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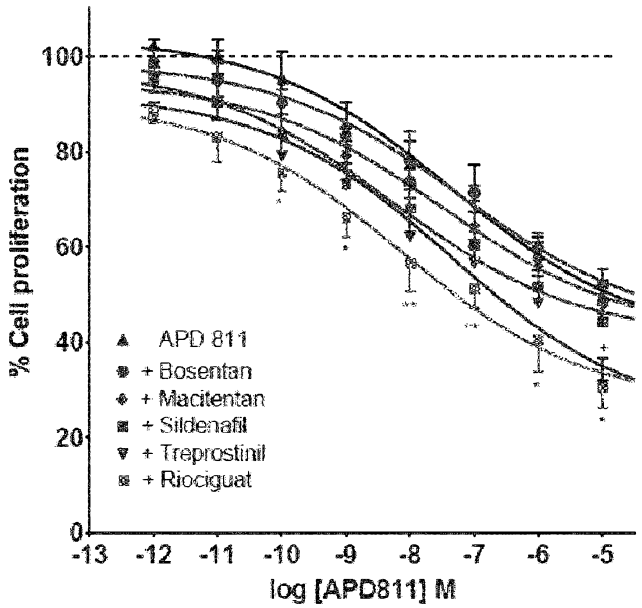

Figure 12

(57) Abstract: The present disclosure encompasses combinations of ralinepag with a cGMP-elevating agent or prostanoid such as riociguat, treprostinil, or iloprost for treating PAH. The disclosed combination therapy provides for advantages such as improved efficacy, improved safety, reduced doses and/or frequency of ralinepag and/or riociguat, reduced doses and/or frequency of ralinepag and/or treprostinil, and reduced doses and/or frequency of ralinepag and/or iloprost. In some embodiments, the clinical effectiveness of a reduced dose combination is additive or synergistic compared to that provided by the corresponding ralinepag, riociguat, treprostinil, and/or iloprost monotherapies.

[Continued on next page]

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METHODS OF TREATING PAH WITH COMBINATIONS OF RALINEPAG AND OTHER AGENTS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of priority of U.S. Provisional Application No. 62/420,515, filed November 10, 2016, and U.S. Provisional Application No. 62/530,533, filed July 10, 2017, the content of both of which are incorporated by reference herein in their entirety.

FIELD OF THE INVENTION

[0001] The present invention relates to methods of treatment and combinations of ralinepag (also known as APD811) and cAMP-elevating agents or cGMP-elevating agents (e.g., soluble guanylate cyclase (sGC) stimulators such as riociguat), combinations of ralinepag and prostanoids (e.g., treprostinil), or combinations of ralinepag and prostacyclin receptor agonists which are useful for the treatment of pulmonary arterial hypertension (PAH); idiopathic PAH; familial PAH; PAH associated with: a collagen vascular disease, a congenital heart disease, portal hypertension, HIV infection, ingestion of a drug or toxin, hereditary hemorrhagic telangiectasia, splenectomy, pulmonary veno-occlusive disease (PVOD) or pulmonary capillary hemangiomatosis (PCH); PAH with significant venous or capillary involvement; platelet aggregation; coronary artery disease; myocardial infarction; transient ischemic attack; angina; stroke; ischemia-reperfusion injury; restenosis; atrial fibrillation; blood clot formation in an angioplasty or coronary bypass surgery individual or in an individual suffering from atrial fibrillation; atherothrombosis; asthma or a symptom thereof; a diabetic-related disorder such as diabetic peripheral neuropathy, diabetic nephropathy or diabetic retinopathy; glaucoma or another disease of the eye with abnormal intraocular pressure; hypertension; inflammation; psoriasis; psoriatic arthritis; rheumatoid arthritis; Crohn's disease; transplant rejection; multiple sclerosis; systemic lupus erythematosus (SLE); ulcerative colitis; atherosclerosis; acne; type 1 diabetes; type 2 diabetes; sepsis; and chronic obstructive pulmonary disorder (COPD).

BACKGROUND

[0002] Pulmonary arterial hypertension (PAH) is a highly proliferative, inflammatory vascular remodeling disease leading to right heart failure and death. The pulmonary pharmacology of prostacyclin (epoprostenol) remains an area of considerable interest because

of the use of epoprostenol and its stable mimetics in the treatment of PAH. Prostanoid compounds iloprost (i.v., inhaled) and treprostinil (subcutaneous, i.v, inhaled) are extensively used in the treatment of this disease. Given the complications posed by the above delivery routes, successful oral therapies are being sought for use earlier in the disease process with improved efficacy and/or outcome with this class of compounds (McLaughlin et al., 2009; Clapp & Gurung, 2015).

[0003] Oral beraprost (which contains several isomers of beraprost), is only licensed in Japan and Korea (Vachiery, 2011) and appears to have limited efficacy clinically (Barst et al., 2003). Oral treprostinil has only recently received FDA approval, though it is not clear at present whether the oral formulation will approach the clinical efficacy seen with either subcutaneous or i.v. administration methods (Tapson et al., 2013). Selexipag (NS-304), is an oral, non-prostanoid IP receptor agonist (Skoro-Sajer & Lang, 2014; Sitbon et al., 2015). It is a non-prostanoid, diphenylpyrazine derivative whose active metabolite, MRE-269 (also known as ACT-333679) is reported to be a highly selective IP receptor agonist (Kuwano et al., 2007). MRE-269 potently binds to the human IP receptor (K_i = 20 nM), while selexipag has much less affinity at this receptor (K_i = 260 nM); both however have little binding affinity for other prostanoid receptors ($K_i \geq 2.6$ μM).

[0004] In normal human pulmonary arterial smooth muscle cells (PASMCs), the IP receptor, through the generation of cyclic AMP (cAMP) appears to be the main mediator of the antiproliferative responses to treprostinil and iloprost (Wharton et al., 2000; Clapp et al., 2002; Falcetti et al., 2010). In contrast, in human PASMCs isolated from IPAH patients, neither the IP receptor nor cAMP appeared to be the main mediator underpinning the antiproliferative effects of treprostinil and iloprost, at least at the concentrations studied (Falcetti et al., 2010). Thus, there is no clear indication in the scientific literature as to how efficacious drugs that are selective for the IP receptors will be in a disease where the IP receptor expression is lower, as it is in patients with PAH (Lai et al., 2008; Falcetti et al., 2010).

[0005] Ralinepag (2-(((1r,4r)-4-(((4-chlorophenyl)(phenyl)carbamoyloxy)methyl)cyclohexyl) methoxy)acetic acid, also known as APD811) is an orally available, non-prostanoid prostacyclin (IP) receptor agonist for treating various conditions as described herein, for example vasospastic diseases such as PAH. Ralinepag is disclosed in US Patent Publication No. 2011/0053958, herein incorporated by reference in its entirety for all purposes.

[0006] Ralinepag is currently being evaluated as an oral monotherapy for treating conditions such as PAH. In a recent clinical trial, patients underwent titration of ralinepag in order to establish the tolerable dose. Potential methods for administering ralinepag comprise dosing at an initially low dose, once or twice daily, then escalating the dose over a period of, typically, weeks, by stepwise increase of the ralinepag dose and/or dosing frequency until the highest tolerable daily dose is achieved.

[0007] The need for dose escalation can delay delivery of the desired highest tolerable therapeutic dose to the patient, thereby delaying potential clinical benefits. In addition, the need to administer multiple daily doses is inconvenient and can reduce patient compliance. It would therefore be desirable to provide methods of administration of ralinepag in which dose titration is reduced or eliminated, and/or the need for multiple daily doses is reduced or eliminated. In addition, it would be desirable to provide methods of enhancing the therapeutic effects of ralinepag and/or a coadministered compound while minimizing side effects, for example by achieving clinical efficacy at a lower dose of one or more compound. Such a method would provide clear clinical benefits, as well as enhance patient compliance. The present disclosure provides improved treatments and methods of administering ralinepag, particularly for treating PAH and related conditions.

SUMMARY OF THE INVENTION

[0008] In its various embodiments, the present invention is directed to a method of decreasing cell proliferation (e.g., of pulmonary arterial smooth muscle cells (PASMCs)), in a patient in need thereof, comprising administering a therapeutically effective amount of ralinepag, or a pharmaceutically acceptable salt, hydrate, or solvate thereof, in combination with a therapeutically effective amount of one or more of a cGMP elevating agent, a cAMP elevating agent, a prostanoid, or a prostacyclin receptor agonist.

[0009] In various embodiments, the one or more cGMP or cAMP elevating agents is a soluble guanylate cyclase modulator.

[0010] In various embodiments, ralinepag is administered in combination with one or more cGMP elevating agents, for example any of the cGMP elevating agents disclosed herein.

[0011] In various embodiments, ralinepag is administered in combination with one or more cAMP elevating agents, for example any of the cAMP elevating agents disclosed herein.

[0012] In various embodiments, ralinepag is administered in combination with a prostanoid,

for example any of the prostanoids disclosed herein.

[0013] In various embodiments, coadministration of ralinepag with one or more cGMP elevating agents, cAMP elevating agents, prostanoids, or prostacyclin receptor agonists does not require titration, e.g., of ralinepag upon initiating said coadministration method. In various embodiments, coadministration of ralinepag with one or more cGMP elevating agents, cAMP elevating agents, prostanoids, or prostacyclin receptor agonists does not require titration, e.g., of the one or more cGMP elevating agents, cAMP elevating agents, prostanoids, or prostacyclin receptor agonists. For example, in some embodiments, coadministration of ralinepag with riociguat does not require titration of ralinepag. In some embodiments, coadministration of ralinepag with riociguat does not require titration of riociguat.

[0014] In various embodiments, coadministration of ralinepag with one or more cGMP elevating agent, cAMP elevating agent, prostanoid, or prostacyclin receptor agonist does not require as many steps as a standard titration scheme (for example, the titration scheme provided on a product label for the monotherapy) for one or both of the coadministered agents. In various embodiments, coadministration of ralinepag with one or more cGMP elevating agent, cAMP elevating agent, prostanoid, or prostacyclin receptor agonist allows for a higher initial dose that a standard titration scheme (for example, the titration scheme provided on a product label for the monotherapy) for one or both of the coadministered agents.

[0015] In various embodiments, the daily dose of ralinepag in combination with the one or more of the cGMP elevating agent, cAMP elevating agent, or prostanoid is at least about 0.01 mg, 0.02 mg, 0.03 mg, 0.04 mg, 0.05 mg, 0.06 mg, 0.07 mg, 0.08 mg, 0.09 mg, 0.1 mg, 0.11 mg, 0.12 mg, 0.13 mg, 0.14 mg, 0.15 mg, 0.16 mg, 0.17 mg, 0.18 mg, 0.19 mg, 0.2 mg, 0.21 mg, 0.22 mg, 0.23 mg, 0.24 mg, 0.25 mg, 0.26 mg, 0.27 mg, 0.28 mg, 0.29 mg, or 0.3 mg (dose equivalent) less than the equivalent therapeutic amount of ralinepag in the absence of coadministration of one or more cGMP elevating agent, cAMP elevating agent, or prostanoid.

[0016] In various embodiments, coadministration of ralinepag with one or more cGMP elevating agents, cAMP elevating agents, prostanoids, or prostacyclin receptor agonists provides a reduced daily dose of ralinepag and/or cGMP elevating agent, cAMP elevating agent, prostanoid, or prostacyclin receptor agonist relative to daily doses of ralinepag and/or cGMP elevating agent, cAMP elevating agent, prostanoid, or prostacyclin receptor agonist

that provides an equivalent clinical effect.

[0017] In various embodiments, ralinepag is coadministered with one or more of riociguat, vericiguat, ataciguat, nelociguat, lificiguat, IW-1701, IW-1973, IWP-051, IWP-121, IWP-427, IWP-953, BAY-60-2770, A-344905; A-350619, A-778935, BI-684067, BI-703704, BAY-41-2272, and BAY-41-8543.

[0018] In various embodiments, the amount of ralinepag is, or is about, 0.02, 0.025 0.04, 0.05, 0.06, 0.075, 0.08, 0.1, 0.12, 0.125, 0.14, 0.15, 0.16, 0.175, 0.18, 0.2, 0.22, 0.24, 0.25, 0.26, 0.275, 0.28, 0.3, 0.32, 0.325, 0.34, 0.35, 0.36, 0.375, 0.38, 0.4, 0.42, 0.44, 0.45, 0.46, 0.48, 0.5, 0.52, 0.54, 0.56, 0.58, 0.6. 0.625, 0.65, 0.675, 0.7, 0.725, 0.75, 0.775, 0.8, 0.825, 0.85, 0.875, 0.9, 0.925, 0.95, 0.975, or 1.0 mg, or a range of any two such amounts. For example, in various embodiments, the amount of ralinepag is, or is about, 0.05-0.6 mg. In various embodiments, the amount of ralinepag is, or is about, 0.05-0.75 mg.

[0019] In various embodiments, the amount of ralinepag is, or is about, 0.02, 0.025, 0.04, 0.05, 0.06, 0.075, 0.08, 0.1, 0.12, 0.125, 0.14, 0.15, 0.16, 0.175, 0.18, 0.2, 0.22, 0.24, 0.25, 0.26, 0.275, 0.28, 0.3, 0.32, 0.325, 0.34, 0.35, 0.36, 0.375, 0.38, 0.4, 0.42, 0.44, 0.45, 0.46, 0.48, 0.5, 0.52, 0.54, 0.56, 0.58, 0.6. 0.625, 0.65, 0.675, 0.7, 0.725, 0.75, 0.775, 0.8, 0.825, 0.85, 0.875, 0.9, 0.925, 0.95, 0.975, or 1.0 mg daily, or a range of any two such amounts. For example, in various embodiments, the amount of ralinepag is, or is about, 0.05-0.6 mg daily.

[0020] In various embodiments, the amount of ralinepag is, or is about, 0.02, 0.025, 0.04, 0.05, 0.06, 0.075, 0.08, 0.1, 0.12, 0.125, 0.14, 0.15, 0.16, 0.175, 0.18, 0.2, 0.22, 0.24, 0.25, 0.26, 0.275, 0.28, 0.3, 0.32, 0.325, 0.34, 0.35, 0.36, 0.375, 0.38, 0.4, 0.42, 0.44, 0.45, 0.46, 0.48, 0.5, 0.52, 0.54, 0.56, 0.58, 0.6 mg, 06.25, 0.65, 0.675, 0.7, 0.725, 0.75, 0.775, 0.8, 0.825, 0.85, 0.875, 0.9, 0.925, 0.95, 0.975, or 1.0 mg once daily (QD), or a range of any two such amounts. For example, in various embodiments, the amount of ralinepag is, or is about, 0.05-0.6 mg QD.

[0021] In various embodiments, the amount of ralinepag is, or is about, 0.01, 0.02, 0.025, 0.03, 0.04, 0.05, 0.06, 0.07, 0.075, 0.08, 0.09, 0.1, 0.11, 0.12, 0.125, 0.13, 0.14, 0.15, 0.16, 0.17, 0.175, 0.18, 0.19, 0.2, 0.21, 0.22, 0.23, 0.24, 0.25, 0.26, 0.27, 0.275, 0.28, 0.29, 0.3, 0.325, 0.35, 0.375, 0.4, 0.425, 0.45, 0.475, or 0.5 mg twice daily (BID), or a range of any two such amounts. For example, in various embodiments, the amount of ralinepag is , or is about, 0.05-0.3 mg BID.

[0022] In various embodiments, the amount of ralinepag is, or is about, 0.01, 0.02, 0.025, 0.03, 0.04, 0.05, 0.06, 0.07, 0.075, 0.08, 0.09, or 0.1 mg every other day.

[0023] In various embodiments, the amount of riociguat is, or is about, 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.25, 2.5, 2.75, 3, 3.25, 3.5, 3.75, 4, 4.25, 4.5, 4.75, or 5 mg, or a range of any two such amounts. For example, in various embodiments, the amount of riociguat is, or is about, 0.5 to 2.5 mg.

[0024] In various embodiments, the amount of riociguat is, or is about, 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.25, 2.5, 2.75, 3, 3.25, 3.5, 3.75, 4, 4.25, 4.5, 4.75, or 5 mg daily, or a range of any two such amounts. For example, in various embodiments, the amount of riociguat is, or is about, 0.5 to 2.5 mg daily.

[0025] In various embodiments, the amount of riociguat is, or is about, 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.25, 2.5, 2.75, 3, 3.25, 3.5, 3.75, 4, 4.25, 4.5, 4.75, or 5 mg once daily (QD), or a range of any two such amounts. For example, in various embodiments, the amount of riociguat is, or is about, 0.5 to 2.5 mg once daily. In various embodiments, the riociguat is administered twice daily. In various embodiments, the riociguat is administered three times daily.

[0026] In various embodiments, the amount of riociguat is, or is about, 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.25, or 2.5 mg twice daily (BID), or a range of any two such amounts.

[0027] In various embodiments, the amount of riociguat is 0.5, 1, 1.5, 2, or 2.5 mg three times daily (TID), or a range of any two such amounts.

[0028] In various embodiments, the amount of riociguat is less than or equal to 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.25, 2.5, 2.75, 3, 3.25, 3.5, 3.75, 4, 4.25, 4.5, 4.75, or 5 mg.

[0029] In various embodiments, the riociguat is in an inhaled form. In various embodiments, the riociguat is in an intravenous form. In various embodiments, the riociguat is in an oral form.

[0030] In various embodiments, the amount of treprostinil is, or is about, 0.1, 0.125, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45, 0.5, 0.6, 0.65, 0.7, 0.75, 0.8, 0.85, 0.9, 0.95, 1, 0.25, 0.5, 1.75, 2, 2.25, 2.5, 2.75, 3, 3.25, 3.5, 3.75, 4, 4.25, 4.5, 4.75, 5, 6, 7, 8, 9, or 10 mg, or a range of any two such amounts. For example, in various embodiments, the amount of treprostinil is, or is about, 0.125-5 mg.

[0031] In various embodiments, the amount of treprostinil is, or is about, 1, 2.5, 3, 4, 5, 6, 7,

7.5,8, 9, 10, 11, 12, 12.5, 13, 14, 15, 16, 17, 17.5, 18, 19, 20, or 25 mg/ml, or a range of any two such amounts. In various embodiments, the amount of treprostinil is 1-10 mg/ml in an IV subcutaneous form.

[0032] In various embodiments, the amount of treprostinil is, or is about, 0.1, 0.2, 0.25, 0.3, 0.4, 0.5, 0.6, 0.7, 0.75, 0.8, 0.9, or 1 mg/ml, or a range of any two such amounts. In various embodiments, the amount of treprostinil is 0.6 mg/ml in an inhaled form.

[0033] In various embodiments, the amount of treprostinil is less than or equal to 0.1, 0.125, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45, 0.5, 0.6, 0.65, 0.7, 0.75, 0.8, 0.85, 0.9, 0.95, 1, 0.25, 0.5, 1.75, 2, 2.25, 2.5, 2.75, 3, 3.25, 3.5, 3.75, 4, 4.25, 4.5, 4.75, or 5 mg. In various embodiments, the amount of treprostinil is less than or equal to 1, 2.5, 3, 4, 5, 6, 7, 7.5,8, 9, or 10 mg/ml. In various embodiments, the amount of treprostinil is less than or equal to 0.1, 0.2, 0.25, 0.3, 0.4, 0.5, or 0.6 mg/ml.

[0034] In various embodiments, the treprostinil is in an inhaled form. In some embodiments, the treprostinil is in an injectable form. In various embodiments, the treprostinil is in an intravenous form. In various embodiments, the treprostinil is in an IV subcutaneous form. In various embodiments, the treprostinil is in an oral form. In various embodiments, the treprostinil is in a transdermal form. In various embodiments, the treprostinil is in a transdermal patch. In various embodiments, the treprostinil is administered once daily. In various embodiments, the treprostinil is administered twice daily. In various embodiments, the treprostinil is administered three times daily.

[0035] In some embodiments, the therapeutically effective amount of ralinepag is a starting dose. In some embodiments, the therapeutically effective amount of ralinepag is a highest tolerated dose. In some embodiments, the therapeutically effective amount of ralinepag is a maximum dose. In some embodiments, the therapeutically effective amount of ralinepag is a maximum tolerated dose. In some embodiments, the therapeutically effective amount of ralinepag is a maintenance dose.

[0036] In some embodiments, the starting dose is for a patient. In some embodiments, the starting dose is for a patient population. In some embodiments, the highest tolerated dose is for a patient. In some embodiments, the highest tolerated dose is for a patient population. In some embodiments, the maximum dose is for a patient. In some embodiments, the maximum dose is for a patient population. In some embodiments, the maximum tolerated dose is for a patient. In some embodiments, the maximum tolerated dose is for a patient population. In

some embodiments, the maintenance dose is for a patient. In some embodiments, the maintenance dose is for a patient population.

[0037] In some embodiments, the starting dose of ralinepag is selected from, or from about, 0.01, 0.02, 0.025, 0.03, 0.04, 0.05, 0.06, 0.07, 0.075, 0.08, 0.09, or 0.1 mg daily. In some embodiments, the starting dose of ralinepag is 0.01 mg daily. In some embodiments, the starting dose of ralinepag is 0.02 mg daily. In some embodiments, the starting dose of ralinepag is 0.05 mg daily.

[0038] In some embodiments, the dose of ralinepag is increased at weekly intervals by 0.05 mg daily to the highest tolerated dose up to 0.8 mg daily.

[0039] In some embodiments, the dose of ralinepag is increased at weekly intervals. In some embodiments, the dose of ralinepag is increased at bimonthly intervals.

[0040] In some embodiments, the dose of ralinepag is increased by an amount selected from 0.02 mg, 0.05 mg, and 0.75 mg and 0.1 mg daily.

[0041] In some embodiments, the dose of ralinepag is increased at weekly intervals by an amount selected from 0.02 mg, 0.05 mg, and 0.75 mg, and 0.1 mg daily.

[0042] In some embodiments, the highest tolerated dose of ralinepag is selected from, or from about, 0.4 mg, 0.45 mg, 0.5 mg, 0.6 mg, 0.65 mg, 0.7 mg, 0.75 mg, 0.8 mg, 0.85 mg, 0.9 mg, 0.95 mg, and 1.0 mg daily. In some embodiments, the highest tolerated dose of ralinepag is 0.6 mg daily. In some embodiments, the highest tolerated dose of ralinepag is 0.75 mg daily. In some embodiments, the highest tolerated dose of ralinepag is 0.8 mg daily. In some embodiments, the highest tolerated dose of ralinepag is from 0.4 to 1.0 mg daily. In some embodiments, the highest tolerated dose of ralinepag is from 0.6 to 1.0 mg daily. In some embodiments, the highest tolerated dose of ralinepag is from 0.6 to 0.8 mg daily. In some embodiments, the highest tolerated dose of ralinepag is from 0.65 to 1.0 mg daily. In some embodiments, the highest tolerated dose of ralinepag is from 0.65 to 0.8 mg daily. In some embodiments, the highest tolerated dose of ralinepag is greater than 0.4 mg daily. In some embodiments, the highest tolerated dose of ralinepag is greater than 0.6 mg daily.

[0043] In some embodiments, the maximum dose of ralinepag is selected from, or from about, 0.4 mg, 0.45 mg, 0.5 mg, 0.6 mg, 0.65 mg, 0.7 mg, 0.75 mg, 0.8 mg, 0.85 mg, 0.9 mg, 0.95 mg, and 1.0 mg daily. In some embodiments, the maximum dose of ralinepag is 0.6 mg daily. In some embodiments, the maximum dose of ralinepag is 0.75 mg daily. In some embodiments, the maximum dose of ralinepag is 0.8 mg daily. In some embodiments, the

maximum dose of ralinepag is from 0.4 to 1.0 mg daily. In some embodiments, the maximum dose of ralinepag is from 0.6 to 1.0 mg daily. In some embodiments, the maximum dose of ralinepag is from 0.6 to 0.8 mg daily. In some embodiments, the maximum dose of ralinepag is from 0.65 to 1.0 mg daily. In some embodiments, the maximum dose of ralinepag is from 0.65 to 0.8 mg daily. In some embodiments, the maximum dose of ralinepag is greater than 0.4 mg daily. In some embodiments, the maximum dose of ralinepag is greater than 0.6 mg daily.

[0044] In some embodiments, the maximum tolerated dose of ralinepag is selected from, or from about, 0.4 mg, 0.45 mg, 0.5 mg, 0.6 mg, 0.65 mg, 0.7 mg, 0.75 mg, 0.8 mg, 0.85 mg, 0.9 mg, 0.95 mg, and 1.0 mg daily. In some embodiments, the maximum tolerated dose of ralinepag is 0.6 mg daily. In some embodiments, the maximum tolerated dose of ralinepag is 0.75 mg daily. In some embodiments, the maximum tolerated dose of ralinepag is 0.8 mg daily. In some embodiments, the maximum tolerated dose of ralinepag is 0.75 mg daily. In some embodiments, the maximum tolerated dose of ralinepag is 0.8 mg daily. In some embodiments, the maximum tolerated dose of ralinepag is from 0.4 to 1.0 mg daily. In some embodiments, the maximum tolerated dose of ralinepag is from 0.6 to 1.0 mg daily. In some embodiments, the maximum tolerated dose of ralinepag is from 0.6 to 0.8 mg daily. In some embodiments, the maximum tolerated dose of ralinepag is from 0.65 to 1.0 mg daily. In some embodiments, the maximum tolerated dose of ralinepag is from 0.65 to 0.8 mg daily. In some embodiments, the maximum tolerated dose of ralinepag is greater than 0.4 mg daily. In some embodiments, the maximum tolerated dose of ralinepag is greater than 0.6 mg daily.

[0045] In some embodiments, the maximum dose of ralinepag in a dosage form is selected from, or from about, 0.4 mg, 0.45 mg, 0.5 mg, 0.6 mg, 0.65 mg, 0.7 mg, 0.75 mg, 0.8 mg, 0.85 mg, 0.9 mg, 0.95 mg, and 1.0 mg. In some embodiments, the maximum dose of ralinepag in a dosage form is 0.6 mg. In some embodiments, the maximum dose of ralinepag in a dosage form is 0.75 mg. In some embodiments, the maximum dose of ralinepag in a dosage form is 0.8 mg. In some embodiments, the maximum dose of ralinepag is from 0.4 to 1.0 mg daily. In some embodiments, the maximum dose of ralinepag is from 0.6 to 1.0 mg daily. In some embodiments, the maximum dose of ralinepag is from 0.6 to 0.8 mg daily. In some embodiments, the maximum dose of ralinepag is from 0.65 to 1.0 mg daily. In some embodiments, the maximum dose of ralinepag is from 0.65 to 0.8 mg daily. In some embodiments, the maximum dose of ralinepag is greater than 0.4 mg daily. In some embodiments, the maximum dose of ralinepag is greater than 0.6 mg daily.

[0046] In some embodiments, the maintenance dose of ralinepag is selected from, or from about, 0.01 mg, 0.02 mg, 0.025 mg, 0.03 mg, 0.04 mg, 0.05 mg, 0.06 mg, 0.065 mg, 0.07 mg, 0.075 mg, 0.08 mg, 0.09 mg, 0.1 mg, 0.12 mg, 0.15 mg, 0.16 mg, 0.2 mg, 0.25 mg, 0.3 mg, 0.35 mg, 0.4 mg, 0.45 mg, 0.5 mg, 0.55 mg, 0.6 mg, 0.65 mg, 0.7 mg, 0.75 mg, 0.8 mg, 0.85 mg, 0.9 mg, 0.95 mg, and 1.0 mg daily. In some embodiments, the maintenance dose of ralinepag is from 0.4 to 1.0 mg daily. In some embodiments, the maintenance dose of ralinepag is from 0.6 to 1.0 mg daily. In some embodiments, the maintenance dose of ralinepag is from 0.6 to 0.8 mg daily. In some embodiments, the maintenance dose of ralinepag is from 0.65 to 1.0 mg daily. In some embodiments, the maintenance dose of ralinepag is from 0.65 to 0.8 mg daily. In some embodiments, the maintenance dose of ralinepag is determined by tolerability. In some embodiments, the maintenance dose of ralinepag is greater than 0.4 mg daily. In some embodiments, the maintenance dose of ralinepag is greater than 0.6 mg daily.

[0047] In some embodiments, in a patient who receives a dose of ralinepag that cannot be tolerated, the dose of ralinepag is reduced to the previous tolerated dose. In some embodiments, the previous tolerated dose is the maximum tolerated dose for the patient.

[0048] In some embodiments, the amount of ralinepag is adjusted to account for a difference in bioequivalence between an immediate-release form and an extended-release form. For example, in some embodiments, 0.8 mg of ralinepag in an extended-release dosage form is provided to equate two 0.3 mg immediate-release dosage forms of ralinepag, where the extended-release dosage form has less than 100% bioequivalence with the immediate-release dosage forms.

[0049] In some embodiments, a therapeutically effective amount is suitable for administration once daily. In some embodiments, a therapeutically effective amount is suitable for administration twice daily. In some embodiments, a therapeutically effective amount is administered once daily. In some embodiments, a therapeutically effective amount is administered twice daily.

[0050] In various embodiments, ralinepag is titrated. In various embodiments, riociguat is titrated. In various embodiments, both ralinepag and riociguat are titrated. In some embodiments, riociguat is titrated in accordance with a product label approved by a regulatory authority (such as the U.S. FDA, see, ADEMPAS® label), which is incorporated herein by reference.

[0051] In various embodiments, ralinepag is titrated. In various embodiments, treprostinil is titrated. In various embodiments, both ralinepag and treprostinil are titrated. In some embodiments, treprostinil is titrated in accordance with a product label approved by a regulatory authority (such as the U.S. FDA, see, TYVASO® label), which is incorporated herein by reference.

[0052] In various embodiments, ralinepag is coadministered with one or more of treprostinil, iloprost, cisaprost, and epoprostenol.

[0053] In various embodiments, the coadministration methods disclosed herein are useful for treating PAH, for example idiopathic PAH; heritable PAH; familial PAH; PAH associated with: a collagen vascular disease, a congenital heart disease, a congenital heart disease with repaired shunts, portal hypertension, connective tissue disease, HIV infection, ingestion of a drug or toxin, hereditary hemorrhagic telangiectasia, splenectomy, pulmonary veno-occlusive disease (PVOD), or pulmonary capillary hemangiomatosis (PCH); and PAH with significant venous or capillary involvement. In some embodiments, the coadministration methods disclosed herein are useful for treating human subjects with symptomatic PAH. In some embodiments, the coadministration methods disclosed herein are useful for treating human subjects with PAH, WHO Functional Class I. In some embodiments, the coadministration methods disclosed herein are useful for treating human subjects with PAH, WHO Functional Class II. In some embodiments, the coadministration methods disclosed herein are useful for treating human subjects with PAH, WHO Functional Class III. In some embodiments, the coadministration methods disclosed herein are useful for treating human subjects with PAH, WHO Functional Class IV. In some embodiments, the coadministration methods disclosed herein are useful for treating human subjects with PAH, WHO Group I. In certain embodiments, the coadministration methods disclosed herein are useful for treating PAH patients with WHO Functional Class II-III symptoms. In various embodiments, the coadministration methods disclosed herein are useful for treating chronic thromboembolic pulmonary hypertension (CTEPH). In various embodiments, the coadministration methods disclosed herein are useful for treating persistent/recurrent CTEPH (WHO Group 4) after surgical treatment. In various embodiments, the coadministration methods disclosed herein are useful for treating inoperable CTEPH to improve exercise capacity and/or WHO functional class. In various embodiments, the coadministration methods disclosed herein are useful for treating PAH (WHO Group 1) to improve exercise capacity. In various embodiments, the coadministration methods disclosed herein are useful for improving WHO

functional class and/or to delay clinical worsening. In various embodiments, the coadministration methods disclosed herein are useful for delaying disease progression and/or reducing the risk of hospitalization for PAH.

