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(54) Title: COMPOSITIONS AND METHODS FOR TREATING RENAL INJURY

(57) Abstract: A method for preventing or treating renal disorder, disease, and/or injury includes administering to the subject and/or kidney a therapeutically effective amount of a 15-PGDH inhibitor.

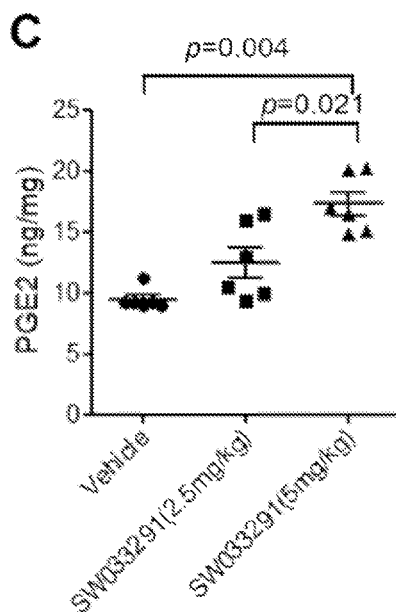


Fig. 1C



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COMPOSITIONS AND METHODS FOR TREATING RENAL INJURY**RELATED APPLICATION**

[0001] This application claims priority from U.S. Provisional Application No. 62/979,813 filed February 21, 2020, the subject matter of which is incorporated herein by reference in its entirety.

BACKGROUND

[0002] Acute kidney injury (AKI) is an important clinical problem associated with high rates of morbidity and mortality (1.7 million deaths annually). Considerable effort has been directed toward the development of preventive strategies for AKI using various agents and animal models. Despite advances in prevention strategies, no specific treatment for AKI has yet been developed.

[0003] The main causes of AKI are hypoxia and oxidative stress due to renal ischemic reperfusion injury (IRI). During periods of transient reduction in renal blood flow (RBF), an insufficient oxygen supply can cause energy impairment (ATP depletion) in the renal outer medulla, resulting in the injury and death of the tubular epithelial cells due to acute tubular necrosis (ATN) and apoptosis. The inflammation due to oxygen-free radicals after reperfusion leads to the extension phase of ischemic AKI. Resistance to hypoxia and the reduction of oxidative stress are treatment targets for ischemic AKI.

SUMMARY

[0004] Embodiments described herein relate to compositions and methods of preventing, treating, or reducing the severity of a renal disorder, disease, and/or injury. It was found that administration of a 15-PGDH inhibitor to a subject prior to and/or after renal injury can induce renal vasodilation, and enhance resistance to hypoxia resulting in a prophylactic and protective effect against renal injury. These benefits can be associated with a prophylactic use of as little as a single dose of a 15-PGDH inhibitor. Administration of a 15-PGDH inhibitor to a subject having or at risk of a renal injury improved renal hemodynamics, decreased induction of oxidative stress, reduced induction of inflammation, attenuated multiple markers of renal damage and preserved renal function. Moreover, administration of a 15-PGDH inhibitor before and/or after administration of a contrast media was found to prevent and/or treat contrast-induced acute kidney injury (CIAKI).

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Accordingly, in some embodiments, compositions and methods of inhibiting 15-PGDH activity can be used to prevent, treat, or reduce the severity of a renal disorder, disease, and/or injury in a subject and/or kidney of a subject in need thereof.

[0005] Examples of renal disorders, diseases, and/or injuries that can be treated include hypotensive injury to the kidney; hypertensive renal disease; diabetic renal disease and diabetic nephropathy; renal disease from vasculitis and autoimmune diseases, including but not limited to lupus erythematosus, polyarteritis, Wegeners' Granulomatosis, and mixed connective tissue disease; ischemic renal injury; acute renal failure; chronic renal failure; glomerulonephritis; nephrotic syndrome; acute tubular necrosis; nephrosclerosis; glomerulosclerosis; minimal change disease; idiopathic membranous nephropathy; membranoproliferative glomerulonephritis; Berger's disease; mesangial proliferative glomerulonephritis; chronic glomerulonephritis; focal glomerulosclerosis; renal effects of Sjogren's syndrome; renal effects of scleroderma; interstitial nephritis; and renal injury to a kidney donor, transplant recipient, and/or transplanted kidney following kidney transplant.

[0006] In other embodiments, the 15-PGDH inhibitor can prevent or treat acute kidney injury (AKI) associated with renal ischemia reperfusion injury (IRI).

[0007] In some embodiments, the amount of 15-PGDH inhibitor administered to a subject can be an amount effective to induce endogenous renal PGE₂ levels of the subject.

[0008] In other embodiments, the amount of 15-PGDH inhibitor administered to a subject can be an amount effective to induce renal vasodilatation, enhance resistance to hypoxia, improve renal hemodynamics, decrease renal oxidative stress, reduce renal inflammation, and preserve renal function.

[0009] In other embodiments, the amount of 15-PGDH inhibitor administered to a subject is an amount effective to reduce malondialdehyde (MDA) and NGAL levels, attenuate medulla tubular damage, reduce medulla acute tubular necrosis (ATN) and apoptosis, reduce induction of high-mobility group box 1 (HMGB1) and proinflammatory cytokines, induce renal EP4 PGE₂ receptors and A_{2A} adenosine receptors in vascular smooth muscle cells that regulate renal arterioles, increase renal cAMP, AMP, and adenosine levels, and/or inhibit induction of creatinine and KIM-1.

[0010] In other embodiments, the 15-PGDH inhibitor can be administered to a subject before a renal injury. For example, the 15-PGDH inhibitor can be administered at a range of

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about 1 minute to about 72 hours, about 10 minutes to about 48 hours, or about 30 minutes to about 36 hours before a renal disorder, disease, and/or injury.

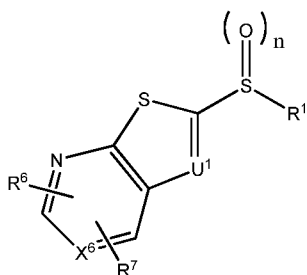
[0011] In other embodiments, the 15-PGDH inhibitor can be administered at a time selected from less than about 2 hours, less than about 8 hours, less than about 24 hours, and less than about 26 hours before the renal disorder, disease, and/or injury.

[0012] In some embodiments, the renal disorder, disease, and/or injury is associated with an organ transplant, such as a kidney transplant, in the subject.

[0013] In other embodiments, the renal disorder, disease, and/or injury is associated with cardiovascular surgery or sepsis.

[0014] In some embodiments, the renal disorder, disease, and/or injury is a contrast-induced acute kidney injury (CIAKI).

[0015] In some embodiments, the 15-PGDH inhibitor can include a compound having the following formula (V):



(V) or a pharmaceutically acceptable salt, tautomer,

or solvate thereof,

wherein n is 0-2

X⁶ is independently is N or CR^c

R¹, R⁶, R⁷, and R^c are the same or different each independently hydrogen or a substituted or unsubstituted group selected from C₁-C₂₄ alkyl, C₂-C₂₄ alkenyl, C₂-C₂₄ alkynyl, C₃-C₂₀ aryl, heteroaryl, heterocycloalkenyl containing from 5-6 ring atoms, C₆-C₂₄ alkaryl, C₆-C₂₄ aralkyl, halo, -Si(C₁-C₃ alkyl)₃, hydroxyl, sulfhydryl, C₁-C₂₄ alkoxy, C₂-C₂₄ alkenyloxy, C₂-C₂₄ alkynyloxy, C₅-C₂₀ aryloxy, acyl, acyloxy, C₂-C₂₄ alkoxycarbonyl, C₆-C₂₀ aryloxy, C₂-C₂₄ alkylcarbonato, C₆-C₂₀ arylcarbonato, carboxy, carboxylato, carbamoyl, C₁-C₂₄ alkyl-carbamoyl, arylcarbamoyl, thiocarbamoyl, carbamido, cyano, isocyano, cyanato, isocyanato, isothiocyanato, azido, formyl, thioformyl, amino, C₁-C₂₄ alkyl amino, C₅-C₂₀ aryl amino, C₂-C₂₄ alkylamido, C₂-C₂₄ alkylamido substituted with a hydroxyl, C₆-C₂₀ arylamido, imino, alkylimino, arylimino, nitro, nitroso, sulfo, sulfonato, C₁-C₂₄

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alkylsulfanyl, arylsulfanyl, C₁-C₂₄ alkylsulfinyl, C₅-C₂₀ arylsulfinyl, C₁-C₂₄ alkylsulfonyl, C₅-C₂₀ arylsulfonyl, sulfonamide, phosphono, phosphonato, phosphinato, phospho, phosphino, polyalkylethers, phosphates, and phosphate esters, groups incorporating amino acids or other moieties expected to bear positive or negative charge at physiological pH, and combinations thereof, and wherein R⁶ and R⁷ may be linked to form a cyclic or polycyclic ring, wherein the ring is a substituted or unsubstituted aryl, a substituted or unsubstituted heteroaryl, a substituted or unsubstituted cycloalkyl, and a substituted or unsubstituted heterocyclyl; and

U¹ is N, C-R², or C-NR³R⁴, wherein R² is selected from the group consisting of a H, a lower alkyl group, O, (CH₂)_{n1}OR' (wherein n1=1, 2, or 3), CF₃, CH₂-CH₂X, O-CH₂-CH₂X, CH₂-CH₂-CH₂X, O-CH₂-CH₂X, X, (wherein X=H, F, Cl, Br, or I), CN, (C=O)-R', (C=O)N(R')₂, O(CO)R', COOR' (wherein R' is H or a lower alkyl group), and wherein R¹ and R² may be linked to form a cyclic or polycyclic ring, wherein R³ and R⁴ are the same or different and are each selected from the group consisting of H, a lower alkyl group, O, (CH₂)_{n1}OR' (wherein n1=1, 2, or 3), CF₃, CH₂-CH₂X, CH₂-CH₂-CH₂X, (wherein X=H, F, Cl, Br, or I), CN, (C=O)-R', (C=O)N(R')₂, COOR' (wherein R' is H or a lower alkyl group), and R³ or R⁴ may be absent.

[0016] In some embodiments, the 15-PGDH inhibitor can inhibit the enzymatic activity of recombinant 15-PGDH at an IC₅₀ of less than 1 μM, or preferably at an IC₅₀ of less than 250 nM, or more preferably at an IC₅₀ of less than 50 nM, or more preferably at an IC₅₀ of less than 10 nM, or more preferably at an IC₅₀ of less than 5 nM at a recombinant 15-PGDH concentration of about 5 nM to about 10 nM.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] Figs. 1(A-I) illustrate a schematic and plots showing 15-hydroxyprostaglandin dehydrogenase (15-PGDH) inhibition with renal ischemia-reperfusion (I/R) injury (IRI) decreases levels of renal injury biomarkers. A: the arachidonic acid prostaglandin (PG) biosynthesis pathway and biological activity of 15-PGDH inhibitor. Cox, cyclooxygenase; NSAIDs, nonsteroidal anti-inflammatory drugs. B: chemical structure of SW033291. C: pharmacological inhibition of 15-PGDH with SW033291 was confirmed by endogenous PGE₂ levels in kidney tissue at 3 h after intraperitoneal injection of 2.5 or 5 mg/kg SW033291 or vehicle. D: PGE₂ levels in kidney tissue at 1 and 3 h after intraperitoneal injection of 5 mg/kg SW033291 or vehicle. E: experimental setup. Mice were subjected to

bilateral renal IRI for 30 min and were injected with vehicle, SW033291, or indo- methacin at 1 h before, immediately after, and 12 h after renal IRI. F I: serum levels of blood urea nitrogen (BUN; F), creatinine (G), neutrophil gelatinase-associ- ated lipocalin (NGAL; H), and kidney injury molecule 1 (KIM-1; I). Renal function was evaluated at 24 h after renal IRI. n = 8–15 animals/group. Data are presented as means $\pm$ SE. Analysis was performed using Student's t test.

[0018] Figs. 2(A-E) illustrate images and plots showing 15-hydroxyprostaglandin dehydrogenase inhibition ameliorates renal tubular cell death in mice with ischemic acute kidney injury. Before and after renal ischemia-reperfusion injury (IRI), mice were injected intraperitoneally three times with vehicle, SW033291 (5 mg/kg), or indomethacin (5 mg/kg). Assessments were performed at 24 h after renal IRI. A: representative gross appearance of the right (Rt) and left (Lt) kidneys of mice injected with vehicle (IRI-vehicle), indomethacin (IRI-indomethacin), or SW033291 (IRI-SW033291) before and after renal IRI. Vascular congestion in the renal medulla is indicated by white arrows. B: representative image of tubular injury in the outer zone of the renal medulla (hematoxylin and eosin staining). Scale bars in the enlarged images = 50 μ m; scale bars in insets = 500 μ m. C: statistical analysis of tubular injury scores (n = 20 per group). D: representative images of apoptosis in the outer zone of the renal medulla (TUNEL staining). Scale bar in the enlarged images = 25 μ m; scale bars in insets = 500 μ m; E: statistical analysis of apoptosis (n = 20 per group). Data are presented as means $\pm$ SE. Analysis was performed using Student's t test.

[0019] Figs. 3(A-H) illustrate plots and a western blot showing 15-hydroxyprostaglandin dehydrogenase inhibitor pretreatment ameliorates the inflammatory response in mice with ischemic acute kidney injury. Before and after renal ischemia-reperfusion injury (IRI), mice were injected intraperitoneally three times with vehicle, SW033291 (5 mg/kg), or indomethacin (5 mg/kg). Assessments were performed at 24 h after renal IRI. A F: proinflammatory cytokine mRNA by real-time PCR (A–C) and protein levels by ELISA (D–F). A and D: IL-17; B and E: TNF- α ; C and F: IL-1 β . G: Western blots of high-mobility group box 1 (HMGB1; 29 kDa) in kidney tissue (representative of three experiments). H: statistical analysis of HMGB1 levels in kidney tissue (n = 9 per group). n = 9 animals/group. Data are presented as means $\pm$ SE. Analysis was performed using Student's t test.

[0020] Figs. 4(A-D) illustrate plots and images showing 15-hydroxyprostaglandin dehydrogenase inhibition induces renal vasodilation in the outer medulla of mice with

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ischemic acute kidney injury. To quantify vasodilation, the inner arteriolar area in the outer medulla was identified by α -smooth muscle actin staining. Assessments were performed at postoperative day 1 (POD1), 24 h after renal ischemia-reperfusion injury (IRI). A: representative images of the change in renal blood flow, as assessed by renal Doppler flux, with administration of vehicle, indomethacin, and SW033291. B: statistical analysis of renal blood flow in sham animals at time 0, in sham animals administered SW033291 (sham-SW033291) at 1 h post-administration of drug, and in cohorts subject to IRI and administered vehicle, indomethacin, and SW033291, which were then assayed at 24 h post-IRI. C: representative images of an arteriole in the outer zone of the renal medulla. Zoomed images are enlargements of the outlined areas. Scale bars in the enlarged images = 50 μ m; scale bars in insets = 500 μ m. D: statistical analysis of the inner arteriolar area of the outer medulla. Data are presented as means $\pm$ SE. Analysis was performed using Student's t test.

[0021] Figs. 5(A-G) illustrate plots, a western blot, and images showing 15-hydroxyprostaglandin dehydrogenase inhibitor promoted adenosine production and upregulated the expression of adenosine A_{2A} receptors in the renal arterioles in the outer medulla via the cAMP/AMP signaling pathway. Assessments were performed at 24 h after renal ischemia-reperfusion injury (IRI). A and B: statistical analysis of cAMP (A) and AMP levels (B) in kidney tissue. n = 12–18 animals/group. C and D: statistical analysis of renal (C) and serum (D) adenosine levels. n = 6–10 animals/group. E: Western blots for A_{2A} receptor protein (45 kDa) in kidney tissue (representative of three experiments). F: statistical analysis of A_{2A} receptor protein levels in kidney tissue (n = 9 per group). G: representative confocal microscopy images of kidneys immunostained for A_{2A} receptor and α -smooth muscle actin (α -SMA). A_{2A} receptor-positive cells were observed in α -SMA-positive cells in the renal arteriolar outer medulla (yellow color). * α -SMA-positive renal arterioles in the outer medullar. Scale bars = 25 μ m. Data are presented as means $\pm$ SE. Analysis was performed using Student's t test.

[0022] Figs. 6(A-L) illustrate plots, a western blot, and images showing 15-hydroxyprostaglandin dehydrogenase inhibitor treatment promoted the expression of EP₄ receptors in the renal arteriolar outer medulla. Assessments were performed at 24 h after renal ischemia-reperfusion injury (IRI). A D: statistical analysis of EP₁ (A), EP₂ (B), EP₃ (C), and EP₄ (D) receptor mRNA levels in kidney tissue by real-time PCR. n = 6–10 animals/group. E: Western blots for EP₄ receptor protein (73 kDa) in kidney tissue

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(representative of three experiments). F: statistical analysis of EP₄ receptor protein levels in kidney tissue (n = 8 per group except sham, where n = 6). G: representative confocal microscopy images for EP₄ receptors (green), α -smooth muscle actin (α -SMA; red), and DAPI (blue)-stained kidney sections. EP₄ receptor-positive cells were observed in α -SMA-positive cells in the renal arteriolar outer medulla (arrow). * α -SMA-positive renal arterioles in the outer medulla. Scale bars = 25 μ m. H–L: effects of the EP₄ inhibitor ONO-AE3-208 on SW033291 amelioration of IRI-induced renal injury. Data are presented as means $\pm$ SE. Analysis was performed using Student's t test.

[0023] Figs. 7(A-F) illustrate plots showing 15-hydroxyprostaglandin dehydrogenase inhibitor pretreatment mitigates renal dysfunction after renal ischemia-reperfusion injury (IRI). A: experimental setup for the three different injection protocols. Mice were injected with vehicle or SW033291 (5 mg/kg) according to three different injection protocols. Renal function was assessed at 24 h after renal IRI. B–D: serum levels of blood urea nitrogen (BUN; B), creatinine (C), neutrophil gelatinase-associated lipocalin (NGAL; D), and kidney injury molecule-1 (KIM-1; E). n = 9–11 animals/group. F: renal blood flow. Data are presented as means $\pm$ SE. Analysis was performed with Student's t test.

[0024] Figs. 8(A-H) illustrate plots showing pretreatment with a single dose of 15-hydroxyprostaglandin dehydrogenase inhibitor attenuates the increase of PGE₂ level and renal damage after renal ischemia-reperfusion injury (IRI). Shown are expression changes of related factors in renal tissue or serum after ischemic acute kidney injury. A: malondialdehyde (MDA) levels in kidney tissue. B–D: neutrophil gelatinase-associated lipocalin (NGAL; B), kidney injury molecule-1 (KIM-1; C), and creatinine (D) levels in serum. E and F: renal PGE₂ levels (E) and serum PGE₂ levels (F). G and H: EP₄ receptor (G) and A_{2A} receptor (H) mRNA levels in kidney tissue. n = 4–8 animals/group. Data are presented as means $\pm$ SE. Analysis was performed using Student's t test.

[0025] Fig. 9 illustrates a schematic mechanism of intrarenal vasodilation by the 15-hydroxyprostaglandin dehydrogenase (15-PGDH) inhibitor (PGDH-i) in ischemic acute kidney injury. 15-PGDH inhibitor pretreatment increases endogenous PGE₂ by inhibiting degradation of PGE₂ prior to an ischemic event in the kidney. Endogenous PGE₂ induces vasodilation through the activated EP₄ receptor. Activation of EP₄ receptors increases the intracellular cAMP level in vascular smooth muscle cells and the effect on vasodilation. Increased cAMP is converted to the adenosine (ADO) substrate AMP, which, in turn,

increases the endovascular adenosine level. ADO activates A_{2A} receptors to induce vasodilation. On the other hand, nonsteroidal anti-inflammatory drugs (NSAIDs), cyclooxygenase (cox) inhibitors, lead to vasoconstriction, in contrast to the hemodynamic effects of 15-PGDH inhibitors. AA, arachidonic acid; ePDE, extracellular phosphodiesterase; RBC, red blood cell.

[0026] Fig. 10 (A-E) illustrate a table, schematic, and graphs showing an establishment of contrast induced acute kidney injury (CIAKI).

[0027] Figs. 11(A-E) illustrate plots and a schematic showing 15-hydroxyprostaglandin dehydrogenase (15-PGDH) inhibition of contrast-induced acute kidney injury decreases the levels of renal injury biomarkers. (A) Prostaglandin E_2 (PGE₂) levels in kidney tissue 1 h after intraperitoneal injection of 5 mg/kg SW033291 or a vehicle. (B) Experimental setup: mice received SW033291, PGE₁, PGE₂, or vehicle at 1 h before, immediately after, and 8, 16, and 24 h after administration of 10 gI/kg iodixanol. (C–E) Serum levels of creatinine, neutrophil gelatinase-associated lipocalin (NGAL), and kidney injury molecule-1 (KIM-1), respectively. Renal function was evaluated at 48 h after contrast medium (CM) injection. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

[0028] Figs. 12(A-E) illustrate images and a plot showing 15-hydroxyprostaglandin dehydrogenase inhibition ameliorates renal tubular cell death in contrast-induced acute kidney injury mice. Before and after contrast medium (CM) administration, mice were injected intraperitoneally with a vehicle, SW033291 (15-PGDH inhibitor; 5 mg/kg), prostaglandin E_1 (PGE₁; 20 mg/kg), or PGE₂ (5 mg/kg). Assessments were performed at 48 h after intravenous CM injection. (A) Representative gross appearances of the left and right kidneys of normal mice, and of those after injection with CM vehicle, CM SW033291, CM PGE₁, or CM PGE₂. Renal congestion in the outer medullary region is indicated by a black arrow. (B) Representative images of tubular injury in the outer zone of the renal medulla (hematoxylin and eosin staining). Scale bars in small panels, 500 μ m and those in enlarged images, 50 μ m. (C) Statistical analyses of tubular injury scores ($n = 20$ per group). (D) Representative images of apoptosis in the outer zone of the renal medulla (terminal deoxynucleotidyl transferase dUTP nick end labeling staining). Scale bars in small panels, 500 μ m, those in enlarged images, 25 μ m. (E) Statistical analyses of apoptosis ($n = 20$ per group). * $p < 0.05$; ** $p < 0.001$.

[0029] Figs. 13(A-C) illustrate images and a graph showing 15-hydroxyprostaglandin dehydrogenase inhibitor effects on iodixanol-induced apoptosis in human renal proximal tubular epithelial cells (hRPTECs). hRPTECs were treated with SW033291 (15-PGDH inhibitor), prostaglandin E₁ (PGE₁), or PGE₂ simultaneously with Visipaque (iodixanol 50 mgI/mL). (A) Representative pictures of hRPTECs viability 24 h after Visipaque (iodixanol 50 mgI/mL) treatment. (B) Quantification of hRPTEC viability by MTT assay. (C) Quantification of hRPTECs apoptosis by flow cytometry; *p < 0.05; **p < 0.001. CM: contrast medium.

[0030] Figs. 14(A-F) illustrate images and plots showing 15-hydroxyprostaglandin dehydrogenase inhibition induces renal vasodilation in the outer medulla via the adenosine monophosphate (AMP)–adenosine signaling pathway. (A) Representative images of arterioles in the outer zone of the renal medulla. Magnified images are enlargements of the outlined areas. (B) Statistical analyses of the inner arteriole area of the outer medulla. (C) Statistical analyses of renal blood flow following administration of a vehicle, SW033291 (inhibitor), prostaglandin E₁ (PGE₁), or PGE₂ in contrast-induced acute kidney injury mice. (D) Representative images of renal blood flux measurements of the study groups. (E, F) Statistical analyses of AMP and adenosine levels in kidney tissue. *p < 0.05; **p < 0.01; ***p < 0.001. Scale bars, 500 μ m; scale bar in the enlarged image, 50 μ m CM: contrast medium.

[0031] Fig. 15(A-H) illustrate plots showing 15-hydroxyprostaglandin dehydrogenase inhibition changes the level of prostaglandin E₂ (PGE₂) receptor (EP) expression in kidney tissue. The protective effects of the 15-PGDH inhibitor, SW033291, can be blocked by an EP4 antagonist, ONO-AE3-208. (A–D) Statistical analyses of the EP expression level. (E–G) Serum levels of creatinine, neutrophil gelatinase-associated lipocalin (NGAL), and kidney injury molecule-1 (KIM-1) due to the absence or presence of ONO-AE3-208 in the contrast-induced acute kidney injury model. (H) Statistical analyses of renal blood flow. p < 0.05; p < 0.01; p < 0.001; CM: contrast medium.

DETAILED DESCRIPTION

[0032] While the following terms are believed to be well understood by one of ordinary skill in the art, the following definitions are set forth to facilitate explanation of the presently disclosed subject matter.

[0033] As used herein, the verb “comprise” as is used in this description and in the claims and its conjugations are used in its non-limiting sense to mean that items following the word are included, but items not specifically mentioned are not excluded. The present invention may suitably “comprise”, “consist of”, or “consist essentially of”, the steps, elements, and/or reagents described in the claims.

[0034] It is further noted that the claims may be drafted to exclude any optional element. As such, this statement is intended to serve as antecedent basis for use of such exclusive terminology as "solely", "only" and the like in connection with the recitation of claim elements, or the use of a "negative" limitation.

[0035] The term “pharmaceutically acceptable” means suitable for use in contact with the tissues of humans and animals without undue toxicity, irritation, allergic response, and the like, commensurate with a reasonable benefit/risk ratio, and effective for their intended use within the scope of sound medical judgment.

[0036] The term “pharmaceutically acceptable salt” include those obtained by reacting the active compound functioning as a base, with an inorganic or organic acid to form a salt, for example, salts of hydrochloric acid, sulfuric acid, phosphoric acid, methanesulfonic acid, camphorsulfonic acid, oxalic acid, maleic acid, succinic acid, citric acid, formic acid, hydrobromic acid, benzoic acid, tartaric acid, fumaric acid, salicylic acid, mandelic acid, carbonic acid, etc. Those skilled in the art will further recognize that acid addition salts may be prepared by reaction of the compounds with the appropriate inorganic or organic acid via any of a number of known methods. The term “pharmaceutically acceptable salts” also includes those obtained by reacting the active compound functioning as an acid, with an inorganic or organic base to form a salt, for example salts of ethylenediamine, N-methyl-glucamine, lysine, arginine, ornithine, choline, N,N'-dibenzylethylenediamine, chloroprocaine, diethanolamine, procaine, N-benzylphenethylamine, diethylamine, piperazine, tris-(hydroxymethyl)-aminomethane, tetramethylammonium hydroxide, triethylamine, dibenzylamine, ephedrine, dehydroabietylamine, N-ethylpiperidine, benzylamine, tetramethylammonium, tetraethylammonium, methylamine, dimethylamine, trimethylamine, ethylamine, basic amino acids, and the like. Non limiting examples of inorganic or metal salts include lithium, sodium, calcium, potassium, magnesium salts and the like.

[0037] Additionally, the salts of the compounds described herein, can exist in either hydrated or unhydrated (the anhydrous) form or as solvates with other solvent molecules. Non-limiting examples of hydrates include monohydrates, dihydrates, etc. Nonlimiting examples of solvates include ethanol solvates, acetone solvates, etc.

[0038] The term "solvates" means solvent addition forms that contain either stoichiometric or non-stoichiometric amounts of solvent. Some compounds have a tendency to trap a fixed molar ratio of solvent molecules in the crystalline solid state, thus forming a solvate. If the solvent is water the solvate formed is a hydrate, when the solvent is alcohol, the solvate formed is an alcoholate. Hydrates are formed by the combination of one or more molecules of water with one of the substances in which the water retains its molecular state as H₂O, such combination being able to form one or more hydrate.

[0039] The compounds and salts described herein can exist in several tautomeric forms, including the enol and imine form, and the keto and enamine form and geometric isomers and mixtures thereof. Tautomers exist as mixtures of a tautomeric set in solution. In solid form, usually one tautomer predominates. Even though one tautomer may be described, the present application includes all tautomers of the present compounds. A tautomer is one of two or more structural isomers that exist in equilibrium and are readily converted from one isomeric form to another. This reaction results in the formal migration of a hydrogen atom accompanied by a switch of adjacent conjugated double bonds. In solutions where tautomerization is possible, a chemical equilibrium of the tautomers will be reached. The exact ratio of the tautomers depends on several factors, including temperature, solvent, and pH. The concept of tautomers that are interconvertible by tautomerizations is called tautomerism.

[0040] Of the various types of tautomerism that are possible, two are commonly observed. In keto-enol tautomerism a simultaneous shift of electrons and a hydrogen atom occurs.

[0041] Tautomerizations can be catalyzed by: Base: 1. deprotonation; 2. formation of a delocalized anion (*e.g.*, an enolate); 3. protonation at a different position of the anion; Acid: 1. protonation; 2. formation of a delocalized cation; 3. deprotonation at a different position adjacent to the cation.

[0042] The terms below, as used herein, have the following meanings, unless indicated otherwise:

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“Amino” refers to the -NH_2 radical.

“Cyano” refers to the -CN radical.

“Halo” or “halogen” refers to bromo, chloro, fluoro or iodo radical.

“Hydroxy” or “hydroxyl” refers to the -OH radical.

“Imino” refers to the =NH substituent.

“Nitro” refers to the -NO_2 radical.

“Oxo” refers to the =O substituent.

“Thioxo” refers to the =S substituent.

[0043] “Alkyl” or “alkyl group” refers to a fully saturated, straight or branched hydrocarbon chain radical having from one to twelve carbon atoms, and which is attached to the rest of the molecule by a single bond. Alkyls comprising any number of carbon atoms from 1 to 12 are included. An alkyl comprising up to 12 carbon atoms is a $\text{C}_1\text{-C}_{12}$ alkyl, an alkyl comprising up to 10 carbon atoms is a $\text{C}_1\text{-C}_{10}$ alkyl, an alkyl comprising up to 6 carbon atoms is a $\text{C}_1\text{-C}_6$ alkyl and an alkyl comprising up to 5 carbon atoms is a $\text{C}_1\text{-C}_5$ alkyl. A $\text{C}_1\text{-C}_5$ alkyl includes C_5 alkyls, C_4 alkyls, C_3 alkyls, C_2 alkyls and C_1 alkyl (i.e., methyl). A $\text{C}_1\text{-C}_6$ alkyl includes all moieties described above for $\text{C}_1\text{-C}_5$ alkyls but also includes C_6 alkyls. A $\text{C}_1\text{-C}_{10}$ alkyl includes all moieties described above for $\text{C}_1\text{-C}_5$ alkyls and $\text{C}_1\text{-C}_6$ alkyls, but also includes C_7 , C_8 , C_9 and C_{10} alkyls. Similarly, a $\text{C}_1\text{-C}_{12}$ alkyl includes all the foregoing moieties, but also includes C_{11} and C_{12} alkyls. Non-limiting examples of $\text{C}_1\text{-C}_{12}$ alkyl include methyl, ethyl, n-propyl, i-propyl, sec-propyl, n-butyl, i-butyl, sec-butyl, t-butyl, n-pentyl, t-amyl, n-hexyl, n-heptyl, n-octyl, n-nonyl, n-decyl, n-undecyl, and n-dodecyl. Unless stated otherwise specifically in the specification, an alkyl group can be optionally substituted.

[0044] “Alkylene” or “alkylene chain” refers to a fully saturated, straight or branched divalent hydrocarbon chain radical, and having from one to twelve carbon atoms. Non-limiting examples of $\text{C}_1\text{-C}_{12}$ alkylene include methylene, ethylene, propylene, *n*-butylene, ethenylene, propenylene, *n*-butenylene, propynylene, *n*-butynylene, and the like. The alkylene chain is attached to the rest of the molecule through a single bond and to the radical group through a single bond. The points of attachment of the alkylene chain to the rest of the molecule and to the radical group can be through one carbon or any two carbons within the chain. Unless stated otherwise specifically in the specification, an alkylene chain can be optionally substituted.

[0045] “Alkenyl” or “alkenyl group” refers to a straight or branched hydrocarbon chain radical having from two to twelve carbon atoms, and having one or more carbon-carbon double bonds. Each alkenyl group is attached to the rest of the molecule by a single bond. Alkenyl group comprising any number of carbon atoms from 2 to 12 are included. An alkenyl group comprising up to 12 carbon atoms is a C₂-C₁₂ alkenyl, an alkenyl comprising up to 10 carbon atoms is a C₂-C₁₀ alkenyl, an alkenyl group comprising up to 6 carbon atoms is a C₂-C₆ alkenyl and an alkenyl comprising up to 5 carbon atoms is a C₂-C₅ alkenyl. A C₂-C₅ alkenyl includes C₅ alkenyls, C₄ alkenyls, C₃ alkenyls, and C₂ alkenyls. A C₂-C₆ alkenyl includes all moieties described above for C₂-C₅ alkenyls but also includes C₆ alkenyls. A C₂-C₁₀ alkenyl includes all moieties described above for C₂-C₅ alkenyls and C₂-C₆ alkenyls, but also includes C₇, C₈, C₉ and C₁₀ alkenyls. Similarly, a C₂-C₁₂ alkenyl includes all the foregoing moieties, but also includes C₁₁ and C₁₂ alkenyls. Non-limiting examples of C₂-C₁₂ alkenyl include ethenyl (vinyl), 1-propenyl, 2-propenyl (allyl), iso-propenyl, 2-methyl-1-propenyl, 1-butenyl, 2-butenyl, 3-butenyl, 1-pentenyl, 2-pentenyl, 3-pentenyl, 4-pentenyl, 1-hexenyl, 2-hexenyl, 3-hexenyl, 4-hexenyl, 5-hexenyl, 1-heptenyl, 2-heptenyl, 3-heptenyl, 4-heptenyl, 5-heptenyl, 6-heptenyl, 1-octenyl, 2-octenyl, 3-octenyl, 4-octenyl, 5-octenyl, 6-octenyl, 7-octenyl, 1-nonenyl, 2-nonenyl, 3-nonenyl, 4-nonenyl, 5-nonenyl, 6-nonenyl, 7-nonenyl, 8-nonenyl, 1-decenyl, 2-decenyl, 3-decenyl, 4-decenyl, 5-decenyl, 6-decenyl, 7-decenyl, 8-decenyl, 9-decenyl, 1-undecenyl, 2-undecenyl, 3-undecenyl, 4-undecenyl, 5-undecenyl, 6-undecenyl, 7-undecenyl, 8-undecenyl, 9-undecenyl, 10-undecenyl, 1-dodecenyl, 2-dodecenyl, 3-dodecenyl, 4-dodecenyl, 5-dodecenyl, 6-dodecenyl, 7-dodecenyl, 8-dodecenyl, 9-dodecenyl, 10-dodecenyl, and 11-dodecenyl. Unless stated otherwise specifically in the specification, an alkyl group can be optionally substituted.

[0046] “Alkenylene” or “alkenylene chain” refers to a straight or branched divalent hydrocarbon chain radical, having from two to twelve carbon atoms, and having one or more carbon-carbon double bonds. Non-limiting examples of C₂-C₁₂ alkenylene include ethene, propene, butene, and the like. The alkenylene chain is attached to the rest of the molecule through a single bond and to the radical group through a single bond. The points of attachment of the alkenylene chain to the rest of the molecule and to the radical group can be through one carbon or any two carbons within the chain. Unless stated otherwise specifically in the specification, an alkenylene chain can be optionally substituted.

[0047] “Alkynyl” or “alkynyl group” refers to a straight or branched hydrocarbon chain radical having from two to twelve carbon atoms, and having one or more carbon-carbon triple bonds. Each alkynyl group is attached to the rest of the molecule by a single bond. Alkynyl group comprising any number of carbon atoms from 2 to 12 are included. An alkynyl group comprising up to 12 carbon atoms is a C₂-C₁₂ alkynyl, an alkynyl comprising up to 10 carbon atoms is a C₂-C₁₀ alkynyl, an alkynyl group comprising up to 6 carbon atoms is a C₂-C₆ alkynyl and an alkynyl comprising up to 5 carbon atoms is a C₂-C₅ alkynyl. A C₂-C₅ alkynyl includes C₅ alkynyls, C₄ alkynyls, C₃ alkynyls, and C₂ alkynyls. A C₂-C₆ alkynyl includes all moieties described above for C₂-C₅ alkynyls but also includes C₆ alkynyls. A C₂-C₁₀ alkynyl includes all moieties described above for C₂-C₅ alkynyls and C₂-C₆ alkynyls, but also includes C₇, C₈, C₉ and C₁₀ alkynyls. Similarly, a C₂-C₁₂ alkynyl includes all the foregoing moieties, but also includes C₁₁ and C₁₂ alkynyls. Non-limiting examples of C₂-C₁₂ alkenyl include ethynyl, propynyl, butynyl, pentynyl and the like. Unless stated otherwise specifically in the specification, an alkyl group can be optionally substituted.

[0048] “Alkynylene” or “alkynylene chain” refers to a straight or branched divalent hydrocarbon chain radical, having from two to twelve carbon atoms, and having one or more carbon-carbon triple bonds. Non-limiting examples of C₂-C₁₂ alkynylene include ethynylene, propargylene and the like. The alkynylene chain is attached to the rest of the molecule through a single bond and to the radical group through a single bond. The points of attachment of the alkynylene chain to the rest of the molecule and to the radical group can be through one carbon or any two carbons within the chain. Unless stated otherwise specifically in the specification, an alkynylene chain can be optionally substituted.

[0049] “Alkoxy” refers to a radical of the formula -OR_a where R_a is an alkyl, alkenyl or alkynyl radical as defined above containing one to twelve carbon atoms. Unless stated otherwise specifically in the specification, an alkoxy group can be optionally substituted.

[0050] “Alkylamino” refers to a radical of the formula -NHR_a or -NR_aR_a where each R_a is, independently, an alkyl, alkenyl or alkynyl radical as defined above containing one to twelve carbon atoms. Unless stated otherwise specifically in the specification, an alkylamino group can be optionally substituted.

[0051] “Alkylcarbonyl” refers to the -C(=O)R_a moiety, wherein R_a is an alkyl, alkenyl or alkynyl radical as defined above. A non-limiting example of an alkyl carbonyl is the methyl carbonyl (“acetal”) moiety. Alkylcarbonyl groups can also be referred to as “C_w-C_z

acyl” where w and z depicts the range of the number of carbon in R_a , as defined above. For example, “ C_1 - C_{10} acyl” refers to alkylcarbonyl group as defined above, where R_a is C_1 - C_{10} alkyl, C_2 - C_{10} alkenyl, or C_2 - C_{10} alkynyl radical as defined above. Unless stated otherwise specifically in the specification, an alkyl carbonyl group can be optionally substituted.

[0052] “Aryl” refers to a hydrocarbon ring system radical comprising hydrogen, 6 to 18 carbon atoms and at least one aromatic ring. For purposes of this invention, the aryl radical can be a monocyclic, bicyclic, tricyclic or tetracyclic ring system, which can include fused or bridged ring systems. Aryl radicals include, but are not limited to, aryl radicals derived from phenyl (benzene), aceanthrylene, acenaphthylene, acephenanthrylene, anthracene, azulene, chrysene, fluoranthene, fluorene, *as*-indacene, *s*-indacene, indane, indene, naphthalene, phenalene, phenanthrene, pleiadene, pyrene, and triphenylene. Unless stated otherwise specifically in the specification, the term “aryl” is meant to include aryl radicals that are optionally substituted.

[0053] “Aralkyl” or “arylalkyl” refers to a radical of the formula $-R_b-R_c$ where R_b is an alkylene group as defined above and R_c is one or more aryl radicals as defined above. Aralkyl radicals include, but are not limited to, benzyl, diphenylmethyl and the like. Unless stated otherwise specifically in the specification, an aralkyl group can be optionally substituted.

[0054] “Aralkenyl” or “arylalkenyl” refers to a radical of the formula $-R_b-R_c$ where R_b is an alkenylene group as defined above and R_c is one or more aryl radicals as defined above. Unless stated otherwise specifically in the specification, an aralkenyl group can be optionally substituted.

[0055] “Aralkynyl” or “arylalkynyl” refers to a radical of the formula $-R_b-R_c$ where R_b is an alkynylene group as defined above and R_c is one or more aryl radicals as defined above. Unless stated otherwise specifically in the specification, an aralkynyl group can be optionally substituted.

[0056] “Carbocyclyl,” “carbocyclic ring” or “carbocycle” refers to a ring structure, wherein the atoms which form the ring are each carbon. Carbocyclic rings can comprise from 3 to 20 carbon atoms in the ring. Carbocyclic rings include aryls and cycloalkyl. Cycloalkenyl and cycloalkynyl as defined herein. Unless stated otherwise specifically in the specification, a carbocyclyl group can be optionally substituted.

[0057] “Cycloalkyl” refers to a stable non-aromatic monocyclic or polycyclic fully saturated hydrocarbon radical consisting solely of carbon and hydrogen atoms, which can include fused, bridged, or spiral ring systems, having from three to twenty carbon atoms, preferably having from three to ten carbon atoms, and which is attached to the rest of the molecule by a single bond. Monocyclic cycloalkyl radicals include, for example, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, and cyclooctyl. Polycyclic cycloalkyl radicals include, for example, adamantyl, norbornyl, decalyl, 7,7-dimethyl-bicyclo[2.2.1]heptyl, and the like. Unless otherwise stated specifically in the specification, a cycloalkyl group can be optionally substituted.

[0058] “Cycloalkenyl” refers to a stable non-aromatic monocyclic or polycyclic hydrocarbon radical consisting solely of carbon and hydrogen atoms, having one or more carbon-carbon double bonds, which can include fused, bridged, or spiral ring systems, having from three to twenty carbon atoms, preferably having from three to ten carbon atoms, and which is attached to the rest of the molecule by a single bond. Monocyclic cycloalkenyl radicals include, for example, cyclopentenyl, cyclohexenyl, cycloheptenyl, cyclooctenyl, and the like. Polycyclic cycloalkenyl radicals include, for example, bicyclo[2.2.1]hept-2-enyl and the like. Unless otherwise stated specifically in the specification, a cycloalkenyl group can be optionally substituted.

[0059] “Cycloalkynyl” refers to a stable non-aromatic monocyclic or polycyclic hydrocarbon radical consisting solely of carbon and hydrogen atoms, having one or more carbon-carbon triple bonds, which can include fused, bridged, or spiral ring systems, having from three to twenty carbon atoms, preferably having from three to ten carbon atoms, and which is attached to the rest of the molecule by a single bond. Monocyclic cycloalkynyl radicals include, for example, cycloheptynyl, cyclooctynyl, and the like. Unless otherwise stated specifically in the specification, a cycloalkynyl group can be optionally substituted.

[0060] “Cycloalkylalkyl” refers to a radical of the formula $-R_b-R_d$ where R_b is an alkylene, alkenylene, or alkynylene group as defined above and R_d is a cycloalkyl, cycloalkenyl, cycloalkynyl radical as defined above. Unless stated otherwise specifically in the specification, a cycloalkylalkyl group can be optionally substituted.

[0061] “Haloalkyl” refers to an alkyl radical, as defined above, that is substituted by one or more halo radicals, as defined above, *e.g.*, trifluoromethyl, difluoromethyl, trichloromethyl, 2,2,2-trifluoroethyl, 1,2-difluoroethyl, 3-bromo-2-fluoropropyl,

1,2-dibromoethyl, and the like. Unless stated otherwise specifically in the specification, a haloalkyl group can be optionally substituted.

[0062] “Haloalkenyl” refers to an alkenyl radical, as defined above, that is substituted by one or more halo radicals, as defined above, *e.g.*, 1-fluoropropenyl, 1,1-difluorobutenyl, and the like. Unless stated otherwise specifically in the specification, a haloalkenyl group can be optionally substituted.

[0063] “Haloalkynyl” refers to an alkynyl radical, as defined above, that is substituted by one or more halo radicals, as defined above, *e.g.*, 1-fluoropropynyl, 1-fluorobutynyl, and the like. Unless stated otherwise specifically in the specification, a haloalkynyl group can be optionally substituted.

[0064] “Heterocyclyl,” “heterocyclic ring” or “heterocycle” refers to a stable 3- to 20-membered non-aromatic, partially aromatic, or aromatic ring radical which consists of two to twelve carbon atoms and from one to six heteroatoms selected from the group consisting of nitrogen, oxygen and sulfur. Heterocyclyl or heterocyclic rings include heteroaryls as defined below. Unless stated otherwise specifically in the specification, the heterocyclyl radical can be a monocyclic, bicyclic, tricyclic or tetracyclic ring system, which can include fused, bridged, and spiral ring systems; and the nitrogen, carbon or sulfur atoms in the heterocyclyl radical can be optionally oxidized; the nitrogen atom can be optionally quaternized; and the heterocyclyl radical can be partially or fully saturated. Examples of such heterocyclyl radicals include, but are not limited to, aziridinyl, oextanyl, dioxolanyl, thienyl[1,3]dithianyl, decahydroisoquinolyl, imidazolyl, imidazolidinyl, isothiazolidinyl, isoxazolidinyl, morpholyl, octahydroindolyl, octahydroisoindolyl, 2-oxopiperazinyl, 2-oxopiperidinyl, 2-oxopyrrolidinyl, oxazolidinyl, piperidinyl, piperazinyl, 4-piperidonyl, pyrrolidinyl, pyrazolidinyl, quinuclidinyl, thiazolidinyl, tetrahydrofuryl, trithianyl, tetrahydropyranyl, thiomorpholyl, thiamorpholyl, 1-oxo-thiomorpholyl, 1,1-dioxo-thiomorpholyl, pyridine-one, and the like. The point of attachment of the heterocyclyl, heterocyclic ring, or heterocycle to the rest of the molecule by a single bond is through a ring member atom, which can be carbon or nitrogen. Unless stated otherwise specifically in the specification, a heterocyclyl group can be optionally substituted.

[0065] “Heterocyclylalkyl” refers to a radical of the formula $-R_b-R_c$ where R_b is an alkylene group as defined above and R_c is a heterocyclyl radical as defined above. Unless

stated otherwise specifically in the specification, a heterocyclylalkyl group can be optionally substituted.

[0066] “Heterocyclylalkenyl” refers to a radical of the formula $-R_b-R_e$ where R_b is an alkenylene group as defined above and R_e is a heterocyclyl radical as defined above. Unless stated otherwise specifically in the specification, a heterocyclylalkenyl group can be optionally substituted.

[0067] “Heterocyclylalkynyl” refers to a radical of the formula $-R_b-R_e$ where R_b is an alkynylene group as defined above and R_e is a heterocyclyl radical as defined above. Unless stated otherwise specifically in the specification, a heterocyclylalkynyl group can be optionally substituted.

[0068] “*N*-heterocyclyl” refers to a heterocyclyl radical as defined above containing at least one nitrogen and where the point of attachment of the heterocyclyl radical to the rest of the molecule is through a nitrogen atom in the heterocyclyl radical. Unless stated otherwise specifically in the specification, a *N*-heterocyclyl group can be optionally substituted.

[0069] “Heteroaryl” refers to a 5- to 20-membered ring system radical one to thirteen carbon atoms and one to six heteroatoms selected from the group consisting of nitrogen, oxygen and sulfur, as the ring member. For purposes of this invention, the heteroaryl radical can be a monocyclic, bicyclic, tricyclic or tetracyclic ring system, which can include fused or bridged ring systems, wherein at least one ring containing a heteroatom ring member is aromatic. The nitrogen, carbon or sulfur atoms in the heteroaryl radical can be optionally oxidized and the nitrogen atom can be optionally quaternized. Examples include, but are not limited to, azepinyl, acridinyl, benzimidazolyl, benzothiazolyl, benzindolyl, benzodioxolyl, benzofuranyl, benzooxazolyl, benzothiazolyl, benzothiadiaazolyl, benzo[*b*][1,4]dioxepinyl, 1,4-benzodioxanyl, benzonaphthofuranyl, benzoxazolyl, benzodioxolyl, benzodioxinyl, benzopyranyl, benzopyranonyl, benzofuranyl, benzofuranonyl, benzothieryl (benzothiophenyl), benzotriazolyl, benzo[4,6]imidazo[1,2-*a*]pyridinyl, carbazolyl, cinnolinyl, dibenzofuranyl, dibenzothiophenyl, furanyl, furanonyl, isothiazolyl, imidazolyl, indazolyl, indolyl, indazolyl, isoindolyl, indolinyl, isoindolinyl, isoquinolyl, indoliziny, isoxazolyl, naphthyridinyl, oxadiazolyl, 2-oxoazepinyl, oxazolyl, oxiranyl, 1-oxidopyridinyl, 1-oxidopyrimidinyl, 1-oxidopyrazinyl, 1-oxidopyridazinyl, 1-phenyl-1*H*-pyrrolyl, phenazinyl, phenothiazinyl, phenoxazinyl, phthalazinyl, pteridinyl, purinyl, pyrrolyl, pyrazolyl, pyridinyl, pyrazinyl, pyrimidinyl, pyridazinyl, pyrazolopyridine, quinazolinyl,

quinoxaliny, quinoliny, quinuclidiny, isoquinoliny, tetrahydroquinoliny, thiazoly, thiadiazoly, triazoly, tetrazoly, triazinyl, and thiophenyl (*i.e.*, thienyl). Unless stated otherwise specifically in the specification, a heteroaryl group can be optionally substituted.

[0070] “*N*-heteroaryl” refers to a heteroaryl radical as defined above containing at least one nitrogen and where the point of attachment of the heteroaryl radical to the rest of the molecule is through a nitrogen atom in the heteroaryl radical. Unless stated otherwise specifically in the specification, an *N*-heteroaryl group can be optionally substituted.

[0071] “Heteroarylalkyl” refers to a radical of the formula $-R_b-R_f$ where R_b is an alkylene chain as defined above and R_f is a heteroaryl radical as defined above. Unless stated otherwise specifically in the specification, a heteroarylalkyl group can be optionally substituted.

[0072] “Heteroarylalkenyl” refers to a radical of the formula $-R_b-R_f$ where R_b is an alkenylene, chain as defined above and R_f is a heteroaryl radical as defined above. Unless stated otherwise specifically in the specification, a heteroarylalkenyl group can be optionally substituted.

[0073] “Heteroarylalkynyl” refers to a radical of the formula $-R_b-R_f$ where R_b is an alkynylene chain as defined above and R_f is a heteroaryl radical as defined above. Unless stated otherwise specifically in the specification, a heteroarylalkynyl group can be optionally substituted.

[0074] “Thioalkyl” refers to a radical of the formula $-SR_a$ where R_a is an alkyl, alkenyl, or alkynyl radical as defined above containing one to twelve carbon atoms. Unless stated otherwise specifically in the specification, a thioalkyl group can be optionally substituted.

[0075] The term “substituted” used herein means any of the above groups (*e.g.*, alkyl, alkylene, alkenyl, alkenylene, alkynyl, alkynylene, alkoxy, alkylamino, alkylcarbonyl, thioalkyl, aryl, aralkyl, carbocyclyl, cycloalkyl, cycloalkenyl, cycloalkynyl, cycloalkylalkyl, haloalkyl, heterocyclyl, *N*-heterocyclyl, heterocyclylalkyl, heteroaryl, *N*-heteroaryl, heteroarylalkyl, heteroarylalkenyl, heteroarylalkynyl, etc) wherein at least one hydrogen atom is replaced by a bond to a non-hydrogen atoms such as, but not limited to: a halogen atom such as F, Cl, Br, and I; an oxygen atom in groups such as hydroxyl groups, alkoxy groups, and ester groups; a sulfur atom in groups such as thiol groups, thioalkyl groups, sulfone groups, sulfonyl groups, and sulfoxide groups; a nitrogen atom in groups such as amines, amides, alkylamines, dialkylamines, arylamines, alkylarylamines, diarylamines, *N*-oxides,

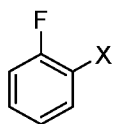
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imides, and enamines; a silicon atom in groups such as trialkylsilyl groups, dialkylarylsilyl groups, alkyl diarylsilyl groups, and triarylsilyl groups; and other heteroatoms in various other groups. “Substituted” also means any of the above groups in which one or more hydrogen atoms are replaced by a higher-order bond (*e.g.*, a double- or triple-bond) to a heteroatom such as oxygen in oxo, carbonyl, carboxyl, and ester groups; and nitrogen in groups such as imines, oximes, hydrazones, and nitriles. For example, “substituted” includes any of the above groups in which one or more hydrogen atoms are replaced with $-NR_gR_h$, $-NR_gC(=O)R_h$, $-NR_gC(=O)NR_gR_h$, $-NR_gC(=O)OR_h$, $-NR_gSO_2R_h$, $-OC(=O)NR_gR_h$, $-OR_g$, $-SR_g$, $-SOR_g$, $-SO_2R_g$, $-OSO_2R_g$, $-SO_2OR_g$, $=NSO_2R_g$, and $-SO_2NR_gR_h$. “Substituted” also means any of the above groups in which one or more hydrogen atoms are replaced with $-C(=O)R_g$, $-C(=O)OR_g$, $-C(=O)NR_gR_h$, $-CH_2SO_2R_g$, $-CH_2SO_2NR_gR_h$. In the foregoing, R_g and R_h are the same or different and independently hydrogen, alkyl, alkenyl, alkynyl, alkoxy, alkylamino, thioalkyl, aryl, aralkyl, cycloalkyl, cycloalkenyl, cycloalkynyl, cycloalkylalkyl, haloalkyl, haloalkenyl, haloalkynyl, heterocyclyl, *N*-heterocyclyl, heterocyclylalkyl, heteroaryl, *N*-heteroaryl and/or heteroarylalkyl. “Substituted” further means any of the above groups in which one or more hydrogen atoms are replaced by a bond to an amino, cyano, hydroxyl, imino, nitro, oxo, thioxo, halo, alkyl, alkenyl, alkynyl, alkoxy, alkylamino, thioalkyl, aryl, aralkyl, cycloalkyl, cycloalkenyl, cycloalkynyl, cycloalkylalkyl, haloalkyl, haloalkenyl, haloalkynyl, heterocyclyl, *N*-heterocyclyl, heterocyclylalkyl, heteroaryl, *N*-heteroaryl and/or heteroarylalkyl group. In addition, each of the foregoing substituents can also be optionally substituted with one or more of the above substituents.

[0076] As used herein, the symbol “” (hereinafter can be referred to as “a point of attachment bond”) denotes a bond that is a point of attachment between two chemical entities, one of which is depicted as being attached to the point of attachment bond and the other of which is not depicted as being attached to the point of attachment bond. For

example, “” indicates that the chemical entity “A” is bonded to another chemical entity via the point of attachment bond. Furthermore, the specific point of attachment to the non-depicted chemical entity can be specified by inference. For example, the compound

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, wherein X is “” infers that the point of attachment bond is the bond by which X is depicted as being attached to the phenyl ring at the ortho position relative to fluorine.

[0077] The phrases "parenteral administration" and "administered parenterally" are art-recognized terms, and include modes of administration other than enteral and topical administration, such as injections, and include, without limitation, intravenous, intramuscular, intrapleural, intravascular, intrapericardial, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intra-articular, subcapsular, subarachnoid, intraspinal and intrastemal injection and infusion.

[0078] The term "treating" is art-recognized and includes inhibiting a disease, disorder or condition in a subject, *e.g.*, impeding its progress; and relieving the disease, disorder or condition, *e.g.*, causing regression of the disease, disorder and/or condition. Treating the disease or condition includes ameliorating at least one symptom of the particular disease or condition, even if the underlying pathophysiology is not affected.

[0079] The term "preventing" is art-recognized and includes stopping a disease, disorder or condition from occurring in a subject, which may be predisposed to the disease, disorder and/or condition but has not yet been diagnosed as having it. Preventing a condition related to a disease includes stopping the condition from occurring after the disease has been diagnosed but before the condition has been diagnosed.

[0080] A "patient," "subject," or "host" to be treated by the subject method may mean either a human or non-human animal, such as a mammal, a fish, a bird, a reptile, or an amphibian. Thus, the subject of the herein disclosed methods can be a human, non-human primate, horse, pig, rabbit, dog, sheep, goat, cow, cat, guinea pig or rodent. The term does not denote a particular age or sex. Thus, adult and newborn subjects, as well as fetuses, whether male or female, are intended to be covered. In one aspect, the subject is a mammal. A patient refers to a subject afflicted with a disease or disorder.

[0081] The terms "prophylactic" or "therapeutic" treatment is art-recognized and includes administration to the host of one or more of the subject compositions. If it is administered prior to clinical manifestation of the unwanted condition (*e.g.*, disease or other

unwanted state of the host animal) then the treatment is prophylactic, *i.e.*, it protects the host against developing the unwanted condition, whereas if it is administered after manifestation of the unwanted condition, the treatment is therapeutic (*i.e.*, it is intended to diminish, ameliorate, or stabilize the existing unwanted condition or side effects thereof).

[0082] The terms "therapeutic agent", "drug", "medicament" and "bioactive substance" are art-recognized and include molecules and other agents that are biologically, physiologically, or pharmacologically active substances that act locally or systemically in a patient or subject to treat a disease or condition. The terms include without limitation pharmaceutically acceptable salts thereof and prodrugs. Such agents may be acidic, basic, or salts; they may be neutral molecules, polar molecules, or molecular complexes capable of hydrogen bonding; they may be prodrugs in the form of ethers, esters, amides and the like that are biologically activated when administered into a patient or subject.

[0083] The phrase "therapeutically effective amount" or "pharmaceutically effective amount" is an art-recognized term. In certain embodiments, the term refers to an amount of a therapeutic agent that produces some desired effect at a reasonable benefit/risk ratio applicable to any medical treatment. In certain embodiments, the term refers to that amount necessary or sufficient to eliminate, reduce or maintain a target of a particular therapeutic regimen. The effective amount may vary depending on such factors as the disease or condition being treated, the particular targeted constructs being administered, the size of the subject or the severity of the disease or condition. One of ordinary skill in the art may empirically determine the effective amount of a particular compound without necessitating undue experimentation. In certain embodiments, a therapeutically effective amount of a therapeutic agent for *in vivo* use will likely depend on a number of factors, including: the rate of release of an agent from a polymer matrix, which will depend in part on the chemical and physical characteristics of the polymer; the identity of the agent; the mode and method of administration; and any other materials incorporated in the polymer matrix in addition to the agent.

[0084] The term "ED50" is art-recognized. In certain embodiments, ED50 means the dose of a drug, which produces 50% of its maximum response or effect, or alternatively, the dose, which produces a pre-determined response in 50% of test subjects or preparations. The term "LD50" is art-recognized. In certain embodiments, LD50 means the dose of a drug,

which is lethal in 50% of test subjects. The term "therapeutic index" is an art-recognized term, which refers to the therapeutic index of a drug, defined as LD50/ED50.

[0085] The terms "IC₅₀," or "half maximal inhibitory concentration" is intended to refer to the concentration of a substance (*e.g.*, a compound or a drug) that is required for 50% inhibition of a biological process, or component of a process, including a protein, subunit, organelle, ribonucleoprotein, etc.

[0086] "Optional" or "optionally" means that the subsequently described circumstance may or may not occur, so that the description includes instances where the circumstance occurs and instances where it does not. For example, the phrase "optionally substituted" means that a non-hydrogen substituent may or may not be present on a given atom, and, thus, the description includes structures wherein a non-hydrogen substituent is present and structures wherein a non-hydrogen substituent is not present.

[0087] Throughout the description, where compositions are described as having, including, or comprising, specific components, it is contemplated that compositions also consist essentially of, or consist of, the recited components. Similarly, where methods or processes are described as having, including, or comprising specific process steps, the processes also consist essentially of, or consist of, the recited processing steps. Further, it should be understood that the order of steps or order for performing certain actions is immaterial so long as the compositions and methods described herein remains operable. Moreover, two or more steps or actions can be conducted simultaneously.

[0088] All percentages and ratios used herein, unless otherwise indicated, are by weight.

[0089] The terms "gene expression" or "protein expression" includes any information pertaining to the amount of gene transcript or protein present in a sample, as well as information about the rate at which genes or proteins are produced or are accumulating or being degraded (*e.g.*, reporter gene data, data from nuclear runoff experiments, pulse-chase data etc.). Certain kinds of data might be viewed as relating to both gene and protein expression. For example, protein levels in a cell are reflective of the level of protein as well as the level of transcription, and such data is intended to be included by the phrase "gene or protein expression information". Such information may be given in the form of amounts per cell, amounts relative to a control gene or protein, in unitless measures, etc.; the term "information" is not to be limited to any particular means of representation and is intended to

mean any representation that provides relevant information. The term "expression levels" refers to a quantity reflected in or derivable from the gene or protein expression data, whether the data is directed to gene transcript accumulation or protein accumulation or protein synthesis rates, etc.

[0090] The terms "healthy" and "normal" are used interchangeably herein to refer to a subject or particular cell or tissue that is devoid (at least to the limit of detection) of a disease condition.

[0091] The term "nucleic acid" refers to polynucleotides such as deoxyribonucleic acid (DNA), and, where appropriate, ribonucleic acid (RNA). The term should also be understood to include analogues of either RNA or DNA made from nucleotide analogues, and, as applicable to the embodiment being described, single-stranded (such as sense or antisense) and double-stranded polynucleotides. In some embodiments, "nucleic acid" refers to inhibitory nucleic acids. Some categories of inhibitory nucleic acid compounds include antisense nucleic acids, RNAi constructs, and catalytic nucleic acid constructs. Such categories of nucleic acids are well-known in the art.

[0092] Embodiments described herein relate to compositions and methods of preventing, treating, or reducing the severity of a renal disorder, disease, and/or injury. It was found that administration of a 15-PGDH inhibitor to a subject prior to and/or after renal injury can induce renal vasodilation, and enhance resistance to hypoxia resulting in a prophylactic and protective effect against renal injury. These benefits can be associated with a prophylactic use of as little as a single dose of a 15-PGDH inhibitor. Administration of a 15-PGDH inhibitor to a subject having or at risk of a renal injury improved renal hemodynamics, decreased induction of oxidative stress, reduced induction of inflammation, attenuated multiple markers of renal damage and preserved renal function. Moreover, administration of a 15-PGDH inhibitor before and/or after administration of a contrast media was found to prevent and/or treat contrast-induced acute kidney injury (CIKI). Advantageously, the administration of a 15-PGDH inhibitor systemically to generate endogenous renal PGE₂ in a subject with a renal injury showed greater effectiveness in treating the renal injury than systemic administration of PGE₁ or PGE₂.

[0093] Accordingly, in some embodiments, compositions and methods of inhibiting 15-PGDH activity can be used to prevent, treat, or reduce the severity of a renal disorder, disease, and/or injury in a subject or kidney of a subject in need thereof. Examples of renal

disorders, diseases, and/or injuries that can be treated include hypotensive injury to the kidney; hypertensive renal disease; diabetic renal disease and diabetic nephropathy; renal disease from vasculitis and autoimmune diseases, including but not limited to lupus erythematosus, polyarteritis, Wegeners' Granulomatosis, and mixed connective tissue disease; ischemic renal injury; acute renal failure; chronic renal failure; glomerulonephritis; nephrotic syndrome; acute tubular necrosis; nephrosclerosis; glomerulosclerosis; minimal change disease; idiopathic membranous nephropathy; membranoproliferative glomerulonephritis; Berger's disease; mesangial proliferative glomerulonephritis; chronic glomerulonephritis; focal glomerulosclerosis; renal effects of Sjogren's syndrome; renal effects of scleroderma; interstitial nephritis; and renal injury to a kidney donor, transplant recipient, and/or transplanted kidney following kidney transplant.

[0094] In certain embodiments, the subject has been identified as having an acute kidney injury (AKI) based on the Acute Kidney Injury Network (AKIN) criteria or Risk/Injury/Failure/Loss/ESRD (RIFLE) criteria.

[0095] In another embodiment, the subject has been identified as having an elevated level of serum creatinine, plasma creatinine, urine creatinine, or blood urea nitrogen (BUN), compared to a healthy control subject.

[0096] In another embodiment, the subject has been identified as having an elevated level of serum or urine neutrophil gelatinase-associated lipocalin, serum or urine interleukin-18, serum or urine cystatin C, or urine KIM-1, compared to a healthy control subject.

[0097] In some embodiments, the renal disorder, disease, and/or injury is an acute kidney injury. In other embodiments, the renal disorder, disease, and/or injury is an ischemic acute kidney injury. In one embodiment, the subject is a human who has been identified as having reduced effective arterial volume. In one embodiment, the subject has been identified as having intravascular volume depletion (*e.g.*, due to hemorrhage, gastrointestinal loss, renal loss, skin and mucous membrane loss, nephrotic syndrome, cirrhosis, or capillary leak). In one embodiment, the subject has been identified as having reduced cardiac output (*e.g.*, due to cardiogenic shock, pericardial disease, congestive heart failure, valvular heart disease, pulmonary disease, or sepsis). In one embodiment, the subject has been identified as having systemic vasodilation (*e.g.*, caused by cirrhosis, anaphylaxis, or sepsis). In one embodiment, the subject has been identified as having renal vasoconstriction (*e.g.*, caused by early sepsis, hepatorenal syndrome, acute hypercalcemia, a drug, or a radiocontrast agent).

[0098] In some embodiments, the renal disorder, disease, and/or injury is a nephrotoxic kidney injury. In one embodiment, the human subject has been exposed to a nephrotoxin. For example, the nephrotoxin can be a nephrotoxic drug selected from the group consisting of an antibiotic (*e.g.*, an aminoglycoside), a chemotherapeutic agent (*e.g.*, cis-platinum), a calcineurin inhibitor, amphotericin B, and a radiographic contrast agent, such as an iodinated contrast media (*e.g.*, Iodixanol or Iobitridol). In another example, the nephrotoxin can be an illicit drug or a heavy metal.

[0099] In certain embodiments, the subject has undergone a trauma injury or a crush injury.

[00100] In certain embodiments, the subject will undergo or has undergone an organ transplant surgery (*e.g.*, a kidney transplant surgery or heart transplant surgery).

