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(54) Title: **ISTAROXIME AND METABOLITE DERIVATIVES THEREOF FOR REDUCING THE RISK OF ACUTE MYOCARDIAL ARRHYTHMIA**

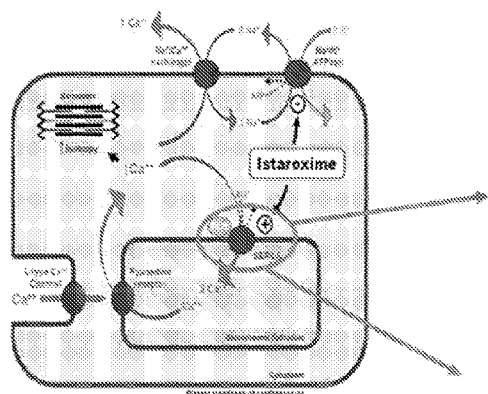


FIG. 1A

(57) Abstract: Compositions for intravenous infusion of istaroxime or derivatives thereof in human patients suffering from AHF with acute myocardial ischemia and/or an acute myocardial infarction are disclosed. The patient may optionally be in early cardiogenic or cardiogenic shock. Likewise, methods of administering istaroxime or derivatives thereof to individuals exhibiting symptoms of, or having been diagnosed with AHF with acute myocardial ischemia and/or an acute myocardial infarction. In particular-, disclosed herein is a method of reducing the risk of or preventing acute myocardial arrhythmias in an individual being treated for AHF with acute myocardial ischemia and/or acute myocardial infarction by administering to the individual a dosage regimen of istaroxime or CVie 216 by intravenous infusion.

ISTAROXIME AND METABOLITE DERIVATIVES THEREOF FOR REDUCING THE RISK OF ACUTE MYOCARDIAL ARRHYTHMIA

Cross-reference to related Application

5 This application claims priority to US Provisional Application No. 63/608,677 filed December 11, 2023, the entire contents being incorporated by reference as though set forth in full.

Filed of the Invention

10 The present invention relates to the field of pharmaceuticals, in particular to formulations of istaroxime or metabolite derivatives thereof, such as Formula II and Formula IV, for reducing the risk of or preventing myocardial arrhythmias in individuals having early cardiac shock, or heart failure with acute myocardial ischemia and/or an acute myocardial infarction and/or during the treatment of these conditions.

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Background

 The prevalence of heart failure (HF) is age-dependent, ranging from less than 2% of people younger than 60 years of age to more than 10% of individuals older than 75 years of age [Metra & Teerlink, 2017, Lancet 390:1981-1995]. In addition, HF is commonly associated with heart rhythm disturbances referred to as myocardial (or cardiac) arrhythmias, which can lead to severe complications that increase the mortality in these individuals. For instance, atrial fibrillation (AF), which is caused by extremely rapid and irregular electrical impulses in the atria of the heart, can result in stroke and/or worsening heart failure if left untreated [January *et al.*, 2019, Circulation 140(2)]. The most life-threatening type of myocardial arrhythmia is ventricular fibrillation (VF), which impacts the ventricles of the heart and causes the heart muscle to fibrillate rather than pump blood normally, requiring immediate medical attention [see, for example, Johns Hopkins Medicine “Ventricular Fibrillation” available at <https://www.hopkinsmedicine.org/health/conditions-and-diseases/ventricular-fibrillation>]. Other types of myocardial arrhythmias include ventricular tachycardia (VT), supraventricular tachycardia (SVT), atrial flutter, and others.

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Most patients with a HF have a history of hypertension, coronary artery disease, cardiomyopathies, valve disease, or a combination of these disorders [Metra & Teerlink, 2017, Lancet 390:1981-1995]. Chronic heart failure (CHF) can be distinguished from individuals exhibiting acute heart failure (AHF), the latter referring generally to a rapid onset or worsening of the symptoms and/or signs of HF, requiring immediate treatment and/or hospitalization. Individuals with CHF or other pre-existing cardiomyopathy, may experience a sudden onset of worsening of their condition, leading to a specific type of AHF referred to as acute decompensated heart failure (ADHF) [Joseph *et al.*, 2009, Tex. Heart Inst. J. 36:510-520]. Importantly, the clinical course and prognosis outcomes for individuals with CHF is much worse after an episode of AHF or ADHF [Solomon *et al.*, 2007, Circulation 116:1482-1487; Teneggi *et al.*, 2018, Heart Failure Rev. 23:667-691; Joseph *et al.*, 2009, Tex. Heart Inst. J. 36:510-520]. Common causes of AHF include acute coronary syndrome, complications due to myocardial infarction (heart attack), and myocardial ischemia, each of which is associated with reduced or blocked blood flow to the heart. Inadequate blood flow to the heart results in the lack of oxygen delivery to the myocardial tissue.

Clinical symptoms in HF are caused by a cardiac double pathological feature that consists in an inotropic abnormality, resulting in diminished systolic emptying (systolic dysfunction) and a compliance abnormality in which the ability of the ventricles to suck blood from the venous system is impaired (diastolic dysfunction), thus reducing the amount of blood available for systolic contraction, which is an impairment of left ventricle (LV) filling. Whatever the initial triggering mechanism of HF, an abnormal distribution of intracellular Ca^{2+} resulting from reduced Ca^{2+} uptake by the sarcoplasmic reticulum (SR), which is the main intracellular Ca^{2+} store [Schwinger *et al.*, 1999, J. Mol. Cell. Cardiol.;31(3):479-91; Bers *et al.*, 2006, Ann N.Y. Acad. Sci. 1080:165-177], underlies the impaired contractility and relaxation. This abnormal Ca^{2+} distribution involves the Ca^{2+} -ATPase of the SR membrane (SERCA2a), an ATP dependent Ca^{2+} transport pump. SERCA2a activity is physiologically limited by its interaction with phospholamban (PLN) [Asahi *et al.*, 1999, J. Biol. Chem. 274:32855–32862; Toyoshima *et al.*, 2003, Proc. Natl Acad. Sci. U S A 100:467–477; Bers, 2008, Annu. Rev. Physiol 70:23-49; MacLennan & Kranias, 2003, Nat. Rev. Mol. Cell. Biol. 4(7):566-577], and SERCA2a restraint is normally relieved by PLN phosphorylation by protein kinase A (PKA), a signaling pathway severely depressed as a consequence of HF remodeling [Karim *et al.*, 2006, J. Mol. Biol.

358:1032–1040; Lohse *et al.*, 2003, *Circ. Res.* 93:896-906; Bers, 2006, *Physiology* 21:380–387; Mann & Bristow, 2005, *Circulation* 111:2837–2849]. A deficiency in cardiac SERCA2a activity is widely recognized as the main cause of the reduced Ca^{2+} uptake in the SR of the failing myocardium [Bers *et al.*, 2006, *Ann. N.Y. Acad. Sci.* 1080:165-177; Bers, 2006, *Physiology* 21:380–387; Minamisawa *et al.*, 1999, *Cell* 99:313–322].

In addition to its consequences on myocyte contractility and relaxation, abnormal Ca^{2+} distribution also facilitates myocardial arrhythmias [Zaza A & Rocchetti, 2015, *Curr. Pharm. Des.* 21:1053-1061] and, in the long term, it accelerates myocytes loss by apoptosis [Nakayama *et al.*, 2007, *J. Clin. Invest.* 117:2431-44]. Reduced SERCA2a function also increases the energy cost of contraction, because it requires a compensatory increase in Ca^{2+} extrusion through the Na-Ca exchanger (NCX), which is less energy efficient [Lipskaya *et al.*, 2010, *Expert Opin. Biol. Ther.* 10:29-41]. Substantial evidence indicates that normalization of SERCA2a function restores intracellular Ca^{2+} homeostasis and improves contractility and relaxation of cardiomyocytes and of the heart *in situ* [Byrne *et al.*, 2008, *Gene Therapy* 15:1550-1557; Sato *et al.*, 2001, *J. Biol. Chem.* 276:9392-99). To summarize, recovery of SERCA2a function in HF may improve cardiac relaxation and contractility while minimizing arrhythmias, myocardial oxygen consumption and myocyte death [Lipskaia *et al.*, 2010, *Expert Opin. Biol Ther.* 10:29-41]. In parallel to SERCA2a activation, inhibition of the Na^+/K^+ -ATPase can further increase intracellular Ca^{2+} content without inducing excessive cytosolic Ca^{2+} accumulation [Shattock *et al.*, 2015, *J Physiol.* 593(6):1361-82]. Therefore, the combination of Na^+/K^+ -ATPase inhibition and SERCA2a stimulation may afford further positive inotropy at a reduced risk of arrhythmogenic Ca^{2+} triggering events.

Another serious AHF condition is cardiogenic shock, which occurs when the heart fails to pump sufficient blood to the brain and other organs. Cardiogenic shock is commonly caused by acute myocardial infarction (AMI), when blood flow to the heart is significantly decreased or stopped and is associated with high rates of morbidity and mortality, posing a therapeutic challenge for clinicians [van Diepen *et al.*, 2017, *Circulation* 136:e232-e268; Hunziker *et al.*, *Circ. Cardiovasc. Interv.* 12(4):e007293; Berg *et al.*, 2019, *Circ. Outcomes* 12:e005618]. The most common intervention for individuals presenting with AMI-induced cardiogenic shock is the revascularization of culprit coronary vessels.

As noted above, individuals suffering AHF and other cardiovascular diseases, such as cardiogenic shock or early cardiogenic shock, often present with myocardial ischemia, which is characterized by reduced or blocked blood flow to the heart. Thus, a common intervention in these patients is to reestablish cardiac blood flow. Increasing blood flow to the heart, or reperfusion, can be performed using various techniques known in the art, including, anticoagulants, thrombolysis, embolectomy, and surgical revascularization. Unfortunately, however, reperfusion after myocardial ischemia has been shown to increase the risk of adverse cardiovascular outcomes, including myocardial arrhythmias. In fact, it has been long observed that rapid reperfusion of blood flow to the myocardial tissue increases the risk for VF, which is one of the most severe types of myocardial arrhythmias and can lead to cardiac death [Wit & Janse, 2001, *Circ. Res.* 89(9):741-743]. Other common types of reperfusion arrhythmias include, AF, VT, and accelerated idioventricular rhythm (AIR) [see, for example, Roland, 2022, Healthline available at <https://www.healthline.com/health/arrhythmia/reperfusion-arrhythmia#bottom-line>].

Unfortunately, some of the most commonly prescribed drugs for the acute improvement of cardiac function, such as norepinephrine, dopamine, dobutamine, and milrinone, all increase the risk for myocardial arrhythmias. Accordingly, there has been a need for therapeutics that can adequately treat AHF, including AHF with ischemia, without increasing the risk for myocardial arrhythmias.

One compound currently being studied for the treatment of patients with AHF and cardiogenic shock (or pre-cardiogenic shock) is istaroxime (PST 2744), which is an androstenedione derivative and chemically unrelated to cardiac glycosides. Istaroxime is disclosed in EP0825197 and in De Munari *et al.*, [2003, *J. Med. Chem.* 46:3644-3654] and is the compound (3Z,5 α)-3-[(2-aminoethoxy)imino]androstane-6,17-dione. Istaroxime may have potential to overcome many of the issues with prior or existing therapies being used to treat patients with these disorders. Istaroxime exerts its effects through dual mechanisms of action: 1) the inhibition of the Na⁺/K⁺-ATPase activity, thereby causing an increase in intracellular calcium, which increases cardiomyocyte contractility (inotropy); and 2) the activation of the sarcoplasmic reticulum calcium ATPase isoform (SERCA2a), thus improving both myocardial relaxation (lusitropy) and contractility. However, compounds that increase intracellular Ca²⁺ also increase afterpotentials of the heart and, therefore, may increase risk of myocardial arrhythmias.

One particular clinical study that examined the effects of istaroxime in patients with AHF is outlined in the HORIZON-HF Study [*see* Gheorghiade *et al.*, 2008, JACC, 51(23):2276-2285]. This study looked at 120 patients hospitalized with ADHF and reduced LV systolic functions administered escalating doses of istaroxime for 6 hours (*i.e.*, 0.5 µg/kg/min, 1.0 µg/kg/min, and 1.5 µg/kg/min). The primary endpoint of the study was the change from baseline in PCWP after 6 hours and revealed dose-dependent improvements in PCWP between the treatment cohort compared to the placebo group. However, this improvement in PCWP plateaued after 3 hours and remained constant from 3 hours to 6 hours following the initiation of the infusion. The results also demonstrated that HR tended to decrease during the 6-hour infusion, while SBP slightly increased in a dose-dependent manner. However, this study excluded ADHF patients presenting with persistent hypotension (the average baseline SBP was 116 mmHg and specifically excluded patients with an SBP lower than 90), cardiogenic shock, acute myocardial infarctions, acute myocardial ischemia, and/or significant myocardial arrhythmias.