BRIEF DESCRIPTION OF THE DRAWINGS

[0054] Figures 1A-1F show antiproliferative effects of ralinepag in combination with 100 nM riociguat. Growth arrested cells were incubated for 96 hours in human smooth muscle basal medium (SMBM) containing either 9% FBS ± 0.1% DMSO, FBS plus ralinepag and DMSO in the absence or presence of 100 nM riociguat and SMBM alone (time control). Cell proliferation was normalized to the growth response induced by FBS and DMSO, which were taken as the FBS and DMSO response minus the time control (= 100% growth at 4 days). Growth responses induced in presence of ralinepag and solvent ± riociguat are shown as % change in cell proliferation relative to the FBS response alone. Data were fit using a variable slope sigmoidal-curve fitting routine in GraphPad and parameters of each fit are shown. Data are from 5 individual patient cell isolates. * = $P < 0.05$, when compared to ralinepag alone; 2 WAY-ANOVA with Bonferroni post hoc test.

[0055] Figure 2 shows that ralinepag is a more effective inhibitor of serum-induced proliferation in human PASMCs from PAH patients in the presence of riociguat. Mean antiproliferative effects of increasing doses of ralinepag in the absence (A) and presence of 100 nM riociguat (B). Human PASMCs were grown in 9% serum (FBS) and 0.1% DMSO for 4 days ± drug(s). Cell proliferation was normalized to the growth response induced by FBS and DMSO, which was taken as the FBS response minus the time control (= 100% growth at 4 days). Growth responses induced in the presence of ralinepag and solvent ± riociguat are shown as % change in cell proliferation relative to the FBS response alone. Shown on the graph also is the effect riociguat (100 nM) in the presence of growth medium containing solvent * = $P < 0.05$, *** = $P < 0.001$ when compared to control (FBS and DMSO); 1 WAY-ANOVA with Bonferroni post hoc test (n = 5).

[0056] Figures 3A-3F show antiproliferative effects of ralinepag in combination with 100 nM sildenafil. Growth arrested cells were incubated for 96 hours in human smooth muscle basal medium (SMBM) containing either 9% FBS ± 0.1% DMSO, FBS plus ralinepag and DMSO in the absence and presence of 100 nM sildenafil or SMBM alone (time control). Cell proliferation was normalized to the growth response induced by FBS alone, which was taken as the FBS response minus the time control (= 100% growth at 4 days). Growth responses

induced in the presence of ralinepag plus solvent are shown as % change in cell proliferation relative to the FBS response alone. Data were fit using a variable slope sigmoidal-curve fitting routine in GraphPad and parameters of each fit are shown. Data are from 5 individual patient cell isolates.

[0057] Figure 4 shows the effect of sildenafil on the antiproliferative response of ralinepag in human PASMCs from PAH patients. Mean anti-proliferative effects of increasing doses of ralinepag in the absence (A) and presence of 100 nM sildenafil (B). Human PASMCs were grown in 9% serum (FBS) and 0.1% DMSO for 4 days ± drug(s). Cell proliferation was normalized to the growth response induced by FBS alone, which was taken as the FBS response minus the time control (= 100% growth at 4 days). Growth responses induced in the presence of ralinepag and solvent ± sildenafil are shown as % change in cell proliferation relative to the FBS response alone. Shown on the graph is the effect sildenafil (Sild; 100 nM) in the presence of growth medium containing solvent. *= $P < 0.05$, **= $P < 0.01$, *** = $P < 0.001$ when compared to control (FBS and DMSO); 1-WAY ANOVA with Bonferroni post hoc test (n = 5).

[0058] Figures 5A-5F show antiproliferative effects of ralinepag in combination with 100 nM treprostinil. Growth arrested cells were incubated for 96 hours in human smooth muscle basal medium (SMBM) containing either 9% FBS ± 0.1% DMSO, FBS plus ralinepag and DMSO in the absence and presence of 100 nM treprostinil or SMBM alone (time control). Cell proliferation was normalized to the growth response induced by FBS alone, which was taken as the FBS response minus the time control (= 100% growth at 4 days). Growth responses induced in the presence of ralinepag plus solvent are shown as % change in cell proliferation relative to the FBS response alone. Data were fit using a variable slope sigmoidal-curve fitting routine in GraphPad and parameters of each fit are shown. Data are from 5 individual patient cell isolates.

[0059] Figure 6 shows ralinepag is a more effective inhibitor of serum-induced proliferation in human PASMCs from PAH patients in the presence of treprostinil. Mean antiproliferative effects of increasing doses of ralinepag in the absence (A) and presence of 100 nM treprostinil (B). Human PASMCs were grown in 9% serum (FBS) and 0.1% DMSO for 4 days ± drug(s). Cell proliferation was normalized to the growth response induced by FBS alone, which was taken as the FBS response minus the time control (= 100% growth at 4 days). Growth responses induced in the presence of ralinepag and solvent ± treprostinil are shown as % change in cell proliferation relative to the FBS response alone. Shown on the

graph also is the effect treprostinil (Trep; 100 nM) in the presence of growth medium containing solvent * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$ when compared to control (FBS and DMSO); 1 WAY-ANOVA with Bonferroni post hoc test (n = 5).

[0060] Figures 7A-7F show antiproliferative effects of ralinepag in combination with 100 nM macitentan. Growth arrested cells were incubated for 96 hours in human smooth muscle basal medium (SMBM) containing either 9% FBS ± 0.1% DMSO, FBS plus ralinepag and DMSO in the absence and presence of 100 nM macitentan or SMBM alone (time control). Cell proliferation was normalized to the growth response induced by FBS alone, which was taken as the FBS response minus the time control (= 100% growth at 4 days). Growth responses induced in the presence of ralinepag plus solvent are shown as % change in cell proliferation relative to the FBS response alone. Data were fit using a variable slope sigmoidal-curve fitting routine in GraphPad and parameters of each fit are shown. Data are from 5 individual patient cell isolates.

[0061] Figure 8 shows the effect of macitentan on the antiproliferative response of ralinepag in human PASMCs from PAH patients. Mean anti-proliferative effects of increasing doses of ralinepag in the absence (A) and presence of 100 nM macitentan (B). Human PASMCs were grown in 9% serum (FBS) and 0.1% DMSO for 4 days ± drug(s). Cell proliferation was normalized to the growth response induced by FBS alone, which was taken as the FBS response minus the time control (= 100% growth at 4 days). Growth responses induced in the presence of ralinepag and solvent ± macitentan are shown as % change in cell proliferation relative to the FBS response alone. Shown on the graph is the effect macitentan (Maci; 100 nM) in the presence of growth medium containing solvent. *= $P < 0.05$, **= $P < 0.01$, *** = $P < 0.001$ when compared to control (FBS and DMSO); 1-WAY ANOVA with Bonferroni post hoc test (n = 5).

[0062] Figures 9A-9F show antiproliferative effects of ralinepag in combination with 100 nM bosentan. Growth arrested cells were incubated for 96 hours in human smooth muscle basal medium (SMBM) containing either 9% FBS ± 0.1% DMSO, FBS plus ralinepag and DMSO in the absence and presence of 100 nM bosentan or SMBM alone (time control). Cell proliferation was normalized to the growth response induced by FBS alone, which was taken as the FBS response minus the time control (= 100% growth at 4 days). Growth responses induced in the presence of ralinepag plus solvent are shown as % change in cell proliferation relative to the FBS response alone. Data were fit using a variable slope sigmoidal-curve fitting routine in GraphPad and parameters of each fit are shown. Data are from 5 individual

patient cell isolates.

[0063] Figure 10 shows the effect of bosentan on the antiproliferative response of ralinepag in human PASMCs from PAH patients. Mean anti-proliferative effects of increasing doses of ralinepag in the absence (A) and presence of 100 nM bosentan (B). Human PASMCs were grown in 9% serum (FBS) and 0.1% DMSO for 4 days ± drug(s). Cell proliferation was normalized to the growth response induced by FBS alone, which was taken as the FBS response minus the time control (= 100% growth at 4 days). Growth responses induced in the presence of ralinepag and solvent ± bosentan are shown as % change in cell proliferation relative to the FBS response alone. Shown on the graph is the effect bosentan (Bos; 100 nM) in the presence of growth medium containing solvent. *= $P < 0.05$, **= $P < 0.01$, *** = $P < 0.001$ when compared to control (FBS and DMSO); 1-WAY ANOVA with Bonferroni post hoc test (n = 5).

[0064] Figures 11A-11E show combinations of PAH therapies with ralinepag on cell proliferation in human PASMC cells. Comparisons were made in cells derived from the same PAH patients and passage and had been grown in 9% serum (FBS) and 0.1% DMSO for 4 days and treated with ralinepag ± riociguat (A), sildenafil (B), treprostinil (C), macitentan (D), or bosentan (E). Cell proliferation was normalized to the growth response induced by FBS plus solvent. Data are shown as mean ± S.E.M. in fit using a variable slope sigmoidal-curve fitting routine in Graph Pad, with parameters of each fit shown. * = $P < 0.05$ when compared to ralinepag alone; 1 WAY-ANOVA with Bonferroni post hoc test (n = 5).

[0065] Figure 12 shows comparisons of different PAH drug combinations with ralinepag on cell proliferation in human PASMCs from PAH patients. Data are shown as mean ± S.E.M. and taken from **Figures 1**, **3**, **5**, 7, and **9**. Concentration dependent effects of ralinepag on serum -induced growth are compared when this drug was combined with 100 nM of either bosentan, macitentan, sildenafil, treprostinil or riociguat. * = $P < 0.05$, ** = $P < 0.01$ when compared to ralinepag; 2 WAY-ANOVA with Bonferroni post hoc test.

DETAILED DESCRIPTION OF THE INVENTION

[0066] The present disclosure provides improved methods of administering ralinepag to patients in need thereof, particularly for patients suffering from PAH in its many forms, for example idiopathic PAH; familial PAH; PAH associated with: a collagen vascular disease, a congenital heart disease, portal hypertension, HIV infection, ingestion of a drug or toxin, hereditary hemorrhagic telangiectasia, splenectomy, pulmonary veno-occlusive disease

(PVOD) or pulmonary capillary hemangiomatosis (PCH); and PAH with significant venous or capillary involvement. The methods of the present disclosure also suitable for treating other conditions such as platelet aggregation; coronary artery disease; myocardial infarction; transient ischemic attack; angina; stroke; ischemia-reperfusion injury; restenosis; atrial fibrillation; blood clot formation in an angioplasty or coronary bypass surgery individual or in an individual suffering from atrial fibrillation; atherothrombosis; asthma or a symptom thereof; a diabetic-related disorder such as diabetic peripheral neuropathy, diabetic nephropathy or diabetic retinopathy; glaucoma or another disease of the eye with abnormal intraocular pressure; hypertension; inflammation; psoriasis; psoriatic arthritis; rheumatoid arthritis; Crohn's disease; transplant rejection; multiple sclerosis; systemic lupus erythematosus (SLE); ulcerative colitis; atherosclerosis; acne; type 1 diabetes; type 2 diabetes; sepsis; and chronic obstructive pulmonary disorder (COPD).

[0067] Pulmonary arterial hypertension (PAH) is a life-threatening disease characterized by a progressive pulmonary vasculopathy leading to right ventricular hypertrophy. Right heart failure occurs if left untreated. Prostacyclin, which has vasodilatory and antiproliferative effects on the pulmonary vasculature has been found to be low in patients with PAH compared with normal controls. Exogenous administration of prostacyclin or an analog of prostacyclin, e.g., prostanoids such as treprostinil, iloprost, and beraprost have been used to treat PAH. However prostacyclin, treprostinil and iloprost are not orally active and must be administered intravenously. Although beraprost is orally active, it has not been approved in Europe and the US.

[0068] Selexipag (2-{4-[(5,6-diphenylpyrazin-2-yl)(propan-2-yl)amino]butoxy}-*N*-(methanesulfonyl)acetamide) and its free carboxylic acid active metabolite, MRE-269, are also known for treating PAH. However, these drugs are expensive and have a relatively short half-life ranging from ~8, 3-4, and 0.5 hours, respectively *in vivo* (Kuwano et al., 2007; Clapp & Gurung, 2015). In contrast, and irrespective of the dose, ralinepag has a relatively long plasma half-life. Thus, ralinepag has the potential to provide improved therapeutic effectiveness in treating PAH (and related conditions as described herein) compared to known therapeutic agents.

[0069] The synthesis of ralinepag, and pharmaceutically acceptable salts, hydrates, polymorphs and solvates thereof, and a general description of pharmaceutical formulations and methods of treatment are disclosed in, e.g., US Appl. No. 12/933,196 (published as US 2011/0053958; issued as US 8,895,776). Methods of treating, including methods of titrating

ralinepag are disclosed in PCT/US 2015/056824 (published as WO 2016/065103). This latter application describes in detail various methods of optimizing the dose for a patient in need of treatment with ralinepag. In various embodiments, the initial dose of ralinepag is equivalent to 0.01 mg of the free acid form (referred to as the dose equivalent to 0.01 mg), and is either dosed once per day (QD) or twice per day (BID). If this dose is tolerated, the frequency of dosing is increased in the second week (e.g., from QD to BID dosing), and/or the amount of ralinepag is increased, e.g., from an initial dose equivalent to 0.01 mg BID, in weekly increments of e.g., 0.01 mg (dose equivalent) up to a BID dose of 0.3 mg ralinepag.

[0070] As used herein, a patient is said to "tolerate" a dose of a compound such as ralinepag if administration of that dose to the patient does not result in an unacceptable adverse event or combination of adverse events. One of skill in the art will appreciate that tolerance is subjective, and the amount that is tolerable by one patient may be intolerable to a different patient. Typically, tolerance reflects a subjective balance between the clinical benefits of the dose relative to any adverse events. As described herein, "adverse events" refers to undesirable or unwanted clinical symptoms associated with treatment with the compound. With regard to ralinepag, typical adverse events include headache, nausea, vomiting, jaw pain, flushing, abnormal pulse rate, abnormal QT interval, a sitting systolic blood pressure greater than about 160 mmHg, a sitting diastolic blood pressure greater than about 100 mmHg, a systolic blood pressure less than about 90 mmHg, abdominal pain, nosebleed, muscle aches, feelings of warmth, palpitations, dizziness, itching, diarrhea, chest pressure, joint aches, prickling or tingling skin sensations, chest pain, chest discomfort, erythema, or combinations of any of the above.

[0071] An "optimized dose" or "optimal dose" refers to a therapeutic dose, typically the highest therapeutic dose of the compound (or pharmaceutically acceptable salts, solvate, hydrates, etc. thereof) which elicits the maximum desired clinical benefits to the patient, while minimizing intolerable side effects (e.g., adverse events). One of skill in the art will recognize that the optimal dose can vary from patient to patient, or even over time for a specific patient.

[0072] The need to titrate ralinepag to minimize side effects and identify optimized or optimal dose can be time-consuming. For example, in many embodiments two to as many as about nine weeks of titration (i.e., gradually increasing the dose and/or frequency of dosing, typically on a weekly basis) may be required to identify the optimal dose of ralinepag. During this titration period, the patient can remain at a suboptimal dose for an appreciable period of

time, which is undesirable. Given the severity of PAH, it would be highly desirable to achieve the optimal dose as quickly as possible. Furthermore, in order to improve patient compliance, it would be desirable to administer ralinepag on a QD schedule rather than BID dosing schedule.

[0073] As described herein, co-administering ralinepag with cGMP or cAMP elevating agents such as riociguat, or co-administering ralinepag with a prostacyclin receptor agonist, or with a prostanoid such as treprostinil or iloprost provides unexpected advantages. Specifically, these combinations provide substantial improvements. In some embodiments, the improvement allows for superior efficacy compared to either monotherapy. In some embodiments, the improvement allows for a reduction in the dosing of ralinepag and/or cAMP elevating agents, cGMP elevating agents, prostacyclin receptor agonists, or prostanoids. Alternatively, or in addition, because such combinations provide substantial improvement, optimal clinical results can be obtained at lower doses of ralinepag and/or the cAMP elevating agents, cGMP elevating agents, prostacyclin receptor agonists, or prostanoids (compared to the respective monotherapies), thereby providing the clinical benefits of a monotherapy treatment method but with reduced side effects. Alternatively, the combinations of the present method can provide both improved clinical benefit and reduced side effects.

[0074] The desired clinical benefit can be measured by any clinical metric suitable or used in the art for measuring improvement in a patient. For example, a clinical benefit could be, e.g., an increase in at least 40 meters measured by the "six-minute walk test" (6MWT) according to the American Thoracic Society guidelines for the Six-minute Walk Test disclosed in *Am. J. Respir. Crit.Care Med.* Vol. 166, p. 111-117, 2002; DOI: 10.1164/rccm.166/1/111. "Equivalent" or substantially similar clinical benefit means a clinical benefit measured by conventional clinical metrics, for example as measured by 6MWT, which provides substantially the same result in an otherwise identical patient, or the same patient, when comparing the combination therapy of ralinepag and at least one cAMP elevating agent, cGMP elevating agent, prostacyclin receptor agonist, or prostanoid described herein, compared to an otherwise identical patient (similar physiological characteristics, clinical presentation, and response to ralinepag) experiencing a similar level of adverse events or side effects.

[0075] In some embodiments, coadministered ralinepag and at least one cAMP elevating agent, cGMP elevating agent, prostacyclin receptor agonist, or prostanoid as described herein

provides an improvement in clinical benefit compared to ralinepag monotherapy using the same or a higher dose of ralinepag. For example, the combination therapy as described herein can exhibit an increase in clinical benefit using the 6MWT metric of at least about 5 m, at least about 10 m, at least about 15 m, at least about 20 m, at least about 25 m, or at least about 30 m compared to the same daily dose administered to an otherwise identical patient using a ralinepag monotherapy (i.e., without coadministered cAMP elevating agent, cGMP elevating agent, prostacyclin receptor agonists, or prostanoids).

[0076] Similarly, as described herein, co-administering ralinepag with a cAMP elevating agent, cGMP elevating agent, prostacyclin receptor agonist, or prostanoid, the dose of such cAMP elevating agent, cGMP elevating agent, prostacyclin receptor agonist, or prostanoid can be reduced relative to the amount required to achieve an equivalent therapeutic effect in an otherwise identical patient administered such cAMP elevating agent, cGMP elevating agent, prostacyclin, or prostanoid as a monotherapy (i.e., without ralinepag). For example, as discussed herein, the dose of the respective cAMP elevating agent, cGMP elevating agent, prostacyclin receptor agonist, or prostanoid can be reduced by about 1%, about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, or about 50%, relative to the dose required without ralinepag, while achieving a similar level of clinical efficacy, for example as measured by 6MWT and/or side effect level. Alternatively, the combination of ralinepag and cAMP elevating agent, cGMP elevating agent, prostacyclin receptor agonists, or prostanoid provides an improvement in efficacy, e.g., as measured by 6MWT (at least about 5 m, at least about 10 m, at least about 15 m, at least about 20 m, at least about 25 m, or at least about 30 m) compared to a patient administered similar levels of cAMP elevating agent, cGMP elevating agent, prostanoid, or a prostacyclin receptor agonist without ralinepag.

[0077] In one embodiment, when co-administering ralinepag with riociguat, prostacyclin, treprostinil, or iloprost (or other prostanoids), significantly lower doses of ralinepag in the combination therapy can be administered compared to the amount required in a ralinepag monotherapy to achieve the same or substantially similar clinical benefit in otherwise identical patients (or in the same patient). That is, the combination of ralinepag and riociguat, prostacyclin, treprostinil, or iloprost act synergistically, so that the clinical effects of ralinepag are potentiated or enhanced by the coadministration of riociguat, prostacyclin, treprostinil, or iloprost. For example, the daily dose of ralinepag required in the combination therapy described herein can be at least about 0.01 mg, 0.02 mg, 0.03 mg, 0.04 mg, 0.05 mg,

0.06 mg, 0.07 mg, 0.08 mg, 0.09 mg, 0.1 mg, 0.15 mg, 0.2 mg, 0.225 mg, 0.25 mg, or 0.275 mg, or 0.3 mg lower than the daily ralinepag dose required in a monotherapy to achieve the equivalent, or substantially the same clinical benefit in an otherwise identical patient (or in the same patient). The daily dose can be provided under a QD or BID dosing protocol.

[0078] In other embodiments, when co-administering ralinepag with riociguat, prostacyclin, treprostinil, or iloprost (or other prostanoids), the same dose of ralinepag can be administered in the combination therapy that could be administered in a ralinepag monotherapy. However, in the combination therapy, a substantially improved clinical benefit is provided, e.g., as measured by 6MWT (at least about 5 m, at least about 10 m, at least about 15 m, at least about 20 m, at least about 25 m, or at least about 30 m) with a substantially similar side effect profile (i.e., comparing the combination therapy with monotherapy in otherwise identical patients, or in the same patient).

[0079] In still other embodiments, equivalent, or substantially similar clinical benefits are provided by the combination of significantly lower doses of ralinepag and reduced doses of riociguat, prostacyclin, treprostinil, or iloprost (or other prostanoids). For example, the daily dose of ralinepag required in the combination therapy described herein can be at least about 0.01 mg, 0.02 mg, 0.03 mg, 0.04 mg, 0.05 mg, 0.06 mg, 0.07 mg, 0.08 mg, 0.09 mg, 0.1 mg, 0.15 mg, 0.2 mg, 0.225 mg, 0.25 mg, or 0.275 mg, or 0.3 mg lower than the ralinepag dose required in a monotherapy to achieve the same, or substantially the same clinical benefit in an otherwise identical patient (or in the same patient). The daily dose of riociguat, prostacyclin, treprostinil, or iloprost (or other prostanoids) can be reduced by about 1%, about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, or about 50%, relative to the dose required in a monotherapy without ralinepag to achieve the equivalent, or substantially the same clinical benefit in an otherwise identical patient (or in the same patient). For example, the daily dose of riociguat required in the combination therapy described herein can be at least about 0.1 mg, 0.2 mg, 0.3 mg, 0.4 mg, 0.5 mg, 0.6 mg, 0.7 mg, 0.8 mg, 0.9 mg, 1.0 mg, 1.25 mg, 1.5 mg, 1.75 mg, or 2 mg lower than the riociguat dose required for an equivalent clinical benefit and/or adverse event profile. The daily dose can be provided under a QD or BID dosing protocols.

[0080] In some embodiments, a therapeutically effective amount of ralinepag is administered contemporaneously with one or more cAMP elevating agent, cGMP elevating agent, prostacyclin receptor agonist, or prostanoid such that therapeutically effective amounts of ralinepag and the one or more cAMP elevating agent, cGMP elevating agent, prostacyclin

receptor agonist, or prostanoid are both present within the patient and exerting their therapeutic effects at the same time. Ralinepag can be administered prior to, simultaneously with, or subsequent to the cAMP elevating agent, cGMP elevating agent, prostacyclin receptor agonists, or prostanoid. If both ralinepag and the cAMP elevating agent, cGMP elevating agent, prostacyclin receptor agonists, or prostanoid administered at the same time, they can be administered separately in different dosage forms, or in some embodiments in a combined dosage form. When administered in separate dosage forms, the ralinepag can be administered orally in the form of a tablet or capsule, and the cAMP elevating agent, cGMP elevating agent, prostacyclin receptor agonists, or prostanoid can be administered orally (if oral dosage forms are available or possible) or as an intravenous or subcutaneous dosage form. In some embodiments, the ralinepag can also be administered as an injectable or IV dosage form.

[0081] In one embodiment, ralinepag is coadministered with riociguat. In another embodiment, ralinepag is coadministered with treprostinil. In yet another embodiment, ralinepag is coadministered with iloprost. In still yet another embodiment, ralinepag is coadministered with prostacyclin. In another embodiment, ralinepag is coadministered with riociguat and treprostinil. In another embodiment, ralinepag is coadministered with riociguat and iloprost.

[0082] Ralinepag is a highly selective IP receptor agonist. In normal human pulmonary arterial smooth muscle cells (PASMCs), the IP receptor, through the generation of cyclic AMP appears to be the main mediator of the antiproliferative responses to treprostinil and iloprost (Wharton *et al.*, 2000; Clapp *et al.*, 2002; Falcetti *et al.*, 2010). In contrast, in human PASMCs isolated from idiopathic pulmonary arterial hypertension (IPAH) patients, neither the IP receptor nor cyclic AMP appeared to be the main mediator underlying the antiproliferative effects of treprostinil and iloprost, at least at the concentrations studied (Falcetti *et al.*, 2010).

[0083] The functional effects of ralinepag in pulmonary arteries or cells from microvessels of normal tissue or from PAH patients was previously unknown, and thus the pharmacological behavior of such selective IP agonists in the pulmonary circulation was unpredictable. Ralinepag was therefore evaluated with other prostacyclin drugs (which are expected to have diverse effects through activation of additional targets, including other prostanoid receptors (Clapp & Gurung, 2015)) to ascertain how it compares in its ability to generate cAMP and inhibit smooth muscle proliferation in PASMCs isolated from PAH patients.

Examples

[0084] The effects of combinations of ralinepag with endothelin-1 receptor antagonists (ERA), phosphodiesterase type 5 (PDE 5) inhibitors, or cAMP/cGMP elevating agents (e.g., soluble guanylate cyclase activators) was evaluated herein.

Source of lung tissue from hypertensive and normal patients

[0085] Lung tissue samples were obtained from patients with group 1 PAH or group 3 PAH (PAH associated with lung diseases) who were either undergoing transplantation after failed treatment, or who had died. For control samples, donor lungs found to be unsuitable for transplantation or from lung resection for suspected malignancy were used (Benyahia *et al.*, 2013).

[0086] Primary cell lines of distal PASMCs from PAH patients were derived from explanted lungs as previously described (Falcetti *et al.*, 2010; Bubb *et al.*, 2014). These cells have an abnormal proliferative capacity when grown in culture (Zhang *et al.*, 2007; Falcetti *et al.*, 2010). Frozen cells were revived and grown in human smooth muscle basal medium-2 (SMBM; Lonza, Slough, UK) supplemented with 9% fetal bovine serum (FBS) at 37°C in a humidified atmosphere of 5% CO2. After reaching confluence, cells were washed with phosphate-buffered saline (PBS) and treated with trypsin-EDTA for further passage. Only cells between passages 3 and 10 were used for experiments.

Cyclic AMP assays

[0087] Human PASMCs from PAH patients were grown to 70-80% confluence in 12 well plates in DMEM/F12 containing 9% foetal bovine serum (FBS), penicillin (50U/ml)/streptomycin (50μg/ml). To assess the time-course of cAMP elevation, cells were stimulated with 100 nM ralinepag for varying times (0.5, 1, 2, 4, 8, 24, 48 hours). This dose of ralinepag was chosen on the basis that it was close to the EC_{50} for cAMP generation in preliminary experiments. In other experiments, the IP receptor agonist was added for a specified time (60 minutes) over a full concentration range (0.1-10,000 nM) in the absence and presence of 1 μM of the IP receptor antagonist, RO-1138452. The antagonist is added 30 minutes beforehand and remained throughout.

[0088] To extract cyclic AMP, the medium was aspirated and PASMCs cells incubated in 0.1 M HCl for 20 minutes on ice followed by centrifugation at 1000 g for 10 minutes at 4°C. The protein concentration in the supernatant was determined using a Bradford based protein assay

(BCA, Novagen, EmD Chemicals, CA, USA). Intracellular cyclic AMP was measured using a competitive enzyme immunoassay 96 well plate kit (ADI-900-163, Enzo Life Sciences, Exeter, UK) and the assay performed according to the manufacturer's instructions. Each sample was run in duplicate and data expressed as pmol of cAMP per mg of total protein for each individual sample. Basal levels were subtracted from each agonist concentration data point. Given the large variation (~15-fold) in cAMP generation from different patient samples, data was also normalized to the peak cyclic AMP response (assigned 100%) in each cell isolate.

Cell proliferation assays

[0089] Comparative concentration-dependent effects of IP receptor agonists on cell proliferation were assessed on human PASMCs derived from PAH patients. Cells were seeded onto 96-well plates at a density of 1 x 10^4 cells/ml and grown at 37 °C in a humidified CO_2 incubator in human smooth muscle basal medium-2 (SMBM; Lonza) containing 9% FBS and penicillin-streptomycin (Pen/Strep; 50 units/ml). After 24 hours, cells were growth arrested by incubating for 48 hours in fresh media containing no added serum. Media was then subsequently removed, replaced with human SMBM containing 9% serum with or without 0.1% DMSO in the absence and presence of the IP receptor agonist (either ralinepag, iloprost, treprostinil or MRE-269) and cells treated with the IP receptor agonist for 4 days over the concentration range (10-12-10-5 M). Responses were directly compared in cells containing the IP receptor antagonist, RO-1138452 (1μM), which was added to cells 30-60 minutes prior to the addition of the IP agonist and remained throughout the experiment. Cells incubated with no added serum over the same time period (4 days) acted as the time control.

[0090] Cell proliferation was obtained using an MTS proliferation kit (Promega), a colorimetric method for determining the number of viable cells which is based on the cleavage of the tetrazolium salt MTS to formazan by cellular mitochondrial dehydrogenases. An increase in cell number leads to a proportional increase in the amount of formazan dye formed which is quantified by measuring the absorbance of the dye solution at 490 nm. In each case background absorbance was corrected by subtracting the average absorbance from the "no cell" control wells from all other absorbance values.

[0091] Cell proliferation was then normalized to the growth response induced by FBS alone, which was taken as the FBS response minus the time control (=100% growth at 4 days). Growth responses induced in presence of solvent ± drugs is shown as % change in cell

proliferation relative to the FBS response alone in all figures.

Materials, reagents, equipment

- Human Smooth Muscle Basal Medium-2 (Lonza, Slough, UK; Cat No. CC-3181)
- DMEM/F12 (Life Technologies, Paisley, UK; Cat No 11320-074)
- Foetal bovine serum South American (FBS; Invitrogen, Cat No 10270106)
- Penicillin-Streptomycin Pen/Strep (5000 units/ml; Life Technologies, Paisley, UK; Cat No 15070-063)
- Sterile Ca2+/Mg2+ free phosphate buffered saline (PBS; Life Technologies, Cat No 10010-056)
- Sterile Trypsin/ EDTA solution (0.05%; Life Technologies, Cat No 25300-054)
- Sterile dimethyl sulfoxide (DMSO; Sigma-Aldrich, Cat No, D2650)
- MRE-269 ([4[(5,6diphenylpyrazinyl)(1methylethyl) amino]butoxy]acetic acid (CAY10010412), iloprost (50:50 R/S isomer; CAY 18215), treprostinil (CAY10162) and RO-1138452 (IP receptor antagonist; CAY 10441), riociguat (Cat No 2644-5), sildenafil citrate (Cat No 2872-10), macitentan (Cat No M009) and treprostinil (CAY10162) were purchased from Cambridge Bioscience, UK (distributor for Cayman Chemical Co). Bosentan (Cat No 11731) was purchased from Cayman Chemicals Company (USA).
- Iloprost came dissolved in methyl acetate which was blown off and replaced with DMSO to give a stock solution of 10 mM. All other stock solutions were made up in DMSO (treprostinil, RO-1138452, ralinepag, MRE-269) to a final concentration of 10 mM. Drugs were serially diluted in growth medium, with the solvent concentration in each well remaining constant at 0.11% regardless of the concentration of the agent added.
- Competitive enzyme immunoassay 96 well plate cAMP kit (ADI-900-163, Enzo Life Sciences, Exeter, UK)
- Cell proliferation assay kit (MTS, Promega, UK, Cat No G5421)
- BCA (bicinchoninic acid) protein assay kit (Cat No. 71285-3; Novagen, Merck Millipore, Nottingham, UK)
- Galaxy R CO2 cell culture incubator (WolfLabs Ltd, York UK)
- Tecan Genios Microplate Reader (Tecan Group Ltd, Männedorf , Germany)
- OpsysMRTM Microplate Reader (Dynex Technology, Chantilly, VA, USA)

Data and statistical analysis

[0092] All data are presented as mean ± standard error of mean (S.E.M) of at least 5 observations. Agonist log-concentration curves were constructed and fitted using the non-linear fitting routine in GraphPad Prism 4 or 6 (San Diego, CA, USA). The concentration of agonist causing 50% of the maximal response (E_{max}) was expressed as the negative log (pEC_{50}) and the mean EC_{50} calculated. Statistical analysis was performed using one or two way ANOVA with post-hoc correction as indicated in the figure legends. *P* values < 0.05 are considered statistically significant.