[00101] In certain embodiments, the subject will undergo or has undergone a surgery complicated by hypoperfusion.

[00102] In certain embodiments, the subject will undergo or has undergone cardiothoracic surgery or a vascular surgery.

[00103] In certain embodiments, the subject will be taking or has taken medication (*e.g.*, an anticholinergic) that interferes with normal emptying of the bladder.

[00104] In certain embodiments, the subject has benign prostatic hypertrophy or a cancer (*e.g.*, prostate cancer, ovarian cancer, or colorectal cancer).

[00105] In certain embodiments, the subject has a kidney stone.

[00106] In certain embodiments, the subject has an obstructed urinary catheter.

[00107] In certain embodiments, the subject has taken a drug that causes or leads to crystalluria, a drug that causes or leads to myoglobinuria, or a drug that causes or leads to cystitis.

[00108] Other embodiments, described herein relate to a method for protecting a kidney from injury in a subject. The method involves administering to the subject an effective amount of 15-PGDH inhibitor to protect the subject's kidney from injury. In some embodiments, the subject has been or will be exposed to an ischemic or nephrotoxic insult. In some embodiments, the human subject has been exposed to oxidative damage (*e.g.*, by free radicals such as reactive oxygen or nitrogen species).

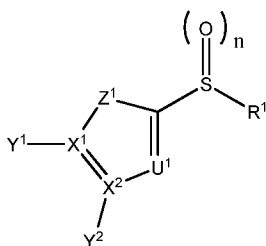
[00109] Still further embodiments relate to a method for protecting a human subject's kidney from kidney injury during organ transplantation, such as kidney transplantation. The

method involves administering to the kidney transplant donor, kidney transplant recipient, and/or transplanted kidney an effective amount of 15-PGDH inhibitor to protect the transplant donor, transplant recipient, and/or transplanted kidney from injury. In certain embodiments, the method further comprises administering to the human subject one or more doses of a 15-PGDH inhibitor before and/or after (*e.g.*, 0.1, 0.2, 0.3, 0.4, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 24, 48, 72, 96, 168 hours, or 1 week, 2 weeks, 3 weeks or 1 month) the kidney transplantation. It will be appreciated that administration of the 15-PGDH inhibitor can protect the human subject's kidney from kidney injury during other non-kidney organ transplantation.

[00110] In some embodiments, 15-PGDH inhibitors potentially used in preventing, treating, or reducing the severity of the renal disorder, disease, and/or injury can be identified using assays in which putative inhibitor compounds are applied to cells expressing 15-PGDH and then the functional effects on 15-PGDH activity are determined. Samples or assays comprising 15-PGDH that are treated with a potential inhibitor are compared to control samples without the inhibitor to examine the extent of effect. Control samples (untreated with modulators) are assigned a relative 15-PGDH activity value of 100%. Inhibition of 15-PGDH is achieved when the 15-PGDH activity value relative to the control is about 80%, optionally 50% or 25%, 10%, 5% or 1%.

[00111] Agents tested as 15-PGDH inhibitors can be any small chemical molecule or compound. Typically, test compounds will be small chemical molecules, natural products, or peptides. The assays are designed to screen large chemical libraries by automating the assay steps and providing compounds from any convenient source to assays, which are typically run in parallel (*e.g.*, in microtiter formats on microtiter plates in robotic assays).

[00112] In some embodiments, the 15-PGDH inhibitor can include a compound having the following formula (I):



(I) or a pharmaceutically acceptable salt, tautomer, or

solvate thereof:

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wherein n is 0-2;

Y^1 , Y^2 , and R^1 are the same or different and are independently hydrogen or a substituted or unsubstituted group selected from C_1 - C_{24} alkyl, C_2 - C_{24} alkenyl, C_2 - C_{24} alkynyl, C_3 - C_{20} aryl, heteroaryl, heterocycloalkenyl containing from 5-6 ring atoms, C_6 - C_{24} alkaryl, C_6 - C_{24} aralkyl, halo, $-\text{Si}(C_1\text{-}C_3 \text{ alkyl})_3$, hydroxyl, sulfhydryl, C_1 - C_{24} alkoxy, C_2 - C_{24} alkenyloxy, C_2 - C_{24} alkynyloxy, C_5 - C_{20} aryloxy, acyl, acyloxy, C_2 - C_{24} alkoxy carbonyl, C_6 - C_{20} aryloxy carbonyl, C_2 - C_{24} alkylcarbonato, C_6 - C_{20} arylcarbonato, carboxy, carboxylato, carbamoyl, C_1 - C_{24} alkyl-carbamoyl, arylcarbamoyl, thiocarbamoyl, carbamido, cyano, isocyano, cyanato, isocyanato, isothiocyanato, azido, formyl, thioformyl, amino, C_1 - C_{24} alkyl amino, C_5 - C_{20} aryl amino, C_2 - C_{24} alkylamido, C_6 - C_{20} arylamido, imino, alkylimino, arylimino, nitro, nitroso, sulfo, sulfonato, C_1 - C_{24} alkylsulfanyl, arylsulfanyl, C_1 - C_{24} alkylsulfinyl, C_5 - C_{20} arylsulfinyl, C_1 - C_{24} alkylsulfonyl, C_5 - C_{20} arylsulfonyl, sulfonamide, phosphono, phosphonato, phosphinato, phospho, phosphino, polyalkylethers, phosphates, and phosphate esters, groups incorporating amino acids or other moieties expected to bear positive or negative charge at physiological pH, and combinations thereof, and wherein Y^1 and Y^2 may be linked to form a cyclic or polycyclic ring, wherein the ring is a substituted or unsubstituted aryl, a substituted or unsubstituted heteroaryl, a substituted or unsubstituted cycloalkyl, and a substituted or unsubstituted heterocyclyl;

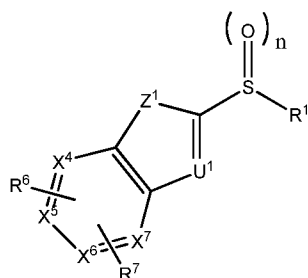
U^1 is N, $C\text{-}R^2$, or $C\text{-}NR^3R^4$, wherein R^2 is selected from the group consisting of a H, a lower alkyl group, O, $(CH_2)_nOR'$ (wherein $n=1, 2$, or 3), CF_3 , $CH_2\text{-}CH_2X$, $O\text{-}CH_2\text{-}CH_2X$, $CH_2\text{-}CH_2\text{-}CH_2X$, $O\text{-}CH_2\text{-}CH_2X$, X, (wherein $X=H, F, Cl, Br$, or I), CN, $(C=O)\text{-}R'$, $(C=O)N(R')_2$, $O(CO)R'$, $COOR'$ (wherein R' is H or a lower alkyl group), and wherein R^1 and R^2 may be linked to form a cyclic or polycyclic ring, wherein R^3 and R^4 are same or different and are each selected from the group consisting of H, a lower alkyl group, O, $(CH_2)_nOR'$ (wherein $n=1, 2$, or 3), CF_3 , $CH_2\text{-}CH_2X$, $CH_2\text{-}CH_2\text{-}CH_2X$, (wherein $X=H, F, Cl, Br$, or I), CN, $(C=O)\text{-}R'$, $(C=O)N(R')_2$, $COOR'$ (wherein R' is H or a lower alkyl group), and R^3 or R^4 may be absent;

X^1 and X^2 are independently N or C, and wherein when X^1 and/or X^2 are N, Y^1 and/or Y^2 , respectively, are absent; and

Z^1 is O, S, CR^aR^b or NR^a , wherein R^a and R^b are independently H or a C_{1-8} alkyl, which is linear, branched, or cyclic, and which is unsubstituted or substituted.

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[00113] In other embodiments, the 15-PGDH inhibitor can include a compound having the following formula (II):



(II) or a pharmaceutically acceptable salt, tautomer,

or solvate thereof;

wherein n is 0-2

X^4 , X^5 , X^6 , and X^7 are independently N or CR^c;

R^1 , R^6 , R^7 , and R^c are the same or different and independently hydrogen or a substituted or unsubstituted group selected from C₁-C₂₄ alkyl, C₂-C₂₄ alkenyl, C₂-C₂₄ alkynyl, C₃-C₂₀ aryl, heteroaryl, heterocycloalkenyl containing from 5-6 ring atoms, C₆-C₂₄ alkaryl, C₆-C₂₄ aralkyl, halo, -Si(C₁-C₃ alkyl)₃, hydroxyl, sulfhydryl, C₁-C₂₄ alkoxy, C₂-C₂₄ alkenyloxy, C₂-C₂₄ alkynyloxy, C₅-C₂₀ aryloxy, acyl, acyloxy, C₂-C₂₄ alkoxy carbonyl, C₆-C₂₀ aryloxy carbonyl, C₂-C₂₄ alkylcarbonato, C₆-C₂₀ arylcarbonato, carboxy, carboxylato, carbamoyl, C₁-C₂₄ alkyl-carbamoyl, arylcarbamoyl, thiocarbamoyl, carbamido, cyano, isocyano, cyanato, isocyanato, isothiocyanato, azido, formyl, thioformyl, amino, C₁-C₂₄ alkyl amino, C₅-C₂₀ aryl amino, C₂-C₂₄ alkylamido, C₂-C₂₄ alkylamido substituted with a hydroxyl, C₆-C₂₀ arylamido, imino, alkylimino, arylimino, nitro, nitroso, sulfo, sulfonato, C₁-C₂₄ alkylsulfanyl, arylsulfanyl, C₁-C₂₄ alkylsulfinyl, C₅-C₂₀ arylsulfinyl, C₁-C₂₄ alkylsulfonyl, C₅-C₂₀ arylsulfonyl, sulfonamide, phosphono, phosphonato, phosphinato, phospho, phosphino, polyalkylethers, phosphates, and phosphate esters, groups incorporating amino acids or other moieties expected to bear positive or negative charge at physiological pH, and combinations thereof, and wherein R^6 and R^7 may be linked to form a cyclic or polycyclic ring, wherein the ring is a substituted or unsubstituted aryl, a substituted or unsubstituted heteroaryl, a substituted or unsubstituted cycloalkyl, and a substituted or unsubstituted heterocyclyl;

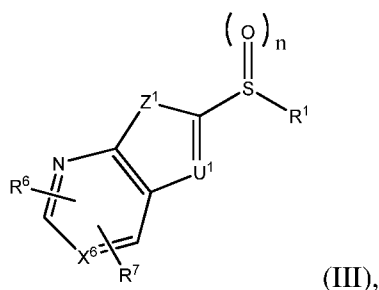
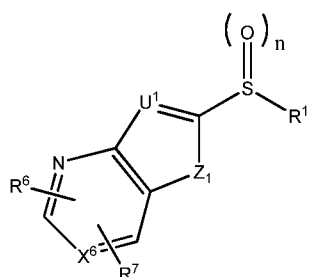
U^1 is N, C-R², or C-NR³R⁴, wherein R² is selected from the group consisting of a H, a lower alkyl group, O, (CH₂)_nOR' (wherein n=1, 2, or 3), CF₃, CH₂-CH₂X, O-CH₂-CH₂X, CH₂-CH₂-CH₂X, O-CH₂-CH₂X, X, (wherein X=H, F, Cl, Br, or I), CN, (C=O)-R', (C=O)N(R')₂, O(CO)R', COOR' (wherein R' is H or a lower alkyl group), and wherein R¹

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and R^2 may be linked to form a cyclic or polycyclic ring, wherein R^3 and R^4 are the same or different and are each selected from the group consisting of H, a lower alkyl group, O, $(CH_2)_nOR'$ (wherein $n=1, 2$, or 3), CF_3 , CH_2-CH_2X , $CH_2-CH_2-CH_2X$, (wherein $X=H, F, Cl, Br$, or I), CN , $(C=O)-R'$, $(C=O)N(R')_2$, $COOR'$ (wherein R' is H or a lower alkyl group), and R^3 or R^4 may be absent; and

Z^1 is O, S, CR^aR^b or NR^a , wherein R^a and R^b are independently H or a C_{1-8} alkyl, which is linear, branched, or cyclic, and which is unsubstituted or substituted.

[00114] In yet other embodiments, the 15-PGDH inhibitor can include a compound having the following formula (III) or (IV):



or solvate thereof;

wherein n is 0-2

X^6 is independently is N or CR^c ;

R^1 , R^6 , R^7 , and R^c are the same or different and independently hydrogen or a substituted or unsubstituted group selected from C_1-C_{24} alkyl, C_2-C_{24} alkenyl, C_2-C_{24} alkynyl, C_3-C_{20} aryl, heteroaryl, heterocycloalkenyl containing from 5-6 ring atoms, C_6-C_{24} alkaryl, C_6-C_{24} aralkyl, halo, $-Si(C_1-C_3 \text{ alkyl})_3$, hydroxyl, sulfhydryl, C_1-C_{24} alkoxy, C_2-C_{24} alkenyloxy, C_2-C_{24} alkynyloxy, C_5-C_{20} aryloxy, acyl, acyloxy, C_2-C_{24} alkoxycarbonyl, C_6-C_{20} aryloxy carbonyl, C_2-C_{24} alkylcarbonato, C_6-C_{20} arylcarbonato, carboxy, carboxylato, carbamoyl, C_1-C_{24} alkyl-carbamoyl, arylcarbamoyl, thiocarbamoyl, carbamido, cyano, isocyano, cyanato, isocyanato, isothiocyanato, azido, formyl, thioformyl, amino, C_1-C_{24} alkyl

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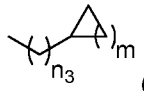
amino, C₂-C₂₄ alkylamido substituted with a hydroxyl, C₅-C₂₀ aryl amino, C₂-C₂₄ alkylamido, C₆-C₂₀ arylamido, imino, alkylimino, arylimino, nitro, nitroso, sulfo, sulfonato, C₁-C₂₄ alkylsulfanyl, arylsulfanyl, C₁-C₂₄ alkylsulfinyl, C₅-C₂₀ arylsulfinyl, C₁-C₂₄ alkylsulfonyl, C₅-C₂₀ arylsulfonyl, sulfonamide, phosphono, phosphonato, phosphinato, phospho, phosphino, polyalkylethers, phosphates, and phosphate esters, groups incorporating amino acids or other moieties expected to bear positive or negative charge at physiological pH, and combinations thereof, and wherein R⁶ and R⁷ may be linked to form a cyclic or polycyclic ring, wherein the ring is a substituted or unsubstituted aryl, a substituted or unsubstituted heteroaryl, a substituted or unsubstituted cycloalkyl, and a substituted or unsubstituted heterocyclyl;

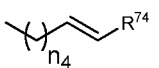
U¹ is N, C-R², or C-NR³R⁴, wherein R² is selected from the group consisting of a H, a lower alkyl group, O, (CH₂)_{n1}OR' (wherein n1=1, 2, or 3), CF₃, CH₂-CH₂X, O-CH₂-CH₂X, CH₂-CH₂-CH₂X, O-CH₂-CH₂X, X, (wherein X=H, F, Cl, Br, or I), CN, (C=O)-R', (C=O)N(R')₂, O(CO)R', COOR' (wherein R' is H or a lower alkyl group), and wherein R¹ and R² may be linked to form a cyclic or polycyclic ring, wherein R³ and R⁴ are the same or different and are each selected from the group consisting of H, a lower alkyl group, O, (CH₂)_{n1}OR' (wherein n1=1, 2, or 3), CF₃, CH₂-CH₂X, CH₂-CH₂-CH₂X, (wherein X=H, F, Cl, Br, or I), CN, (C=O)-R', (C=O)N(R')₂, COOR' (wherein R' is H or a lower alkyl group), and R³ or R⁴ may be absent;

Z¹ is O, S, CR^aR^b or NR^a, wherein R^a and R^b are independently H or a C₁₋₈ alkyl, which is linear, branched, or cyclic, and which is unsubstituted or substituted.

[00115] In some embodiments, R¹ is selected from the group consisting of branched,

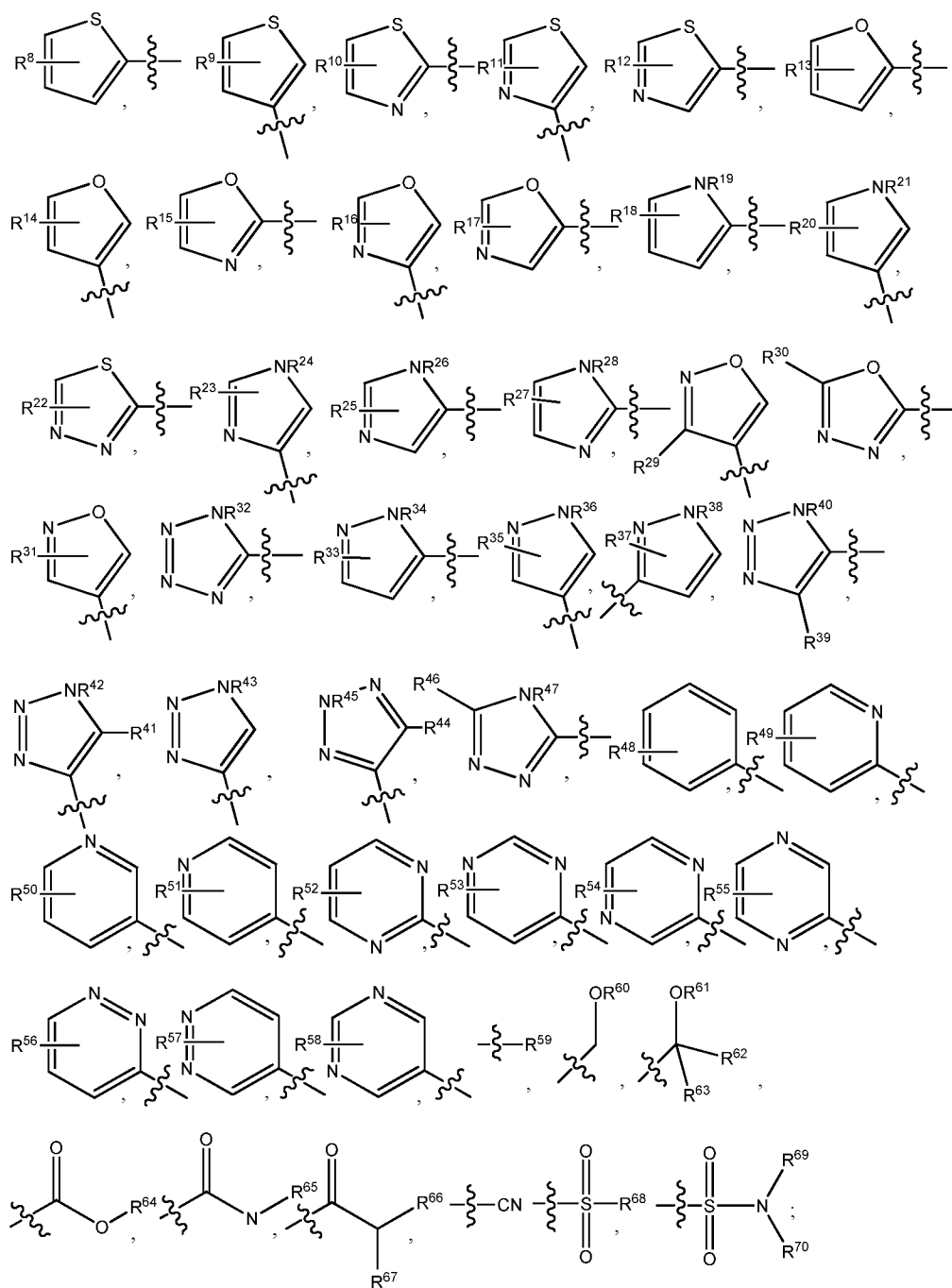
linear, or cyclic alkyl,  wherein n₂=0-6 and X is any of the following: CF_yH_z (y + z =

3), CCl_yH_z (y + z = 3), OH, OAc, OMe, R⁷¹, OR⁷², CN, N(R⁷³)₂,  (n₃=0-5, m=1-5),

and  (n₄=0-5).

[00116] In other embodiments, R⁶ and R⁷ can each independently be one of the following:

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each R⁸, R⁹, R¹⁰, R¹¹, R¹², R¹³, R¹⁴, R¹⁵, R¹⁶, R¹⁷, R¹⁸, R¹⁹, R²⁰, R²¹, R²², R²³, R²⁴, R²⁵, R²⁶, R²⁷, R²⁸, R²⁹, R³⁰, R³¹, R³², R³³, R³⁴, R³⁵, R³⁶, R³⁷, R³⁸, R³⁹, R⁴⁰, R⁴¹, R⁴², R⁴³, R⁴⁴, R⁴⁵, R⁴⁶, R⁴⁷, R⁴⁸, R⁴⁹, R⁵⁰, R⁵¹, R⁵², R⁵³, R⁵⁴, R⁵⁵, R⁵⁶, R⁵⁷, R⁵⁸, R⁵⁹, R⁶⁰, R⁶¹, R⁶², R⁶³, R⁶⁴, R⁶⁵, R⁶⁶, R⁶⁷, R⁶⁸, R⁶⁹, R⁷⁰, R⁷¹, R⁷², R⁷³, and R⁷⁴ are the same or different and are independently selected from the group consisting of hydrogen, substituted or unsubstituted C₁-C₂₄ alkyl, C₂-C₂₄ alkenyl, C₂-

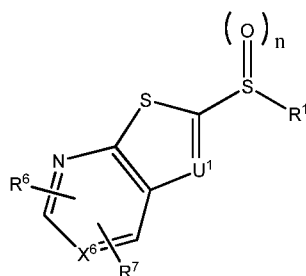
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C₂₄ alkynyl, C₃-C₂₀ aryl, heterocycloalkenyl containing from 5-6 ring atoms, (wherein from 1-3 of the ring atoms is independently selected from N, NH, N(C₁-C₆ alkyl), NC(O)(C₁-C₆ alkyl), O, and S), heteroaryl or heterocyclyl containing from 5-14 ring atoms, (wherein from 1-6 of the ring atoms is independently selected from N, NH, N(C₁-C₃ alkyl), O, and S), C₆-C₂₄ alkaryl, C₆-C₂₄ aralkyl, halo, silyl, hydroxyl, sulfhydryl, C₁-C₂₄ alkoxy, C₂-C₂₄ alkenyloxy, C₂-C₂₄ alkynyloxy, C₅-C₂₀ aryloxy, acyl (including C₂-C₂₄ alkylcarbonyl (--CO-alkyl) and C₆-C₂₀ arylcarbonyl (-CO-aryl)), acyloxy (-O-acyl), C₂-C₂₄ alkoxycarbonyl (-(CO)-O-alkyl), C₆-C₂₀ aryloxycarbonyl (-(CO)-O-aryl), C₂-C₂₄ alkylcarbonato (-O-(CO)-O-alkyl), C₆-C₂₀ arylcarbonato (-O-(CO)-O-aryl), carboxy (-COOH), carboxylato (-COO⁻), carbamoyl (-(CO)--NH₂), C₁-C₂₄ alkyl-carbamoyl (-(CO)-NH(C₁-C₂₄ alkyl)), arylcarbamoyl (-(CO)-NH-aryl), thiocarbamoyl (-(CS)-NH₂), carbamido (-NH-(CO)-NH₂), cyano(-CN), isocyano (-N⁺C⁻), cyanato (-O-CN), isocyanato (-O-N⁺=C⁻), isothiocyanato (-S-CN), azido (-N=N⁺=N⁻), formyl (--CO--H), thioformyl (--CS--H), amino (--NH₂), C₁-C₂₄ alkyl amino, C₁-C₂₄ alkyl amino substituted with hydroxyl, C₅-C₂₀ aryl amino, C₂-C₂₄ alkylamido (-NH-(CO)-alkyl), C₆-C₂₀ arylamido (-NH-(CO)-aryl), sulfanamido (-SO₂N(R)₂ where R is independently H, alkyl, aryl or heteroaryl), imino (-CR=NH where R is hydrogen, C₁-C₂₄ alkyl, C₅-C₂₀ aryl, C₆-C₂₄ alkaryl, C₆-C₂₄ aralkyl, etc.), alkylimino (-CR=N(alkyl), where R=hydrogen, alkyl, aryl, alkaryl, aralkyl, etc.), arylimino (-CR=N(aryl), where R=hydrogen, alkyl, aryl, alkaryl, etc.), nitro (-NO₂), nitroso (-NO), sulfo (-SO₂-OH), sulfonato (-SO₂-O⁻), C₁-C₂₄ alkylsulfanyl (-S-alkyl; also termed "alkylthio"), arylsulfanyl (-S-aryl; also termed "arylthio"), C₁-C₂₄ alkylsulfinyl (-SO-alkyl), C₅-C₂₀ arylsulfinyl (-SO-aryl), C₁-C₂₄ alkylsulfonyl (-SO₂-alkyl), C₅-C₂₀ arylsulfonyl (-SO₂-aryl), sulfonamide (-SO₂-NH₂, -SO₂NY₂ (wherein Y is independently H, aryl or alkyl), phosphono (-P(O)(OH)₂), phosphonato (-P(O)(O⁻)₂), phosphinato (-P(O)(O⁻)), phospho (-PO₂), phosphino (--PH₂), polyalkyl ethers (-[(CH₂)_nO]_m), phosphates, phosphate esters [-OP(O)(OR)₂ where R = H, methyl or other alkyl], groups incorporating amino acids or other moieties expected to bear positive or negative charge at physiological pH, and combinations thereof, or a pharmaceutically acceptable salt, tautomer, or solvate thereof.

[00117] In still other embodiments, R⁶ and R⁷ can independently be a group that improves aqueous solubility, for example, a phosphate ester (-OPO₃H₂), a phenyl ring linked to a phosphate ester (-OPO₃H₂), a phenyl ring substituted with one or more methoxyethoxy groups, or a morpholine, or an aryl or heteroaryl ring substituted with such a group.

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[00118] In other embodiments, the 15-PGDH inhibitor can include a compound having the following formula (V):



(V) or a pharmaceutically acceptable salt, tautomer,

or solvate thereof;

wherein n is 0-2

X⁶ is independently is N or CR^c

R¹, R⁶, R⁷, and R^c are the same or different each independently hydrogen or a substituted or unsubstituted group selected from C₁-C₂₄ alkyl, C₂-C₂₄ alkenyl, C₂-C₂₄ alkynyl, C₃-C₂₀ aryl, heteroaryl, heterocycloalkenyl containing from 5-6 ring atoms, C₆-C₂₄ alkaryl, C₆-C₂₄ aralkyl, halo, -Si(C₁-C₃ alkyl)₃, hydroxyl, sulfhydryl, C₁-C₂₄ alkoxy, C₂-C₂₄ alkenyloxy, C₂-C₂₄ alkynyloxy, C₅-C₂₀ aryloxy, acyl, acyloxy, C₂-C₂₄ alkoxycarbonyl, C₆-C₂₀ aryloxy, C₂-C₂₄ alkylcarbonato, C₆-C₂₀ arylcarbonato, carboxy, carboxylato, carbamoyl, C₁-C₂₄ alkyl-carbamoyl, arylcarbamoyl, thiocarbamoyl, carbamido, cyano, isocyano, cyanato, isocyanato, isothiocyanato, azido, formyl, thioformyl, amino, C₁-C₂₄ alkyl amino, C₅-C₂₀ aryl amino, C₂-C₂₄ alkylamido, C₂-C₂₄ alkylamido substituted with a hydroxyl, C₆-C₂₀ arylamido, imino, alkylimino, arylimino, nitro, nitroso, sulfo, sulfonato, C₁-C₂₄ alkylsulfanyl, arylsulfanyl, C₁-C₂₄ alkylsulfinyl, C₅-C₂₀ arylsulfinyl, C₁-C₂₄ alkylsulfonyl, C₅-C₂₀ arylsulfonyl, sulfonamide, phosphono, phosphonato, phosphinato, phospho, phosphino, polyalkylethers, phosphates, and phosphate esters, groups incorporating amino acids or other moieties expected to bear positive or negative charge at physiological pH, and combinations thereof, and wherein R⁶ and R⁷ may be linked to form a cyclic or polycyclic ring, wherein the ring is a substituted or unsubstituted aryl, a substituted or unsubstituted heteroaryl, a substituted or unsubstituted cycloalkyl, and a substituted or unsubstituted heterocyclyl; and

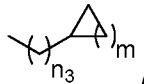
U¹ is N, C-R², or C-NR³R⁴, wherein R² is selected from the group consisting of a H, a lower alkyl group, O, (CH₂)_nOR' (wherein n=1, 2, or 3), CF₃, CH₂-CH₂X, O-CH₂-CH₂X, CH₂-CH₂-CH₂X, O-CH₂-CH₂X, X, (wherein X=H, F, Cl, Br, or I), CN, (C=O)-R', (C=O)N(R')₂, O(CO)R', COOR' (wherein R' is H or a lower alkyl group), and wherein R¹

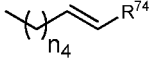
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and R^2 may be linked to form a cyclic or polycyclic ring, wherein R^3 and R^4 are the same or different and are each selected from the group consisting of H, a lower alkyl group, O, $(CH_2)_{n1}OR'$ (wherein $n1=1, 2$, or 3), CF_3 , CH_2-CH_2X , $CH_2-CH_2-CH_2X$, (wherein $X=H, F, Cl, Br$, or I), CN , $(C=O)-R'$, $(C=O)N(R')_2$, $COOR'$ (wherein R' is H or a lower alkyl group), and R^3 or R^4 may be absent.

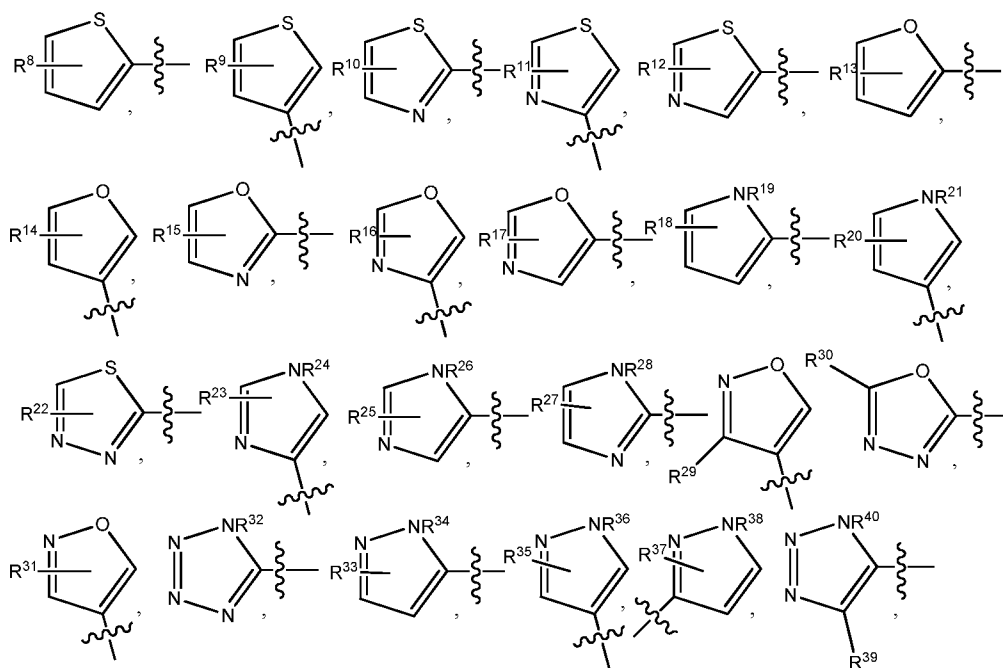
[00119] In some embodiments, R^1 is selected from the group consisting of branched,

linear, or cyclic alkyl,  wherein $n_2=0-6$ and X is any of the following: CF_yH_z ($y+z=$

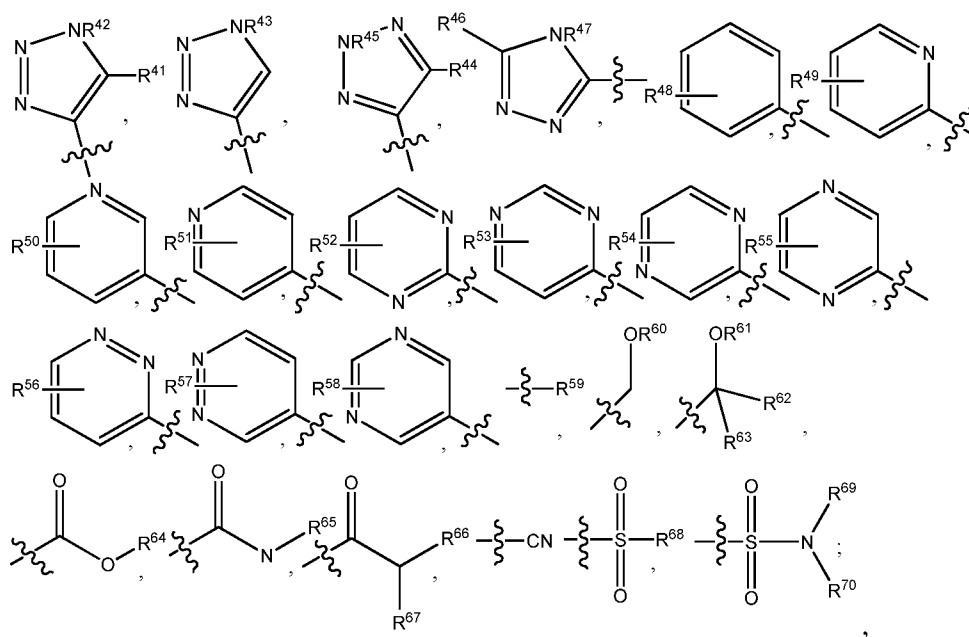
3), CCl_yH_z ($y+z=3$), OH , OAc , OMe , R^{71} , OR^{72} , CN , $N(R^{73})_2$,  ($n_3=0-5$, $m=1-5$),

and  ($n_4=0-5$).

[00120] In other embodiments, R^6 and R^7 can each independently be one of the following:



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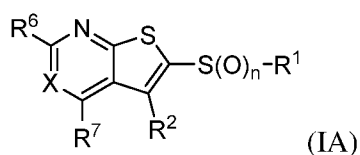
each $R^8, R^9, R^{10}, R^{11}, R^{12}, R^{13}, R^{14}, R^{15}, R^{16}, R^{17}, R^{18}, R^{19}, R^{20}, R^{21}, R^{22}, R^{23}, R^{24}, R^{25}, R^{26}, R^{27}, R^{28}, R^{29}, R^{30}, R^{31}, R^{32}, R^{33}, R^{34}, R^{35}, R^{36}, R^{37}, R^{38}, R^{39}, R^{40}, R^{41}, R^{42}, R^{43}, R^{44}, R^{45}, R^{46}, R^{47}, R^{48}, R^{49}, R^{50}, R^{51}, R^{52}, R^{53}, R^{54}, R^{55}, R^{56}, R^{57}, R^{58}, R^{59}, R^{60}, R^{61}, R^{62}, R^{63}, R^{64}, R^{65}, R^{66}, R^{67}, R^{68}, R^{69}, R^{70}, R^{71}, R^{72}, R^{73}$, and R^{74} , are the same or different and are independently selected from the group consisting of hydrogen, substituted or unsubstituted C_1 - C_{24} alkyl, C_2 - C_{24} alkenyl, C_2 - C_{24} alkynyl, C_3 - C_{20} aryl, heterocycloalkenyl containing from 5-6 ring atoms, (wherein from 1-3 of the ring atoms is independently selected from N, NH, $N(C_1-C_6 \text{ alkyl})$, $NC(O)(C_1-C_6 \text{ alkyl})$, O, and S), heteroaryl or heterocyclyl containing from 5-14 ring atoms, (wherein from 1-6 of the ring atoms is independently selected from N, NH, $N(C_1-C_3 \text{ alkyl})$, O, and S), C_6 - C_{24} alkaryl, C_6 - C_{24} aralkyl, halo, silyl, hydroxyl, sulfhydryl, C_1 - C_{24} alkoxy, C_2 - C_{24} alkenyloxy, C_2 - C_{24} alkynyloxy, C_5 - C_{20} aryloxy, acyl (including C_2 - C_{24} alkylcarbonyl ($--CO-$ alkyl) and C_6 - C_{20} arylcarbonyl ($-CO-$ aryl)), acyloxy ($-O-$ acyl), C_2 - C_{24} alkoxy carbonyl ($-(CO)-O-$ alkyl), C_6 - C_{20} aryloxy carbonyl ($-(CO)-O-$ aryl), C_2 - C_{24} alkylcarbonato ($-O-(CO)-O-$ alkyl), C_6 - C_{20} arylcarbonato ($-O-(CO)-O-$ aryl), carboxy ($-COOH$), carboxylato ($-COO^-$), carbamoyl ($-(CO)--NH_2$), C_1 - C_{24} alkyl-carbamoyl ($-(CO)-NH(C_1-C_{24} \text{ alkyl})$), arylcarbamoyl ($-(CO)-NH-$ aryl), thiocarbamoyl ($-(CS)-NH_2$), carbamido ($-NH-(CO)-NH_2$), cyano ($-CN$), isocyano ($-N^+C^-$), cyanato ($-O-CN$), isocyanato ($-O-N^+=C^-$), isothiocyanato ($-S-CN$), azido ($-N=N^+=N^-$), formyl ($-(CO)--H$), thioformyl ($-(CS)--H$), amino ($--NH_2$), C_1 - C_{24} alkyl amino, C_1 - C_{24} alkyl amino substituted with hydroxyl, C_5 - C_{20} aryl amino, C_2 - C_{24} alkylamido ($-NH-$

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(CO)-alkyl), C₆-C₂₀ arylamido (-NH-(CO)-aryl), sulfanamido (-SO₂N(R)₂ where R is independently H, alkyl, aryl or heteroaryl), imino (-CR=NH where R is hydrogen, C₁-C₂₄ alkyl, C₅-C₂₀ aryl, C₆-C₂₄ alkaryl, C₆-C₂₄ aralkyl, etc.), alkylimino (-CR=N(alkyl), where R=hydrogen, alkyl, aryl, alkaryl, aralkyl, etc.), arylimino (-CR=N(aryl), where R=hydrogen, alkyl, aryl, alkaryl, etc.), nitro (-NO₂), nitroso (-NO), sulfo (-SO₂-OH), sulfonato (-SO₂-O⁻), C₁-C₂₄ alkylsulfanyl (-S-alkyl; also termed "alkylthio"), arylsulfanyl (-S-aryl; also termed "arylthio"), C₁-C₂₄ alkylsulfinyl (-SO-alkyl), C₅-C₂₀ arylsulfinyl (-SO-aryl), C₁-C₂₄ alkylsulfonyl (-SO₂-alkyl), C₅-C₂₀ arylsulfonyl (-SO₂-aryl), sulfonamide (-SO₂-NH₂, -SO₂NY₂ (wherein Y is independently H, aryl or alkyl), phosphono (-P(O)(OH)₂), phosphonato (-P(O)(O⁻)₂), phosphinato (-P(O)(O⁻)), phospho (-PO₂), phosphino (-PH₂), polyalkyl ethers (-[(CH₂)_nO]_m), phosphates, phosphate esters [-OP(O)(OR)₂ where R = H, methyl or other alkyl], groups incorporating amino acids or other moieties expected to bear positive or negative charge at physiological pH, and combinations thereof, or a pharmaceutically acceptable salt, tautomer, or solvate thereof.

[00121] In still other embodiments, R⁶ and R⁷ can independently be a group that improves aqueous solubility, for example, a phosphate ester (-OPO₃H₂), a phenyl ring linked to a phosphate ester (-OPO₃H₂), a phenyl ring substituted with one or more methoxyethoxy groups, or a morpholine, or an aryl or heteroaryl ring substituted with such a group.

[00122] In other embodiments, the 15-PGDH inhibitor can include a compound having a structure of formula (IA):



or a pharmaceutically acceptable salt, tautomer, or solvate thereof, wherein:

R¹ is alkyl, haloalkyl, cycloalkyl, alkylene-cycloalkyl, alkylene-alkoxy, heterocyclyl, or alkylene-heterocyclyl;

R² is -NH₂, CN, or -NHC(O)alkyl;

R⁶ is heterocyclyl or heteroaryl, each of which is optionally substituted with one or more R³;

R⁷ is alkyl, haloalkyl, cycloalkyl, aryl, heterocyclyl, heteroaryl, -C(O)-alkyl,

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-C(O)O-alkyl, or -C(O)NR⁵-alkyl, each of which is optionally substituted with one or more R⁴;

R³ is oxo, -OH, -O-alkylene-OH, -O-alkylene-N(R⁵)₂, -N(R⁵)₂, -N(R⁵)(alkylene-OH), -N(R⁵)(alkylene-O-alkyl), alkyl, -alkylene-OH, haloalkyl, cycloalkyl, heterocyclyl, -C(O)N(R⁵)₂, -C(O)N(R⁵)(alkylene-OH), -C(O)-alkyl, -C(O)O-alkyl, or -S(O)_m-alkyl, wherein the cycloalkyl and the heterocyclyl is each optionally substituted with R¹⁰;

R⁴ is oxo, halogen, -CN, -N(R⁵)₂, -OH, -O-alkylene-OH, -S(O)_m-alkyl, -C(O)-alkyl, -C(O)-cycloalkyl, alkyl, -alkylene-O-alkyl, alkoxy, haloalkyl, cycloalkyl, heterocyclyl, or -alkylene-aryl optionally substituted with R⁸;

each R⁵ is independently, H, alkyl, -alkylene-OH optionally substituted with -OH, -alkylene-NH₂, -alkylene-N(R⁹)₂, -alkylene-O-alkylene-OH, -alkylene-O-alkylene-NH₂, -C(O)-alkyl, -C(O)O-alkyl, -alkylene-COOH, or -S(O)_m-alkyl;

or alternatively, two R⁵ together with the N atom to which they are attached can form a 4- to 7-membered heterocycle, optionally containing an additional heteroatom selected from O, S, or N, and wherein the heterocycle is optionally substituted with R⁸;

R⁸ is halogen, alkyl, or alkoxy;

R⁹ is H or alkyl, or two R⁹ together with the N atom to which they are attached can form a 4- to 7-membered heterocycle, optionally containing an additional heteroatom selected from O, S(O)_t, or N;

R¹⁰ is -OH, halogen, alkyl, or alkoxy;

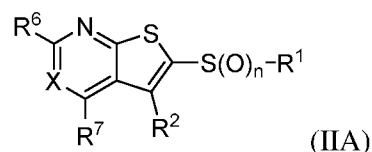
X is N or CH;

m is 0, 1, or 2;

n is 0, 1, or 2; and

t is 0, 1, or 2.

[00123] In some embodiment, the 15-PGDH inhibitor can include a compound having a structure of formula (IIA):



or a pharmaceutically acceptable salt, tautomer, or solvate thereof, wherein:

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R^1 is alkyl, haloalkyl, cycloalkyl, alkylene-cycloalkyl, alkylene-alkoxy, heterocyclyl, or alkylene-heterocyclyl;

R^2 is $-NH_2$, CN, or $-NHC(O)alkyl$;

R^6 is heterocyclyl or heteroaryl, each of which is optionally substituted with one or more R^3 ; R^7 is alkyl, haloalkyl, cycloalkyl, aryl, heterocyclyl, heteroaryl, $-C(O)-alkyl$, $-C(O)O-alkyl$, or $-C(O)NR^5-alkyl$, each of which is optionally substituted with one or more R^4 ;

R^3 is oxo, $-OH$, $-O-alkylene-N(R^5)_2$, $-N(R^5)_2$, $-N(R^5)(alkylene-OH)$, alkyl, haloalkyl, cycloalkyl, heterocyclyl, $-C(O)N(R^5)_2$, $-C(O)N(R^5)(alkylene-OH)$, $-C(O)-alkyl$, $-C(O)O-alkyl$, or $-S(O)_m-alkyl$;

R^4 is oxo, halogen, $-CN$, $-N(R^5)_2$, $-OH$, $-O-alkylene-OH$, $-S(O)_m-alkyl$, $-C(O)-alkyl$, $-C(O)-cycloalkyl$, alkyl, alkoxy, haloalkyl, cycloalkyl, heterocyclyl, or $-alkylene-aryl$ optionally substituted with R^8 ;

each R^5 is independently, H, alkyl, $-alkylene-OH$ optionally substituted with $-OH$, $-alkylene-NH_2$, $-alkylene-N(R^9)_2$, $-alkylene-O-alkylene-OH$, $-alkylene-O-alkylene-NH_2$, $-C(O)-alkyl$, $-C(O)O-alkyl$, $-alkylene-COOH$, or $-S(O)_m-alkyl$;

or alternatively, two R^5 together with the N atom to which they are attached can form a 4- to 7-membered heterocycle, optionally containing an additional heteroatom selected from O, S, or N, and wherein the heterocycle is optionally substituted with R^8 ;

R^8 is halogen, alkyl, or alkoxy;

R^9 is H or alkyl, or two R^9 together with the N atom to which they are attached can form a 4- to 7-membered heterocycle, optionally containing an additional heteroatom selected from O, S(O), or N;

X is N or CH;

m is 0, 1, or 2;

n is 0, 1, or 2; and

t is 0, 1, or 2.

[00124] In some embodiments, R^1 is C_1-C_6 alkyl, C_1-C_6 haloalkyl, 3- to 6-membered cycloalkyl, $-(C_1-C_6 alkylene)-(3- to 6-membered cycloalkyl)$, $-(C_1-C_6 alkylene)-(C_1-C_6 alkoxy)$, 3- to 6-membered heterocyclyl, or $-(C_1-C_6 alkylene)-(3- to 6-membered heterocyclyl)$.

[00125] In other embodiments, R^1 is cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl,

-(CH₂)_p-cyclopropyl, -(CH₂)_p-cyclobutyl, -(CH₂)_p-cyclopentyl, or -(CH₂)_p-cyclohexyl; wherein p is 1, 2, or 3.

[00126] In still other embodiments, R² is -NH₂.

[00127] In some embodiments, R⁶ is 5- to 6-membered heterocyclyl or 5- to 10-membered heteroaryl, each of which is optionally substituted with one or more R³.

[00128] In other embodiments, R⁶ is 5- to 6-membered heteroaryl optionally substituted with one or more R³.

[00129] In still other embodiments, R⁶ is 8- to 10-membered bicyclic heteroaryl optionally substituted with one or more R³.

[00130] In some embodiments, R⁷ is C₁-C₆ alkyl, C₁-C₆ haloalkyl, 3- to 6-membered cycloalkyl, 6- to 10-membered aryl, 3- to 6-membered heterocyclyl, 5- to 10-membered heteroaryl, -C(O)(C₁-C₆ alkyl), -C(O)O(C₁-C₆ alkyl), or -C(O)NR⁵(C₁-C₆ alkyl), each of which is optionally substituted with one or more R⁴.

[00131] In other embodiments, R⁷ is C₁-C₆ alkyl, C₁-C₆ haloalkyl, 3- to 6-membered cycloalkyl, phenyl, 3- to 6-membered heterocyclyl, or 5- to 10-membered heteroaryl, each of which is optionally substituted with one or more R⁴.

[00132] In still other embodiments, R⁷ is C₁-C₆ haloalkyl, 3- to 6-membered cycloalkyl, phenyl, 5- to 10-membered heteroaryl each of which is optionally substituted with one or more R⁴.

[00133] In some embodiments, R³ is -O-(C₁-C₆ alkylene)-N(R⁵)₂, -N(R⁵)₂, -N(R⁵)(C₁-C₆ alkylene-OH), -C(O)N(R⁵)₂, -C(O)N(R⁵)(C₁-C₆ alkylene-OH), -C(O)(C₁-C₆ alkyl), -C(O)O(C₁-C₆ alkyl), or -S(O)_m(C₁-C₆ alkyl).

[00134] In other embodiments, R³ is -(C₁-C₃ alkyl)OH, -NH₂, -N(C₁-C₃ alkyl)₂, -NHCH₂CH₂OH, -N(C₁-C₃ alkyl)CH₂CH₂OH, N(CH₂CH₂OH)₂, -NHCH₂CH(CH₂OH)₂, -N(C₁-C₃ alkyl)CH₂CH(CH₂OH)₂, -NHCH₂CH₂OCH₂CH₂OH, -NHCH₂CH₂OCH₂CH₂NH₂, -NHCH₂CH₂NH₂, -N(C₁-C₃ alkyl)CH₂CH₂NH₂, -NHCH₂CH₂NH(C₁-C₃ alkyl), -NHCH₂CH₂N(C₁-C₃ alkyl)₂, -N(C₁-C₃ alkyl)CH₂CH₂NH(C₁-C₃ alkyl), -N(C₁-C₃ alkyl)CH₂CH₂N(C₁-C₃ alkyl)₂, -NHSO₂CH₃, -N(C₁-C₃ alkyl)SO₂CH₃, -OCH₂CH₂OH, -OCH₂CH₂NH₂, -OCH₂CH₂NH(C₁-C₃ alkyl), or -OCH₂CH₂N(C₁-C₃ alkyl)₂.

[00135] In other embodiments, R³ is -NH₂, -N(C₁-C₃ alkyl)₂, -NHCH₂CH₂OH, -N(C₁-C₃ alkyl)CH₂CH₂OH, N(CH₂CH₂OH)₂, -NHCH₂CH(CH₂OH)₂, -N(C₁-C₃ alkyl)CH₂CH(CH₂OH)₂, -NHCH₂CH₂OCH₂CH₂OH, -NHCH₂CH₂OCH₂CH₂NH₂,

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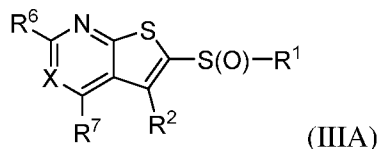
-NHCH₂CH₂NH₂, -N(C₁-C₃ alkyl)CH₂CH₂NH₂, -NHCH₂CH₂NH(C₁-C₃ alkyl),
 -NHCH₂CH₂N(C₁-C₃ alkyl)₂, -N(C₁-C₃ alkyl)CH₂CH₂NH(C₁-C₃ alkyl), -N(C₁-C₃
 alkyl)CH₂CH₂N(C₁-C₃ alkyl)₂, -NHSO₂CH₃, -N(C₁-C₃ alkyl)SO₂CH₃, -OCH₂CH₂OH,
 -OCH₂CH₂NH₂, -OCH₂CH₂NH(C₁-C₃ alkyl), or -OCH₂CH₂N(C₁-C₃ alkyl)₂.

[00136] In still other embodiments, R³ is -NHCH₂CH₂OH or -N(CH₃)CH₂CH₂OH.

[00137] In some embodiments, R⁴ is halogen, -CN, -N(R⁵)₂, -OH, -O-(C₁-C₆ alkylene)-
 OH, -S(O)_m(C₁-C₆ alkyl), -C(O)(C₁-C₆ alkyl), -C(O)-(3- to 6-membered cycloalkyl), C₁-C₆
 alkyl, C₁-C₆ haloalkyl, 3- to 6-membered cycloalkyl, or 3- to 6-membered heterocyclyl.

[00138] In some embodiments, n is 1.

[00139] In other embodiments, the compound has the structure of formula (IIIA):



or a pharmaceutically acceptable salt, tautomer, or solvate thereof, wherein:

R¹ is 3- to 6-membered cycloalkyl, -(C₁-C₆ alkylene)-(3- to 6-membered
 cycloalkyl), 3- to 6-membered heterocyclyl, or -(C₁-C₆ alkylene)-(3- to 6-membered
 heterocyclyl);

R² is -NH₂, CN, or -NHC(O)alkyl;

R⁶ is heterocyclyl or heteroaryl, each of which is optionally substituted with
 one or more R³;

R⁷ is alkyl, haloalkyl, cycloalkyl, aryl, heterocyclyl, heteroaryl, -C(O)-alkyl, -
 C(O)O-alkyl, or -C(O)NR⁵-alkyl, each of which is optionally substituted with one or more
 R⁴;

R³ is oxo, -OH, -O-alkylene-N(R⁵)₂, -N(R⁵)₂, -N(R⁵)(alkylene-OH), alkyl,
 haloalkyl, cycloalkyl, heterocyclyl, -C(O)N(R⁵)₂, -C(O)N(R⁵)(alkylene-OH), -C(O)-alkyl, -
 C(O)O-alkyl, or -S(O)_m-alkyl,

R⁴ is oxo, halogen, -CN, -N(R⁵)₂, -OH, -O-alkylene-OH, -S(O)_m-alkyl, -C(O)-
 alkyl, -C(O)-cycloalkyl, alkyl, haloalkyl, cycloalkyl, heterocyclyl, or -alkylene-aryl
 optionally substituted with R⁸;

each R⁵ is independently, H, alkyl, -alkylene-OH optionally substituted with

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-OH, -alkylene-NH₂, -alkylene-N(R⁹)₂, -alkylene-O-alkylene-OH, -alkylene-O-alkylene-NH₂, -C(O)-alkyl, -C(O)O-alkyl, -alkylene-COOH, or -S(O)_m-alkyl;

or alternatively, two R⁵ together with the N atom to which they are attached can form a 4- to 7-membered heterocycle, optionally containing an additional heteroatom selected from O, S, or N, and wherein the heterocycle is optionally substituted with R⁸;

R⁸ is halogen, alkyl, or alkoxy;

R⁹ is H or alkyl, or two R⁹ together with the N atom to which they are attached can form a 4- to 7-membered heterocycle, optionally containing an additional heteroatom selected from O, S(O)_t, or N;

X is N or CH;

m is 0, 1, or 2; and

t is 0, 1, or 2.

[00140] In some embodiments, R¹ is 3- to 5-membered cycloalkyl or -(C₁-C₆ alkylene)-(3- to 5-membered cycloalkyl).

[00141] In other embodiments, R¹ is cyclobutyl.

[00142] In still other embodiments, R¹ is a bicyclic 4- to 6-membered cycloalkyl.

[00143] In some embodiments, R⁷ is C₁-C₆ alkyl, C₁-C₆ haloalkyl, 3- to 6-membered cycloalkyl, 6- to 10-membered aryl, 3- to 6-membered heterocyclyl, 5- to 10-membered heteroaryl, -C(O)(C₁-C₆ alkyl), -C(O)O(C₁-C₆ alkyl), or -C(O)NR⁵(C₁-C₆ alkyl), each of which is optionally substituted with one or more R⁴.

[00144] In other embodiments, R⁷ is C₁-C₄ alkyl, C₁-C₆ haloalkyl, 3- to 6-membered cycloalkyl, phenyl, 3- to 6-membered heterocyclyl, or 5- to 10-membered heteroaryl, each of which is optionally substituted with one or more R⁴. In other embodiments, R⁷ is C₁-C₆ haloalkyl, 3- to 6-membered cycloalkyl, phenyl, 3- to 6-membered heterocyclyl, or 5- to 10-membered heteroaryl, each of which is optionally substituted with one or more R⁴.

[00145] In other embodiments, R⁷ is C₁-C₃ alkyl, C₁-C₃ haloalkyl, 3-membered cycloalkyl, phenyl, 4-membered heterocyclyl, or 5- to 6-membered heteroaryl, each of which is optionally substituted with one or more R⁴. In other embodiments, R⁷ is C₁-C₃ haloalkyl, 3-membered cycloalkyl, phenyl, 4-membered heterocyclyl, or 5- to 6-membered heteroaryl, each of which is optionally substituted with one or more R⁴.

[00146] In other embodiments, R⁷ is -CF₃, isopropyl, cyclopropyl, phenyl, pyridyl, pyrazole, or triazole, each of which is optionally substituted with one or more R⁴. In other

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embodiments, R^7 is $-CF_3$, cyclopropyl, phenyl, pyridyl, pyrazole, or triazole, each of which is optionally substituted with one or more R^4 .

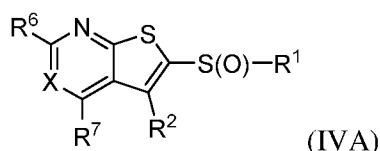
[00147] In some embodiments, R^3 is $-O-(C_1-C_6 \text{ alkylene})-N(R^5)_2$, $-N(R^5)_2$, $-N(R^5)(C_1-C_6 \text{ alkylene-OH})$, $-C(O)N(R^5)_2$, $-C(O)N(R^5)(C_1-C_6 \text{ alkylene-OH})$, $-C(O)(C_1-C_6 \text{ alkyl})$, $-C(O)O(C_1-C_6 \text{ alkyl})$, or $-S(O)_m(C_1-C_6 \text{ alkyl})$.

[00148] In other embodiments, R^3 is $-NH_2$, $-N(C_1-C_3 \text{ alkyl})_2$, $-NHCH_2CH_2OH$, $-N(C_1-C_3 \text{ alkyl})CH_2CH_2OH$, $N(CH_2CH_2OH)_2$, $-NHCH_2CH(CH_2OH)_2$, $-N(C_1-C_3 \text{ alkyl})CH_2CH(CH_2OH)_2$, $-NHCH_2CH_2OCH_2CH_2OH$, $-NHCH_2CH_2OCH_2CH_2NH_2$, $-NHCH_2CH_2NH_2$, $-N(C_1-C_3 \text{ alkyl})CH_2CH_2NH_2$, $-NHCH_2CH_2NH(C_1-C_3 \text{ alkyl})$, $-NHCH_2CH_2N(C_1-C_3 \text{ alkyl})_2$, $-N(C_1-C_3 \text{ alkyl})CH_2CH_2NH(C_1-C_3 \text{ alkyl})$, $-N(C_1-C_3 \text{ alkyl})CH_2CH_2N(C_1-C_3 \text{ alkyl})_2$, $-NHSO_2CH_3$, $-N(C_1-C_3 \text{ alkyl})SO_2CH_3$, $-OCH_2CH_2OH$, $-OCH_2CH_2NH_2$, $-OCH_2CH_2NH(C_1-C_3 \text{ alkyl})$, or $-OCH_2CH_2N(C_1-C_3 \text{ alkyl})_2$.

[00149] In still other embodiments, R^3 is $-NHCH_2CH_2OH$ or $-N(CH_3)CH_2CH_2OH$.

[00150] In some embodiments and without being limited by theory, Applicants surprisingly and unexpectedly discovered that substituents at the R^7 position could be modified to improve hERG activity, including hERG inhibition (IC_{50}), blockade, and efflux ratio. For example, in some embodiments, certain 6- to 10-membered aryls (*e.g.*, optionally substituted phenyl) and 5- to 10-membered heteroaryls (*e.g.*, optionally substituted pyridyls, pyrazoles, and triazoles) were observed to have beneficial hERG properties. In some embodiments, certain C_1-C_6 haloalkyls (*e.g.*, $-CF_3$) exhibited improved hERG inhibition (IC_{50}), while also improving half-life and solubility.

[00151] In other embodiments, the compound has the structure of formula (IVA):



or a pharmaceutically acceptable salt, tautomer, or solvate thereof, wherein:

R^1 is cycloalkyl, alkylene-cycloalkyl, alkylene-alkoxy, heterocyclyl, or alkylene-heterocyclyl;

R^2 is $-NH_2$, CN, or $-NHC(O)alkyl$;

R^6 is heterocyclyl or heteroaryl, each of which is optionally substituted with one or more R^3 ;

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R^7 is C_1 - C_6 haloalkyl, aryl or heteroaryl, each of which is optionally substituted with one or more R^4 ;

R^3 is oxo, $-OH$, $-O$ -alkylene- $N(R^5)_2$, $-N(R^5)_2$, $-N(R^5)$ (alkylene- OH), alkyl, haloalkyl, cycloalkyl, heterocyclyl, $-C(O)N(R^5)_2$, $-C(O)N(R^5)$ (alkylene- OH), $-C(O)$ -alkyl, $-C(O)O$ -alkyl, or $-S(O)_m$ -alkyl;

each R^5 is independently, H, alkyl, $-alkylene-OH$ optionally substituted with $-OH$, $-alkylene-NH_2$, $-alkylene-N(R^9)_2$, $-alkylene-O-alkylene-OH$, $-alkylene-O-alkylene-NH_2$, $-C(O)$ -alkyl, $-alkylene-COOH$, $-C(O)O$ -alkyl, or $-S(O)_m$ -alkyl;

or alternative, two R^5 together with the N atom to which they are attached can form a 4- to 7-membered heterocycle, optionally containing an additional heteroatom selected from O, S, or N, and wherein the heterocycle is optionally substituted with R^8 ;

R^4 is halogen, alkyl, or alkoxy;

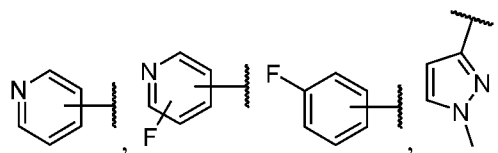
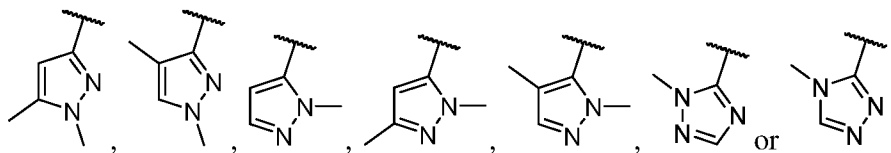
X is N or CH; and

m is 0, 1, or 2.

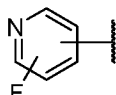
[00152] In some embodiments, R^7 is $-CF_3$, pyridyl, pyrazole, phenyl, or triazole, each of which is optionally substituted with R^4 .

[00153] In other embodiments, R^7 is $-CF_3$, pyridyl, fluorophenyl, or a triazole optionally substituted with halogen or methyl.

[00154] In other embodiments, R^7 is $-CF_3$,



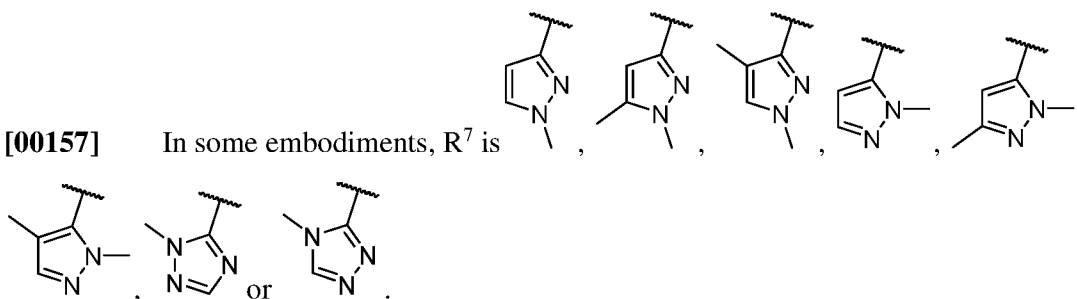
[00155] In still other embodiments, R^7 is $-CF_3$.



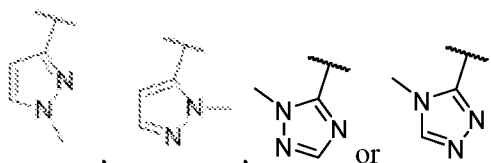
[00156] In still other embodiments, R^7 is

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[00157] In some embodiments, R⁷ is



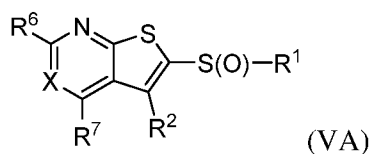
[00158] In some embodiments, R⁷ is



[00159] In other embodiments, R⁶ is 8- to 10-membered bicyclic heteroaryl optionally substituted with one or more R³.

[00160] In some embodiments and without being limited by theory, Applicants surprisingly and unexpectedly discovered that 3- to 6-membered cycloalkyls at the R⁷ position can improve solubility while maintaining PDGH activity.

[00161] In other embodiments, the compound has the structure of formula (VA):



or a pharmaceutically acceptable salt, tautomer, or solvate thereof, wherein:

R¹ is cycloalkyl, -alkylene-cycloalkyl, -alkylene-alkoxy, heterocyclyl, or -alkylene-heterocyclyl;

R² is -NH₂, CN, or -NHC(O)alkyl;

R⁶ is heterocyclyl or heteroaryl, each of which is optionally substituted with one or more R³;

R⁷ is 3- to 6-membered cycloalkyl, optionally substituted with one or more R⁴;

R³ is oxo, -OH, -O-alkylene-N(R⁵)₂, -N(R⁵)₂, -N(R⁵)(alkylene-OH), alkyl, haloalkyl, cycloalkyl, heterocyclyl, -C(O)N(R⁵)₂, -C(O)N(R⁵)(alkylene-OH), -C(O)-alkyl, -C(O)O-alkyl, or -S(O)_m-alkyl;

R⁴ is halogen, -CN, -NH₂, -OH, or C₁-C₃ alkyl;

each R⁵ is independently, H, alkyl, -alkylene-OH optionally substituted with

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-OH, -alkylene-NH₂, -alkylene-N(R⁹)₂, -alkylene-O-alkylene-OH, -alkylene-O-alkylene-NH₂, -C(O)-alkyl, -alkylene-COOH, -C(O)O-alkyl, or -S(O)_m-alkyl;

or alternative, two R⁵ together with the N atom to which they are attached can form a 4- to 7-membered heterocycle, optionally containing an additional heteroatom selected from O, S, or N, and wherein the heterocycle is optionally substituted with R⁸;

R⁸ is halogen, alkyl, or alkoxy;

X is N or CH;

m is 0, 1, or 2.

[00162] In some embodiments, R⁷ is cyclopropyl.

[00163] In other embodiments, R¹ is 3- to 6-membered cycloalkyl, -(C₁-C₆ alkylene)-(3- to 6-membered cycloalkyl), -(C₁-C₆ alkylene)-(C₁-C₆ alkoxy), 3- to 6-membered heterocyclyl, or -(C₁-C₆ alkylene)-(3- to 6-membered heterocyclyl).

[00164] In some embodiments, R¹ is cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, -(CH₂)_p-cyclopropyl, -(CH₂)_p-cyclobutyl, -(CH₂)_p-cyclopentyl, or -(CH₂)_p-cyclohexyl; wherein p is 1, 2, or 3.