WO 2020/180356 A9 discloses another clinical study that examined prolonged istaroxime infusion in 120 patients with ADHF [*see also* Carubelli *et al.*, 2020, Euro. J. Heart Failure 22:1684-1693]. This study involved two doses of istaroxime (0.5 µg/kg/min and 1.0 µg/kg/min) infused for up to 24 hours. The primary endpoint of this study was E/Ea ratio and revealed an improvement in both treatment groups compared to the placebo groups. In contrast to what the results of the Horizon Study suggested, prolonged infusion with Istaroxime showed significant improvements for most of the diastolic function parameters measured after 6 hours of infusion. However, as with the HORIZON-HF study, patients with cardiogenic shock, acute myocardial infarctions, acute myocardial ischemia, and/or significant myocardial arrhythmias were excluded.

The SEISMiC study examined the effects of istaroxime in patients with AHF-related pre-cardiogenic shock [Metra *et al.*, 2022, Euro. J. Heart Failure 24:1967-1977]. The primary endpoint of this study was SBP AUC through 6 hours and revealed patients administered istaroxime had improved blood pressure. Again, patients with overt cardiogenic shock, acute myocardial infarctions, acute myocardial ischemia, and/or significant myocardial arrhythmias were excluded.

While istaroxime has been studied extensively in AHF patients, it has not been shown to be effective in significantly protecting against the risk of serious myocardial arrhythmias,

especially in patients presenting with acute myocardial ischemia and/or acute myocardial infarction. The exclusion of these subjects was due, in part, to the concern that istaroxime's ability to inhibit Na^+/K^+ -ATPase activity would increase the risk of patient susceptibility to myocardial arrhythmias and other deleterious side effects. Accordingly, conventional wisdom dictated that the risk could be even more elevated in patients already presenting with acute myocardial ischemia and/or acute myocardial infarction, especially when reperfusion is accomplished.

Thus, there is a clear but unmet need to treat patients exhibiting AHF with acute myocardial ischemia and/or acute myocardial infarction, including those having a pre-existing and elevated risk for serious myocardial arrhythmias, such as VF. The ability to administer a compound to treat serious cardiovascular disorders while protecting against significant myocardial arrhythmias would meaningfully extend the profile of such compounds with respect to safety and efficacy for a broader spectrum of patients with AHF and cardiogenic shock.

Summary of the Invention

Described herein are pharmaceutical compositions containing istaroxime, or a derivative thereof, formulated for administration to a subject for use in a treatment method for AHF with acute myocardial ischemia and/or an acute myocardial infarction. In particular embodiments, the administration is by intravenous infusion, whereby the E wave, E wave deceleration time, A wave, E/A ratio, e' , E/e' ratio, stroke volume, heart rate, cardiac index, systolic blood pressure (SBP), diastolic blood pressure (DBP), mean blood pressure, and/or other parameters of heart function in the individual are improved. Most significantly administration of istaroxime to the individual does induce a significant increase in the incidence of serious acute myocardial arrhythmias, or a significant increase in the duration of serious acute myocardial arrhythmias, even during perfusion following acute myocardial ischemia. The pharmaceutical compositions and methods herein include dual mechanism compounds and pure SERCA2a activators derived from istaroxime or its metabolites. This surprising result enables the use of this life-saving medication to be administered to a broader spectrum of AHF patients, including those exhibiting pre-cardiogenic shock or cardiogenic shock who are at risk for arrhythmias from having clinical ischemia-reperfusion to the myocardium. One aspect of the invention features a method of treating acute heart failure in a human subject, which includes the steps of treating the human

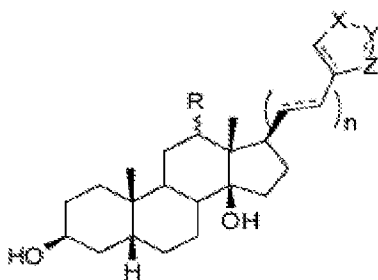
subject suffering from early cardiogenic shock with myocardial ischemia and/or an acute myocardial infarction, via intravenous infusion a pharmaceutical composition comprising istaroxime, or a pharmaceutically acceptable salt, solvate, or hydrate thereof, at a dose of about 0.1 mcg/kg/min to about 2.5 mcg/kg/min, for an infusion period of at least about 3 hours. In this method, the administering of the istaroxime or derivative thereof results in a measurable improvement in heart function and an absence or reduction of acute myocardial arrhythmias. In some human subjects, the absence of acute myocardial arrhythmias continues for at least 3 hours following initiation of infusion. A reduction of acute myocardial arrhythmias can be compared to the average number of acute myocardial arrhythmias or the average duration of acute myocardial arrhythmias in a cohort of individuals not administered istaroxime or derivative thereof. The infusion period can be at least about 6 hours, at least about 12 hours, at least about 24 hours, at least about 48 hours, or at least about 60 hours. In some embodiments, the acute heart failure comprises pre-cardiogenic shock or cardiogenic shock. In other embodiments, istaroxime is administered at a dose of about 0.5 mcg/kg/min to about 1.5 mcg/kg/min and istaroxime or derivatives thereof are infused for about 60 hours.

In some embodiments, the acute myocardial ischemia is detected by: (i) ECG monitoring, echocardiogram, stress test, coronary angiogram, nuclear perfusion imaging, positron emission tomography, cardiovascular magnetic resonance, a chest X-ray, or a heart MRI; and/or (ii) laboratory blood tests for proteins and enzymes. For instance, the acute myocardial ischemia may be detected by ECG monitoring. In some embodiments of the method, reperfusion is performed/induced following diagnoses of the acute myocardial ischemia. For instance, reperfusion may be initiated within 3 hours of the diagnosis of the acute myocardial ischemia. In some embodiments, the reperfusion comprises administration of recombinant tissue plasminogen activator (rTPA), reteplase, anistreplase, desmoteplase, nattokinase, lumbrokinase, serrapeptase, papain, DNase, bromelain, honiol, tenecteplase, alteplase, urokinase, streptokinase, anticoagulation with heparin, calcium channel blockers, ranolazine, beta blockers, nitrates, aspirin, or cholesterol-lowering compounds. In other embodiments, the reperfusion comprises balloon angioplasty, stent placement, coronary artery bypass graft, or enhanced external counterpulsation.

In an embodiment, the absence or reduction of acute myocardial arrhythmias is measured by electrocardiogram. The acute myocardial arrhythmias can be a ventricular tachycardia,

ventricular fibrillation, or a combination thereof. In some embodiments, the absence or reduction of acute myocardial arrhythmias comprises a reduction in the duration of the acute myocardial arrhythmias. In others, the improvement in heart function is diastolic heart function measured by echocardiography, such as, but not limited to decreased E wave, increased E wave deceleration time, increased A wave, decreased E/A ratio, increased e', or decreased E/e' ratio. Further, the improvement in heart function may include one or more of improved diastolic relaxation, increased diastolic blood pressure, decreased heart rate, a decrease in dyspnea, increased stroke volume, increased cardiac index, increased cardiac output, decreased pulmonary capillary wedge pressure, or increased stroke volume index. In an embodiment, the improvement in heart function is measured by sphygmomanometer, an electrocardiogram, cardiac catheterization, a radionuclide ventriculography scan, or any combination thereof.

Another aspect of the invention features a method of treating acute heart failure in a human subject having acute heart failure associated with acute myocardial ischemia and/or an acute myocardial infarction, and administering to the human subject by intravenous infusion a pharmaceutical composition comprising a dual mechanism compound at a dose of about 0.1 mcg/kg/min to about 2.5 mcg/kg/min, for an infusion period of at least about 3 hours. In this method, administration of the dual mechanism compound results in a measurable improvement in heart function and an absence or reduction of acute myocardial arrhythmias, such as (i) the absence of acute myocardial arrhythmias continues for at least 3 hours following initiation of infusion; or (ii) the reduction of acute myocardial arrhythmias is compared to the average number of acute myocardial arrhythmias or the average duration of acute myocardial arrhythmias in a cohort of individuals not administered the dual mechanism compound. In such embodiments, the dual mechanism compound has a structure formula (II)



Formula (II)

wherein X, Y, Z are annular atoms comprised in a five-membered carbocyclic or heterocyclic ring, selected from the group consisting of CH, NH, N, O, S; and wherein n is 0 or 1 and said heterocyclic ring selected from the group consisting of imidazolyl, pyrazolyl, thiazolyl, isoxazolyl, and the corresponding dihydro- and tetrahydro derivatives;

R is H or OH; and

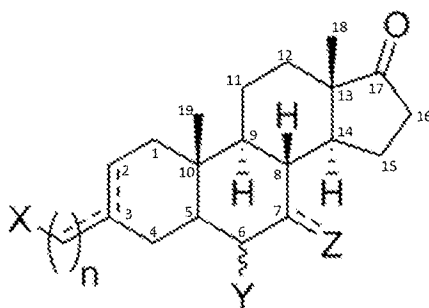
the dotted line represents an optional double bond C=C; the thick line represents a bond in the β configuration; the wavy line represents a bond both in the α and β configuration; or

a pharmaceutically acceptable salt, solvate, or hydrate thereof.

In some embodiments, the dual mechanism compound may be 3-beta-hydroxy-5-beta-10-beta-methyl-13-beta-methyl-14-beta-hydroxy-17-beta-(imidazol-4-yl)androstane; 3-beta-hydroxy-5-beta-10-beta-methyl-13-beta-methyl-14-beta-hydroxy-17-beta-(2-guanidino-thiazol-4-yl)androstane; 3-beta-hydroxy-5-beta-10-beta-methyl-13-beta-methyl-14-beta-hydroxy-17-beta-(pyrazol-3-yl)androstane; 3-beta-hydroxy-5-beta-10-beta-methyl-12-beta-hydroxy-13-beta-methyl-14-beta-hydroxy-17-beta-(imidazol-4-yl)androstane; 3-beta-hydroxy-5-beta-10-beta-methyl-12-beta-hydroxy-13-beta-methyl-14-beta-hydroxy-17-beta-(2-guanidino-thiazol-4-yl)androstane; 3-beta-hydroxy-5-beta-10-beta-methyl-13-beta-methyl-14-beta-hydroxy-17-beta-(N-(3-aminopropyl)-imidazol-4-yl)androstane; 3-beta-hydroxy-5-beta-10-beta-methyl-13-beta-methyl-14-beta-hydroxy-17-beta-((5-(3-aminopropyl)-isoxazol-3-yl))androstane; 3-beta-hydroxy-5-beta-10-beta-methyl-13-beta-methyl-14-beta-hydroxy-17-beta-((5-(3-aminopropyl)-isoxazol-3-yl)-ethyl)androstane; 3-beta-hydroxy-5-beta-10-beta-methyl-13-beta-methyl-14-beta-hydroxy-17-beta-(5-(2-aminoethyl)-isoxazol-3-yl)-ethyl)androstane; or 3-beta-hydroxy-5-beta-10-beta-methyl-13-beta-methyl-14-beta-hydroxy-17-beta-(5-(2-aminomethyl)-isoxazol-3-yl)-ethenyl)androstane.

Another aspect of the invention features a method of treating acute heart failure in a human subject having acute myocardial ischemia and/or an acute myocardial infarction comprises administering to the human subject by intravenous infusion a pharmaceutical composition comprising a pure SERCA2a activator at a dose of about 0.1 mcg/kg/min to about 2.5 mcg/kg/min, for an infusion period of at least about 3 hours. In this method, the administering of the pure SERCA2a activator results in a measurable improvement in heart

function and an absence or reduction of acute myocardial arrhythmias, such as (i) the absence of acute myocardial arrhythmias continues for at least 3 hours following initiation of infusion; or (ii) the reduction of acute myocardial arrhythmias is compared to the average number of acute myocardial arrhythmias or the average duration of acute myocardial arrhythmias in a cohort of individuals not administered the pure SERCA2a activator. In such embodiments, the pure SERCA2a activator compound has a structure formula (IV):



Formula (IV)

wherein X is selected from the group consisting of a carboxylic acid, carboxylic ester or a bioisoster thereof, primary alcohol, ether, and an amine group, wherein the bioisoster consists of a sulfate, sulfonic acid, phosphate, phosphonate, or a nitrogen-containing etherocyclic ring, and wherein the amine group optionally comprises a primary amine, secondary amine, or a cyclic amine;

n is 1, 2, 3, 4, or 5;

a C3-C1' dashed line represents an optional exocyclic double bond C=C at position C3-C1';

a C2-C3 dashed line represents an optional endocyclic double bond C=C;

Y at C6 is a hydroxyl (OH) in the alpha- or beta-configuration or a hydroxymethyl (CH₂OH) in the alpha-configuration; and

Z at C7 is a -H or -OH in an alpha-configuration or a ketone, wherein a dashed line represents an optional carbonyl group (C=O) at Z,

or

a pharmaceutically acceptable salt, solvate, or hydrate thereof.