Example 1: Antiproliferative effects of ralinepag in combination with cyclic GMP and cyclic AMP elevating agents or endothelin-1 antagonists

[0093] The antiproliferative effects of ralinepag in human pulmonary smooth muscle cells (PASMCs) from PAH patients were compared with other prostacyclin mimetics, and compared in combination with an endothelin-1 receptor antagonist (ERA), a phosphodiesterase type 5 (PDE5) inhibitor or a soluble guanylate cyclase (sGC) activator.

[0094] Ralinepag and MRE-269 behaved as selective IP receptor agonists in cyclic AMP and cell proliferation assays in human PASMCs from PAH patients, with ralinepag producing 2-fold more cyclic AMP and 10-fold more antiproliferation effects. Both ralinepag and MRE-269 produced weaker maximal effects in the cyclic AMP and cell proliferation assays than treprostinil. Treprostinil and iloprost exhibited inhibition of cell proliferation through IP-independent mechanisms.

Antiproliferative effects of ralinepag in combination with riociguat

[0095] The concentration-dependent antiproliferative effects of ralinepag in the absence and presence of 100 nM riociguat in human PASMCs grown in 9% serum and 0.1% DMSO for four days are shown in **Figure 1**. In four out of the five cell isolates, there was a greater inhibition of cell growth when riociguat was combined with ralinepag compared to ralinepag alone. From the mean data, overall, ralinepag was more effective in the presence of riociguat across the entire concentration range (0.01-10,000 nM), being significantly more effective at 10 nM and above ($P<0.05$, two way ANOVA, with Bonferroni post-hoc correction).

[0096] Riociguat alone (100 nM) caused significant inhibition (~15%) of cell proliferation compared to FBS and DMSO. At 100 nM, ralinepag inhibited growth by 28% (**Figure 2A**),

which was almost double the inhibition induced by the same dose of riociguat (**Figure 2B**). However, in the presence of 100 nM riociguat, significant inhibition of cell growth occurred at a 100-fold lower dose (0.01 nM) of ralinepag. This suggests that the agents work on separate pathways to enhance inhibition of proliferation—riociguat through cGMP (Lang *et al.*, 2012) and ralinepag through the IP receptor and cAMP generation (proliferation was completely inhibited by the IP receptor antagonist RO1138452 in experiments provided herein). Potentiation occurred at sub nanomolar concentrations of ralinepag. At the higher doses of ralinepag, inhibition of cell growth appeared to be more than predicted from additive effects.

[0097] Riociguat has been shown to induce apoptosis and inhibit proliferation of pulmonary artery cells associated with an up-regulation of soluble guanylyl expression and increased cyclic GMP production (Lang *et al.*, 2012). Thus, potentiation of ralinepag effects by riociguat may result from elevated cyclic AMP levels in response to cyclic GMP-dependent inhibition of PDE3, an isoform known to regulate cAMP generation induced by IP receptor agonists (Knebel *et al.*, 2013). Previous experiments showed that riociguat was the most effective antiproliferative combination with treprostinil compared to either PDE5 inhibitors or ERAs in human PASMCs from PAH patients (Patel *et al.*, 2014). However, the combined effects were less than predicted if additive. Both agents may work on separate pathways (cyclic GMP and the IP receptor/cAMP pathway) with some crossover in terms of mechanism of growth inhibition. For example, inhibition of cell growth through inhibition of the calcium-dependent phosphatase, calcineurin, could occur through both cyclic GMP and cyclic AMP (Jabr *et al.*, 2007; Lu *et al.*, 2013).

Antiproliferative effects of ralinepag in combination with sildenafil

[0098] The concentration-dependent antiproliferative effects of ralinepag in the absence and presence of 100 nM sildenafil in human PASMCs grown in 9% serum and 0.1% DMSO for four days are shown in **Figure 3**. In four out of the five cell isolates, there was a greater inhibition of cell growth when sildenafil was combined with ralinepag compared to ralinepag alone. Ralinepag appeared significantly ($P<0.001$, two-way ANOVA) more effective in the presence of sildenafil across the entire concentration range (0.01 -10,000 nM), though significance at individual drug doses was not found with a Bonferroni post-hoc analysis. The mean antiproliferative effects of ralinepag in the absence and presence of 100 nM sildenafil compared with responses to 9% serum and 0.1% DMSO alone are shown in **Figure 4**. Unlike riociguat, sildenafil (100 nM) did not significantly inhibit growth induced by serum.

However, when combined with ralinepag, there was further inhibition of cell growth compared to ralinepag alone. At 1 nM ralinepag, growth was inhibited by 14% rising to 27% in the presence of sildenafil, though the magnitude of these changes were smaller at the highest (10μM) dose tested (56% inhibition of cell growth as opposed to 50%).

[0099] The potentiating effect of sildenafil was found to be less than was observed with riociguat. This may due to riociguat being a direct activator of guanylate cyclase, producing a greater rise in cyclic GMP than sildenafil, which would be expected to increase basal cyclic GMP levels. Furthermore, in three of five cell isolates, the antiproliferative effects of the drug combination tapered off at higher doses of ralinepag, consistent with some overlap of downstream mechanisms of PDE5 inhibitors and IP receptor agonists. Indeed, sildenafil inhibits cell proliferation in part through activation of PPARγ (Wang *et al.*, 2013), a mechanism known to underlie the antiproliferative effects of treprostinil in these pulmonary smooth muscle cells (Falcetti *et al.*, 2010). It is likely for sildenafil that the dose used in these experiments is below the therapeutic dose, as the upper plasma levels of this drug in patients was 1500 nM (Burgess *et al.*, 2008). It is, however, well above the K_i for PDE5 inhibition reported for sildenafil, which is reported to be 1-3 nM (Ballard *et al.*, 1998). At 100 nM, sildenafil is also likely to inhibit PDE1 and PDE6 activity (K_i 40 nM and 10 nM, respectively), so its mode of action therapeutically may not entirely be related to inhibition of PDE5 (Bischoff, 2004).

Antiproliferative effects of ralinepag in combination with treprostinil

[0100] The antiproliferative effects of ralinepag in the absence and presence of 100 nM treprostinil in human PASMCs grown in 9% serum and 0.1% DMSO for 4 days is shown in **Figure 5**. In four out of the five cell isolates, there was a greater inhibition of cell growth when treprostinil was combined with ralinepag compared to ralinepag alone. From the mean data, overall ralinepag appeared more effective in the presence of treprostinil across the entire concentration range ($P<0.001$, two-way ANOVA), though only the highest dose (10 μM) of ralinepag was significantly enhanced by treprostinil in a post hoc test ($P<0.05$, two-way ANOVA with Bonferroni correction).

[0101] Mean antiproliferative effects of ralinepag in the absence and presence of 100 nM treprostinil compared with responses to 9% serum and 0.1% DMSO alone are shown in **Figure 6**. Treprostinil significantly inhibited growth at 100 nM compared to serum and DMSO alone. When combined with ralinepag, significant inhibition of cell growth occurred

at a 10-fold lower dose (0.1 nM) of ralinepag. At the highest dose tested (10μM), ralinepag produced a 69% inhibition of cell growth as opposed to 50% in the absence of treprostinil. This greater inhibition of serum-induced cell proliferation can be accounted for in part by the magnitude of the inhibition induced by treprostinil alone, and suggests that both agents are inhibiting cell proliferation via separate pathways.

[0102] That treprostinil enhanced ralinepag responses might be surprising given that both agents are IP agonists and would presumably activate the same pathway to inhibit cell proliferation. Treprostinil is also a potent activator of EP2 and DP1 receptors (reviewed in Clapp & Gurung, 2015), which may explain the greater response to ralinepag in the presence of treprostinil. In experiments provided herein, it was observed that the antiproliferative responses to treprostinil were only weakly inhibited by an IP receptor antagonist, whereas those responses to ralinepag were completely inhibited.

Antiproliferative effects of ralinepag in combination with endothelin-1 receptor antagonists (ERAs)

[0103] The antiproliferative effects of ralinepag in the absence and presence of 100 nM macitentan in human PASMCs grown in 9% serum and 0.1% DMSO for 4 days are shown in **Figure 7**. Macitentan (100 nM) only weakly affected responses to ralinepag when combined, and in two out of the five cell isolates, did not enhance the antiproliferative effects at any concentration of ralinepag investigated. In the other three cell isolates, there was a trend to a greater inhibition of cell growth, which was more apparent at the lower doses of ralinepag when combined with 100 nM macitentan. However, overall from the mean data, ralinepag was not significantly more effective in the presence of macitentan compared to ralinepag alone.

[0104] Mean antiproliferative effects of ralinepag in the absence and presence of 100 nM macitentan compared with responses to 9% serum and 0.1% DMSO alone are shown in **Figure 8**. Macitentan alone (100 nM) caused a significant inhibition (~12%) of cell proliferation compared to serum and DMSO. Furthermore, when combined with macitentan, ralinepag inhibited cell proliferation at a 10-fold lower dose (0.1 nM) compared to ralinepag alone. At higher concentrations of ralinepag (>10 nM), effects converged, such that responses to ralinepag were similar in the absence or presence of macitentan.

[0105] The modes of action of these two drugs may overlap, and thus provide no real added benefit when combined. Macitentan is a mixed ET-1 antagonist, inhibiting binding to ETA

and ETB receptors with a mean IC_{50} value of 0.5 nM and 391 nM, respectively (Iglarz *et al.*, 2014). Thus at the concentration used, macitentan would predominately inhibit ETA receptors, receptors known to contribute to ET-1 induced cell proliferation of distal human PASMCs (Zamora *et al.*, 1993; Davie *et al.*, 2002). On the other hand, prostacyclin analogues inhibit serum or transforming growth factor β (TGF-β) induced release of ET-1 in human distal PASMCs, and such a mechanism is postulated in part to underlie the antiproliferative effects of prostacyclin analogues in normal human PASMCs (Wort *et al.*, 2001; Davie *et al.*, 2002).

[0106] The antiproliferative effects of ralinepag in the absence and presence of 100 nM bosentan in human PASMCs grown in 9% serum and 0.1% DMSO for 4 days are shown in **Figure 9**. In contrast to the other PAH drugs, bosentan did not enhance the antiproliferative response to ralinepag in four out of the five cell isolates, and in one cell isolate, actually decreased its response. Overall, from the mean data, ralinepag was not more effective in the presence of bosentan across the entire concentration. Shown in **Figure 10** are the mean antiproliferative effects of ralinepag in the absence and presence of 100 nM bosentan compared with responses to 9% serum and 0.1% DMSO alone. Unlike macitentan, bosentan (100 nM) did not cause a significant inhibition of cell proliferation when compared to serum and DMSO alone. Furthermore, when combined with ralinepag, bosentan did not enhance inhibition of cell proliferation compared to ralinepag alone, nor did it lower the concentration (1nM), at which ralinepag started to significantly inhibit cell proliferation as observed with all other agents (riociguat, treprostinil, and macitentan). Thus, bosentan appears to have less of an inhibitory effect on cell proliferation than macitentan, possibly related to its lower potency at the ETA receptor and/or differential receptor ET-1 receptor selectivity. While bosentan is like macitentan a mixed ET-1 receptor antagonist, its potency and selectivity ratio against ETA and ETB receptors is somewhat different as are its receptor kinetics. Bosentan has a 10-fold lower potency than macitentan with a Ki of 4 nM for the ETA receptor, but only has a selectivity ratio of ETA/ETB of ~20 (Davie *et al.*, 2009), compared to a selectivity ratio of 780 for macitentan (Iglarz *et al.*, 2014).

Comparisons of different PAH drug combinations with ralinepag

[0107] It appears that cGMP elevating agents and treprostinil combine with ralinepag to provide a greater antiproliferative effect, whereas ETRAs do not significantly enhance ralinepag effects on cell proliferation (**Figure 11**). At 10 nM ralinepag, growth was inhibited by 45% when combined with riociguat and by only 21% in the presence of bosentan (**Figure**

12). Compared to riociguat, and to a lesser extent with treprostinil, there was however a smaller potentiating effect with sildenafil (no significant difference with a post-hoc test, even though there is a significant drug interaction). A summary of the antiproliferative effects of ralinepag in the absence and presence of different PAH therapies, where EC_{50} and E_{Max} values were obtained for each individual fit (using data contained in **Figures 1**, **3**, **5**, 7, and **9** are presented as the mean ± S.E.M in **Table 1**. **Table 1** shows that ralinepag is ~2.5-5 times more potent in the presence of riociguat and sildenafil, respectively (EC_{50} = 10 nM and 5 nM). Overall, ralinepag produces a significantly greater maximum response in the presence of riociguat or treprostinil, but not when combined with other PAH therapies. An analysis where EC_{50} and E_{Max} values were obtained from fits to mean data (**Figure 11**) are presented in **Table 2**. The EC_{50} for ralinepag is slightly higher than from individual fits (44 nM as opposed to 25 nM), though potency was still enhanced 8-fold in the presence of riociguat and sildenafil. Ralinepag still produced a significantly greater maximum response in the presence of riociguat or treprostinil, but not with the other PAH therapies.

Table 1. Mean anti-proliferative effects of ralinepag in combination with other PAH drugs (extrapolated log EC_{50} and Emax from individual fits)

APD811 + 100nM Drug	Log EC_{50} (n=5)	EC_{50}	E_{max} (% inhibition)
APD811	-7.60 ± 0.52	25 nM	58.2 ± 4.1%
+ Bosentan	-7.69 ± 0.47	20 nM	52.8 ± 4.3%
+ Macitentan	-7.42 ± 0.62	38 nM	65.3 ± 3.8%
+ Sildenafil	-8.30 ± 0.53	5 nM	58.5 ± 4.7%
+ Treprostinil	-7.29 ± 0.35	51 nM	77.2 ± 6.6%
+ Riociguat	-8.01 ± 0.55	10 nM	76.0 ± 4.7%

Table 2. Mean anti-proliferative effects of ralinepag in combination with other PAH drugs (extrapolated log EC_{50} and Emax from mean fits)

Drug	Log EC_{50} (n=5)	EC_{50}	E_{max} (% Inhibition)
APD811	-7.35 ± 0.79	44 nM	59.6 ± 17.3%
+ Bosentan	-7.21 ± 0.83	61 nM	57.1 ± 15.6%
+ Macitentan	-7.49 ± 0.78	32 nM	58.5 ± 13.9%
+ Sildenafil	-8.26 ± 0.72	5 nM	59.0 ± 13.1%
+ Treprostinil	-7.37 ± 0.86	42 nM	76.1 ± 20.1%
+ Riociguat	-8.12 ± 0.72	6 nM	73.1 ± 13.1%

Example 2: Clinical Trial

[0108] A 22-week randomized, double-blind, placebo-controlled study with a dose titration period of up to 9 weeks was conducted. Sixty-one patients were randomized 2:1 ralinepag to placebo. Right Heart Catheterization (RHC) measurements were obtained prior to study Day 1 of the dose titration period and at Week 22. The following values were obtained and recorded: pulmonary artery pressure (PAP) (systolic, diastolic, and mean), heart rate (HR), right atrial pressure (RAP), pulmonary capillary wedge pressure (PCWP) right ventricular pressure (RVP) and cardiac output (CO), pulmonary vascular resistance (PVR), arterial and mixed venous oxygen saturation (FiO2) (if applicable). Systemic vascular resistance (SVR) was estimated from blood pressure measurements. All patients were receiving background PAH treatment with an endothelin receptor antagonist, phosphodiesterase type-5-inhibitor, or soluble guanylate cyclase activator, alone or in combination.

[0109] The primary efficacy endpoint for the study was change from baseline in PVR after 22 weeks of treatment. Additional analyses included change from baseline in 6 MWD after 22 weeks of treatment, hemodynamics, and safety and tolerability. Ralinepag was administered as a capsule in 0.01, 0.02, 0.03, 0.04, and 0.10 mg dose strengths.

[0110] The starting dose of ralinepag was 0.01 mg twice daily. The dose of ralinepag was titrated according to patient tolerability. If the initial dose was tolerated (0.01 mg twice daily), then the dose was increased once a week in the following fashion: 0.02 mg twice daily, 0.03 mg twice daily, 0.04 mg twice daily, 0.06 mg twice daily, 0.08 mg, 0.1 mg twice daily, 0.2 mg twice daily and 0.3 mg twice daily. The dose was optionally escalated to a possible maximum total daily dose of 0.6 mg (0.3 mg twice daily), pending tolerability. If a dose was not tolerated, ralinepag was optionally decreased to the previous dose level. If the initial dose of 0.01 mg twice daily was not tolerated, dosing was optionally decreased to 0.01 mg once daily.

[0111] Ralinepag achieved the primary endpoint with a statistically significant change from baseline in pulmonary vascular resistance (PVR) compared to placebo. Ralinepag also demonstrated numerical improvement in 6-minute walk distance (6MWD). Adverse events observed in the study were consistent with other prostacyclin treatments for the management of PAH. The distribution of maintenance doses for patients receiving ralinepag was as follows: 0.02 mg (n=1), 0.03 mg (n=1), 0.04 mg (n=0), 0.06 mg (n=3), 0.08 mg (n=3), 0.12 mg (n=5), 0.16 mg (n=4), 0.2 mg (n=6), 0.4 mg (n=12), and 0.6 mg (n=5).

[0112] All documents, including patent and nonpatent literature cited herein are each incorporated herein by reference in their entirety for all purposes.

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CLAIMS

What is claimed is:

1. A method of decreasing cell proliferation in a patient in need thereof, comprising: administering a therapeutically effective amount of ralinepag, or a pharmaceutically acceptable salt, hydrate, or solvate thereof, in combination with a therapeutically effective amount of one or more of a cGMP elevating agent, a cAMP elevating agent, or a prostanoid.

2. The method of claim 1, wherein the one or more cGMP or cAMP elevating agents is a soluble guanylate cyclase (sGC) stimulator.

3. The method of claims 1 or 2, wherein the ralinepag is administered in combination with one or more cGMP elevating agents.

4. The method of claims 1 or 2, wherein the ralinepag is administered in combination with one or more cAMP elevating agents.

5. The method of claim 1, wherein the ralinepag is administered in combination with a prostanoid.

6. The method of claim 5, wherein the ralinepag is administered in combination with a synthetic prostacyclin analog.

7. The method of any of claims 1-6, wherein the dose of ralinepag does not require standard titration upon initiating said method.

8. The method of any of claims 1-7, wherein the therapeutically effective amount of ralinepag in combination with the one or more of the cGMP elevating agent, cAMP elevating agent, or prostanoid is less than the equivalently therapeutically effective amount of ralinepag in the absence of coadministration of one or more of the cGMP elevating agent, cAMP elevating agent, or prostanoid.

9. The method of claim 8, wherein the daily dose of ralinepag in combination with the one or more of the cGMP elevating agent, cAMP elevating agent, or prostanoid is at least about 0.01 mg (dose equivalent) less than the equivalent therapeutic amount of ralinepag in the absence of coadministration of one or more cGMP elevating agent, cAMP elevating agent, or prostanoid.

10. The method of claim 8, wherein the daily dose of ralinepag in combination with the one or more of the cGMP elevating agent, cAMP elevating agent, or prostanoid is at least about 0.05 mg (dose equivalent) less than the equivalent therapeutic amount of ralinepag in the absence of coadministration of one or more cGMP elevating agent, cAMP elevating agent, or prostanoid.

11. The method of claim 8, wherein the daily dose of ralinepag in combination with the one or more of the cGMP elevating agent, cAMP elevating agent, or prostanoid is at least about 0.1 mg (dose equivalent) less than the equivalent therapeutic amount of ralinepag in the absence of coadministration of one or more cGMP elevating agent, cAMP elevating agent, or prostanoid.

12. The method of any of claims 1- 11, wherein the therapeutically effective amount of the one or more cGMP elevating agent, cAMP elevating agent, or prostanoid is less than the equivalently therapeutically effective amount of the one or more cGMP elevating agents in the absence of coadministration of ralinepag.

13. The method of claim 12, wherein the therapeutically effective amount of the one or more cGMP elevating agent, cAMP elevating agent, or prostanoid is at least about 1% less than the equivalently therapeutically effective amount of the one or more cGMP elevating agents in the absence of coadministration of ralinepag.

14. The method of claim 12, wherein the therapeutically effective amount of the one or more cGMP elevating agent, cAMP elevating agent, or prostanoid is at least about 10% less than the equivalently therapeutically effective amount of the one or more cGMP elevating agents in the absence of coadministration of ralinepag.

15. The method of any of claims 1-14, wherein the frequency of administering a therapeutically effective amount of ralinepag in combination with one or more of the cGMP elevating agent, cAMP elevating agent, or prostanoid is less than the frequency of administering an equivalently therapeutically effective amount of ralinepag in the absence of coadministration of one or more of the cGMP elevating agent, cAMP elevating agent, or prostanoid.

16. The method of claim 15, wherein the therapeutically effective amount of ralinepag in combination with one or more of the cGMP elevating agent, cAMP elevating agent, or prostanoid is administered once per day.

17. The method of any of claims 1-16, wherein the frequency of administering a therapeutically effective amount of the one or more of the cGMP elevating agent, cAMP elevating agent, or prostanoid in combination with ralinepag is less than the frequency of administering an equivalently therapeutically effective amount of the one or more of the cGMP elevating agent, cAMP elevating agent, or prostanoid in the absence of coadministration of ralinepag.

18. The method of claim 17, wherein the therapeutically effective amount of the one or more of the cGMP elevating agent, cAMP elevating agent, or prostanoid in combination with ralinepag is administered once per day.

19. The method of any of claims 1-18, wherein the one or more cGMP elevating agents is selected from the group consisting of riociguat, vericiguat, ataciguat, nelociguat, lificiguat, IW-1701, IW-1973, IWP-051, IWP-121, IWP-427, IWP-953, BAY-60-2770, A-344905, A-350619, A-778935, BI-684067, BI-703704, BAY-41-2272, and BAY-41-8543.

20. The method of claim 1, wherein the cGMP elevating agent is riociguat.

21. The method of claim 1, wherein the one or more prostanoids is treprostinil.

22. The method of claim 1, wherein the one or more prostanoids is iloprost.

23. The method of any of claims 1-22, wherein the method comprises coadministration of ralinepag and riociguat.

24. The method of claim 23, wherein the amount of riociguat is selected from 0.5, 1, 1.5, 2, or 2.5 mg.

25. The method of claim 24, wherein the amount of riociguat is administered once daily.

26. The method of claim 24, wherein the amount of riociguat is administered twice daily.

27. The method of claim 24, wherein the amount of riociguat is administered three times daily.

28. The method of any of claims 23 to 27, wherein the riociguat is titrated.

29. The method of any of claims 1-22, wherein the method comprises coadministration of ralinepag and treprostinil.

30. The method of any of claims 1-22, wherein the method comprises coadministration of ralinepag and iloprost.

31. The method of any of claims 1-22, wherein the method comprises coadministration of ralinepag and a synthetic prostacyclin analog.

32. The method of any of claims 1-31, wherein said patient in need thereof is treated for a condition selected from the group consisting of pulmonary arterial hypertension (PAH), idiopathic PAH; familial PAH; PAH associated with: a collagen vascular disease, a congenital heart disease, portal hypertension, HIV infection, ingestion of a drug or toxin, hereditary hemorrhagic telangiectasia, splenectomy, pulmonary veno-occlusive disease (PVOD), or pulmonary capillary hemangiomatosis (PCH); PAH with significant venous or capillary involvement; and chronic thromboembolic pulmonary hypertension (CTEPH).

33. The method of any of claims 1-31, wherein said patient in need thereof is treated for PAH.

34. The method of any of claims 1-31, wherein the method of decreasing cell proliferation is a method of treating PAH.

35. The method of any of claims 1-31, wherein the method of decreasing cell proliferation is a method of treating CTEPH.

36. The method of any of claims 1-35, wherein the amount of ralinepag is selected from 0.02, 0.04, 0.06, 0.08, 0.1, 0.12, 0.14, 0.16, 0.18, 0.2, 0.22, 0.24, 0.26, 0.28, 0.3, 0.32, 0.34, 0.36, 0.38, 0.4, 0.42, 0.44, 0.46, 0.48, 0.5, 0.52, 0.54, 0.56, 0.58, or 0.6 mg.

37. The method of any of claims 1-35, wherein the amount of ralinepag is selected from 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.11, 0.12, 0.13, 0.14, 0.15, 0.16, 0.17, 0.18, 0.19, 0.2, 0.21, 0.22, 0.23, 0.24, 0.25, 0.26, 0.27, 0.28, 0.29, or 0.3 mg.

38. The method of any of claims 1-35, wherein the amount of ralinepag selected from 0.01 mg, 0.02 mg, 0.025 mg, 0.03 mg, 0.04 mg, 0.05 mg, 0.06 mg, 0.065 mg, 0.07 mg, 0.075 mg, 0.08 mg, 0.09 mg, 0.1 mg, 0.12 mg, 0.15 mg, 0.16 mg, 0.2 mg, 0.25 mg, 0.3 mg, 0.35 mg, 0.4 mg, 0.45 mg, 0.5 mg, 0.55 mg, 0.6 mg, 0.65 mg, 0.7 mg, 0.75 mg, 0.8 mg, 0.85 mg, 0.9 mg, 0.95 mg, and 1.0 mg daily.

39. The method of any of claims 1-35, wherein the amount of ralinepag is a starting dose selected from 0.01, 0.02, 0.025, 0.03, 0.04, 0.05, 0.06, 0.07, 0.075, 0.08, 0.09, and 0.1 mg daily.

40. The method of any of claims 1-35, wherein the amount of ralinepag is a highest tolerated dose selected from 0.4 mg, 0.45 mg, 0.5 mg, 0.55 mg, 0.6 mg, 0.65 mg, 0.7 mg, 0.75 mg, 0.8 mg, 0.85 mg, 0.9 mg, 0.95 mg, and 1.0 mg daily.

41. The method of any of claims 1-35, wherein the amount of ralinepag is a maximum dose selected from 0.4 mg, 0.45 mg, 0.5 mg, 0.55 mg, 0.6 mg, 0.65 mg, 0.7 mg, 0.75 mg, 0.8 mg, 0.85 mg, 0.9 mg, 0.95 mg, and 1.0 mg daily.

42. The method of any of claims 1-35, wherein the amount of ralinepag is a maximum tolerated dose selected from 0.4 mg, 0.45 mg, 0.5 mg, 0.55 mg, 0.6 mg, 0.65 mg, 0.7 mg, 0.75 mg, 0.8 mg, 0.85 mg, 0.9 mg, 0.95 mg, and 1.0 mg daily.

43. The method of any of claims 1-35, wherein the amount of ralinepag is a maintenance dose selected from 0.01 mg, 0.02 mg, 0.025 mg, 0.03 mg, 0.04 mg, 0.05 mg, 0.06 mg, 0.065 mg, 0.07 mg, 0.075 mg, 0.08 mg, 0.09 mg, 0.1 mg, 0.12 mg, 0.15 mg, 0.16 mg, 0.2 mg, 0.25 mg, 0.3 mg, 0.35 mg, 0.4 mg, 0.45 mg, 0.5 mg, 0.55 mg, 0.6 mg, 0.65 mg, 0.7 mg, 0.75 mg, 0.8 mg, 0.85 mg, 0.9 mg, 0.95 mg, and 1.0 mg daily.

44. The method of any of claims 36 to 43, wherein the amount of ralinepag is administered once daily.

45. The method of any of claims 36 to 43, wherein the amount of ralinepag is administered twice daily.

46. The method of any of claims 1 to 45, wherein the ralinepag is titrated.

47. A method of treating pulmonary arterial hypertension, comprising administering to an individual in need thereof ralinepag and riociguat.

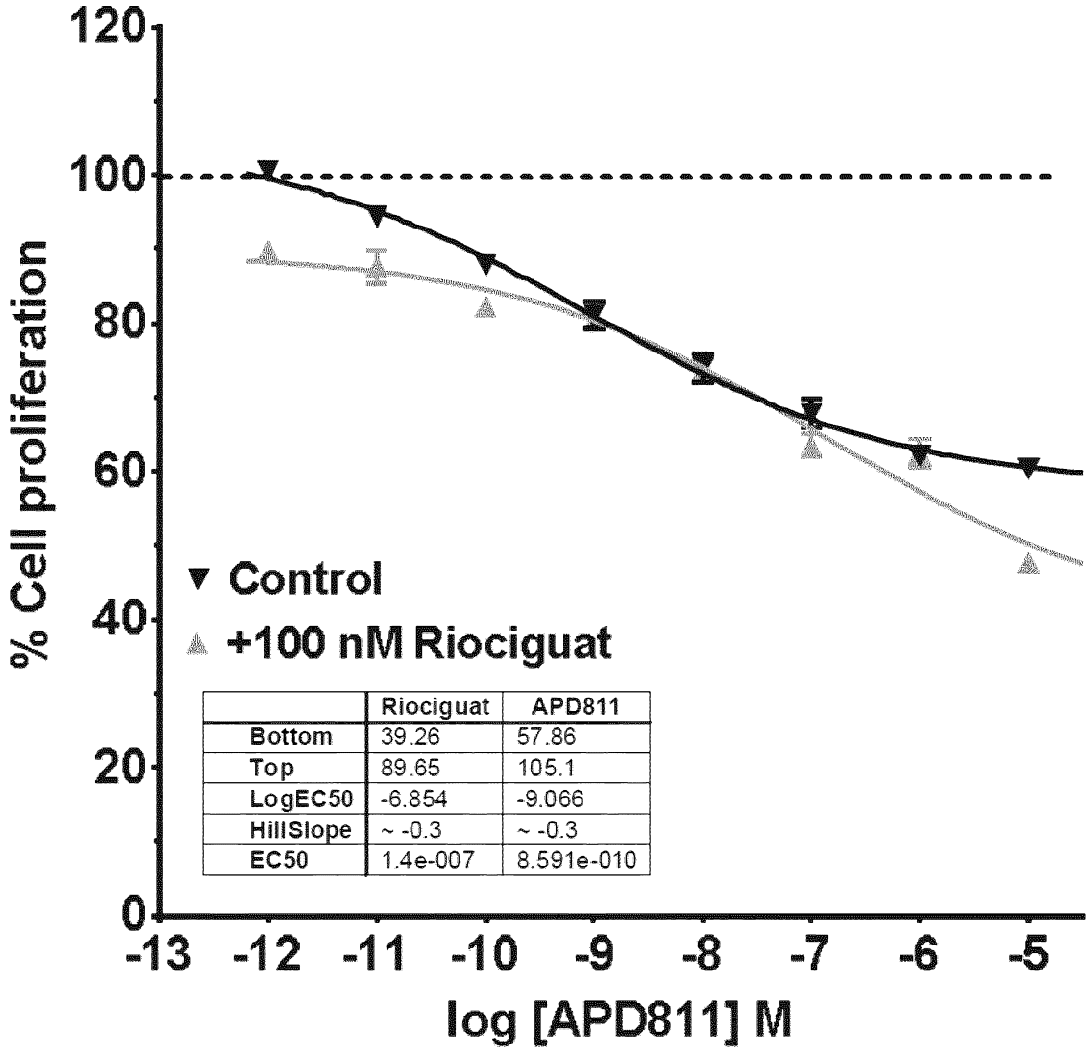
48. The method of claim 47, wherein the amount of riociguat is selected from about 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.25, 2.5, 2.75, 3, 3.25, 3.5, 3.75, 4, 4.25, 4.5, 4.75, and 5 mg.

49. A method of treating pulmonary arterial hypertension, comprising administering to an individual in need thereof ralinepag and treprostinil.

50. The method of claim 49, wherein the amount of treprostinil is selected from 0.1, 0.125, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45, 0.5, 0.6, 0.65, 0.7, 0.75, 0.8, 0.85, 0.9, 0.95, 1, 0.25, 0.5, 1.75, 2, 2.25, 2.5, 2.75, 3, 3.25, 3.5, 3.75, 4, 4.25, 4.5, 4.75, and 5 mg.