[00165] In some embodiments, R³ is -O-(C₁-C₆ alkylene)-N(R⁵)₂, -N(R⁵)₂, -N(R⁵)(C₁-C₆ alkylene-OH), -C(O)N(R⁵)₂, -C(O)N(R⁵)(C₁-C₆ alkylene-OH), -C(O)(C₁-C₆ alkyl), -C(O)O(C₁-C₆ alkyl), or -S(O)_m(C₁-C₆ alkyl).

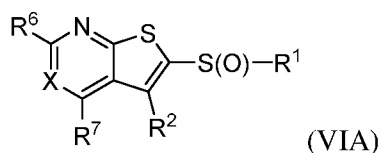
[00166] In some embodiments, R³ is -NH₂, -N(C₁-C₃ alkyl)₂, -NHCH₂CH₂OH, -N(C₁-C₃ alkyl)CH₂CH₂OH, N(CH₂CH₂OH)₂, -NHCH₂CH(CH₂OH)₂, -N(C₁-C₃ alkyl)CH₂CH(CH₂OH)₂, -NHCH₂CH₂OCH₂CH₂OH, -NHCH₂CH₂OCH₂CH₂NH₂, -NHCH₂CH₂NH₂, -N(C₁-C₃ alkyl)CH₂CH₂NH₂, -NHCH₂CH₂NH(C₁-C₃ alkyl), -NHCH₂CH₂N(C₁-C₃ alkyl)₂, -N(C₁-C₃ alkyl)CH₂CH₂NH(C₁-C₃ alkyl), -N(C₁-C₃ alkyl)CH₂CH₂N(C₁-C₃ alkyl)₂, -NHSO₂CH₃, -N(C₁-C₃ alkyl)SO₂CH₃, -OCH₂CH₂OH, -OCH₂CH₂NH₂, -OCH₂CH₂NH(C₁-C₃ alkyl), or -OCH₂CH₂N(C₁-C₃ alkyl)₂.

[00167] In other embodiments, R³ is -NHCH₂CH₂OH or -N(CH₃)CH₂CH₂OH.

[00168] In some embodiments and without being limited by theory, Applicants surprisingly and unexpectedly discovered that the R⁶ position can be substituted with certain R³ groups to improve solubility and activity.

[00169] In other embodiments, the compound has the structure of formula (VIA):

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or a pharmaceutically acceptable salt, tautomer, or solvate thereof, wherein:

R^1 is cycloalkyl, -alkylene-cycloalkyl, -alkylene-alkoxy, heterocyclyl, or -alkylene-heterocyclyl;

R^2 is $-NH_2$, CN, or $-NHC(O)alkyl$;

R^6 is heterocyclyl or heteroaryl, each of which is substituted with one or more R^3 ;

R^7 is haloalkyl, cycloalkyl, aryl, heterocyclyl, heteroaryl, $-C(O)-alkyl$, $-C(O)O-alkyl$, or $-C(O)NR^5-alkyl$, each of which is optionally substituted with one or more R^4 ;

R^3 is $-O-(C_1-C_6 \text{ alkylene})-N(R^5)_2$, $-N(R^5)_2$, $-N(R^5)(C_1-C_6 \text{ alkylene}-OH)$, $-C(O)N(R^5)_2$, $-C(O)N(R^5)(C_1-C_6 \text{ alkylene}-OH)$, $-C(O)(C_1-C_6 \text{ alkyl})$, $-C(O)O(C_1-C_6 \text{ alkyl})$, or $-S(O)_m(C_1-C_6 \text{ alkyl})$;

R^4 is oxo, halogen, $-CN$, $-N(R^5)_2$, $-OH$, $-O-alkylene-OH$, $-S(O)_m-alkyl$, $-C(O)-alkyl$, $-C(O)-cycloalkyl$, $alkyl$, $haloalkyl$, $cycloalkyl$, $heterocyclyl$, or $-alkylene-aryl$ optionally substituted with R^8 ;

each R^5 is independently H, $C_1-C_6 \text{ alkyl}$, $-(C_1-C_6 \text{ alkylene})-OH$ optionally substituted with $-OH$, $-alkylene-NH_2$, $-alkylene-N(R^9)_2$, $-alkylene-O-alkylene-OH$, $-alkylene-O-alkylene-NH_2$, $-C(O)(C_1-C_6 \text{ alkyl})$, $-C(O)O(C_1-C_6 \text{ alkyl})$, $alkylene-COOH$, or $-S(O)_m(C_1-C_6 \text{ alkyl})$;

or alternative, two R^5 together with the N atom to which they are attached can form a 4- to 7-membered heterocycle, optionally containing an additional heteroatom selected from O, S, or N, and wherein the heterocycle is optionally substituted with R^8 ;

R^8 is halogen, $alkyl$, or $alkoxy$;

X is N or CH;

m is 0, 1, or 2.

[00170] In some embodiments, R^3 is $-O-(C_1-C_6 \text{ alkylene})-N(R^5)_2$, $-N(R^5)_2$ or $-N(R^5)(C_1-C_6 \text{ alkylene}-OH)$.

[00171] In other embodiments, R^5 is H, C₁-C₆ alkyl, -(C₁-C₆ alkylene)-OH, or -S(O)₂(C₁-C₃ alkyl).

[00172] In some embodiments, R^3 is -NH₂, -N(C₁-C₃ alkyl)₂, -NHCH₂CH₂OH, -N(C₁-C₃ alkyl)CH₂CH₂OH, N(CH₂CH₂OH)₂, -NHCH₂CH(CH₂OH)₂, -N(C₁-C₃ alkyl)CH₂CH(CH₂OH)₂, -NHCH₂CH₂OCH₂CH₂OH, -NHCH₂CH₂OCH₂CH₂NH₂, -NHCH₂CH₂NH₂, -N(C₁-C₃ alkyl)CH₂CH₂NH₂, -NHCH₂CH₂NH(C₁-C₃ alkyl), -NHCH₂CH₂N(C₁-C₃ alkyl)₂, -N(C₁-C₃ alkyl)CH₂CH₂NH(C₁-C₃ alkyl), -N(C₁-C₃ alkyl)CH₂CH₂N(C₁-C₃ alkyl)₂, -NHSO₂CH₃, -N(C₁-C₃ alkyl)SO₂CH₃, -OCH₂CH₂OH, -OCH₂CH₂NH₂, -OCH₂CH₂NH(C₁-C₃ alkyl), or -OCH₂CH₂N(C₁-C₃ alkyl)₂.

[00173] In other embodiments, R^3 is -NHCH₂CH₂OH or -N(CH₃)CH₂CH₂OH.

[00174] In still other embodiments, R^3 is -NHCH₂CH₂OH.

[00175] In some embodiments, R^6 is 5- to 6-membered heterocyclyl or 5- to 10-membered heteroaryl, each of which is optionally substituted with one or more R^3 .

[00176] In other embodiments, R^6 is 5- to 6-membered heteroaryl optionally substituted with one or more R^3 .

[00177] In some embodiments, R^6 is furan, thiophene, pyrrole, thiazole, isothiazole, oxazole, isooxazole, pyrazole, imidazole, triazole, pyridine, pyrimidine, pyridazine, or pyrazine, each optionally substituted with one or more R^3 .

[00178] In other embodiments, R^6 is thiazole, imidazole, oxazole, pyridine, or pyrimidine.

[00179] In some embodiments, R^6 is 8- to 10-membered bicyclic heteroaryl optionally substituted with one or more R^3 .

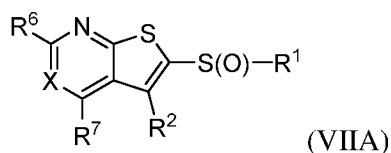
[00180] In other embodiments, R^6 is 5- to 6-membered heterocyclyl, optionally substituted with one or more R^3 , selected from morpholine, pyridine-one, or piperidine.

[00181] In some embodiments, R^7 is C₁-C₃ haloalkyl, 3-membered cycloalkyl, phenyl, 4-membered heterocyclyl, or 5- to 6-membered heteroaryl, each of which is optionally substituted with one or more R^4 .

[00182] In other embodiments, R^7 is -CF₃, cyclopropyl, phenyl, pyrrole, pyridyl, or triazole, each of which is optionally substituted with one or more R^4 .

[00183] In some embodiments, the compound has the structure of formula (VIIA):

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or a pharmaceutically acceptable salt, tautomer, or solvate thereof, wherein:

R^1 is cycloalkyl, alkylene-cycloalkyl, alkylene-alkoxy, heterocyclyl, or alkylene-heterocyclyl;

R^2 is $-NH_2$, CN, or $-NHC(O)alkyl$;

R^6 is fused bicyclic heterocyclyl or fused bicyclic heteroaryl, each of which is optionally substituted with one or more R^3 ;

R^7 is alkyl, haloalkyl, cycloalkyl, aryl, heterocyclyl, heteroaryl, $-C(O)-alkyl$, $-C(O)O-alkyl$, or $-C(O)NR^5-alkyl$, each of which is optionally substituted with one or more R^4 ;

R^3 is oxo, $-OH$, $-O-alkylene-N(R^5)_2$, $-N(R^5)_2$, $-N(R^5)(alkylene-OH)$, alkyl, haloalkyl, cycloalkyl, heterocyclyl, $-C(O)N(R^5)_2$, $-C(O)-alkyl$, $-C(O)O-alkyl$, or $-S(O)_m-alkyl$;

R^4 is oxo, halogen, $-CN$, $-N(R^5)_2$, $-OH$, $-O-alkylene-OH$, $-S(O)_m-alkyl$, $-C(O)-alkyl$, $-C(O)-cycloalkyl$, alkyl, haloalkyl, cycloalkyl, heterocyclyl, or $-alkylene-aryl$ optionally substituted with R^8 ;

each R^5 is independently, H, alkyl, $-alkylene-OH$ optionally substituted with $-OH$, $-alkylene-O-alkylene-OH$, $-alkylene-O-alkylene-NH_2$, $-C(O)-alkyl$, $-C(O)O-alkyl$, or $-S(O)_m-alkyl$;

or alternatively, two R^5 together with the N atom to which they are attached can form a 4- to 7-membered heterocycle, optionally containing an additional heteroatom selected from O, S, or N, and wherein the heterocycle is optionally substituted with R^8 ;

R^8 is halogen, alkyl, or alkoxy;

X is N or CH; and

m is 0, 1, or 2.

[00184] In some embodiments, R^6 is 8- to 10-membered fused bicyclic heteroaryl, each of which is optionally substituted with one or more R^3 .

[00185] In some embodiments, R^7 is C_1-C_6 alkyl, C_1-C_6 haloalkyl, 3- to 6-membered cycloalkyl, 6- to 10-membered aryl, 3- to 6-membered heterocyclyl, 5- to 10-membered

heteroaryl, -C(O)(C₁-C₆ alkyl), -C(O)O(C₁-C₆ alkyl), or -C(O)NR⁵(C₁-C₆ alkyl), each of which is optionally substituted with one or more R⁴.

[00186] In other embodiments, R⁷ is C₁-C₄ alkyl, C₁-C₆ haloalkyl, 3- to 6-membered cycloalkyl, phenyl, 3- to 6-membered heterocyclyl, or 5- to 10-membered heteroaryl, each of which is optionally substituted with one or more R⁴. In other embodiments, R⁷ is C₁-C₆ haloalkyl, 3- to 6-membered cycloalkyl, phenyl, 3- to 6-membered heterocyclyl, or 5- to 10-membered heteroaryl, each of which is optionally substituted with one or more R⁴.

[00187] In still other embodiments, R⁷ is C₁-C₃ alkyl, C₁-C₆ haloalkyl, 3- to 6-membered cycloalkyl, phenyl, 3- to 6-membered heterocyclyl, or 5- to 6-membered heteroaryl, each of which is optionally substituted with one or more R⁴.

[00188] In other embodiments, R⁷ is C₁-C₃ alkyl, C₁-C₆ haloalkyl, 3- to 6-membered cycloalkyl, phenyl, pyrazole, pyridyl, or triazole, each of which is optionally substituted with one or more R⁴. In other embodiments, R⁷ is C₁-C₆ haloalkyl, 3- to 6-membered cycloalkyl, phenyl, pyrazole, pyridyl, or triazole, each of which is optionally substituted with one or more R⁴.

[00189] In other embodiments, R⁷ is -CF₃, isopropyl, cyclopropyl, phenyl, pyridyl, or triazole, each of which is optionally substituted with one or more R⁴. In other embodiments, R⁷ is -CF₃, cyclopropyl, phenyl, pyridyl, or triazole, each of which is optionally substituted with one or more R⁴.

[00190] In some embodiments, R¹ is C₁-C₆ alkyl, C₁-C₆ haloalkyl, 3- to 6-membered cycloalkyl, -(C₁-C₆ alkylene)-(3- to 6-membered cycloalkyl), -(C₁-C₆ alkylene)-(C₁-C₆ alkoxy), 3- to 6-membered heterocyclyl, or -(C₁-C₆ alkylene)-(3- to 6-membered heterocyclyl).

[00191] In other embodiments, R¹ is cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, -(CH₂)_p-cyclopropyl, -(CH₂)_p-cyclobutyl, -(CH₂)_p-cyclopentyl, or -(CH₂)_p-cyclohexyl; wherein p is 1, 2, or 3.

[00192] In some embodiments, R³ is -O-(C₁-C₆ alkylene)-N(R⁵)₂, -N(R⁵)₂, -N(R⁵)(C₁-C₆ alkylene-OH), -C(O)N(R⁵)₂, -C(O)N(R⁵)(C₁-C₆ alkylene-OH), -C(O)(C₁-C₆ alkyl), -C(O)O(C₁-C₆ alkyl), or -S(O)_m(C₁-C₆ alkyl).

[00193] In other embodiments, R³ is -NH₂, -N(C₁-C₃ alkyl)₂, -NHCH₂CH₂OH, -N(C₁-C₃ alkyl)CH₂CH₂OH, N(CH₂CH₂OH)₂, -NHCH₂CH(CH₂OH)₂, -N(C₁-C₃ alkyl)CH₂CH(CH₂OH)₂, -NHCH₂CH₂OCH₂CH₂OH, -NHCH₂CH₂OCH₂CH₂NH₂,

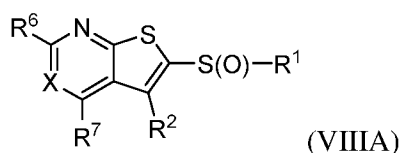
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-NHCH₂CH₂NH₂, -N(C₁-C₃ alkyl)CH₂CH₂NH₂, -NHCH₂CH₂NH(C₁-C₃ alkyl),
 -NHCH₂CH₂N(C₁-C₃ alkyl)₂, -N(C₁-C₃ alkyl)CH₂CH₂NH(C₁-C₃ alkyl), -N(C₁-C₃
 alkyl)CH₂CH₂N(C₁-C₃ alkyl)₂, -NHSO₂CH₃, -N(C₁-C₃ alkyl)SO₂CH₃, -OCH₂CH₂OH,
 -OCH₂CH₂NH₂, -OCH₂CH₂NH(C₁-C₃ alkyl), or -OCH₂CH₂N(C₁-C₃ alkyl)₂.

[00194] In some embodiments, R³ is -NHCH₂CH₂OH or -N(CH₃)CH₂CH₂OH.

[00195] In other embodiments, R⁴ is halogen, -CN, -N(R⁵)₂, -OH, -O-(C₁-C₆ alkylene)-
 OH, -S(O)_m(C₁-C₆ alkyl), -C(O)(C₁-C₆ alkyl), -C(O)-(3- to 6-membered cycloalkyl), C₁-C₆
 alkyl, C₁-C₆ haloalkyl, 3- to 6-membered cycloalkyl, or 3- to 6-membered heterocyclyl.

[00196] In some embodiments, the compound has the structure of formula (VIII A):

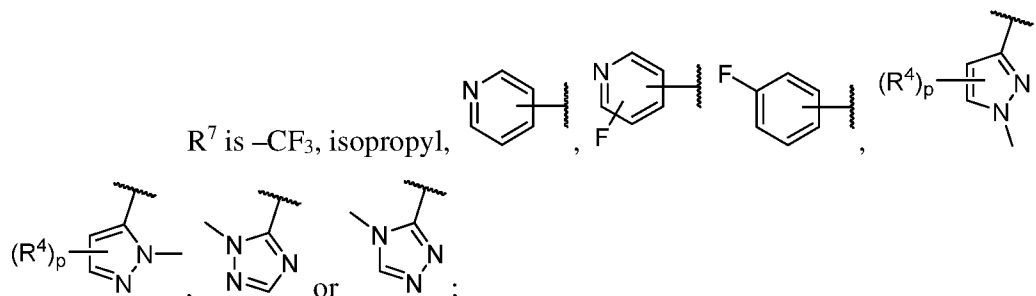


or a pharmaceutically acceptable salt, tautomer, or solvate thereof, wherein:

R¹ is cyclobutyl or -(C₁-C₄ alkylene)-(C₁-C₃ alkoxy);

R² is -NH₂, CN, or -NHC(O)alkyl;

R⁶ is heterocyclyl or heteroaryl, each of which is optionally substituted with
 one or more R³;



R³ is oxo, -OH, -O-alkylene-OH, -O-alkylene-N(R⁵)₂, -N(R⁵)₂, -
 N(R⁵)(alkylene-OH), -N(R⁵)(alkylene-O-alkyl), alkyl, -alkylene-OH, haloalkyl, cycloalkyl,
 heterocyclyl, -C(O)N(R⁵)₂, -C(O)N(R⁵)(alkylene-OH), -C(O)-alkyl, -C(O)O-alkyl, or -S(O)_m-
 alkyl, wherein the cycloalkyl and the heterocyclyl is each optionally substituted with R¹⁰;

R⁴ is C₁-C₃ alkyl;

each R⁵ is independently, H, alkyl, -alkylene-OH optionally substituted with
 -OH, -alkylene-NH₂, -alkylene-N(R⁹)₂, -alkylene-O-alkylene-OH, -alkylene-O-alkylene-NH₂,
 -C(O)-alkyl, -C(O)O-alkyl, -alkylene-COOH, or -S(O)_m-alkyl;

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or alternatively, two R⁵ together with the N atom to which they are attached can form a 4- to 7-membered heterocycle, optionally containing an additional heteroatom selected from O, S, or N, and wherein the heterocycle is optionally substituted with R⁸;

R⁸ is halogen, alkyl, or alkoxy;

R⁹ is H or alkyl, or two R⁹ together with the N atom to which they are attached can form a 4- to 7-membered heterocycle, optionally containing an additional heteroatom selected from O, S(O)_t, or N;

R¹⁰ is -OH, halogen, alkyl, or alkoxy;

X is N or CH;

m is 0, 1, or 2;

p is 0 or 1; and

t is 0, 1, or 2.

[00197] In still other embodiments, R² is -NH₂.

[00198] In some embodiments, R⁶ is 5- to 6-membered heterocyclyl or 5- to 10-membered heteroaryl, each of which is optionally substituted with one or more R³.

[00199] In other embodiments, R⁶ is 5- to 6-membered heteroaryl optionally substituted with one or more R³.

[00200] In still other embodiments, R⁶ is 8- to 10-membered bicyclic heteroaryl optionally substituted with one or more R³.

[00201] In some embodiments, R³ is -O-(C₁-C₆ alkylene)-N(R⁵)₂, -N(R⁵)₂, -N(R⁵)(C₁-C₆ alkylene-OH), -C(O)N(R⁵)₂, -C(O)N(R⁵)(C₁-C₆ alkylene-OH), -C(O)(C₁-C₆ alkyl), -C(O)O(C₁-C₆ alkyl), or -S(O)_m(C₁-C₆ alkyl).

[00202] In other embodiments, R³ is -(C₁-C₃ alkyl)OH, -NH₂, -N(C₁-C₃ alkyl)₂, -NHCH₂CH₂OH, -N(C₁-C₃ alkyl)CH₂CH₂OH, N(CH₂CH₂OH)₂, -NHCH₂CH(CH₂OH)₂, -N(C₁-C₃ alkyl)CH₂CH(CH₂OH)₂, -NHCH₂CH₂OCH₂CH₂OH, -NHCH₂CH₂OCH₂CH₂NH₂, -NHCH₂CH₂NH₂, -N(C₁-C₃ alkyl)CH₂CH₂NH₂, -NHCH₂CH₂NH(C₁-C₃ alkyl), -NHCH₂CH₂N(C₁-C₃ alkyl)₂, -N(C₁-C₃ alkyl)CH₂CH₂NH(C₁-C₃ alkyl), -N(C₁-C₃ alkyl)CH₂CH₂N(C₁-C₃ alkyl)₂, -NHSO₂CH₃, -N(C₁-C₃ alkyl)SO₂CH₃, -OCH₂CH₂OH, -OCH₂CH₂NH₂, -OCH₂CH₂NH(C₁-C₃ alkyl), or -OCH₂CH₂N(C₁-C₃ alkyl)₂.

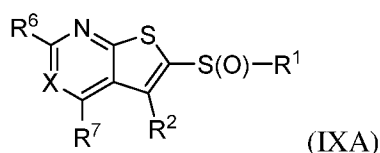
[00203] In still other embodiments, R³ is -NHCH₂CH₂OH or -N(CH₃)CH₂CH₂OH.

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[00204] In some embodiments, R^4 is halogen, $-\text{CN}$, $-\text{N}(\text{R}^5)_2$, $-\text{OH}$, $-\text{O}-(\text{C}_1-\text{C}_6 \text{ alkylene})-\text{OH}$, $-\text{S}(\text{O})_m(\text{C}_1-\text{C}_6 \text{ alkyl})$, $-\text{C}(\text{O})(\text{C}_1-\text{C}_6 \text{ alkyl})$, $-\text{C}(\text{O})-(3\text{- to } 6\text{-membered cycloalkyl})$, $\text{C}_1-\text{C}_6 \text{ alkyl}$, $\text{C}_1-\text{C}_6 \text{ haloalkyl}$, $3\text{- to } 6\text{-membered cycloalkyl}$, or $3\text{- to } 6\text{-membered heterocyclyl}$.

[00205] In some embodiments, n is 1.

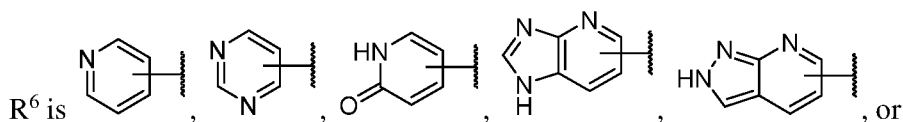
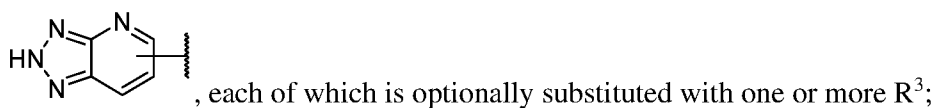
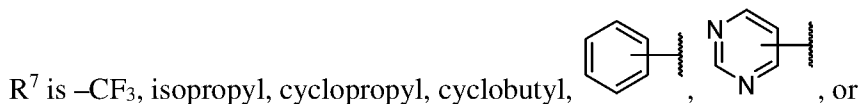
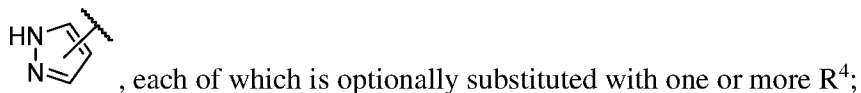
[00206] In some embodiments, the compound has the structure of formula (IXA):



or a pharmaceutically acceptable salt, tautomer, or solvate thereof, wherein:

R^1 is cyclobutyl or $-(\text{C}_1-\text{C}_4 \text{ alkylene})-(\text{C}_1-\text{C}_3 \text{ alkoxy})$;

R^2 is $-\text{NH}_2$, CN , or $-\text{NHC}(\text{O})\text{alkyl}$;



R^3 is $-\text{NH}_2$, $-\text{NH}(\text{C}_1-\text{C}_3 \text{ alkyl})$, $-\text{NH}(\text{C}_1-\text{C}_4 \text{ alkylene})-\text{OH}$, or $\text{C}_1-\text{C}_3 \text{ alkyl}$;

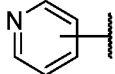
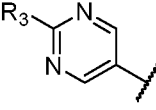
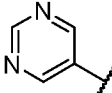
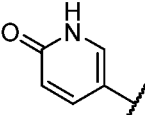
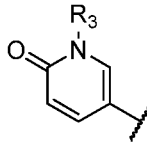
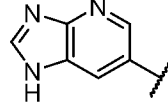
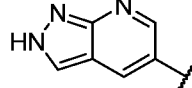
R^4 is $\text{C}_1-\text{C}_3 \text{ alkyl}$; and

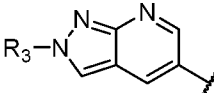
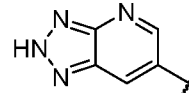
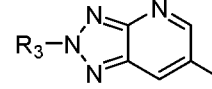
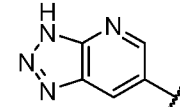
X is N or CH .

[00207] In some embodiments of Formula (IXA), R^1 is cyclobutyl. In some embodiments of Formula (IXA), R^1 $-(\text{C}_1-\text{C}_4 \text{ alkylene})-(\text{C}_1-\text{C}_3 \text{ alkoxy})$. In some embodiments of Formula (IXA), the R^1 $-(\text{C}_1-\text{C}_4 \text{ alkylene})-(\text{C}_1-\text{C}_3 \text{ alkoxy})$ is $-(\text{C}_2-\text{C}_3 \text{ alkylene})-(\text{C}_1 \text{ alkoxy})$.

[00208] In some embodiments of Formula (IXA), R^2 is $-\text{NH}_2$.

[00209] In some embodiments of Formula (IXA), R⁶ is


or

[00210] In some embodiments of Formula (IXA), R³ is -NH₂. In some embodiments of Formula (IXA), R³ is -NH(C₁-C₃ alkyl). In some embodiments of Formula (IXA), R³ is -NH(C₁-C₄ alkylene)-OH (*e.g.*, -NH(C₂-C₄ alkylene)-OH). In some embodiments of Formula (IXA), R³ is C₁-C₃ alkyl (*e.g.*, methyl or ethyl).

[00211] In some embodiments, of Formula (IXA), R⁷ is -CF₃, isopropyl, cyclopropyl, or cyclobutyl. In some embodiments, of Formula (IXA), R⁷ is isopropyl. In some embodiments

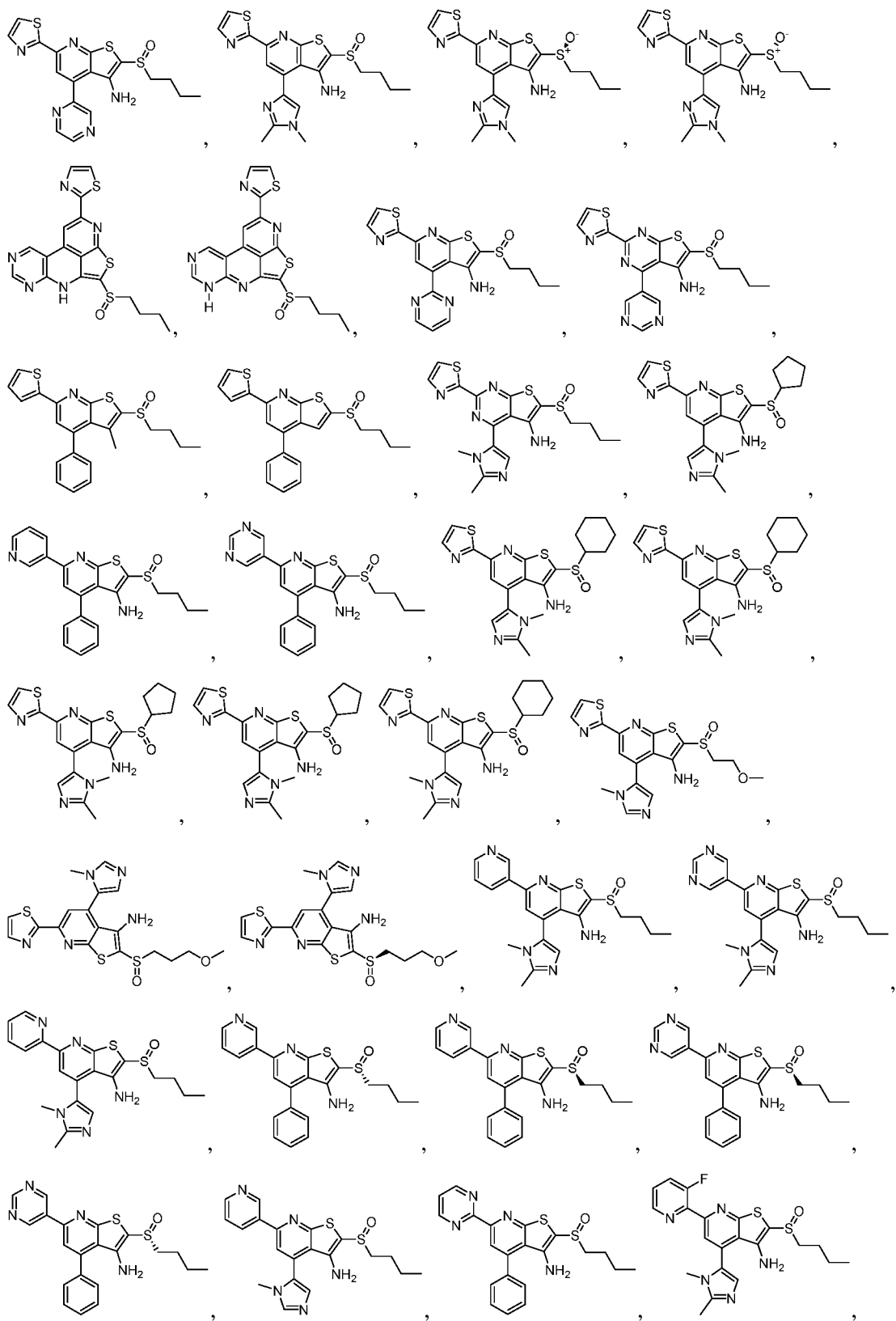
of Formula (VII), R⁷ is , , or , each of which is optionally substituted with one or more R⁴. In some embodiments, each R⁴ is independently selected from methyl or ethyl.

[00212] In some embodiments of Formula (IXA), X is $-CH$.

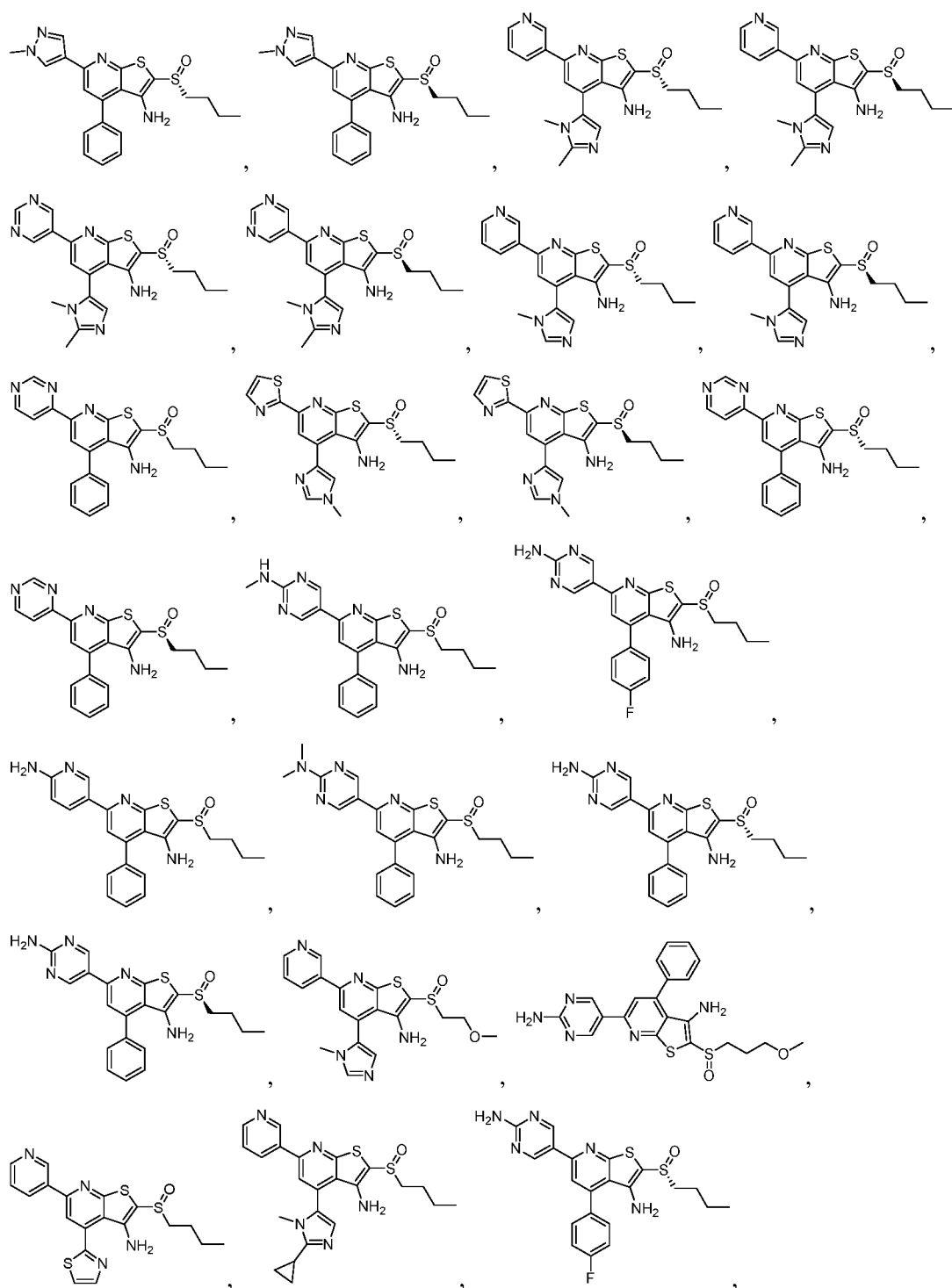
[00213] Examples of compounds having formulas (I), (II), (III), (IV), (V), (IA), (IIA), (IIIA), (IVA), (VA), (VIA), (VIIA), (VIII A), and (IXA) are described in U.S. Patent Application Publication Nos. 2015/0072998, 2017/0165241, 2017/0173028, 2018/0118756, WO2018/218251, and WO2020/106998, all of which are incorporated herein by reference in their entirety.

[00214] For example, the 15-PGDH inhibitor can include a compound selected from:

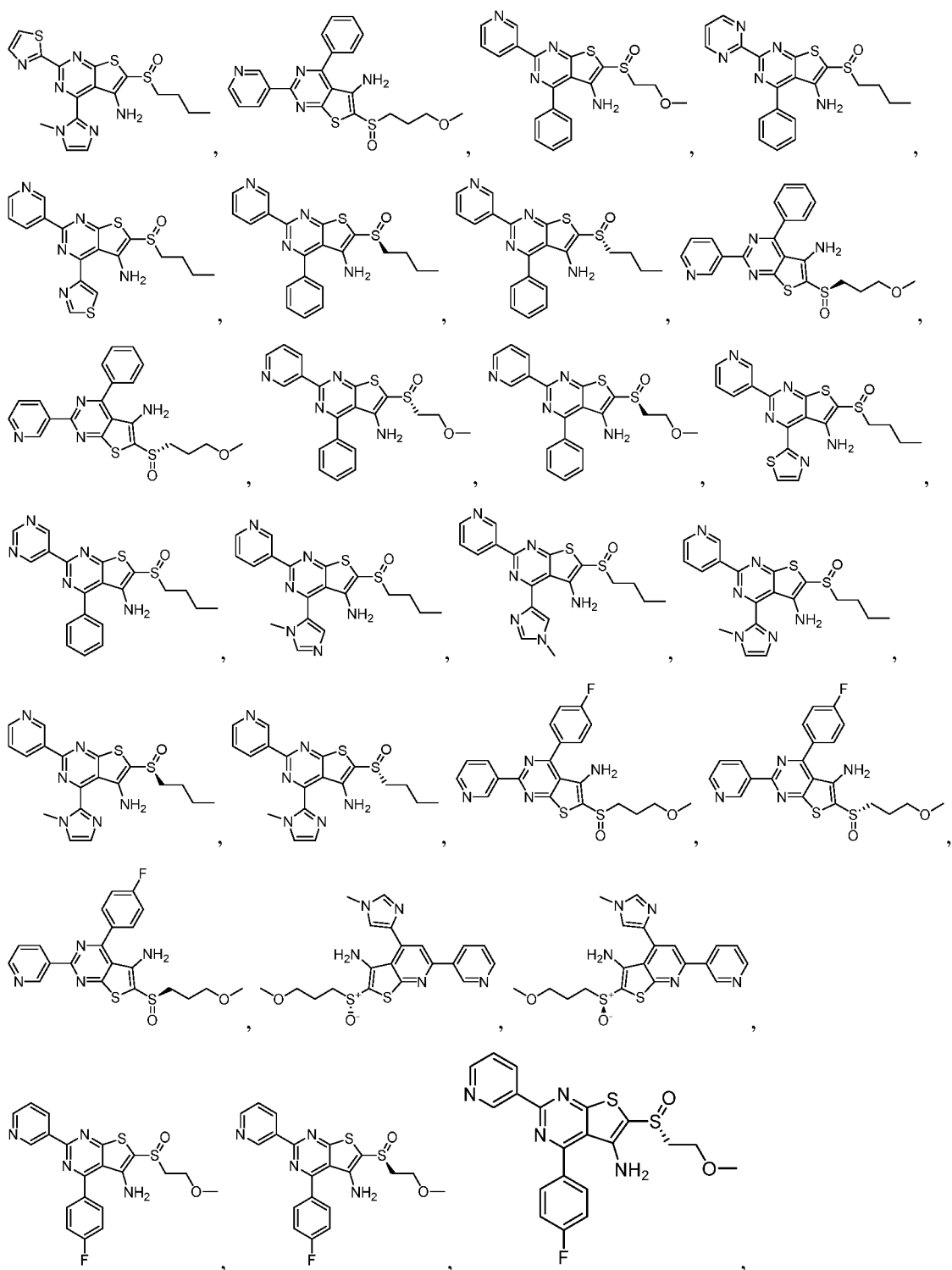
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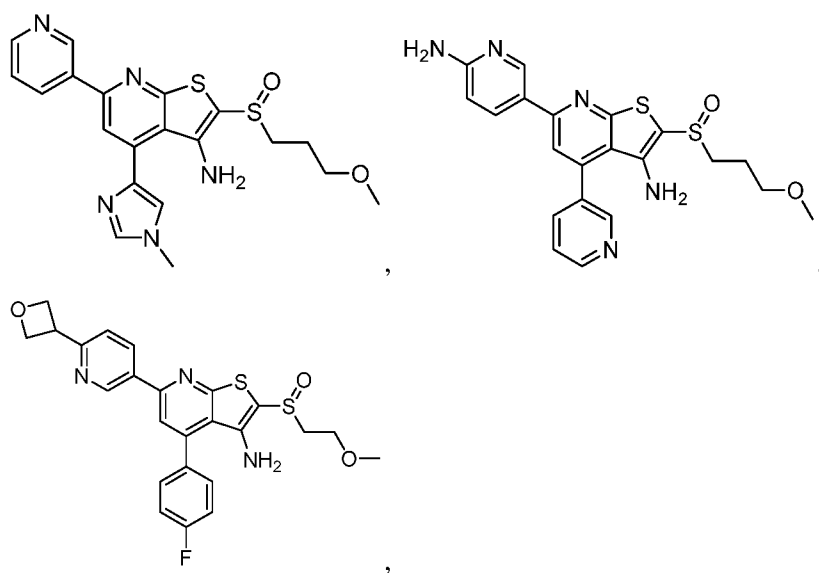
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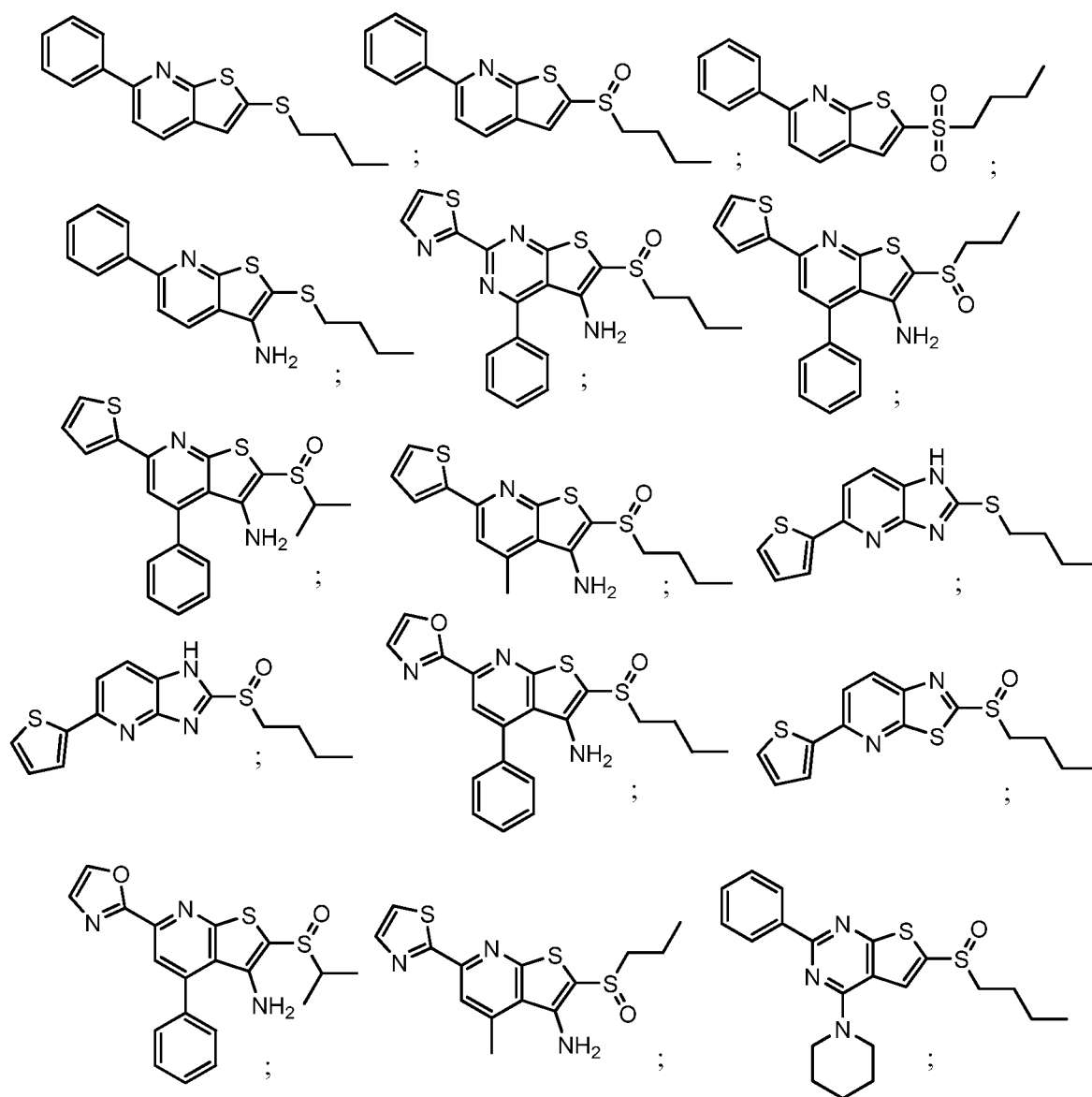
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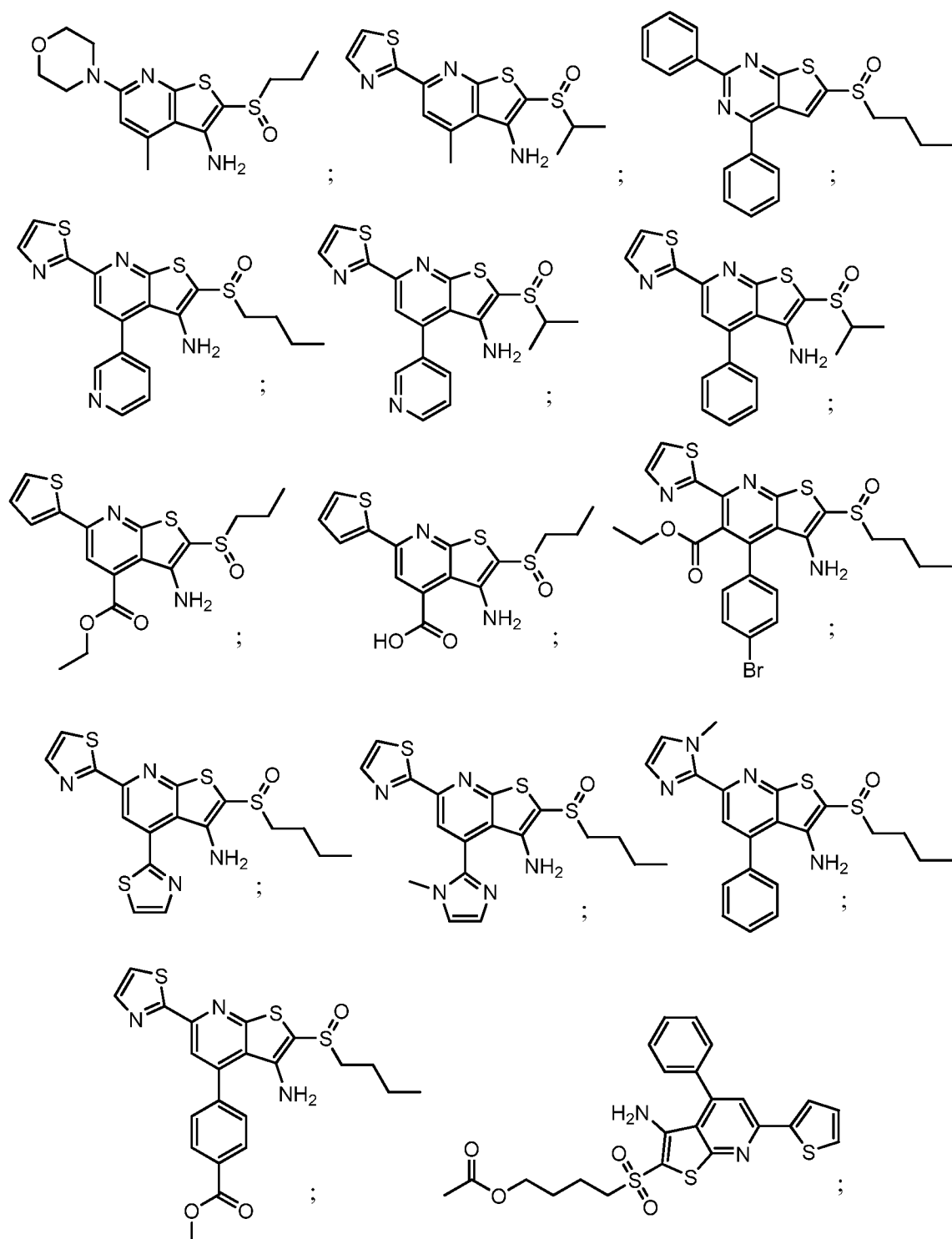
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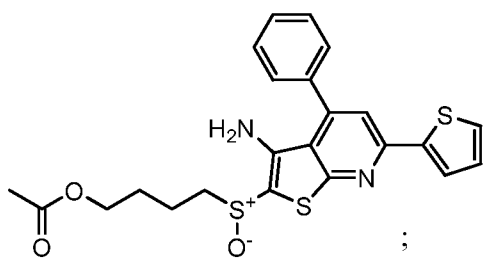
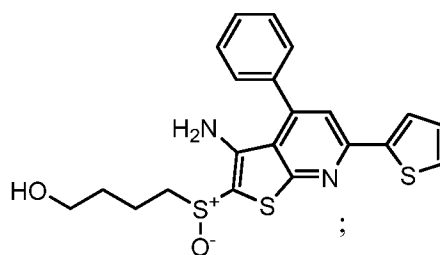
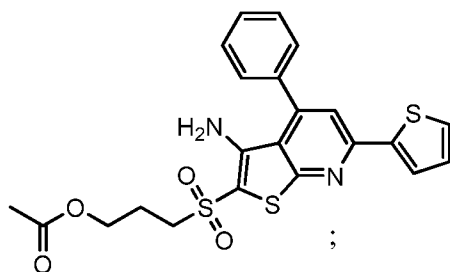
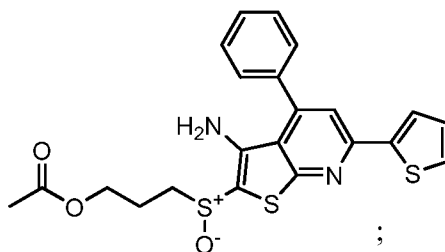
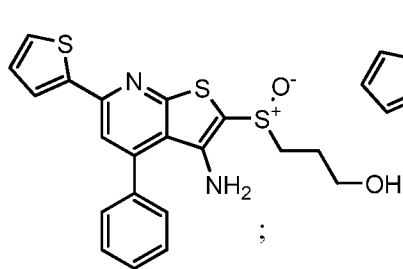
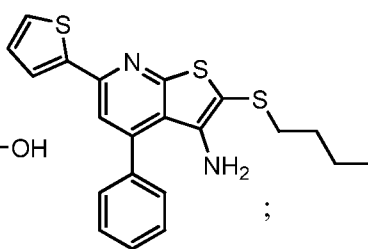
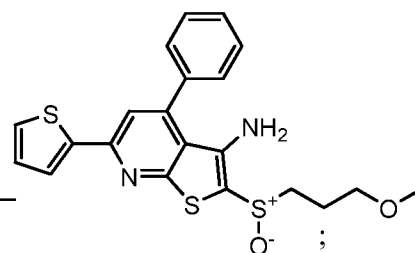
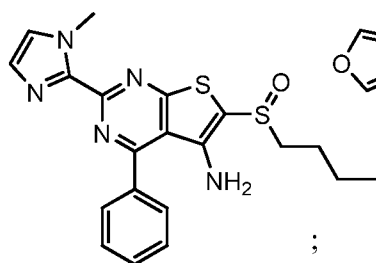
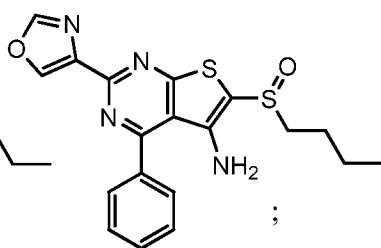
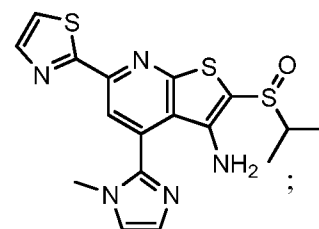
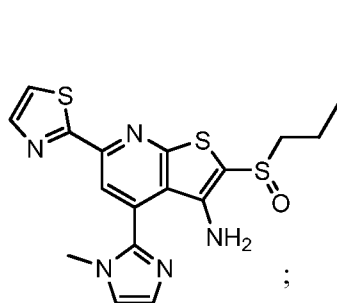
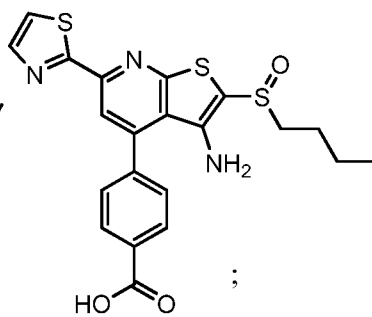
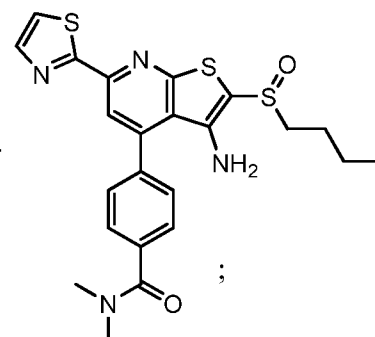
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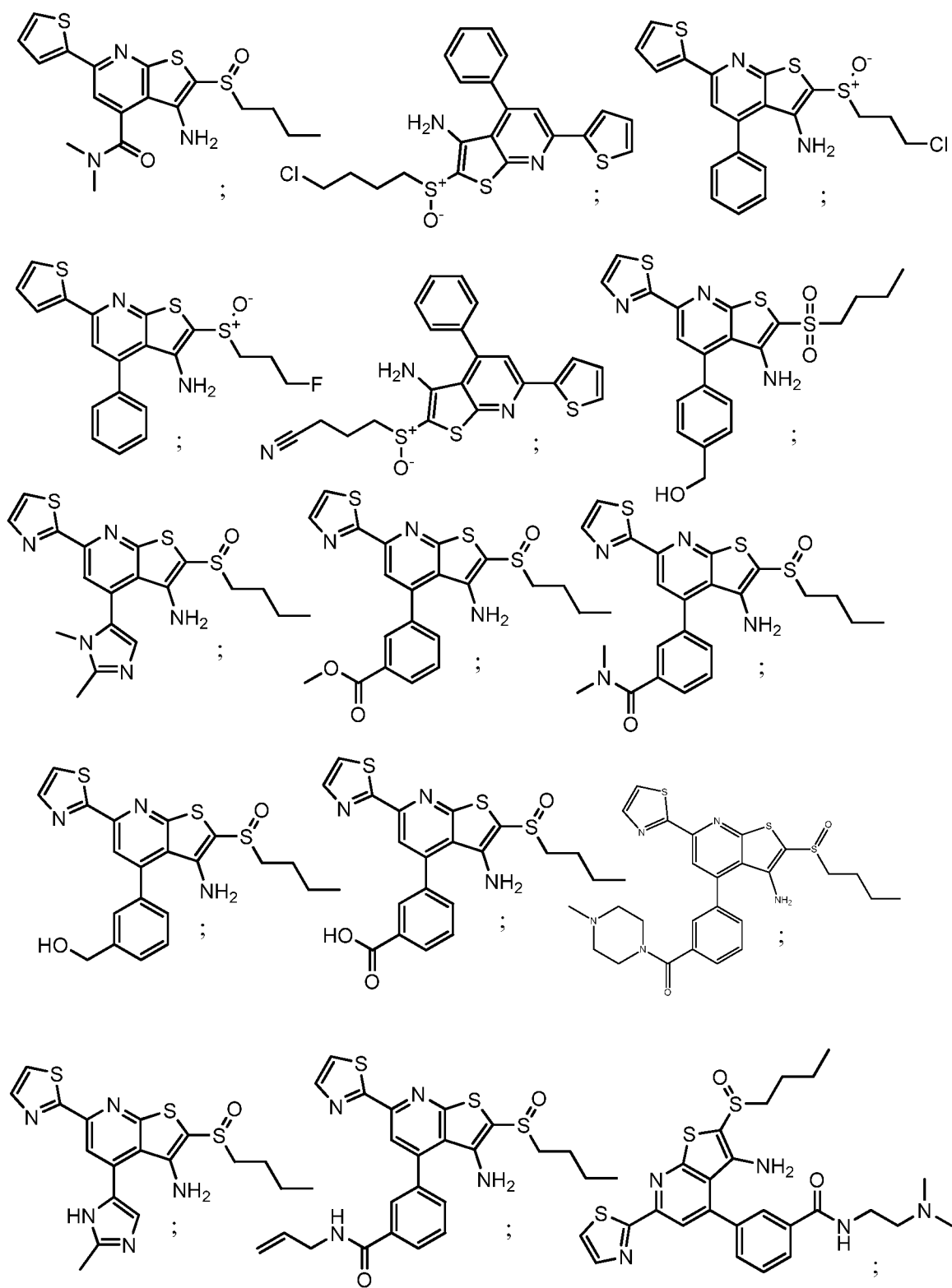
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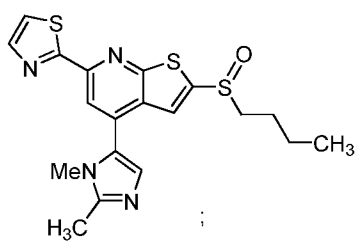
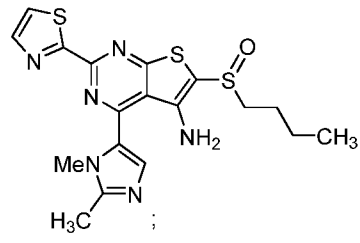
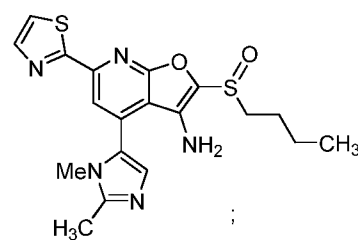
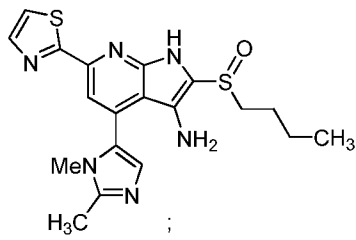
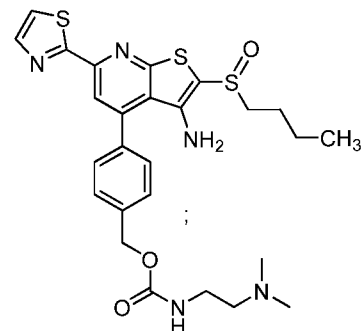
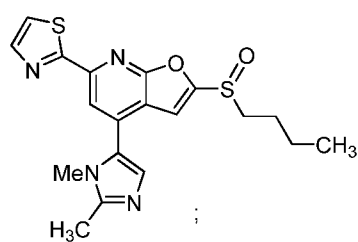
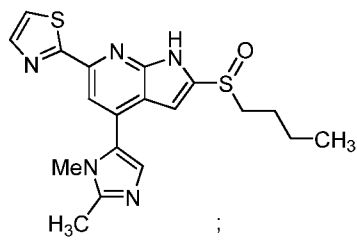
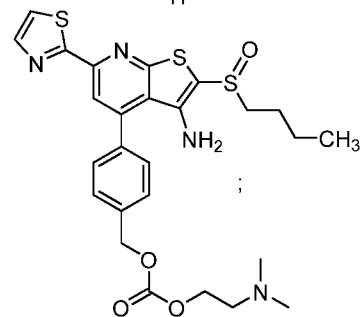
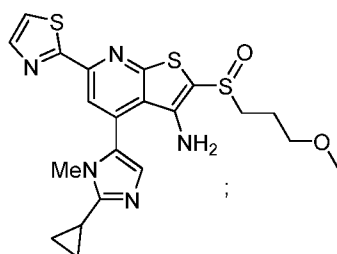
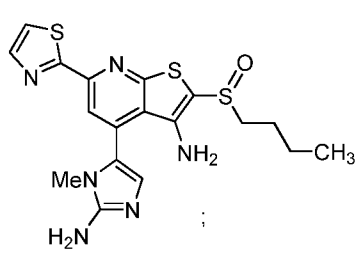
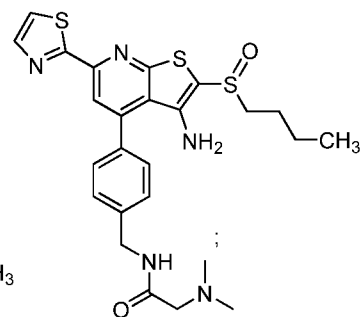
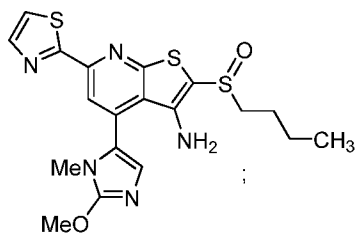
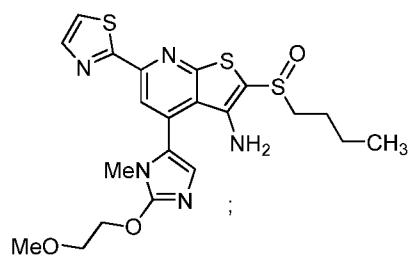


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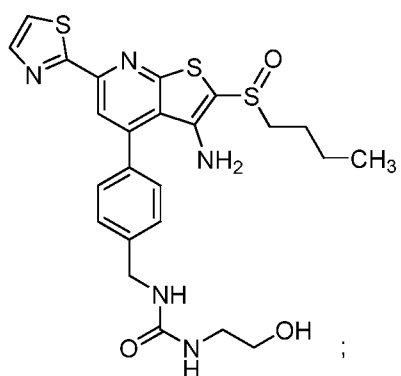
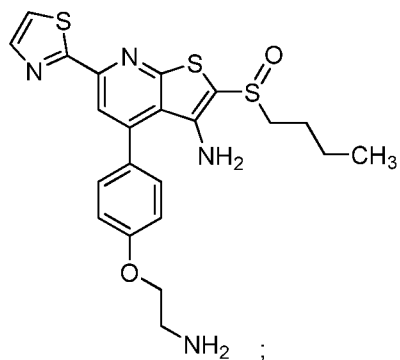
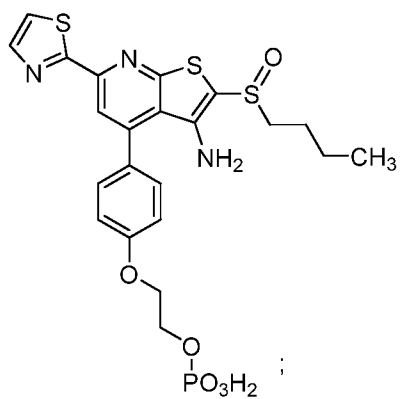
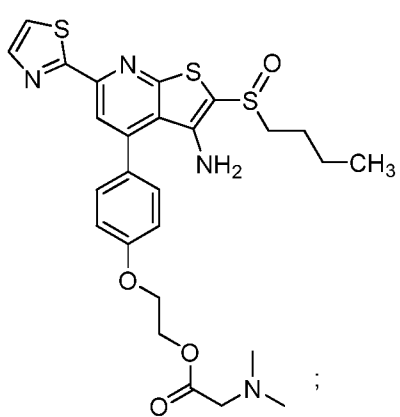
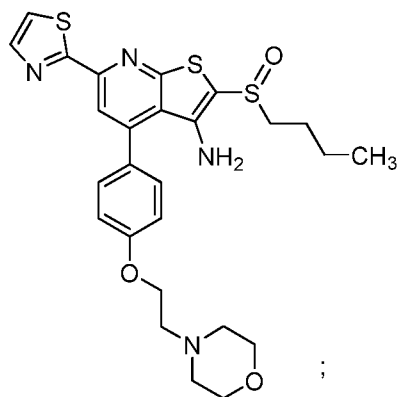
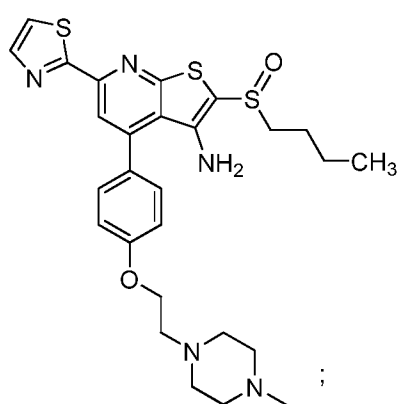


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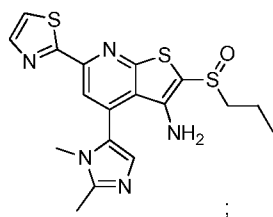
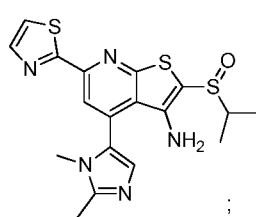
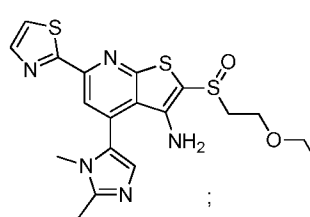
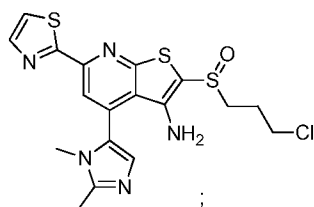
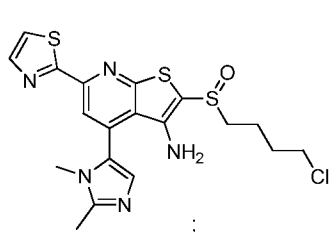
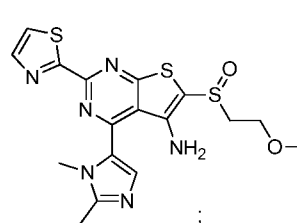
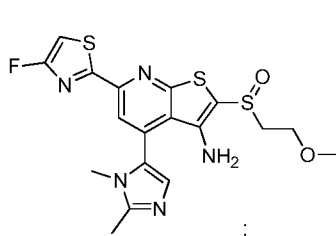
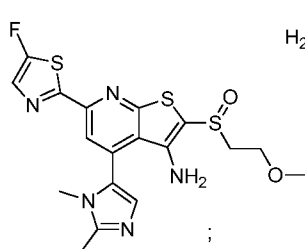
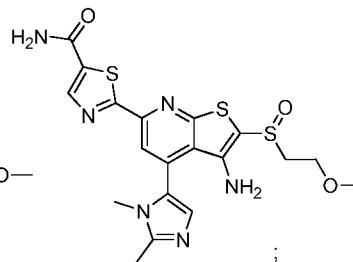
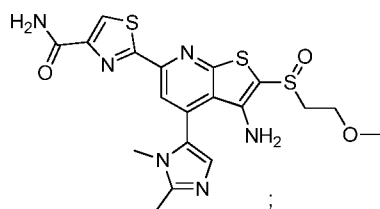
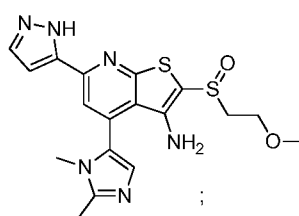
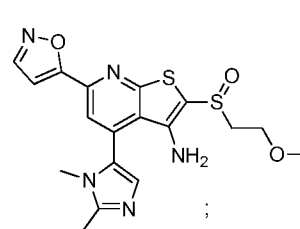
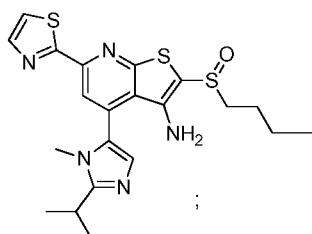
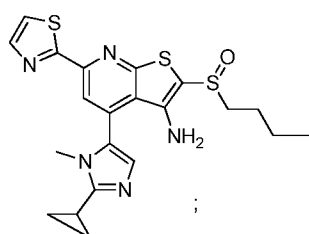
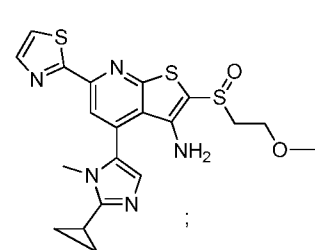
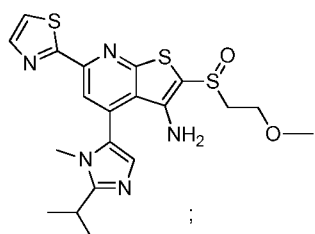