In an embodiment, the pure SERCA2a activator may be (E)-4-(6alpha-hydroxy-17-oxoandrostane-3-yliden)butyric acid; (Z)-4-(6alpha-hydroxy-17-oxoandrostane-3-yliden)butyric

acid; (E)-4-(6beta-hydroxy-17-oxoandrostane-3-yliden)butyric acid; (Z)-4-(6beta-hydroxy-17-oxoandrostane-3-yliden)butyric acid; (E)-3-[2-(azetidin-3-yl)ethyliden]-6alpha-hydroxyandrostane-17-one; (Z)-3-[2-(azetidin-3-yl)ethyliden]-6alpha-hydroxyandrostane-17-one; (E)-3-(4-aminobutyl)-6alpha-hydroxyandrost-2-ene-17-one hydroiodide; 3-[2-(piperidin-4-yl)ethyl]-6alpha-hydroxyandrost-2-ene-17-one hydroiodide; (EZ)-3-(4-aminobutyliden)-6alpha-hydroxyandrostane-17-one; (E)-3-[2-(piperidin-4-yl)ethyliden]-6alpha-hydroxyandrostane-17-one; (Z)-3-[2-(piperidin-4-yl)ethyliden]-6alpha-hydroxyandrostane-17-one; 3 beta-[2-(piperidin-4-yl)ethyl]-6alpha-hydroxyandrostane-17-one; Ethyl (6alpha-hydroxy-17-ketoandrostane-3beta-yl) acetate; 4-(6alpha-hydroxy-17-oxoandrostane-3-yl) butyric acid; 4-(6beta-hydroxy-17-oxoandrostane-3-yl) butyric acid; 2-(6beta-hydroxy-17-oxoandrostane-3-yl) acetic acid; Ethyl 4-(6alpha-hydroxy-17-oxoandrostane-3-yl) butyrate; Ethyl 4-(6alpha-hydroxy-17-oxoandrostane-3-yl) caproate; 6-(6beta-hydroxy-17-oxoandrostane-3-yl) caproic acid; (E,Z)-3-(5-N-methylaminopentyliden)-6alpha-hydroxymethylandrostandane-7,17-dione; (E,Z)-3-[2-(pyrrolidine-3yl)ethyliden]-6alpha-hydroxymethylandrostandane-7,17-dione; (E,Z)-3-[2-(azetidine-3-yl)ethyliden]-6alpha-hydroxymethylandrostandane-7,17-dione; (E,Z)-3-[2-(piperidin-4-yl)ethyliden]-6alpha-hydroxymethylandrostandane-7,17-dione; (E,Z)-3-(5-N-methylaminopentyliden)-6alpha-hydroxymethyl-7alpha-hydroxyandrostane-17-one; 3 beta-[2-(azetidine-3-yl)ethyl]-6alpha-hydroxymethylandrostandane-7,17-dione; 3 beta-[2-(azetidine-3-yl)ethyl]-6alpha-hydroxymethyl-7alpha-hydroxyandrostane-17-one; 3 beta-[2-(pyrrolidine-3yl)ethyl]-6alpha-hydroxymethylandrostandane-7,17-dione; 3 beta-[2-(pyrrolidine-3yl)ethyl]6alpha-hydroxymethyl-7alpha-hydroxyandrostane-17-one; 3 beta-[2-(piperidin-4-yl)ethyl]-6alpha-hydroxymethylandrostandane-7,17-dione; or 3 beta-[2-(piperidin-4-yl)ethyl]-6alpha-hydroxymethyl-7alpha-hydroxyandrostane-17-one. In one particular embodiments, the pure SERCA2a activator is 2-(6beta-hydroxy-17-oxoandrostane-3-yl) acetic acid.

These and other features and advantages of the present disclosure will become better understood with regard to the following description, appended claims, and accompanying drawings.

Brief Description of the Drawings

Figures 1A – 1B. Mode of action of Istaroxime and its follow-on compounds on calcium hemostasis regulation. Fig. 1A) The steroid derivative istaroxime, which is endowed with a

double mechanism of action, that is, Na⁺/K⁺ ATPase inhibition and SERCA2a activation. Fig. 1B. CVie216 behaves as a selective SERCA2a activator showing a longer half-life than istaroxime and it enhanced in vitro SERCA2a activity in healthy guinea pig preparations and in diseased (STZ) rat preparations; furthermore, the stimulatory effect on SERCA2a depended on the presence of PLN.

Figures 2A -2B. Triggered activity incidence in left ventricular papillary muscles isolated from various group hearts. Fig. 2A) Representative tracings of action potential driven at 2, 3.3, and 5 Hz in preparations isolated from various group hearts exposed to high calcium (5.4 mmol/L) + isoproterenol (1 μ M). Triggered activity (spontaneous action potentials; red double arrow line) could be induced in a preparation from DM+Veh group heart following 1-minute stimulation at all frequencies. Fig. 2B) Incidence of triggered activity in preparations isolated from various group hearts at driven frequencies of 2, 3.3, and 5 Hz. Results are shown as the percentage of preparations (n) inducible into triggered activity/total of preparations. *P<0.05 vs Ctr group by Fisher exact test. DM indicates STZ injection; Ista, Istaroxime; and Veh, vehicle.

Figures 3A -3D. Effect of 50 μ M Istaroxime and CVie216 infusion on reperfusion-induced arrhythmias in the isolated, perfused Control (Ctr) and Diabetic (DM) rats' hearts following 10 min regional ischemia. Fig. 3A) Raw ECG recordings for ventricular tachycardia/fibrillation (VT/VF) in DM rats isolated heart under I/R. Fig. 3B) Incidence of reperfusion induced VT/VF, and the incidence of VF significant decreased in DM rats' hearts. Fig. 3C) The scoring system assigns a numeric value based upon the severity of arrhythmia, with larger values indicating greater severity. Fig. 3D) arrhythmias score in different experimental groups. *P < 0.05 vs DM + Veh by one-way ANOVA.

Figures 4A – 4C. Effect of 10 μ M Istaroxime and CVie216 infusion on reperfusion-induced arrhythmias in the isolated, perfused Diabetic rats' hearts following 10 min regional ischemia.

Fig. 4A) Raw ECG recordings for ventricular tachycardia/fibrillation (VT/VF) in DM rats isolated heart under I/R. Fig. 4B) Mean data for incidence of Reperfusion induced VT/VF which significant decreased in 10 μ M CVie216 group, compared with the Veh group. Fig. 4C) Arrhythmias score in different experimental groups. * P < 0.05 vs Veh by one-way ANOVA. ★ P < 0.05 vs Veh by one-way ANOVA with post-hoc Dunnett test, one side.

Detailed Description

The compositions and methods disclosed herein provide, for the first time, a treatment regimen that includes administering istaroxime or derivatives thereof to individuals with acute heart failure (AHF) (with or without early cardiogenic shock or cardiogenic shock) that present with acute myocardial ischemia and/or an acute myocardial infarction in order to treat the underlying AHF condition while, at the same time, reducing the risk of preventing or reducing the development of acute myocardial arrhythmias. Surprisingly, preclinical work in diabetic rat models of heart failure with ischemia and reperfusion shows significantly reduced incidence of serious arrhythmia events, including reduced incidence of ventricular fibrillation (VF), ventricular tachycardia (VT), and arrhythmia score in cohorts administered istaroxime derivatives or its pure SERCA2a derivatives, as compared to controls. Accordingly, this demonstrates that istaroxime and its progeny can be used safely and effectively in patients with AHF and who are presenting with acute myocardial ischemia potentially with reperfusion and/or acute myocardial infarction. This result is surprising due to one of the two mechanisms of action of istaroxime. Importantly, istaroxime inhibits the Na^+/K^+ -ATPase, which, in turn increases intracellular concentrations of Ca^{2+} in the myocardium. This process was believed in the art to increase the risk for after-depolarizations and myocardial arrhythmias. Therefore, this result shows the potential for istaroxime administration to reduce the risk of arrhythmias resulting from heart failure. This includes patients with AHF-related pre-cardiogenic shock or cardiogenic shock resulting from acute myocardial infarction, which is a common cause of this deadly disorder.

In some embodiments, individuals with AHF and acute myocardial ischemia are administered istaroxime and exhibit an improvement in one or more parameters of heart function with decreased incidence of (or reduced duration of) serious acute myocardial arrhythmias, such as VF or VT. In another embodiment, individuals presenting with AHF-related pre-cardiogenic shock or cardiogenic shock resulting from an acute myocardial infarction or patients exhibiting decreased cardiac blood flow resulting in ischemia are administered istaroxime and exhibit an improvement in one or more parameters of heart function with decreased incidence of (or reduced duration of) serious acute myocardial arrhythmias. In yet other embodiments, individuals with AHF and acute myocardial ischemia are administered istaroxime before, during, or after cardiac reperfusion and exhibit an improvement in one or more parameters of heart function with reduced incidence of (or reduced duration of) serious acute myocardial arrhythmias. For some

individuals with AHF, istaroxime administration decreases the risk that arrhythmia will even occur. For others, the duration of the arrhythmia is reduced due to the administration of istaroxime (or an istaroxime derivative, e.g. Formula II or Formula IV). For example, the ventricular defibrillation may still occur, but the duration of the event is shortened as compared to placebo groups or the mean duration seen in the population. The administration of istaroxime or istaroxime derivative may reduce the occurrence of ventricular arrhythmias in a larger percentage of individuals as compared to placebo and/or control groups. In still other embodiments, individuals with heart failure and acute myocardial ischemia and/or acute myocardial infarction are administered a derivative of istaroxime, such as Formula II or Formula IV, and exhibit an improvement in one or more parameters of heart function with no incidence of (or reduced duration of) serious acute myocardial arrhythmias. Each of these scenarios represents a decreased risk of serious acute arrhythmias.

The compositions and methods disclosed herein will be described in more detail below.

Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as those commonly understood by one of ordinary skill in the art to which this invention belongs. Standard techniques are used unless otherwise specified. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, suitable methods and materials are described below. The materials, methods and examples are illustrative only, and are not intended to be limiting. All publications, patents and other documents mentioned herein are incorporated by reference in their entirety.

As used herein, the singular forms “a,” “an,” and “the” include the plural referents unless the context clearly indicates otherwise.

The term “about” refers to the variation in the numerical value of a measurement, e.g., volume, time, pressure, concentration, etc., due to typical error rates of the device used to obtain that measure. In one embodiment, the term “about” means within 5% of the reported numerical value, preferably, the term “about” means within 3% of the reported numerical value.

The term “cardiogenic shock” as used herein refers to a condition in an individual, such as a human patient, where the heart cannot pump enough blood and oxygen to the brain, kidneys, and other vital organs leading to end-organ hypoperfusion. Overt “cardiogenic shock” or SCAI

Stage C “cardiogenic shock” is defined by a systolic blood pressure of less than about 90 mmHg (hypotension), a cardiac index of less than about 2.2 L/min/m², pulmonary capillary wedge pressure of greater than 15 mmHg, and evidence of end-organ hypoperfusion. “Pre-cardiogenic shock”, SCAI Stage B “pre-cardiogenic shock”, or “early cardiogenic shock” refer to a condition where the individual has not yet developed overt “cardiogenic shock” and is defined by a systolic blood pressure of less than about 90 mmHg without evidence of end-organ hypoperfusion.

The term “heart failure” refers to a clinical syndrome characterized by typical symptoms (*e.g.*, breathlessness, ankle swelling and fatigue) that may be accompanied by signs (*e.g.*, elevated jugular venous pressure, pulmonary crackles and peripheral edema) caused by a structural and/or functional cardiac abnormality, resulting in a reduced cardiac output and/or elevated intracardiac pressures at rest or during stress.

The terms “acute heart failure” or “AHF” are used interchangeably herein and refer generally to a rapid onset or worsening of symptoms and/or signs of HF requiring immediate treatment and hospitalization. The current definition of “acute heart failure” is rather nonspecific and may include a broad spectrum of conditions with several phenotypes characterized by different clinical presentation, etiology, precipitating factors, therapeutic approach, and prognosis. In addition, a large proportion of human patients have a subacute course of the disease with a progressive worsening of signs and symptoms of HF which could develop days before hospital admission.

The term “acute decompensated heart failure” as used herein refers to a sudden worsening of the signs and symptoms of heart failure, which, in humans, typically includes difficulty breathing (dyspnea), leg or feet swelling, and fatigue.

The term “acute coronary syndrome” as used herein refers to a sudden reduction or blockage of blood flow to the heart. A common example of “acute coronary syndrome” is a myocardial infarction (heart attack).

The terms “arrhythmia”, “cardiac arrhythmia”, and “myocardial arrhythmia” are used interchangeably herein and refer to an irregular heartbeat. A resting heart rate that is too fast (*e.g.*, greater than about 100 beats per minute in an adult human) is referred to as “tachycardia”, while a resting heart rate that is too slow (*e.g.*, less than about 60 beats per minute in an adult human) is referred to as “bradycardia”.

The term “atrial fibrillation” or “AF” is a cardiac arrhythmia characterized by rapid and irregular beating in the atrial chambers of the heart.