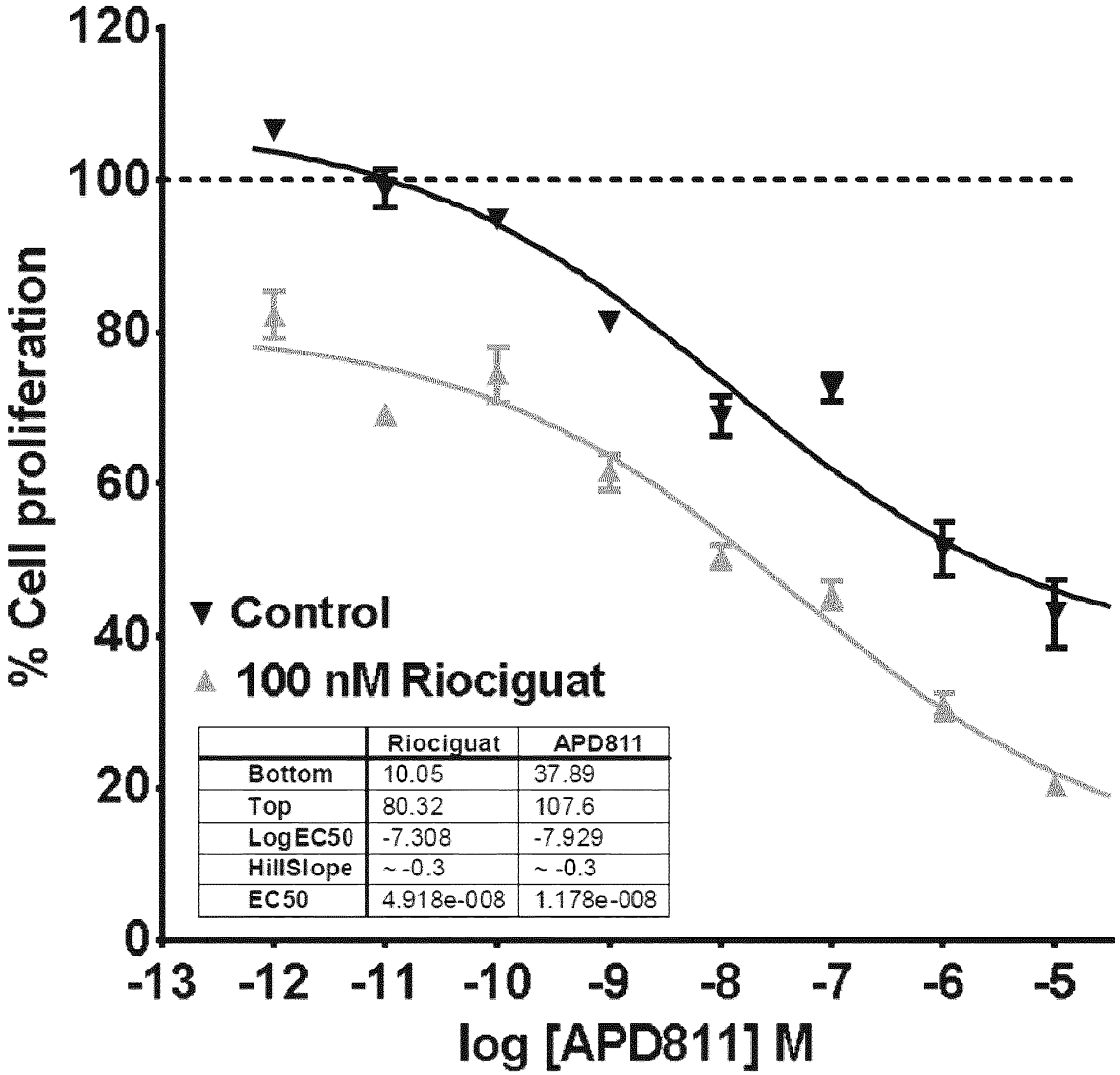
51. The method of claim 49, wherein the amount of treprostinil is selected from0.1, 0.2, 0.25, 0.3, 0.4, 0.5, 0.6, 0.7, 0.75, 0.8, 0.9, 1, 2.5, 3, 4, 5, 6, 7, 7.5,8, 9, and 10 mg/ml.

52. The method of any of claims 47-51, wherein the amount of ralinepag is selected from about 0.01 mg, 0.02 mg, 0.025 mg, 0.03 mg, 0.04 mg, 0.05 mg, 0.06 mg, 0.065 mg, 0.07 mg, 0.075 mg, 0.08 mg, 0.09 mg, 0.1 mg, 0.12 mg, 0.15 mg, 0.16 mg, 0.2 mg, 0.25 mg, 0.3 mg, 0.35 mg, 0.4 mg, 0.45 mg, 0.5 mg, 0.55 mg, 0.6 mg, 0.65 mg, 0.7 mg, 0.75 mg, 0.8 mg, 0.85 mg, 0.9 mg, 0.95 mg, and 1.0 mg.



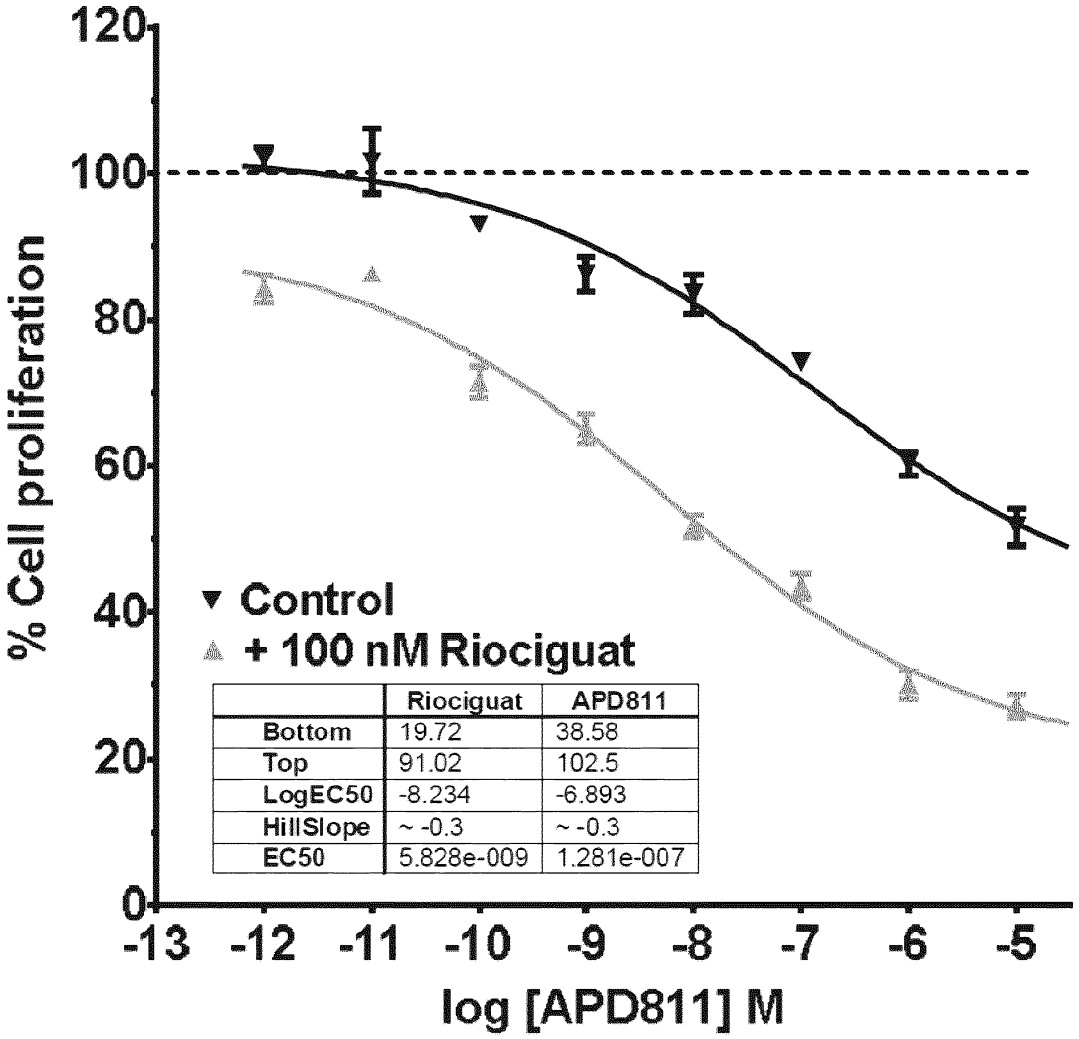
	Riociguat	APD811
Bottom	39.26	57.86
Top	89.65	105.1
LogEC50	-6.854	-9.066
HillSlope	~ -0.3	~ -0.3
EC50	1.4e-007	8.591e-010

Figure 1A



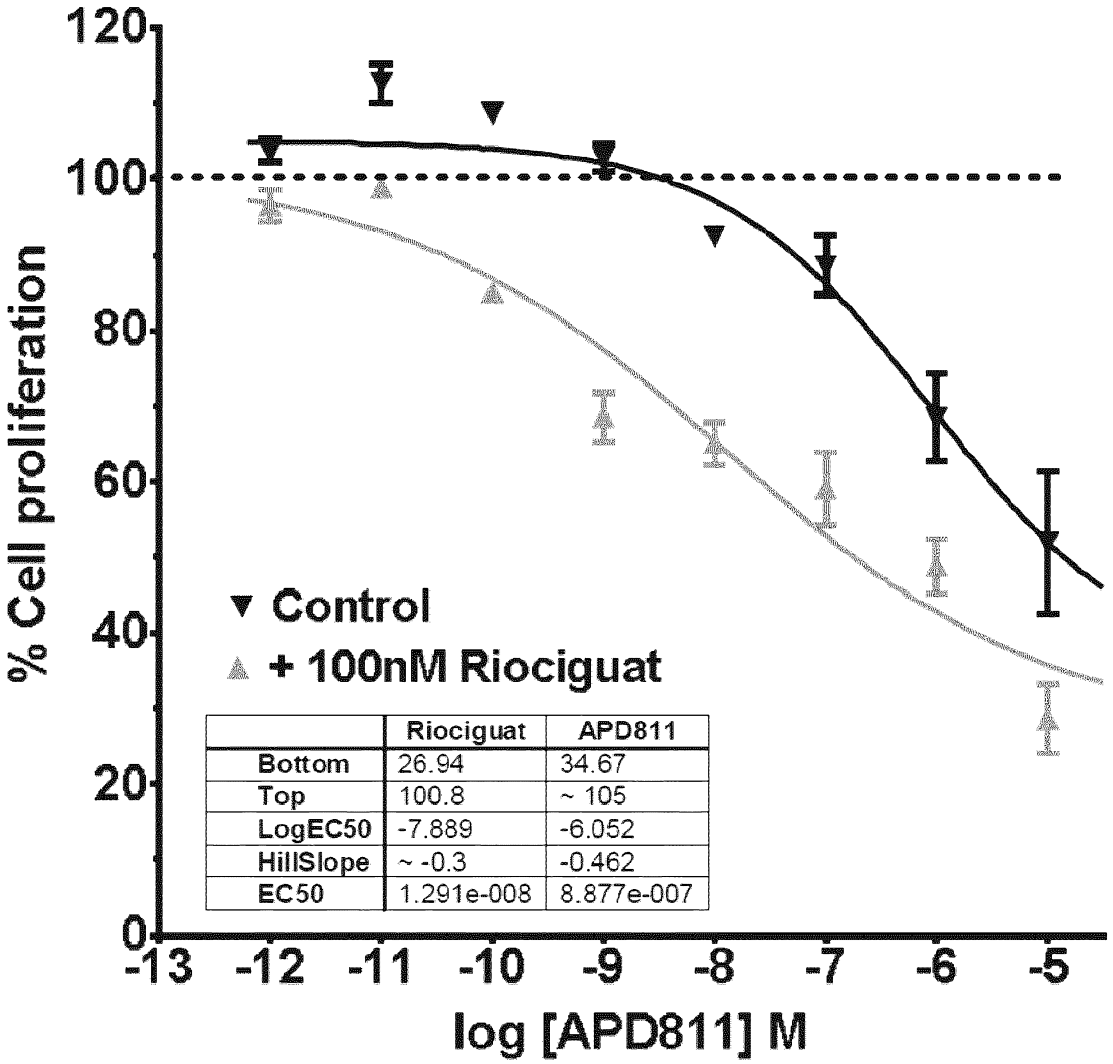
	Riociguat	APD811
Bottom	10.05	37.89
Top	80.32	107.6
LogEC50	-7.308	-7.929
HillSlope	~ -0.3	~ -0.3
EC50	4.918e-008	1.178e-008

Figure 1B



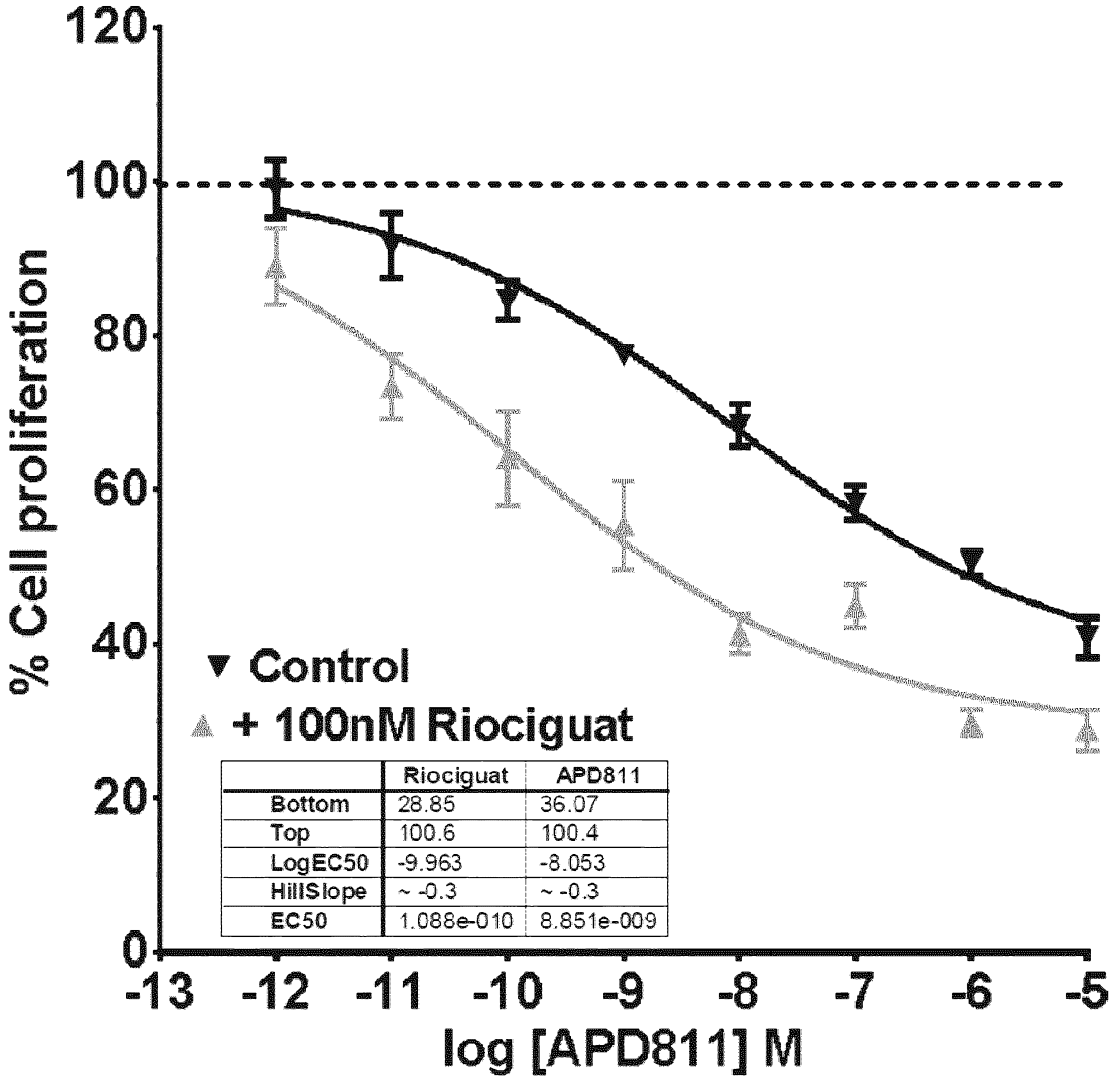
	Riociguat	APD811
Bottom	19.72	38.58
Top	91.02	102.5
LogEC50	-8.234	-6.893
HillSlope	~ -0.3	~ -0.3
EC50	5.828e-009	1.281e-007

Figure 1C



	Riociguat	APD811
Bottom	26.94	34.67
Top	100.8	~ 105
LogEC50	-7.889	-6.052
HillSlope	~ -0.3	-0.462
EC50	1.291e-008	8.877e-007

Figure 1D



	Riociguat	APD811
Bottom	28.85	36.07
Top	100.6	100.4
LogEC50	-9.963	-8.053
HillSlope	~ -0.3	~ -0.3
EC50	1.088e-010	8.851e-009

Figure 1E

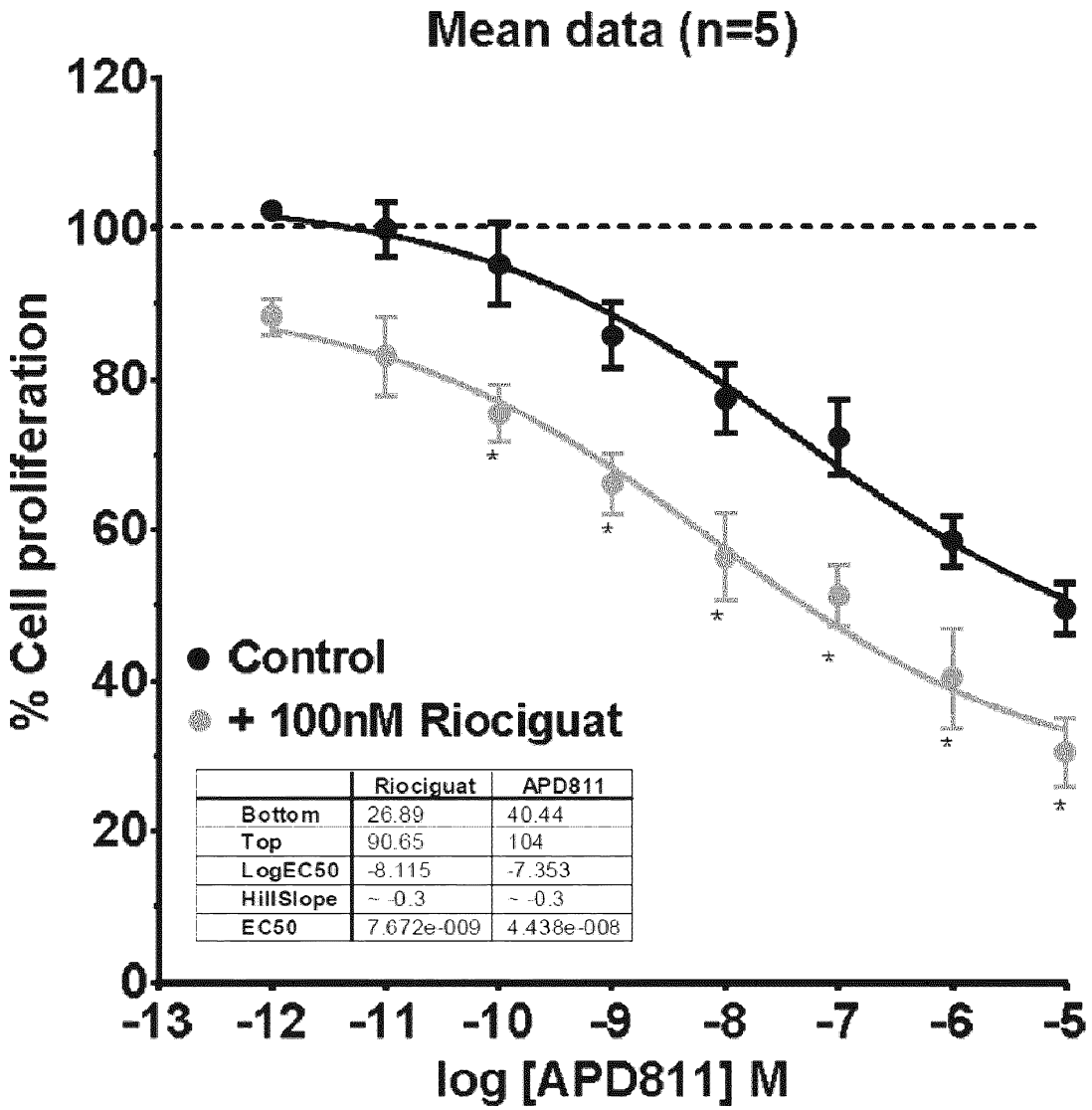

Mean data (n=5)
% Cell proliferation
120
100
80
60
40
20
0
-13
-12
-11
-10
-9
-8
-7
-6
-5
log [APD811] M
Control
+ 100nM Riociguat
Riociguat
APD811
Bottom
26.89
40.44
Top
90.65
104
LogEC50
-8.115
-7.353
HillSlope
~ -0.3
~ -0.3
EC50
7.672e-009
4.438e-008

Figure 1F

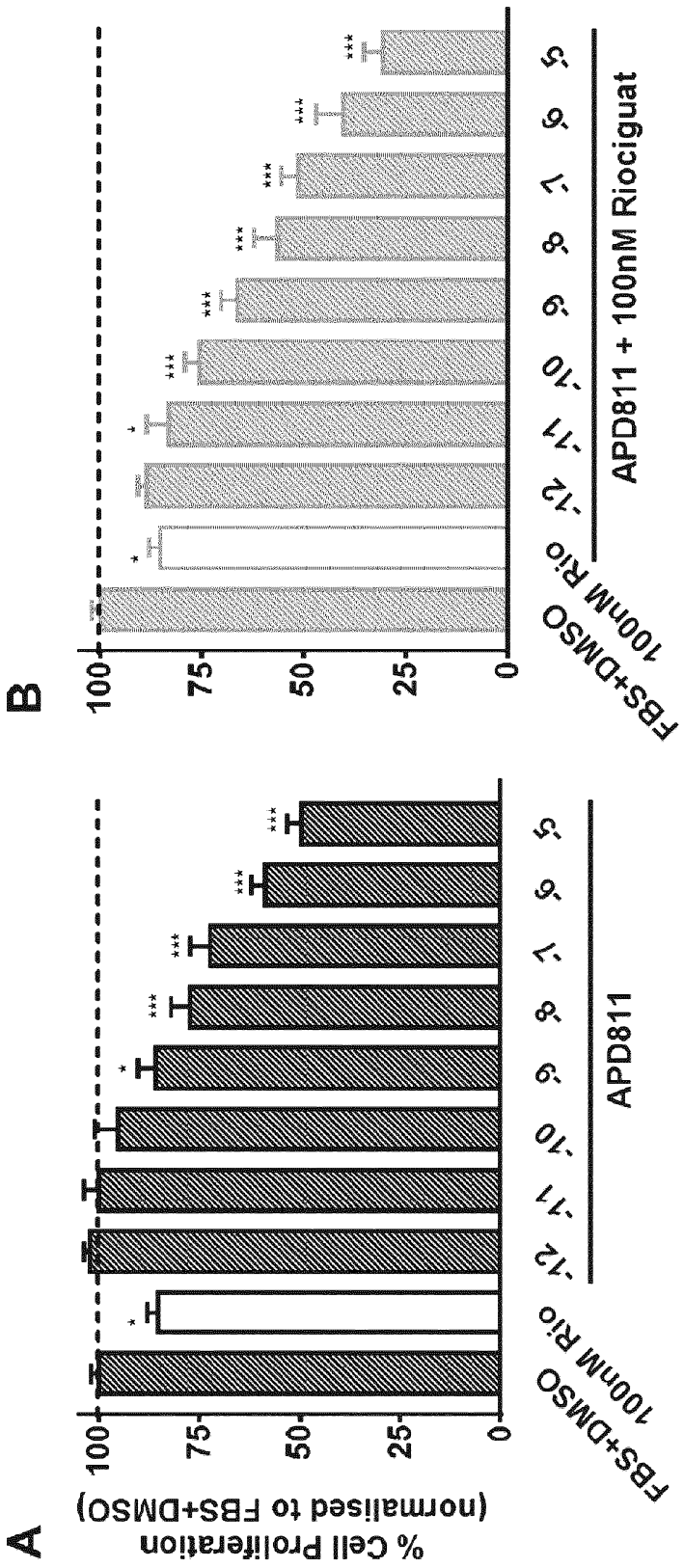
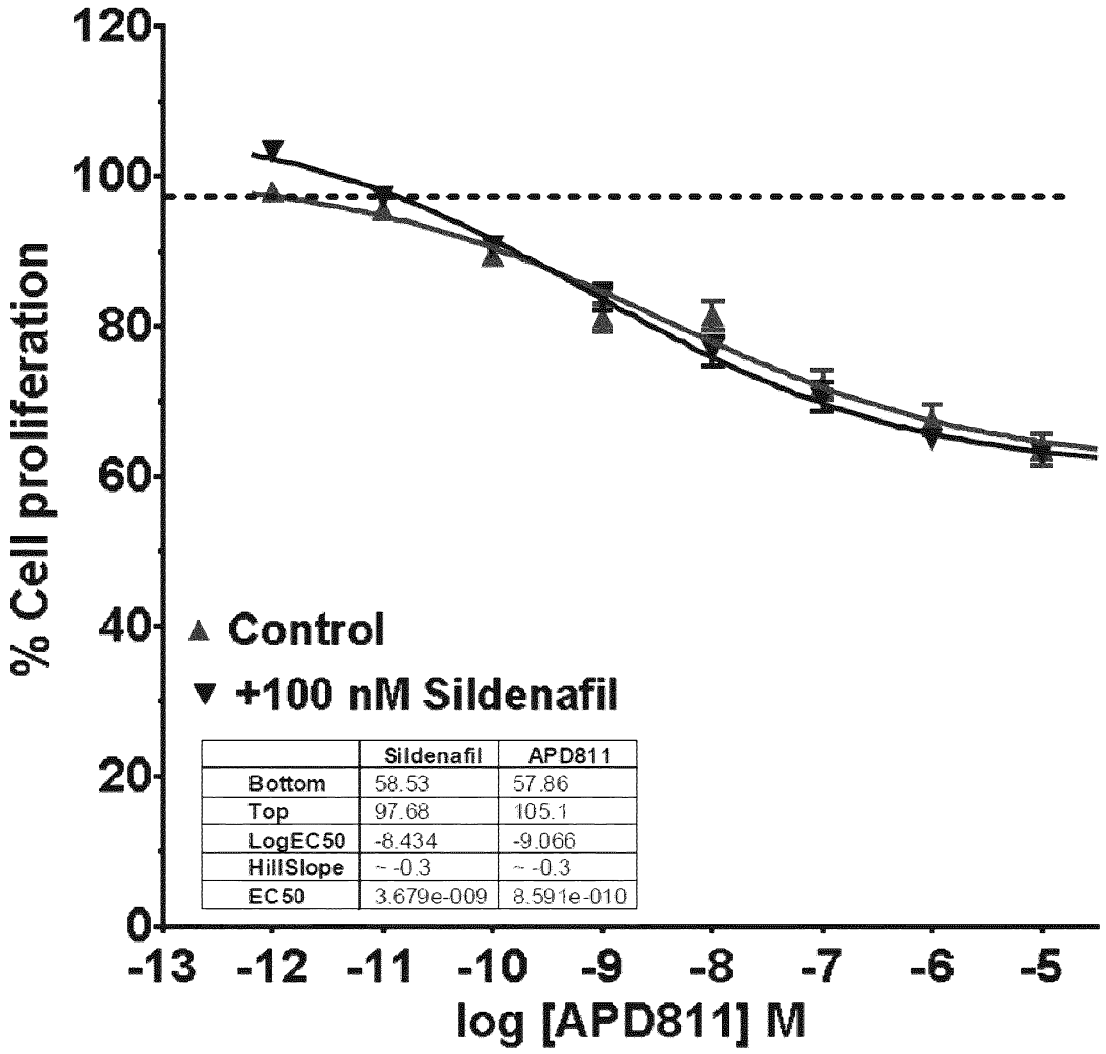
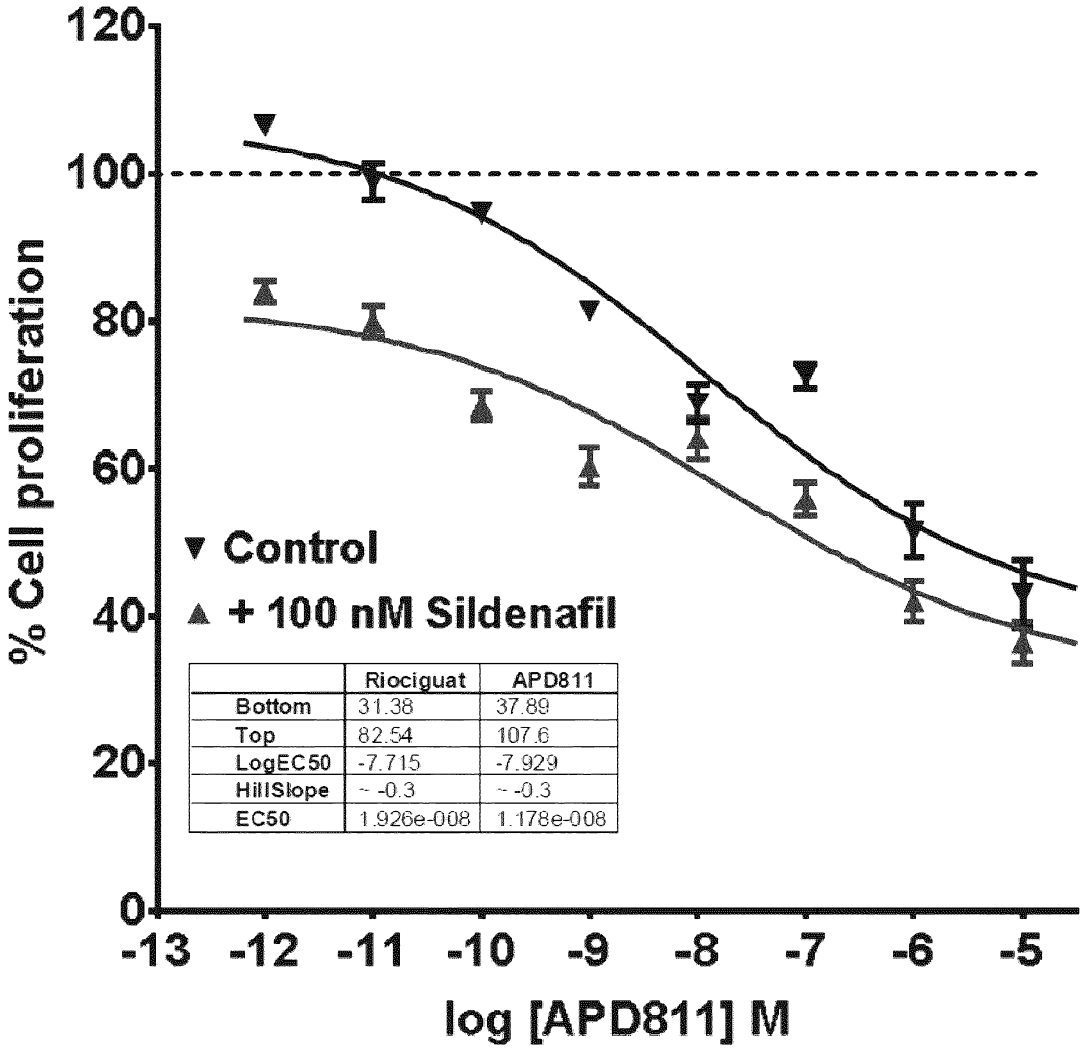