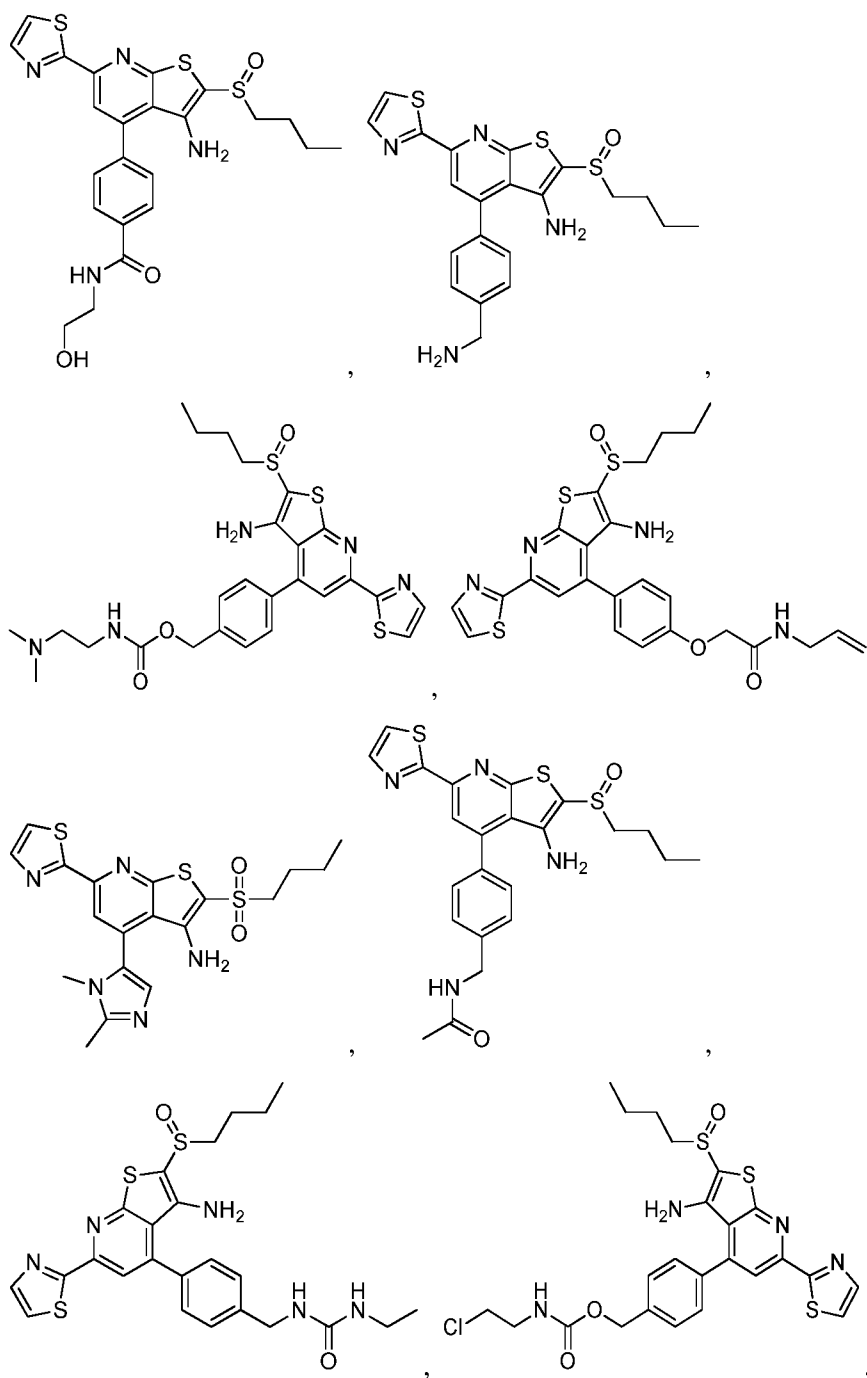
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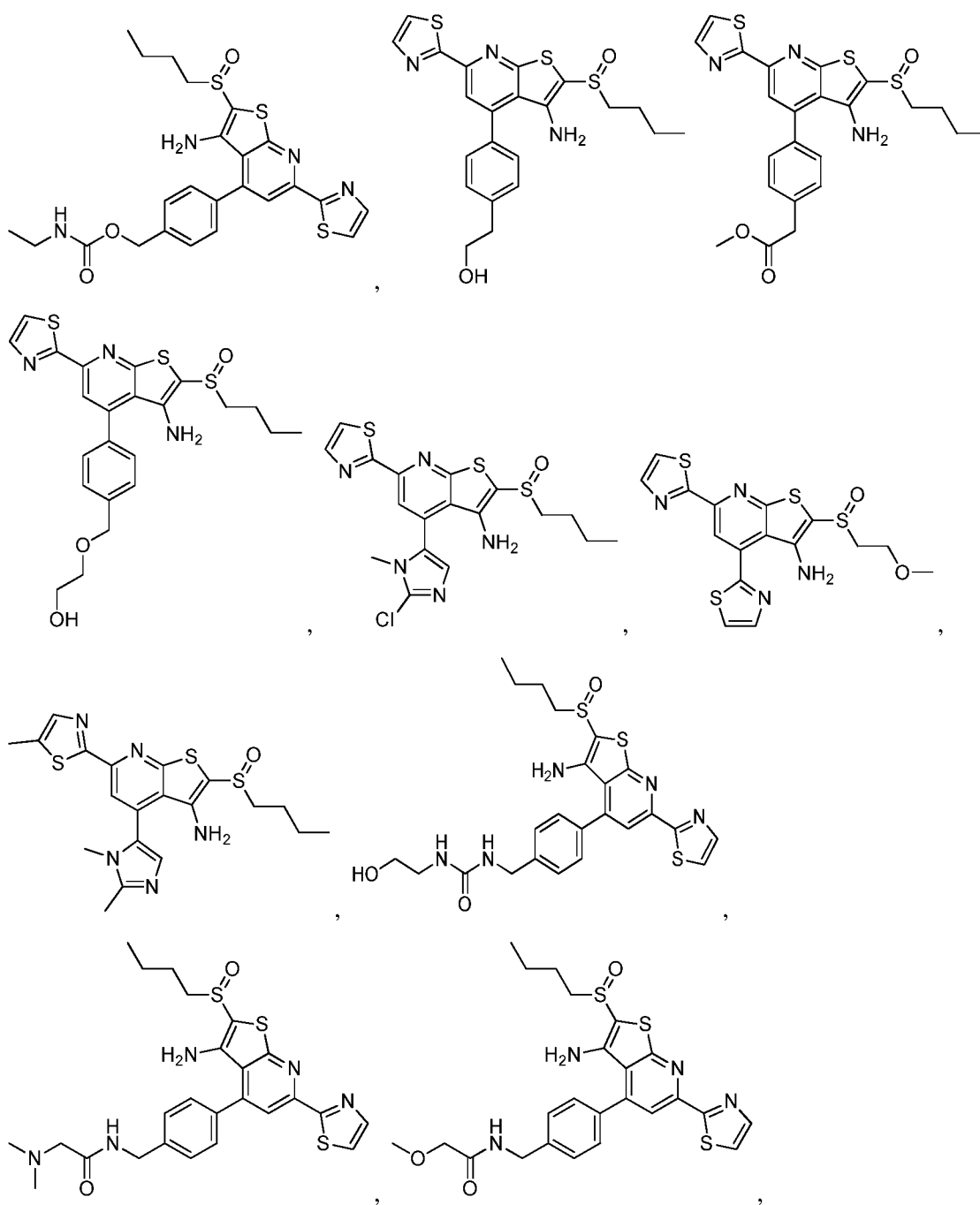
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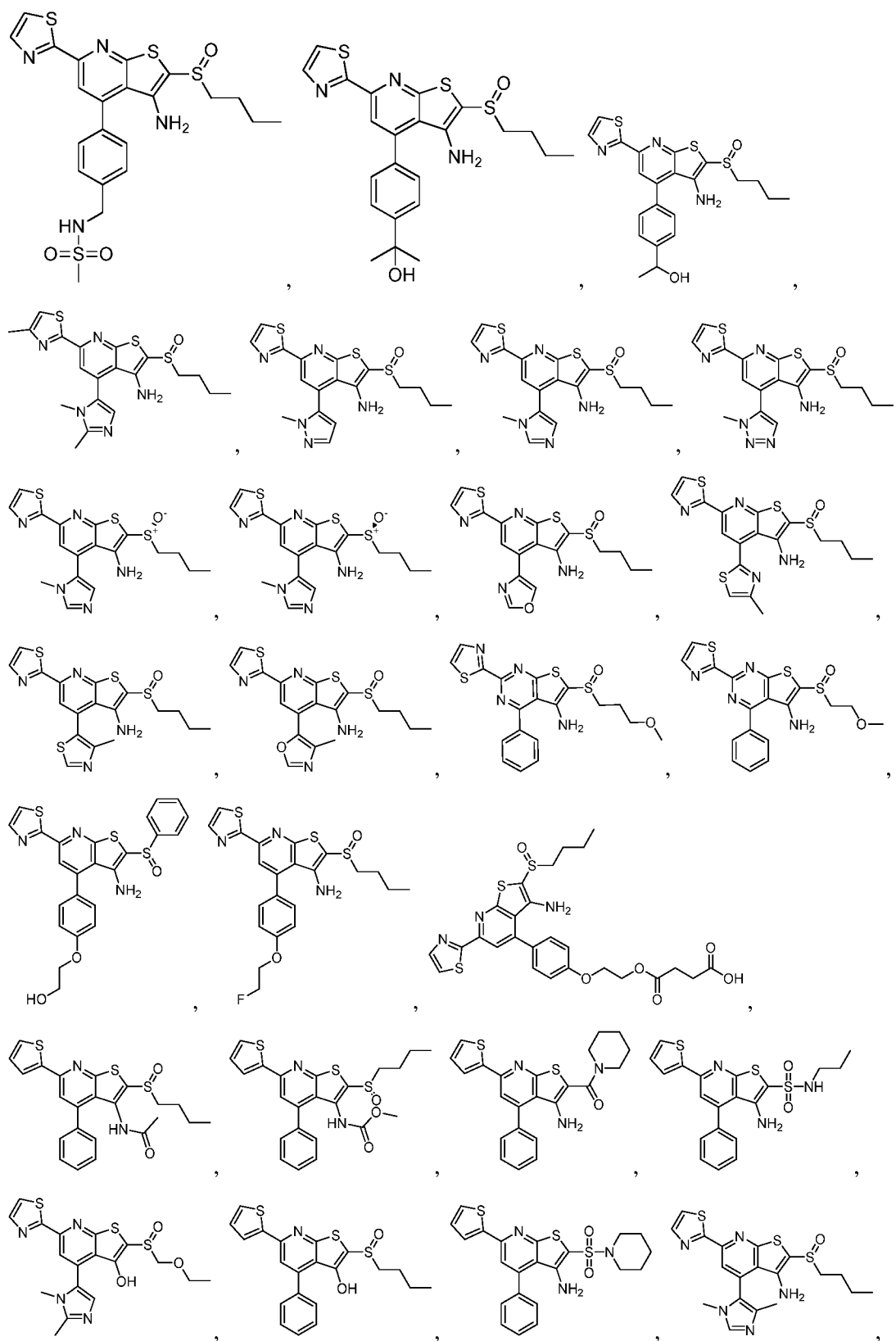
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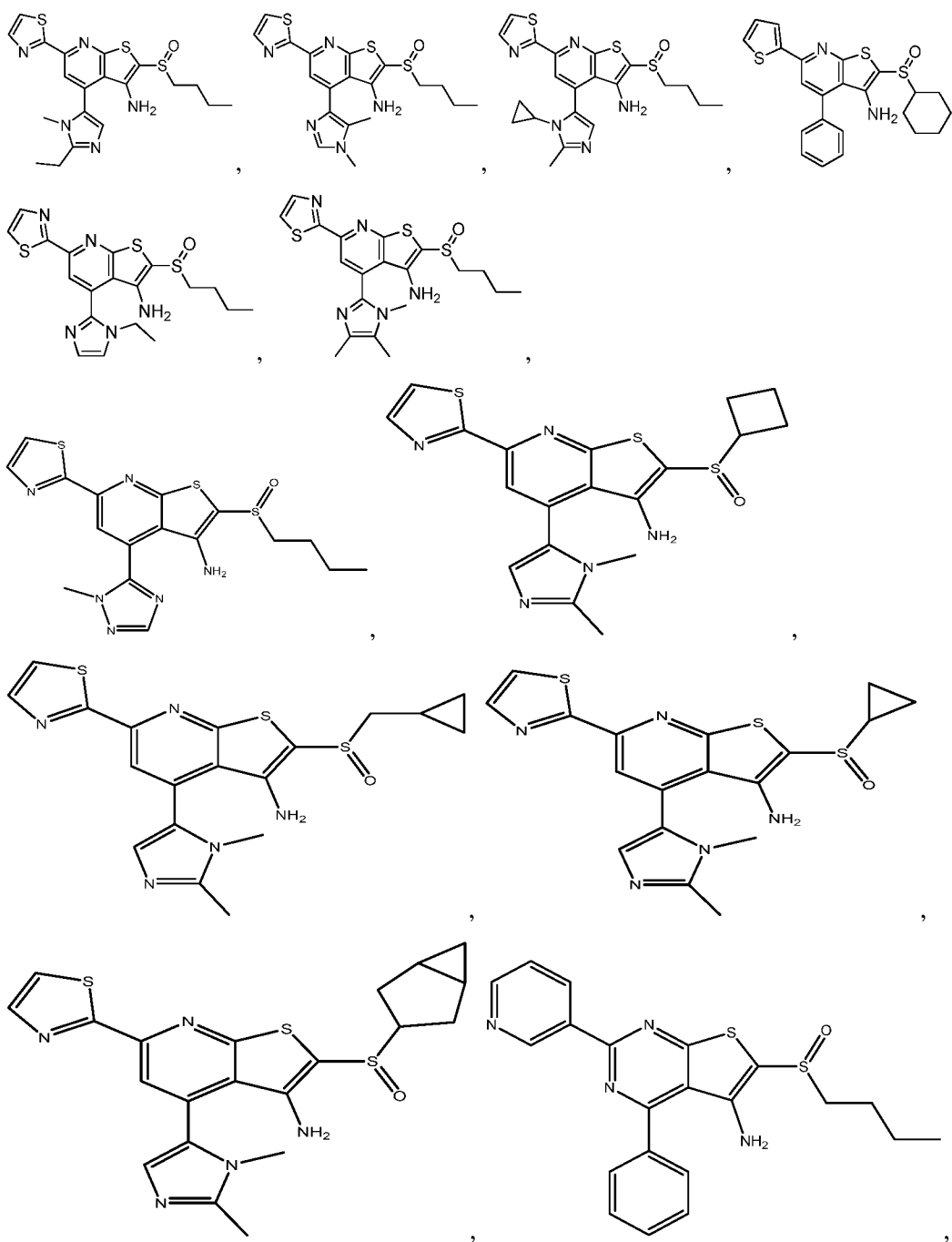
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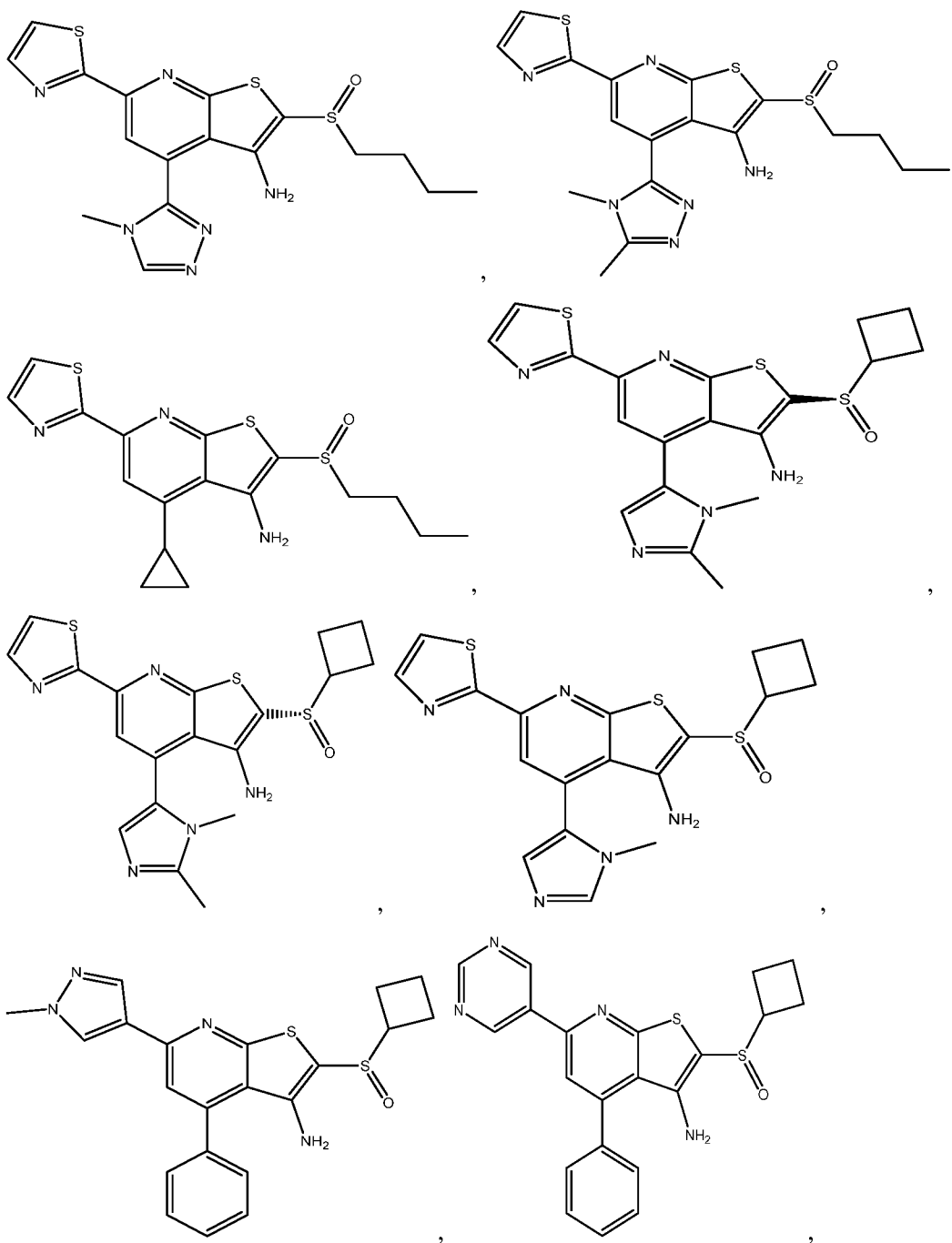
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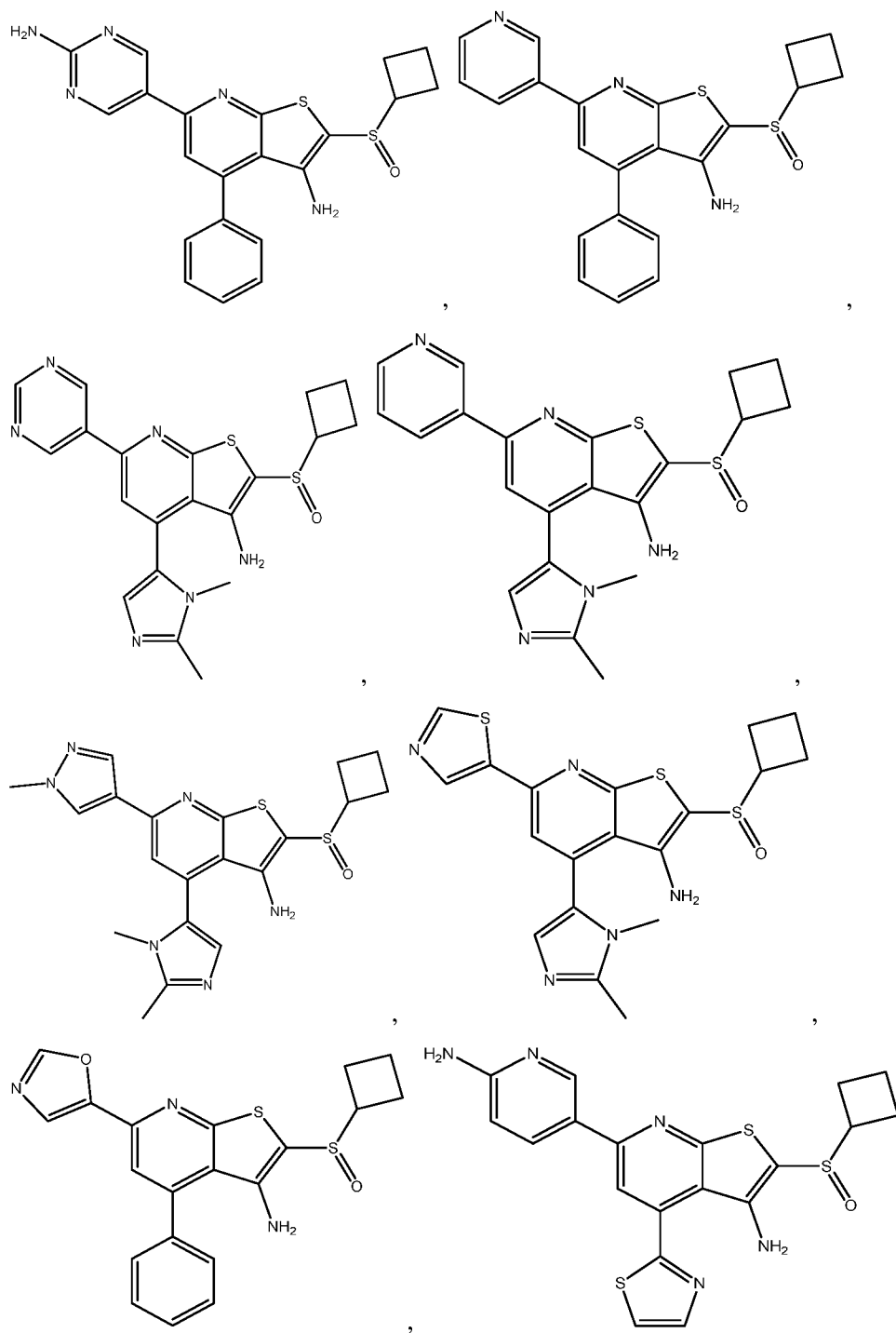
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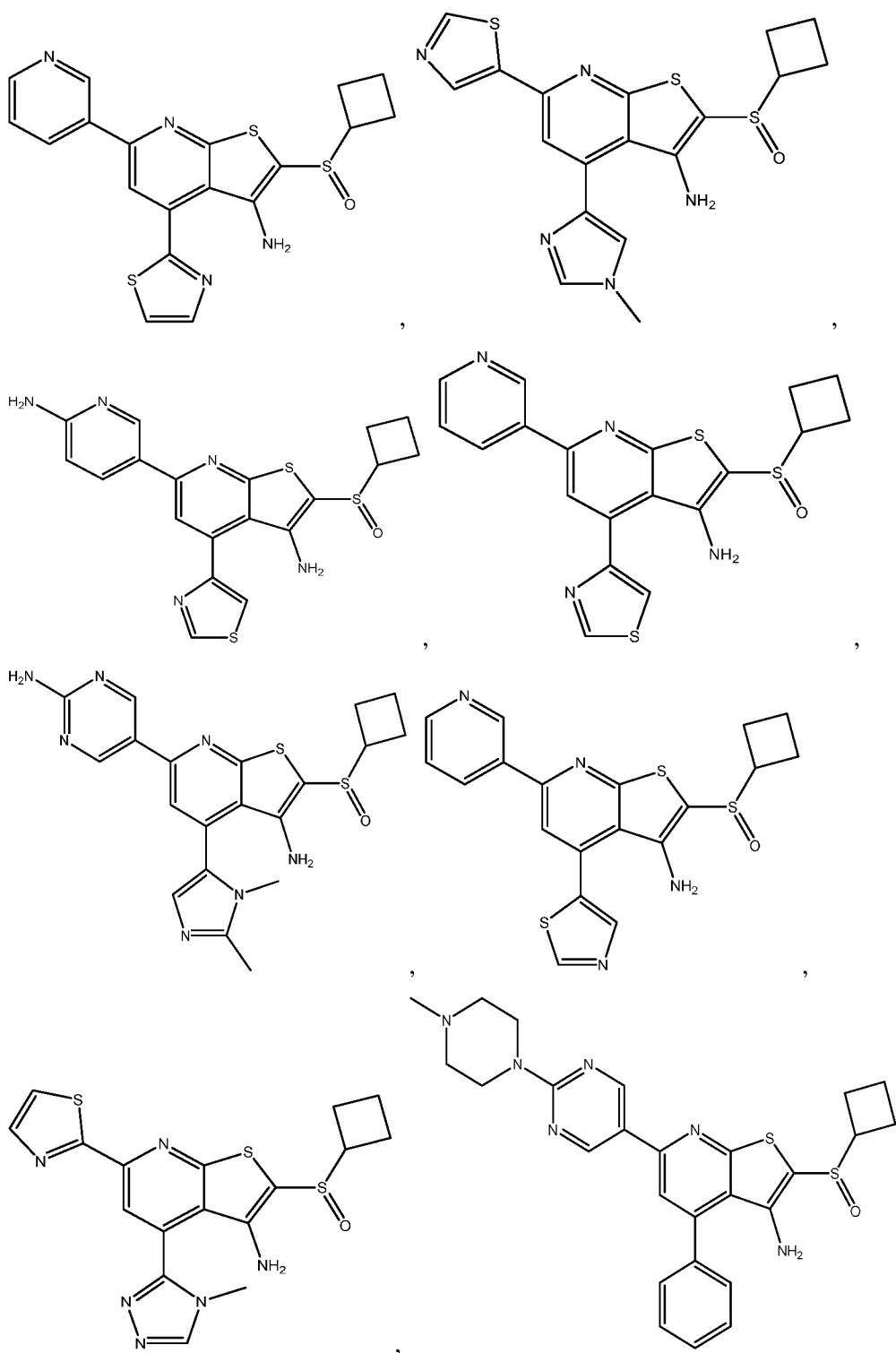
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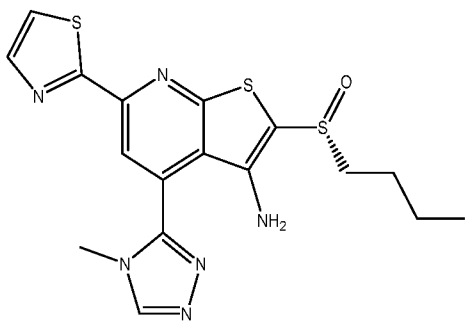
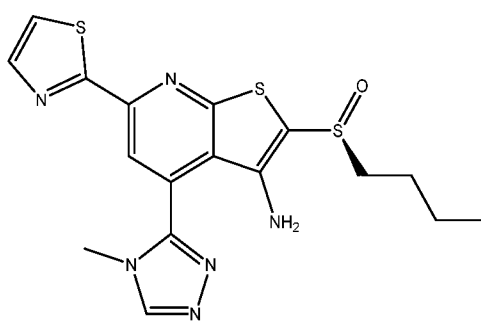
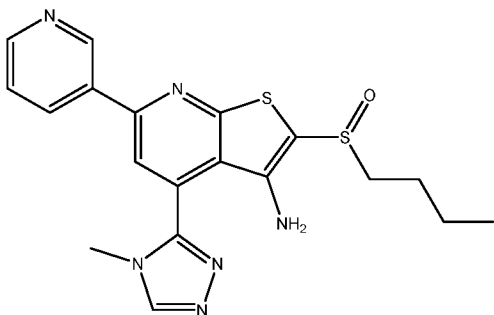
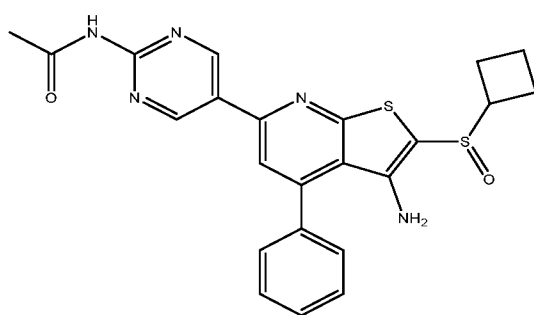
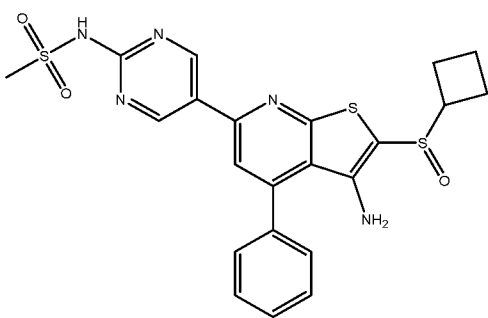
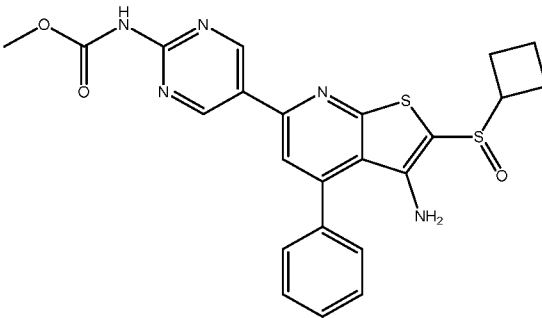
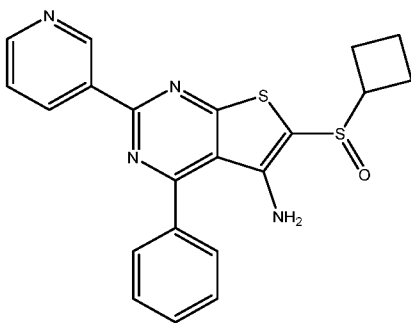
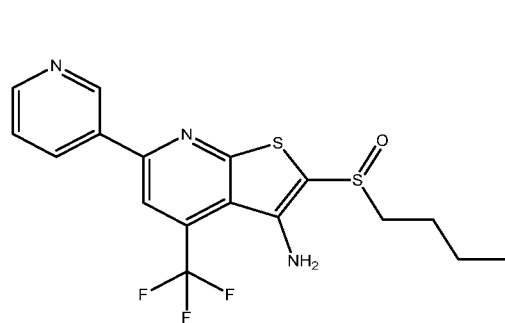
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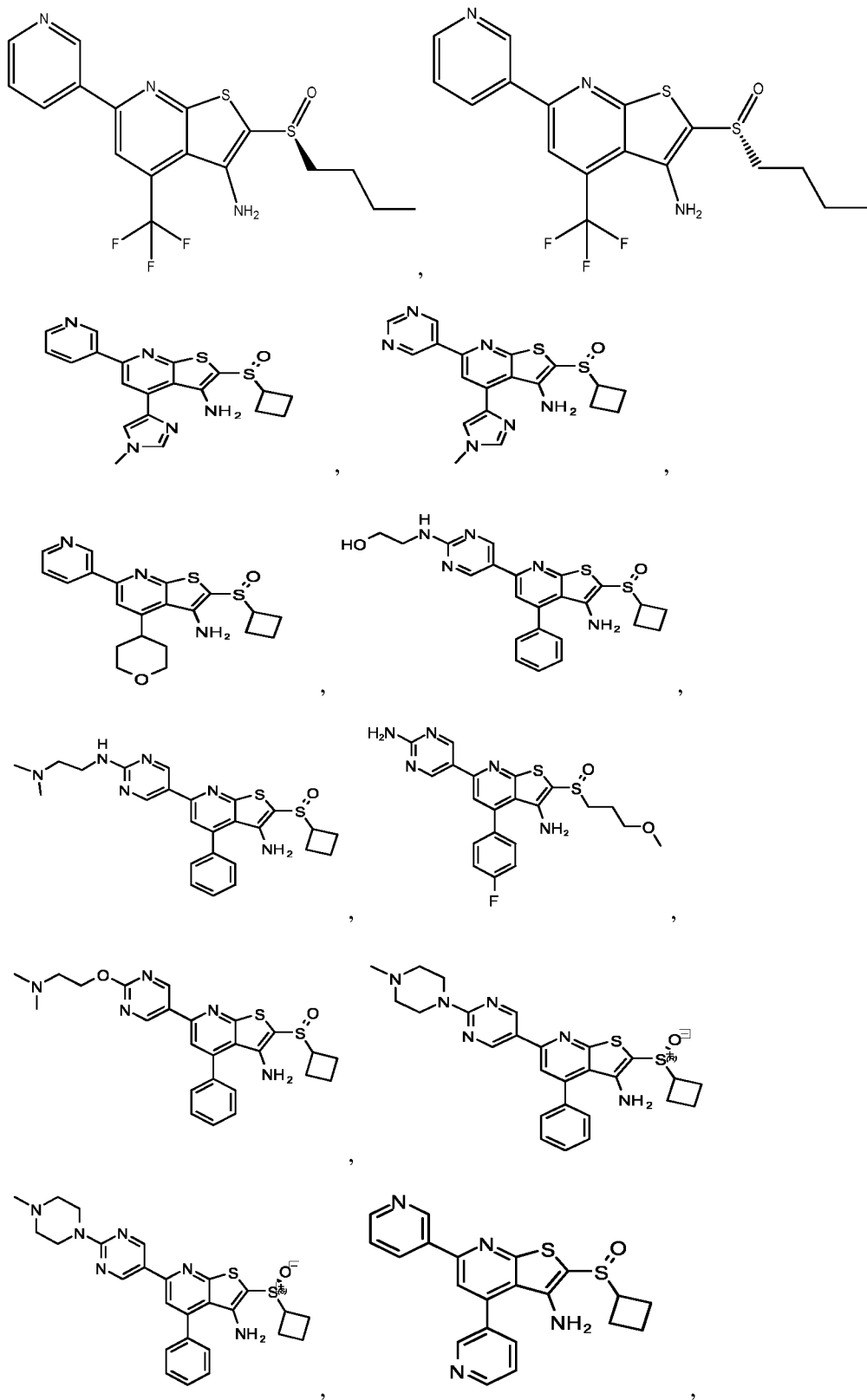
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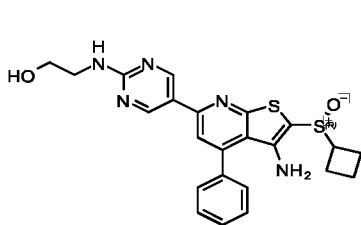
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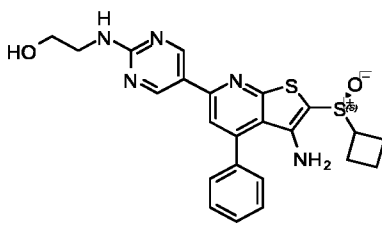
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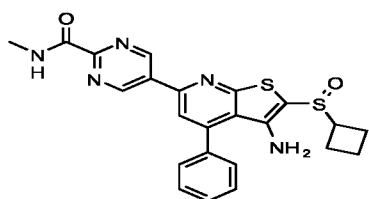
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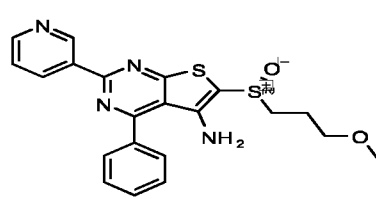
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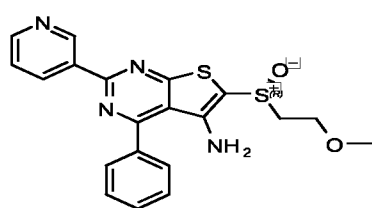
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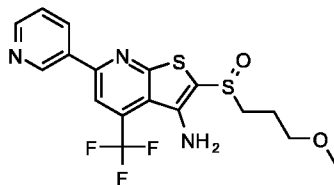
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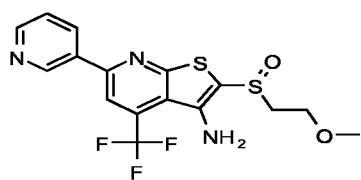
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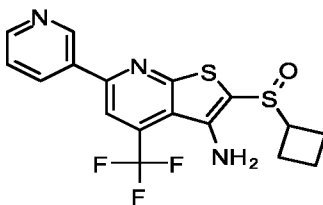
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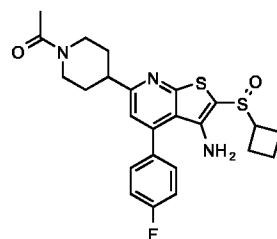
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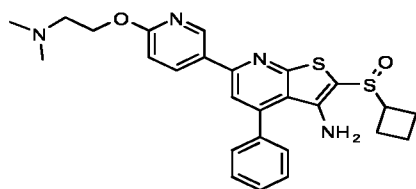
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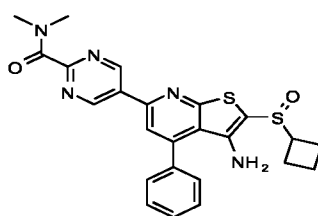
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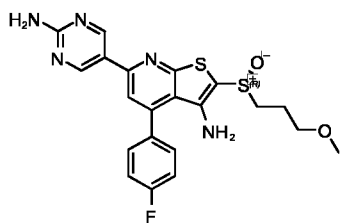
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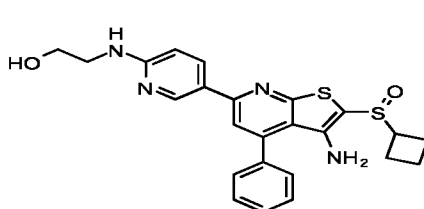
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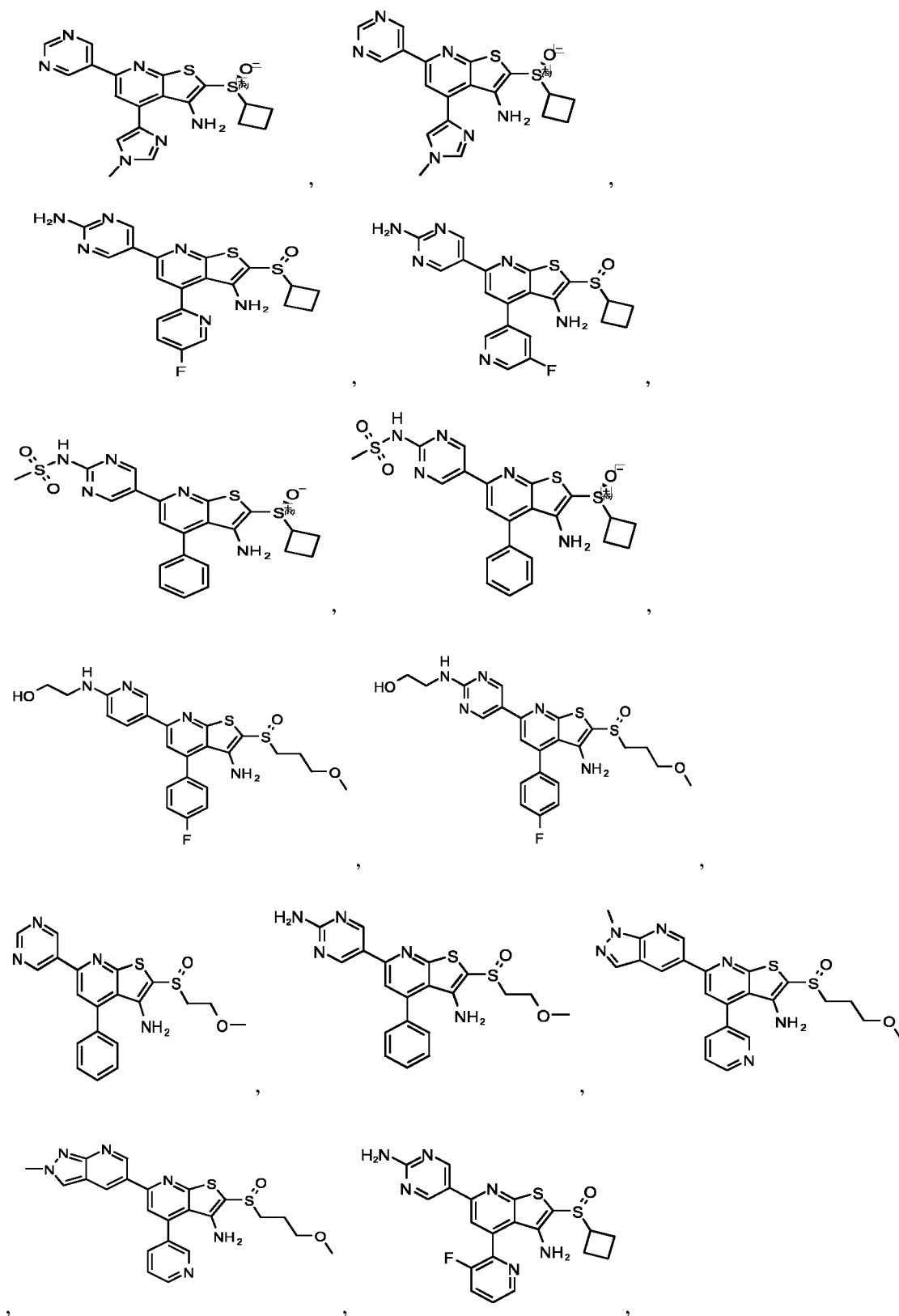


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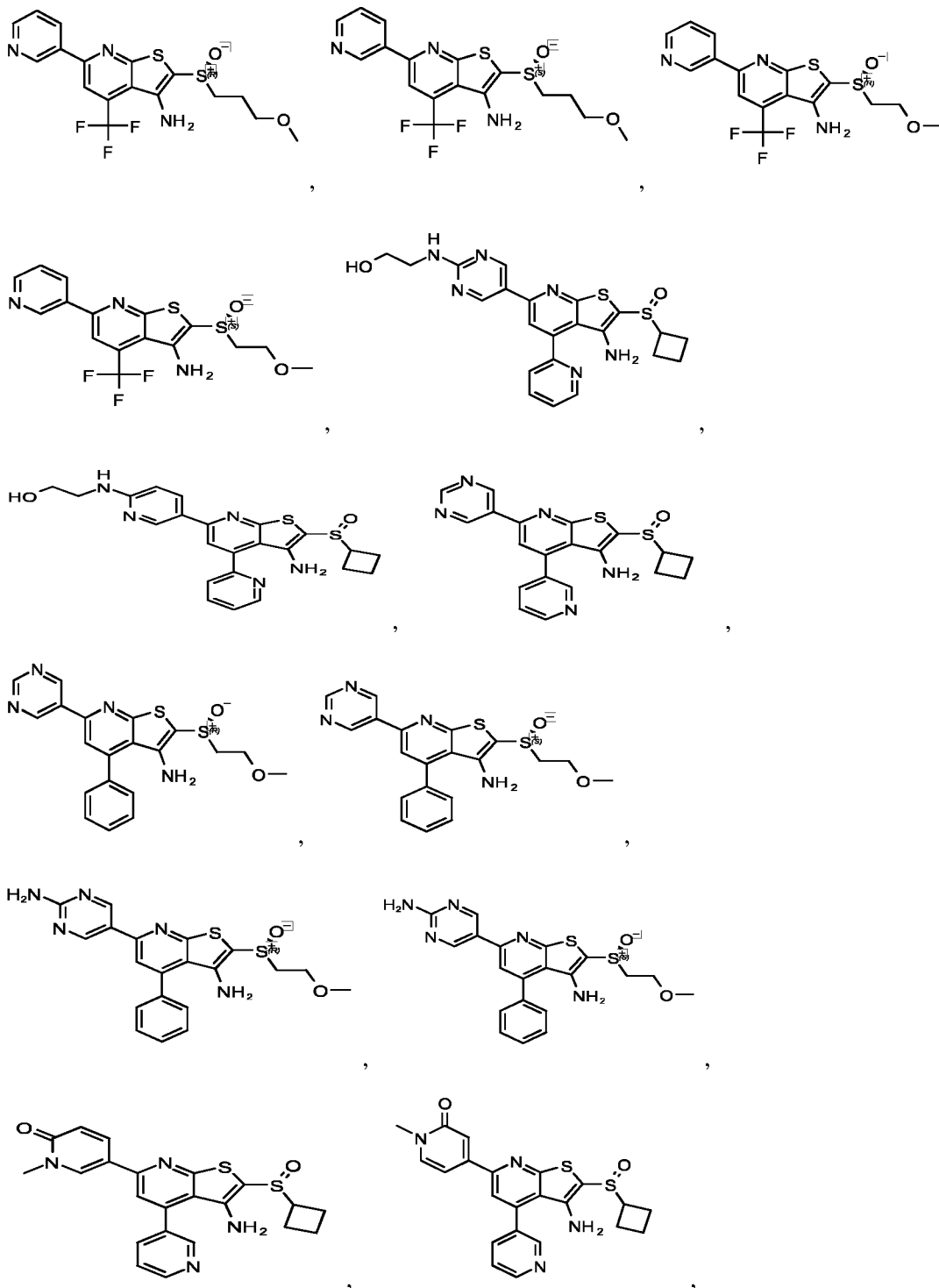


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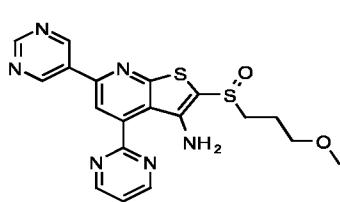
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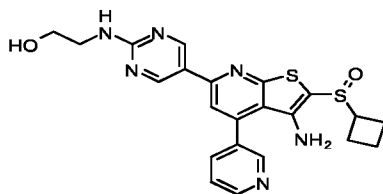
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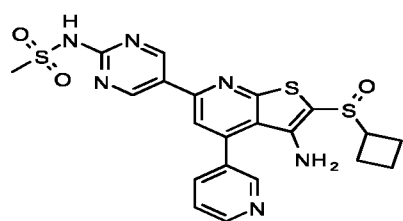
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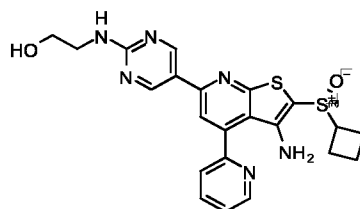
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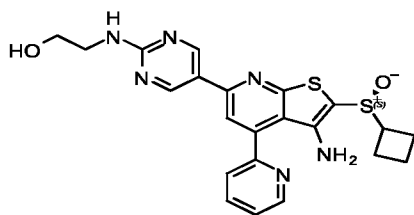
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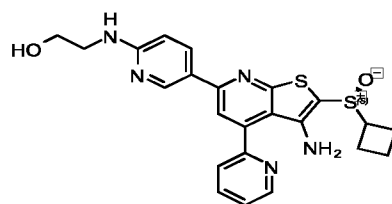
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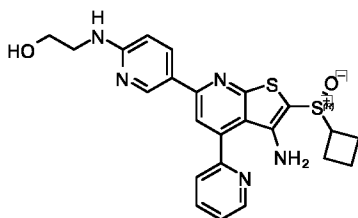
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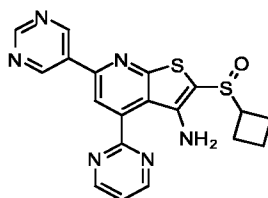
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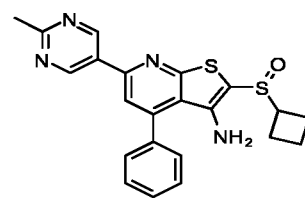
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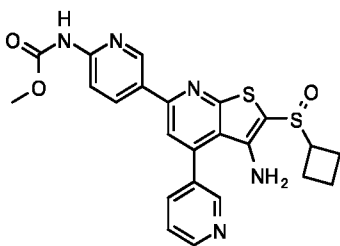
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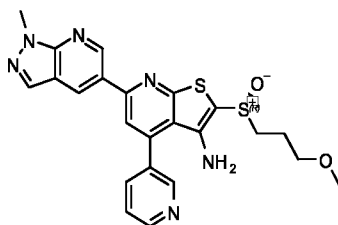
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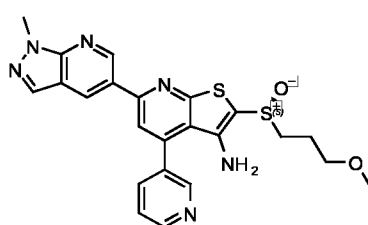
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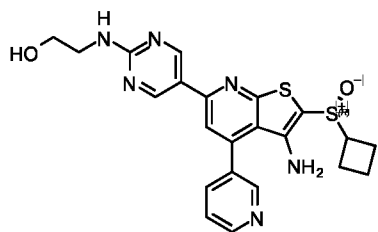


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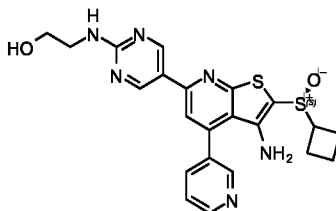


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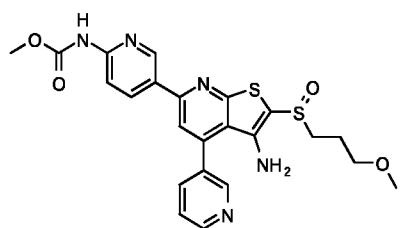
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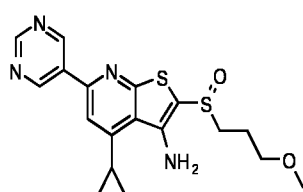
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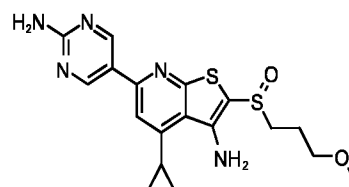
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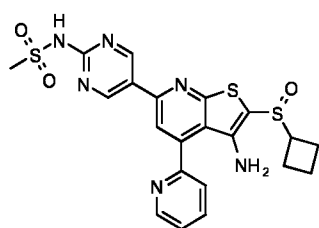
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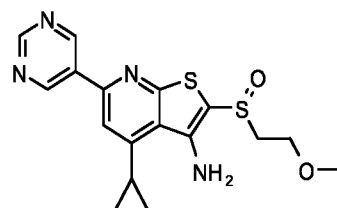
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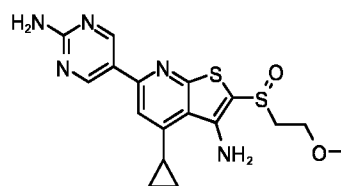
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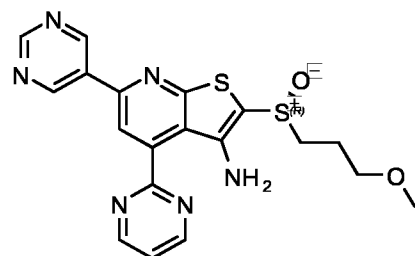
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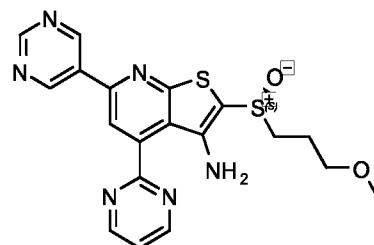
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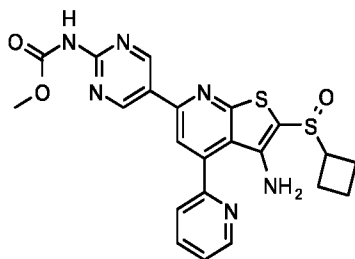
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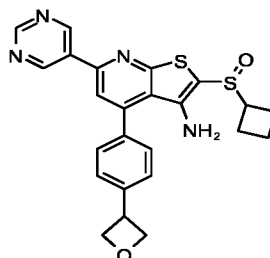
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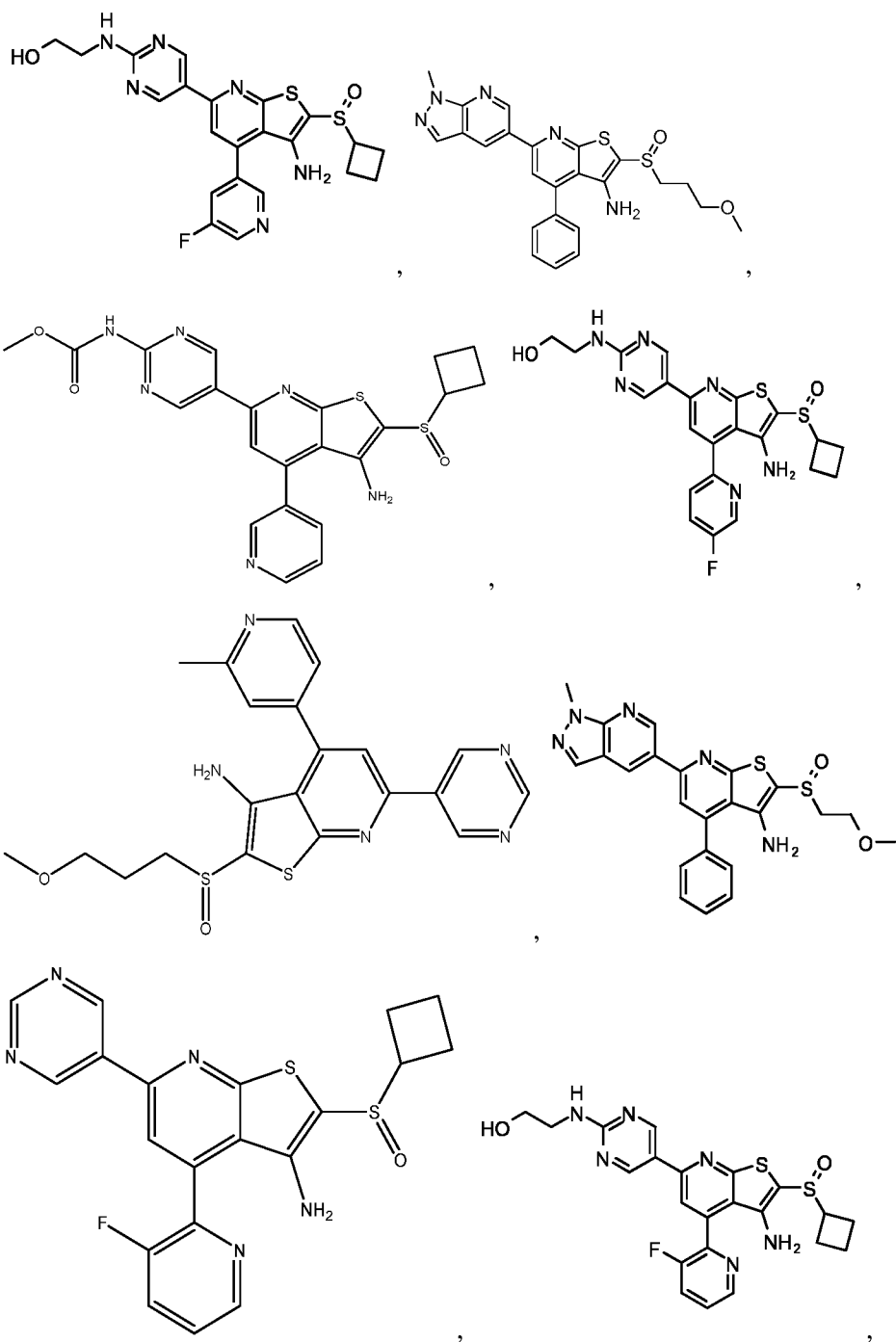


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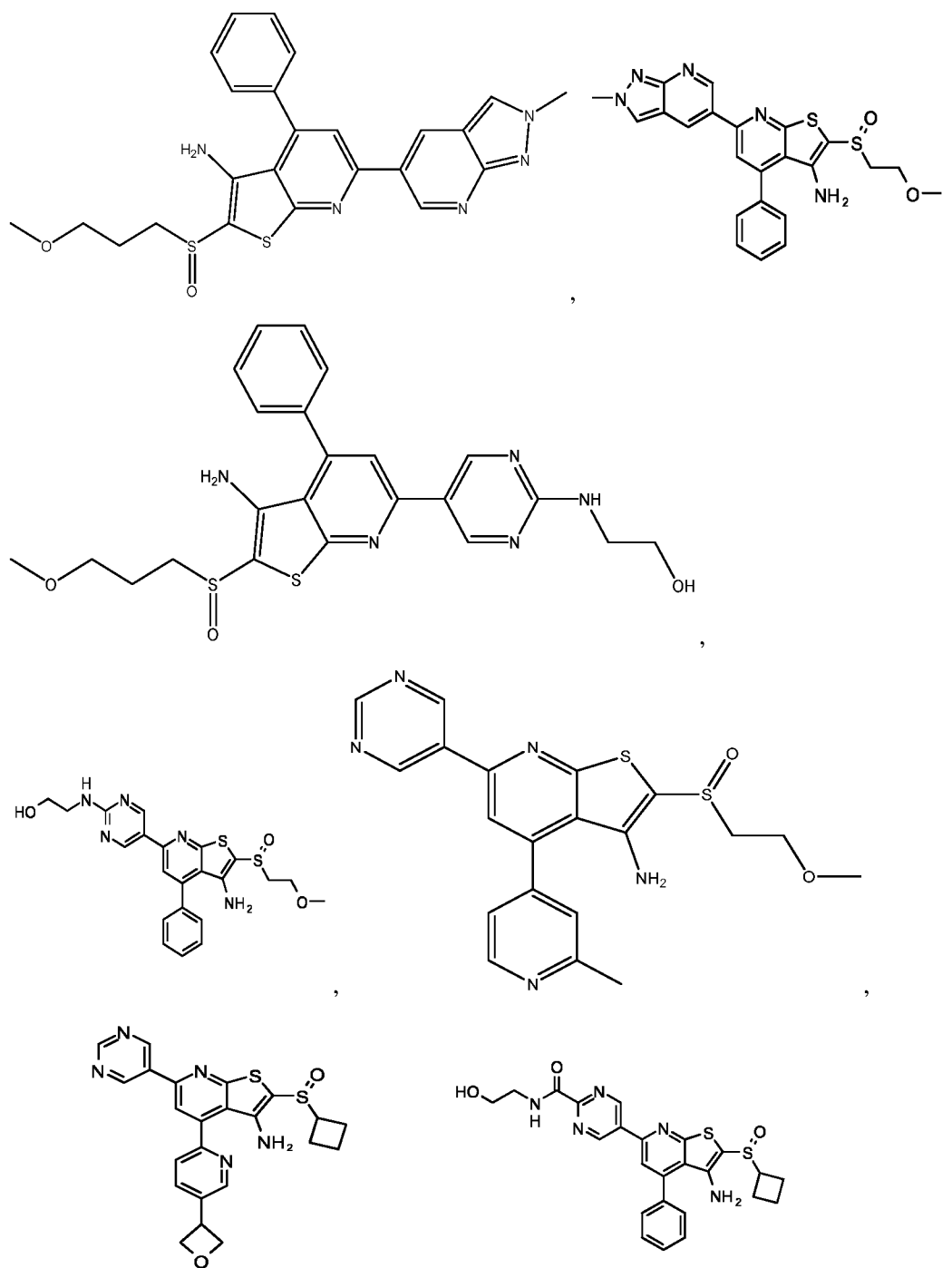