The terms “chronic heart failure” or “CHF” are used interchangeably herein and refer to the current clinical classification of chronic HF based on the presence of signs and symptoms of HF and left ventricular ejection fraction (LVEF) in an individual, such as a human patient, recognizing three categories: “heart failure with reduced ejection fraction” or “HFrEF,” which is characterized by an LVEF of less than about 40%; “heart failure with mid-range ejection fraction” or “HFmEF” or “HFmrEF,” which is characterized by an LVEF from about 40% to about 49%; and “heart failure with preserved ejection fraction” or “HFpEF,” which is characterized by an LVEF of equal to or greater than about 50%. The terms “HFmrEF” and “HFpEF” include two additional criteria, namely increased natriuretic peptides levels (BNP >35 pg/ml and/or NT-proBNP >125 pg/mL) associated with the evidence of structural and/or functional heart disease (left ventricular hypertrophy and/or left atrium enlargement and/or evidence of diastolic dysfunction).

The term “hypotension” as used herein is defined as systolic blood pressure (SBP) that is less than 90 mmHg or mean arterial blood pressure (MAP) less than about 60 mmHg or greater than 30 mmHg drop from baseline.

The term “treating” refers to any indicia of success in the treatment or amelioration of the disease or condition in an individual. Treating can include, for example, reducing or alleviating the severity of one or more symptoms of the disease or condition, or it can include reducing the frequency with which symptoms of a disease, defect, disorder, or adverse condition, and the like, are experienced by an individual, such as a human patient.

The term “preventing” refers to the prevention of the disease or condition, *e.g.*, pre-myocardial arrhythmia, in an individual, such as a human patient. For example, if an individual at risk of developing a serious acute myocardial arrhythmia is treated with the methods of the present invention and does not later develop a serious acute myocardial arrhythmia, then the disease has been prevented in that individual.

The term “treat or prevent” is sometimes used herein to refer to a method that results in some level of treatment or amelioration of the disease or condition and contemplates a range of results directed to that end, including but not restricted to prevention of the condition entirely.

As used herein, the term “pharmaceutically acceptable” salt, solvate, hydrate, or ester means a salt, solvate, hydrate, or ester form of the active ingredient which is compatible with any other ingredients of the pharmaceutical composition, which is not deleterious to the subject to which the composition is to be administered.

5 As used herein, the term “pharmaceutically acceptable carrier” means a chemical composition with which an istaroxime compound or a derivative or metabolite of the istaroxime compound may be combined and which, following the combination, can be used to administer the compound to a mammal.

The term “intravenous infusion” refers to the administration or delivery of liquid
10 substances directly into a vein of a mammal. Some “infusions” use only the pressure supplied by gravity, whereas other “infusions” use an infusion pump or other device to control the rate of administration and/or the dose of liquid substances into the vein of the mammal.

The term “parameter” as used herein to refer to measuring heart function means any heart function that is observable or measurable using suitable measuring techniques available in the
15 art. A non-limiting list of exemplary “parameters” of heart function include heart rate (HR), blood pressure (BP), diastolic relaxation, systolic contraction, dyspnea, chest congestion, diastolic blood pressure (DBP), systolic blood pressure (SBP), systolic blood pressure area under the curve (SBP AUC), creatine clearance, deceleration slope, mitral inflow velocity, mean arterial pressure, brain natriuretic peptide levels (BNP), NT-pro-BNP levels, troponin levels,
20 venous lactate levels, echocardiographic measurements, left ventricle end diastolic diameter (EDD), left ventricle end systolic diameter (ESD), left ventricle end diastolic volume (EDV), left ventricle end systolic volume (ESV), left atrium diameter (LAD), left atrium area (LAA), left atrium volume (LAV), E wave, A wave, E wave deceleration time (EDT), E/A ratio, Ea, Aa, E/Ea ratio, left ventricle ejection fraction (LVEF), Sa, stroke volume (SV), cardiac output (CO),
25 stroke volume index (SVI), cardiac index (CI), pulmonary arterial systolic pressure (PASP), tricuspid annular plane systolic excursion (TAPSE), right ventricle Sa, mitral regurgitation (MR), inferior vena cava diameter (IVC), and the like. As one having ordinary skill in the art will appreciate, measuring one or more “parameters” of heart function can be used to detect heart dysfunction as compared to the average normal parameters and can also be used to determine
30 whether heart function has improved following or during treatment.

The terms “therapeutically active” or “active” ingredient or compound refer to a substance that provides a beneficial effect to the individual to whom the substance is administered. A “therapeutically effective amount” or “therapeutically effective dose” is the amount of a composition or active ingredient sufficient to provide a beneficial effect to the individual to whom the composition or active ingredient is administered.

The term “ventricular fibrillation” or “VF” is a cardiac arrhythmia that initiates in the ventricle as opposed to the normal electrical conduction system in the heart. It is characterized by rapid uncoordinated electrical depolarization of the heart ventricle and characterized by a quivering or fibrillating heart with no consistent heartbeat.

The term “ventricular tachycardia” or “VT” is a cardiac arrhythmia is characterized by 3 or more heartbeats in a row at a heart rate of greater than 100 beats per minute originating in the ventricle as opposed to the normal cardiac electrical conduction system in the heart.

Description

The present invention is directed to administration of istaroxime, or derivatives thereof, to individuals, such as human patients, to treat AHF with acute myocardial ischemia and/or acute myocardial infarction. Surprisingly, and despite the belief in the art that the Na^+/K^+ -ATPase inhibitory function of istaroxime could increase the risk of myocardial arrhythmias, the data presented herein shows that istaroxime can reduce the risk of serious myocardial arrhythmias in individuals with AHF, even if that individual is exhibiting acute myocardial ischemia and even after reperfusion of the heart. As such, istaroxime can be used to treat AHF and improve heart function while reducing the risk of serious myocardial arrhythmias. This finding has profound implications in the treatment of cardiovascular disorders since the number of individuals with AHF that can be treated with istaroxime is substantially increased. Indeed, previous clinical trials with istaroxime expressly excluded individuals presenting with acute myocardial ischemia and/or myocardial infarction [*see, e.g., Gheorghiade et al.*, 2008, JACC 51(2):2276-2285; Carubelli *et al.*, 2020, Euro. J. Heart Failure. 22(9):1684-1693; Metra *et al.*, 2022, Euro. J. Heart Failure 24:1967-1977; WO 2020/180356 A9; the entire content of each of which is incorporated by reference herein].

In a preferred embodiment, istaroxime (or derivative thereof), is administered to the individual by intravenous infusion. In a more preferred embodiment, the istaroxime is

administered within 24 hours of the onset of symptoms of acute myocardial ischemia, or within 12 hours of the onset of symptoms of acute myocardial ischemia, or within 6 hours of the onset of symptoms of acute myocardial ischemia, or within 3 hours, or within 2 hours, or within 1

embodiment, the istaroxime (or derivative thereof) is administered within 24 hours of the onset of symptoms of an acute myocardial infarction, or within 12 hours of the onset of symptoms of an acute myocardial infarction, or within 6 hours of the onset of symptoms of an acute myocardial infarction, or within 3 hours, or within 2 hours, or within 1 hour, or within 30 minutes of the onset of symptoms of an acute myocardial infarction. For individuals exhibiting

symptoms of AHF and acute myocardial ischemia and/or an acute myocardial infarction, istaroxime infusion is administered to treat the underlying AHF and improve heart function, but also prevents or reduces the risk/duration of acute myocardial arrhythmias, such as, but not limited to VF or VT. In other embodiments, administration of istaroxime (or derivative thereof)

to the individual with acute myocardial ischemia reduces the duration and/or severity of the acute myocardial arrhythmia as compared to an individual or cohort of individuals with acute

myocardial ischemia, but who are not administered istaroxime. In another embodiment, an individual with AHF-related pre-cardiogenic shock or cardiogenic shock in combination with acute myocardial ischemia and/or an acute myocardial infarction is administered istaroxime (or derivative thereof) by intravenous infusion to improve heart function while preventing or

reducing the risk/severity of acute myocardial arrhythmias.

In some embodiments, the istaroxime infusion (or infusion of an istaroxime derivative) is between about 30 minutes and up to 24 hours or more, *e.g.*, 30 m, 45 m, 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, 7 h, 8 h, 9 h, 10 h, 11 h, 12 h, 13 h, 14 h, 15 h, 16 h, 17 h, 18 h, 19 h, 20 h, 21 h, 22 h, 23 h, 24 h, or more hours. For instance, the infusion can be for up to 6 hours or up to 24 hours, or longer.

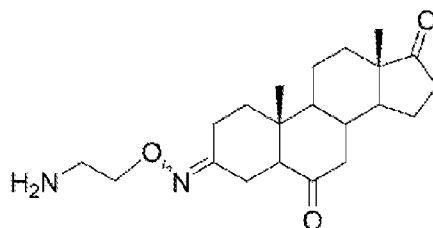
In some embodiments, the duration of the infusion is from about 6 hours to about 24 hours, or from about 12 hours to about 24 hours, or about 18 hours to about 24 hours. In other

embodiments, the duration of the infusion is greater than about 24 hours, *e.g.*, 25 h, 26 h, 27 h, 28 h, 29 h, 30 h, 31 h, 32 h, 33 h, 34 h, 35 h, 36 h, 37 h, 38 h, 39 h, 40 h, 41 h, 42 h, 43 h, 44 h, 45 h, 46 h, 47 h, 48 h, 54 h, 60 h, 66 h, 72 h, 78 h, 84 h, 90 h, 96 h, 5 days, 6 days, 7 days, 8

days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, or longer. In particular embodiments, infusion is for at least about 6 hours, or at least about 12 hours, or at least about 24 hours, or at

least about 48 hours, or at least about 60 hours. It being understood that treatment with istaroxime (or derivative thereof) not only improves heart function in the individual, but additionally prevents or reduces the risk/severity of acute myocardial arrhythmias.

Istaroxime is an inotropic compound having the following structural Formula (I):



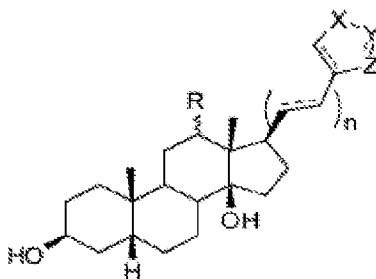
Formula (I)

As noted above, this compound, and the method of making this compound, is disclosed in EP0828197, the entire content of which is incorporated herein by reference. Istaroxime has two known mechanisms of action: 1) the inhibition of the Na^+/K^+ -ATPase activity; and 2) the activation of the sarcoplasmic reticulum calcium ATPase isoform (SERCA2a). Accordingly, istaroxime and other istaroxime metabolites or derivatives with the dual mechanism of action are sometimes referred to herein as “dual mechanism compounds.” It is an object of the present invention to utilize istaroxime, or its pharmaceutically acceptable salt or ester, hydrate, solvate, or polymorphic form in a medicament to treat or prevent AHF (or AHF-related pre- or overt cardiogenic shock) in an individual while reducing or eliminating the risk of developing acute myocardial arrhythmias. In a preferred embodiment, istaroxime is administered to an individual for the treatment of AHF (or AHF-related pre- or overt cardiogenic shock) with or without acute myocardial ischemia and/or acute myocardial infarction to improve heart functioning while reducing or eliminating the risk of developing acute myocardial arrhythmias.

In an alternative embodiment, other dual mechanism compounds derived from istaroxime are suitable for use in the pharmaceutical compositions and methods provided herein. It is an object of the present invention to utilize these dual mechanism compounds, or their pharmaceutically acceptable salts or esters, hydrates, solvates, or polymorphic forms in a medicament to treat or prevent AHF (or AHF-related pre- or overt cardiogenic shock) in an individual while reducing or eliminating the risk of developing acute myocardial arrhythmias. In a preferred embodiment, a dual mechanism compound is administered to an individual for the treatment of AHF (or AHF-related pre- or overt cardiogenic shock) with or without acute

myocardial ischemia and/or acute myocardial infarction to improve heart functioning while reducing or eliminating the risk of developing acute myocardial arrhythmias.

These dual mechanism compounds, and methods of making these compounds, are disclosed in WO 2020/020728 A1, the entire content of which is incorporated herein by
 5 reference. These dual mechanism compounds have the following structural Formula (II):



Formula (II)

wherein X, Y, Z are annular atoms comprised in a five-membered carbocyclic or heterocyclic ring, selected from the group consisting of CH, NH, N, O, S; and wherein n
 10 is 0 or 1 and said heterocyclic ring selected from the group consisting of imidazolyl, pyrazolyl, thiazolyl, isoxazolyl, and the corresponding dihydro- and tetrahydro derivatives;

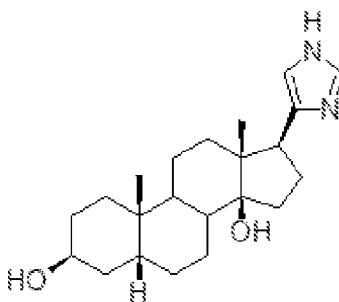
R is H or OH; and

the dotted line represents an optional double bond C=C; the thick line represents a bond in the β configuration; the wavy line represents a bond both in the α and β configuration;
 or

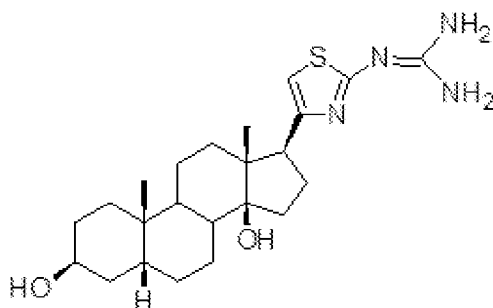
the pharmaceutically acceptable salts, solvates, or hydrates thereof.