A
% Cell Proliferation
(normalised to FBS+DMSO)
100
75
50
25
0
FBS+DMSO
100nM Rio
-12
-11
-10
-9
-8
-7
-6
-5
APD811
B
100
75
50
25
0
FBS+DMSO
100nM Rio
-12
-11
-10
-9
-8
-7
-6
-5
APD811 + 100nM Riociguat

Figure 2



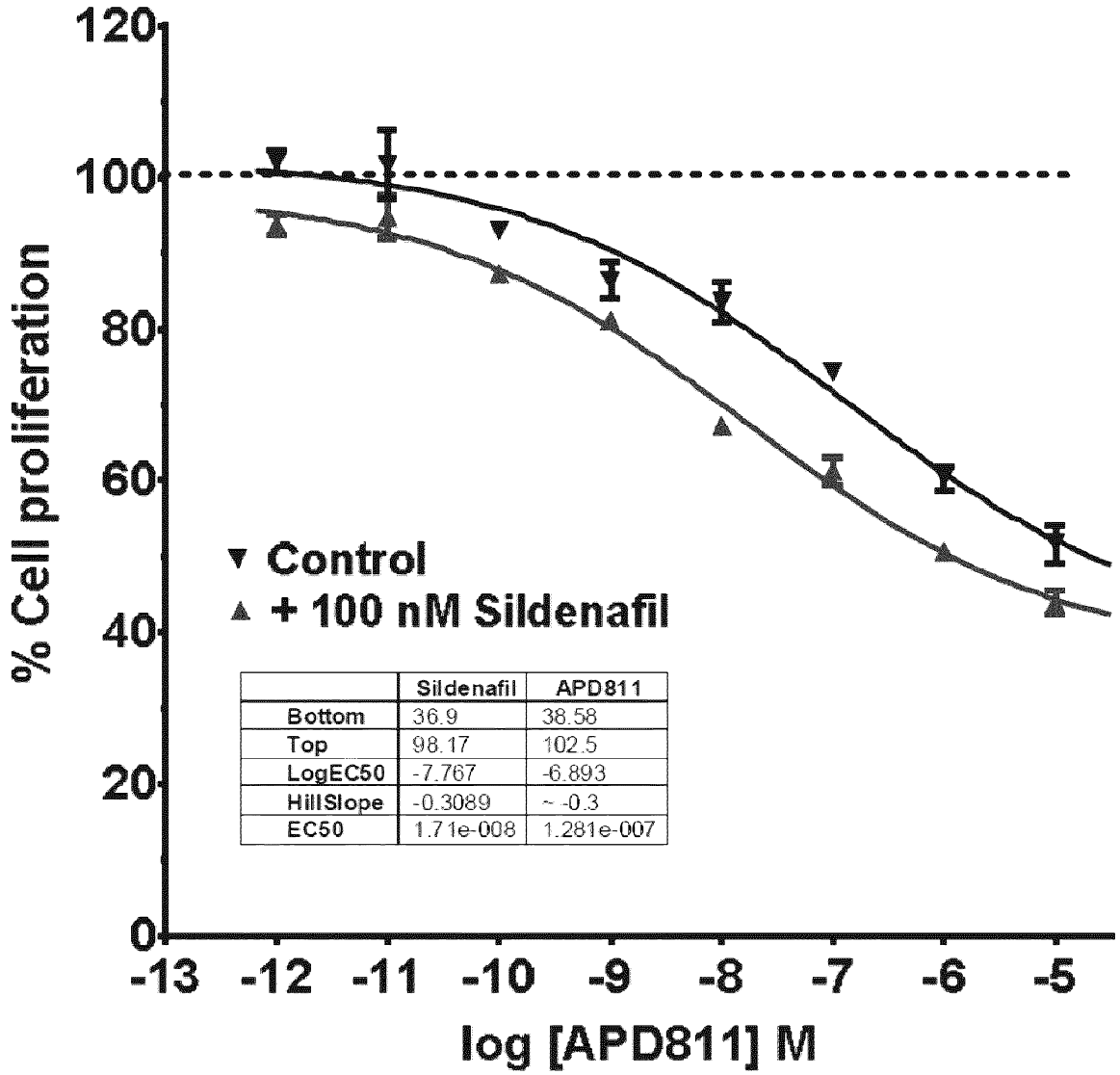
	Sildenafil	APD811
Bottom	58.53	57.86
Top	97.68	105.1
LogEC50	-8.434	-9.066
HillSlope	~ -0.3	~ -0.3
EC50	3.679e-009	8.591e-010

Figure 3A



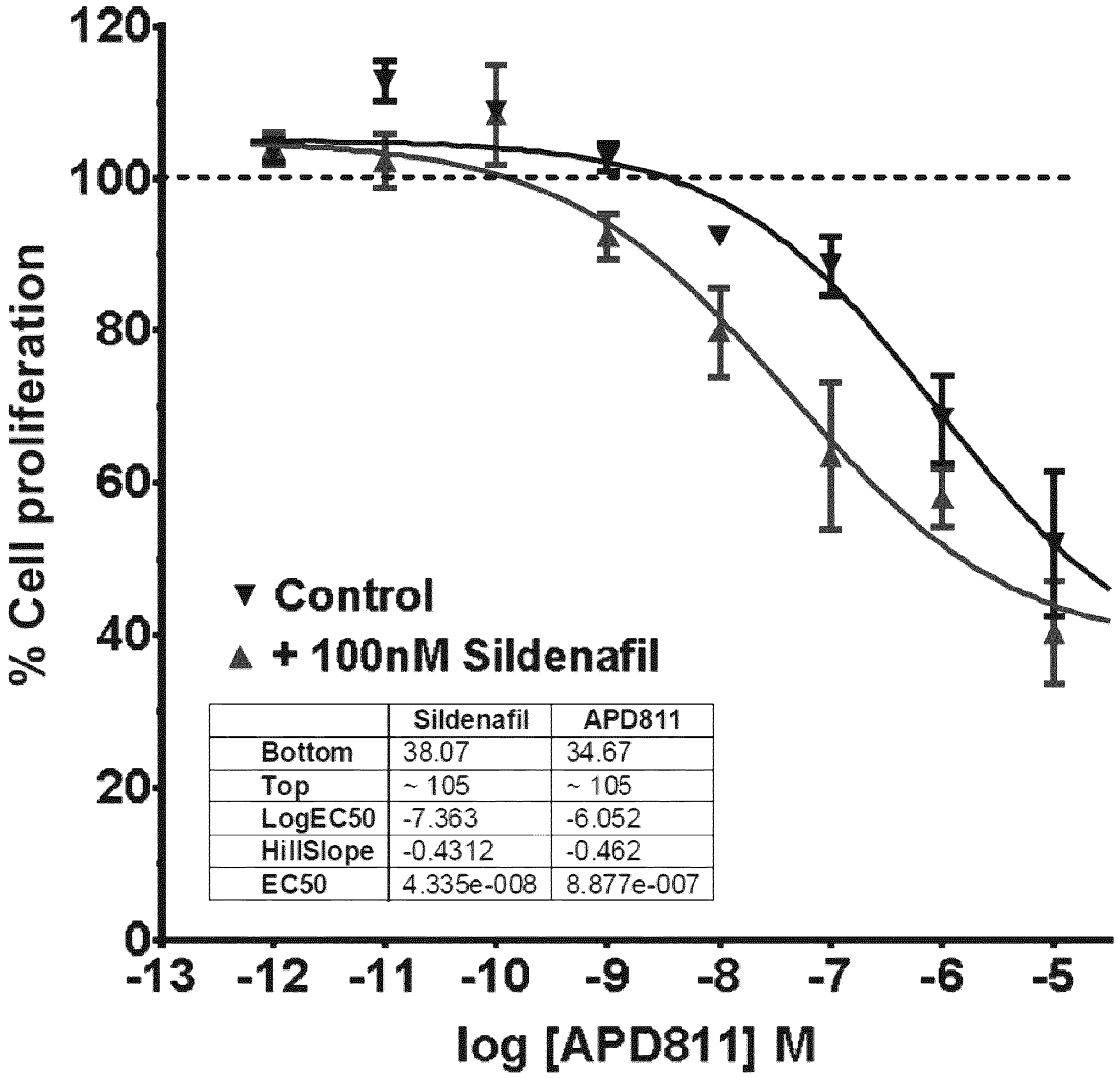
	Riociguat	APD811
Bottom	31.38	37.89
Top	82.54	107.6
LogEC50	-7.715	-7.929
HillSlope	-0.3	-0.3
EC50	1.926e-008	1.178e-008

Figure 3B



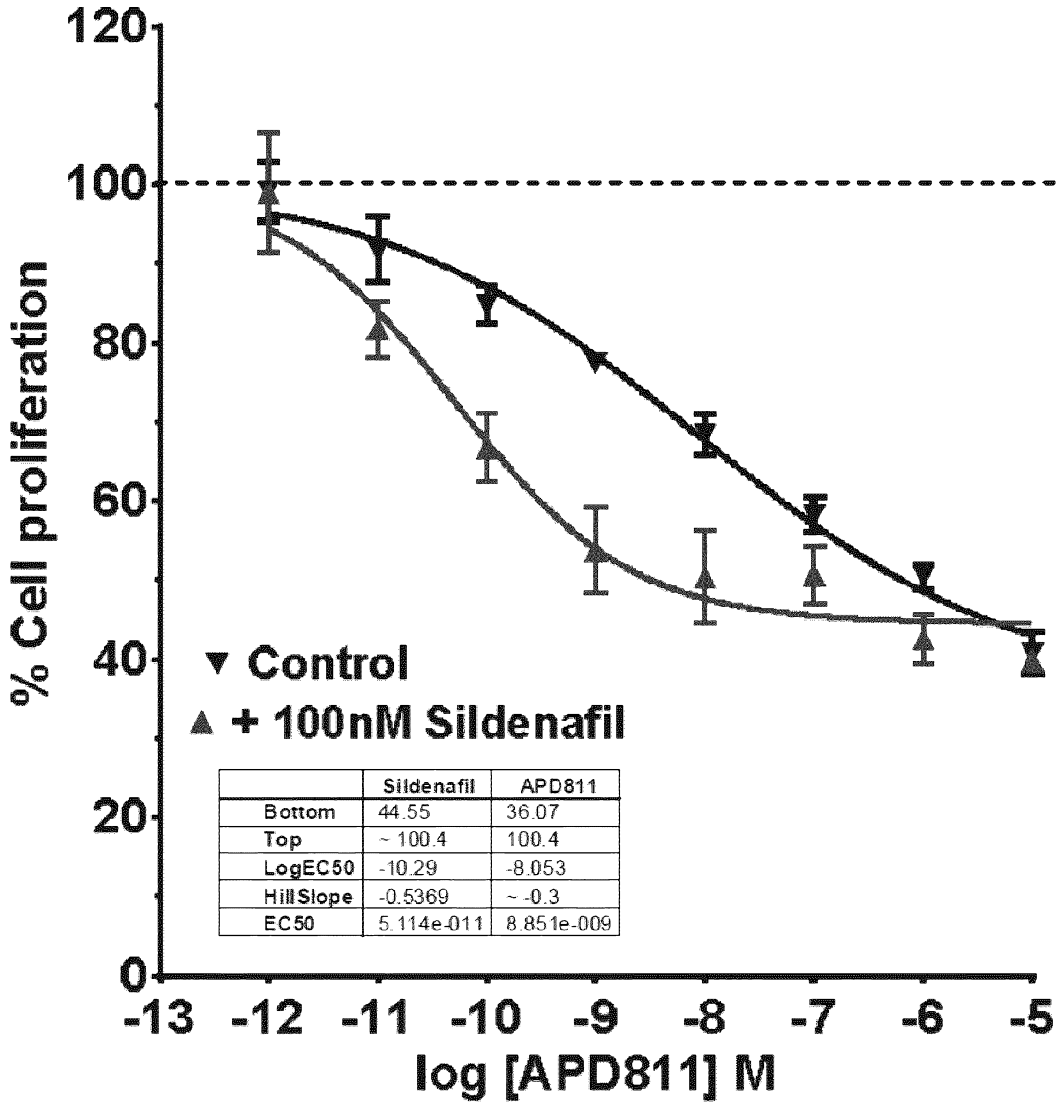
	Sildenafil	APD811
Bottom	36.9	38.58
Top	98.17	102.5
LogEC50	-7.767	-6.893
HillSlope	-0.3089	~ -0.3
EC50	1.71e-008	1.281e-007

Figure 3C



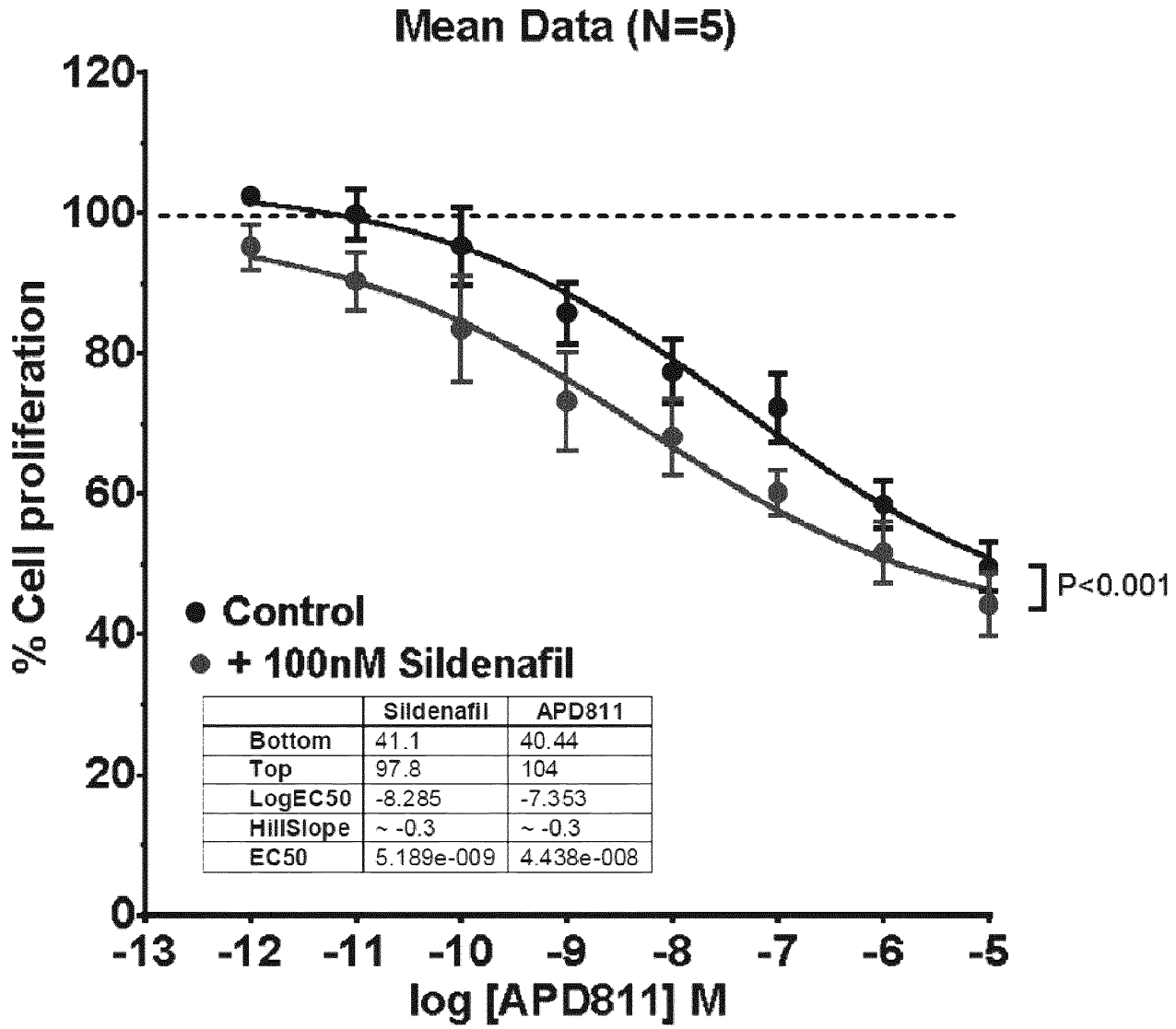
	Sildenafil	APD811
Bottom	38.07	34.67
Top	~ 105	~ 105
LogEC50	-7.363	-6.052
HillSlope	-0.4312	-0.462
EC50	4.335e-008	8.877e-007

Figure 3D



	Sildenafil	APD811
Bottom	44.55	36.07
Top	~ 100.4	100.4
LogEC50	-10.29	-8.053
HillSlope	-0.5369	~ -0.3
EC50	5.114e-011	8.851e-009

Figure 3E



	Sildenafil	APD811
Bottom	41.1	40.44
Top	97.8	104
LogEC50	-8.285	-7.353
HillSlope	~ -0.3	~ -0.3
EC50	5.189e-009	4.438e-008

Figure 3F

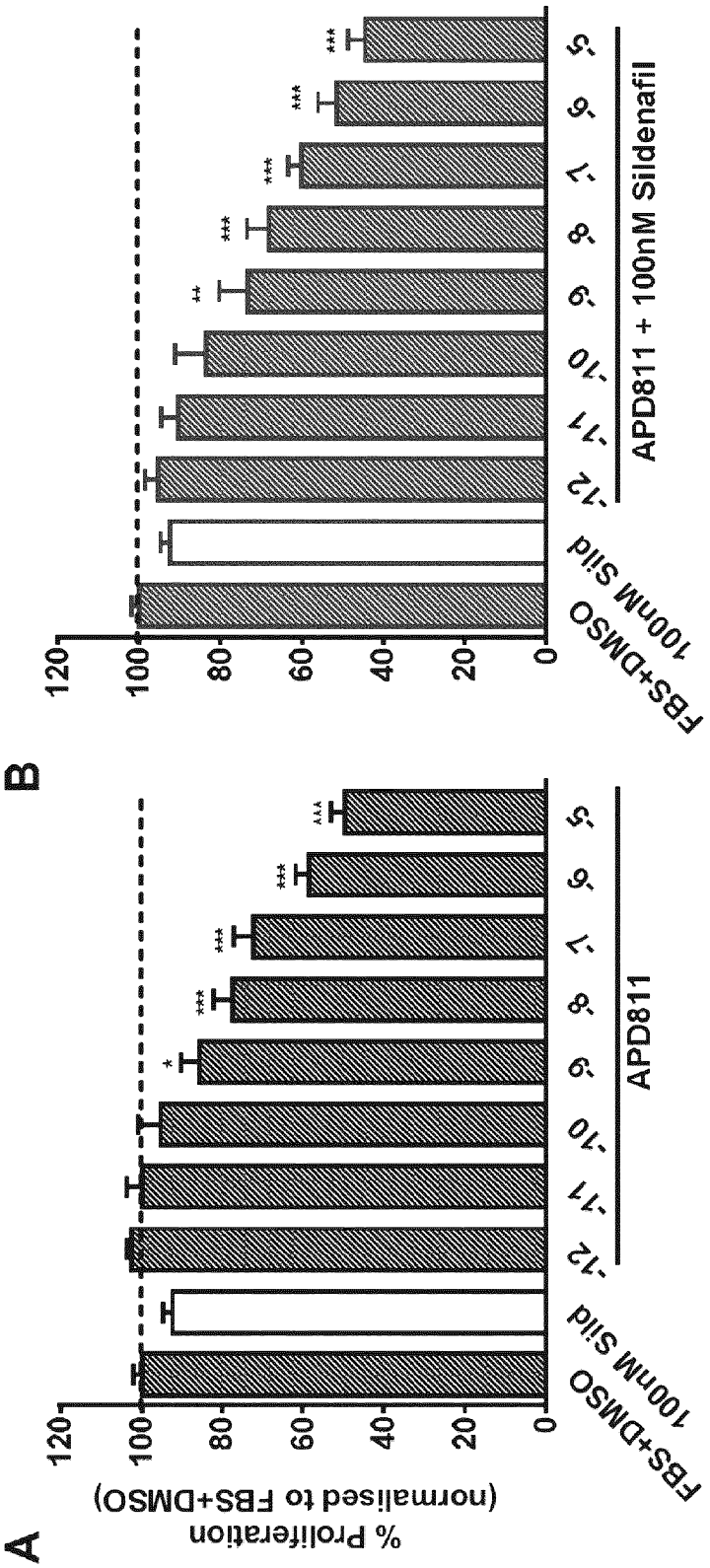
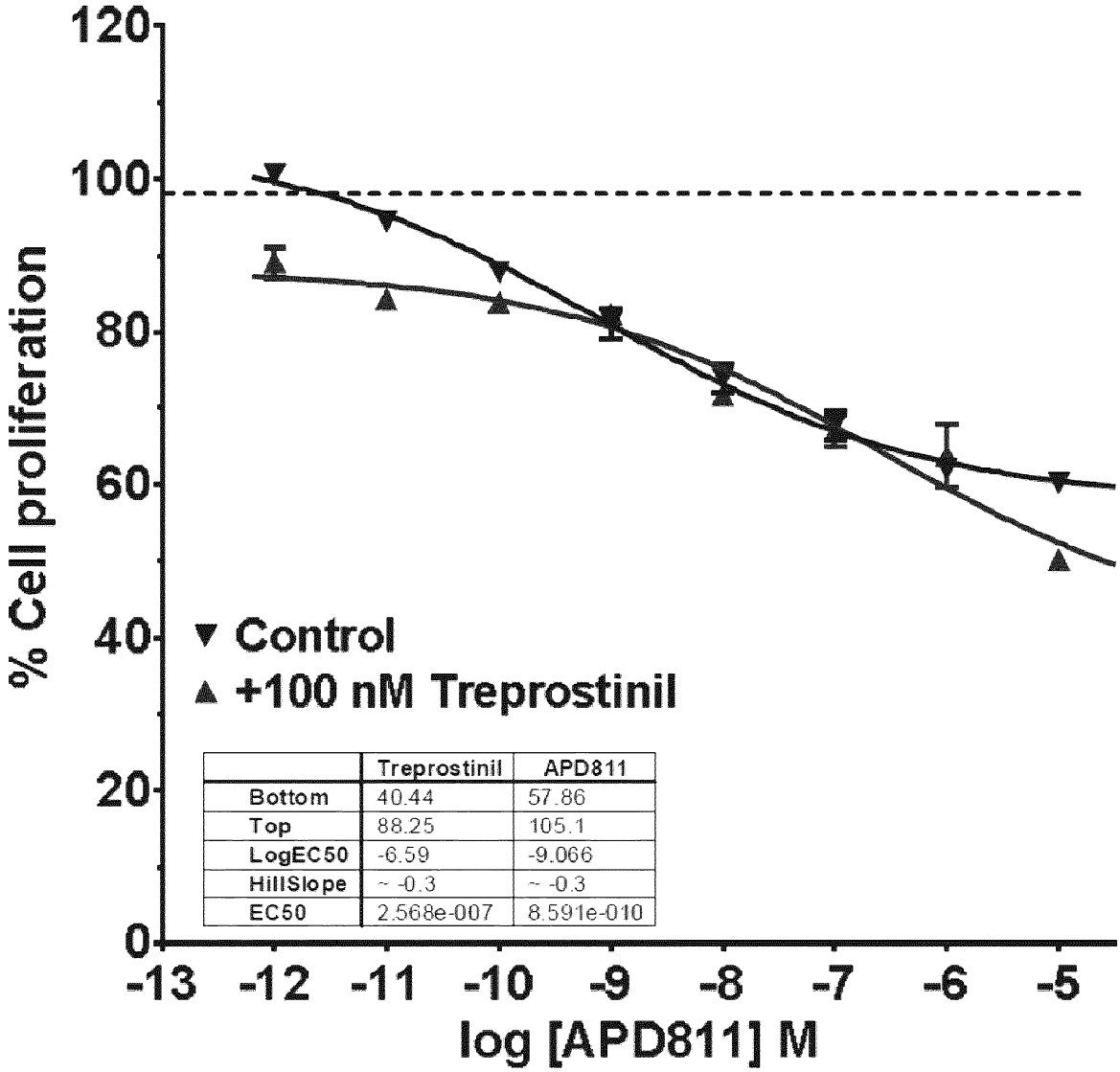
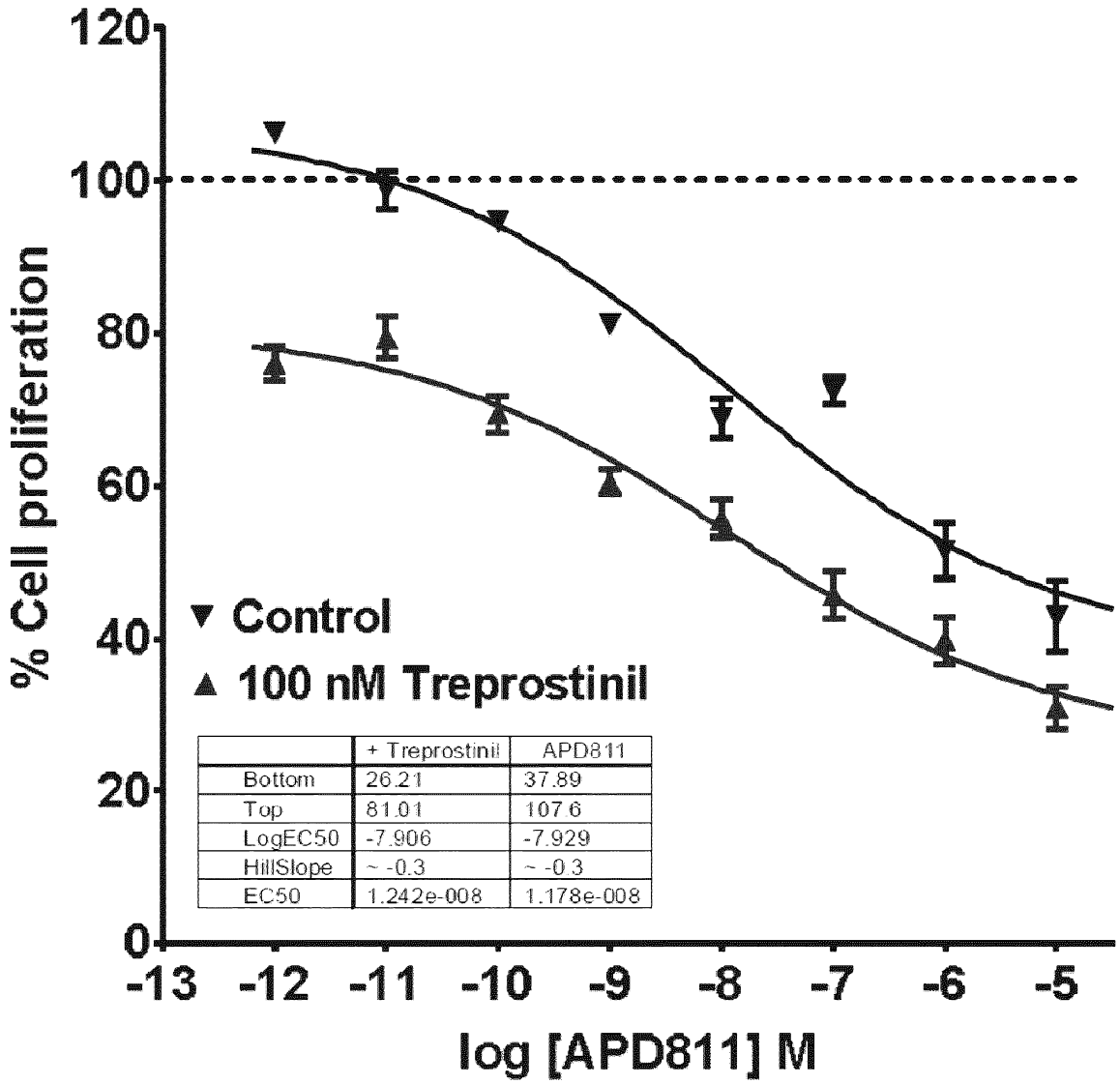

A
% Proliferation
(normalised to FBS+DMSO)
120
100
80
60
40
20
0
FBS+DMSO
100nM Sild
APD811
B
APD811 + 100nM Sildenafil

Figure 4



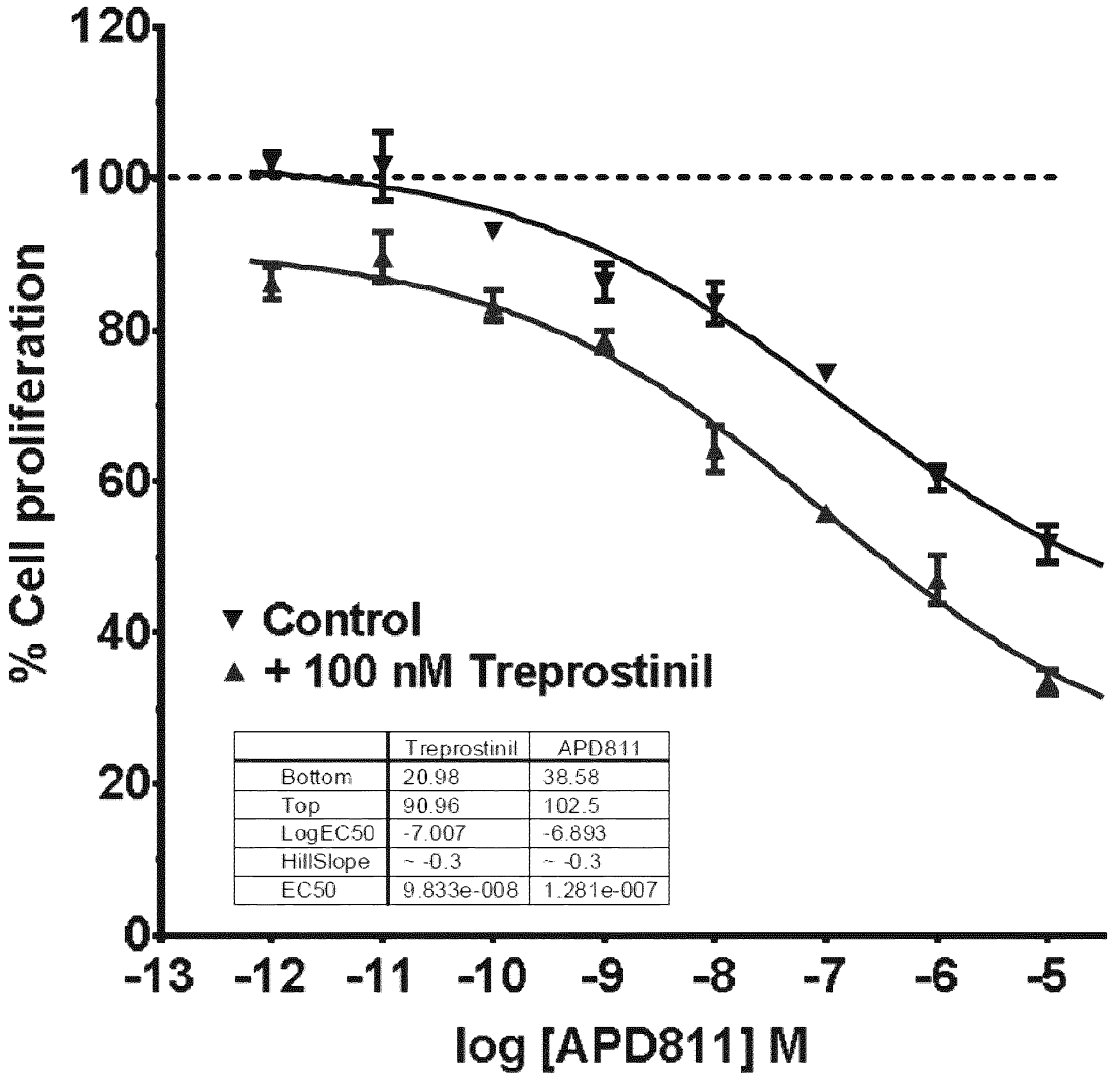
	Treprostinil	APD811
Bottom	40.44	57.86
Top	88.25	105.1
LogEC50	-6.59	-9.066
HillSlope	-0.3	-0.3
EC50	2.568e-007	8.591e-010