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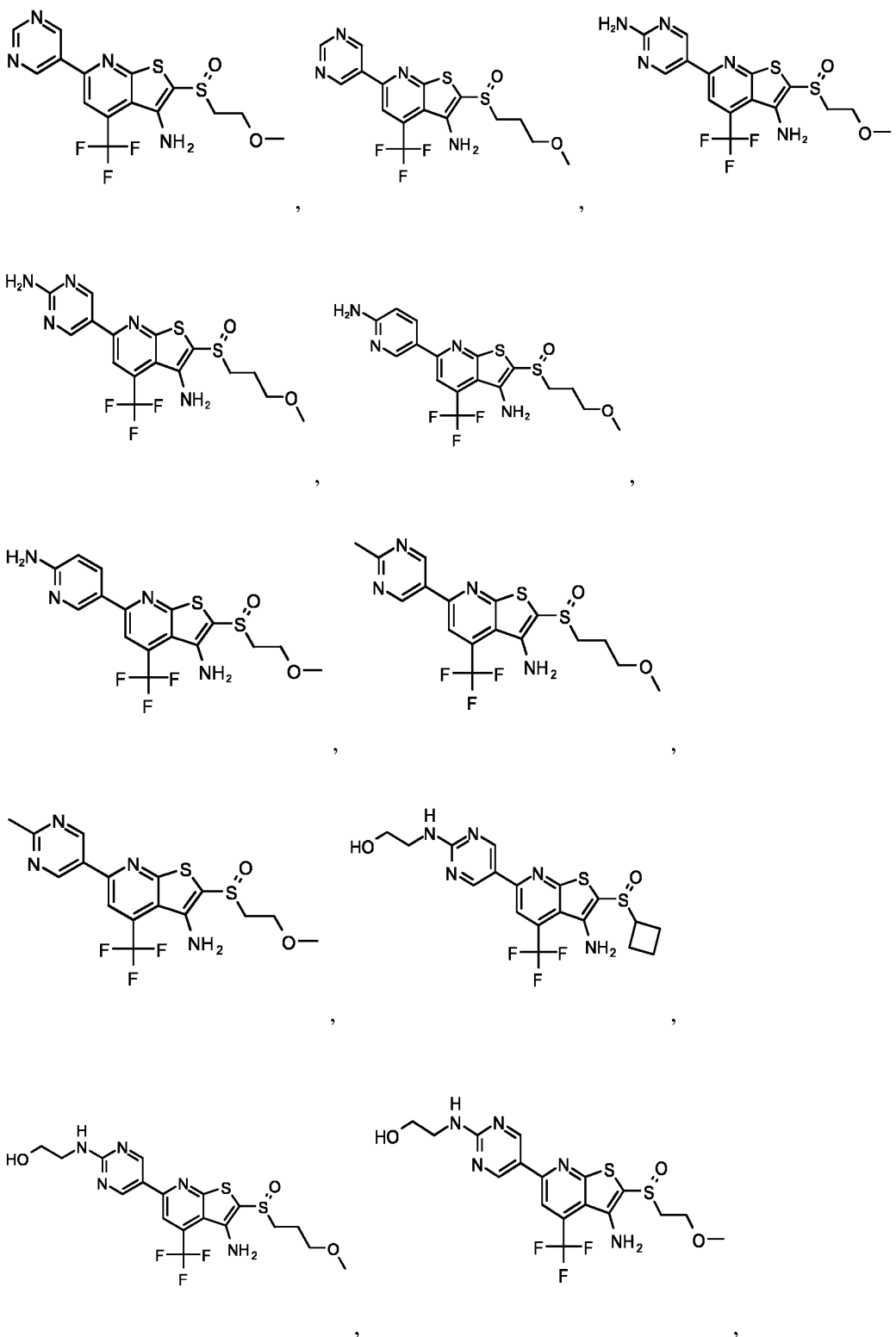
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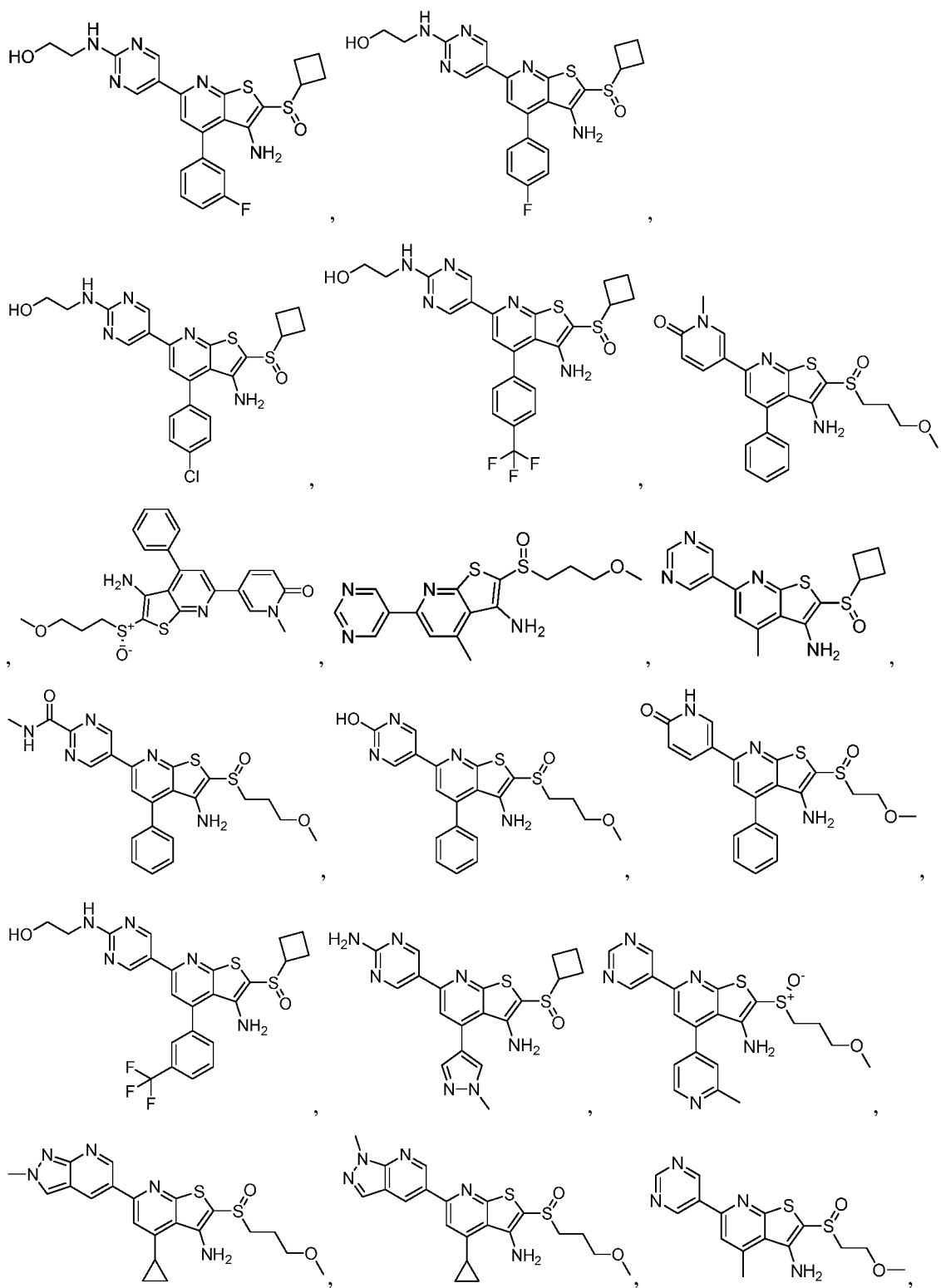
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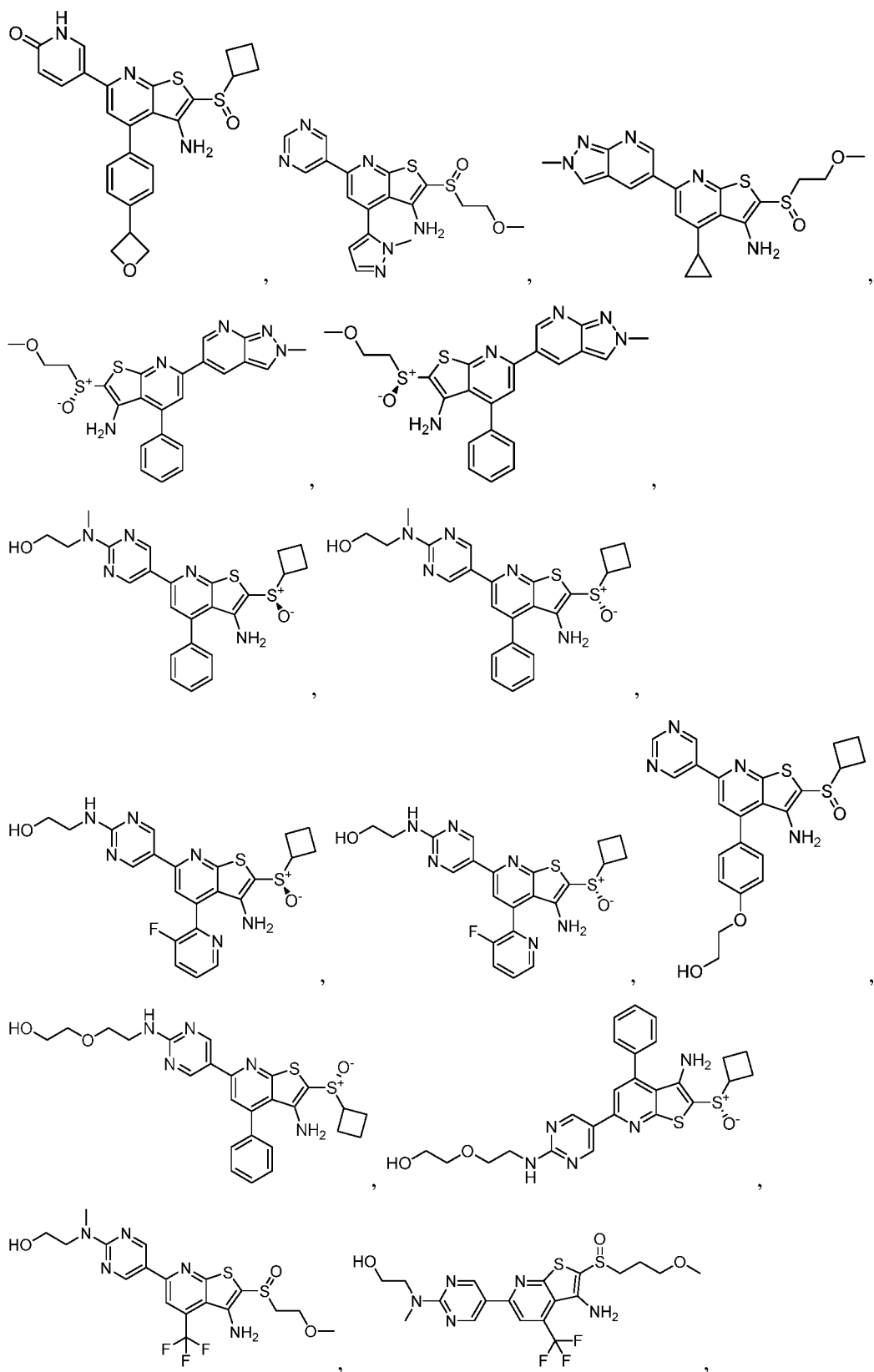
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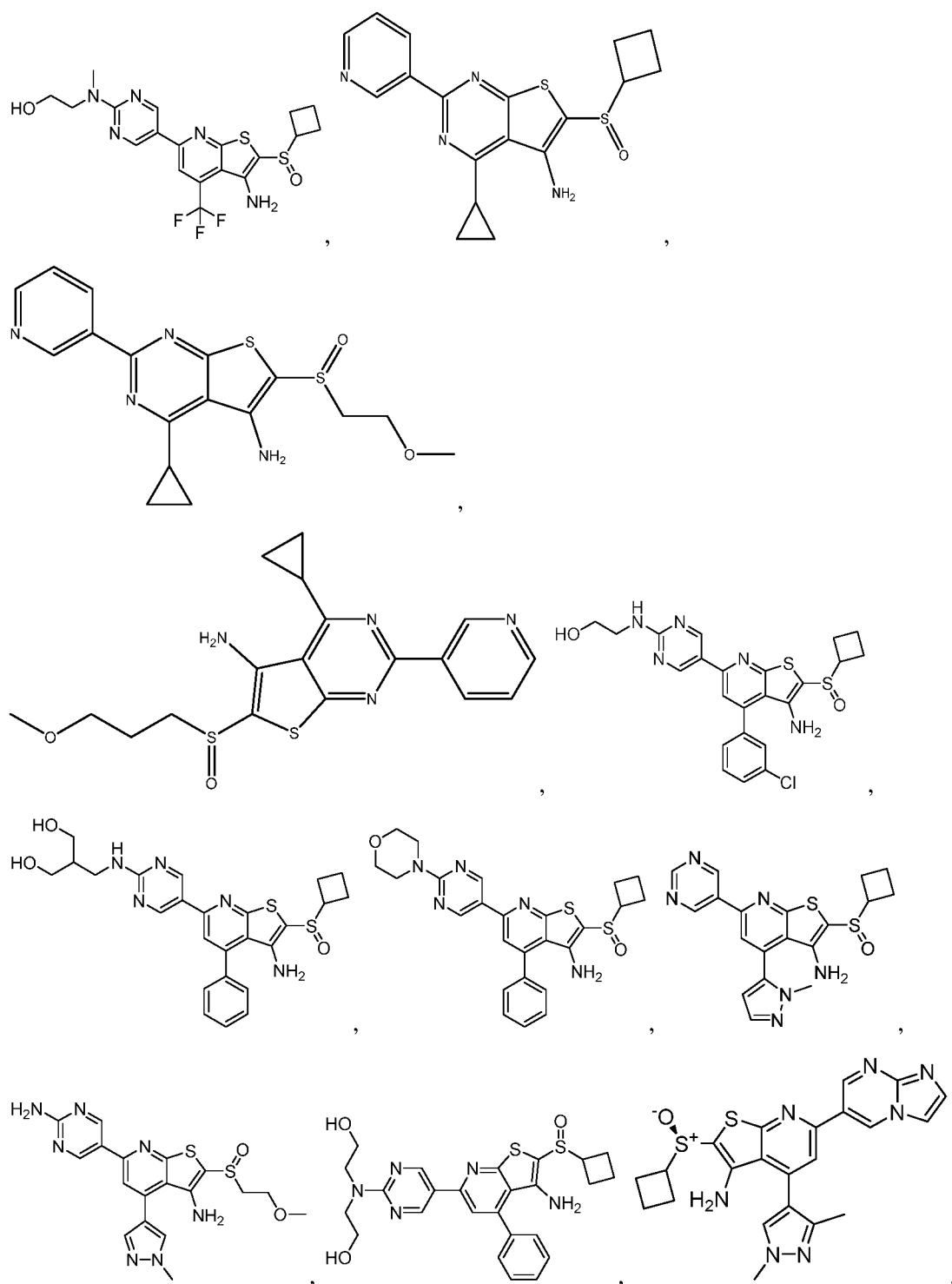
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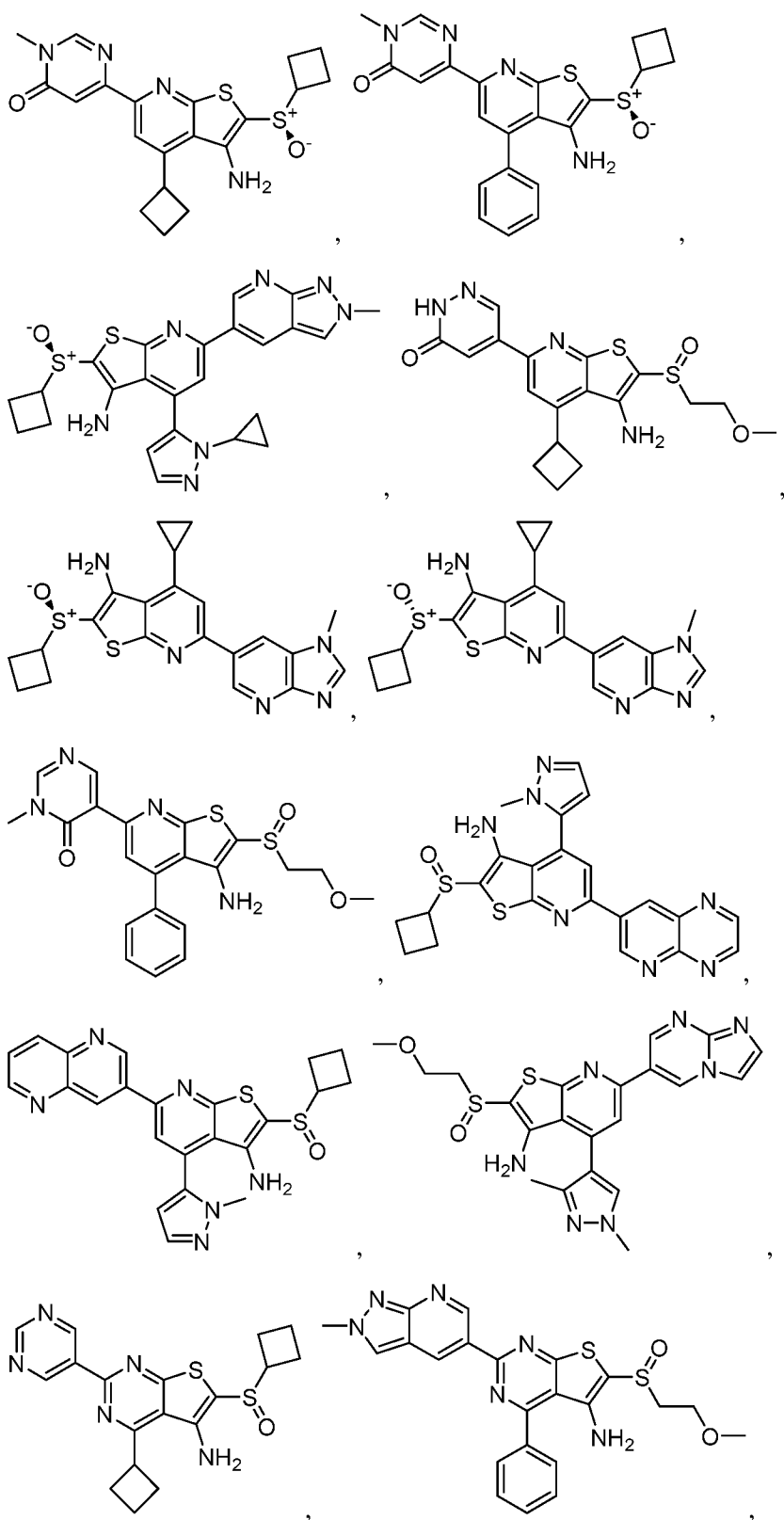
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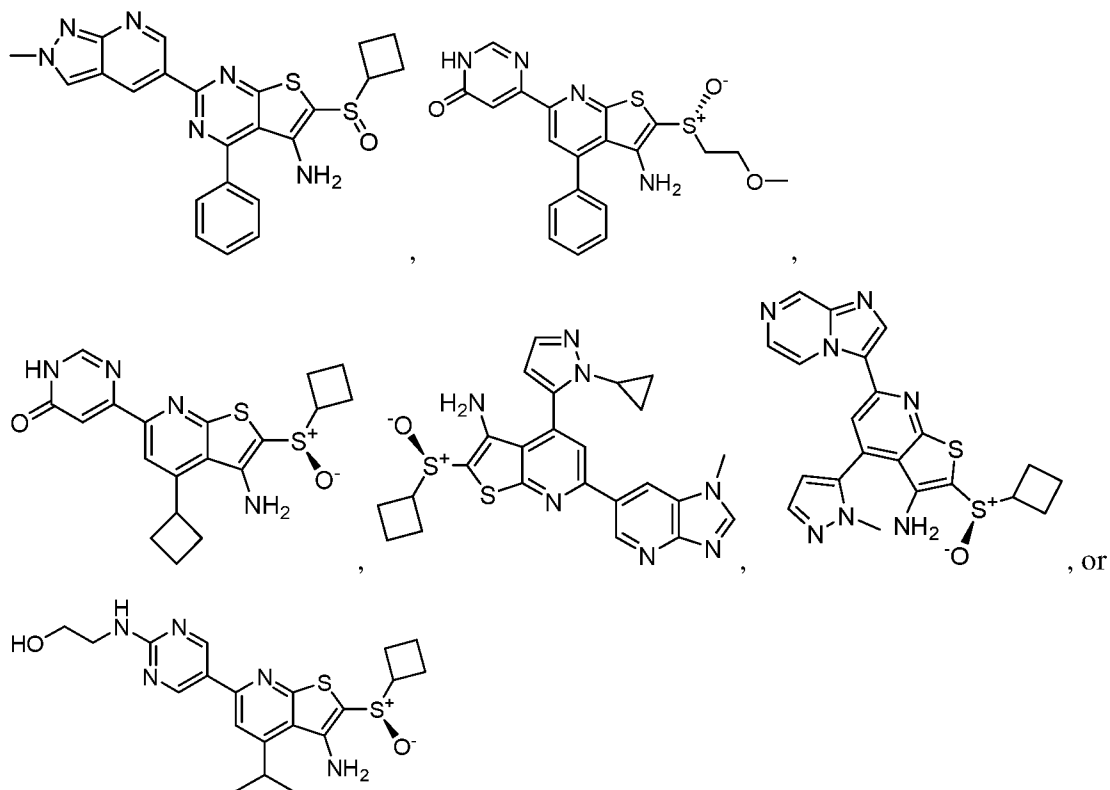
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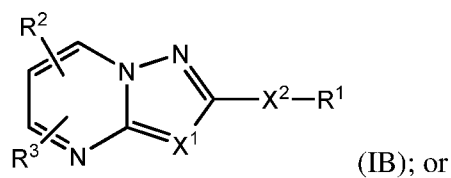
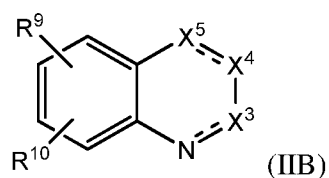


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or a pharmaceutically acceptable salt, tautomer, or solvate thereof.

[00215] In other embodiments, the 15-PGDH inhibitor can include compound having at least one of the formulas (IB) or (IIB), or a pharmaceutically acceptable salt, tautomer, or solvate thereof:



wherein X^1 is N or CR^4 ;

X^2 is S, S=O, S(=O)₂, or C=O;

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X^3 is CR^8 , the compound forming a polycyclic heteroaryl with 10 ring atoms, or absent, the compound forming a polycyclic heteroaryl with 9 ring atoms;

X^4 is N, NH, or CR^7 ;

X^5 is N, C=O, or CR^{16} , and X^5 is N if X^4 is CR^7 , or X^3 is absent, X^4 is NH if X^5 is C=O, and X^5 is CR^{16} if X^4 is N and X^3 is CR^8 ;

$R^1, R^2, R^3, R^4, R^9, R^{10}$, and R^{16} are the same or different and are independently selected from the group consisting of hydrogen, substituted or unsubstituted C_1 - C_{24} alkyl, C_2 - C_{24} alkenyl, C_2 - C_{24} alkynyl, C_3 - C_{20} aryl, heterocycloalkenyl containing from 5-7 ring atoms, (wherein from 1-3 of the ring atoms is independently selected from N, NH, N(C_1 - C_6 alkyl), NC(O) (C_1 - C_6 alkyl), O, and S), heteroaryl or heterocyclyl containing from 5-14 ring atoms, (wherein from 1-6 of the ring atoms is independently selected from N, NH, N(C_1 - C_3 alkyl), O, and S), C_6 - C_{24} alkaryl, C_6 - C_{24} aralkyl, halo, silyl, hydroxyl, sulfhydryl, C_1 - C_{24} alkoxy, C_2 - C_{24} alkenyloxy, C_2 - C_{24} alkynyloxy, C_5 - C_{20} aryloxy, acyl (including C_2 - C_{24} alkylcarbonyl (--CO-alkyl) and C_6 - C_{20} arylcarbonyl (--CO-aryl)), acyloxy (-O-acyl), C_2 - C_{24} alkoxycarbonyl (-(CO)-O-alkyl), C_6 - C_{20} aryloxycarbonyl (-(CO)-O-aryl), C_2 - C_{24} alkylcarbonato (-O-(CO)-O-alkyl), C_6 - C_{20} arylcarbonato (-O-(CO)-O-aryl), carboxy (-COOH), carboxylato (-COO⁻), carbamoyl (-(CO)--NH₂), C_1 - C_{24} alkyl-carbamoyl (-(CO)-NH(C_1 - C_{24} alkyl)), arylcarbamoyl (-(CO)-NH-aryl), thiocarbamoyl (-(CS)-NH₂), carbamido (-NH-(CO)-NH₂), cyano(-CN), isocyano (-N⁺C⁻), cyanato (-O-CN), isocyanato (-O-N⁺=C⁻), isothiocyanato (-S-CN), azido (-N=N⁺=N⁻), formyl (--(CO)--H), thioformyl (--(CS)--H), amino (--NH₂), C_1 - C_{24} alkyl amino, C_5 - C_{20} aryl amino, C_2 - C_{24} alkylamido (-NH-(CO)-alkyl), C_6 - C_{20} arylamido (-NH-(CO)-aryl), sulfanamido (-SO₂N(R)₂ where R is independently H, alkyl, aryl or heteroaryl), imino (-CR=NH where R is hydrogen, C_1 - C_{24} alkyl, C_5 - C_{20} aryl, C_6 - C_{24} alkaryl, C_6 - C_{24} aralkyl, etc.), alkylimino (-CR=N(alkyl), where R=hydrogen, alkyl, aryl, alkaryl, aralkyl, etc.), arylimino (-CR=N(aryl), where R=hydrogen, alkyl, aryl, alkaryl, etc.), nitro (-NO₂), nitroso (-NO), sulfo (-SO₂-OH), sulfonato (-SO₂-O⁻), C_1 - C_{24} alkylsulfanyl (-S-alkyl; also termed "alkylthio"), arylsulfanyl (-S-aryl; also termed "arylthio"), C_1 - C_{24} alkylsulfanyl (-(SO)-alkyl), C_5 - C_{20} arylsulfanyl (-(SO)-aryl), C_1 - C_{24} alkylsulfonyl (-SO₂-alkyl), C_5 - C_{20} arylsulfonyl (-SO₂-aryl), sulfonamide (-SO₂-NH₂, -SO₂NY₂ (wherein Y is independently H, aryl or alkyl), phosphono (-P(O)(OH)₂), phosphonato (-P(O)(O⁻)₂), phosphinato (-P(O)(O⁻)), phospho (-PO₂), phosphino (--PH₂), polyalkyl ethers (-[(CH₂)_nO]_m), phosphates, phosphate esters [-OP(O)(OR)₂ where R = H,

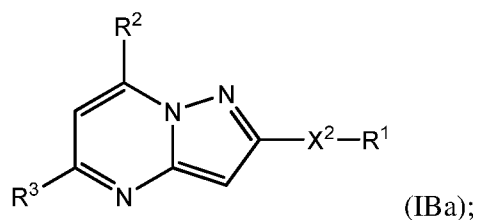
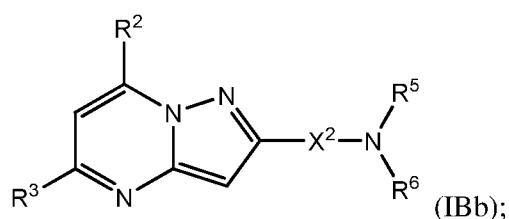
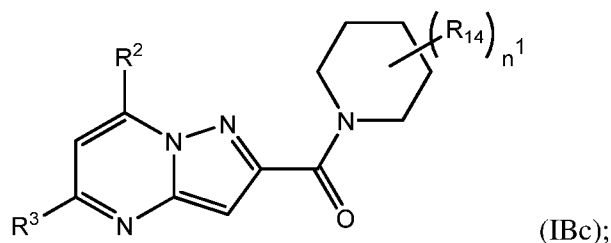
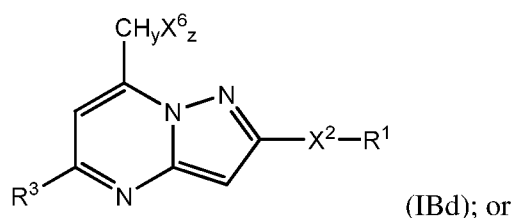
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methyl or other alkyl], groups incorporating amino acids or other moieties expected to bear positive or negative charge at physiological pH, and combinations thereof;

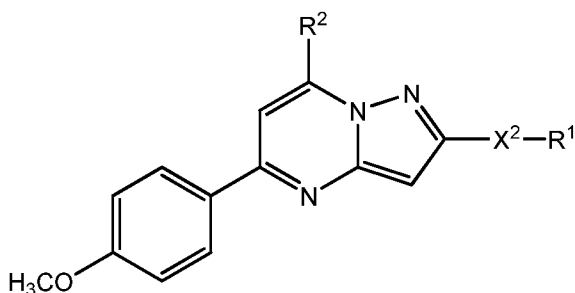
R^7 and R^8 are same or different and are each independently selected from the group consisting of H, a substituted or unsubstituted aryl, a substituted or unsubstituted heteroaryl, a substituted or unsubstituted cycloalkyl, and a substituted or unsubstituted heterocyclyl, and at least one of R^7 or R^8 is not H.

[00216] In some embodiments, at least one of R^2 or R^3 is not H, and at least one of R^9 or R^{10} is not H.

[00217] In some embodiments, the 15-PGDH inhibitor can include a compound having at least one of the following formulas:



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(IBe); or a pharmaceutically acceptable salt, tautomer, or solvate thereof;

wherein X^2 is S, S=O, S(=O)₂, or C=O;

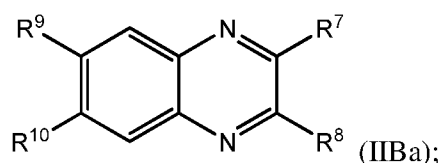
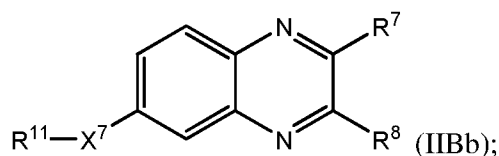
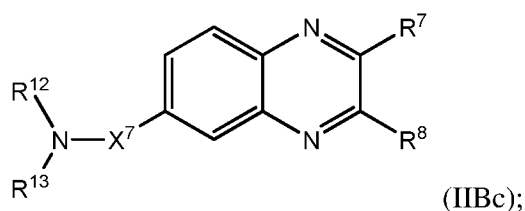
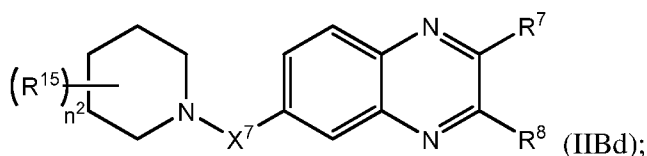
X^6 is Cl, Br, or F, and $y + z = 3$;

R^1 , R^2 , R^3 , R^5 , R^6 , and R^{14} are the same or different and are independently selected from the group consisting of hydrogen, substituted or unsubstituted C₁-C₂₄ alkyl, C₂-C₂₄ alkenyl, C₂-C₂₄ alkynyl, C₃-C₂₀ aryl, heterocycloalkenyl containing from 5-7 ring atoms, (wherein from 1-3 of the ring atoms is independently selected from N, NH, N(C₁-C₆ alkyl), NC(O) (C₁-C₆ alkyl), O, and S), heteroaryl or heterocyclyl containing from 5-14 ring atoms, (wherein from 1-6 of the ring atoms is independently selected from N, NH, N(C₁-C₃ alkyl), O, and S), C₆-C₂₄ alkaryl, C₆-C₂₄ aralkyl, halo, silyl, hydroxyl, sulfhydryl, C₁-C₂₄ alkoxy, C₂-C₂₄ alkenyloxy, C₂-C₂₄ alkynyloxy, C₅-C₂₀ aryloxy, acyl (including C₂-C₂₄ alkylcarbonyl (--CO-alkyl) and C₆-C₂₀ arylcarbonyl (-CO-aryl)), acyloxy (-O-acyl), C₂-C₂₄ alkoxycarbonyl (-(CO)-O-alkyl), C₆-C₂₀ aryloxycarbonyl (-(CO)-O-aryl), C₂-C₂₄ alkylcarbonato (-O-(CO)-O-alkyl), C₆-C₂₀ arylcarbonato (-O-(CO)-O-aryl), carboxy (-COOH), carboxylato (-COO⁻), carbamoyl (-(CO)--NH₂), C₁-C₂₄ alkyl-carbamoyl (-(CO)-NH(C₁-C₂₄ alkyl)), arylcarbamoyl (-(CO)-NH-aryl), thiocarbamoyl (-(CS)-NH₂), carbamido (-NH-(CO)-NH₂), cyano(-CN), isocyano (-N⁺C⁻), cyanato (-O-CN), isocyanato (-O-N⁺=C⁻), isothiocyanato (-S-CN), azido (-N=N⁺=N⁻), formyl (--(CO)--H), thioformyl (--(CS)--H), amino (--NH₂), C₁-C₂₄ alkyl amino, C₅-C₂₀ aryl amino, C₂-C₂₄ alkylamido (-NH-(CO)-alkyl), C₆-C₂₀ arylamido (-NH-(CO)-aryl), sulfanamido (-SO₂N(R)₂ where R is independently H, alkyl, aryl or heteroaryl), imino (-CR=NH where R is hydrogen, C₁-C₂₄ alkyl, C₅-C₂₀ aryl, C₆-C₂₄ alkaryl, C₆-C₂₄ aralkyl, etc.), alkylimino (-CR=N(alkyl), where R=hydrogen, alkyl, aryl, alkaryl, aralkyl, etc.), arylimino (-CR=N(aryl), where R=hydrogen, alkyl, aryl, alkaryl, etc.), nitro (-NO₂), nitroso (-NO), sulfo (-SO₂-OH), sulfonato (-SO₂-O⁻), C₁-C₂₄ alkylsulfanyl (-S-alkyl; also termed "alkylthio"), arylsulfanyl (-S-aryl; also termed

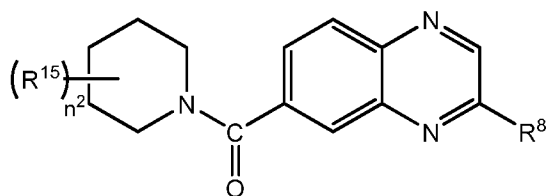
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"arylthio"), C₁-C₂₄ alkylsulfinyl (-(SO)-alkyl), C₅-C₂₀ arylsulfinyl (-(SO)-aryl), C₁-C₂₄ alkylsulfonyl (-SO₂-alkyl), C₅-C₂₀ arylsulfonyl (-SO₂-aryl), sulfonamide (-SO₂-NH₂, -SO₂NY₂ (wherein Y is independently H, aryl or alkyl), phosphono (-P(O)(OH)₂), phosphonato (-P(O)(O⁻)₂), phosphinato (-P(O)(O⁻)), phospho (-PO₂), phosphino (-PH₂), polyalkyl ethers (-[(CH₂)_nO]_m), phosphates, phosphate esters [-OP(O)(OR)₂ where R = H, methyl or other alkyl], groups incorporating amino acids or other moieties expected to bear positive or negative charge at physiological pH, and combinations thereof; wherein R⁵ and R⁶ may be linked to form a cyclic or polycyclic ring, wherein the ring is a substituted or unsubstituted aryl, a substituted or unsubstituted heteroaryl, a substituted or unsubstituted cycloalkyl, and a substituted or unsubstituted heterocyclyl, n¹ is 0-4, and each R¹⁴ is the same or different.

[00218] In other embodiments, the 15-PGDH inhibitor can include a compound having at least one of the following formulas:



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(IIBf); or a pharmaceutically acceptable salt,

tautomer, or solvate thereof;

wherein X^7 is S, S=O, S(=O)₂, or C=O;

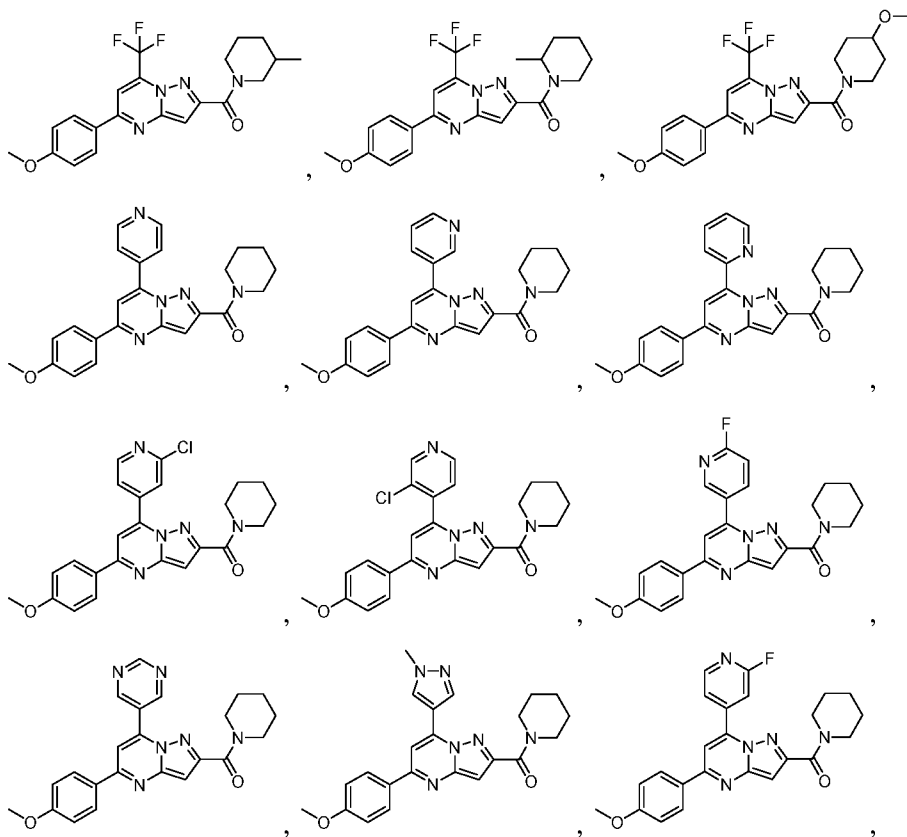
R^7 and R^8 are same or different and are each independently selected from the group consisting of H, a substituted or unsubstituted aryl, a substituted or unsubstituted heteroaryl, a substituted or unsubstituted cycloalkyl, and a substituted or unsubstituted heterocyclyl, and at least one of R^7 or R^8 is not H;

R^9 , R^{10} , R^{11} , R^{12} , R^{13} , and R^{15} are the same or different and are independently selected from the group consisting of hydrogen, substituted or unsubstituted C₁-C₂₄ alkyl, C₂-C₂₄ alkenyl, C₂-C₂₄ alkynyl, C₃-C₂₀ aryl, heterocycloalkenyl containing from 5-7 ring atoms, (wherein from 1-3 of the ring atoms is independently selected from N, NH, N(C₁-C₆ alkyl), NC(O) (C₁-C₆ alkyl), O, and S), heteroaryl or heterocyclyl containing from 5-14 ring atoms, (wherein from 1-6 of the ring atoms is independently selected from N, NH, N(C₁-C₃ alkyl), O, and S), C₆-C₂₄ alkaryl, C₆-C₂₄ aralkyl, halo, silyl, hydroxyl, sulfhydryl, C₁-C₂₄ alkoxy, C₂-C₂₄ alkenyloxy, C₂-C₂₄ alkynyloxy, C₅-C₂₀ aryloxy, acyl (including C₂-C₂₄ alkylcarbonyl (--CO-alkyl) and C₆-C₂₀ arylcarbonyl (-CO-aryl)), acyloxy (-O-acyl), C₂-C₂₄ alkoxycarbonyl (-(CO)-O-alkyl), C₆-C₂₀ aryloxycarbonyl (-(CO)-O-aryl), C₂-C₂₄ alkylcarbonato (-O-(CO)-O-alkyl), C₆-C₂₀ arylcarbonato (-O-(CO)-O-aryl), carboxy (-COOH), carboxylato (-COO⁻), carbamoyl (-(CO)--NH₂), C₁-C₂₄ alkyl-carbamoyl (-(CO)-NH(C₁-C₂₄ alkyl)), arylcarbamoyl (-(CO)-NH-aryl), thiocarbamoyl (-(CS)-NH₂), carbamido (-NH-(CO)-NH₂), cyano(-CN), isocyano (-N⁺C⁻), cyanato (-O-CN), isocyanato (-O-N⁺=C⁻), isothiocyanato (-S-CN), azido (-N=N⁺=N⁻), formyl (--(CO)--H), thioformyl (--(CS)--H), amino (--NH₂), C₁-C₂₄ alkyl amino, C₅-C₂₀ aryl amino, C₂-C₂₄ alkylamido (-NH-(CO)-alkyl), C₆-C₂₀ arylamido (-NH-(CO)-aryl), sulfanamido (-SO₂N(R)₂ where R is independently H, alkyl, aryl or heteroaryl), imino (-CR=NH where R is hydrogen, C₁-C₂₄ alkyl, C₅-C₂₀ aryl, C₆-C₂₄ alkaryl, C₆-C₂₄ aralkyl, etc.), alkylimino (-CR=N(alkyl), where R=hydrogen, alkyl, aryl, alkaryl, aralkyl, etc.), arylimino (-CR=N(aryl), where R=hydrogen, alkyl, aryl, alkaryl, etc.), nitro (-NO₂), nitroso (-NO), sulfo (-SO₂-OH), sulfonato (-SO₂-O⁻),

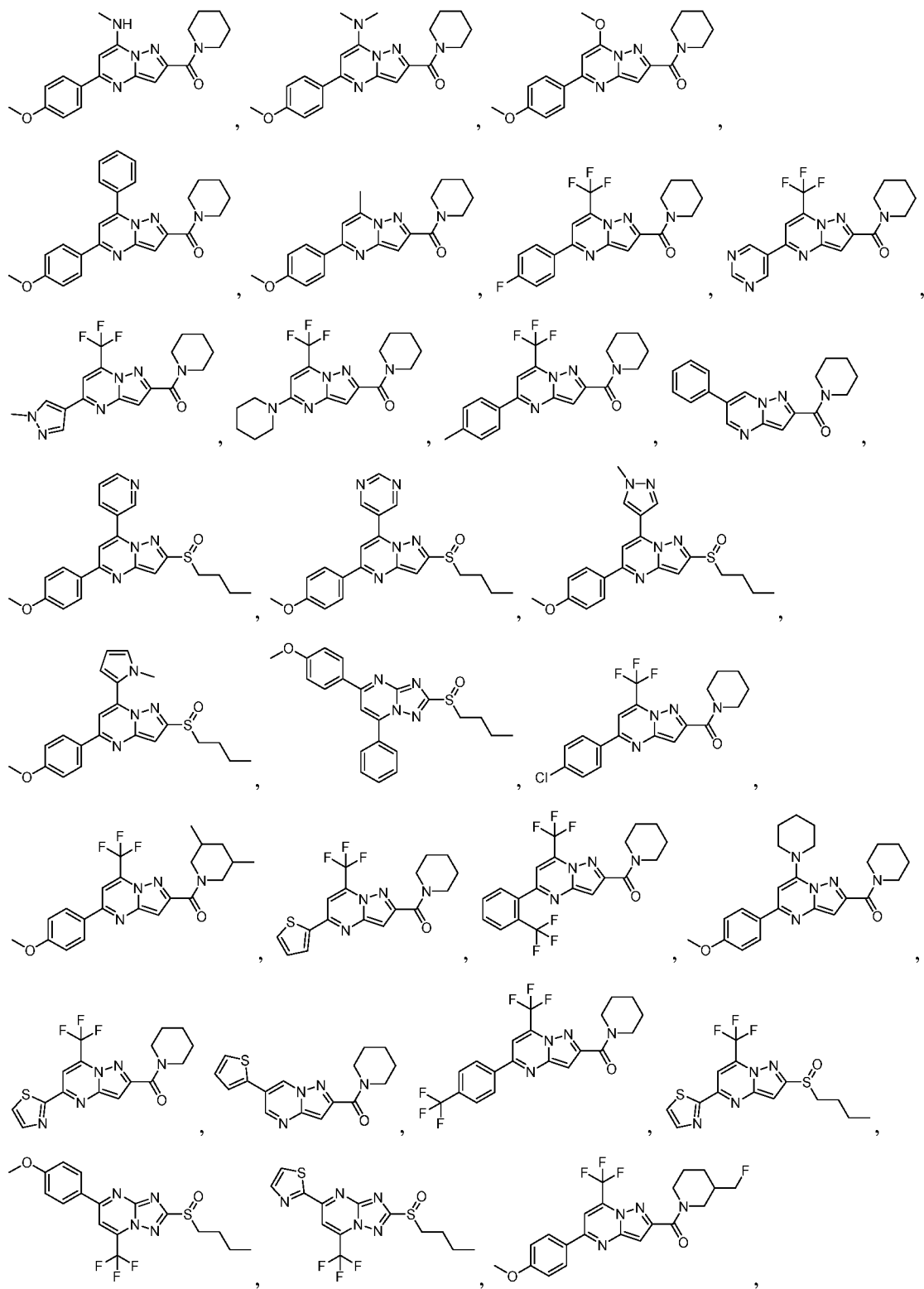
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C₁-C₂₄ alkylsulfanyl (-S-alkyl; also termed "alkylthio"), arylsulfanyl (-S-aryl; also termed "arylthio"), C₁-C₂₄ alkylsulfinyl (-SO-alkyl), C₅-C₂₀ arylsulfinyl (-SO-aryl), C₁-C₂₄ alkylsulfonyl (-SO₂-alkyl), C₅-C₂₀ arylsulfonyl (-SO₂-aryl), sulfonamide (-SO₂-NH₂, -SO₂NY₂ (wherein Y is independently H, aryl or alkyl), phosphono (-P(O)(OH)₂), phosphonato (-P(O)(O⁻)₂), phosphinato (-P(O)(O⁻)), phospho (-PO₂), phosphino (-PH₂), polyalkyl ethers (-[(CH₂)_nO]_m), phosphates, phosphate esters [-OP(O)(OR)₂ where R = H, methyl or other alkyl], groups incorporating amino acids or other moieties expected to bear positive or negative charge at physiological pH, and combinations thereof; wherein R¹² and R¹³ may be linked to form a cyclic or polycyclic ring, wherein the ring is a substituted or unsubstituted aryl, a substituted or unsubstituted heteroaryl, a substituted or unsubstituted cycloalkyl, and a substituted or unsubstituted heterocyclyl, n² is 0-4, and each R¹⁵ is the same or different.

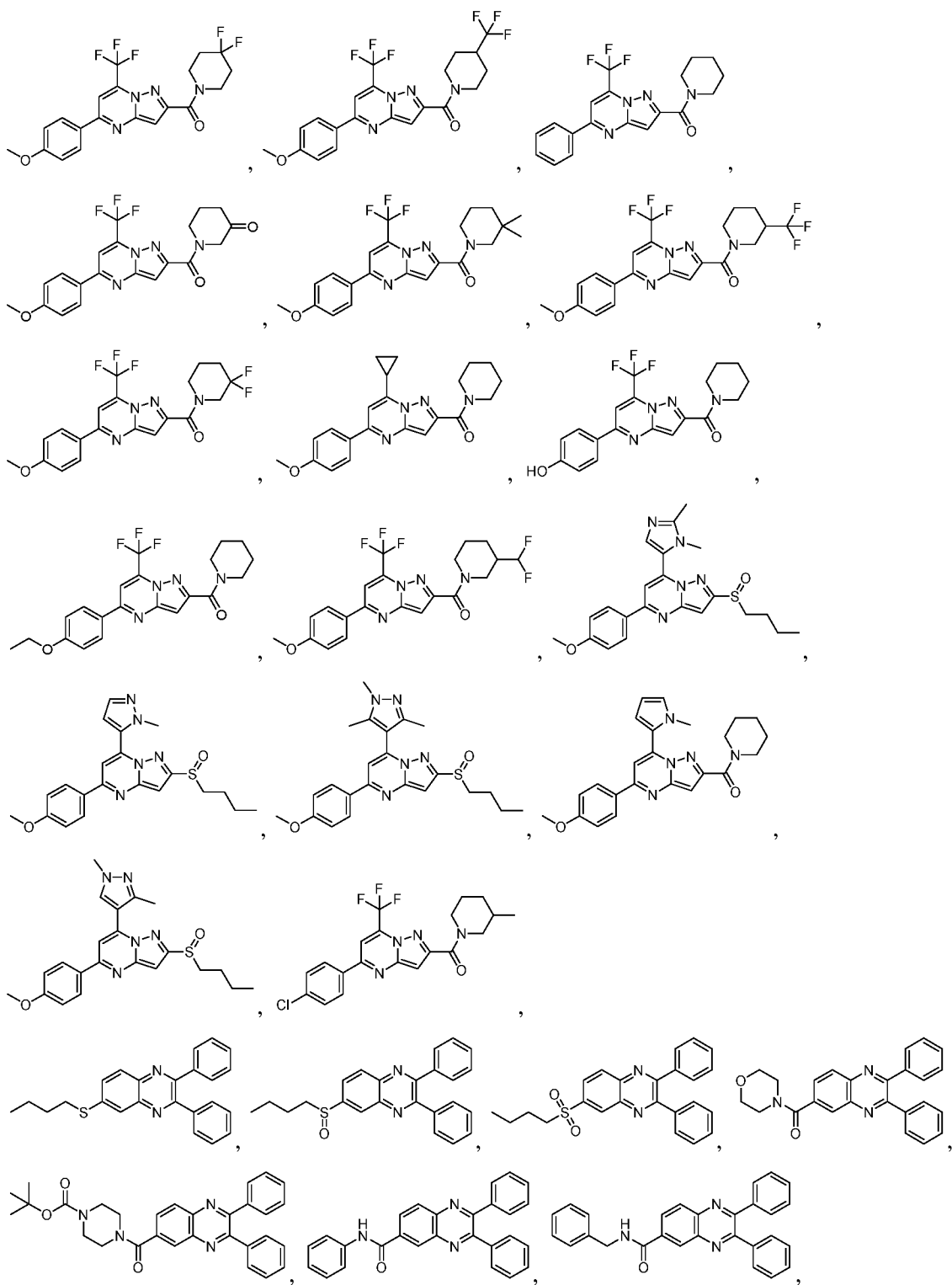
[00219] Examples of 15-PGDH inhibitors having formulas (IB), (IBa), (IBb), (IBc), (IBd), (IBe), (IIB), (IIBa), (IIBb), (IIBc), (IIBd), (IIBe), or (IIBf) can include the following compounds:

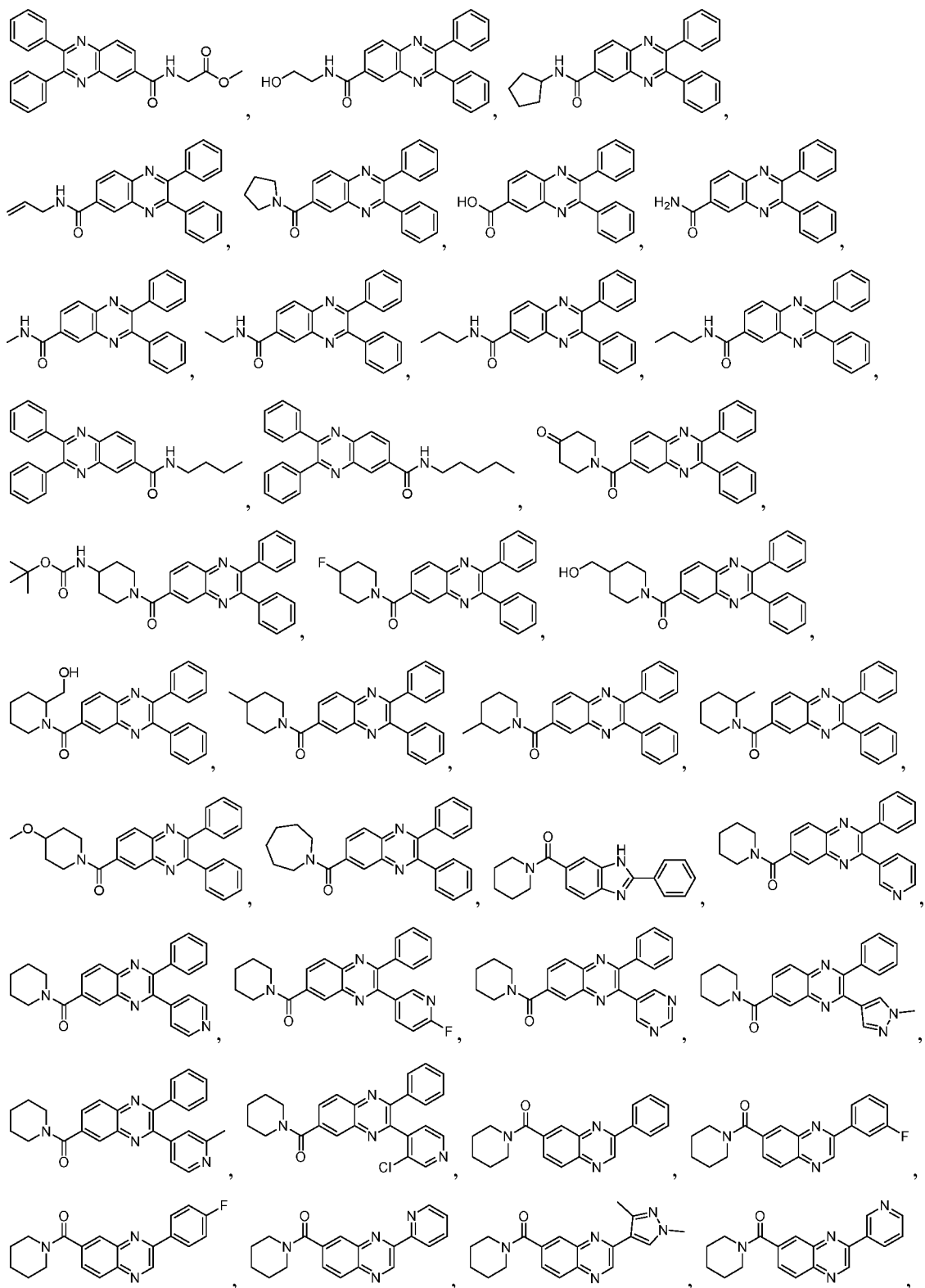


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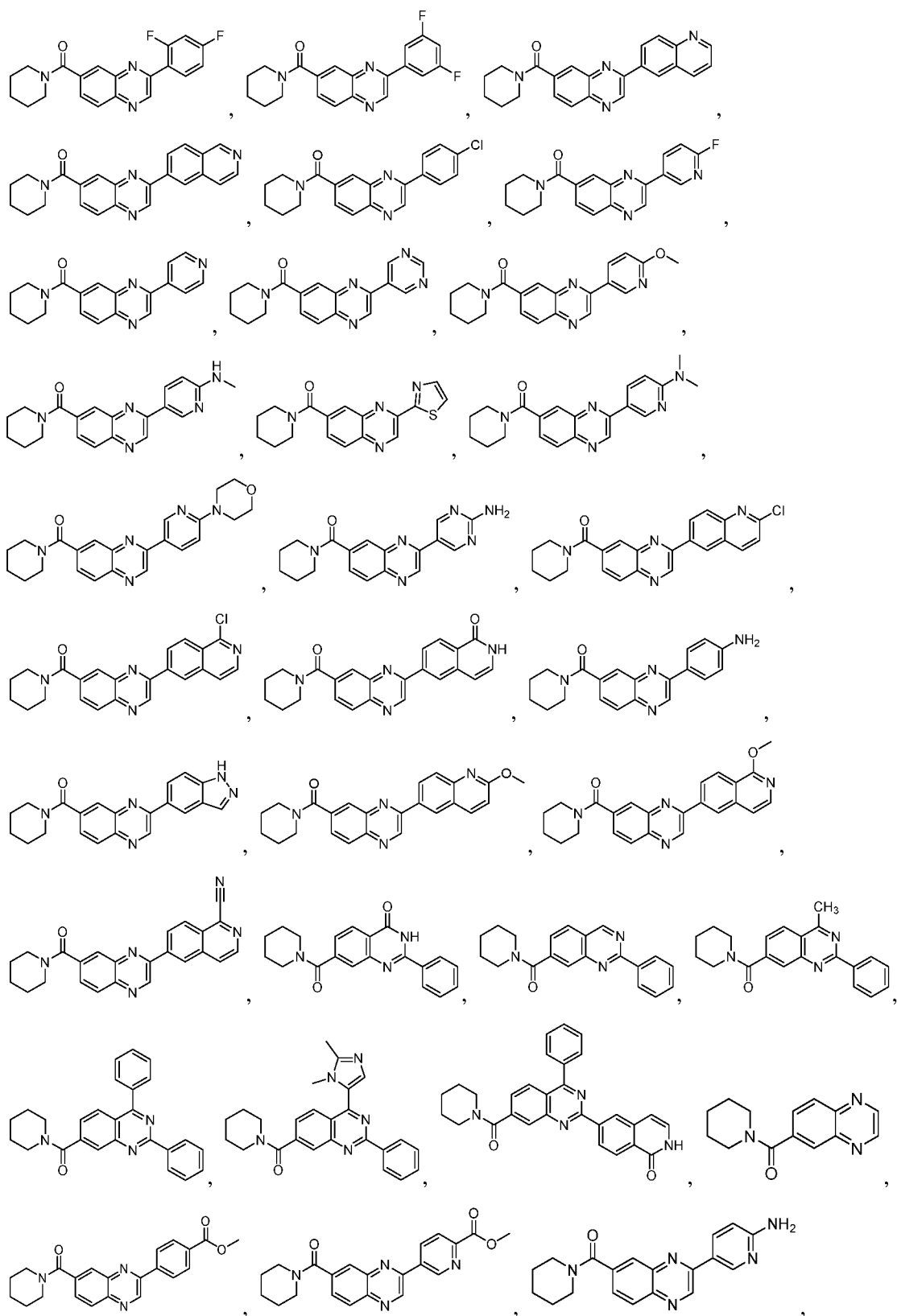


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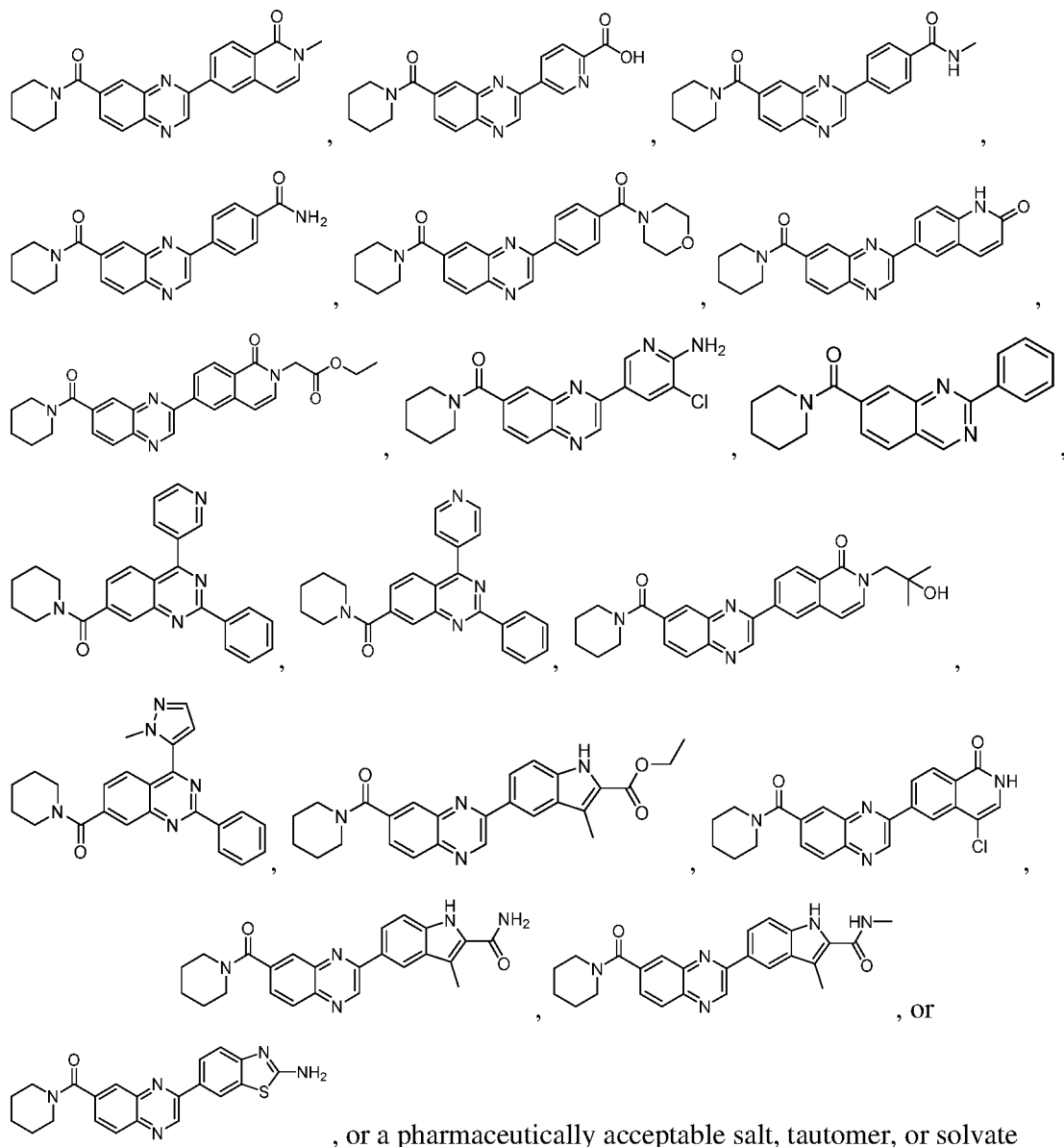




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thereof.

[00220] Still other example of 15-PGDH inhibitors include compounds described in WO2018/145080, which is incorporated by reference in its entirety.

[00221] In certain embodiments, the 15-PGDH inhibitor can be selected that can ia) at 2.5 μ M concentration, stimulate a Vaco503 reporter cell line expressing a 15-PGDH luciferase fusion construct to a luciferase output level of greater than 70 (using a scale on which a value of 100 indicates a doubling of reporter output over baseline); iia) at 2.5 μ M concentration stimulate a V9m reporter cell line expressing a 15-PGDH luciferase fusion

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construct to a luciferase output level of greater than 75; iia) at 7.5 μ M concentration stimulate a LS174T reporter cell line expressing a 15-PGDH luciferase fusion construct to a luciferase output level of greater than 70; and iva) at 7.5 μ M concentration, does not activate a negative control V9m cell line expressing TK-renilla luciferase reporter to a level greater than 20; and va) inhibits the enzymatic activity of recombinant 15-PGDH protein at an IC_{50} of less than 1 μ M.

[00222] In other embodiments, the 15-PGDH inhibitor can ib) at 2.5 μ M concentration, stimulate a Vaco503 reporter cell line expressing a 15-PGDH luciferase fusion construct to increase luciferase output; iib) at 2.5 μ M concentration stimulate a V9m reporter cell line expressing a 15-PGDH luciferase fusion construct to increase luciferase output; iiib) at 7.5 μ M concentration stimulate a LS174T reporter cell line expressing a 15-PGDH luciferase fusion construct to increase luciferase output; ivb) at 7.5 μ M concentration, does not activate a negative control V9m cell line expressing TK-renilla luciferase reporter to a luciferase level greater than 20% above background; and vb) inhibits the enzymatic activity of recombinant 15-PGDH protein at an IC_{50} of less than 1 μ M.

[00223] In other embodiments, the 15-PGDH inhibitor can inhibit the enzymatic activity of recombinant 15-PGDH at an IC_{50} of less than 1 μ M, or preferably at an IC_{50} of less than 250 nM, or more preferably at an IC_{50} of less than 50 nM, or more preferably at an IC_{50} of less than 10 nM, or more preferably at an IC_{50} of less than 5 nM at a recombinant 15-PGDH concentration of about 5 nM to about 10 nM.

[00224] It will be appreciated that other 15-PGDH inhibitors can be used in the methods described herein. These other 15-PGDH inhibitors can include known 15-PGDH inhibitors including, for example, tetrazole compounds of formulas (I) and (II), 2-alkylideneaminoxyacetamide compounds of formula (I), heterocyclic compounds of formulas (VI) and (VII), and pyrazole compounds of formula (III) described in U.S. Patent Application Publication No. 2006/0034786 and U.S. Patent No. 7,705,041; benzylidene-1,3-thiazolidine compounds of formula (I) described in U.S. Patent Application Publication No. 2007/0071699; phenylfurylmethylthiazolidine-2,4-dione and phenylthienylmethylthiazolidine-2,4-dione compounds described in U.S. Patent Application Publication No. 2007/0078175; thiazolidenedione derivatives described in U.S. Patent Application Publication No. 2011/0269954; phenylfuran, phenylthiophene, or phenylpyrazole compounds described in U.S. Patent No. 7,294,641, 5-(3,5-disubstituted

phenylazo)-2-hydroxybenzene-acetic acids and salts and lactones described in U.S. Patent No. 4,725,676, and azo compounds described in U.S. Patent No. 4,889,846.

[00225] Still other examples of 15-PGDH inhibitors are described in the following publications: Seo SY et al. Effect of 15-hydroxyprostaglandin dehydrogenase inhibitor on wound healing. *Prostaglandins Leukot Essent Fatty Acids*. 2015;97:35-41. doi: 10.1016/j.plefa.2015.03.005. PubMed PMID: 25899574; Piao YL et al. Wound healing effects of new 15-hydroxyprostaglandin dehydrogenase inhibitors. *Prostaglandins Leukot Essent Fatty Acids*. 2014;91(6):325-32. doi: 10.1016/j.plefa.2014.09.011. PubMed PMID: 25458900; Choi D et al. Control of the intracellular levels of prostaglandin E(2) through inhibition of the 15-hydroxyprostaglandin dehydrogenase for wound healing. *Bioorg Med Chem*. 2013;21(15):4477-84. doi: 10.1016/j.bmc.2013.05.049. PubMed PMID: 23791868; Wu Y et al. Synthesis and biological evaluation of novel thiazolidinedione analogues as 15-hydroxyprostaglandin dehydrogenase inhibitors. *J Med Chem*. 2011;54(14):5260-4. Epub 2011/06/10. doi: 10.1021/jm200390u. PubMed PMID: 21650226; Duveau DY et al. Structure-activity relationship studies and biological characterization of human NAD(+)-dependent 15-hydroxyprostaglandin dehydrogenase inhibitors. *Bioorg Med Chem Lett*. 2014;24(2):630-5. doi: 10.1016/j.bmcl.2013.11.081. PubMed PMID: 24360556; PMCID: PMC3970110; Duveau DY et al. Discovery of two small molecule inhibitors, ML387 and ML388, of human NAD+-dependent 15-hydroxyprostaglandin dehydrogenase. *Probe Reports from the NIH Molecular Libraries Program*. Bethesda (MD)2010; Wu Y et al. Synthesis and SAR of thiazolidinedione derivatives as 15-PGDH inhibitors. *Bioorg Med Chem*. 2010;18(4):1428-33. doi: 10.1016/j.bmc.2010.01.016. PubMed PMID: 20122835; Wu Y et al. Synthesis and biological evaluation of novel thiazolidinedione analogues as 15-hydroxyprostaglandin dehydrogenase inhibitors. *J Med Chem*. 2011;54(14):5260-4. Epub 2011/06/10. doi: 10.1021/jm200390u. PubMed PMID: 21650226; Jadhav A et al. Potent and selective inhibitors of NAD+-dependent 15-hydroxyprostaglandin dehydrogenase (HPGD). *Probe Reports from the NIH Molecular Libraries Program*. Bethesda (MD)2010; Niesen FH et al. High-affinity inhibitors of human NAD-dependent 15-hydroxyprostaglandin dehydrogenase: mechanisms of inhibition and structure-activity relationships. *PLoS One*. 2010;5(11):e13719. Epub 2010/11/13. doi: 10.1371/journal.pone.0013719. PubMed PMID: 21072165; PMCID: 2970562; Michelet, J. et al. Composition comprising at least one 15-PGDH inhibitor. US20080206320 A1, 2008; and Rozot, R et al. Care/makeup compositions

comprising a 2-alkylideneaminoxyacetamide compound for stimulating the growth of the hair or eyelashes and/or slowing loss thereof. US7396525 B2, 2008.

[00226] The 15-PGDH inhibitors described herein can be used to treat, prevent, or reduce the symptoms or severity of the renal disorder, disease, and/or injury in a subject (*e.g.*, a human subject) in need thereof. The 15-PGDH inhibitors are also useful in preventing the development of chronic kidney disease in a subject in need thereof. In certain embodiments, the 15-PGDH inhibitors are useful in preventing the development of chronic kidney disease in a subject in need thereof following an insult that can cause or causes acute kidney injury. In addition, the 15-PGDH inhibitors described herein can be used in methods for protecting a kidney from acute or chronic kidney injury in a subject in need thereof. Furthermore, the 15-PGDH inhibitors described herein can be used in methods for treating patients with renal insufficiency or renal failure, attributable at least in part to use of a drug or chemical.

[00227] Acute kidney injury is commonly divided into two major categories based on the type of insult. The first category is ischemic acute kidney injury (alternatively referred to as kidney hypoperfusion) and the second category is nephrotoxic acute kidney injury. The former results from impaired blood flow (kidney hypoperfusion) and oxygen delivery to the kidney; whereas, the latter results from a toxic insult to the kidney. Both of these categories of insults can lead to a secondary condition called acute tubular necrosis (ATN).

[00228] The most common causes of ischemic acute kidney injury are intravascular volume depletion, reduced cardiac output, systemic vasodilatation, and renal vasoconstriction. Intravascular volume depletion can be caused by hemorrhage (*e.g.*, following surgery, postpartum, or trauma); gastrointestinal loss (*e.g.*, from diarrhea, vomiting, nasogastric loss); renal losses (*e.g.*, caused by diuretics, osmotic diuresis, diabetes insipidus); skin and mucous membrane losses (*e.g.*, burns, hyperthermia); nephrotic syndrome; cirrhosis; or capillary leak. Reduced cardiac output can be due to cardiogenic shock, pericardial disease (*e.g.*, restrictive, constrictive, tamponade), congestive heart failure, valvular heart disease, pulmonary disease (*e.g.*, pulmonary hypertension, pulmonary embolism), or sepsis. Systemic vasodilation can be the result of cirrhosis, anaphylaxis, or sepsis. Finally, renal vasoconstriction can be caused by early sepsis, hepatorenal syndrome, acute hypercalcemia, drug-related (*e.g.*, norepinephrine, vasopressin, nonsteroidal anti-inflammatory drugs, angiotensin-converting enzyme inhibitors, calcineurin inhibitors), or use

of a radiocontrast agent. The 15-PGDH inhibitors described herein can be used to treat or reduce the symptoms or severity of acute kidney injury or any other kidney injury caused by any of the above mentioned causes of ischemic acute kidney injury. In addition, the 15-PGDH inhibitors thereof described herein can be used to prevent the development of acute kidney injury or any other kidney injury following exposure to the above-mentioned causes of ischemic acute kidney injury.