Particular dual mechanism compounds having formula II are shown here:

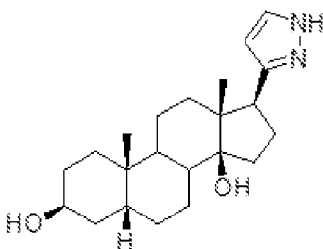
3-beta-hydroxy-5-beta-10-beta-methyl-13-beta-methyl-14-beta-hydroxy-17-beta-(imidazol-4-yl)androstane (**CVie 101**):



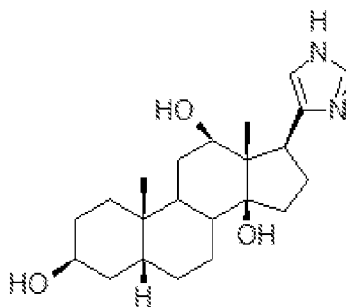
3-beta-hydroxy-5-beta-10-beta-methyl-13-beta-methyl-14-beta-hydroxy-17-beta-(2-guanidinothiazol-4-yl)androstane (**CVie 102**):



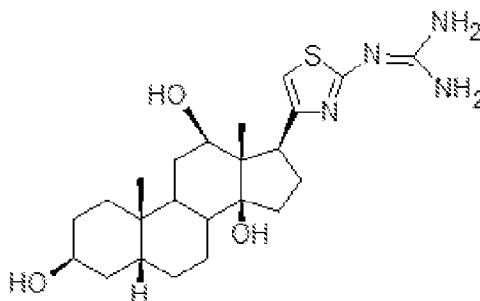
- 5 3-beta-hydroxy-5-beta-10-beta-methyl-13-beta-methyl-14-beta-hydroxy-17-beta-(pyrazol-3-yl)androstane (**CVie 103**):



- 10 3-beta-hydroxy-5-beta-10-beta-methyl-12-beta-hydroxy-13-beta-methyl-14-beta-hydroxy-17-beta-(imidazol-4-yl)androstane (**CVie 104**):

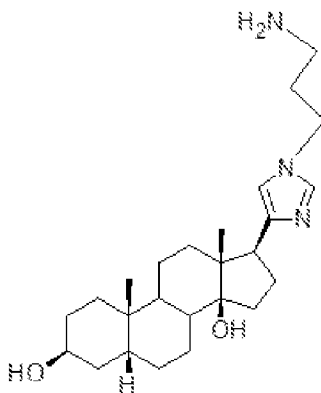


3-beta-hydroxy-5-beta-10-beta-methyl-12-beta-hydroxy-13-beta-methyl-14-beta-hydroxy-17-beta-(2-guanidino-thiazol-4-yl)androstane (**CVie 105**):

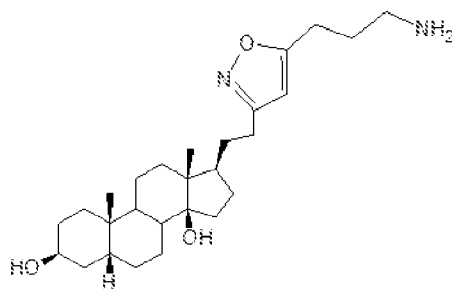


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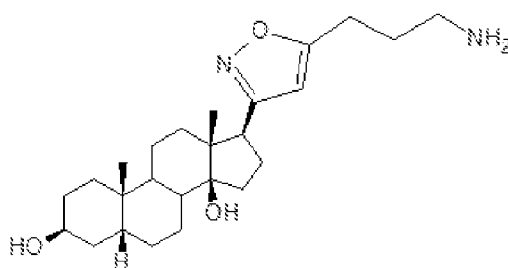
3-beta-hydroxy-5-beta-10-beta-methyl-13-beta-methyl-14-beta-hydroxy-17-beta-(N-(3-aminopropyl)-imidazol-4-yl)androstane (**CVie 106**):



10 3-beta-hydroxy-5-beta-10-beta-methyl-13-beta-methyl-14-beta-hydroxy-17-beta-((5-(3-aminopropyl)-isoxazol-3-yl))androstane (**CVie 107**):

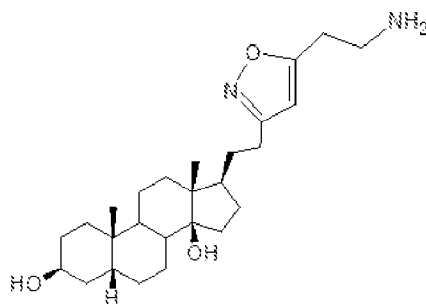


3-beta-hydroxy-5-beta-10-beta-methyl-13-beta-methyl-14-beta-hydroxy-17-beta-((5-(3-aminopropyl)-isoxazol-3-yl)-ethyl)androstane (**CVie 108**):

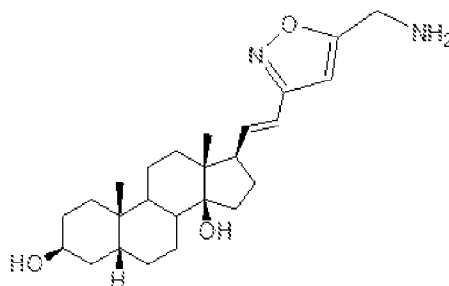


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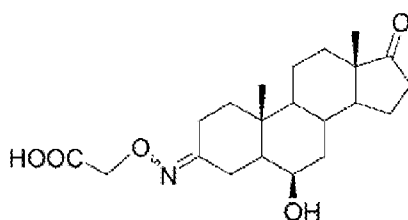
3-beta-hydroxy-5-beta-10-beta-methyl-13-beta-methyl-14-beta-hydroxy-17-beta-(5-(2-aminoethyl)-isoxazol-3-yl)-ethyl)androstane (**CVie 109**):



10 3-beta-hydroxy-5-beta-10-beta-methyl-13-beta-methyl-14-beta-hydroxy-17-beta-(5-(2-aminomethyl)-isoxazol-3-yl)-ethenyl)androstane (**CVie 110**):



Also disclosed herein is a metabolite of istaroxime having the following structural formula (III):



PST 3093

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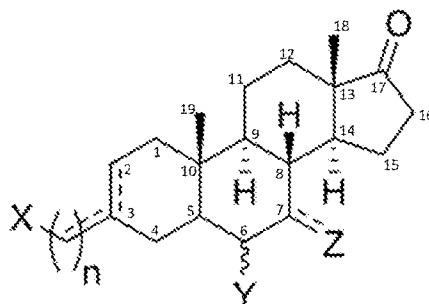
Formula (III)

The compound, and the method of making this compound, is disclosed in WO 2020/180356 A9, the entire content of which is incorporated herein by reference. The activity of PST 3093 is different from that of istaroxime in that PST 3093 can stimulate SERCA2a, but does not inhibit the Na⁺/K⁺-ATPase. Compounds with only the SERCA2a-stimulating activity are sometimes referred to herein as “pure SERCA2a activators”. It is therefore an object of the present invention to utilize PST 3093, or its pharmaceutically acceptable salt or ester, hydrate, solvate, or polymorphic form in a medicament to treat or prevent AHF (or AHF-related pre- or overt cardiogenic shock) in an individual while reducing or eliminating the risk of developing acute myocardial arrhythmias. In a preferred embodiment, PST 3093 is administered directly or via administration of istaroxime to an individual for the treatment of AHF (or AHF-related pre- or overt cardiogenic shock) with or without acute myocardial ischemia and/or acute myocardial infarction to improve heart functioning while reducing or eliminating the risk of developing acute myocardial arrhythmias.

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In another alternative embodiments, other pure SERCA2a activators are used in the medicaments, pharmaceutical compositions and methods provided herein. These pure SERCA2a activators have the general formula (IV):



Formula (IV)

wherein X is selected from the group consisting of a carboxylic acid, carboxylic ester or a bioisoster thereof, primary alcohol, ether, and an amine group, wherein the bioisoster consists of a sulfate, sulfonic acid, phosphate, phosphonate, or a nitrogen-containing etherocyclic ring, and wherein the amine group optionally comprises a primary amine, secondary amine, or a cyclic amine;

n is 1, 2, 3, 4, or 5;

a C3-C1' dashed line represents an optional exocyclic double bond C=C at position C3-C1';

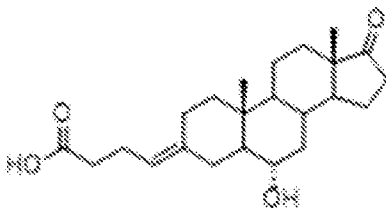
a C2-C3 dashed line represents an optional endocyclic double bond C=C;

Y at C6 is a hydroxyl (OH) in the alpha- or beta-configuration or a hydroxymethyl (CH₂OH) in the alpha-configuration; and

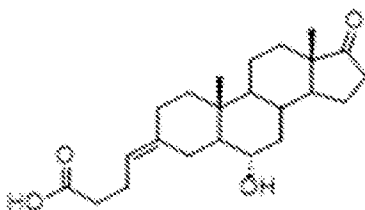
Z at C7 is a -H or -OH in an alpha-configuration or a ketone, wherein a dashed line represents an optional carbonyl group (C=O) at Z.

Particular pure SERCA2a activators having formula IV are shown here:

(E)-4-(6 α -hydroxy-17-oxoandrostane-3-yliden)butyric acid (**CVie201**):

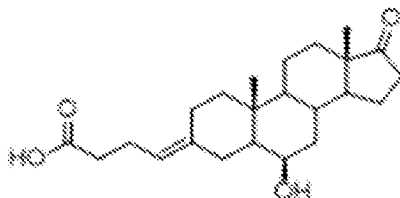


(Z)-4-(6 α -hydroxy-17-oxoandrostane-3-yliden)butyric acid (**CVie202**):

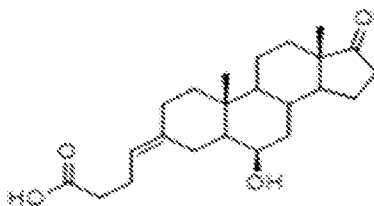


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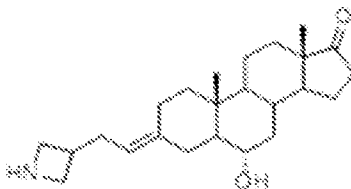
(E)-4-(6 β -hydroxy-17-oxoandrostane-3-yliden)butyric acid (**CVie203**):



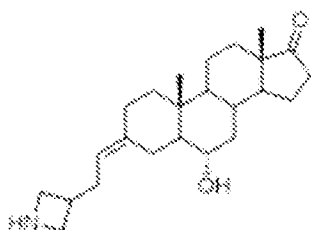
(Z)-4-(6 β -hydroxy-17-oxoandrostane-3-yliden)butyric acid (**CVie204**):



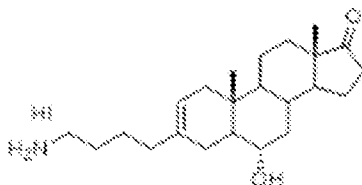
10 (E)-3-[2-(azetidin-3-yl)ethyliden]-6 α -hydroxyandrostane-17-one (**CVie205**):



(Z)-3-[2-(azetidin-3-yl)ethyliden]-6alpha-hydroxyandrostane-17-one (**CVie206**):

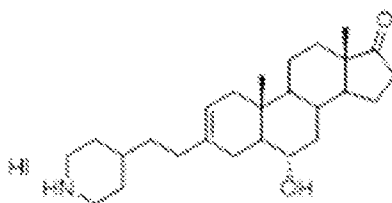


(E)-3-(4-aminobutyl)-6alpha-hydroxyandrost-2-ene-17-one hydroiodide (**CVie207**):

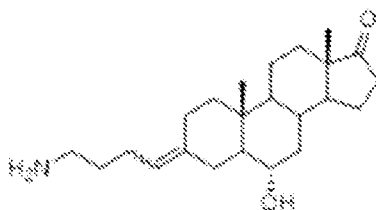


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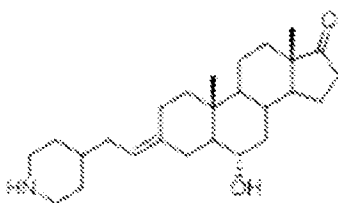
3-[2-(piperidin-4-yl)ethyl]-6alpha-hydroxyandrost-2-ene-17-one hydroiodide (**CVie208**):