Figure 5A



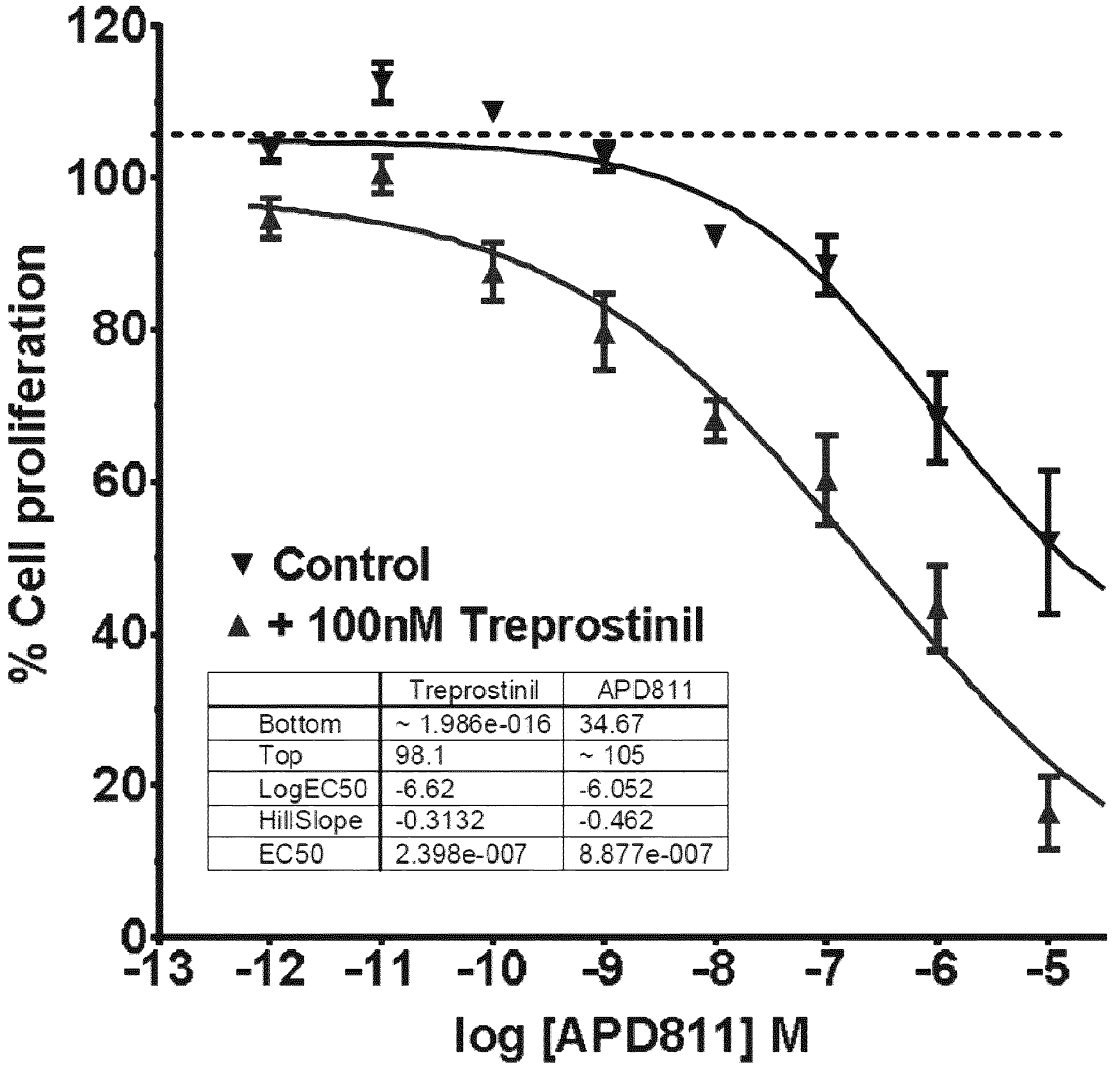
	+ Treprostinil	APD811
Bottom	26.21	37.89
Top	81.01	107.6
LogEC50	-7.906	-7.929
HillSlope	~ -0.3	~ -0.3
EC50	1.242e-008	1.178e-008

Figure 5B



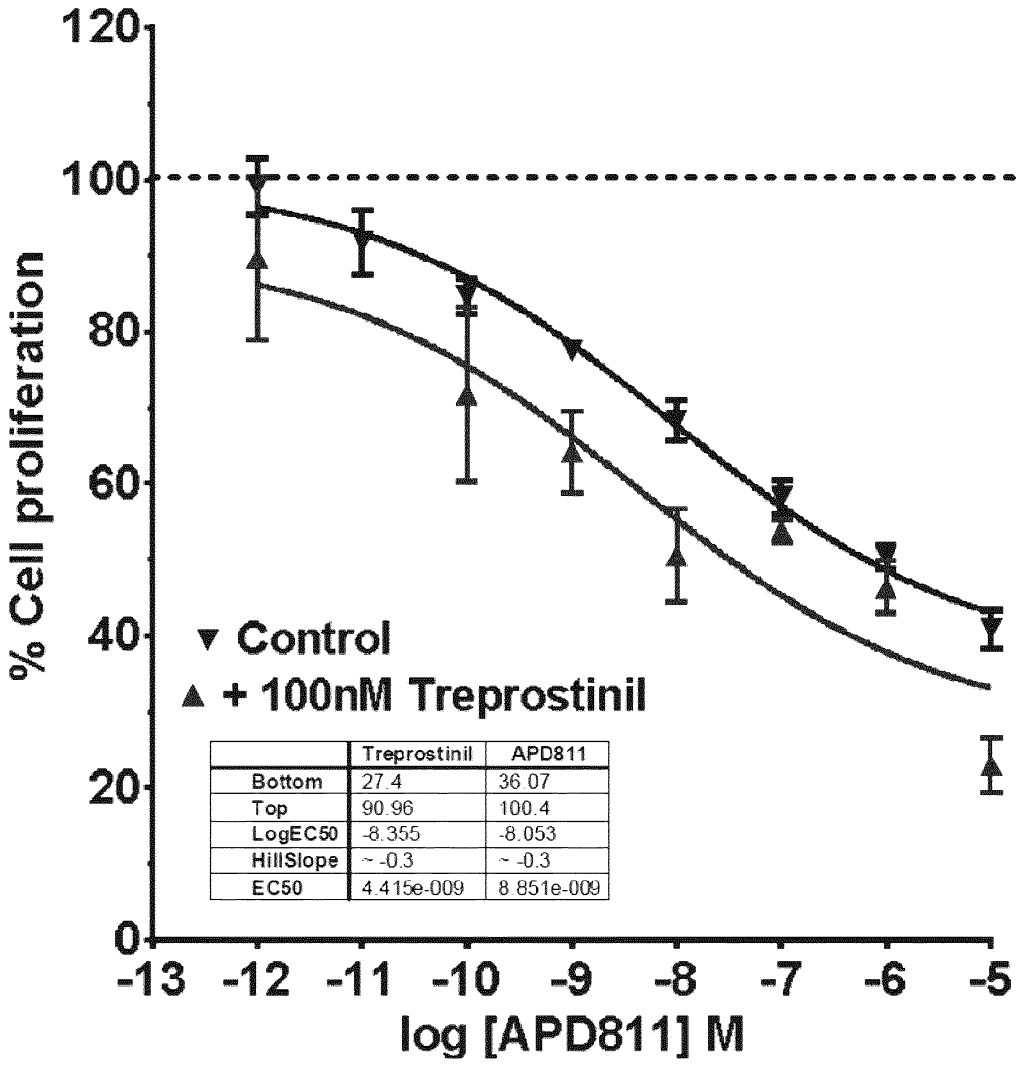
	Treprostinil	APD811
Bottom	20.98	38.58
Top	90.96	102.5
LogEC50	-7.007	-6.893
HillSlope	~ -0.3	~ -0.3
EC50	9.833e-008	1.281e-007

Figure 5C



	Treprostinil	APD811
Bottom	~ 1.986e-016	34.67
Top	98.1	~ 105
LogEC50	-6.62	-6.052
HillSlope	-0.3132	-0.462
EC50	2.398e-007	8.877e-007

Figure 5D



	Treprostinil	APD811
Bottom	27.4	36.07
Top	90.96	100.4
LogEC50	-8.355	-8.053
HillSlope	~ -0.3	~ -0.3
EC50	4.415e-009	8.851e-009

Figure 5E

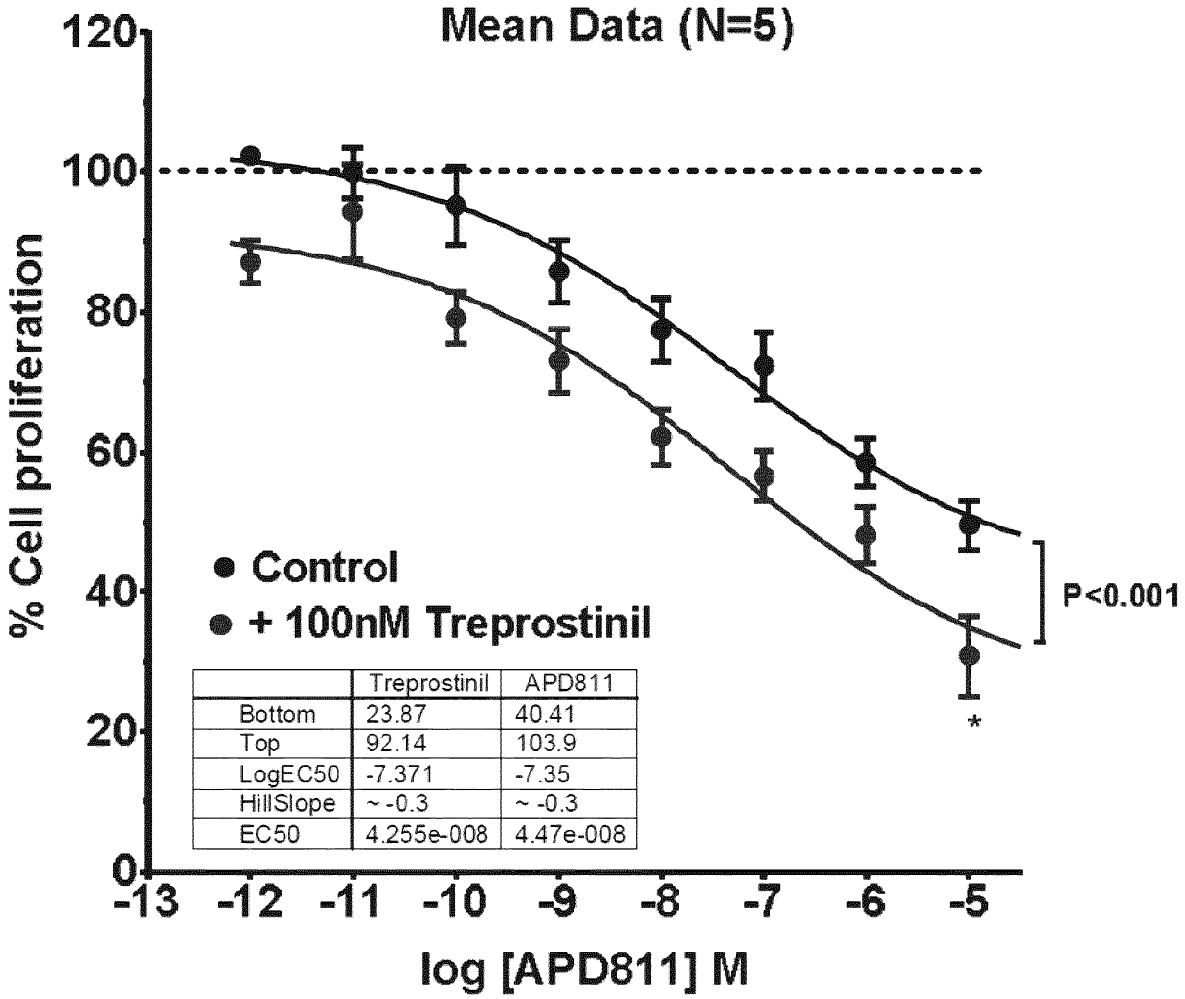

Mean Data (N=5)
% Cell proliferation
120
100
80
60
40
20
0
-13
-12
-11
-10
-9
-8
-7
-6
-5
log [APD811] M
Control
+ 100nM Treprostinil
P<0.001
*
Treprostinil
APD811
Bottom
23.87
40.41
Top
92.14
103.9
LogEC50
-7.371
-7.35
HillSlope
~ -0.3
~ -0.3
EC50
4.255e-008
4.47e-008

Figure 5F

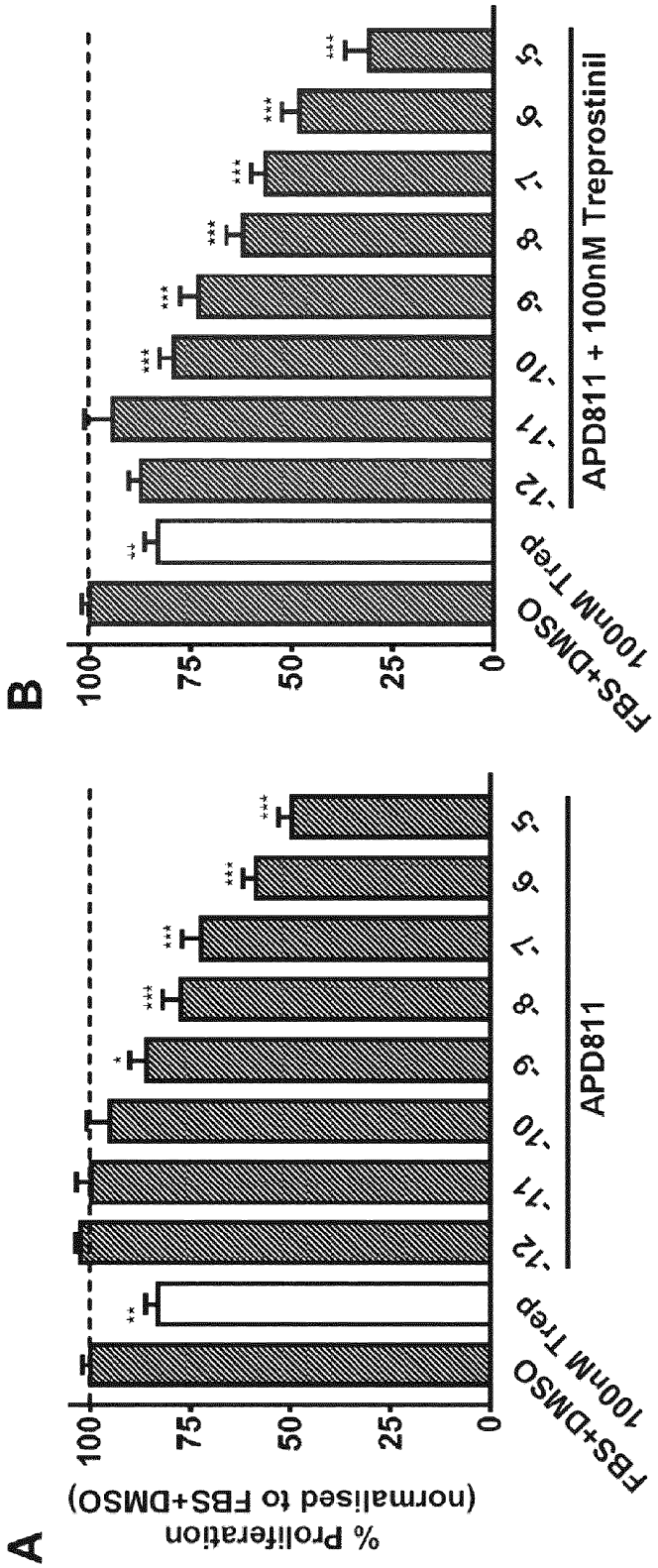
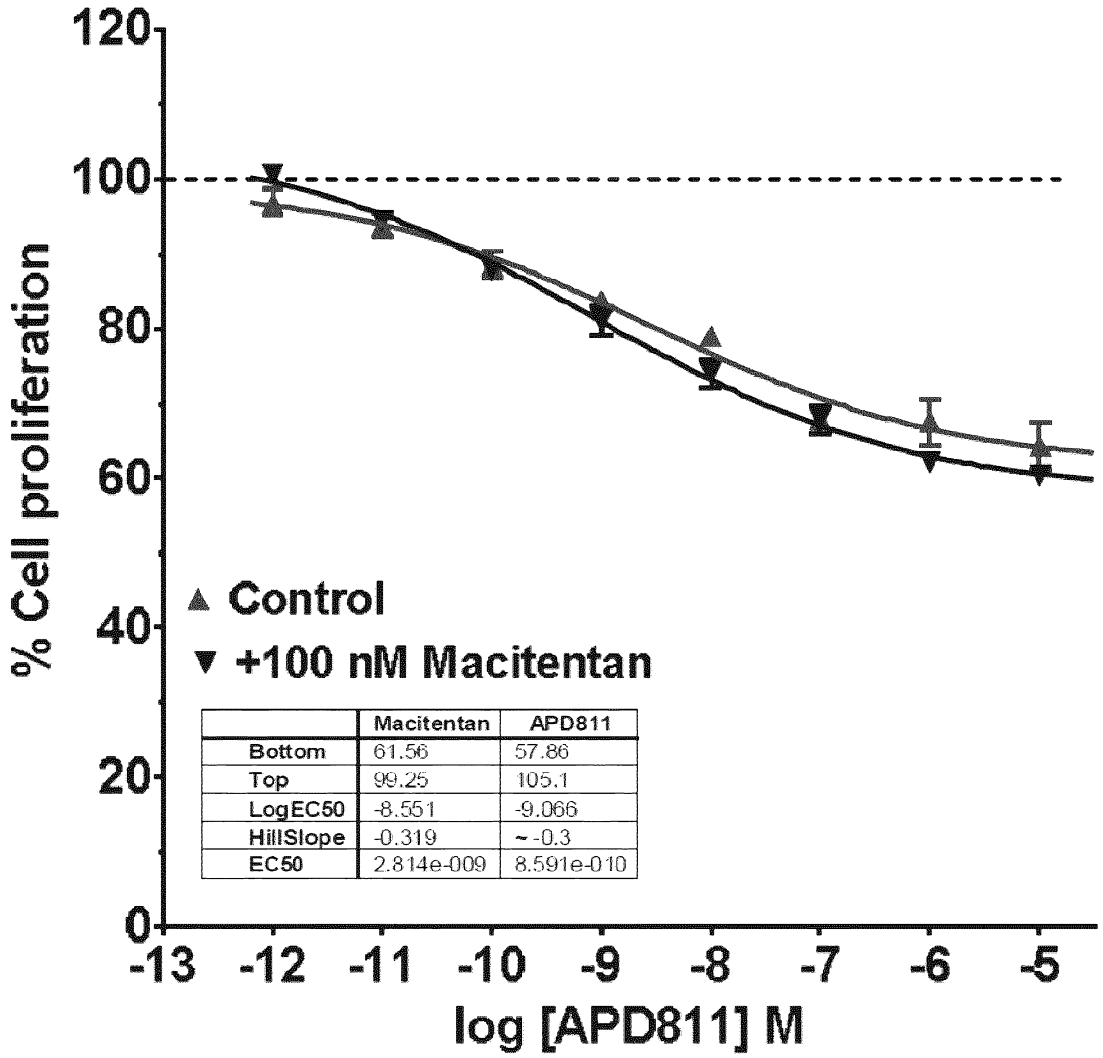
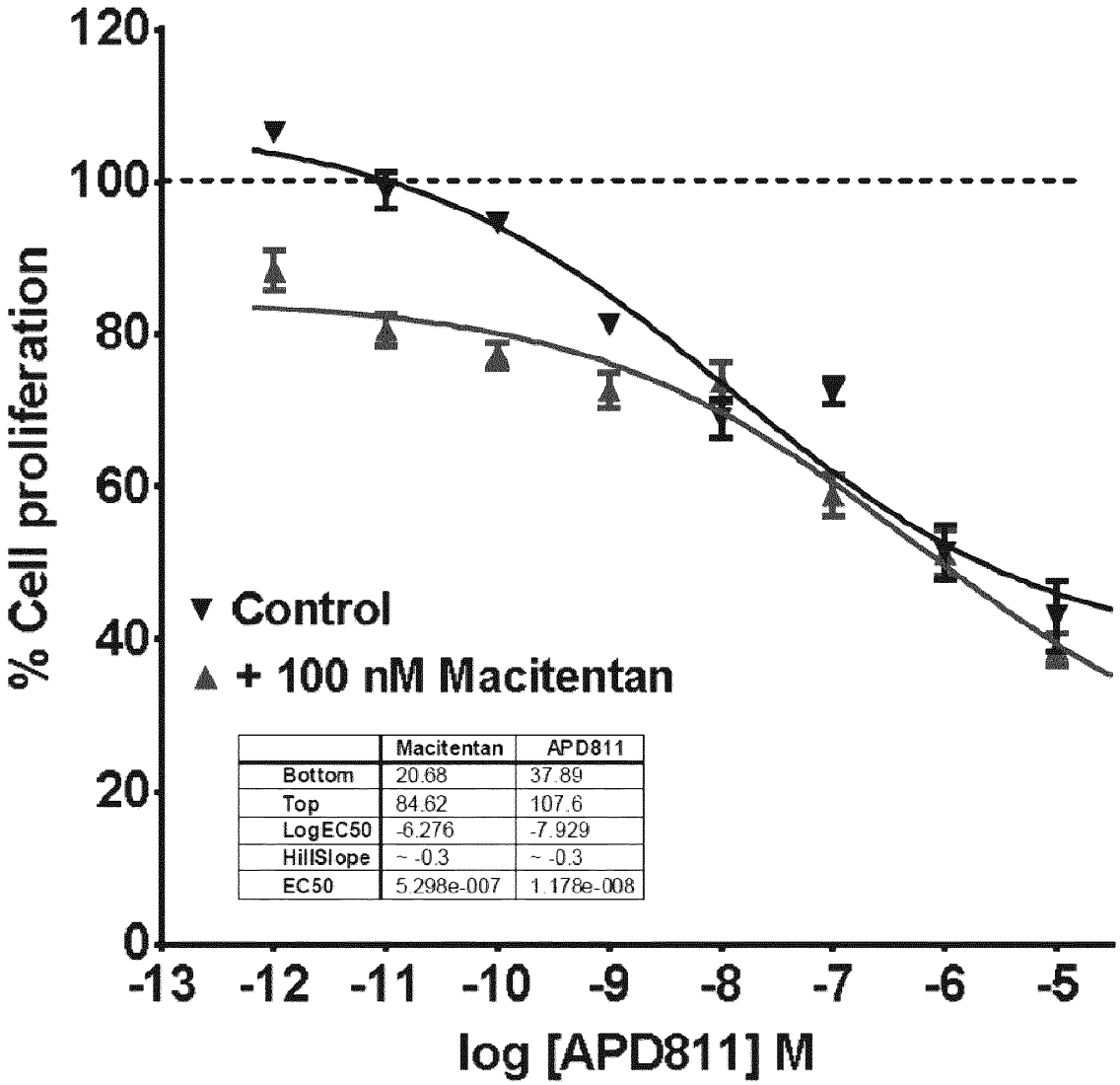

A
% Proliferation
(normalised to FBS+DMSO)
100
75
50
25
0
FBS+DMSO
100nM Trep
-12
-11
-10
-9
-8
-7
-6
-5
APD811
B
APD811 + 100nM Treprostinil

Figure 6



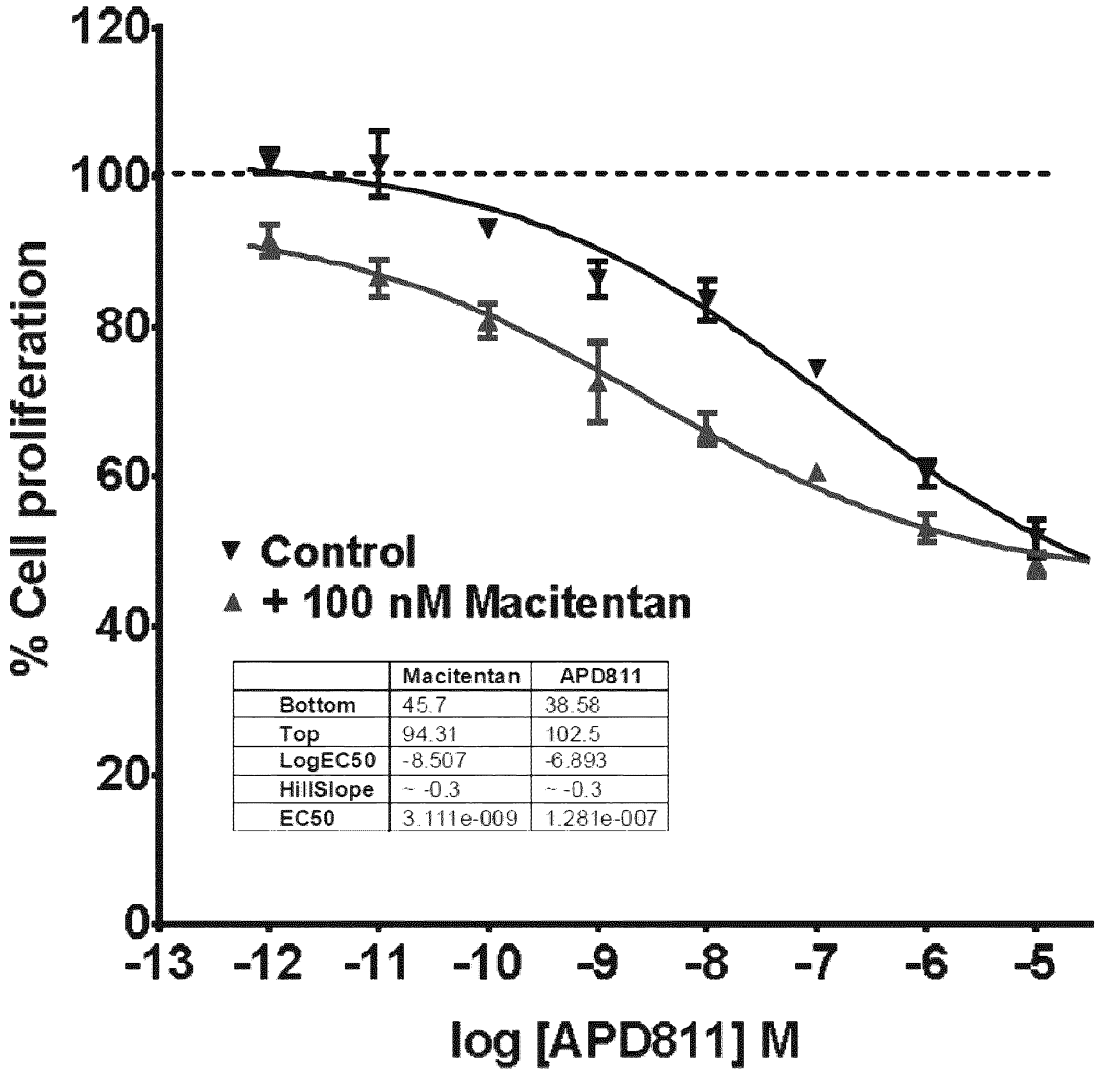
	Macitentan	APD811
Bottom	61.56	57.86
Top	99.25	105.1
LogEC50	-8.551	-9.066
HillSlope	-0.319	~-0.3
EC50	2.814e-009	8.591e-010

Figure 7A



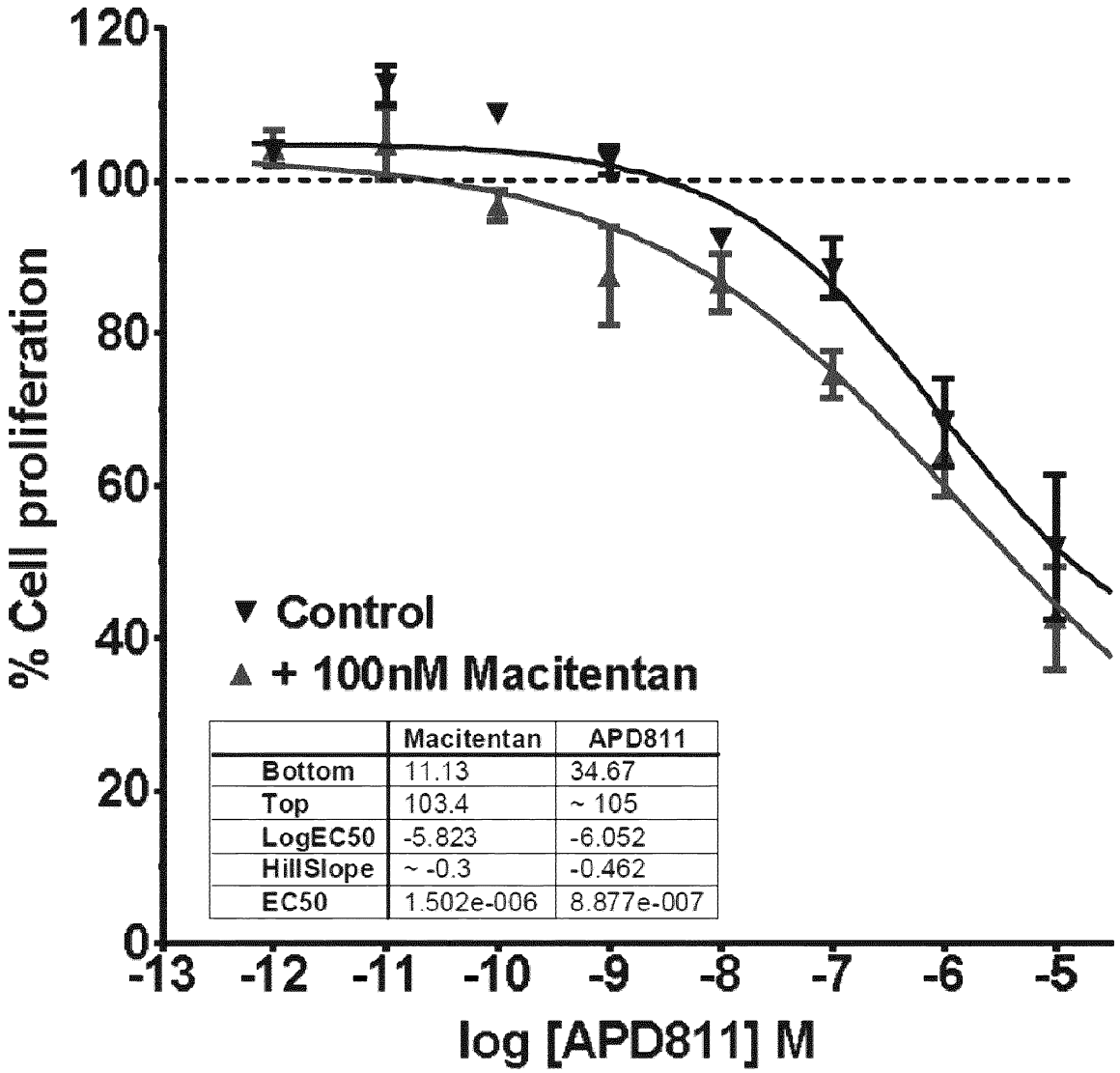
	Macitentan	APD811
Bottom	20.68	37.89
Top	84.62	107.6
LogEC50	-6.276	-7.929
HillSlope	~ -0.3	~ -0.3
EC50	5.298e-007	1.178e-008