[00229] Nephrotoxic acute kidney injury is often associated with exposure to a nephrotoxin such as a nephrotoxic drug. Examples of nephrotoxic drugs include an antibiotic (*e.g.*, aminoglycosides such as gentamicin), a chemotherapeutic agent (*e.g.*, cis-platinum), a calcineurin inhibitor (*e.g.*, tacrolimus, cyclosporine), cephalosporins such as cephaloridine, cyclosporin, pesticides (*e.g.*, paraquat), environmental contaminants (*e.g.*, trichloroethylene, dichloroacetylene), amphotericin B, puromycin, aminonucleoside (PAN), a radiographic contrast agent (*e.g.*, acetrizoate, diatrizoate, iodamide, ioglicate, iothalamate, ioxithalamate, metrizoate, metrizamide, iohexol, iopamidol, iopentol, iopromide, iodixanol, iobitridol, and ioversol), a nonsteroidal anti-inflammatory, an anti-retroviral, an immunosuppressant, an oncological drug, or an ACE inhibitor. A nephrotoxin can be, for example, a trauma injury, a crush injury, an illicit drug, analgesic abuse, a gunshot wound, or a heavy metal. The 15-PGDH inhibitors described herein can be used to treat or reduce the symptoms or severity of acute kidney injury or any other kidney injury caused by any of the above mentioned causes of nephrotoxic acute kidney injury. In addition, the 15-PGDH inhibitors described herein can be used to prevent the development of acute kidney injury or any other kidney injury following exposure to the above mentioned causes of nephrotoxic acute kidney injury.

[00230] In certain embodiments, the 15-PGDH inhibitors described herein can be used to prevent the development of ATN following exposure to an insult such as ischemia or nephrotoxins/nephrotoxic drugs. In certain embodiments, the 15-PGDH inhibitors described herein can be used to treat or reduce the symptoms or severity of ATN following ischemia or exposure to nephrotoxins/nephrotoxic drugs.

[00231] In certain embodiments, the 15-PGDH inhibitors described herein can be used to prevent a drop in glomerular filtration following ischemia or exposure to nephrotoxins/nephrotoxic drugs. In some embodiments, the 15-PGDH inhibitors can be used to prevent tubular epithelial injury and/or necrosis following ischemia or exposure to nephrotoxins/nephrotoxic drugs. In some embodiments, the 15-PGDH inhibitors can be used

to decrease the microvascular permeability, improve vascular tone, and/or reduce inflammation of endothelial cells. In other embodiments, the 15-PGDH inhibitors described herein can be used to restore blood flow in the kidney following ischemia or exposure to nephrotoxins/nephrotoxic drugs. In further embodiments, the 15-PGDH inhibitors described herein can be used to prevent chronic renal failure.

[00232] The 15-PGDH inhibitors described herein can also be used to treat or prevent acute kidney injury resulting from surgery complicated by hypoperfusion. In certain specific embodiments, the surgery is one of cardiac surgery, major vascular surgery, major trauma, or surgery associated with treating a gunshot wound. In one embodiment, the cardiac surgery is coronary artery bypass grafting (CABG). In another embodiment, the cardiac surgery is valve surgery.

[00233] In some embodiments, the 15-PGDH inhibitors described herein can be used to treat or prevent acute kidney injury following organ transplantation such as kidney transplantation or heart transplantation.

[00234] In some embodiments, the 15-PGDH inhibitors described herein can be used to treat or prevent acute kidney injury following reduced effective arterial volume and kidney hypoperfusion.

[00235] In some embodiments, the 15-PGDH inhibitors described herein can be used to treat or prevent acute kidney injury in a subject who is taking medication (*e.g.*, an anticholinergic) that interferes with normal emptying of the bladder. In certain embodiments, the 15-PGDH inhibitors described herein can be used to treat or prevent acute kidney injury in a subject who has an obstructed urinary catheter. In some embodiments, the 15-PGDH inhibitors described herein can be used to treat or prevent acute kidney injury in a subject who is taking a drug that causes crystalluria. In some embodiments, the 15-PGDH inhibitors described herein can be used to treat or prevent acute kidney injury in a subject who is taking a drug that causes or leads to myoglobinuria. In some embodiments, the 15-PGDH inhibitors described herein can be used to treat or prevent acute kidney injury in a subject who is taking a drug that causes or leads to cystitis.

[00236] In some embodiments, the 15-PGDH inhibitors described herein can be used to treat or prevent acute kidney injury in a subject who has benign prostatic hypertrophy or prostate cancer.

[00237] In some embodiments, the 15-PGDH inhibitors described herein can be used to treat or prevent acute kidney injury in a subject who has a kidney stone.

[00238] In some embodiments, the 15-PGDH inhibitors described herein can be used to treat or prevent acute kidney injury in a subject who has an abdominal malignancy (*e.g.*, ovarian cancer, colorectal cancer).

[00239] In certain embodiments, the 15-PGDH inhibitors described herein can be used to treat or prevent acute kidney injury, wherein sepsis does not cause or result in the acute kidney injury.

[00240] Acute kidney injury typically occurs within hours to days following the original insult (*e.g.*, ischemia or nephrotoxin insult). Thus, 15-PGDH inhibitors described herein can be administered before the insult, or within an hour to 30 days (*e.g.*, 0.5 hours, 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 9 hours, 10 hours, 11 hours, 12 hours, 13 hours, 14 hours, 15 hours, 16 hours, 17 hours, 18 hours, 19 hours, 20 hours, 21 hours, 22 hours, 23 hours, 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 15 days, 20 days, 25 days, 28 days, or 30 days) after the insult (*e.g.*, a surgery or nephrotoxin insult described herein).

[00241] A subject can be determined to have, or have the risk of developing, acute kidney injury based on, *e.g.*, the Risk Injury Failure Loss ESRD (RIFLE) criteria or the Acute Kidney Injury Network criteria (Bagshaw et al., *Nephrol. Dial. Transplant.*, 23 (5):1569-1574 (2008); Lopes et al., *Clin. Kidney J.*, 6(1):8-14 (2013)).

[00242] In certain embodiments, the methods of this disclosure involve determining measuring the levels of one or more of: serum, plasma or urine creatinine or blood urea nitrogen (BUN); measuring the levels of serum or urine neutrophil gelatinase-associated lipocalin (NGAL), serum or urine interleukin-18 (IL-18), serum or urine cystatin C, or urine KIM-1, compared to a healthy control subject, to assess whether the subject has, or has a risk of developing, acute kidney injury.

[00243] The efficacy of the 15-PGDH inhibitors can be assessed in various animal models. Animal models for acute kidney injury include those disclosed in *e.g.*, Heyman et al., *Contrin. Nephrol.*, 169:286-296 (2011); Heyman et al., *Exp. Opin. Drug Disc.*, 4(6): 629-641 (2009); Morishita et al., *Ren. Fail.*, 33(10):1013-1018 (2011); Wei Q et al., *Am. J. Physiol. Renal Physiol.*, 303(11):F1487-94 (2012).

[00244] The efficacy of treatments may be measured by a number of available diagnostic tools, including physical examination, blood tests, measurements of blood systemic and capillary pressure, proteinuria (*e.g.*, albuminuria), microscopic and macroscopic hematuria, assessing serum creatinine levels, assessment of the glomerular filtration rate, histological evaluation of renal biopsy, urinary albumin creatinine ratio, albumin excretion rate, creatinine clearance rate, 24-hour urinary protein secretion, and renal imaging (*e.g.*, MRI, ultrasound).

[00245] In some embodiments, the amount of 15-PGDH inhibitor administered to the subject can be an amount effective to induce endogenous renal PGE2 levels of the subject.

[00246] In other embodiments, the amount of 15-PGDH inhibitor administered to the subject can be an amount effective to induce renal vasodilatation, enhance resistance to hypoxia, improve renal hemodynamics, decrease renal oxidative stress, reduce renal inflammation, and preserve renal function.

[00247] In other embodiments, the amount of 15-PGDH inhibitor administered to the subject is an amount effective to reduce malondialdehyde (MDA) and NGAL levels, attenuate medulla tubular damage, reduce medulla acute tubular necrosis (ATN) and apoptosis, reduces induction of high-mobility group box 1 (HMGB1) and proinflammatory cytokines, induce renal EP4 PGE2 receptors and A2A adenosine receptors in vascular smooth muscle cells that regulate renal arterioles, increase renal cAMP, AMP, and adenosine levels, and/or inhibit induction of creatinine and KIM-1.

[00248] In some embodiments, the pharmaceutical composition may be formulated into a parenteral or oral dosage form. The solid dosage form for oral administration may be manufactured by adding excipient, if necessary, together with binder, disintegrants, lubricants, coloring agents, and/or flavoring agents, to the 15-PGDH inhibitors and shaping the resulting mixture into the form of tablets, sugar-coated pills, granules, powder or capsules. The additives that can be added in the composition may be ordinary ones in the art. For example, examples of the excipient include lactose, sucrose, sodium chloride, glucose, starch, calcium carbonate, kaolin, microcrystalline cellulose, silicate and the like. Exemplary binders include water, ethanol, propanol, sweet syrup, sucrose solution, starch solution, gelatin solution, carboxymethylcellulose, hydroxypropyl cellulose, hydroxypropyl starch, methylcellulose, ethylcellulose, shellac, calcium phosphonate and polypyrrolidone. Examples of the disintegrant include dry starch, sodium arginate, agar powder, sodium bicarbonate, calcium carbonate, sodium lauryl sulfate, stearic monoglyceride and lactose.

Further, purified talc, stearates, sodium borate, and polyethylene glycol may be used as a lubricant; and sucrose, bitter orange peel, citric acid, tartaric acid, may be used as a flavoring agent. In some embodiments, the pharmaceutical composition can be made into aerosol formulations (*e.g.*, they can be nebulized) to be administered via inhalation.

[00249] The 15-PGDH inhibitors described herein may be combined with flavoring agents, buffers, stabilizing agents, and the like and incorporated into oral liquid dosage forms such as solutions, syrups or elixirs in accordance with conventional methods. One example of the buffers may be sodium citrate. Examples of the stabilizing agents include tragacanth, acacia and gelatin.

[00250] In some embodiments, the 15-PGDH inhibitors may be incorporated into an injection dosage form, for example, for a subcutaneous, intramuscular or intravenous route by adding thereto pH adjusters, buffers, stabilizing agents, relaxants, topical anesthetics. Examples of the pH adjusters and the buffers include sodium citrate, sodium acetate and sodium phosphate. Examples of the stabilizing agents include sodium pyrosulfite, EDTA, thioglycolic acid and thiolactic acid. The topical anesthetics may be procaine HCl, lidocaine HCl and the like. The relaxants may be sodium chloride, glucose and the like.

[00251] In other embodiments, the 15-PGDH inhibitors may be incorporated into suppositories in accordance with conventional methods by adding thereto pharmaceutically acceptable carriers that are known in the art, for example, polyethylene glycol, lanolin, cacao butter or fatty acid triglycerides, if necessary, together with surfactants such as Tween.

[00252] The pharmaceutical composition may be formulated into various dosage forms as discussed above and then administered through various routes including an oral, inhalational, transdermal, subcutaneous, intravenous or intramuscular route. The dosage can be a pharmaceutically or therapeutically effective amount.

[00253] Therapeutically effective dosage amounts of the 15-PGDH inhibitor may be present in varying amounts in various embodiments. For example, in some embodiments, a therapeutically effective amount of the 15-PGDH inhibitor may be an amount ranging from about 10-1000 mg (*e.g.*, about 20 mg-1,000 mg, 30 mg-1,000 mg, 40 mg-1,000 mg, 50 mg-1,000 mg, 60 mg-1,000 mg, 70 mg-1,000 mg, 80 mg-1,000 mg, 90 mg-1,000 mg, about 10-900 mg, 10-800 mg, 10-700 mg, 10-600 mg, 10-500 mg, 100-1000 mg, 100-900 mg, 100-800 mg, 100-700 mg, 100-600 mg, 100-500 mg, 100-400 mg, 100-300 mg, 200-1000 mg, 200-900 mg, 200-800 mg, 200-700 mg, 200-600 mg, 200-500 mg, 200-400 mg, 300-1000 mg,

300-900 mg, 300-800 mg, 300-700 mg, 300-600 mg, 300-500 mg, 400 mg-1,000 mg, 500 mg-1,000 mg, 100 mg-900 mg, 200 mg-800 mg, 300 mg-700 mg, 400 mg-700 mg, and 500 mg-600 mg). In some embodiments, the 15-PGDH inhibitor is present in an amount of or greater than about 10 mg, 50 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. In some embodiments, the 15-PGDH inhibitor is present in an amount of or less than about 1000 mg, 950 mg, 900 mg, 850 mg, 800 mg, 750 mg, 700 mg, 650 mg, 600 mg, 550 mg, 500 mg, 450 mg, 400 mg, 350 mg, 300 mg, 250 mg, 200 mg, 150 mg, or 100 mg.

[00254] In other embodiments, a therapeutically effective dosage amount may be, for example, about 0.001 mg/kg weight to 500 mg/kg weight, *e.g.*, from about 0.001 mg/kg weight to 400 mg/kg weight, from about 0.001 mg/kg weight to 300 mg/kg weight, from about 0.001 mg/kg weight to 200 mg/kg weight, from about 0.001 mg/kg weight to 100 mg/kg weight, from about 0.001 mg/kg weight to 90 mg/kg weight, from about 0.001 mg/kg weight to 80 mg/kg weight, from about 0.001 mg/kg weight to 70 mg/kg weight, from about 0.001 mg/kg weight to 60 mg/kg weight, from about 0.001 mg/kg weight to 50 mg/kg weight, from about 0.001 mg/kg weight to 40 mg/kg weight, from about 0.001 mg/kg weight to 30 mg/kg weight, from about 0.001 mg/kg weight to 25 mg/kg weight, from about 0.001 mg/kg weight to 20 mg/kg weight, from about 0.001 mg/kg weight to 15 mg/kg weight, from about 0.001 mg/kg weight to 10 mg/kg weight.

[00255] In still other embodiments, a therapeutically effective dosage amount may be, for example, about 0.0001 mg/kg weight to 0.1 mg/kg weight, *e.g.* from about 0.0001 mg/kg weight to 0.09 mg/kg weight, from about 0.0001 mg/kg weight to 0.08 mg/kg weight, from about 0.0001 mg/kg weight to 0.07 mg/kg weight, from about 0.0001 mg/kg weight to 0.06 mg/kg weight, from about 0.0001 mg/kg weight to 0.05 mg/kg weight, from about 0.0001 mg/kg weight to about 0.04 mg/kg weight, from about 0.0001 mg/kg weight to 0.03 mg/kg weight, from about 0.0001 mg/kg weight to 0.02 mg/kg weight, from about 0.0001 mg/kg weight to 0.019 mg/kg weight, from about 0.0001 mg/kg weight to 0.018 mg/kg weight, from about 0.0001 mg/kg weight to 0.017 mg/kg weight, from about 0.0001 mg/kg weight to 0.016 mg/kg weight, from about 0.0001 mg/kg weight to 0.015 mg/kg weight, from about 0.0001 mg/kg weight to 0.014 mg/kg weight, from about 0.0001 mg/kg weight to 0.013 mg/kg weight, from about 0.0001 mg/kg weight to 0.012 mg/kg weight, from about 0.0001 mg/kg weight to 0.011 mg/kg weight, from about

0.0001 mg/kg weight to 0.01 mg/kg weight, from about 0.0001 mg/kg weight to 0.009 mg/kg weight, from about 0.0001 mg/kg weight to 0.008 mg/kg weight, from about 0.0001 mg/kg weight to 0.007 mg/kg weight, from about 0.0001 mg/kg weight to 0.006 mg/kg weight, from about 0.0001 mg/kg weight to 0.005 mg/kg weight, from about 0.0001 mg/kg weight to 0.004 mg/kg weight, from about 0.0001 mg/kg weight to 0.003 mg/kg weight, from about 0.0001 mg/kg weight to 0.002 mg/kg weight. In some embodiments, the therapeutically effective dose may be 0.0001 mg/kg weight, 0.0002 mg/kg weight, 0.0003 mg/kg weight, 0.0004 mg/kg weight, 0.0005 mg/kg weight, 0.0006 mg/kg weight, 0.0007 mg/kg weight, 0.0008 mg/kg weight, 0.0009 mg/kg weight, 0.001 mg/kg weight, 0.002 mg/kg weight, 0.003 mg/kg weight, 0.004 mg/kg weight, 0.005 mg/kg weight, 0.006 mg/kg weight, 0.007 mg/kg weight, 0.008 mg/kg weight, 0.009 mg/kg weight, 0.01 mg/kg weight, 0.02 mg/kg weight, 0.03 mg/kg weight, 0.04 mg/kg weight, 0.05 mg/kg weight, 0.06 mg/kg weight, 0.07 mg/kg weight, 0.08 mg/kg weight, 0.09 mg/kg weight, or 0.1 mg/kg weight. The effective dose for a particular individual can be varied (*e.g.*, increased or decreased) over time, depending on the needs of the individual.

[00256] In some embodiments, a therapeutically effective dosage may be a dosage of 10 µg/kg/day, 50 µg/kg/day, 100 µg/kg/day, 250 µg/kg/day, 500 µg/kg/day, 1000 µg/kg/day or more. In various embodiments, the amount of the 15-PGDH inhibitor or pharmaceutical salt thereof is sufficient to provide a dosage to a patient of between 0.01 µg/kg and 10 µg/kg; 0.1 µg/kg and 5 µg/kg; 0.1 µg/kg and 1000 µg/kg; 0.1 µg/kg and 900 µg/kg; 0.1 µg/kg and 900 µg/kg; 0.1 µg/kg and 800 µg/kg; 0.1 µg/kg and 700 µg/kg; 0.1 µg/kg and 600 µg/kg; 0.1 µg/kg and 500 µg/kg; or 0.1 µg/kg and 400 µg/kg.

[00257] Various embodiments may include differing dosing regimen. In some embodiments, the 15-PGDH inhibitor can be administered via continuous infusion. In some embodiments, the continuous infusion is intravenous. In other embodiments, the continuous infusion is subcutaneous. The dosing regimen for a single subject need not be at a fixed interval, but can be varied over time, depending on the needs of the subject.

[00258] In one aspect, a pharmaceutical composition comprising an effective amount of the 15-PGDH inhibitor is administered at least twice. In another aspect, a pharmaceutical composition is administered at least five times. In yet another aspect, a pharmaceutical composition is administered at least 10 times. One of ordinary skill in the art can determine how often to administer the composition based on the particular disease or disorder being

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treated or how the subject has responded to prior treatments. One of ordinary skill in the art can also determine when to administer a treatment relative to the time that renal injury occurs, including before, after, or both.

[00259] In one embodiment, the subject is treated with the 15-PGDH inhibitor prior to the renal injury. In one aspect, the subject can be treated starting at least several days before the injury or as close to several minutes before the renal injury. For example, the 15-PGDH inhibitor therapy can begin at about 2 hours, 8 hours, 24 hours, or 26 hours prior to ischemic reperfusion injury. One of ordinary skill in the art will appreciate that the 15-PGDH inhibitor can be administered at varying times and not just at about 2, 8, 24, or 26 hours prior to the renal injury. In one aspect, the range of time for treating prior to the renal injury can be from about 1.0 minutes to about 72 hours. In another aspect, the range of time for treating prior to the renal injury can be from about 10 minutes to about 48 hours. In another aspect, the range of time for treating prior to the renal injury event can be from about 30 minutes to about 24 hours.

[00260] In one embodiment, the subject is treated with the 15-PGDH inhibitor after the renal injury event or both before and after as described above. In one aspect, the subject can be treated starting immediately after such as several minutes after the renal injury. For example, the 15-PGDH inhibitor therapy can begin at about 30 minutes, 2 hours, 8 hours, 24 hours, or 48 hours after the ischemic reperfusion injury. One of ordinary skill in the art will appreciate that the 15-PGDH inhibitor can be administered at varying times as well.

Example 1

[00261] In this Example, we examined SW033291, a potent small-molecule inhibitor of 15- PGDH, for effects in the kidney, demonstrating that SW033291 shows potent in vivo activity in increasing endogenous renal PGE₂, in mediating renal vasodilation, and in conferring renal protection against ischemic acute kidney injury (AKI).

Materials and Methods

Animals

[00262] Male C57/BL6 mice (10 wk) were purchased from Orient Bio. The care of and experimental procedures involving animals were approved by the Institutional Animal Care and Use Committee of Inje University (protocol no. 2016-010).

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Induction of renal IRI

[00263] Briefly, mice were anesthetized with isoflurane using a vaporizer, and bilateral renal arteries were clamped for 30 min (2, 49). After the ischemic time, clamps were released to induce blood reperfusion. SW033291 (5 mg/kg, Cayman, Ann Arbor, MI), indomethacin (5 mg/kg, Sigma-Aldrich), or vehicle [10% ethanol, 5% cremophor EL, and 85% D5W (5% dextrose in water)] were intraperitoneally administered three times at 1 h before, immediately after, and 12 h after AKI. Serum and kidney tissue were collected 24 h after renal IRI. These dose levels of SW033291 and indomethacin have been demonstrated as biologically effective in C57/BL6 mice in our prior published study. As an additional comparator, celecoxib (100 mg/kg, Pfizer), a selective COX-2 inhibitor, was also orally administered two times at 2 h before and 12 h after renal IRI. Additionally, an EP₄ receptor antagonist, ONO-AE3-208 (10 mg/kg, Cayman), was administered for three doses, either concurrently with SW033291 or individually on the same schedule.

Measurement of PGE₂ levels and renal function

[00264] After reperfusion for 24 h, kidney tissues (~20 mg) were homogenized in cold PBS containing indomethacin (10 µg/mL) and then centrifuged for 10 min at 12,000 rpm. Protein concentrations of supernatant were determined by BCA assay (ThermoFisher Scientific, Rockford, IL). The PGE₂ level was measured using a PGE₂ ELISA kit (R&D Systems, Minneapolis, MN) in triplicate. PGE₂ levels were expressed as nanograms of PGE₂ per milligram of protein. Renal function was assessed by determining the serum levels of blood urea nitrogen (BUN; Abcam), serum creatinine (Agilent 6410 LC-MS/MS, Agilent, Santa Clara, CA), lipocalin-2 (R&D Systems), and kidney injury molecule-1 (KIM-1; R&D Systems) after reperfusion for 24 h. Measurement of serum creatinine was by HPLC. Serum creatinine was assessed using an Agilent 6410 LC-MS/MS system coupled with an Agilent 1200 series HPLC system (Agilent). The sample preparation for serum creatinine was that 50 µL of serum samples were mixed with 100 µL of acetonitrile that contained creatinine-d₃ as an internal standard (5 µg/mL). The mixture was centrifuged at 13,000 rpm for 10 min after vigorous vortex, and 1 µL of supernatant was then injected into the LC-MS/MS system. The chromatography separation was performed on a Hypersil GOLD C18 column (150 X 2.1 mm, 3 µm, Thermo Scientific) using a mobile phase of 0.1% formic acid solution and acetonitrile [70:30 (vol/vol)]. The flow rate was 0.2 mL/min. Electrospray ionization was performed in

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the positive ion mode. The capillary voltage was set at 4,000 V. Nitrogen as the nebulizing gas was set at 15 psi, and the drying gas temperature was set at 300°C, with a flow rate of 6 L/ min. The multiple-reaction monitoring mode using specific precursor/production transition was used for quantification. Detection of ions was performed by monitoring m/z 114.1 $\rightarrow$ 44.1 for creatinine and 117.1 $\rightarrow$ 77.1 for creatinine-d3. The collision energies were 10 and 15 eV for creatinine and creatinine-d3, respectively. The peak areas for all analytes were integrated automatically using Agilent Mass Hunter Analysis software (version B.01.04).

Necrotic and apoptotic cell death assays

[00265] To evaluate necrosis, 5- μ m-thick paraffin sections were stained with hematoxylin and eosin. Tubular injury of individual slides was scored semi-quantitatively according to a scoring system by a pathologist in a blinded manner. The scoring system was as follows: 0, no damage; 1, patchy isolated unicellular necrosis; 2, tubular necrosis < 25%; 3, tubular necrosis 25– 50%; and 4, tubular necrosis > 50%. To analyze the frequency of apoptosis, 5- μ m-thick paraffin sections were subjected to a TUNEL assay (Millipore, Temecula, CA) according to the manufacturer's protocol. TUNEL-positive cells were counted in at least five separate fields (64 magnification) in the outer medulla, and the apoptosis index (in %, number of apoptosis cells/total number of cells) was calculated using GENASIS software.

Measurement of proinflammatory cytokine levels

[00266] Inflammatory cytokine mRNA and protein levels were measured by real-time PCR and ELISA. Kidney tissue and serum were harvested after reperfusion for 24 h. Total RNA was extracted from frozen kidney tissue using TRIzol reagent (ThermoFisher Scientific) according to the manufacturer's protocol. RNA was converted to cDNA using oligo-dT primers. IL-17, TNF- α , and IL-1 β mRNA levels were determined by real-time PCR. Serum IL-17 (R&D Systems), TNF- α (R&D Systems), and IL-1 β (R&D Systems) were measured using commercial ELISA kits according to the manufacturer's instructions.

Assessment of renal vasodilation

[00267] The inner arteriolar area of the outer medulla was determined using α -smooth muscle actin (α -SMA)- stained sections. Kidneys were removed without perfusion under inhalational isoflurane anesthesia, divided into three pieces horizontally, immediately fixed in

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10% formalin, and then embedded in paraffin. The inner area of α -SMA-positive vessels in the outer medulla was measured using ImageJ. The results are expressed as average areas of renal arteries in the outer medulla. As a surrogate for RBF, we used noninvasive laser Doppler flowmetry (periflux system 5000, Perimed) to measure flux, placing laser-Doppler probes at a fixed position on the kidney surface and recording renal cortical measurements. Renal-Doppler flux (RDF) was measured before (1 h after administration of vehicle, indomethacin, and SW033291), during, and 24 h after renal IRI. The relative increase represents the percent increase in RDF from baseline to peak for each test.

Measurement of cAMP, AMP, and adenosine levels

[00268] After reperfusion for 24 h, kidney tissues were harvested, homogenized in 10 volumes of 0.1 M HCl, and centrifuged for 10 min at 12,000 rpm. cAMP levels in kidney tissues and serum were measured using a cAMP Complete ELISA kit (Enzo Life Science, Farmingdale, NY). AMP and adenosine in kidney tissue were assessed by Agilent 6410 LC-MS/MS. Serum adenosine was measured using an assay kit (Abnova, Walnut, CA).

Assessment of PGE₂ and adenosine receptor

[00269] PGE₂ receptors (EP₁, EP₂, EP₃, and EP₄) and adenosineA_{2A} receptor mRNA levels were determined by real-time PCR. Their protein levels were determined by Western blot assay and immunofluorescence analysis.

Measurement of reactive oxygen species levels

[00270] Reactive oxygen species (ROS) levels were determined by measurement of malondialdehyde (MDA), the end product of lipid peroxidation in kidney lysates. Free MDA reacts with thiobarbituric acid (TBA) at 95°C to generate an MDA-TBA adduct, which can be quantified colorimetrically at a wavelength of 532 nm. MDA levels were measured in kidney lysates using a lipid peroxidation (MDA) assay kit (Abcam, Cambridge, UK). Results were corrected for total protein level and are expressed as μ M MDA/g protein.

Statistical analysis

[00271] Statistical analyses were performed with one-way ANOVA followed by a Bonferroni post hoc test when three or more experimental groups were compared. Data are

presented as means $\pm$ SE, using Student's *t*-test for two-group analysis. Values of $P < 0.05$ were considered indicative of statistical significance.

Results

15-PGDH inhibition ameliorates renal dysfunction in mice with ischemic AKI

[00272] Endogenous PGE₂ levels are decreased by blocking PGE₂ synthesis with NSAIDs that inhibit COX-1 and/ or COX-2 but are increased by blocking PGE₂ degradation by inhibiting 15-PGDH (Fig. 1A). SW033291 is a potent and specific chemical inhibitor of 15-PGDH with a subnanomolar IC₅₀ value (Fig. 1B). Treatment of mice with SW033291 induced a dose-dependent increase in renal PGE₂ at 3 h after intraperitoneal injection of 2.5 or 5 mg/kg SW033291 (Fig. 1C) and showed a peak at 1 h with 5 mg/kg SW033291 tripling endogenous renal PGE₂ (Fig. 1D). To interrogate effects of inhibition of 15-PGDH on protection from IRI, mice were subjected to 30 min of IRI and administered three doses of SW033291 (IRI-SW033291) versus vehicle (IRI-vehicle), versus indomethacin (IRI-indomethacin), or versus celecoxib (IRI-celecoxib). Treatments were administered beginning 1 h before, immediately after, and 12 h after renal IRI (Fig. 1E) (with the exception that the celecoxib dose was administered 2 h before and 12 h after renal IRI). IRI-vehicle mice exhibited significant ischemic AKI, as indicated by increases in BUN, creatinine, neutrophil gelatinase-associated lipocalin (NGAL), and KIM-1 (Fig. 1, F–I). However, IRI-SW033291 markedly protected the kidney from IRI, significantly reducing BUN, creatinine, NGAL, and KIM-1 compared with IRI-vehicle mice (Fig. 1, F–I). In contrast, inhibition of endogenous PGE₂ production with administration of either indomethacin or celecoxib significantly aggravated IRI, as reflected by the increase in BUN of the indomethacin-treated group (Fig. 1F) and the increase of BUN and creatinine in the celecoxib-treated group. In summary, inhibition of 15-PGDH increased endogenous renal PGE₂ levels and ameliorated renal dysfunction from ischemic AKI compared with renal dysfunction being worsened by inhibiting COX-1 and COX-2 with indomethacin or COX-2 alone with celecoxib.

15-PGDH inhibition attenuates renal necrosis and apoptosis in mice with ischemic AKI

[00273] During renal IRI, tubular epithelial cells undergo injury, apoptosis, and acute tubular necrosis. Postischemic congestion that persists in the outer medulla further exacerbates renal injury by worsening hypoxia. By gross pathology, IRI-vehicle mice

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showed increased tissue congestion in the outer medulla versus sham mice. This congestion was ameliorated by treatment with SW033291 but was worsened by treatment with indomethacin (Fig. 2A). Histopathological assessment of IRI-vehicle revealed features of acute tubular damage with tubular dilatation, extensive tubular necrosis, and apoptosis (Fig. 2, B–E). In contrast, SW033291 treatment markedly alleviated renal injury, reducing the histological renal injury score and the count of TUNEL-positive apoptotic cells (Fig. 2, C and E). Additionally, high-mobility group box 1 (HMGB1) promotes kidney damage after IRI and induces proinflammatory cytokines. Similarly, compared with IRI-vehicle mice, SW033291 treatment reduced HMGB1 levels (Fig. 3, G and H) and blocked induction of IL-17, TNF- α , and IL-1 β (protein only) (Fig. 3, A–F). In contrast to the effects of SW033291, treatment of IRI mice with indomethacin increased HMGB1 (Fig. 3, G and H). In summary, prophylactic SW033291 protects from AKI, reducing tubular damage in the outer medulla, ATN, apoptosis, HMGB1, and downstream inflammatory cytokines that promote kidney damage after IRI.

15-PGDH inhibition induces renal vasodilatation in the outer medulla concomitant with induction of a PGE₂/EP₄ receptor and adenosine/A_{2A} receptor signaling pathway

[00274] To investigate the mechanism of the renal protective effect of the 15-PGDH inhibitor shown in Figs. 1–3, we interrogated the effects of inhibition of 15-PGDH on renal hemodynamics using measurement of renal cortical Doppler flux as a surrogate to infer RBF. SW033291 increased inferred RBF above baseline within 1 h after the first injection, reflecting elevated RBF just before when AKI was initiated (Fig. 4, A and B). These benefits persisted throughout the 24-h course of study. Thus, at 24 h following IRI, vehicle-treated mice demonstrated a nearly 40% decrease in RDF-inferred RBF (vs. sham mice) (Fig. 4, A and B), whereas, in marked contrast, treatment of mice with SW033291 completely preserved RDF-inferred RBF at this time point (Fig. 4, A and B). SW033291 increases in cortical RDF-inferred RBF at 24 h were paralleled by findings of significantly increased renal arteriolar area in the outer medulla of SW033291-treated mice at 24 h post-IRI (compared with both post-IRI-vehicle-treated mice and sham mice; Fig. 4, C and D). Again, in contrast, both indomethacin or celecoxib administration reduced RDF (Fig. 4, A and B). In summary, these findings suggest that SW033291 increased RBF via a PGE₂-mediated vasodilatory mechanism.

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[00275] PGE₂ induces cAMP levels by signaling via EP₂ and EP₄ receptors, a pathway that is well characterized as inducing vasodilation in the renal afferent arteriole. Thus, we interpreted the coincident induction of PGE₂ and of increased RDF at 1 h after SW033291 as likely reflecting this established biology (Figs. 1D and 4B). This biology further motivated us to examine cAMP as a potential downstream mechanism for the persistent SW033291-induced increase in RDF-inferred RBF at 24 h post-IRI. At 24 h post-IRI, both vehicle- and indomethacin-treated mice showed reduced renal cAMP versus sham mice (Fig. 5A). However, in parallel with RDF-inferred increased RBF, cAMP levels at 24 h were also elevated over sham mice in IRI-SW033291-treated mice (Fig. 5A). Moreover, at 24 h post-IRI, SW033291-treated mice also showed increased levels of the cAMP breakdown product AMP (Fig. 5B). Furthermore, an additional vasodilatory molecule, adenosine, was also decreased in the kidney at 24 h after IRI, and, like cAMP, this decrease was also reversed in mice treated with SW033291 (Fig. 5C). Increased adenosine was, furthermore, detectable at 24 h post-IRI in serum SW033291-treated mice (Fig. 5D). Moreover, at 24 h, SW033291 also induced the adenosine A_{2A} receptor (compared with both sham and IRI mice) (Fig. 5, E and F), with immunohistochemistry localizing the upregulated adenosine A_{2A} receptors to the membrane of α -SMA-positive vascular smooth muscle cells that directly regulate constriction or dilation of renal arterioles (Fig. 5G).

[00276] To inspect for upstream elements of the PGE₂ signaling pathway that might correlate with SW033291-induced signaling events at the 24-h post IRI time point, we looked for simultaneous effects of SW033291 on the expression of PGE₂ receptors. Intriguingly, we found that at 24 h post-IRI, mice treated with SW033291 had significantly increased EP₄ receptor expression, at both mRNA and protein levels, with EP₄ receptor protein more than twice as high in SW033291- versus vehicle-treated mice (Fig. 6, D–F) (compared with both sham and vehicle-treated IRI mice). Indomethacin, in contrast, reduced EP₄ receptor mRNA levels (Fig. 6, D–F) and moreover increased EP₁ receptor mRNA levels, a receptor known to be involved in vasoconstriction (Fig. 6A). SW033291 and indomethacin had no effect on levels of EP₂ and EP₃ receptors (Fig. 6, B and C). EP₄ receptors induced by SW033291 were localized to the membrane of α -SMA-positive vascular smooth muscle cells that directly regulate constriction or dilation of renal arterioles (Fig. 6G).

[00277] To interrogate the functional role of EP₄ receptors in mediating SW033291 effects, we conducted experiments of IRI mice co-administered the three-dose schedule of

SW033291 together with an EP₄ receptor antagonist, ONO-AE3-208. Co-administration of ONO-AE3-208 substantially blocked the beneficial effects of SW033291 on reversing IRI-induced increases of BUN, serum creatinine, and serum Kim-1 and on reversing IRI-induced decreases in RDF-inferred RBF (Fig. 6, H–L).

[00278] In overview, induction of renal vasodilation by 15-PGDH inhibition was well correlated with increased PGE₂ levels at 1 h post administration of drug, and, at 24 h, it was well correlated with induction of downstream mediators that included cAMP, AMP, adenosine, and adenosine A_{2A} and EP₄ receptors, with induction of both EP₄ and A_{2A} receptors targeted to vascular smooth muscle cells and with EP₄ function required for drug activity.

Pretreatment with a single 15-PGDH inhibitor dose mitigates renal dysfunction

[00279] To interrogate the contributions of SW033291 administered just before versus just after AKI, we compared the effects of administration of a single dose of SW033291 given 1 h before IRI (Pre) versus two doses given immediately after IRI (Post) versus our standard three-dose regimen given 1 h before, immediately after, and 12 h after IRI (Pre + Post) (Fig. 7A). Surprisingly, IRI-SW033291 (Pre) was as effective in ameliorating AKI as IRI-SW033291 (Pre + Post) (Fig. 7, B–E), as assessed by BUN, creatinine, NGAL, and KIM-1, although IRI-SW033291 (Pre) provided somewhat less protection from reduction of RDF-inferred RBF versus IRI-SW033291 (Pre + Post) (Fig. 7F). IRI-SW033291 (Post) was insufficient to ameliorate AKI in the absence of concomitant pretreatment with drug. These findings suggest that a single dose of SW033291 administered before IRI can provide prophylaxis from inducing AKI.

Pretreatment with a single 15-PGDH inhibitor dose attenuates AKI-induced oxidative stress and blocks injury-induced increases in renal PGE₂

[00280] MDA, a marker of oxidative stress, began to increase immediately after renal IRI of vehicle-treated mice and peaked at 2 h at 48% above baseline. A single pre-IRI dose of SW033291 blunted and reduced this increase to only 14% (Fig. 8A). IRI-SW033291 (Pre) mice also showed notable protection from the induction of NGAL, KIM-1, and creatinine (Fig. 8, B–D). In vehicle-treated mice, IRI induced two peaks of renal PGE₂, an immediate post-IRI peak plus a 14-h post-IRI peak (Fig. 8E). Intriguingly, a single pre-IRI dose of SW033291 induced an early PGE₂ peak just before IRI and was then sufficient to

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substantially block both of the later post-IRI peaks of PGE₂ (Fig. 8E). Moreover, the single pretreatment dose of SW033291 also significantly decreased both renal and serum PGE₂ levels at 24 h (Fig. 8, E and F). Furthermore, pretreatment with SW033291 increased pre-IRI levels of both EP₄ and A_{2A} receptors (Fig. 8, G and H). In overview, induction of endogenous PGE₂ by administration of a prophylactic 15-PGDH inhibitor before IRI increases EP₄ and A_{2A} receptors, induces vasodilatation, attenuates post-IRI oxidative stress, and, thereby, reduces multiple markers of renal injury.

[00281] In this example, we showed that pretreatment with a 15-PGDH inhibitor (SW033291) markedly protected the kidney from induction of the classic injury hallmarks of ischemic AKI. In particular, we demonstrated that administration of SW033291 improved renal hemodynamics (Fig. 4), decreased induction of oxidative stress (Fig. 8A), reduced induction of inflammation (Fig. 3), attenuated multiple markers of renal damage, and preserved renal function (Figs. 1 and 2). These benefits were also associated with the prophylactic use of a single pre-IRI dose of SW033291, which showed notable protection from induction of NGAL, KIM-1, and creatinine (Fig. 7, C–E). The activity of SW033291 in preventing renal injury is likely to be an on-target effect due to modulation of PGE₂, as efficacy of SW033291 was blocked by inhibiting EP₄ signaling with ONO-AE3-208 and as treatment with indomethacin, a COX antagonist, and celecoxib, a COX-2 antagonist, acted opposite to SW033291 and further exacerbated renal injury (Figs. 1, 2, and 6, H–L).

[00282] PGE₂ is known to regulate renal hemodynamics and inflammation, but its rapid degradation in vivo has been an obstacle to therapeutic applications. Our data demonstrate that pretreatment with a single dose of SW033291 can induce PGE₂ and reduce renal damage from ischemic AKI (Fig. 7). Specifically, inhibition of 15-PGDH and increasing endogenous PGE₂ before ischemic IRI provides a protective effect in the kidney via increasing renal vasodilation and RBF that would be expected to enhance oxygen delivery capacity and increase resistance to hypoxia. These results suggest that the renal protective effect of PGE₂, in part, relates to the timing of its increase. RBF is determined by the vasodilator-vasoconstriction balance, with PGE₂ and adenosine both acting as significant endogenous vasoactive mediators. PGE₂ exerts a vasodilatory effect in the kidney, and the use of NSAIDs in the early postoperative period is associated with renal dysfunction due to reduced RBF from suppression of synthesis of endogenous renal PGE₂.

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[00283] Our findings are consistent with observations that in most blood vessels, the vasodilation effect of PGE₂ is mediated by the EP₄ receptor, which, via coupling to G_{as}, can directly activate adenylate cyclase and cAMP production. Our results are also consistent with the role of adenosine and its A_{2A} receptor as important regulators of renal vasodilatation and mediators of protection from ischemic AKI. Induction of cAMP by SW033291 is, thus, likely the direct result of induction of renal PGE₂ and activation of EP₄ receptors.

[00284] Our results support the role of 15-PGDH inhibition in renal vasodilation due to PGE₂-dependent increases in PGE₂/EP₄ and adenosine/A_{2A} receptors plus the induction of cAMP/AMP (Fig. 9). Most significantly, our results demonstrate that enhancement of renal PGE₂ by 15-PGDH inhibition before IRI induces renal vasodilation, which we suggest enhances resistance to hypoxia and results in prophylaxis against ischemic AKI. Inhibition of 15-PGDH may provide a novel pharmacological approach for prophylaxis against ischemic AKI in various clinical settings, including renal transplantation, shock, and cardiovascular surgery.

Example 2

[00285] In this Example, we analyzed whether a 15-PGDH inhibitor in a contrast-induced acute kidney injury (CIAKI) mouse model has a protective effect against renal deterioration and histological damage by contrast media (CM), and against direct tubular cell toxicity by culturing renal tubular cells in vitro. In addition, we investigated the changes in intrarenal hemodynamic and renal blood flow (RBF) as a mechanism, and the role of receptors related to PGE₂.

Materials and Methods

Mice and reagents

[00286] Female C57/BL6 mice (age, 10 weeks; body weight, 20–25 g) were purchased from Orient Bio Inc. (Daejeon, Republic of Korea). Before the experiments, all mice were housed individually in standard cages and were allowed to acclimate under specific-pathogen-free conditions in the animal care facility of the College of Medicine of Inje University, Republic of Korea. The care of and experimental procedures involving the animals were approved by the Institutional Animal Care and Use Committee of Inje University (protocol no. 2018-019).

Induction of CIAKI

[00287] The two different radioactive iodine in terms of viscosity and osmolality were administered intravenously to 5 mice for each group. Ten grams of iodine per body weight (gI/kg) of each CM was injected by a 26-gauge syringe through the tail vein. The blood samples for the analysis of functional assessment of kidney injury such as Cr, NGAL, and KIM-1 were taken at 48 h after administration (Figs. 10(A,B)).

Study design of each group with drug administration

[00288] Female mice were injected with 10 gI/kg of Visipaque (iodixanol) via the tail vein. SW033291 (5 mg/kg; Cayman, Ann Arbor, MI, USA), PGE1 (20 mg/kg; Cayman), or PGE2 (5 mg/kg; Sigma-Aldrich, St. Louis, MO, USA) or a vehicle (10%, ethanol; 5%, cremophor EL; and 85%, dextrose 5% in water) were intraperitoneally administered 1 h before, immediately after, and 8, 16, and 24 h after the iodixanol injection (Figure 11(C)). For inhibition of the EP4s, mice were treated with 0.2 mg/kg/day of ONO-AE3-208 by subcutaneous injection for 14 days. Serum and kidney tissue were collected 48 h after the iodixanol injection.

Measurement of PGE2 levels

[00289] Aside from the CIAKI experiment design, we first confirmed whether SW033291 administration actually increases PGE2 levels in kidney tissue in normal mice. Kidney tissues were harvested 1 h after the SW033291 injection, rinsed in ice-cold phosphate-buffered saline (PBS) containing indomethacin (10 µg/mL), and snap-frozen in liquid nitrogen. Next, 20 mg kidney tissue was homogenized in 500 mL cold PBS containing indomethacin (10 µg/mL) using a tissue homogenizer. The suspension was sonicated in an ice-water bath for 1 min using cycles of 10 s of sonication with 10 s of cooling, and then they were centrifuged for 10 min at 12,000 rpm. The supernatant was collected for PGE-2 assay. Protein concentrations were determined by bicinchoninic acid assay (Cat. #23225, Thermo Scientific). The PGE2 level in the supernatant was measured using a PGE2 enzyme-linked immunosorbent assay kit (R&D Systems, Minneapolis, MN, USA) in triplicate. PGE-2 levels were expressed as ng of PGE2/mg protein.

Assessment of renal function

[00290] Renal function was assessed by determining the serum levels of creatinine (Arbor Assays, Ann Arbor, MI, USA), NGAL (R&D Systems), and KIM-1 (R&D Systems) at 48 h after the iodixanol injection.

Necrotic and apoptotic cell death assays

[00291] Kidneys were harvested at 48 h after the CM injection, fixed in 4% phosphate-buffered formalin, and embedded in paraffin. To evaluate necrosis, 5 mm thick paraffin sections were stained with hematoxylin and eosin. Tubular injury was scored semi-quantitatively according to a system by a pathologist who examined at least 20 separate fields (400x magnification) in the outer medulla, which is the zone most sensitive to ischemic injury. The scoring system was as follows: 0, no damage; 1, patchy isolated unicellular necrosis; 2, tubular necrosis < 25%; 3, tubular necrosis 25–50%; and 4, tubular necrosis > 50%. At least 20 consecutive high-power fields per section were scored by two operators blind to the details of the experiment. To analyze the frequency of apoptosis, 5 mm thick paraffin sections were subjected to terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) assay (Millipore, Temecula, CA, USA) according to the manufacturer's protocol. Four 5 mm thick paraffin sections were incubated with the TUNEL reaction mixture at 37°C for 1 h, followed by incubation with a horseradish peroxidase-conjugated detection antibody. The signals were visualized using diaminobenzidine (Sigma-Aldrich). After counterstaining with Mayer's hematoxylin, TUNEL-positive cells were counted in at least five separate fields (640x magnification) in the outer medulla, and the apoptosis index (% , number of apoptosis cells/total number of cells) was calculated using GENASIS software.

Human renal proximal tubular epithelial cells culture

[00292] Human renal proximal tubular epithelial cells (hRPTECs) were purchased from the American Type Culture Collection (#PCS-400-010TM, Manassas, VA, USA). These cells were grown in 75 cm² flasks in Renal Epithelial Cell Basal Medium (PCS-400-030TM, ATCCVR) supplemented with Renal Epithelial Cell Growth Kit (PCS-400- 040TM, ATCCVR).

Cell viability assay

[00293] 3-(4,5-Dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay was used to assess cell viability. The hRPTECs were cultivated in 96-well plates at a density of 10^5 cells/mL and then incubated for 24 h. They were treated with SW033291 (1 μ M), PGE1 (100 ng/mL), or PGE2 (100 nM) simultaneously with Visipaque (iodixanol, 50 mgI/mL) for 24 h. Then, 10 μ M MTT (Sigma-Aldrich) was added to each well for an additional 4 h. The blue MTT formazan precipitate was dissolved in 100 μ L dimethyl sulfoxide. The absorbance at 540 nm was measured with a multi-well plate reader. Cell viability was expressed as a percentage of the no- treatment cells, as the mean value $\pm$ standard deviation of the six independent experiments.

Analyses of apoptosis by flow cytometry

[00294] After the treatment described in the section above, the hRPTECs were pelleted by centrifugation at 1800 rpm for 10 min and incubated with annexin V fluorescein isothiocyanate and propidium iodide using an Apoptosis Detection Kit I (#556547, BD Biosciences, San Jose, CA, USA) according to the manufacture's instruction. Then quantification was conducted using a FACSC flow cytometer with Cell Quest software (BD Biosciences).

Assessment of renal vasodilation in the outer medulla

[00295] 48 h after CM injection, kidney tissues were harvested, fixed in 4% phosphate-buffered formalin, and embedded in paraffin. To quantify vasodilation, the inner arteriole area of the outer medulla was determined using α -SMA-stained sections. They were incubated for 1 h with an α -SMA antibody. 3,3'-diamino- benzidine (0.7 g/tablet; Sigma-Aldrich) was added, followed by washing three times with PBS for 1 min each. After counterstaining with Mayer's hematoxylin, the inner areas of α -SMA-positive vessels in the outer medulla (25x magnification) were measured using ImageJ software. The results were expressed as the average area of all of the renal arteries in each outer medulla section.

Renal blood flow (RBF) assessment

[00296] Total RBF was assessed by measuring renal Doppler flux using noninvasive laser Doppler flowmetry (PeriFlux System 5000, Perimed AB, Sweden). Laser Doppler probes were placed on the kidney surface to measure the renal flux. The flux was measured

48 h after CM administration. The relative increase represented the percentage increase in renal blood flow from baseline to peak for each test. Statistical significance was set as $p < 0.05$.

Measurement of adenosine monophosphate (AMP) and adenosine levels

[00297] 48 h after CM administration, serum and kidney tissues were harvested. The adenosine levels in the serum and kidney tissues were measured using high-performance liquid chromatography.

Statistical analyses

[00298] Results are presented as mean $\pm$ standard error of the mean. Statistical analyses were performed with one- way analysis of variance followed by the Bonferroni post-test when three or more experimental groups were compared. Values of $p < 0.05$ were considered indicative of statistical significance.

Results

CIAKI is occurred in mice with intravenous injection of visipaqueVR (iodixanol)

[00299] While Xenetix and Visipaque have the same iodine concentration, Visipaque has a relatively lower osmolality and a higher viscosity than Xenetix (Fig. 10(A)). When compared with normal control, Xenetix showed a significant increase in Cr only; NGAL and KIM-1 did not show significant differences. On the other hand, Visipaque showed elevation in all three renal damage markers, Cr, NGAL and KIM-1, when compared with normal control (Figure 10(C–E)). Furthermore, Visipaque showed a significantly higher necrosis and apoptosis shown by Renal injury score and TUNEL assay than normal control (Fig. 12). Therefore, it can be said that Visipaque, an isosmolar and high viscosity agent, successfully induced CIAKI in mouse, and thus we chose Visipaque as the contrast media for CIAKI mouse model.

15-PGDH inhibitor attenuates renal dysfunction in CIAKI mouse model

[00300] Renal PGE2 levels measured 1 h after administration of a 15-PGDH inhibitor (SW033291) increased by an average of three times compared to vehicle-administered, normal, control mice (9.29 ± 0.67 ng/mg in normal vs. 29.71 ± 2.79 ng/mg in SW033291, $p < 0.01$; Fig. 11(A)). Compared to the control group, creatinine, neutrophil gelatinase-

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associated lipocalin (NGAL), and kidney injury molecule-1 (KIM-1) levels were significantly increased in CM-treated mice (CM þ vehicle; creatinine, 62 ± 0.02 mg/dL [normal] vs. 1.94 ± 0.24 mg/dL [iodixa- nol (10 gI/kg)], $p < 0.001$; NGAL, 63.55 ± 8.88 ng/mL [normal] vs. 299.71 ± 38.64 ng/mL [iodixanol (10 gI/kg)], $p < 0.001$; KIM-1, 0.03 ± 0.001 ng/mL [normal] vs. 1.41 ± 0.41 ng/mL [iodixanol (10 gI/kg)], $p < 0.001$; Fig. 11(C–E)).

[00301] SW033291 treatment in CIAKI mice led to marked reductions in the levels of creatinine, NGAL, and KIM-1 (creatinine, 1.94 ± 0.24 mg/dL [CM þ vehicle] vs. 1.10 ± 0.11 mg/dL [CM þ SW033291], $p < 0.05$; NGAL, 299.71 ± 38.64 ng/mL [CM þ vehicle] vs. 140.41 ± 25.52 ng/mL [CM þ SW033291], $p < 0.001$; KIM-1, 1.41 ± 0.41 ng/mL [CM þ vehicle] vs. 0.43 ± 0.30 ng/mL [CM þ SW033291], $p < 0.05$; Fig. 11(C–E)). In addition, in a group treated with PGE2, the reduction in renal injury biomarkers was similar to that of the SW033291 administration group. However, PGE1 decreased serum levels of creatinine in CIAKI mice, but not NGAL and KIM-1.

15-PGDH inhibitor ameliorates renal necrosis and apoptosis in CIAKI

[00302] In the gross findings of the mice kidneys, two groups, those treated with CM þ vehicle and CM þ PGE1, showed prominent vascular congestion (red blood cells sludging and medullary hyperemia) in the outer medullary region compared to the normal group, whereas two other groups, those treated with CM þ SW033291 and CM þ PGE2, showed less-congested outer medullary areas (Fig. 12A). In the microscopic findings of the mouse kidneys, CM þ vehicle mice showed distinct tubular dilation, necrotic cells, and loss of brush border compared to normal renal tissue (Fig. 3B). The microscopic renal injury score of the SW033291 administration group was significantly lower than that of the CM þ vehicle mice (1.90 ± 0.10 (CM þ vehicle) vs. 0.53 ± 0.16 (CM þ SW033291), $p < 0.001$; Fig. 12(B,C)). The CM þ PGE2 group also showed significantly lower renal injury scores than the CM þ vehicle group, but significantly higher than the SW033291 administration group (0.53 ± 0.16 [CM þ SW033291] vs. 1.55 ± 0.14 [CM þ PGE2], $p < 0.001$). No reduction in renal injury score was observed in the PGE1 group (Fig. 12(B,C)). The frequency of apoptosis detected by terminal deoxy-nucleotidyl transferase dUTP nick end labeling (TUNEL) staining was higher in the CIAKI mice than normal ones, and administration of SW033291 reduced the proportion of TUNEL-positive cells caused by CM ($46.77 \pm 4.77\%$ [CM þ vehicle] vs. $24.01 \pm 2.90\%$ [CM þ SW033291], $p < 0.001$). The mice treated with PGE1 and PGE2 also

showed significantly lower TUNEL-positive cell proportions compared to the CIAKI mice (Figs. 12(D,E)).

15-PGDH inhibitor protects human renal proximal tubular epithelial cells from iodixanol-induced apoptosis

[00303] We assessed the viability of hRPTECs using a MTT assay. This was significantly lower in the CM group than in the normal, control group. However, SW033291 treatment significantly increased cell viability compared to the CM þ vehicle group (44.77 ± 3.01 [CM þ vehicle] vs. 70.87 ± 1 [CM þ SW033291], $p < 0.05$), but the PGE1 and PGE2 treatment groups did not show any difference in cell viability from the CM þ vehicle group (Figs. 13(A,B)). The apoptosis rate of hRPTECs also increased more than 2.5 times in the CM group compared to the normal group, but when SW033291 was added, the apoptosis rate caused by CM significantly decreased ($25.78 \pm 2.17\%$ [CM þ vehicle] vs. $10.06 \pm 0.85\%$ [CM þ SW033291], $p < 0.001$). The PGE2 group tended to have a lower apoptotic rate than the CM þ vehicle group ($25.78 \pm 2.17\%$ [CM þ vehicle] vs. $19.43 \pm 1.38\%$ [CM þ PGE2], $p = 0.069$), but this was not statistically significant. However, comparing the SW033291 and PGE2 treatment groups, SW033291 had a significantly higher anti-apoptosis effect ($10.06 \pm 0.85\%$ [CM þ SW033291] vs. 19.43 ± 1.38 [CM þ PGE2], $p < 0.05$; Fig. 13C). There were no differences in apoptotic rate between the PGE1 treatment and CM þ vehicle groups.

15-PGDH inhibitor induces renal vasodilation via the PGE2 receptor 4–adenosine monophosphate–adenosine pathway in the outer medulla

[00304] SW033291 treatment in CIAKI mice significantly increased the average renal arteriole area in the outer medulla compared to untreated CIAKI mice ($683.63 \pm 111.11 \mu\text{m}^2$ [CM þ vehicle] vs. $1132.97 \pm 159.86 \mu\text{m}^2$ [CM þ SW033291], $p < 0.05$; Fig. 14(A,B)). CIAKI mice exhibited a decrease in RBF, but SW033291 treatment significantly prevented the reduction of RBF by CM injection (360.0 ± 24.86 [CM þ vehicle] vs. 635.2 ± 11.10 [CM þ SW033291], $p < 0.001$; Figs. 14(C,D)). PGE2 treatment also significantly preserved RBF from the CM-induced reduction of RBF (360.0 ± 24.86 [CM þ vehicle] vs. 541.4 ± 22.65 [CM þ PGE2], $p = 0.001$), but PGE1 did not. Levels of AMP and adenosine in renal tissue were all significantly decreased in CIAKI mice compared to the sham, but these changes were substantially reversed by SW033291-treated mice (AMP, 10.64 ± 1.27 ng/mL [CM þ vehicle] vs. 15.60 ± 1.26 ng/mL [CM þ SW033291], $p < 0.05$; adenosine, 1.65 ± 0.14 ng/mL

[CM þ vehicle] vs. 2.65 ± 0.15 ng/mL [CM þ SW033291], $p < 0.01$; Figs. 14(E,F)).

Administering PGE2 also increased AMP and adenosine levels compared to CIAKI mice (Figs. 14(E,F)).

[00305] Next, we analyzed PGE2 receptor (EP) expression. EP1 increased in the CM þ vehicle group, but not in the SW033291, PGE1, or PGE2 groups (Fig. 15(A)). There were no differences in the expression level of EP2 between the groups (Fig. 15(B)). However, the SW033291 group showed significantly lower EP3 expression than the CM group (1.10 ± 0.03 -fold [CM þ vehicle] vs. 1.00 ± 0.02 -fold [CM þ SW033291], $p < 0.05$; Fig. 15(C)). The EP4 expression level was significantly increased only in the SW033291 group (1.09 ± 0.02 -fold [CM þ vehicle] vs. 1.25 ± 0.02 -fold [CM þ SW033291], $p < 0.01$; Fig. 15(D)). An EP4 antagonist, ONO-AE3-208, attenuated the renoprotective effects of SW033291, as indicated by increases in creatinine, NGAL, and KIM-1 (creatinine, 0.50 ± 0.10 mg/dL [CM þ SW033291] vs. 1.51 ± 0.31 mg/dL [CM þ ONO-AE3-208 þ SW033291], $p < 0.05$; NGAL, 140.41 ± 25.52 ng/mL [CM þ SW033291] vs. 229.85 ± 27.12 ng/mL [CM þ ONO-AE3-208 þ SW033291], $p < 0.05$; KIM-1, 0.09 ± 0.05 ng/mL [CM þ SW033291] vs. 0.85 ± 0.26 ng/mL [CM þ ONO-AE3-208 þ SW033291], $p < 0.05$; Fig. 15(E–G)). Moreover, we assessed RBF, and ONO-AE3-208 blocked the increase in RBF by SW033291 treatment in CIAKI mice (Fig. 15(H)).

[00306] To establish a CIAKI animal model, we considered the conventional rat models using water depletion and furosemide to induce a severe dehydration, and then administering CM to achieve CIAKI; however, this method is rather complicated and time-consuming, and most importantly, far from clinical settings. We specifically chose mouse over rat to establish a CIAKI animal model, and using a number of different CM with varying osmolality and viscosity, we aimed to find the appropriate CM that induces CIAKI. Visipaque (iodixanol), a CM with high viscosity and iso-osmolality, showed a significant functional and histopathological renal damage. This example shows that CM with a high viscosity can induce CIAKI in mouse on its own, without dehydration or using other drugs. CM viscosity is a key element in the pathophysiology of CIAKI, and when CM is administered in a dehydrated state, fluid viscosity increases exponentially and flow through medullary tubules and vessels decreases, which increases the duration of contact of CM to tubular cells, thereby increasing renal tubular toxicity.

[00307] We analyzed whether functional and histological protection against CIAKI is possible by administering a 15-PGDH inhibitor, PGE1, or PGE2. Biomarker analyses were used to detect the functional changes of the kidneys; the 15-PGDH inhibitor, PGE1, and PGE2 all showed a similar effect by reducing serum creatinine levels in the CIAKI model (Fig. 11C). On the other hand, NGAL and KIM-1 showed varying results; with 15-PGDH inhibitor and PGE2, the concentration of the two markers decreased, whereas PGE1 did not decrease them (Figs. 11(D, E)). Creatinine is a biomarker that reflects the kidney's ability to excrete waste, while NGAL and KIM-1 suggest that the kidney has undergone a tubular injury. Therefore, we can assume that PGE1, PGE2, and the 15-PGDH inhibitor all have a protective effect in the excretory function against CM, but only PGE2 and the 15-PGDH inhibitor have a protective effect against tubular toxicity.

[00308] To assess the degree of histological renal injury, we used the renal injury score to evaluate necrosis, and TUNEL staining for apoptosis. The 15-PGDH inhibitor effectively inhibited both necrosis and apoptosis of renal tissues by CM, whereas PGE2 inhibited apoptosis but failed to successfully reduce necrosis. PGE1 decreased apoptosis but increased necrosis (Fig. 12). Interestingly, the 15-PGDH inhibitor showed a greater effect in reducing renal necrosis than PGE2. The estimated half-life of PGE2 is less than 15 s because PGE2 is rapidly degraded by 15-PGDH. Therefore, increasing the level and extending the half-life of intrinsic PGE2 induced by blocking 15-PGDH (Figs. 11(A, B)) would be more effective for reducing the renal injury score than directly administering PGE2, which has a short half-life.

[00309] We performed cell culture experiments using hRPTECs to determine whether CM has a direct cellular toxicity to renal tubular cells and whether the 15-PGDH inhibitor, PGE1, and PGE2 have protective effects. CM showed a decrease in cellular viability and increase in apoptosis on the hRPTECs, as reported by other previous studies. However, such harmful effects of CM were significantly reversed by the administration of the 15-PGDH inhibitor (Fig. 13); PGE1 had no protective effect against cellular toxicity, and PGE2 showed only a minimal effect, much less than that of the 15-PGDH inhibitor. Therefore, the protective effect on the renal tubular cells against CM could be better achieved by inhibiting the 15-PGDH to block the catabolism of the endogenous PGE2, rather than administering exogenous PGE2.

[00310] CM is also known as a toxic agent that decreases renal blood flow and increases vasoconstriction in many studies. Our renal hemodynamic study also showed that CM has

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obviously decreased renal blood flow (Fig. 14). However, this deteriorating effect of CM on renal hemodynamics was definitely reversed by the administration of PGE2 or 15-PGDH inhibitor, but not by PGE1. Moreover, 15-PGDH inhibitor has increased renal vasodilation more than that of CM group, confirmed by measuring the size of arterioles. Furthermore, the analysis of vasoactive substances to increase the renal vasodilation showed that both adenosine, involved in renal blood vessel expansion, and its precursor, AMP, were increased in the 15-PGDH inhibitor or PGE2 administration group than CM group. Thus, we can suggest that the protective effects of PGE2 and 15-PGDH inhibitor from renal vasoconstriction of CM are induced by increasing adenosine and AMP, which cause renal vasodilation.

[00311] To investigate the mechanism behind the renal protective effect of 15-PGDH inhibitor, we examined the change in expression of PGE2 receptors. According to many studies regarding the contribution of prostaglandin EP receptors to renal microvascular reactivity, EP1 and EP3 mediate renal vasoconstrictor responses, whereas EP2 and EP4 mediate vasodilation. Our results are consistent with these observations that vasodilatory effect of 15-PGDH inhibitor is mediated by the decreased EP 3 expression and increased EP4 expression. Moreover, ONO-AE3-208, an EP4 antagonist, clearly offsets the renal protective effect of the 15-PGDH inhibitor. We can assume that the 15-PGDH inhibitor maximizes the protective action against renal damage by CM through the EP4.

[00312] In summary, administering the 15-PGDH inhibitor, SW033291, before and after CM in the CIAKI mouse model had a functional and histological protection against CIAKI. This was achieved by providing protection against both hemodynamic and tubular toxic effects, the two main mechanisms of CIAKI. The intrarenal vasodilation and increased renal blood flow induced by SW033291 were via the EP4s, which were strongly associated with the increase in AMP and adenosine levels. The protective effect of SW033291 on the tubular cell toxicity of CM was achieved by inhibiting tubular cell apoptosis. Therefore, we suggest that 15-PGDH inhibitor may be a novel prophylactic agent for CIAKI, and further studies are needed for clinical implications.

[00313] From the above description of the invention, those skilled in the art will perceive improvements, changes and modifications. Such improvements, changes and modifications within the skill of the art are intended to be covered by the appended claims. All references,

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publications, and patents cited in the present application are herein incorporated by reference in their entirety.

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Having described the invention, we claim:

1. A method for preventing or treating a renal disorder, disease, and/or injury, the method comprising:

administering to a subject and/or kidney of the subject having or at risk of the renal disorder, disease, and/or injury a therapeutically effective amount of a 15-PGDH inhibitor.

2. The method of claim 1, wherein the method prevents or treats at least one of: acute kidney injury; hypotensive injury to the kidney; hypertensive renal disease; diabetic renal disease; diabetic nephropathy; renal disease from vasculitis and autoimmune diseases; ischemic renal injury; acute renal failure; chronic renal failure; glomerulonephritis; nephrotic syndrome; acute tubular necrosis; nephrosclerosis; glomerulosclerosis; minimal change disease; idiopathic membranous nephropathy; membranoproliferative glomerulonephritis; Berger's disease; mesangial proliferative glomerulonephritis; chronic glomerulonephritis; focal glomerulosclerosis; renal effects of Sjogren's syndrome; renal effects of scleroderma; interstitial nephritis; and renal injury following kidney transplant to a kidney donor, transplant recipient, and/or transplanted kidney.

3. The method of claim 1, wherein the amount of 15-PGDH inhibitor administered to the subject is an amount effective to induce endogenous renal PGE2 levels of the subject.