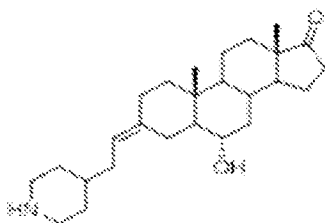
(EZ)-3-(4-aminobutyliden)-6alpha-hydroxyandrostane-17-one (**CVie209**):



(E)-3-[2-(piperidin-4-yl)ethyliden]-6alpha-hydroxyandrostane-17-one (**CVie210**):

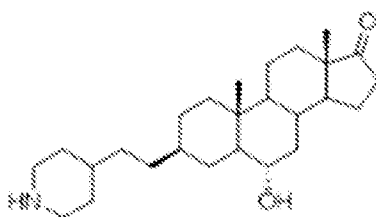


(Z)-3-[2-(piperidin-4-yl)ethyliden]-6alpha-hydroxyandrostane-17-one (**CVie211**):

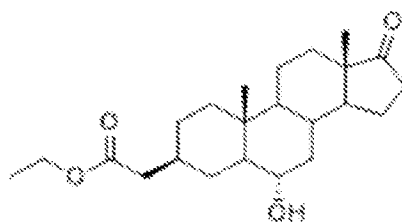


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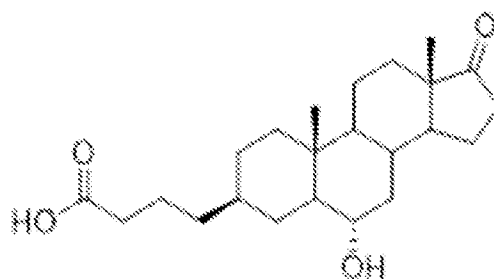
3 beta-[2-(piperidin-4-yl)ethyl]-6alpha-hydroxyandrostane-17-one (**CVie212**):



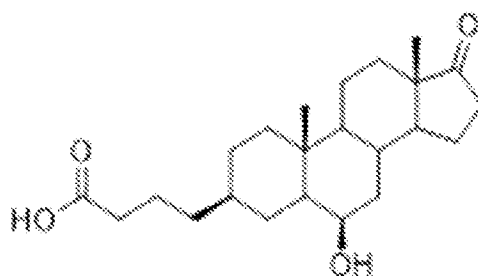
Ethyl (6alpha-hydroxy-17-ketoandrostane-3beta-yl) acetate (**CVie213**):



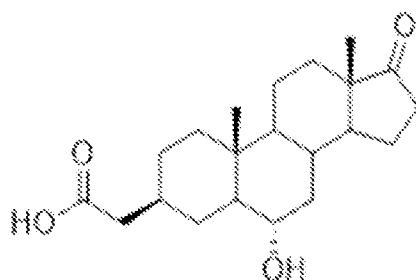
4-(6alpha-hydroxy-17-oxoandrosta-3-yl) butyric acid (**CVie214**):



5 4-(6beta-hydroxy-17-oxoandrosta-3-yl) butyric acid (**CVie215**):

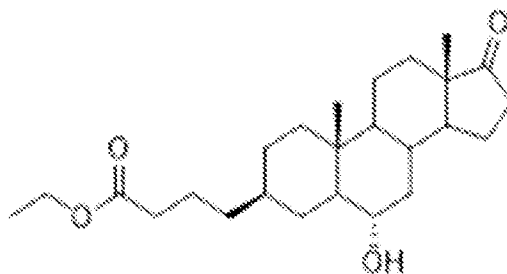


2-(6beta-hydroxy-17-oxoandrosta-3-yl) acetic acid (**CVie216**):

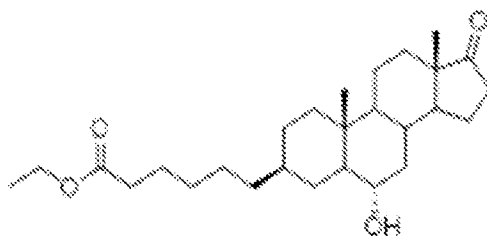


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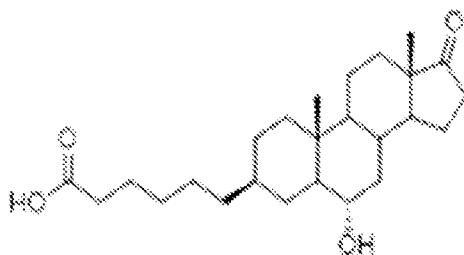
Ethyl 4-(6alpha-hydroxy-17-oxoandrosta-3-yl) butyrate (**CVie217**):



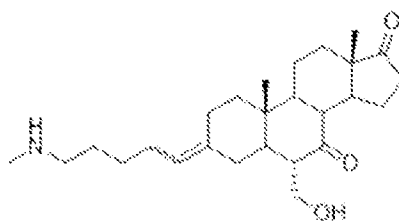
Ethyl 4-(6 α -hydroxy-17-oxoandrostan-3-yl) caproate (**CVie218**):



5 6-(6 β -hydroxy-17-oxoandrostan-3-yl) caproic acid (**CVie219**):

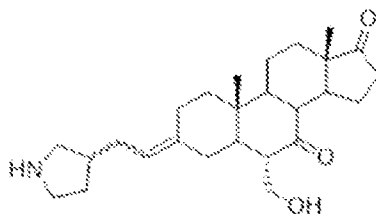


(E,Z)-3-(5-N-methylaminopentyliden)-6 α -hydroxymethylandrostan-7,17-dione (**CVie401**);

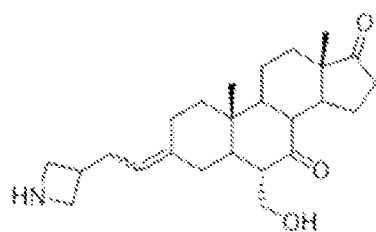


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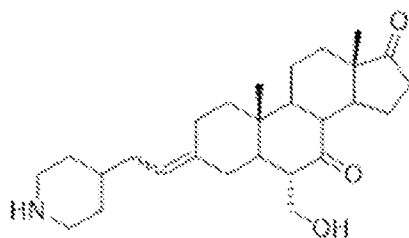
(E,Z)-3-[2-(pyrrolidine-3-yl)ethyliden]-6 α -hydroxymethylandrostan-7,17-dione (**CVie402**):



(E,Z)-3-[2-(azetidine-3-yl)ethyliden]-6alpha-hydroxymethylandrostand-7,17-dione (**CVie403**):

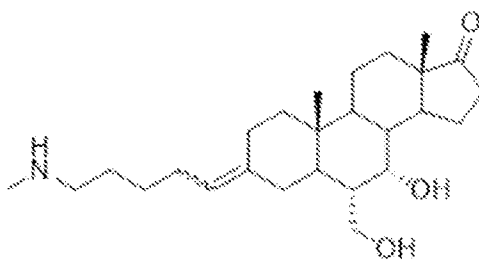


(E,Z)-3-[2-(piperidin-4-yl)ethyliden]-6alpha-hydroxymethylandrostand-7,17-dione (**CVie405**):

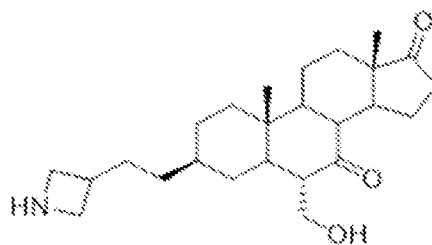


5

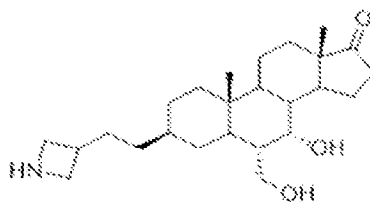
(E,Z)-3-(5-N-methylaminopentyliden)-6alpha-hydroxymethyl-7alpha-hydroxyandrostand-17-one (**CVie406**):



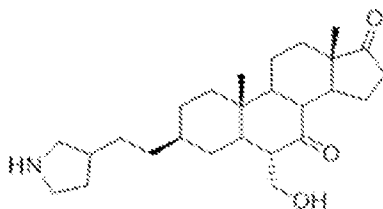
3 beta-[2-(azetidine-3-yl)ethyl]-6alpha-hydroxymethylandrostande-7,17-dione (**CVie407**):



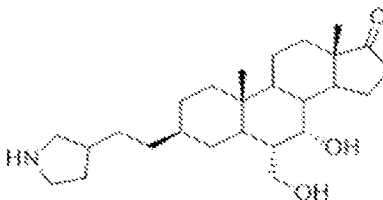
3 beta-[2-(azetidine-3-yl)ethyl]-6alpha-hydroxymethyl-7alpha-hydroxyandrostande-17-one
5 (**CVie408**)



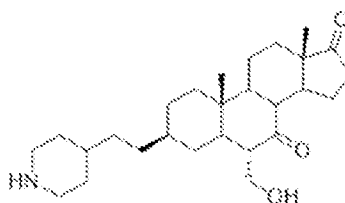
3 beta-[2-(pyrrolidine-3-yl)ethyl]-6alpha-hydroxymethylandrostande-7,17-dione (**CVie409**):



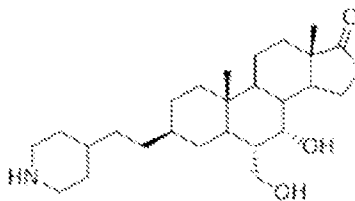
3 beta-[2-(pyrrolidine-3-yl)ethyl]-6 alpha-hydroxymethyl-7 alpha-hydroxyandrostane-17-one
(**CVie410**):



5 3 beta-[2-(piperidin-4-yl)ethyl]-6 alpha-hydroxymethyl-7 alpha-hydroxyandrostane-17-one
(**CVie411**):



3 beta-[2-(piperidin-4-yl)ethyl]-6 alpha-hydroxymethyl-7 alpha-hydroxyandrostane-17-one
(**CVie412**):



Each of the particular pure SERCA2a activator compounds, and methods of making these compounds, are disclosed in WO 2021/069570 A1, the entire content of which is herein incorporated by reference. In a particular embodiment of the present invention, Formulas I, II, III and IV, or pharmaceutically acceptable salt or ester, hydrate, solvate, or polymorphic form is
5 utilized in a medicament to treat or prevent AHF, particularly AHF-related pre- or overt cardiogenic shock, in an individual while reducing or eliminating the risk of developing acute myocardial arrhythmias. In a preferred embodiment, these agents are administered to an individual for the treatment of AHF or AHF-related pre- or overt cardiogenic shock with or without acute myocardial ischemia and/or acute myocardial infarction to improve heart
10 functioning while reducing or eliminating the risk of developing acute myocardial arrhythmias.

Also disclosed herein is a pharmaceutical composition comprising istaroxime or one of the dual mechanism compounds or pure SERCA2a activators described above in an admixture with at least one pharmaceutically acceptable vehicle and/or excipient. In preferred
15 embodiments, the pharmaceutical composition is formulated for administering to an individual by infusion, preferably, it is by intravenous infusion.

To determine efficacy of istaroxime administration (or administration with one of the dual mechanism compounds or pure SERCA2a activators described above) in an individual with AHF, there are a number of parameters of heart function that can be measured to determine whether or not the condition has been improved or prevented. For instance, in some
20 embodiments, art-standard equipment (*e.g.*, echocardiogram, pulmonary artery catheter, transpulmonary thermodilution, sphygmomanometer, EKG, and others) is used to measure one or more parameters of cardiac function, such as, but not limited to cardiac output, cardiac index, stroke volume, stroke volume index, E/A ratio, e' , E/e' ratio, Sa, S, left ventricle ejection fraction (LVEF), Aa, Ea, and the like.

For instance, echocardiography can be performed on individuals according to international standards [*see*, for example, Lang *et al.*, 2005, J. Am. Soc. Echocardiogr. 18(12):1440-1463; Negueh *et al.*, 2009, Eur. J. Echocardiogr. 10(2):165-193; Evangelista *et al.*, 2008, Eur. J. Echocardiogr. 9(4):438-448]. Echocardiography is within the purview of skilled
25 physicians or sonographers. For instance, echocardiography can be performed during screening of potential clinical study participants or upon admission of an individual to a health care facility.
30 In other embodiments, echocardiography is done prior to treatment and/or at baseline and then 1

h, 2 h, 3 h, 4 h, 5 h, 6 h, 7 h, 8 h, 9 h, 10 h, 11 h, 12 h, 13 h, 14 h, 15 h, 16 h, 17 h, 18 h, 19 h, 20 h, 21 h, 22 h, 23 h, 24 h, 25 h, 26 h, 27 h, 28 h, 29 h, 30 h, 31 h, 32 h, 33 h, 34 h, 35 h, 36 h, 37 h, 38 h, 39 h, 40 h, 41 h, 42 h, 43 h, 44 h, 45 h, 46 h, 47 h, 48 h, 72 h, 96 h, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days 16 days 17 days, 18 days 19 days, 20 days, 21 days 22 days, 23 days, 24 days, 25 days, 26 days, 27 days, 28 days, 29 days, or 30 days following the start of infusion. In one embodiment, echocardiography is monitored prior to, during , and immediately following the initiation of infusion, and values are recorded. Echocardiographic values may continue to be monitored and recorded at 24 and 30 hours post-infusion. In other embodiments, echocardiographic values are recorded prior to, during, immediately following infusion, and 12, 24, 48, 72, and 96 hours post-infusion. Table 1 summarizes exemplary echocardiographic and other heart function parameters.