Figure 7B



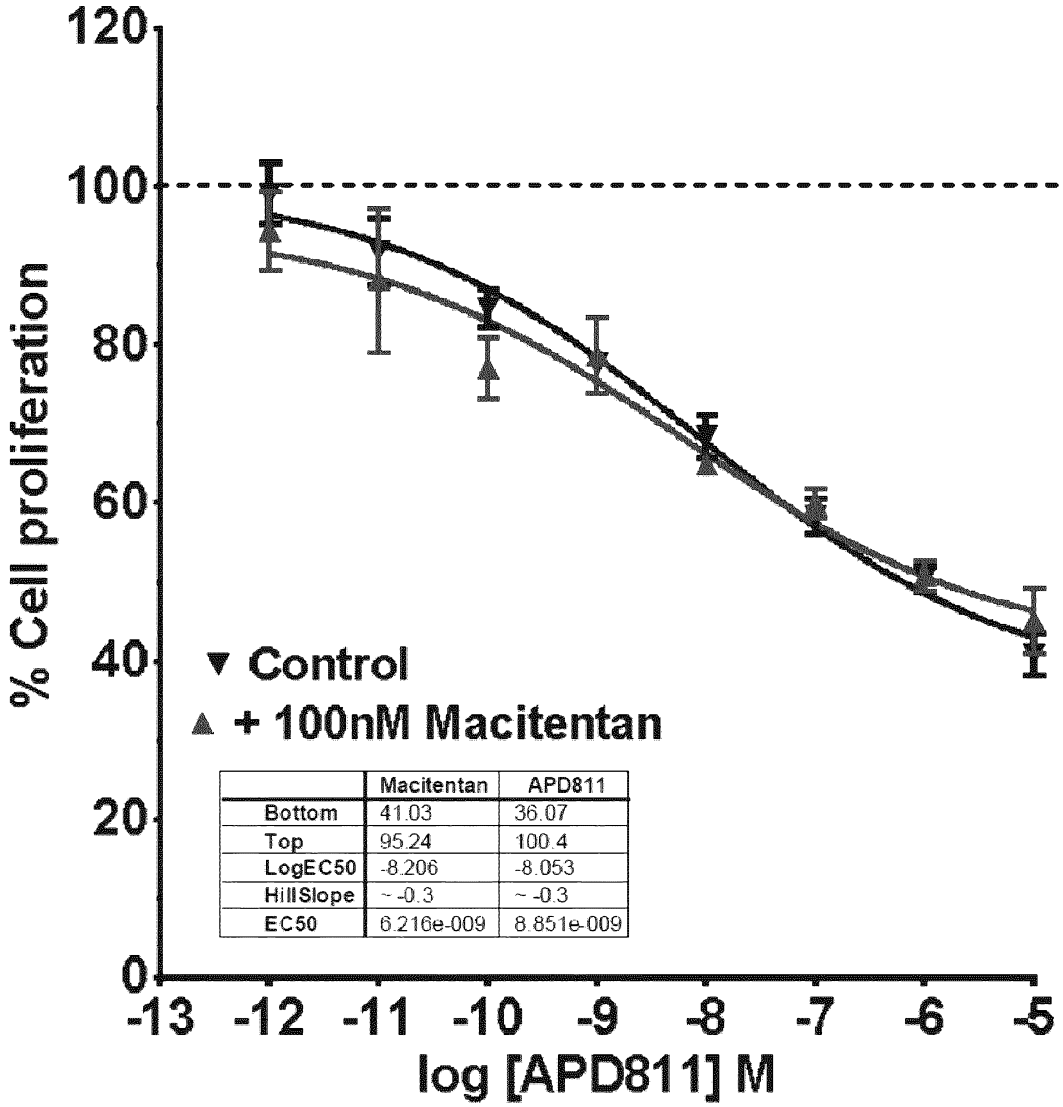
	Macitentan	APD811
Bottom	45.7	38.58
Top	94.31	102.5
LogEC50	-8.507	-6.893
HillSlope	~ -0.3	~ -0.3
EC50	3.111e-009	1.281e-007

Figure 7C



	Macitentan	APD811
Bottom	11.13	34.67
Top	103.4	~ 105
LogEC50	-5.823	-6.052
HillSlope	~ -0.3	-0.462
EC50	1.502e-006	8.877e-007

Figure 7D



	Macitentan	APD811
Bottom	41.03	36.07
Top	95.24	100.4
LogEC50	-8.206	-8.053
HillSlope	~ -0.3	~ -0.3
EC50	6.216e-009	8.851e-009

Figure 7E

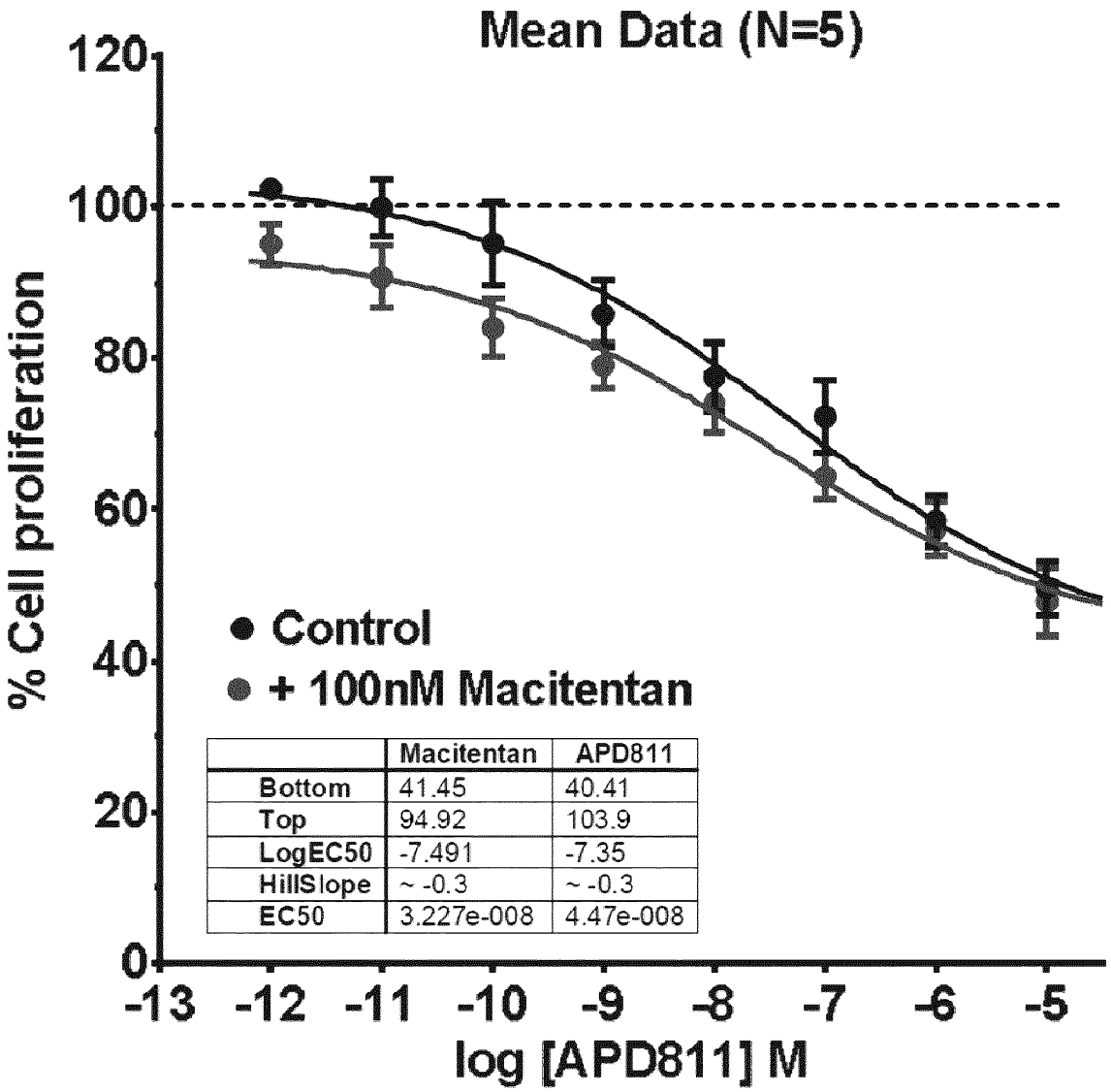

Mean Data (N=5)
% Cell proliferation
120
100
80
60
40
20
0
-13
-12
-11
-10
-9
-8
-7
-6
-5
log [APD811] M
Control
+ 100nM Macitentan
Macitentan
APD811
Bottom
41.45
40.41
Top
94.92
103.9
LogEC50
-7.491
-7.35
HillSlope
~ -0.3
~ -0.3
EC50
3.227e-008
4.47e-008

Figure 7F

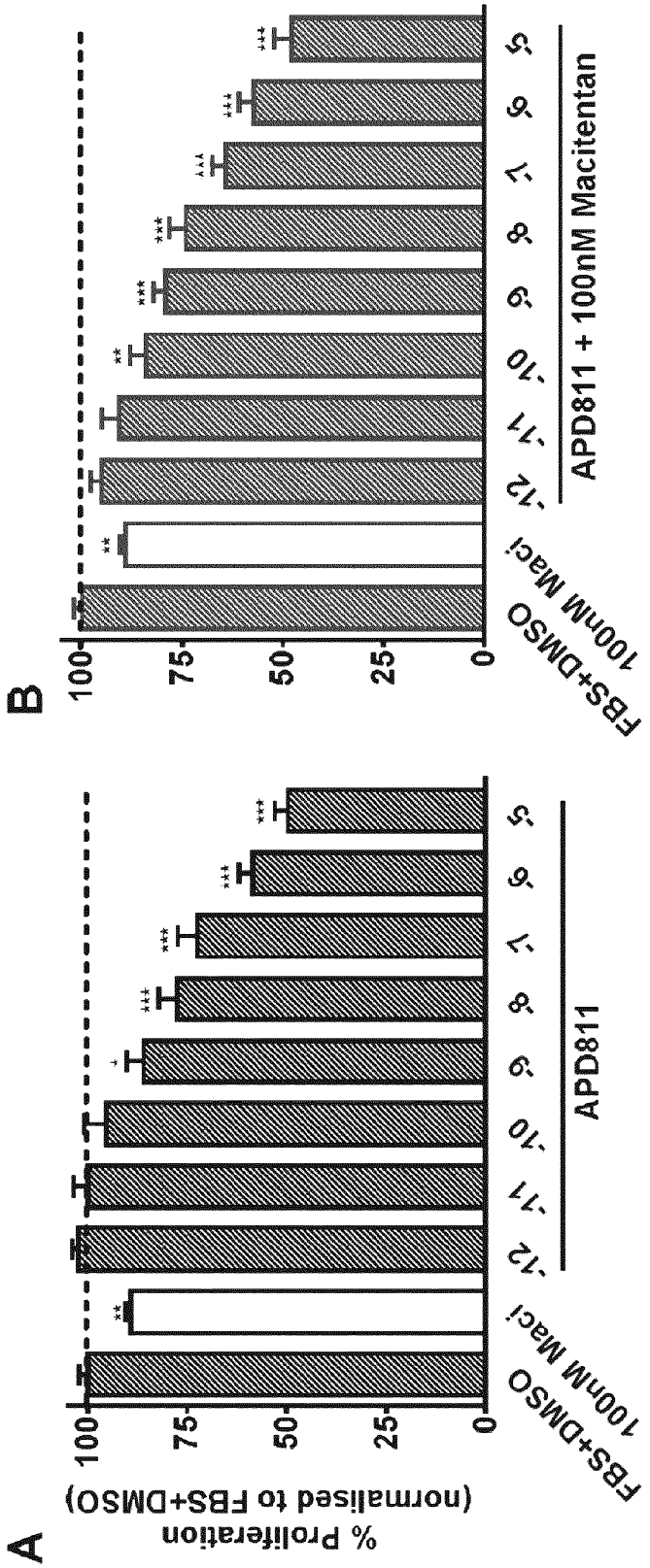
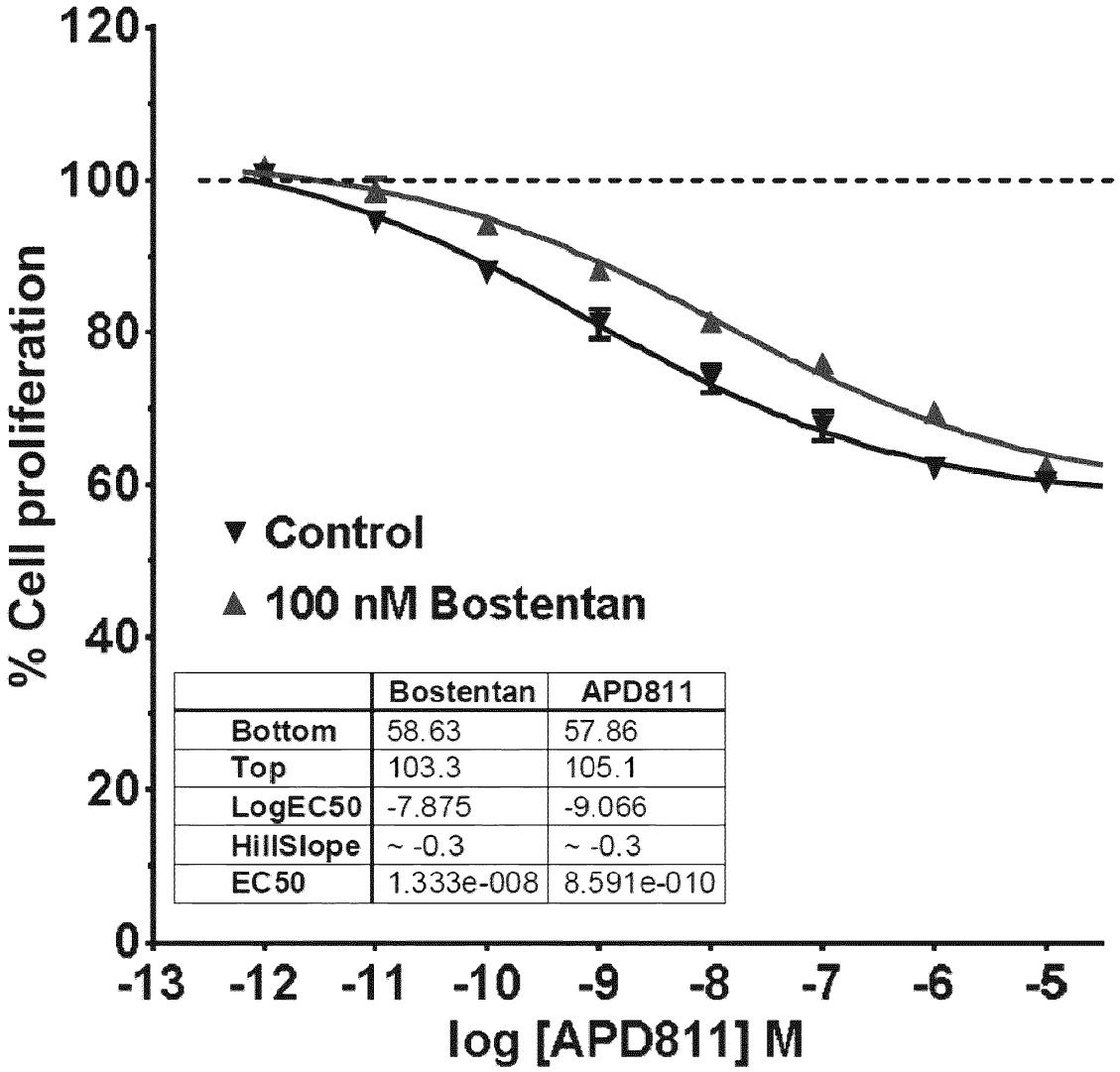
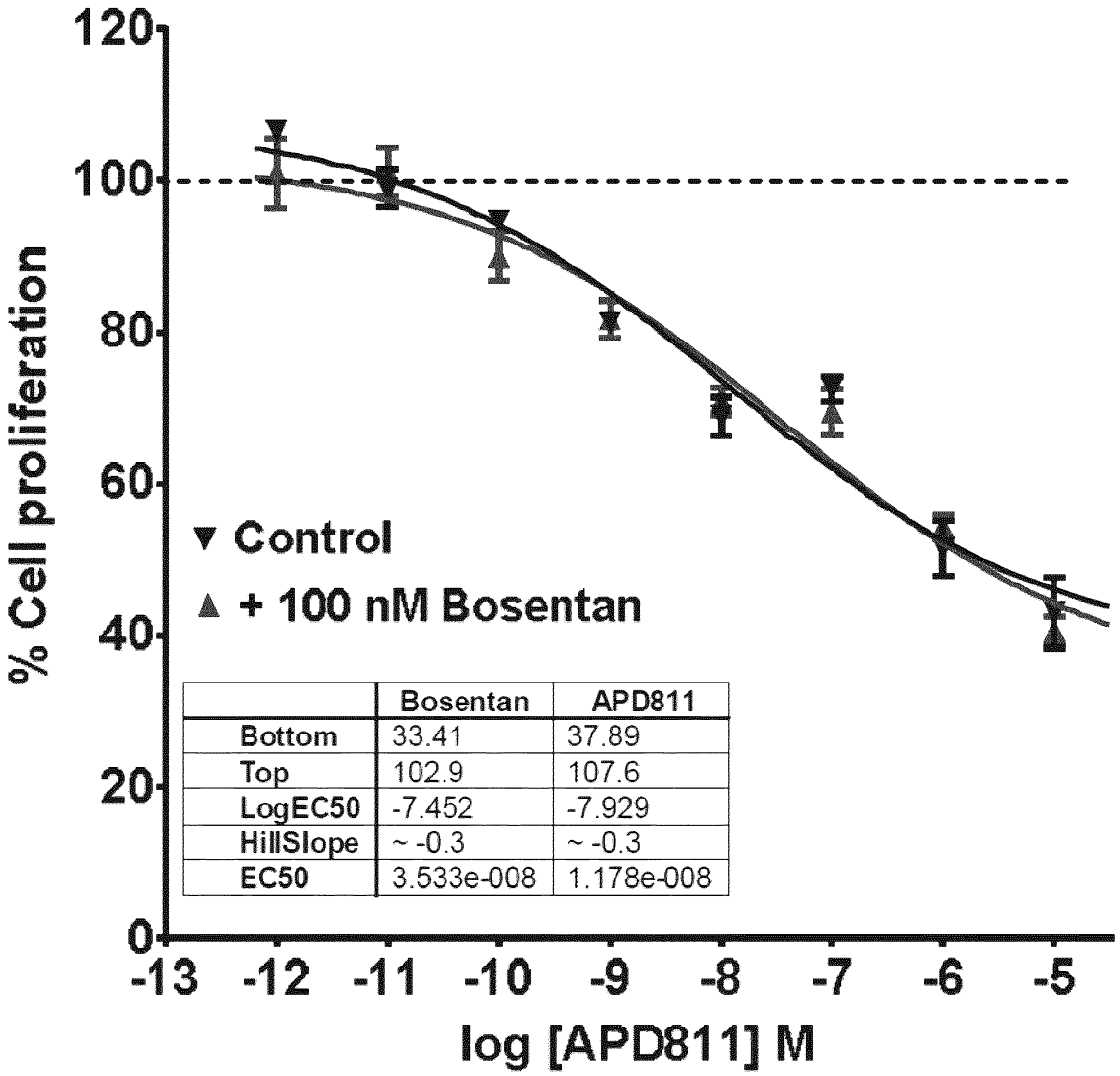

A
% Proliferation
(normalised to FBS+DMSO)
100
75
50
25
0
FBS+DMSO
100nM Macitentan
-12
-11
-10
-9
-8
-7
-6
-5
APD811
B
100
75
50
25
0
FBS+DMSO
100nM Macitentan
-12
-11
-10
-9
-8
-7
-6
-5
APD811 + 100nM Macitentan

Figure 8



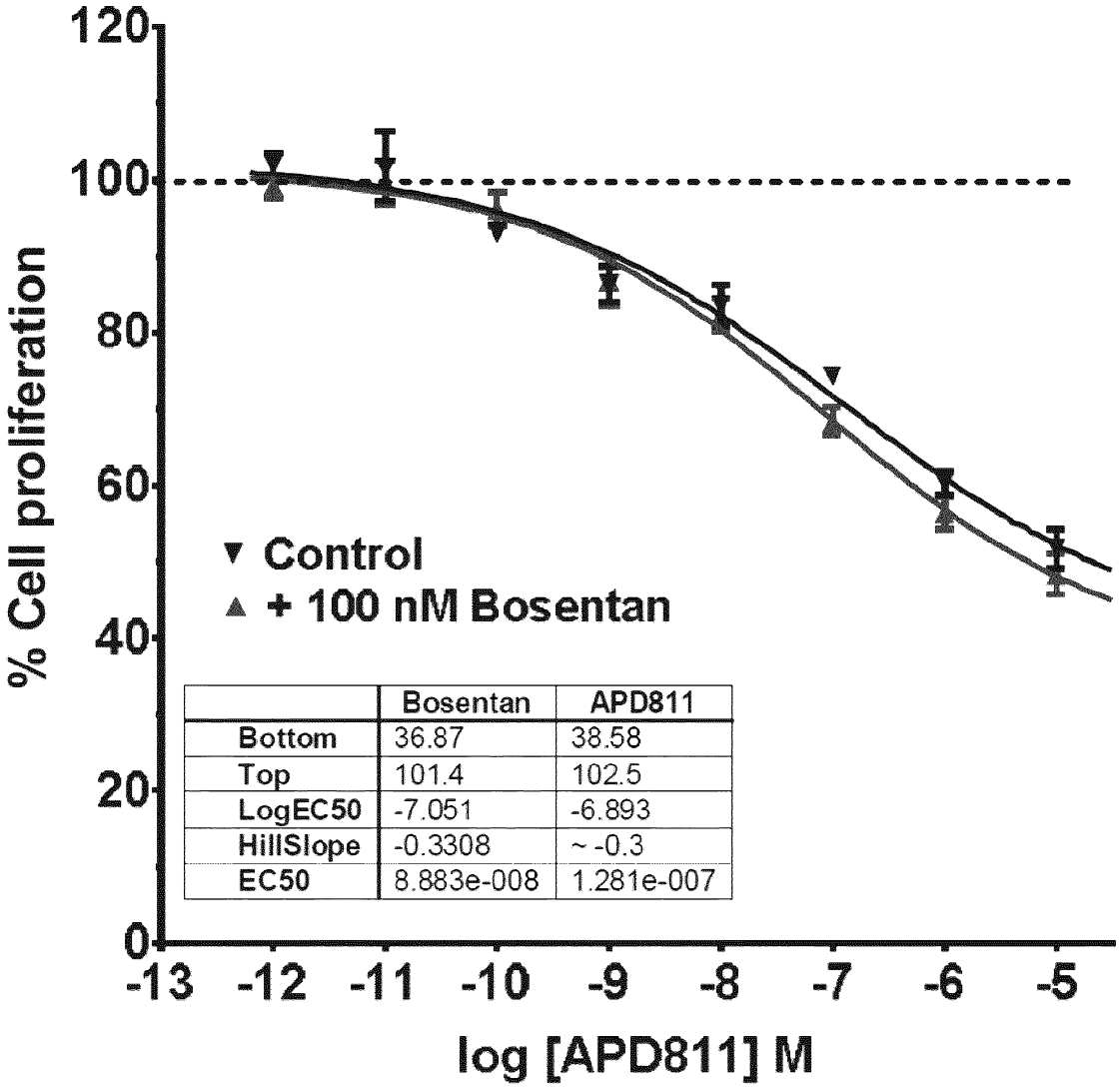
	Bostentan	APD811
Bottom	58.63	57.86
Top	103.3	105.1
LogEC50	-7.875	-9.066
HillSlope	~ -0.3	~ -0.3
EC50	1.333e-008	8.591e-010

Figure 9A



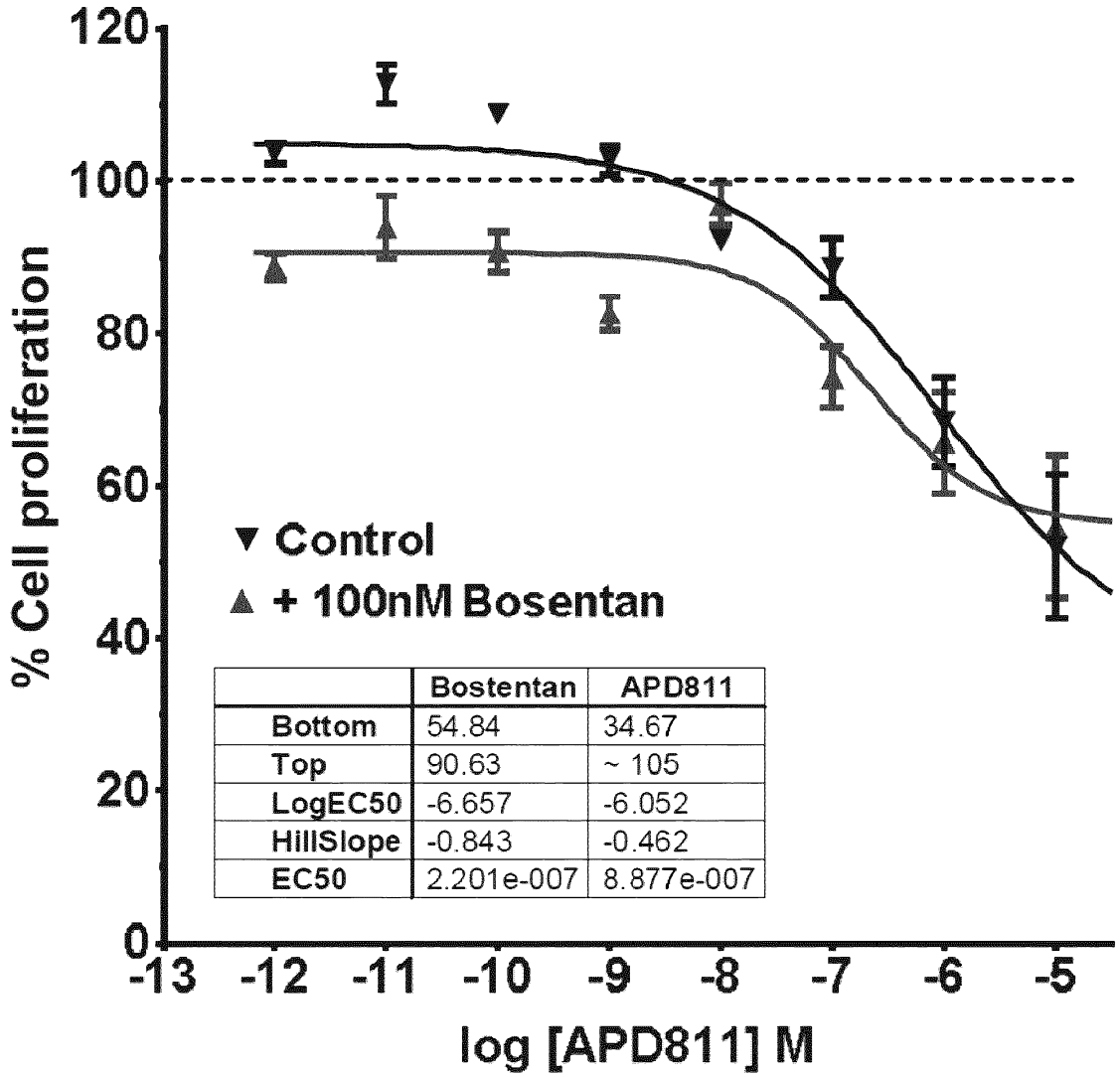
	Bosentan	APD811
Bottom	33.41	37.89
Top	102.9	107.6
LogEC50	-7.452	-7.929
HillSlope	~ -0.3	~ -0.3
EC50	3.533e-008	1.178e-008

Figure 9B



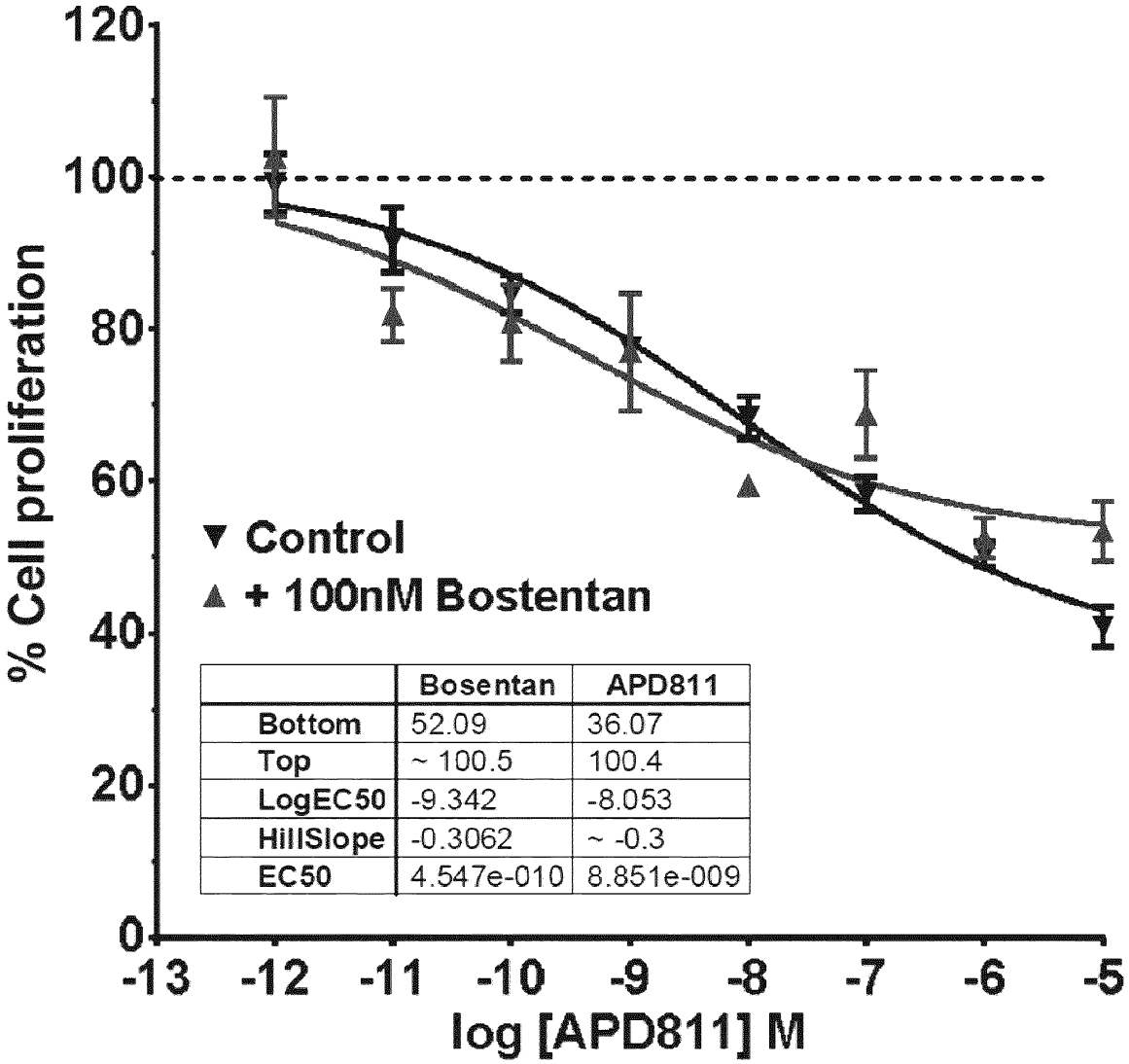
	Bosentan	APD811
Bottom	36.87	38.58
Top	101.4	102.5
LogEC50	-7.051	-6.893
HillSlope	-0.3308	~ -0.3
EC50	8.883e-008	1.281e-007