4. The method of claim 1, wherein the amount of 15-PGDH inhibitor administered to the subject is an amount effective to induce renal vasodilatation, enhance resistance to hypoxia, improve renal hemodynamics, decrease renal oxidative stress, reduce renal inflammation, and/or preserve renal function.

5. The method of claim 1, the amount of 15-PGDH inhibitor administered to the subject is an amount effective to reduce malondialdehyde (MDA) and NGAL levels, attenuate medulla tubular damage, reduce medulla acute tubular necrosis (ATN) and apoptosis, reduces induction of high-mobility group box 1 (HMGB1) and proinflammatory

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cytokines, induce renal EP4 PGE2 receptors and A2A adenosine receptors in vascular smooth muscle cells that regulate renal arterioles, increase renal cAMP, AMP, and adenosine levels, and/or inhibit induction of creatinine and KIM-1.

6. The method of claim 1, wherein the 15-PGDH inhibitor is administered before the renal injury.

7. The method of claim 6, wherein the 15-PGDH inhibitor is administered at a range of about 1 minute to about 72 hours before the renal injury.

8. The method of claim 6, wherein the 15-PGDH inhibitor is administered at a range of about 10 minutes to about 48 hours before the renal injury.

9. The method of claim 6, wherein the 15-PGDH inhibitor is administered at a range of about 30 minutes to about 36 hours before the renal injury.

10. The method of claim 6, wherein the 15-PGDH inhibitor is administered at time less than 2 hours, 8 hours, 24 hours, or 26 hours before the renal injury.

11. The method of claim 1, wherein the renal injury is associated with a transplant in said subject.

12. The method of claim 11, wherein said transplant is a kidney transplant.

13. The method of claim 1, wherein renal injury is associated with cardiovascular surgery or sepsis.

14. The method of claim 1, wherein the renal injury is contrast induced acute kidney injury.

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and R^2 may be linked to form a cyclic or polycyclic ring, wherein R^3 and R^4 are the same or different and are each selected from the group consisting of H, a lower alkyl group, O, $(CH_2)_{n1}OR'$ (wherein $n1=1, 2, \text{ or } 3$), CF_3 , CH_2-CH_2X , $CH_2-CH_2-CH_2X$, (wherein $X=H, F, Cl, Br, \text{ or } I$), CN , $(C=O)-R'$, $(C=O)N(R')_2$, $COOR'$ (wherein R' is H or a lower alkyl group), and R^3 or R^4 may be absent.

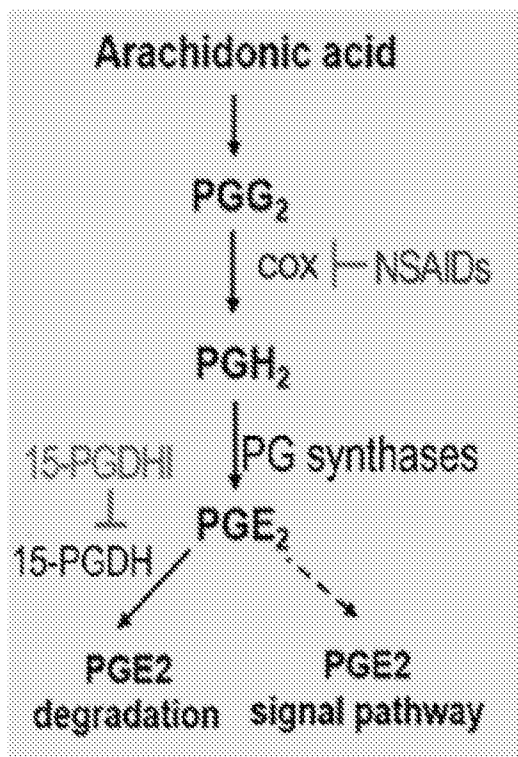
A

Fig. 1A

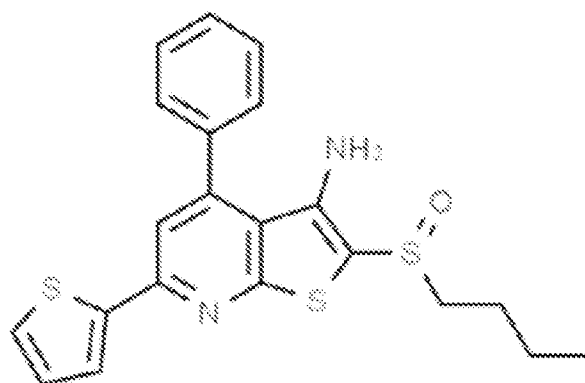
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Fig. 1B

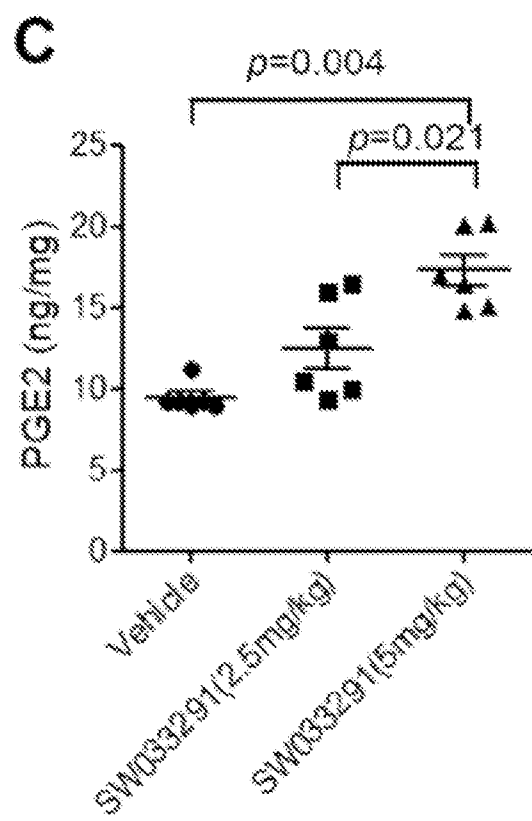


Fig. 1C

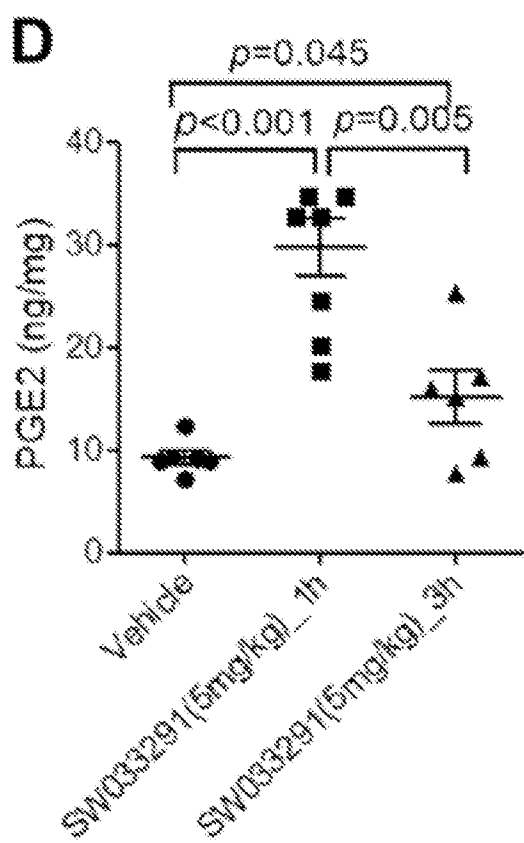


Fig. 1D

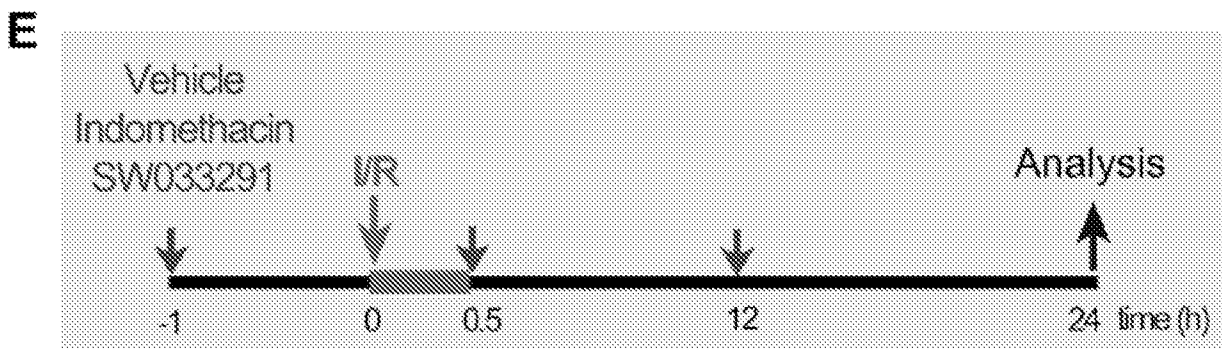


Fig. 1E

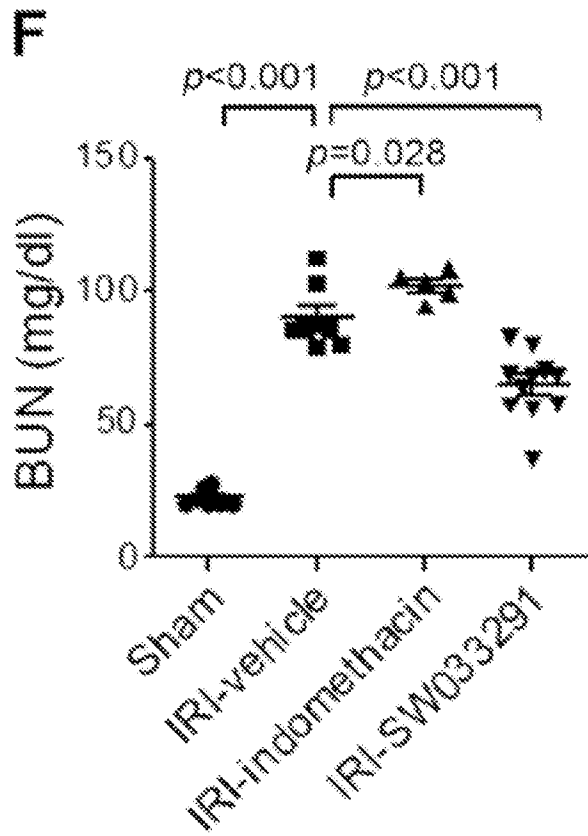


Fig. 1F

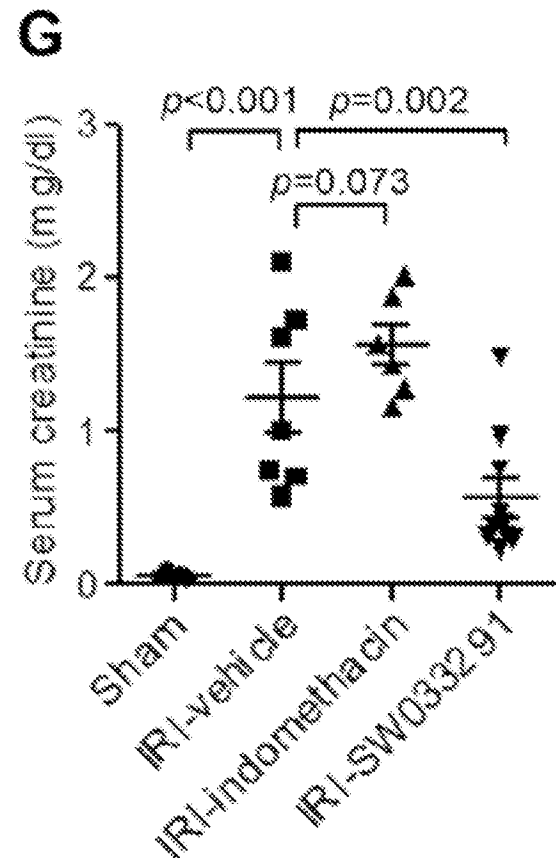


Fig. 1G

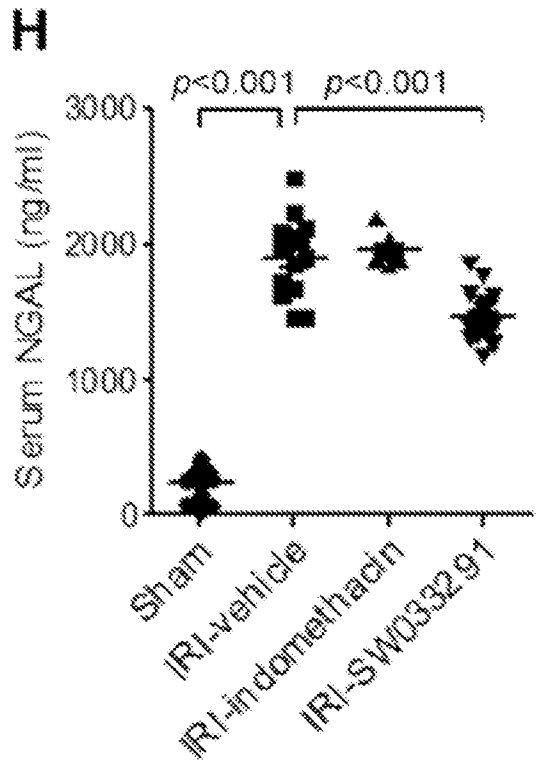


Fig. 1H

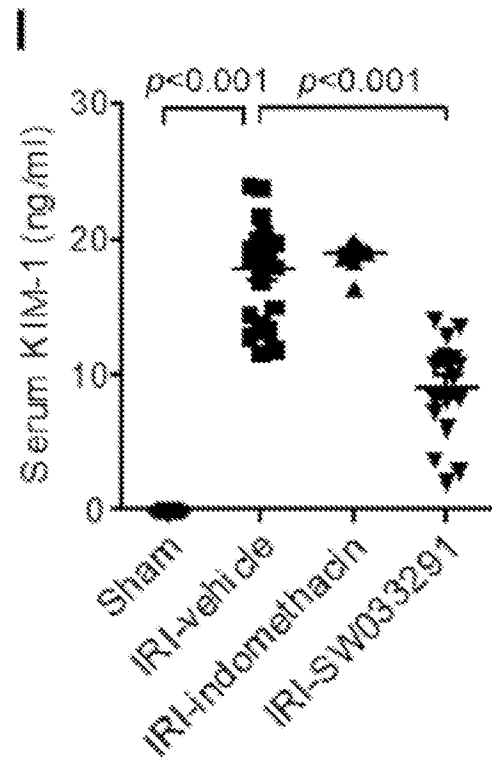


Fig. 1I

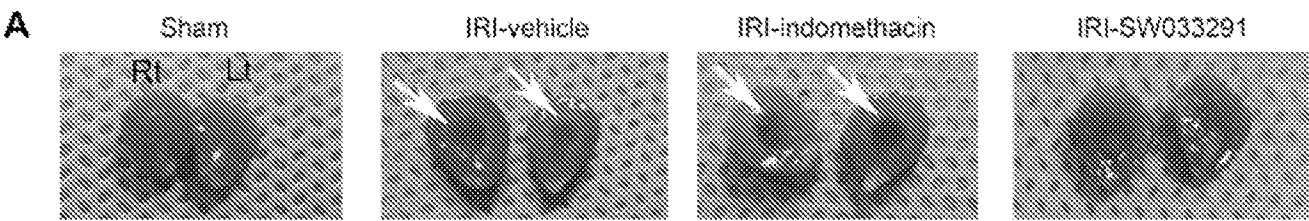


Fig. 2A

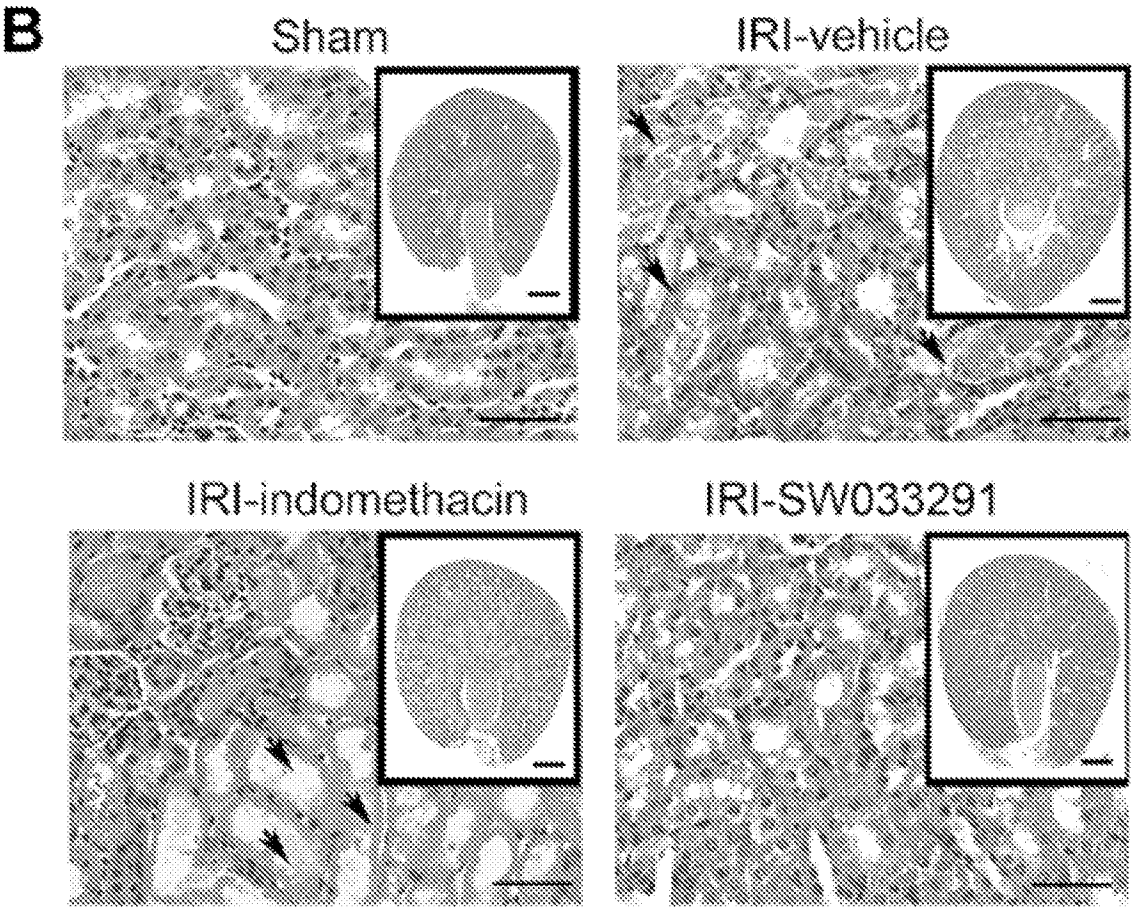


Fig. 2B

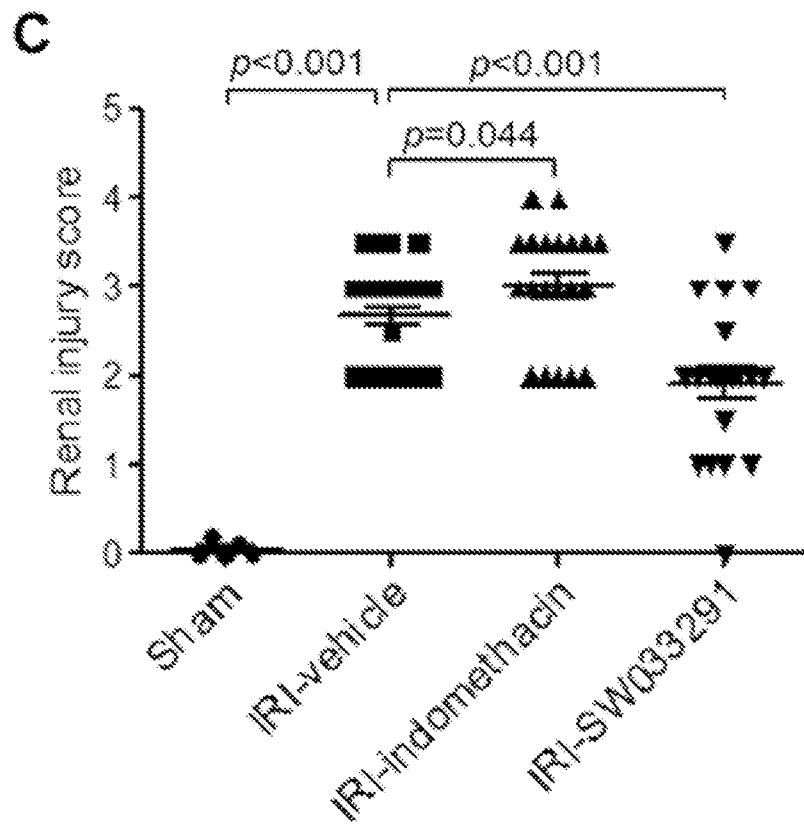


Fig. 2C

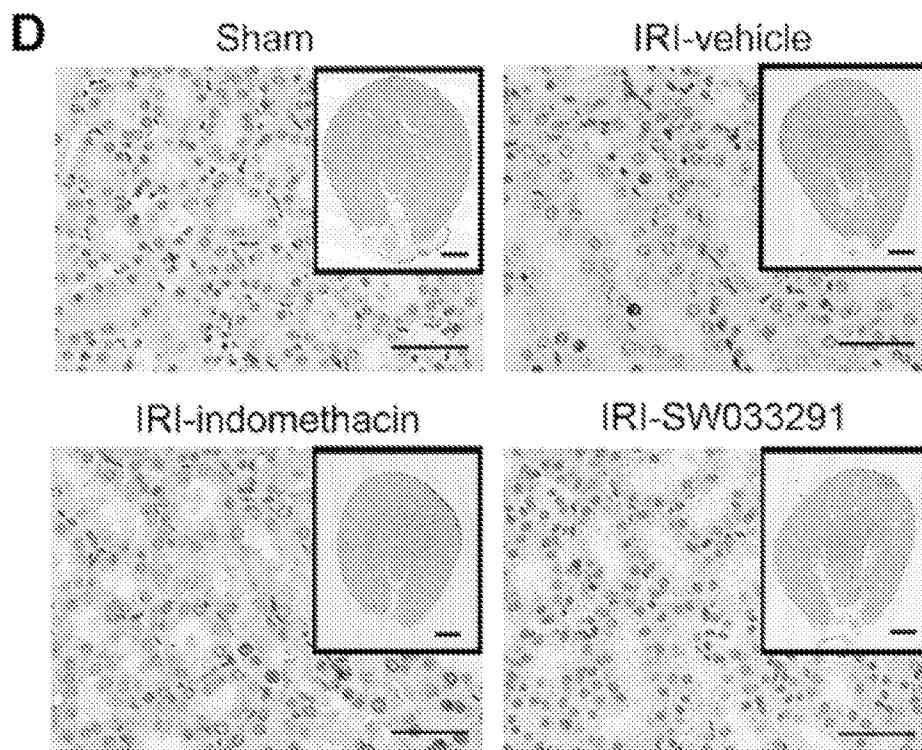
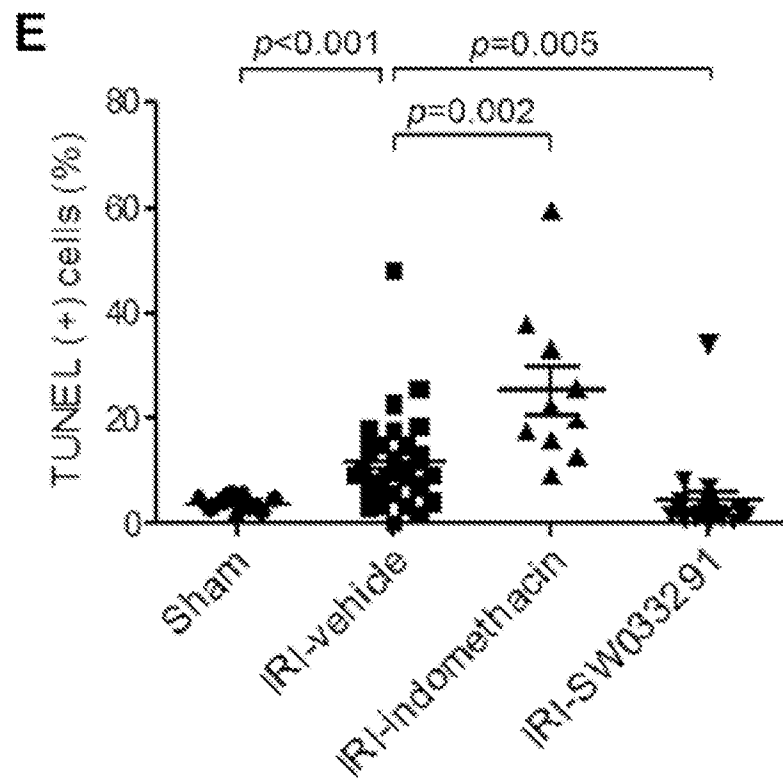
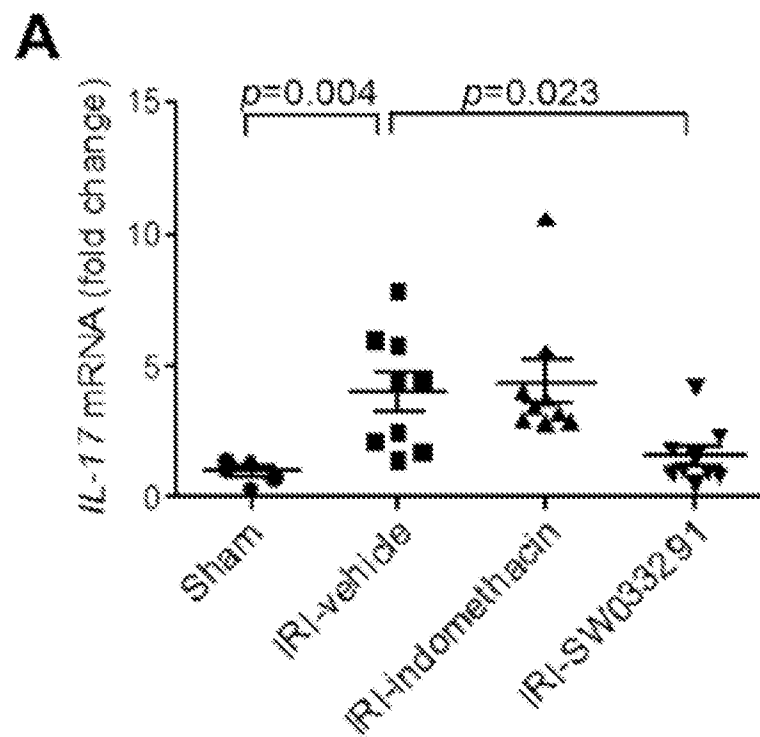