Table 1. Heart Function Parameters

Parameter	Definition
<i>Cardiac dimension measures</i>	
Left ventricle end diastolic diameter (EDD)	measured with M-mode echocardiography at the level of mitral valve (MV) leaflets from parasternal long axis view (PLAX) (NR: 42-59 mm human males and 39-53 mm human females)
Left ventricle end systolic diameter (ESD)	measured with M-mode echocardiography at the level of mitral valve (MV) leaflets from PLAX (NR: 25-35 mm)
Left ventricle end diastolic volume (EDV)	measured with M-mode echocardiography at the level of mitral valve (MV) leaflets from PLAX (NR: 67-155 mL human males and 56-104 mL human females)
Left ventricle end systolic volume (ESV)	measured with M-mode echocardiography at the level of mitral valve (MV) leaflets from PLAX (NR: 22-58 mL human males and 19-49 mL human females)
Left atrium diameter (LAD)	measured at end-ventricular systole with M-mode echocardiography from PLAX. (NR: 30-40 mm human males and 27-38 mm human females)
Left atrium area (LAA)	measured from apical four chamber view (NR: ≤ 20 cm ²)
Left atrium volume (LAV)	derived from area-length measured from apical four chamber view (NR: 18-58 mL human males and 22-52 mL human females)
<i>Left ventricle diastolic function parameters</i>	

E wave	measured from mitral valve pulsed wave Doppler, is the peak velocity of early filling. Normal range for all the diastolic parameters significantly changes with age.
A wave	measured from mitral valve pulsed wave Doppler is the peak velocity of late atrial filling. Not evaluable in patients with AF.
E wave deceleration time (EDT)	measured from mitral valve pulsed wave Doppler represent the slope of the descending part of E wave.
E/A ratio	determines the type of diastolic filling pattern (normal E/A= 1-2 and EDT= 150-200 ms, abnormal relaxation E/A<1 and EDT≥240 ms, pseudonormal E/A=0.8-1.5; restrictive E/A≥2 and EDT <160 ms). Not evaluable in patients with AF.
Ea	measured with tissue Doppler method at the lateral and septal side of the mitral annulus from apical four chamber view is the early diastolic velocity. The value has been calculated as the average between Ea lateral and Ea septal. (NR≥10 cm/s).
Aa	measured with tissue Doppler method at the lateral and septal side of the mitral annulus from apical four chamber view is the late atrial diastolic velocity. The value has been calculated as the average between Aa lateral and Aa septal. Not evaluable in patients with AF
E/Ea ratio	this is a derived measure from E and Ea value. This is highly correlated with left ventricle filling pressure and with prognosis in patients with HF.
<i>Left ventricle systolic function parameters</i>	
Left ventricle ejection fraction (LVEF)	measured with Simpson biplane method according to international recommendations from apical four chamber view and apical two chamber view. (NR ≥55%)
Sa	measured with tissue Doppler method at the lateral and septal side of the mitral annulus from apical four chamber view. The value has been calculated as the average between Sa lateral and Sa septal. Validation studies demonstrated that Sa correlates with LVEF (NR ≥6 cm/s).
<i>Overall cardiac contraction parameters</i>	
Stroke volume (SV)	is a derived measure obtained with the application of Bernoulli's formula using the dimension of left ventricle outflow tract (LVOT) as diameter and LVOT time velocity integral as velocity. (NR >60 mL/beat)
Cardiac output (CO)	is derived by the multiplication of SV x heart rate (HR) (NR: >4 L/min).

Stroke volume index (SVI)	is a derived parameter obtained by the adjustment of SV by body surface area (BSA) (NR: 33-47 mL/beat/m ²).
Cardiac index (CI)	is a derived parameter obtained by the adjustment of CO by body surface area (BSA) (NR: 2.5-4 L/min/m ²).
<i>Right ventricle function parameters</i>	
Pulmonary arterial systolic pressure (PASP)	estimated by the sum of the peak velocity at tricuspidal continuous wave Doppler and a fixed value derived from inferior vena cava diameter and respiratory change. (NR<35 mmHg).
Tricuspid annular plane systolic excursion (TAPSE)	measured from M-mode echocardiography from apical four chamber view. TAPSE correlates with right ventricle ejection fraction and its reduction associated with worse prognosis in HF. (NR>16 mm).
Right ventricle Sa	measured with tissue Doppler method at right ventricle free wall from apical four chamber view. Sa is a derived parameter of systolic function and correlated with right ventricle ejection fraction. (NR >10 cm/s).
<i>Other parameters</i>	
Mitral regurgitation (MR)	evaluated with a visual qualitative assessment and graded in four categories: none, mild, moderate, and severe.
Inferior vena cava diameter (IVC)	measured with M-mode echocardiography from subcostal view at 1-2 cm from the junction with right atrium. This parameter has been used to estimate systolic pulmonary artery pressure. It correlated with right atrium pressure indicating the grade of congestion. Increased IVC diameter is associated with prognosis in patients with HF (NR: ≤1.5 cm).

NR = normal range.

In one exemplary embodiment, an individual presenting with AHF with acute myocardial ischemia and/or an acute myocardial infarction is administered istaroxime (or one of the dual mechanism compounds or pure SERCA2a activators described above), whereby the heart rate is decreased by about 3 to about 9 beats per minute, *e.g.*, 3, 4, 5, 6, 7, 8, or 9, beats per minute.

In another embodiment, individuals with AHF and acute myocardial ischemia and/or an acute myocardial infarction who are administered istaroxime (or one of the dual mechanism compounds or pure SERCA2a activators described above) will exhibit an improvement in one or

more additional parameters of cardiac function, including, but not limited to, increased cardiac index (CI), decreased left atrial area, increased stroke volume, decreased left ventricular end-systolic volume, decreased pulmonary capillary wedge pressure, increased cardiac output, increased diastolic blood pressure, and decreased left ventricular end-diastolic volume.

- 5 Interestingly, istaroxime administration improved CI by increasing the stroke volume, but not by increasing the heart rate. As such, istaroxime increases cardiac output in these individuals without additional strain of increased heart rate on the heart.

In another embodiment, blood is drawn from the individual, *e.g.*, during prescreening, at baseline and/or at various timepoints post-infusion, *e.g.*, 12, 24, 48, 72, 96 hours, 5 days, 10
10 days, 30 days after the start of istaroxime, dual mechanism compound, or pure SERCA2a activator administration. This blood can be used for various laboratory testing to assess the individual's body chemistry, hematology, heart protein levels, and the pharmacokinetics of the istaroxime infusion. Exemplary chemistry includes, but is not limited to, electrolytes, liver function tests, lipids, creatinine (and estimated glomerular filtration rate (eGFR)), urea, blood
15 urea nitrogen (BUN), glucose, albumin, and protein. Exemplary hematology includes, but is not limited to, complete blood count with differential. Suitable heart proteins include cardiac troponin I (cTNI), cardiac troponin T (cTNT), N-terminal pro b-type natriuretic peptide (NT-proBNP), and brain natriuretic peptide (BNP). The levels of most of these proteins provide information to the practitioner about heart function and the measurement and/or interpretation of
20 these levels is well within the purview of the skilled artisan. For example, in one embodiment, the level of NT-proBNP is decreased as compared to baseline within about 6 hour of infusion, or within about 24 hours of administration. The levels of some of parameters are not a direct measure of heart function, but rather are used to assess kidney function. While many inotropic or vasopressor drugs have been shown to have a detrimental effect on renal function, individuals
25 administered istaroxime exhibit improved cardiac function without the deleterious impact on renal function. For instance, in one embodiment, in an individual with AHF and acute myocardial ischemia and/or an acute myocardial infarction who are administered istaroxime for a period of 24 hours, the level of eGFR, which is a measure of kidney function, is increased as compared to baseline within about 6 hours of infusion, or within about 24 hours of infusion.

- 30 Yet other parameters of heart function include chest congestion, which can be measured by use of a chest x-ray, lung ultrasound, or other art-standard equipment, wherein an

improvement in the level of chest congestion following istaroxime infusion or infusion of one of the dual mechanism compound or SERCA2a activators described above indicates treatment or prevention of AHF. Yet others include heart rate, mean arterial pressure (MAP), dyspnea or labored breathing, need for administration of intravenous vasopressors, other inotropes, and/or mechanical cardiac or renal support, symptoms of worsening heart failure, incident of adverse events (AEs) or serious adverse events (SAEs), need for admission to intensive care unit, discharge from the intensive care unit, and death. In some embodiments, MAP is increased as compared to baseline within about 24 hours of infusion.

As noted above, it is an object of the invention to, for the first time, administer the compounds described above to individuals with AHF and acute myocardial ischemia to treat the AHF while reducing the risk of or preventing serious acute myocardial arrhythmias. Ischemia, or decreased cardiac blood flow, in heart failure subjects can be detected using a variety of techniques, such as, but not limited to, with ECG monitoring, echocardiogram, stress test, coronary angiogram, nuclear perfusion imaging, positron emission tomography, cardiovascular magnetic resonance, a chest X-ray, or a heart MRI. For instance, the most common ECG sign of acute myocardial ischemia is flat or down sloping ST-segment depression of 1.0 mm or greater followed by elevation of the ST segment as ischemia progresses to infarction.

In addition, laboratory blood tests for proteins and enzymes that appear or increase with the heart muscle is damaged, such as elevated troponin or creatine kinase. The use of these techniques to diagnose and monitor ischemia is well within the purview of the skilled artisan. Symptoms of ischemia manifest in a variety of ways, such as, but not limited to, chest pressure or pain (typically on the left side of the body), neck or jaw pain, shoulder or arm pain, rapid heartbeat, shortness of breath, nausea or vomiting, sweating, and fatigue. Pre-existing conditions, such as diabetes, may exacerbate these symptoms.

Treatment for acute myocardial ischemia may include reperfusion, which is the reintroduction of blood flow to the heart. Reperfusion can be performed medically and/or mechanically. As one having ordinary skill in the art will appreciate, drug-induced reperfusion may include the administration of certain drugs that initiate the breakdown of blood clots (also referred to as thrombolytic therapy), such as, but not limited to recombinant tissue plasminogen activator (rTPA), reteplase, anistreplase, desmoteplase, nattokinase, lumbrokinase, serrapeptase, papain, DNase, bromelain, honiol, tenecteplase, alteplase, urokinase, and streptokinase.

Additional anticoagulation with heparin may also be employed. Other compounds for medically induced reperfusion include calcium channel blockers, ranolazine, beta blockers, nitrates, aspirin, and cholesterol-lowering compounds. Thrombolytic/fibrinolytic therapy is typically begun within 12 hours of the onset of ischemia symptoms; preferably, within 6 hours of the onset of symptoms; more preferably, within the first 2 hours or even 90 minutes of the onset of ischemia symptoms. Mechanical reperfusion includes, but is not limited to, balloon angioplasty, stent placement, coronary artery bypass graft, enhanced external counterpulsation, and the like. As with medical reperfusion, mechanical reperfusion therapy is typically begun within 12 hours of the onset of ischemia symptoms; preferably, within 6 hours of the onset of symptoms; more preferably, within the first 2 hours or even 90 minutes of the onset of ischemia symptoms.

As noted above, reperfusion following acute myocardial ischemia in AHF subjects increases the risk of arrhythmias, which can lead to worsening condition and even death. For instance, VF is the most dangerous of myocardial arrhythmias and requires immediate medical intervention. Therefore, to avoid arrhythmias associated with reperfusion during AHF treatment, great care should be taken to avoid administration of compounds that increase the risk for myocardial arrhythmias. For example, catecholamines (norepinephrine, dopamine, epinephrine) can increase calcium release from myocardial sarcoplasmic reticulum resulting in delayed after depolarizations leading to arrhythmias.

Myocardial arrhythmias can be detected and characterized using art-standard techniques as readily understood by the skilled artisan. One of the most common techniques used to measure myocardial arrhythmias is via an electrocardiogram or ECG. An ECG records the electrical signal from the heart and displays these signals as waves on a monitor or other viewing device. ECG monitoring, such as with a Holter monitor, can be used to assess and classify the different types of myocardial arrhythmias based on their site of origin (atrial, ventricular, or supraventricular) and by the pattern (tachycardia, multifocal tachycardia, bradycardia, fibrillation, flutter). In general, tachycardia refers to a resting heart rate that is elevated relative to normal (greater than about 100 beats per minute), bradycardia refers to a resting heart rate that is lower relative to normal (less than about 60 beats per minute), fibrillation refers to a form of electrical chaos resulting in the absence of definable P waves or QRS complexes (the first negative deviation Q wave followed by an upward positive deviation R wave, and a negative deflection after the R portion referred to as the S wave), and flutter refers to a highly organized

reentrant rhythm. In addition to identification/classification of the type of arrhythmia, the duration of the episode is also recorded to measure the severity of the arrhythmia. Some of these classification measurements are summarized in Table 2.