Figure 9C



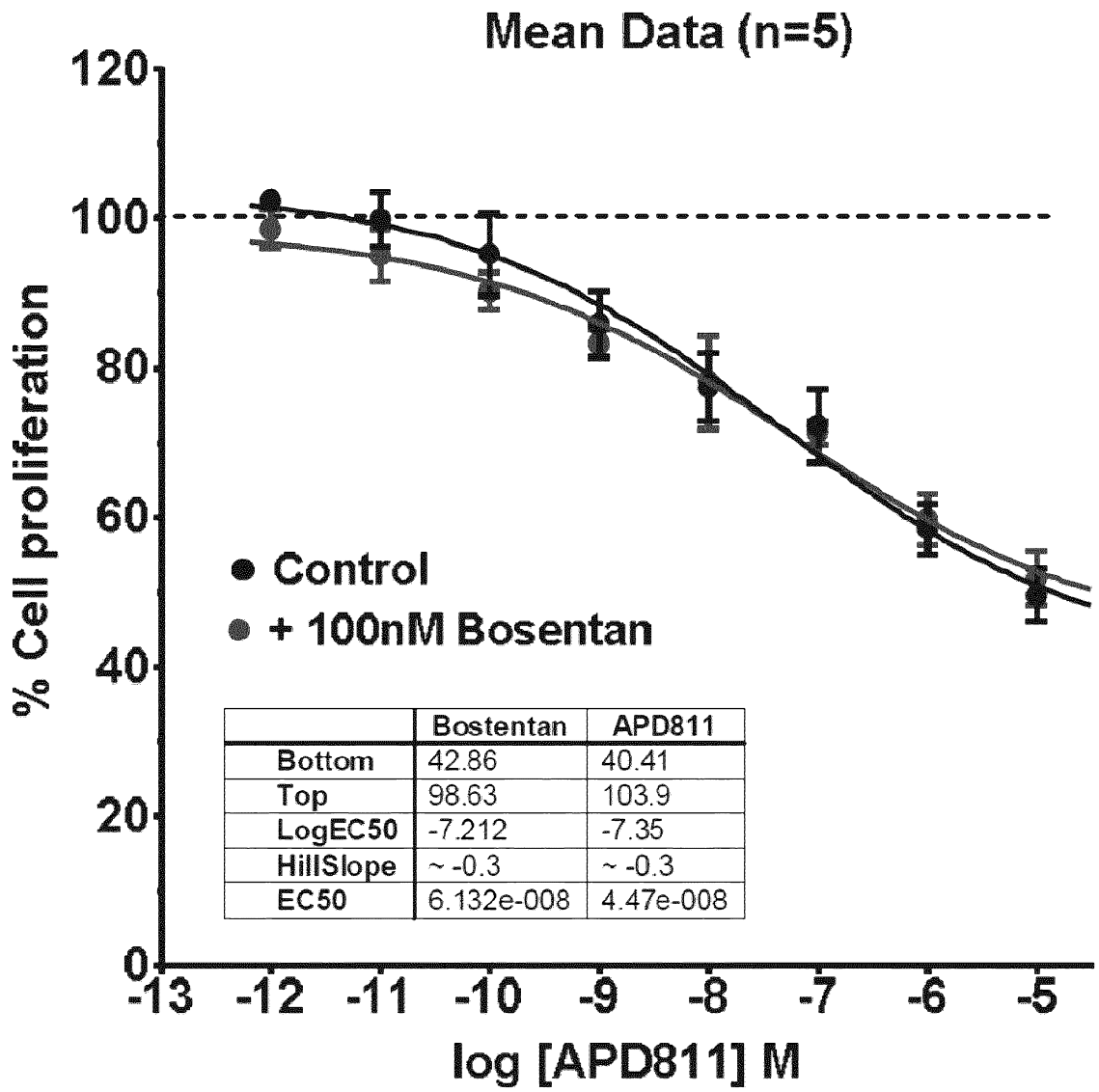
	Bostentan	APD811
Bottom	54.84	34.67
Top	90.63	~ 105
LogEC50	-6.657	-6.052
HillSlope	-0.843	-0.462
EC50	2.201e-007	8.877e-007

Figure 9D



	Bosentan	APD811
Bottom	52.09	36.07
Top	~ 100.5	100.4
LogEC50	-9.342	-8.053
HillSlope	-0.3062	~ -0.3
EC50	4.547e-010	8.851e-009

Figure 9E



	Bostentan	APD811
Bottom	42.86	40.41
Top	98.63	103.9
LogEC50	-7.212	-7.35
HillSlope	~ -0.3	~ -0.3
EC50	6.132e-008	4.47e-008

Figure 9F

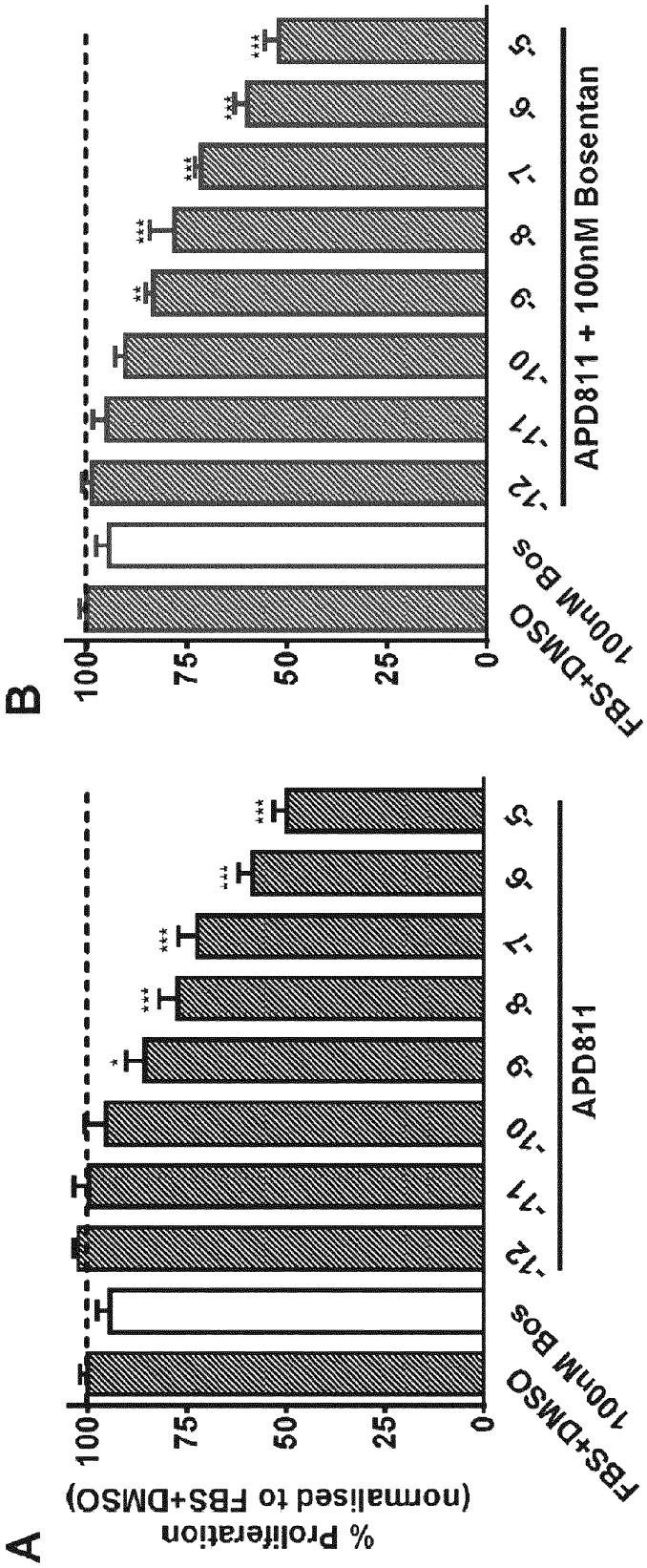

A
% Proliferation
(normalised to FBS+DMSO)
100
75
50
25
0
FBS+DMSO
100nM Bos
1-12
1-11
1-10
1-9
1-8
1-7
1-6
1-5
APD811
B
100
75
50
25
0
FBS+DMSO
100nM Bos
2-12
2-11
2-10
2-9
2-8
2-7
2-6
2-5
APD811 + 100nM Bosentan

Figure 10

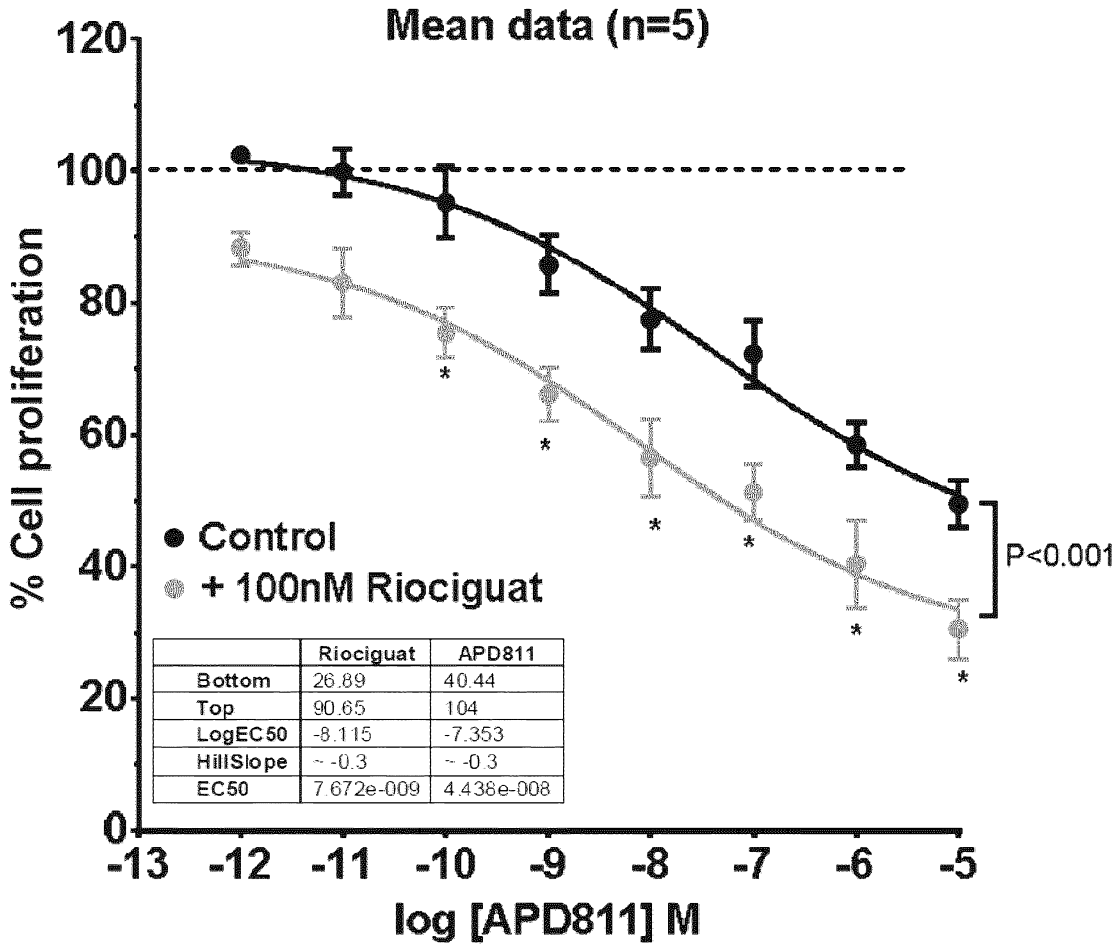

Mean data (n=5)
% Cell proliferation
120
100
80
60
40
20
0
-13
-12
-11
-10
-9
-8
-7
-6
-5
log [APD811] M
Control
+ 100nM Riociguat
P<0.001
Riociguat APD811
Bottom 26.89 40.44
Top 90.65 104
LogEC50 -8.115 -7.353
HillSlope ~ -0.3 ~ -0.3
EC50 7.672e-009 4.438e-008

Figure 11A

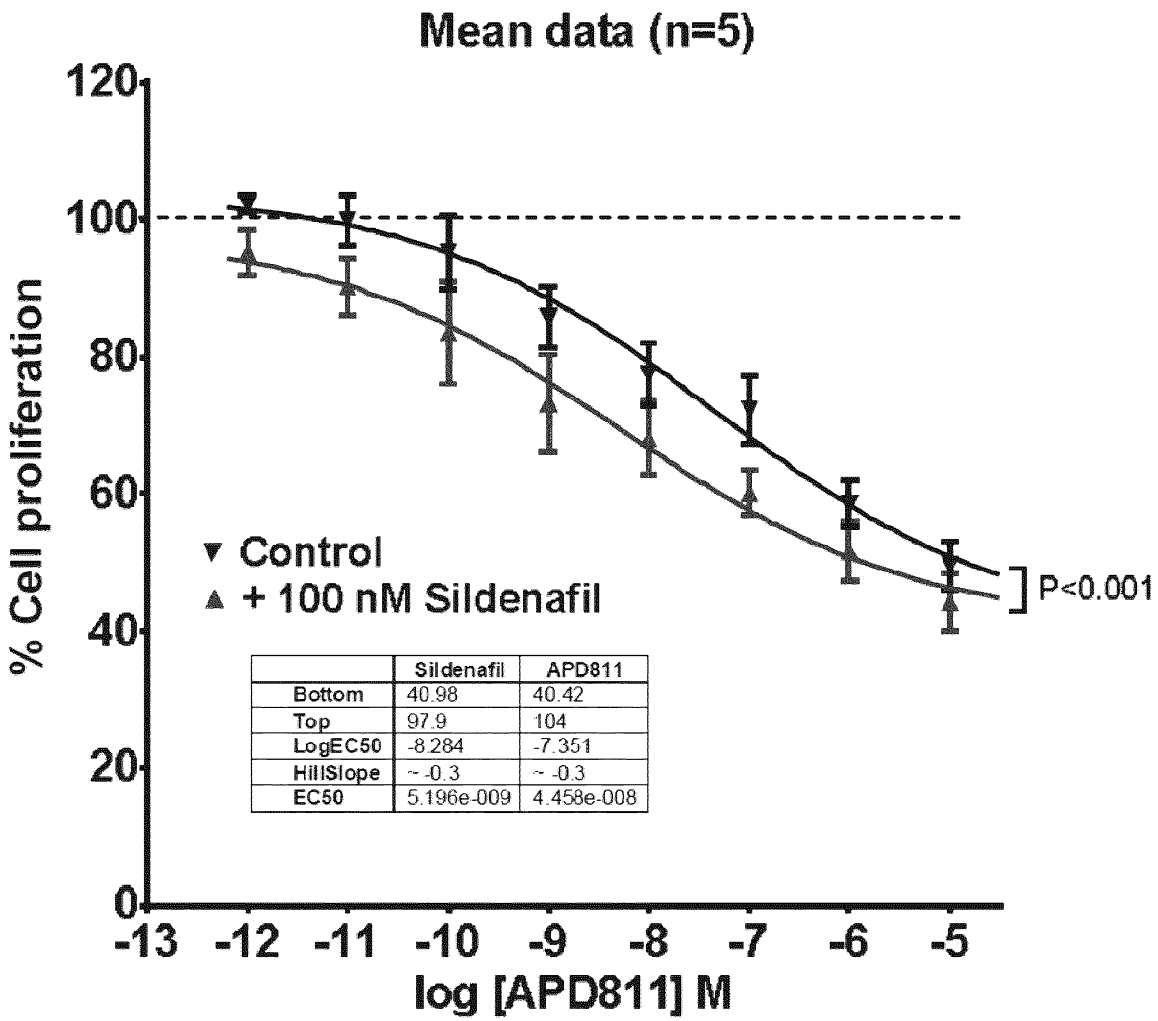

Mean data (n=5)
% Cell proliferation
120
100
80
60
40
20
0
-13
-12
-11
-10
-9
-8
-7
-6
-5
log [APD811] M
Control
+ 100 nM Sildenafil
P<0.001
Sildenafil
APD811
Bottom
40.98
40.42
Top
97.9
104
LogEC50
-8.284
-7.351
HillSlope
~ -0.3
~ -0.3
EC50
5.196e-009
4.458e-008

Figure 11B

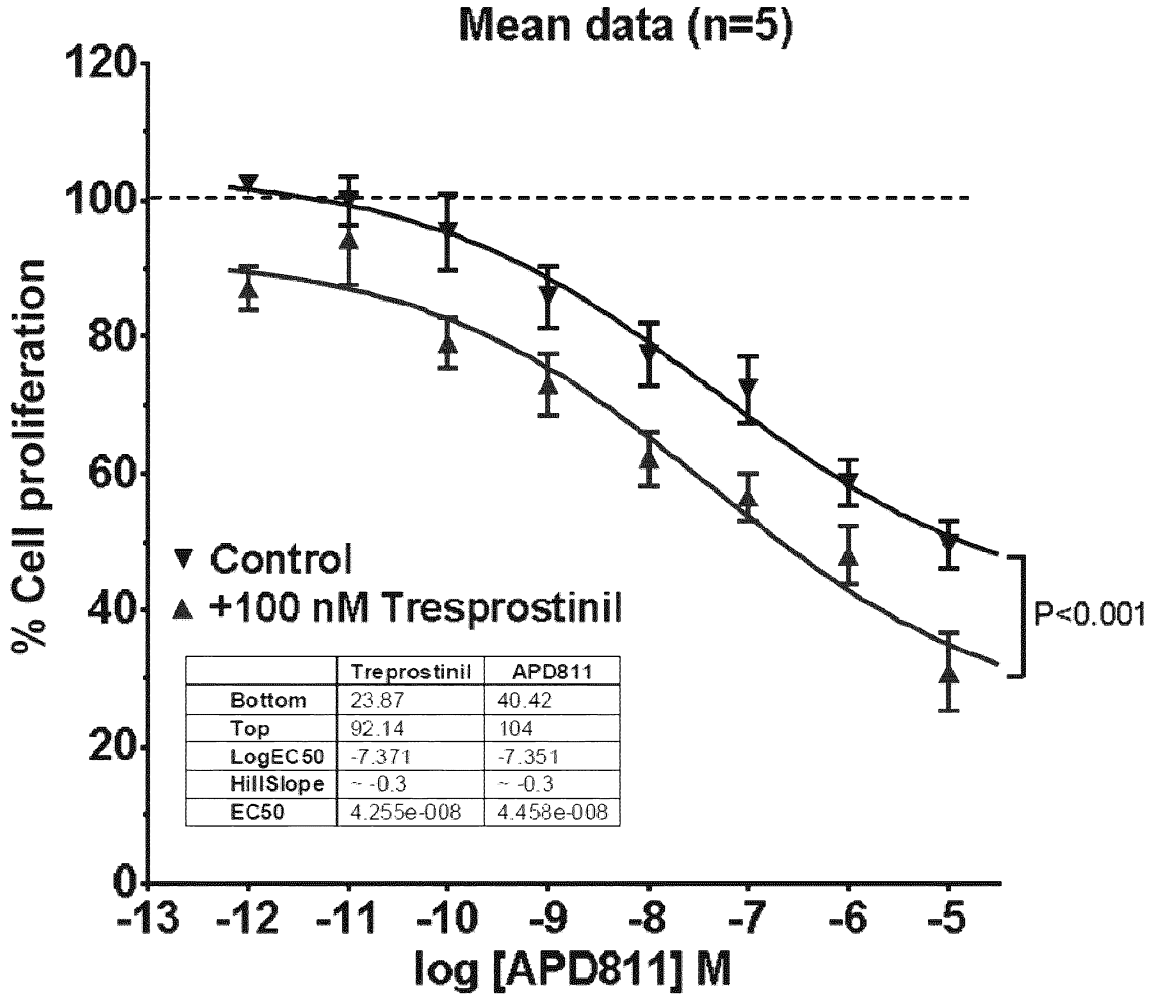

Mean data (n=5)
% Cell proliferation
120
100
80
60
40
20
0
-13 -12 -11 -10 -9 -8 -7 -6 -5
log [APD811] M
▼ Control
▲ +100 nM Tresprostinil
P<0.001
Treprostinil APD811
Bottom 23.87 40.42
Top 92.14 104
LogEC50 -7.371 -7.351
HillSlope ~ -0.3 ~ -0.3
EC50 4.255e-008 4.458e-008

Figure 11C

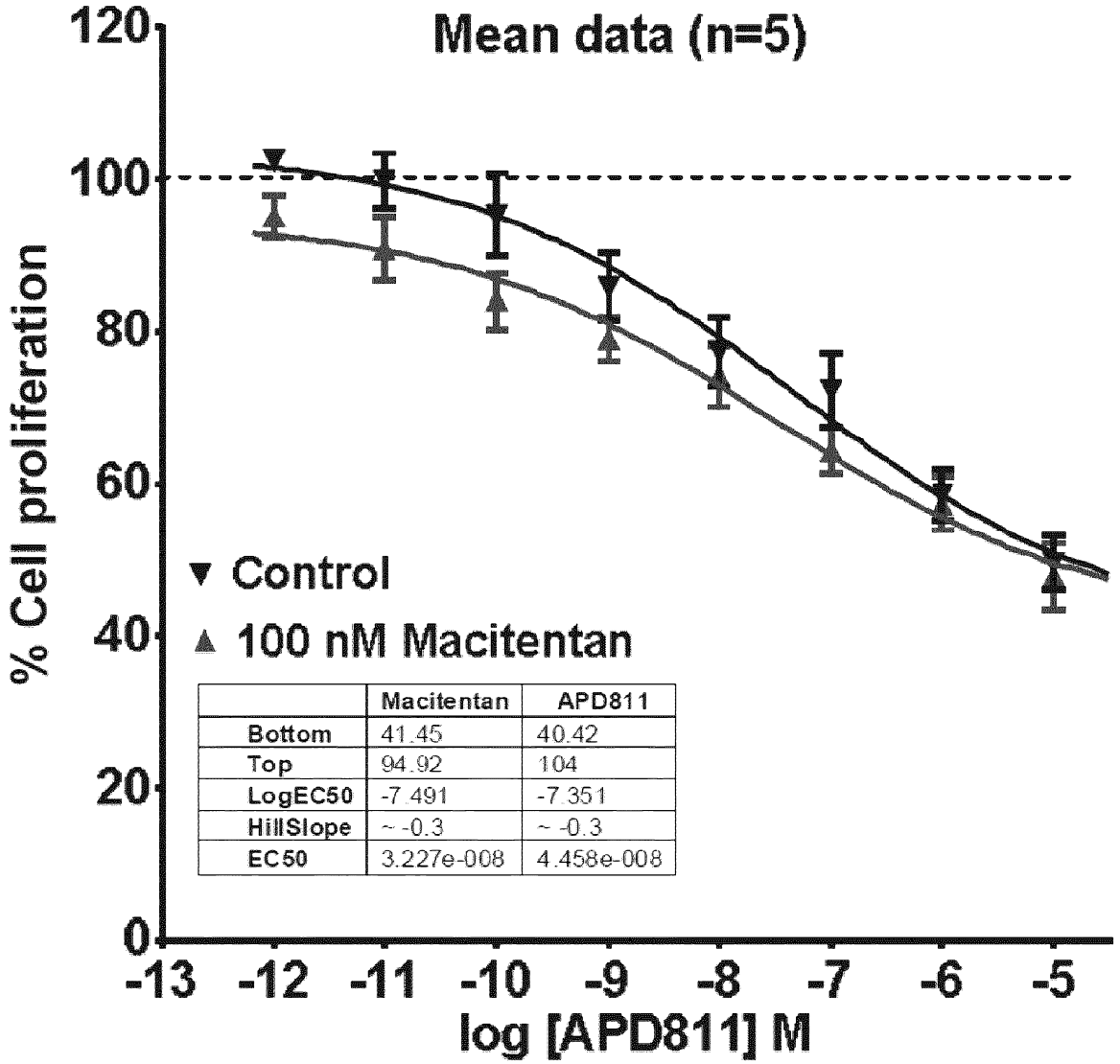

Mean data (n=5)
% Cell proliferation
120
100
80
60
40
20
0
-13
-12
-11
-10
-9
-8
-7
-6
-5
log [APD811] M
▼ Control
▲ 100 nM Macitentan
Macitentan
APD811
Bottom
41.45
40.42
Top
94.92
104
LogEC50
-7.491
-7.351
HillSlope
~ -0.3
~ -0.3
EC50
3.227e-008
4.458e-008

Figure 11D

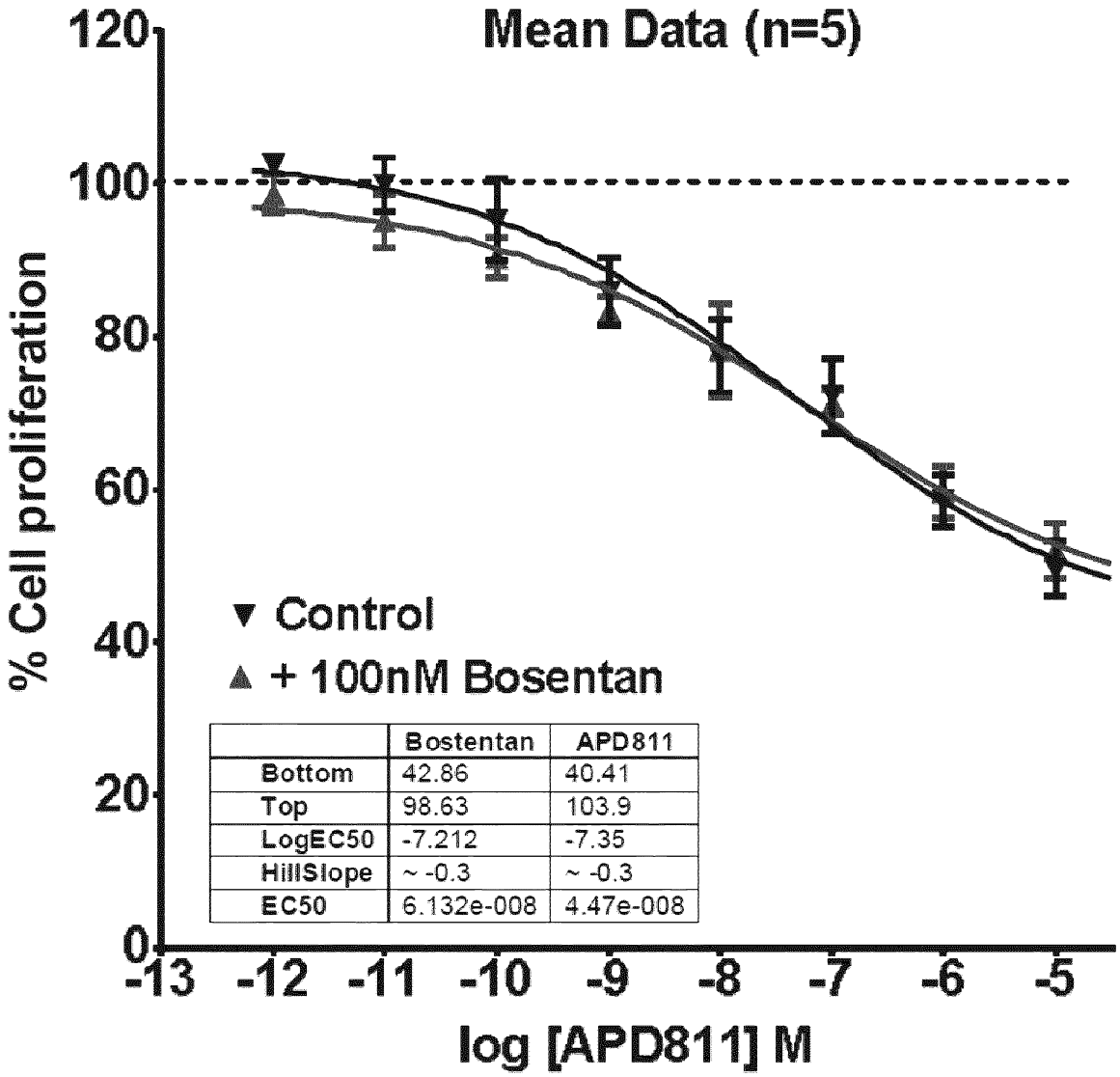

Mean Data (n=5)
% Cell proliferation
120
100
80
60
40
20
0
-13
-12
-11
-10
-9
-8
-7
-6
-5
log [APD811] M
▼ Control
▲ + 100nM Bosentan
Bostentan
APD811
Bottom
42.86
40.41
Top
98.63
103.9
LogEC50
-7.212
-7.35
HillSlope
~ -0.3
~ -0.3
EC50
6.132e-008
4.47e-008

Figure 11E

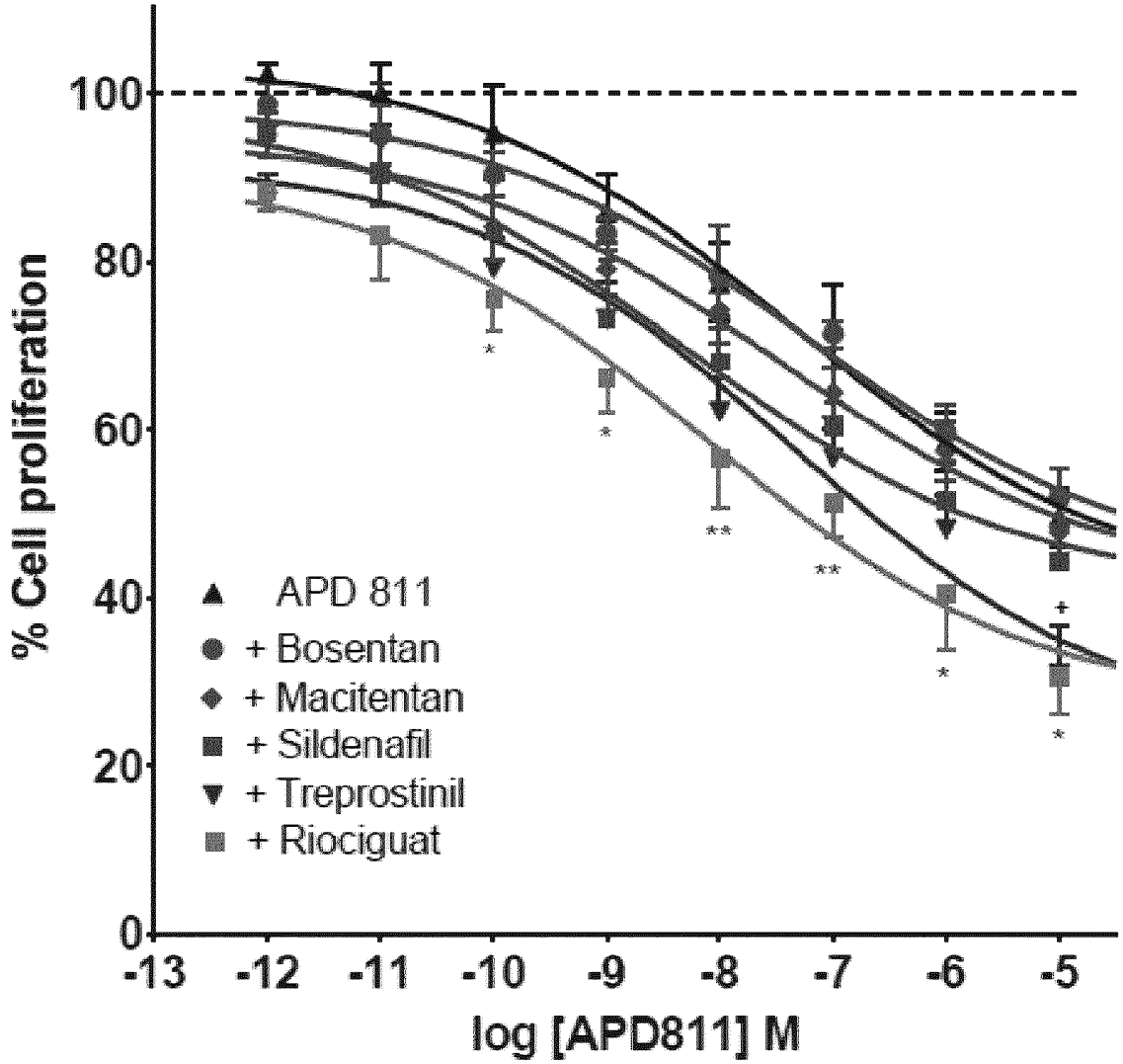

% Cell proliferation
100
80
60
40
20
0
-13
-12
-11
-10
-9
-8
-7
-6
-5
log [APD811] M
APD 811
+ Bosentan
+ Macitentan
+ Sildenafil
+ Treprostinil
+ Riociguat
*
**
+

Figure 12