Fig. 2D



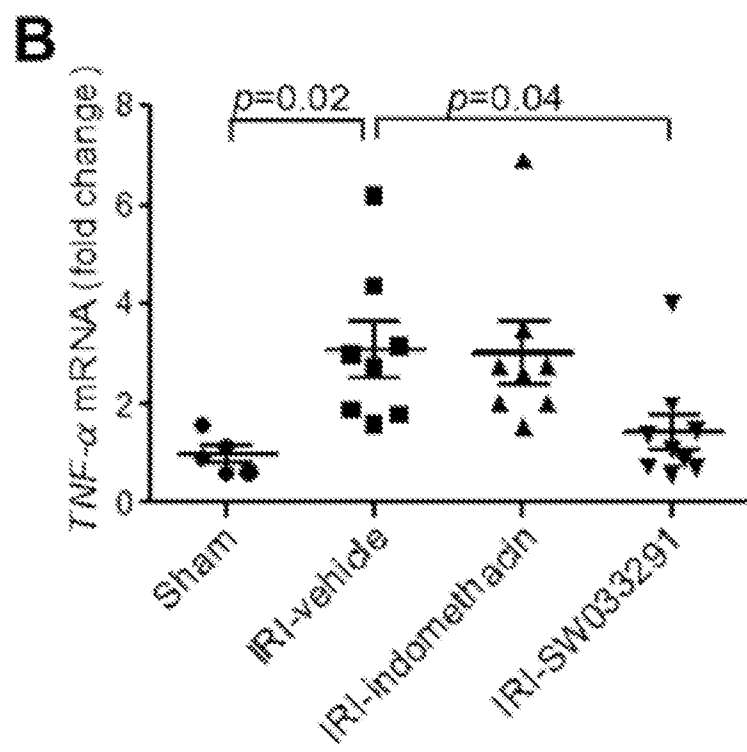


Fig. 3B

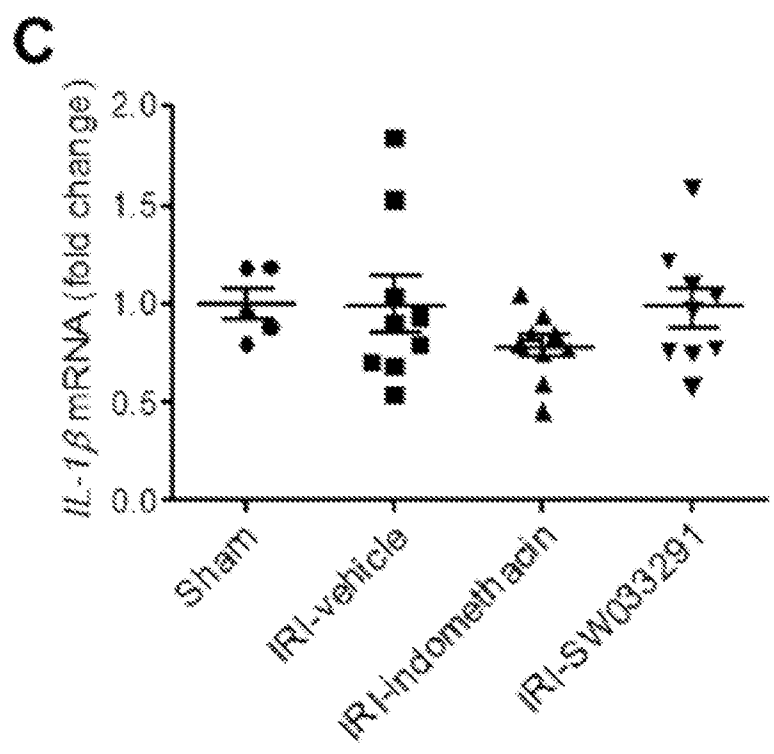


Fig. 3C

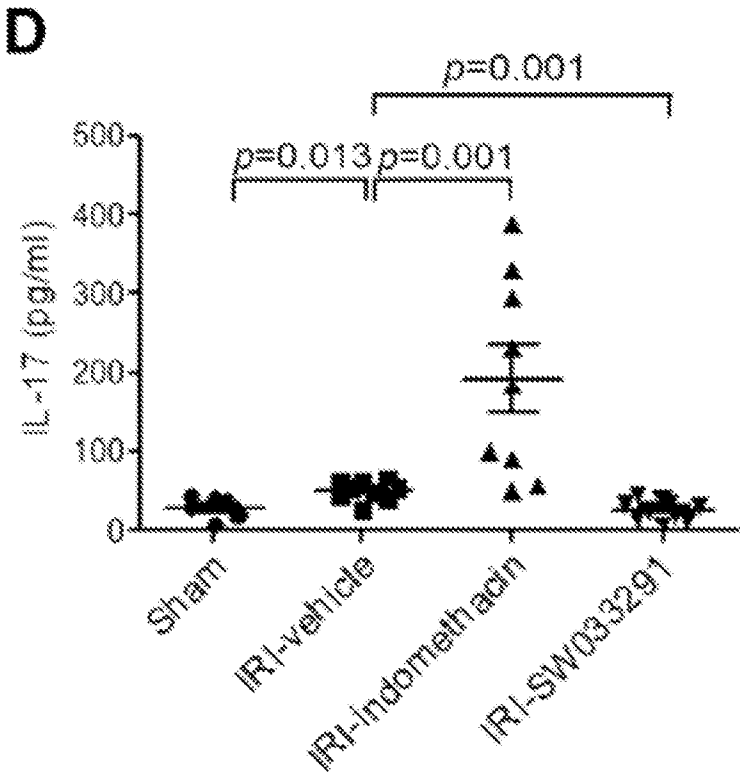


Fig. 3D

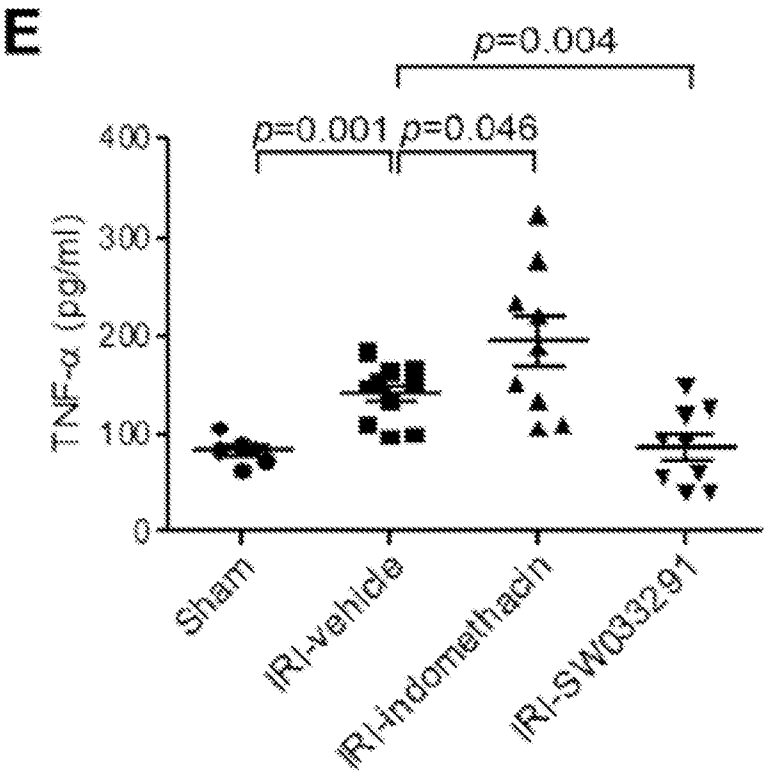


Fig. 3E

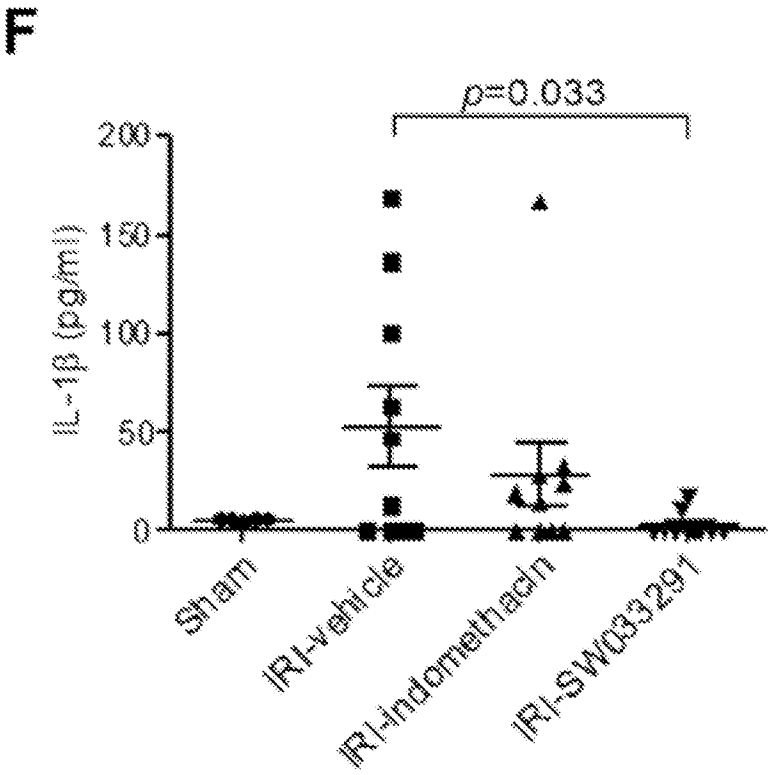


Fig. 3F

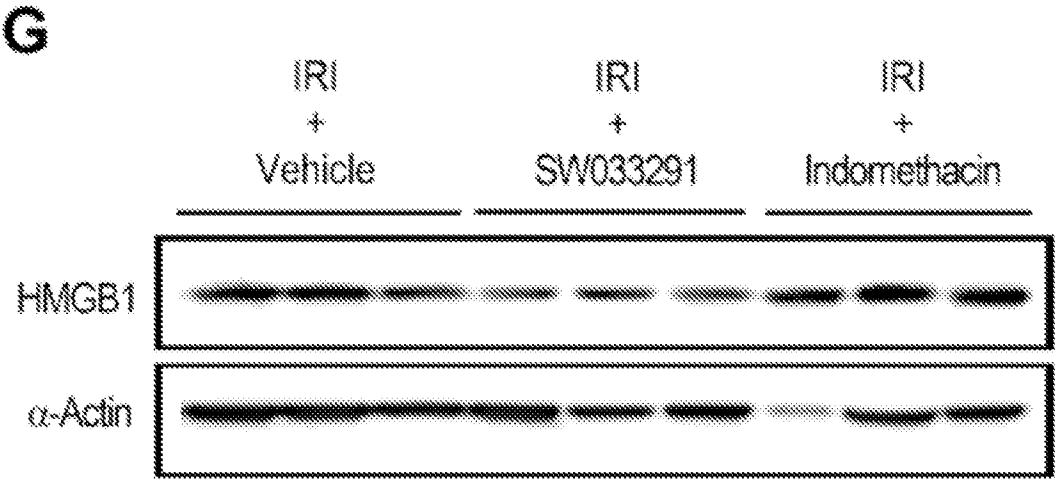


Fig. 3G

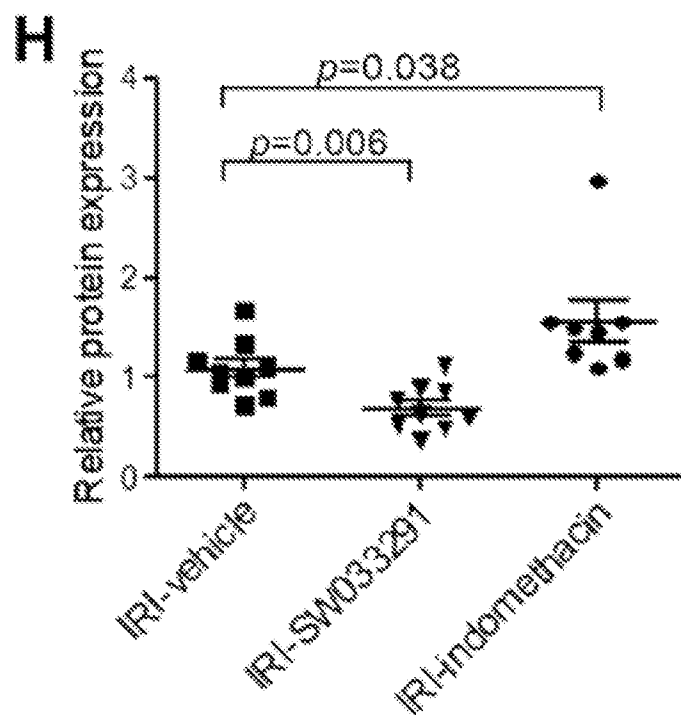


Fig. 3H

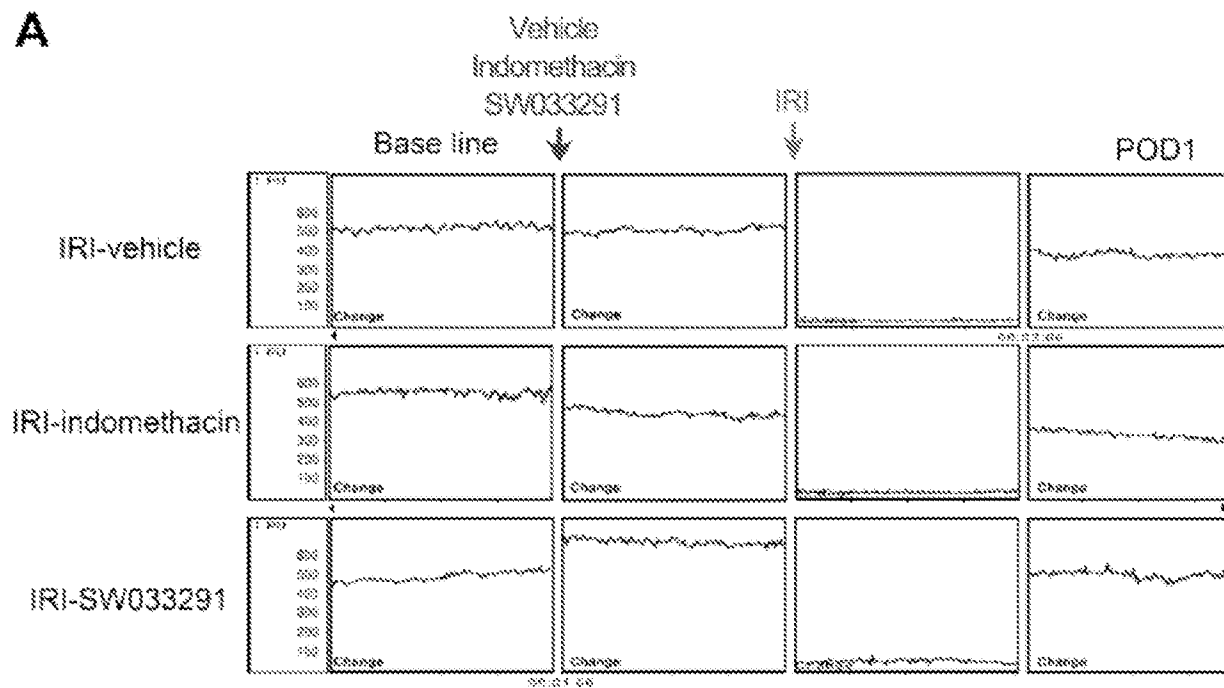
A

Fig. 4A

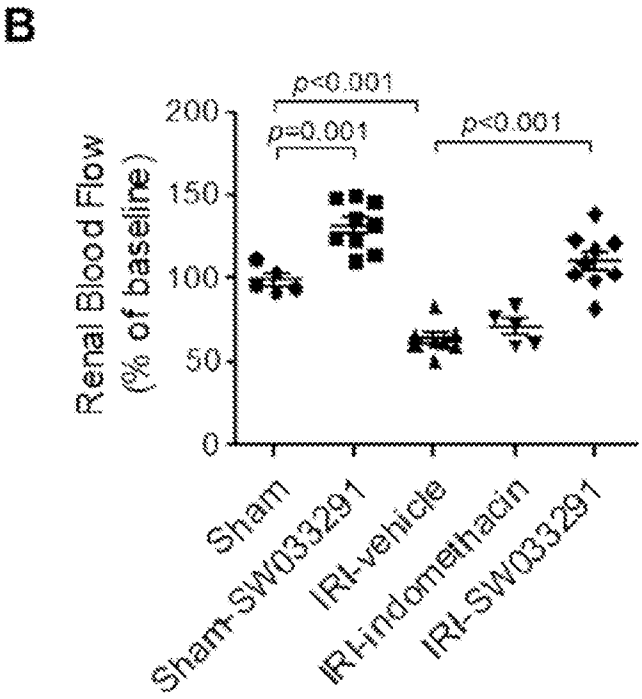


Fig. 4B

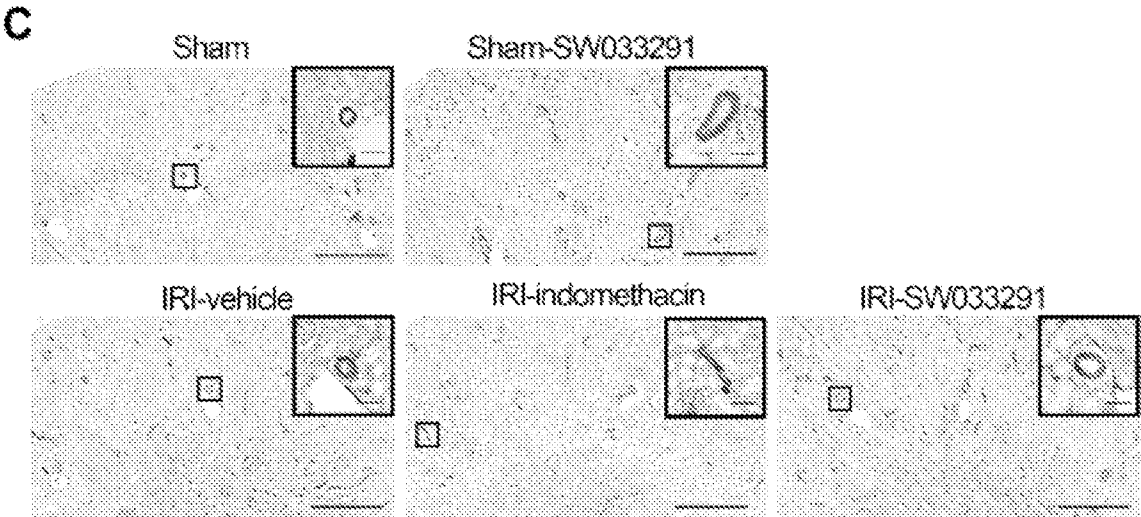


Fig. 4C

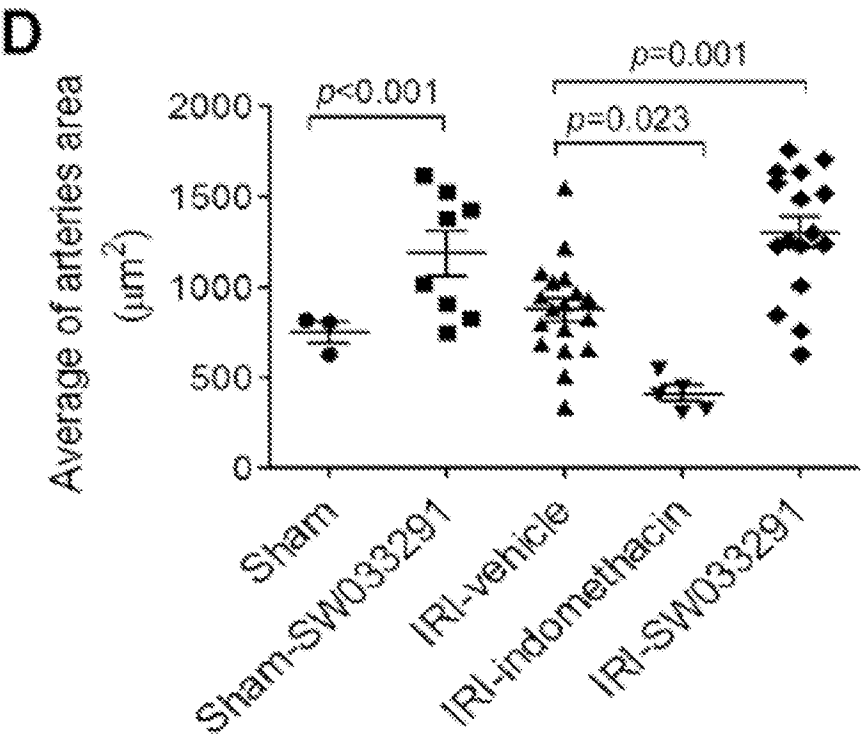


Fig. 4D

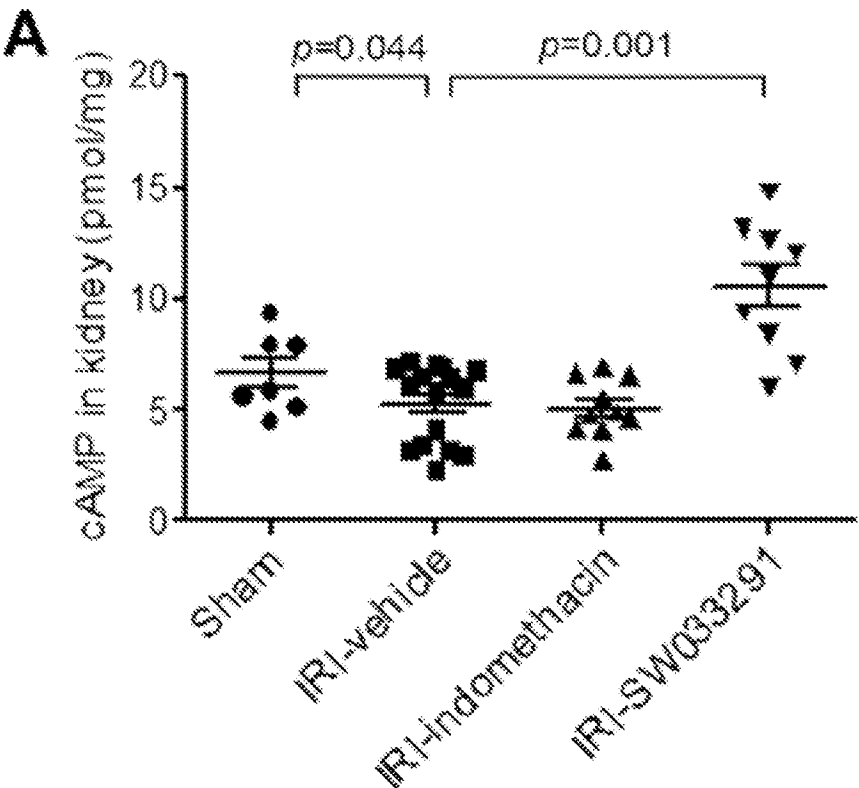


Fig. 5A

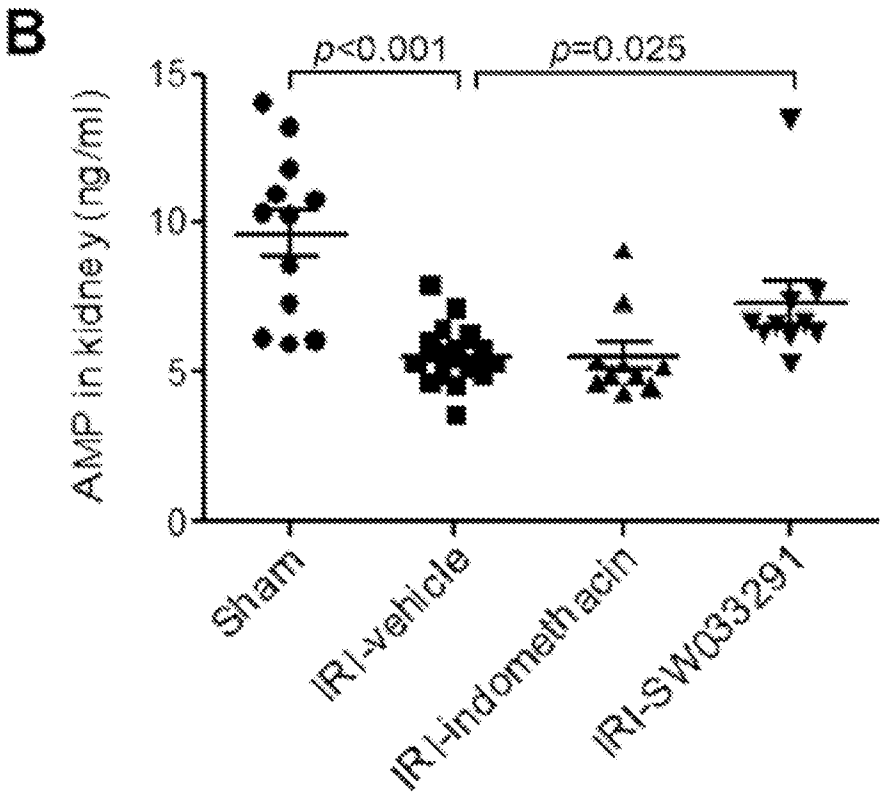


Fig. 5B

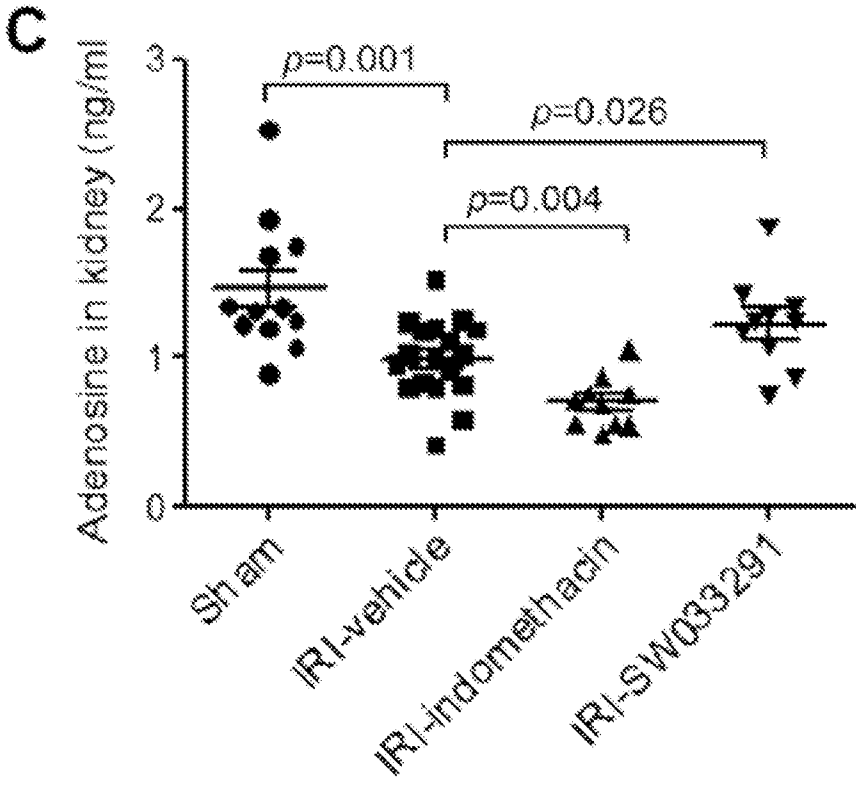


Fig. 5C

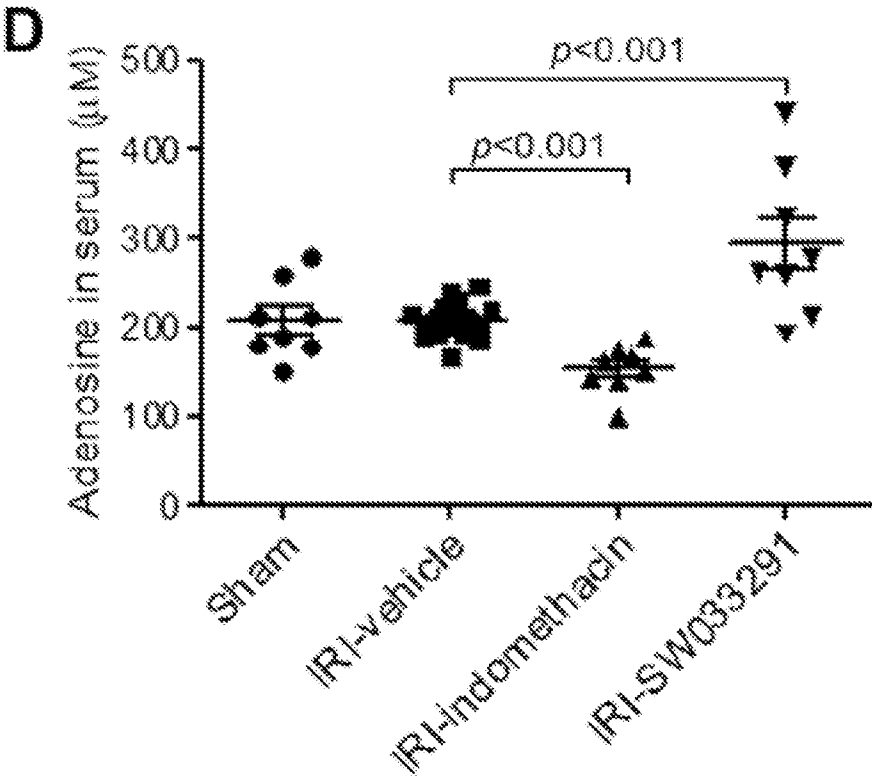


Fig. 5D

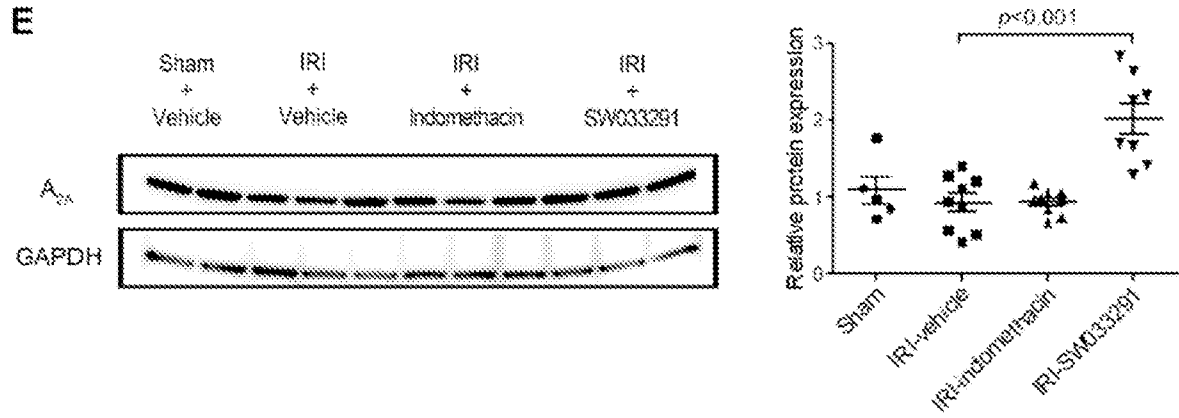


Fig. 5E

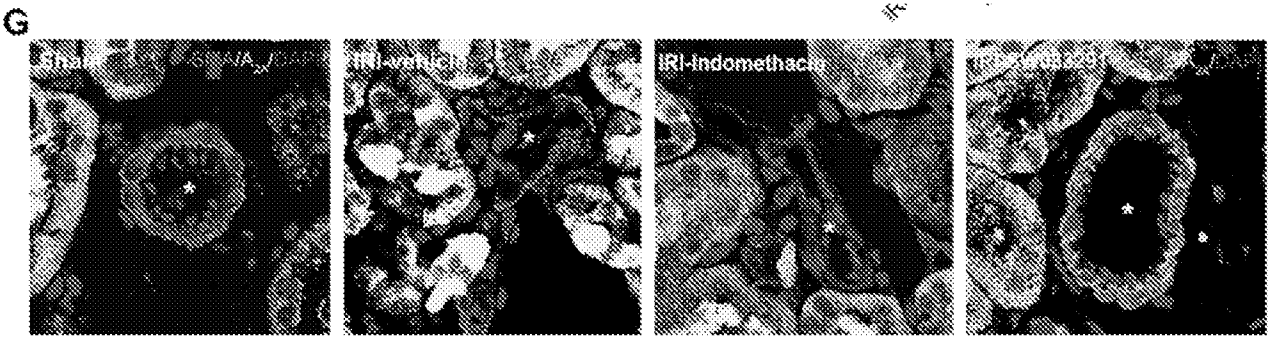


Fig. 5G

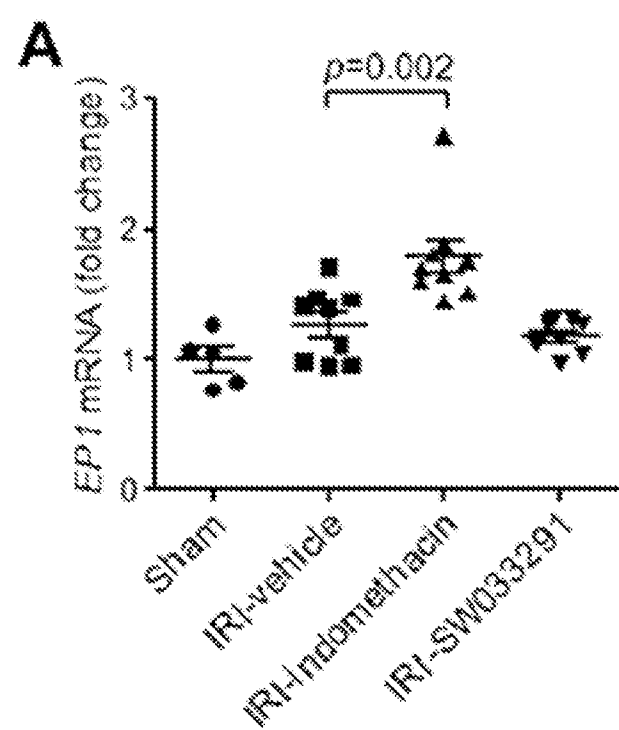


Fig. 6A

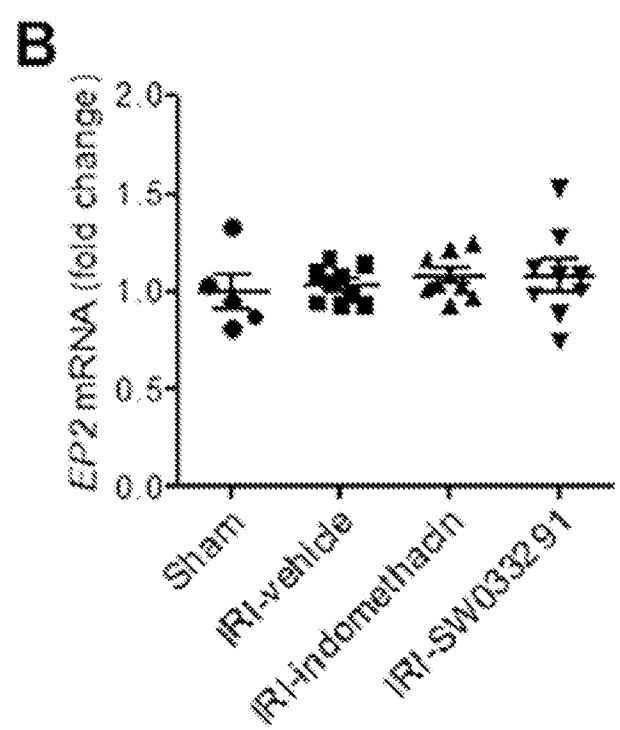


Fig. 6B

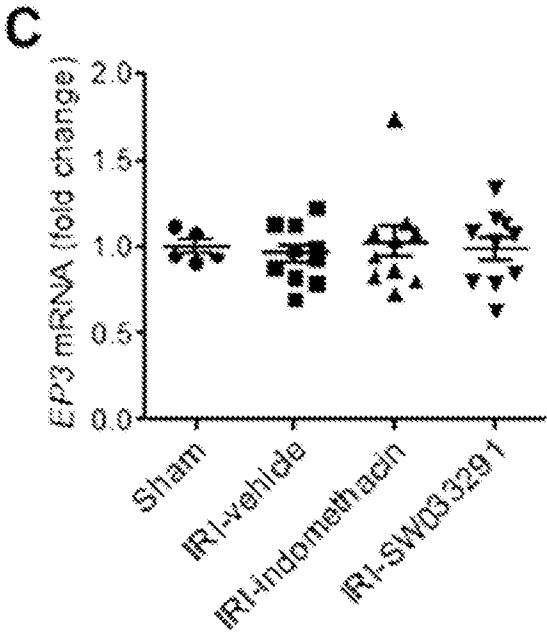


Fig. 6C

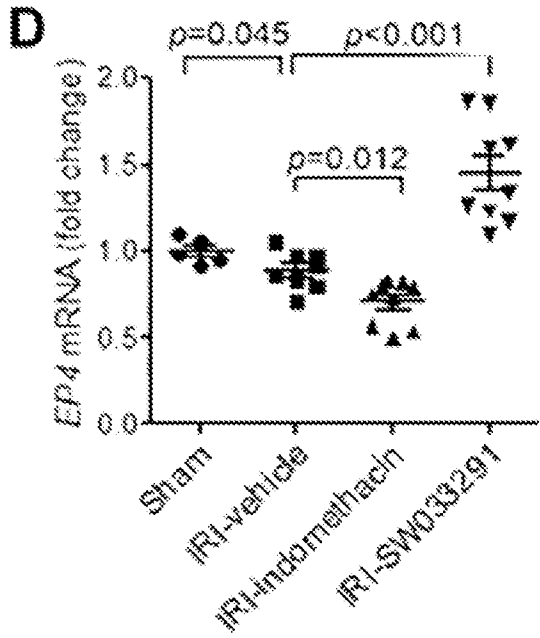


Fig. 6D

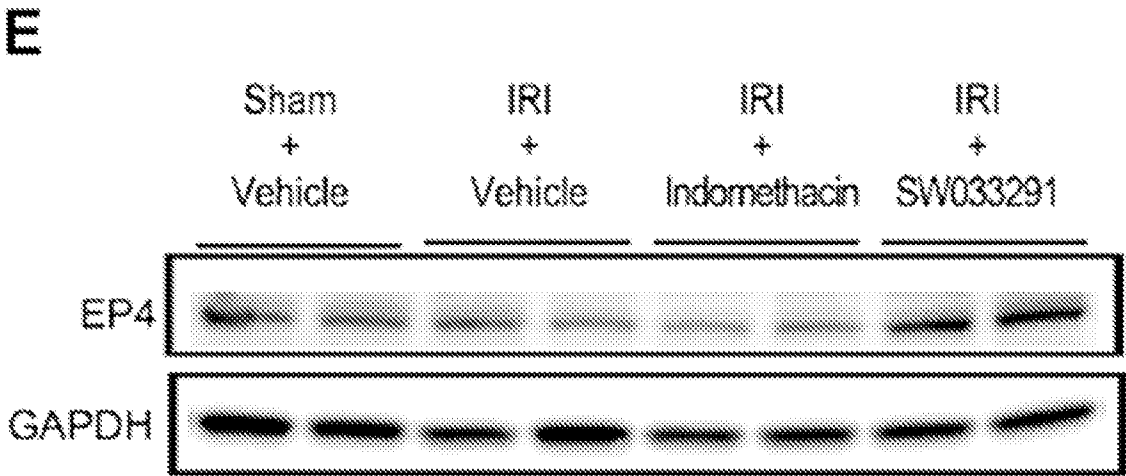


Fig. 6E

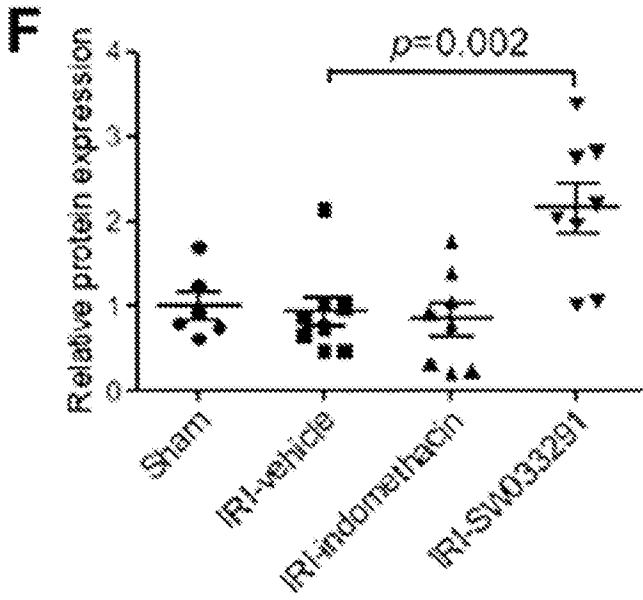


Fig. 6F

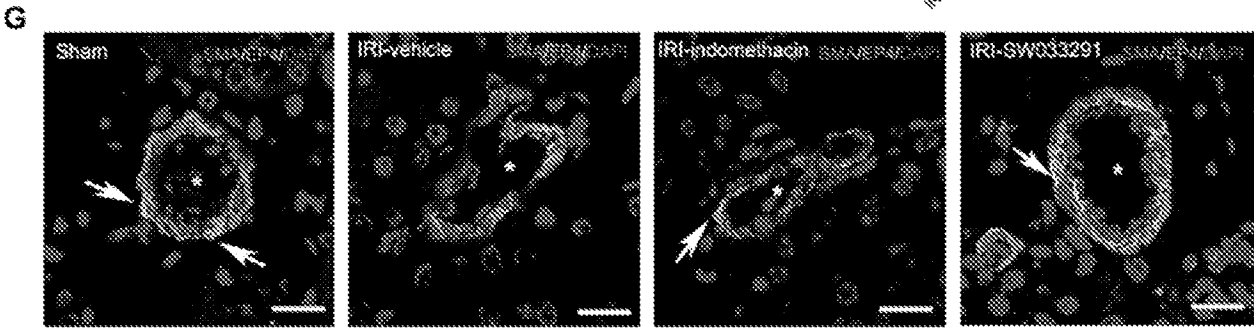


Fig. 6G

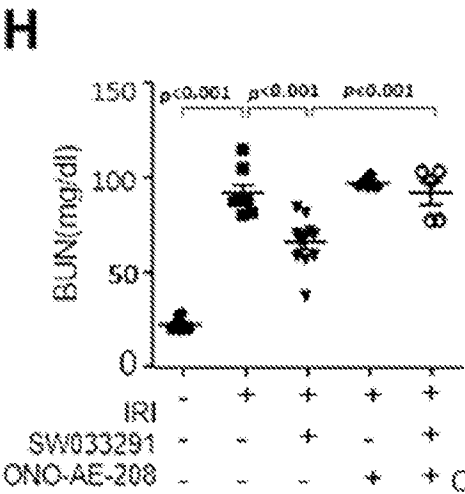


Fig. 6H

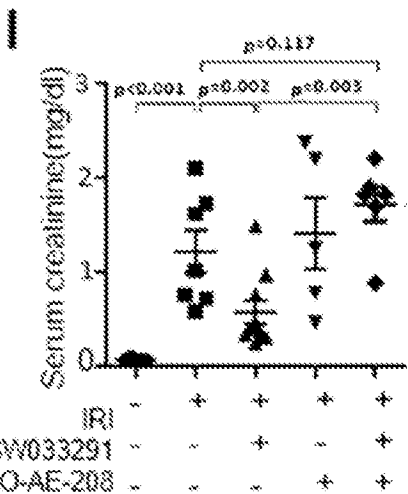


Fig. 6I

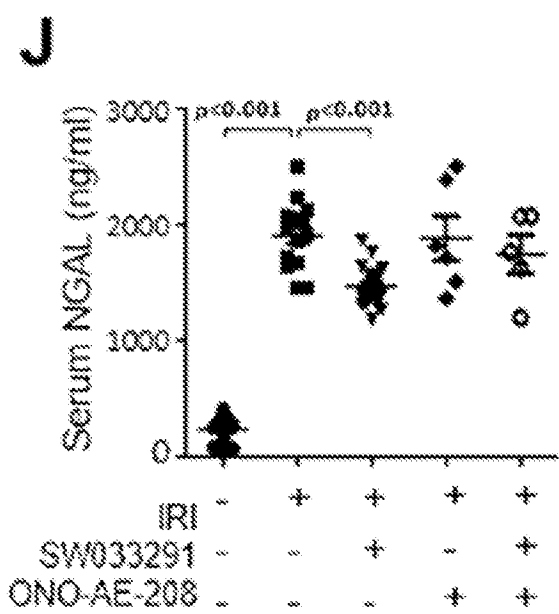


Fig. 6J

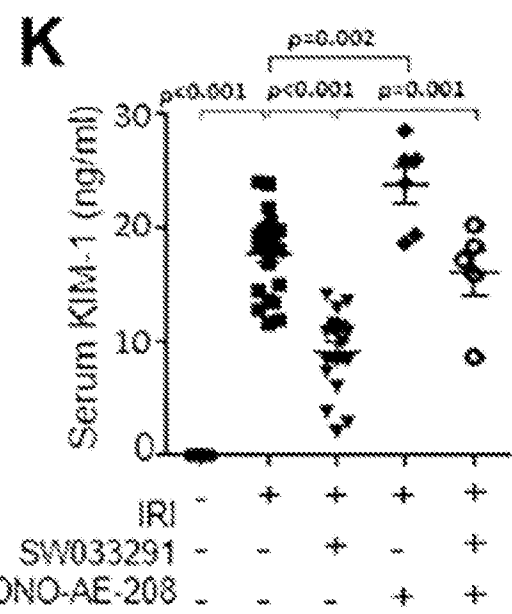


Fig. 6K

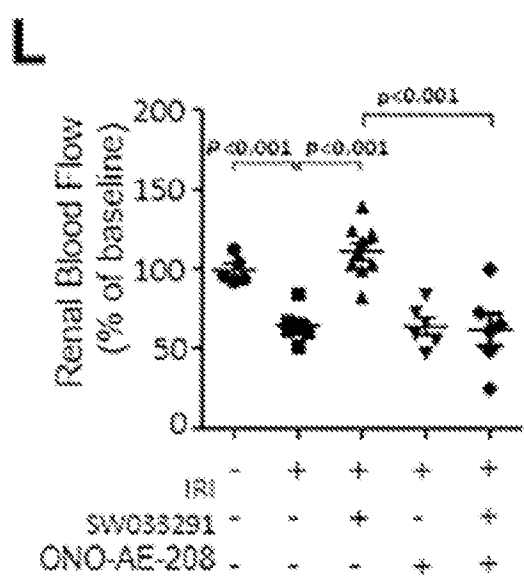


Fig. 6L

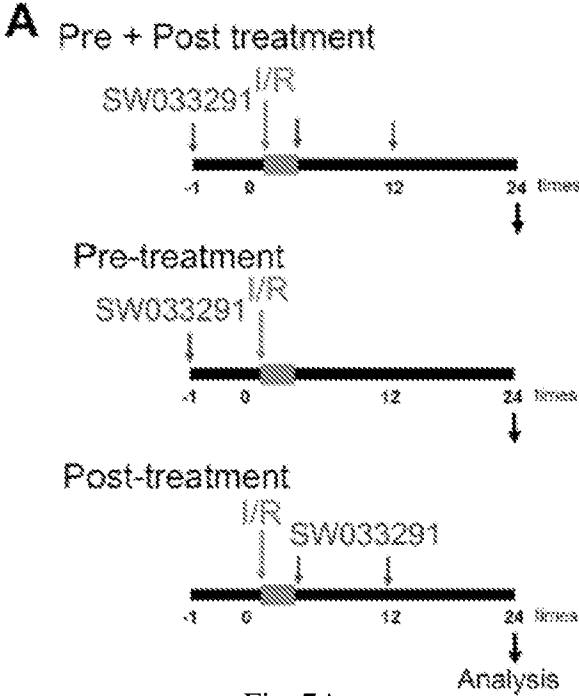


Fig. 7A

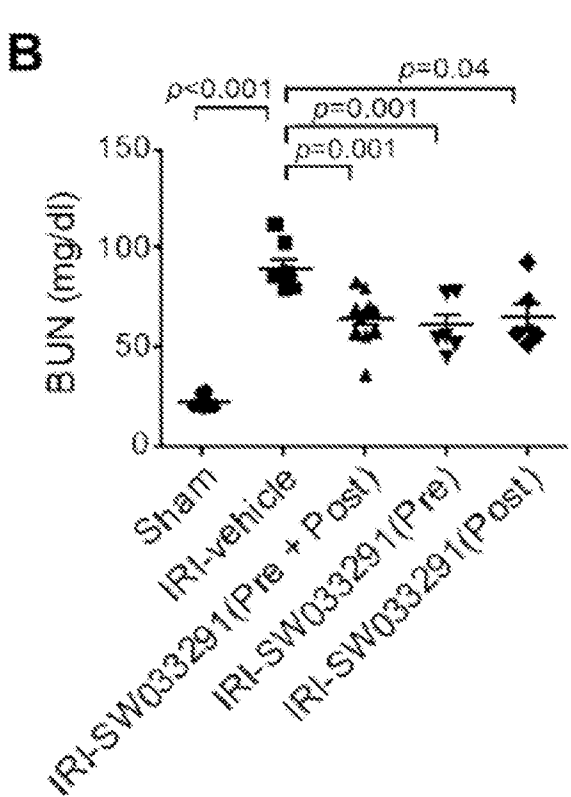


Fig. 7B

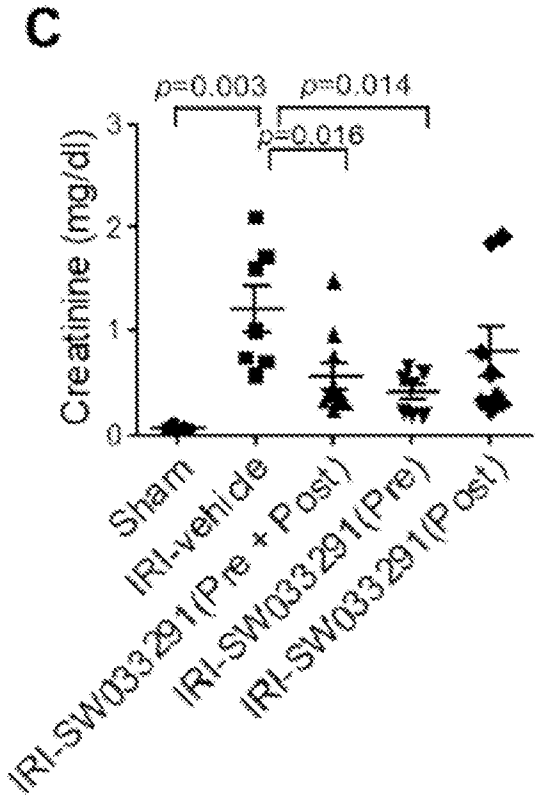


Fig. 7C

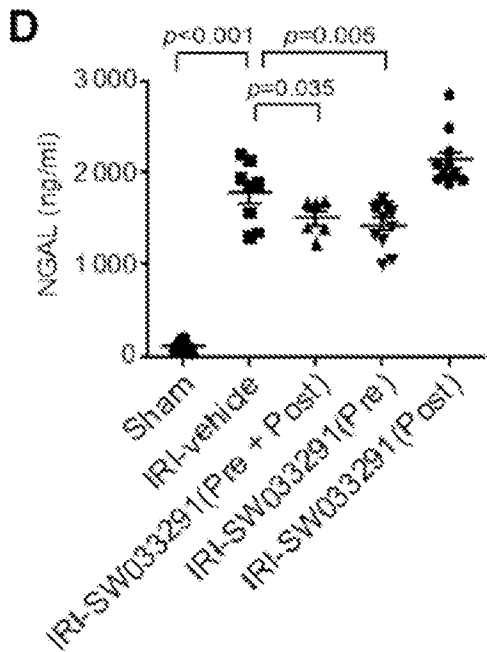


Fig. 7D

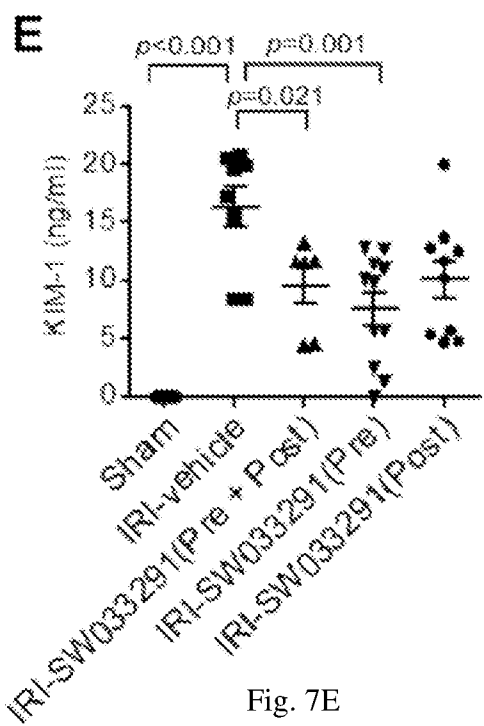


Fig. 7E

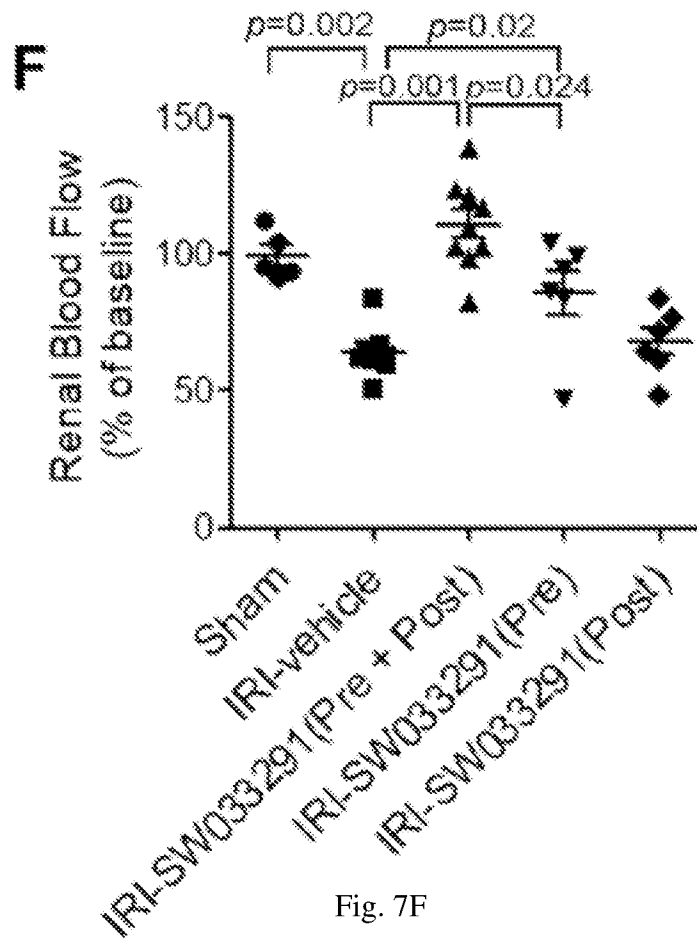


Fig. 7F

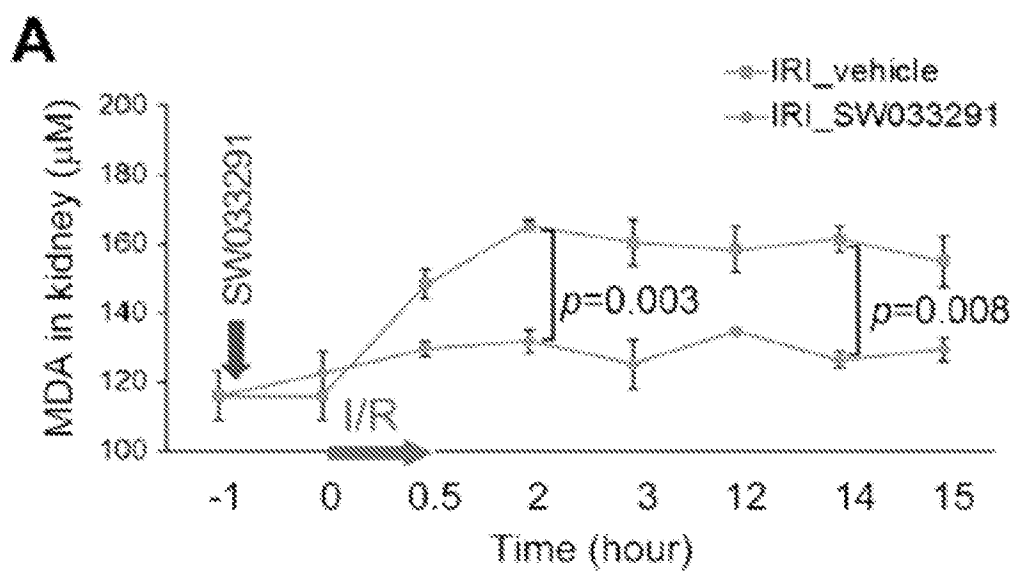


Fig. 8A

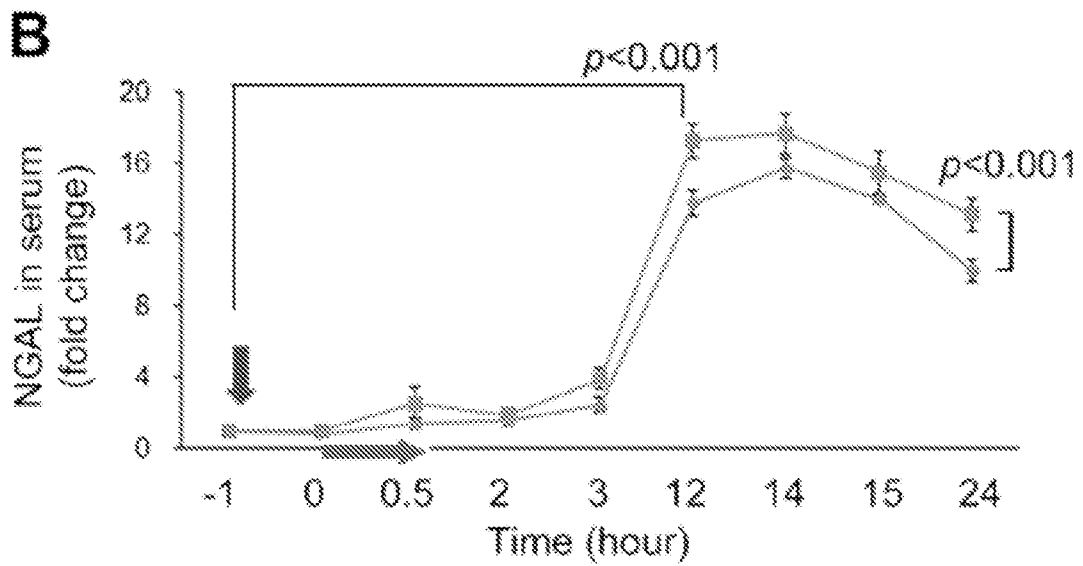


Fig. 8B

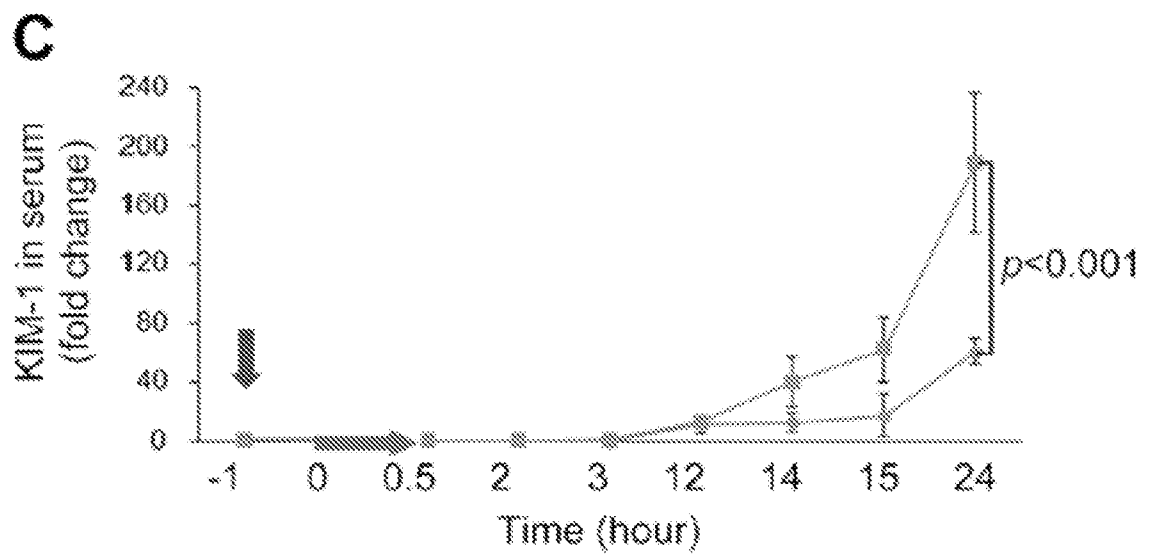


Fig. 8C

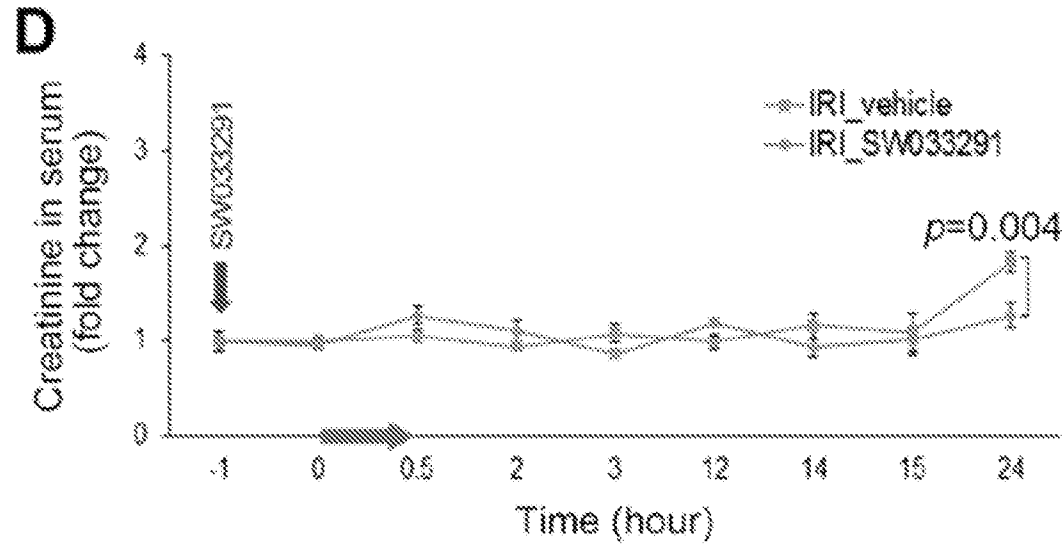


Fig. 8D

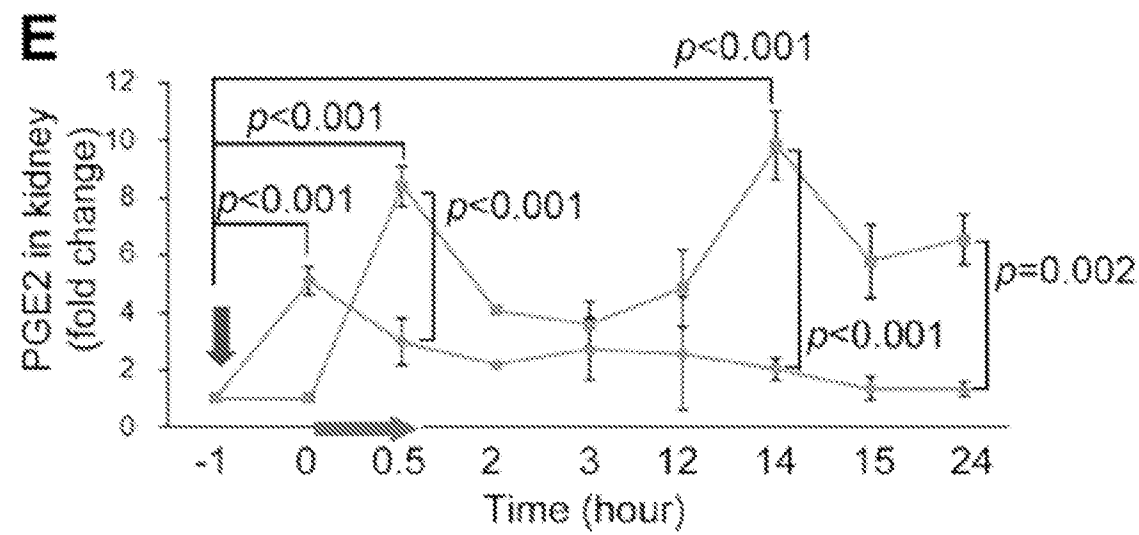


Fig. 8E

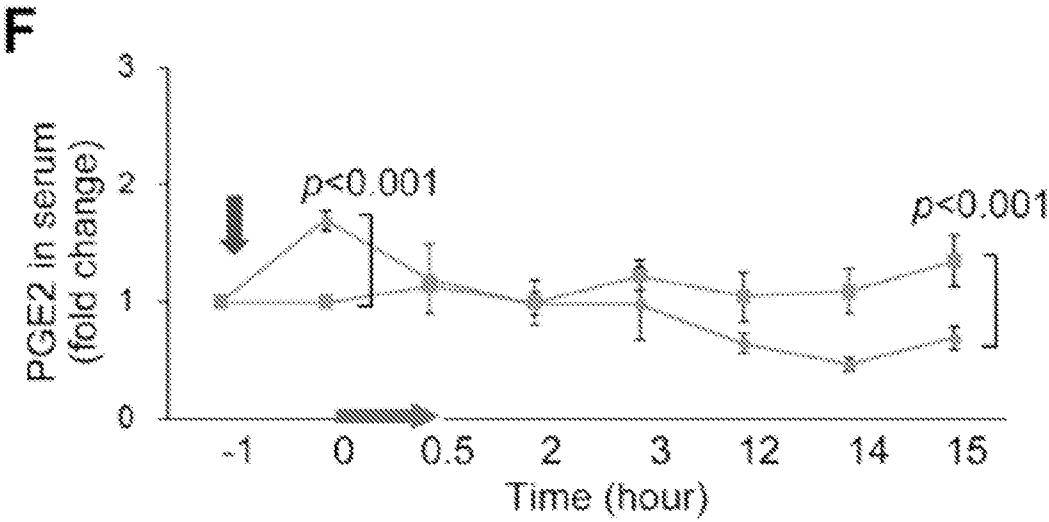


Fig. 8F

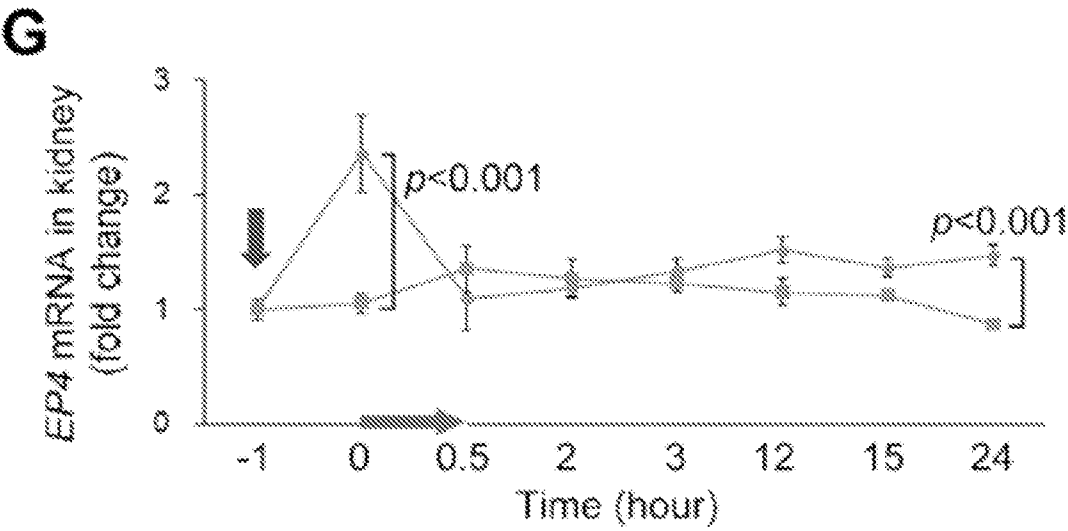


Fig. 8G

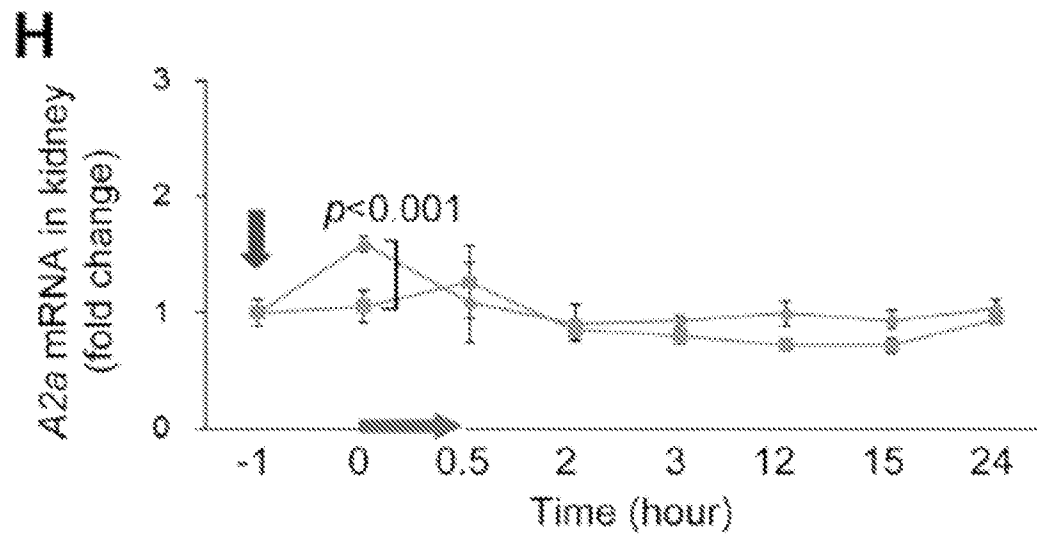


Fig. 8H

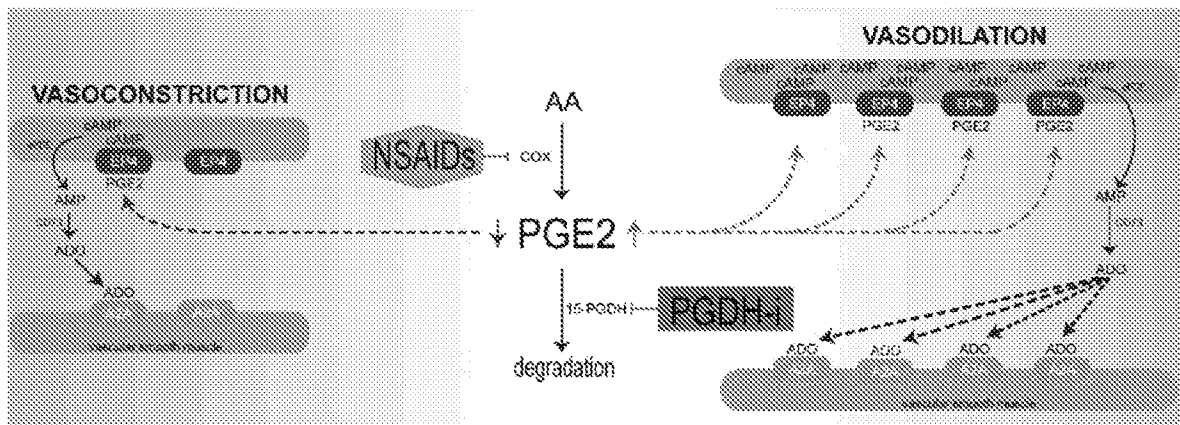


Fig. 9

(A)

CM	Iodine Con.	Viscosity (mPa.s, 37 °C)	Osmolality (mOsm/kg H ₂ O)	Hydrophilicity (OH-number)
Iodixanol (Visipaque)	320	11.8	290	9
Iobitridol (Xenetix)	300	6.0	695	6

Fig. 10A

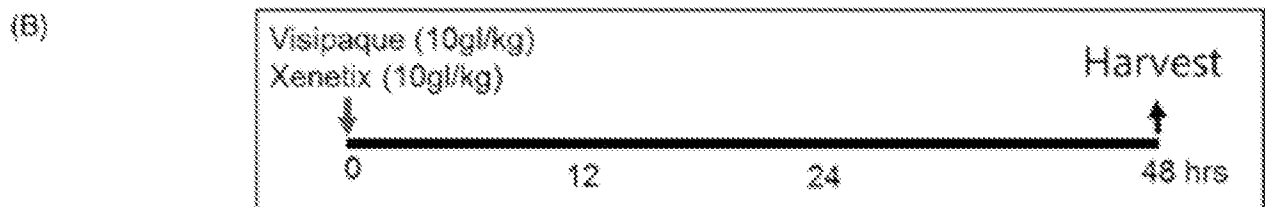


Fig. 10B

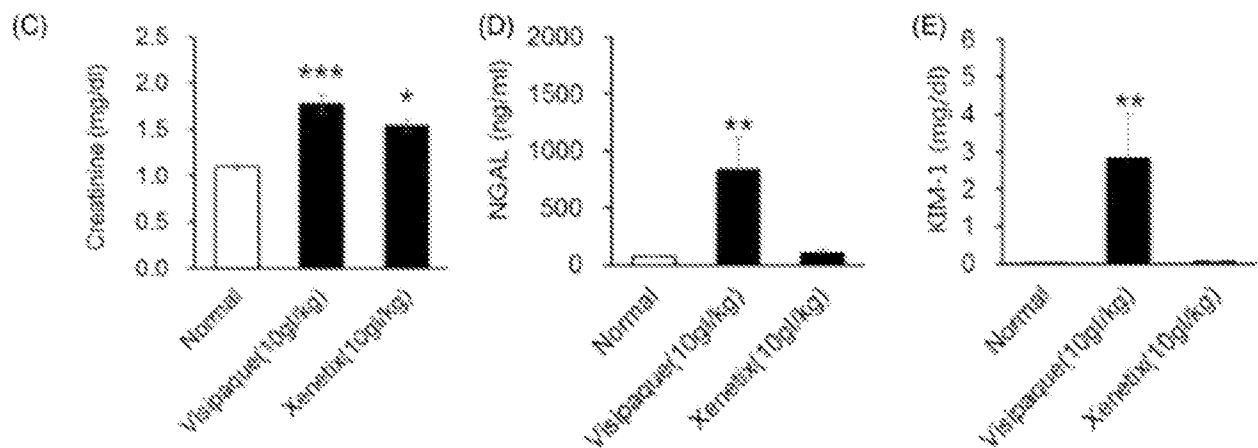


Fig. 10C

Fig. 10D

Fig. 10E

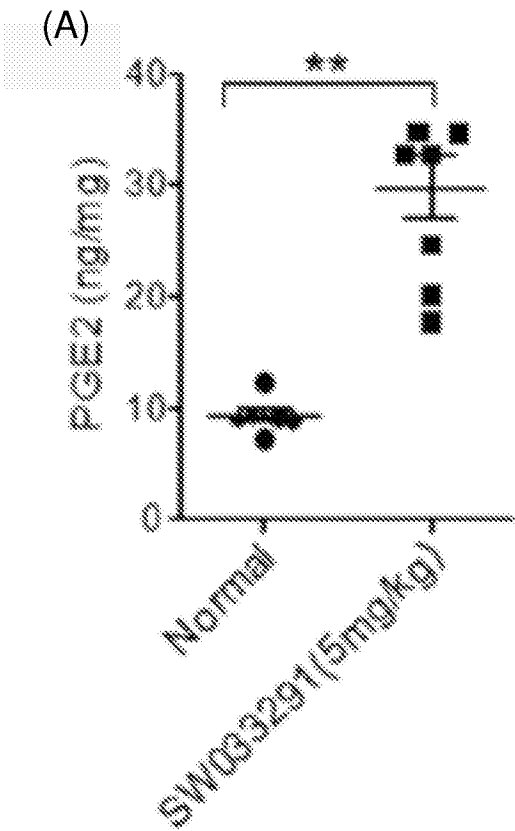


Fig. 11A

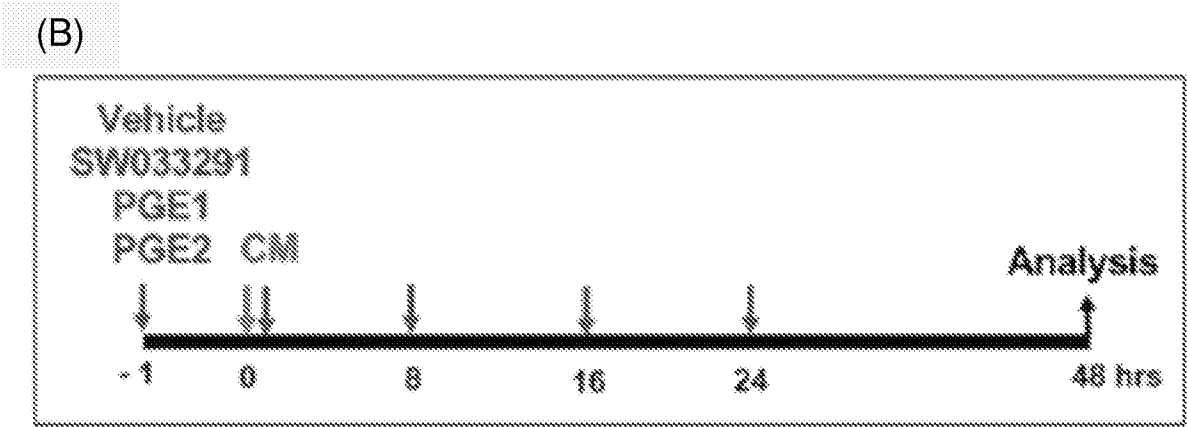


Fig. 11B

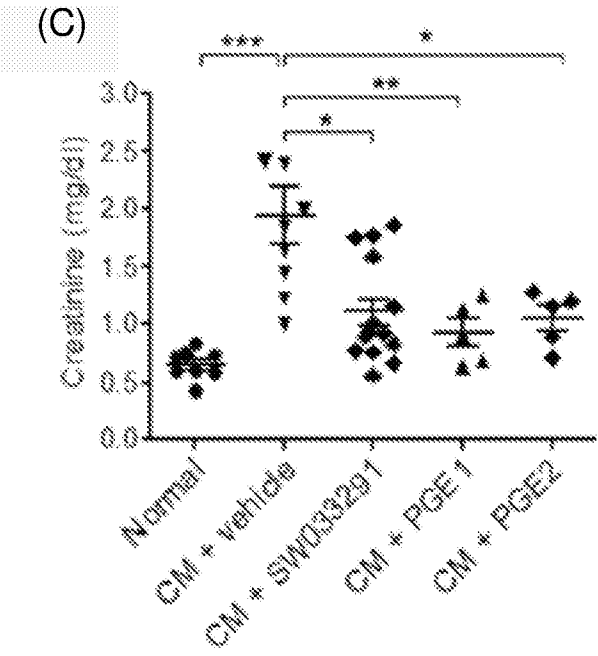


Fig. 11C

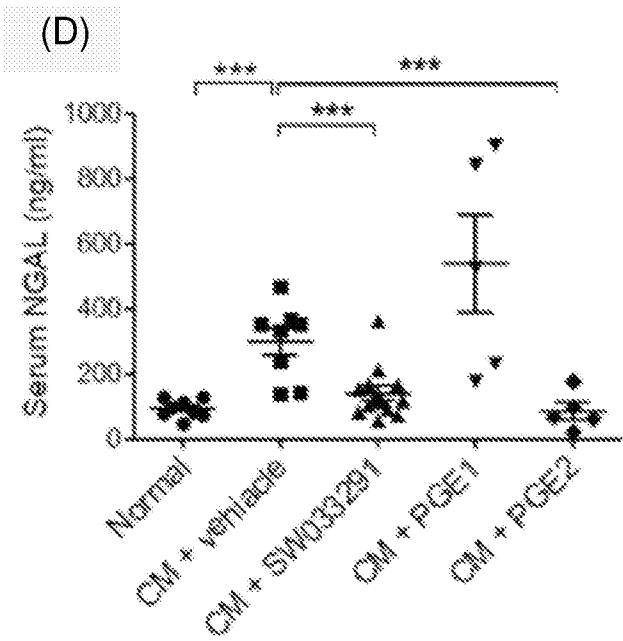


Fig. 11D

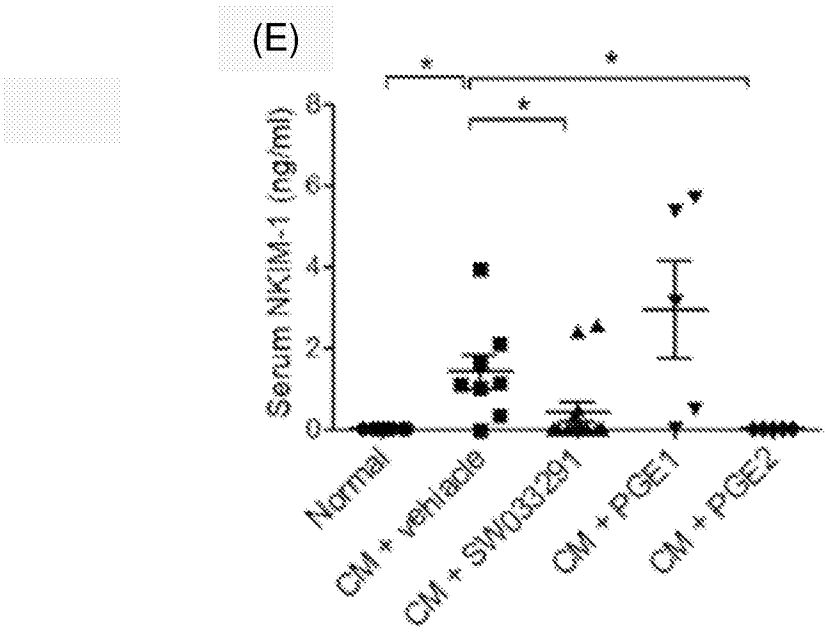


Fig. 11E

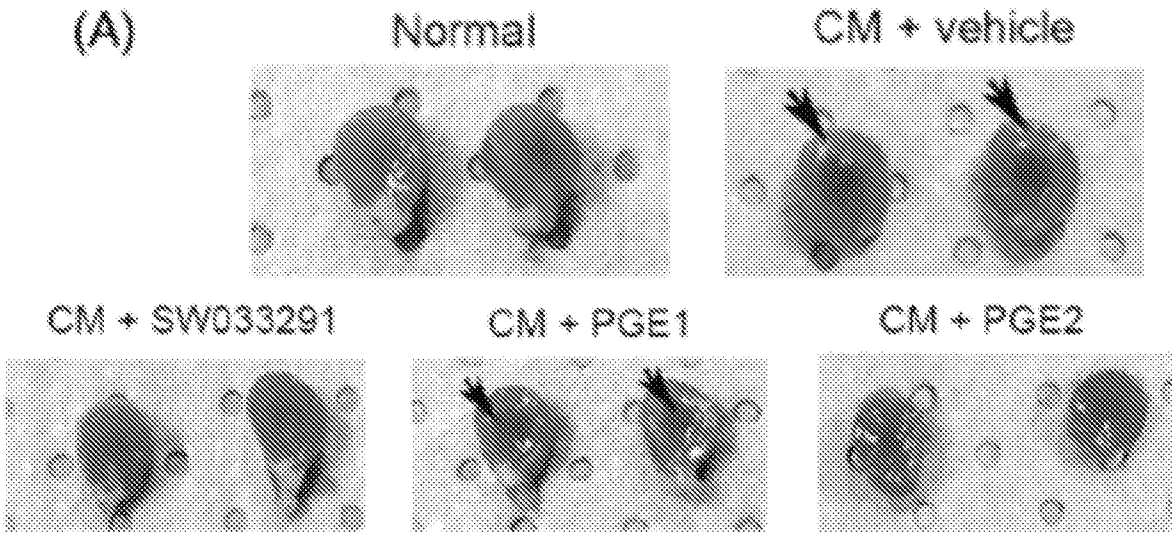


Fig. 12A

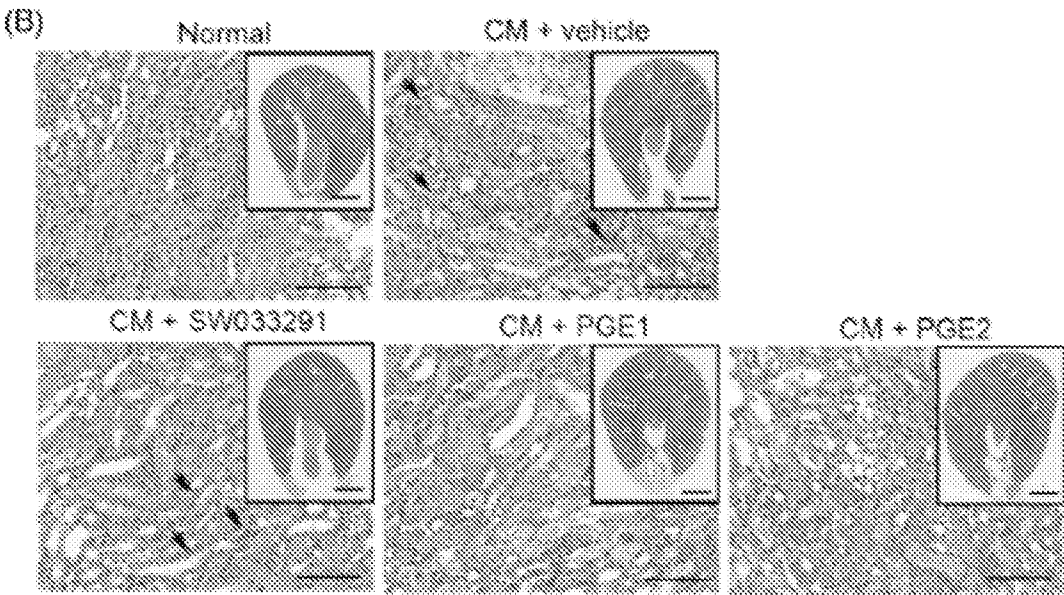


Fig. 12B

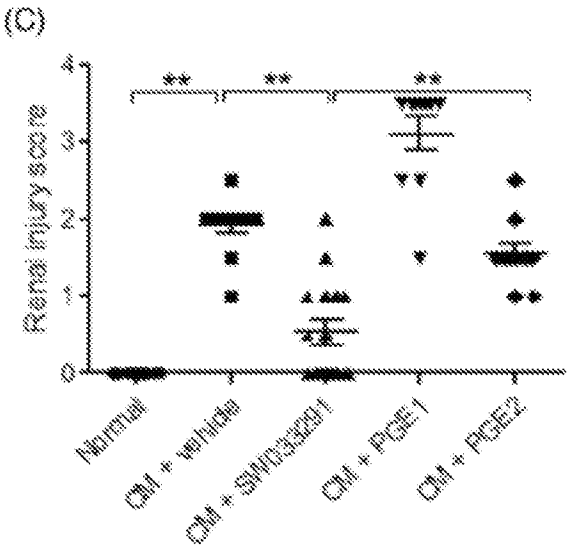


Fig. 12C

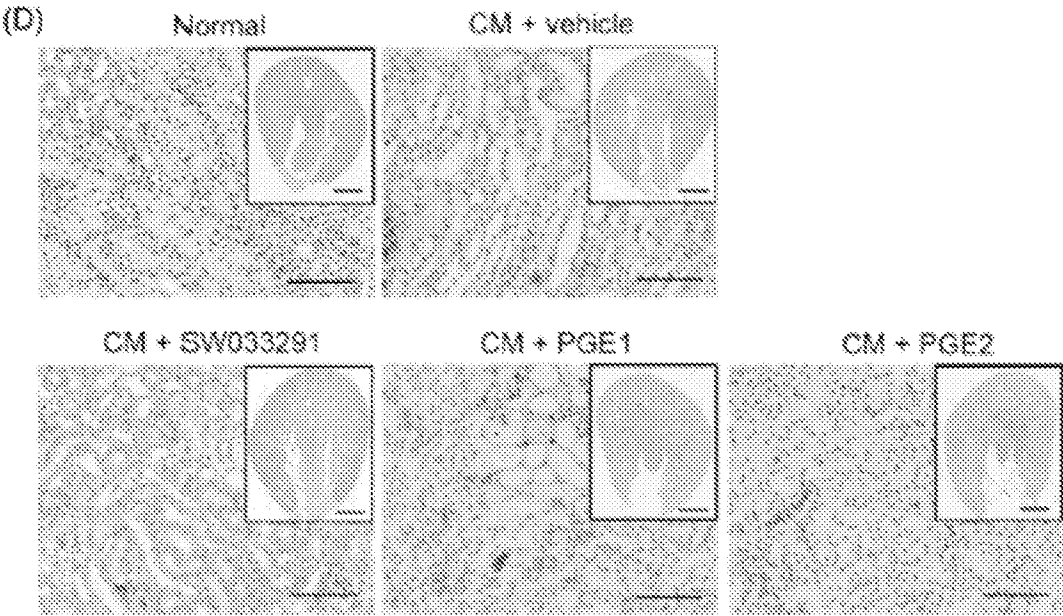


Fig. 12D

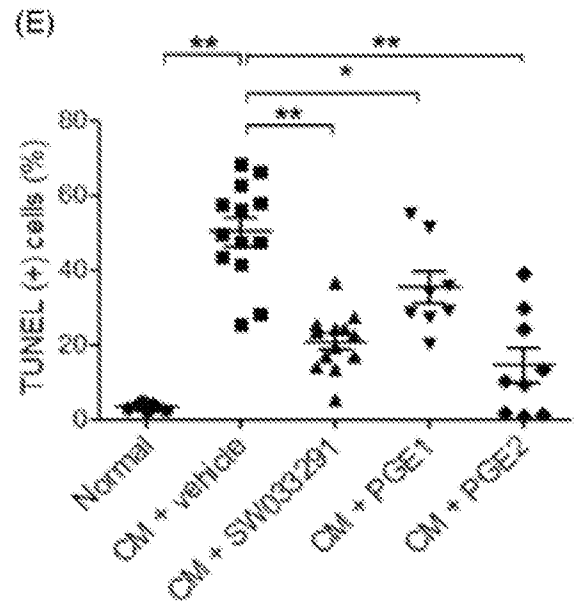


Fig. 12E

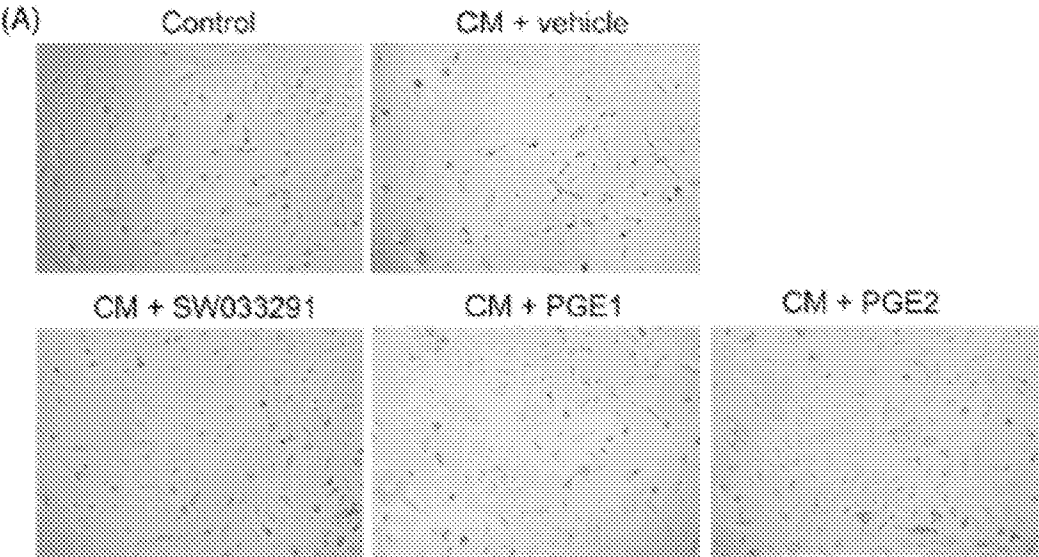


Fig. 13A

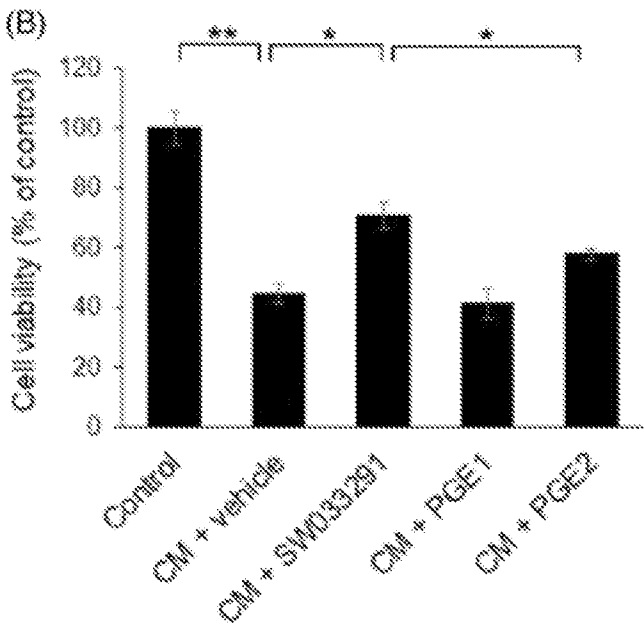


Fig. 13B

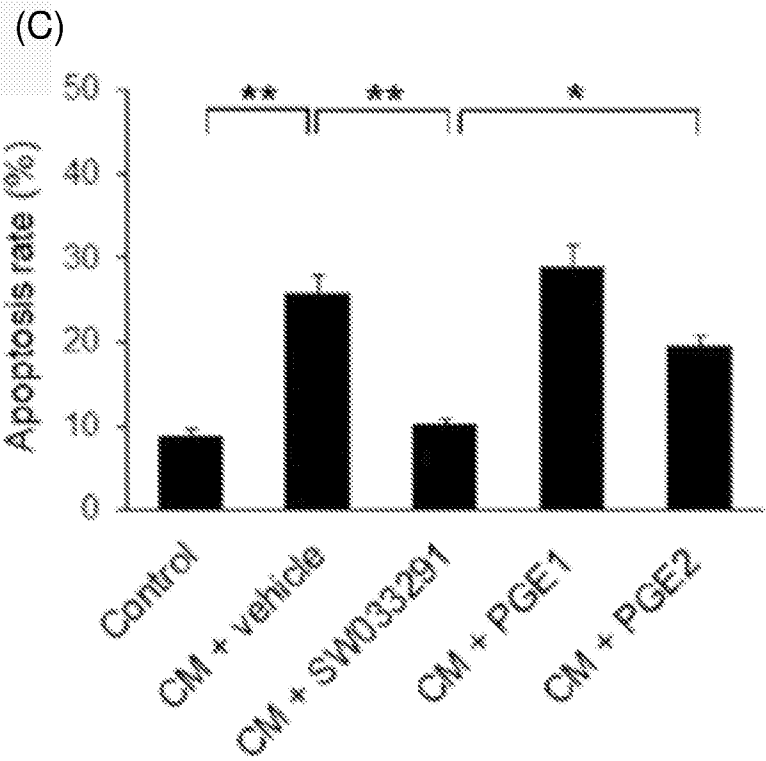


Fig. 13C

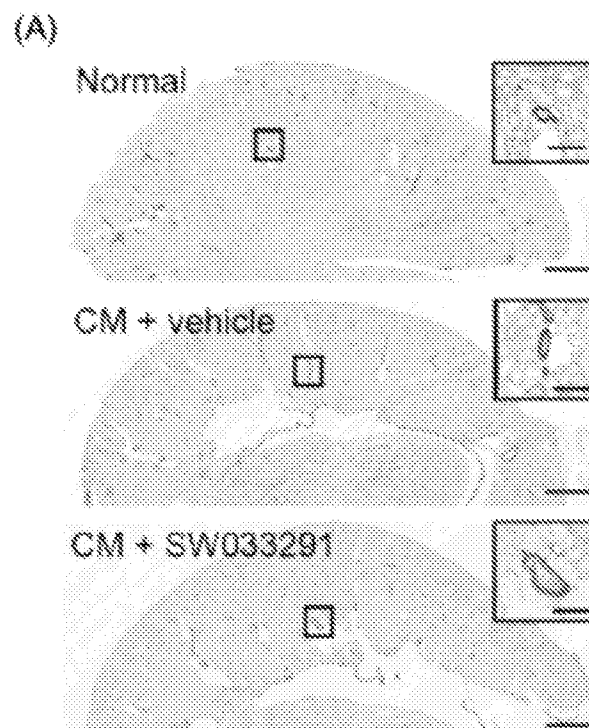


Fig. 14A

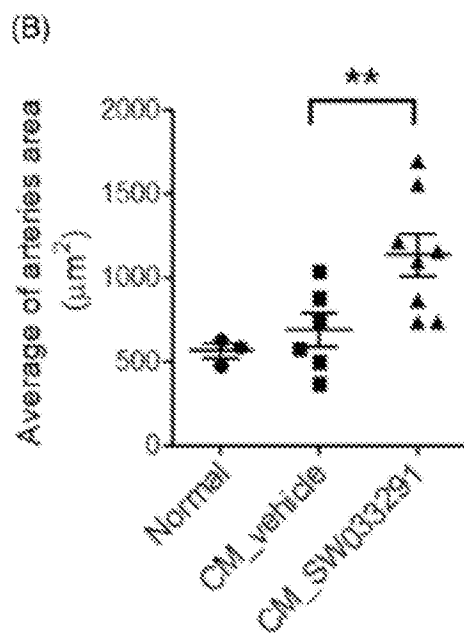


Fig. 14B

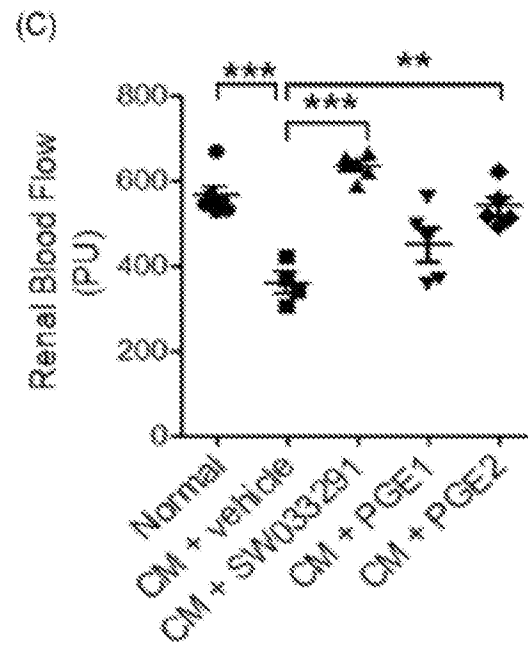


Fig. 14C

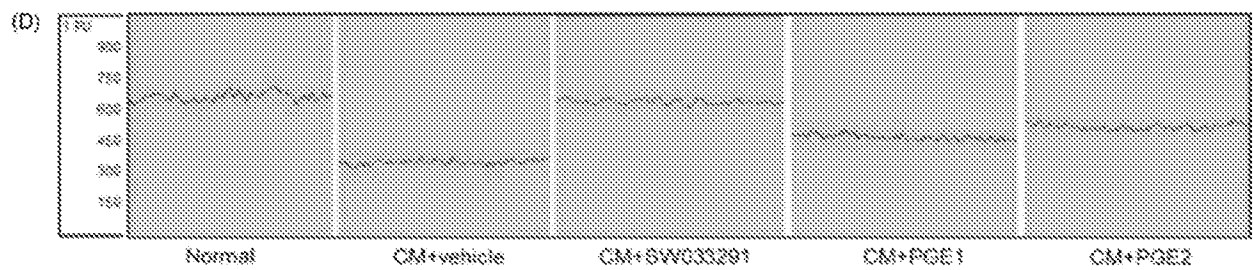


Fig. 14D

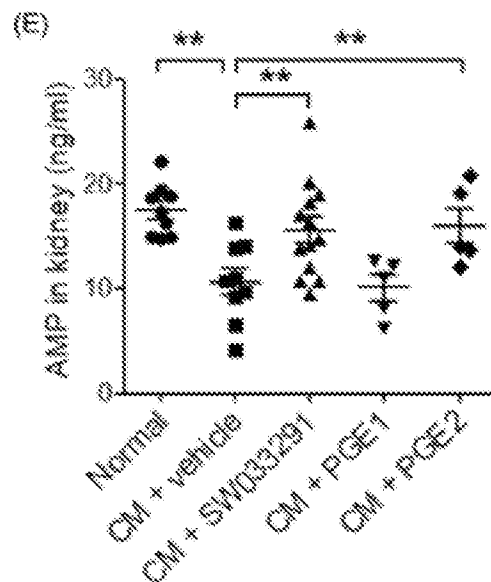


Fig. 14E

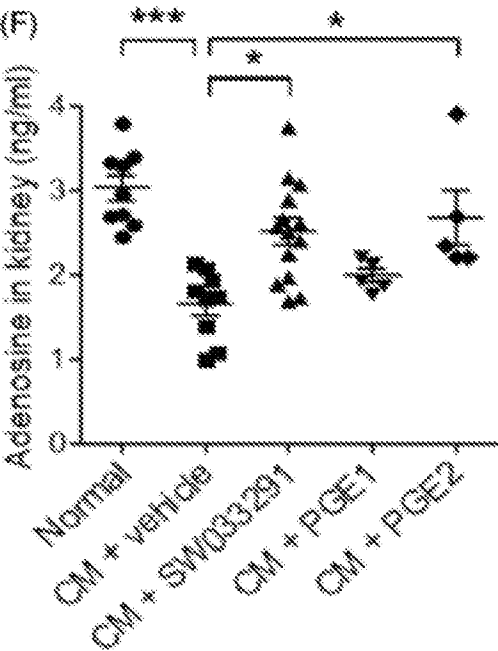


Fig. 14F

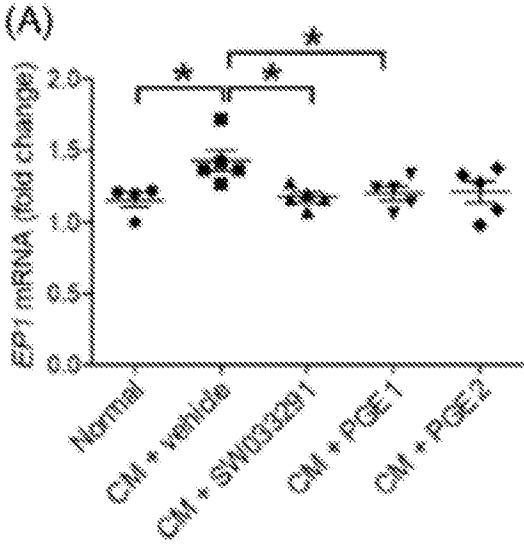


Fig. 15A

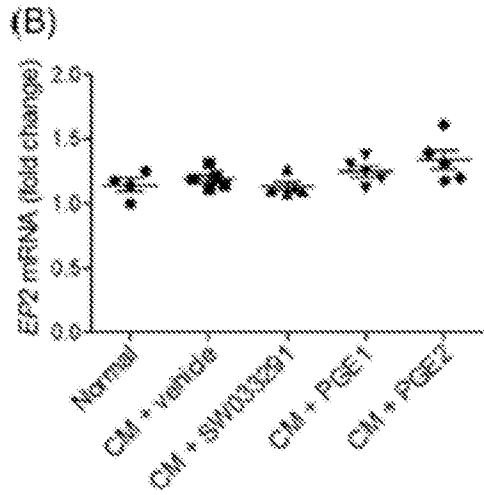


Fig. 15B

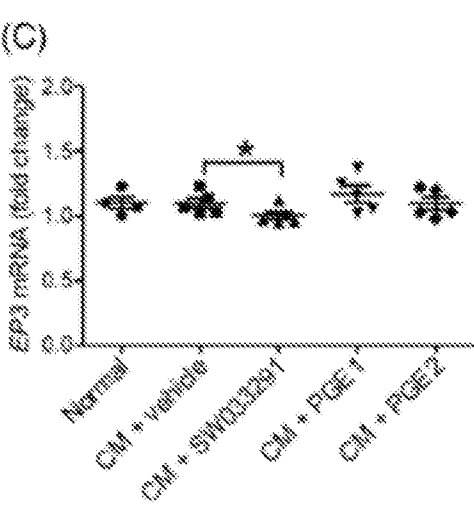


Fig. 15C