5 **Table 2: ECG Patterns.**

Type	ECG patterns
Atrial Fibrillation (AF)	Absence of P waves; Irregular ventricular rate; Narrow QRS with normal morphology.
Paroxysmal Supraventricular Tachycardia (PSVT)	Sudden onset (paroxysmal); Narrow QRS complex (less than about 120 msec) tachycardia (about 140 to 250 beats/min; P waves absent or inverted; Regular ventricular rate.
Ventricular Tachycardia (VT)	Wide amplitude QRS complex; QRS complexes not preceded by P waves; T waves with the opposite polarity compared to the QRS complex; T waves may be difficult to separate from the QRS complex; RR interval with less than 3 large boxes or a pulse rate greater than about 100 beats/min.
Ventricular Fibrillation (VF)	Rapid uncoordinated electrical depolarization of the ventricle; An undulating baseline with no consistent pattern of waves or complexes.

Further still, some AHF patients have increased risk for arrhythmias. The more severe the heart failure, the higher the risk for arrhythmias from the disease and/or from medications given to treat the disease. As one having ordinary skill in the art will appreciate, high risk individuals can be identified as having a family history of arrhythmias and heart failure, physical examination,

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and laboratory assessments, including blood tests for elevated brain natriuretic peptide (BNP) N-terminal-pro brain natriuretic peptide (NT-proBNP).

In summary, art-standard medical assessments can be used to identify subjects or cohorts of subjects that present with HF (including AHF and pre/overt cardiogenic shock) with ischemia, HF with ischemia-reperfusion injury, and HF with elevated risk of myocardial arrhythmias.

As shown in the examples, administration of both istaroxime and CVie 216 to rat hearts isolated from a diabetic rat model used in the art to induce myocardial arrhythmias and mimic the clinical condition, showed a significant reduction in arrhythmia score (see Table 3 and FIG. 1B). Moreover, CVie 216 administration to intact diabetic rats with induced ischemia and reperfusion showed a reduction in duration of ventricular premature contractions and arrhythmia scored compared to rats administered saline (see Table 4). Importantly, the protective effect attributed to istaroxime does not rely on its metabolism to PST 3093 or other pure SERCA2a metabolite given the short time frame of action shown in the examples. Therefore, these results demonstrate that istaroxime and its metabolites and derivatives, whether they be dual mechanism compounds or pure SERCA2a activators, can be used to treat AHF and AHF-related disorders (such as pre-cardiogenic shock and cardiogenic shock) while protecting against acute myocardial arrhythmias, including arrhythmias due to reperfusion injury. The latter has important implications for the treatment of patients who exhibit sudden ischemia or myocardial infarction and need immediate reperfusion. Thus, the inventors demonstrate, for the first time, that istaroxime and its progeny can be used to treat a much wider range of AHF patients without significantly increasing the risk of arrhythmias.

Pharmaceutical compositions

Pharmaceutical compositions and formulations for intravenous infusion comprising istaroxime, CVie 216, or one of the other dual mechanism compounds or pure SERCA2a activators described herein, or a pharmaceutically acceptable salt, solvate, or hydrate thereof in an admixture with at least one conventional pharmaceutically acceptable carrier and/or vehicle and/or excipient are commonly known in the art.

The pharmaceutical compositions and formulations for intravenous infusion can be formulated in any way and can be administered in a variety of unit dosage forms depending upon the condition or disease and the degree of illness, the general medical condition of each patient,

the resulting preferred method of administration and the like. Details on techniques for formulation and administration are well described in the scientific and patent literature [*see, e.g.*, the latest edition of Remington's Pharmaceutical Sciences, Mack Publishing Co, Easton PA ("Remington's")].

5 The formulations may conveniently be presented in unit dosage form and may be prepared by any method known in the art of pharmacy. The amount of active ingredient which can be combined with a carrier or vehicle material to produce a single dosage form will vary depending upon the subject being treated and the particular mode of administration. The amount of active ingredient that can be combined with a carrier material to produce a single dosage form
10 will generally be the amount of the compound which produces a therapeutic effect.

Pharmaceutical formulations as provided herein can be prepared according to any method known to the art for the manufacture of pharmaceuticals. Such formulations can contain additional agents, such as preserving or stabilizing agents. A formulation can be admixed with nontoxic pharmaceutically acceptable carriers or excipients which are suitable for manufacture.

15 Formulations may comprise one or more diluents, emulsifiers, preservatives, buffers, excipients, etc. and may be provided in such forms as liquids, powders, emulsions, lyophilized powders, etc.

Aqueous suspensions can contain an active agent (*e.g.*, a composition used to practice the uses and methods as provided herein) in an admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients include a suspending agent, such as
20 sodium carboxymethylcellulose, methylcellulose, hydroxypropyl-methylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia, and dispersing or wetting agents, such as a naturally-occurring phosphatide (*e.g.*, lecithin), a condensation product of an alkylene oxide with a fatty acid (*e.g.*, polyoxyethylene stearate), a condensation product of ethylene oxide with a long chain aliphatic alcohol (*e.g.*, heptadecaethylene oxycetanol), a
25 condensation product of ethylene oxide with a partial ester derived from a fatty acid and a hexitol (*e.g.*, polyoxyethylene sorbitol mono-oleate), or a condensation product of ethylene oxide with a partial ester derived from fatty acid and a hexitol anhydride (*e.g.*, polyoxyethylene sorbitan mono-oleate). The aqueous suspension can also contain one or more preservatives such as ethyl or n-propyl p-hydroxybenzoate. Formulations can be adjusted for osmolarity.

30 According to the present invention, istaroxime, CVie 216, or one of the other compounds described herein is given by intravenous (IV) administration. These formulations can comprise a

solution of active agent dissolved in a pharmaceutically acceptable carrier. Acceptable vehicles and solvents that can be employed are water, dextrose in water, and Ringer's solution, an isotonic sodium chloride. These solutions are sterile and generally free of undesirable matter. These formulations may be sterilized by conventional, well known sterilization techniques. The formulations may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents, *e.g.*, sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate and the like. The concentration of active agent in these formulations can vary widely, and will be selected primarily based on fluid volumes, viscosities, body weight, and the like, in accordance with the particular mode of administration selected and the patient's needs. The administration is by bolus or continuous infusion (*e.g.*, substantially uninterrupted introduction into a blood vessel for a specified period of time).

The compounds as provided herein can be lyophilized. Provided herein is a stable lyophilized formulation comprising a composition as provided herein, which can be made by lyophilizing a solution comprising a pharmaceutical as provided herein and a bulking agent, *e.g.*, mannitol, trehalose, raffinose, lactose, and sucrose or mixtures thereof. There are many other conventional lyophilizing agents. Among the sugars, lactose is the most common. Also used are citric acid, sodium carbonate, EDTA, Benzyl alcohol, glycine, sodium chloride, etc. [*see*, for example, Journal of Excipients and Food Chemistry Vol. 1, Issue 1 (2010) pp 41-54; U.S. patent app. no. 20040028670]. For instance, istaroxime can be prepared as a lyophilized powder for injection according to the teaching of CN103315968.

The compounds provided herein can be formulated for oral administration. Formulations of a pharmaceutical composition suitable for oral administration comprise at least one of the compounds described herein combined with a pharmaceutically acceptable carrier, in a variety of dosage forms, including but not limited to pills, tablets, granules, powders, capsules, dispersions, suspensions, solutions, emulsions, microemulsions, gels and films, to name a few. Such dosage forms typically include carriers, excipients, and or permeation enhancers to facilitate formulation and delivery of the active ingredients.

The pharmaceutically acceptable carriers for oral formulations are selected from proteins, carbohydrates, lipids, organic and inorganic molecules, and combinations thereof. The active ingredients can be combined with the carrier in an appropriate diluent to form a solution or a

suspension. Such liquid formulations can be viscous or non-viscous depending on the amount and the carrier used. The liquid formulations can be used directly or can be further formulated into an appropriate capsule, gel capsule or solid by methods known to those skilled in the art.

Alternatively, solid formulations can be made by combining solid components. Such solid formulations can be used as a powder or formulated into granules, capsules, tablets or films any one of which can be made as a time release formulation.

Suitable proteins for use as carriers in oral dosage forms include milk proteins such as casein, sodium caseinate, whey, reduced lactose whey, whey protein concentrate, gelatin, soy protein (isolated), brown algae protein, red algae protein, baker's yeast extract and albumins.

Suitable carbohydrates include celluloses such as methylcellulose, sodium carboxymethylcellulose, carboxymethylcellulose, hydroxypropylcellulose, hydroxypropylmethylcellulose, cellulose acetate and ethyl cellulose, starches such as cornstarch, potato starch, tapioca starch, wheat starch, acid modified starch, pregelatinized starch and unmodified starch, alginates such as ammonium alginate, sodium alginate, and calcium alginate, glutens such as corn gluten and wheat gluten, gums such as acacia (gum Arabic), gum ghatti, guar gum, karaya gum (sterculia gum) and gum (tragacanth), insoluble glucose isomerase enzyme preparations, sugars such as corn sugar, invert sugar, corn syrup, high fructose corn syrup, and sodium gluconate. Suitable lipids include tocopherols such as α -tocopherol acetate, short-, medium- and long-chain fatty acids and esters thereof, fatty alcohols and ethers thereof, oils such as coconut oil (refined), soybean oil (hydrogenated) and rapeseed oil, aluminum palmitate, dilauryl thiodipropionate, enzyme-modified lecithin, calcium stearate, enzyme-modified fats, glyceryl palmitostereate, lecithin, mono- and diglycerides, glycerin and waxes such as beeswax (yellow and white), candelilla wax and carnauba wax and vegetable oil. Suitable organic and inorganic substances include methyl and vinyl pyrrolidones such as polyvinylpyrrolidone, methylsulfonyl methane, dimethylsulfoxide and related compounds, hydroxy and polyhydroxy acids such as polylactic acid, among many others.

According to the present invention, the compounds as provided herein can be administered for prophylactic and/or therapeutic treatments. In therapeutic applications, compositions are administered to a subject already suffering from a condition, or disease in an amount sufficient to treat, prevent, cure, alleviate or partially arrest the clinical manifestations of the condition, or disease and its complications (*i.e.*, a "therapeutically effective amount"). For

example, in alternative embodiments, pharmaceutical compositions as provided herein are administered in an amount sufficient to treat, prevent or ameliorate in an individual in need thereof. The amount of pharmaceutical composition adequate to accomplish this is defined as a “therapeutically effective dose.” The dosage schedule and amounts effective for this use, *i.e.*, the “dosing regimen,” will depend upon a variety of factors, including the stage of the disease or condition, the severity of the disease or condition, the general state of the patient's health, the patient's physical status, age and the like. In calculating the dosage regimen for a patient, the mode of administration also is taken into consideration.

The dosage regimen also takes into consideration pharmacokinetics parameters well known in the art, *i.e.*, the active agents' bioavailability, metabolism, clearance, and the like [*see, e.g.*, Hidalgo-Aragones, 1996, Steroid Biochem. Mol. Biol. 58:611-617; Groning, 1996, Pharmazie 51:337-341; Fotherby, 1996, Contraception 54:59-69; Johnson, 1995, J. Pharm. Sci. 84:1144-1146; Rohatagi, 1995, Pharmazie 50:610-613; Brophy, 1983, Eur. J. Clin. Pharmacol. 24:103-108; the latest Remington's, *supra*]. The state of the art allows the clinician to determine the dosage regimen for each individual patient, active agent and disease or condition treated. Guidelines provided for similar compositions used as pharmaceuticals can be used as guidance to determine the dosage regimen, *i.e.*, dose schedule and dosage levels, administered practicing the methods as provided herein are correct and appropriate.

Single or multiple administrations of formulations can be given depending on the dosage and frequency as required by the AHF symptoms of patient. The formulations should provide a sufficient quantity of active agent to effectively treat or prevent or ameliorate a conditions, diseases or symptoms as described herein. A correct treatment of AHF or related disease, by selectively normalizing a depressed biochemical activity underlying the symptoms of subset of patients, may be expected to selectively improve the symptoms and to reduce the incidence of unwanted side effects produced by the available drugs either during hospital staying or after discharge. The term prevention is applicable when the continuous monitoring of the pulmonary pressure is possible with the appropriate chronic implantable devices that provide measurements of heart function parameters.

In one embodiment, an effective amount of istaroxime or equivalent of a pharmaceutically acceptable salt, solvate, or hydrate thereof, is administered to an individual with AHF symptoms and who is presenting with acute myocardial ischemia at a dosing schedule,

e.g., from about 0.1 µg/kg/min to about 3.0 µg/kg/min, *e.g.*, 0.1 µg/kg/min, 0.15 µg/kg/min, 0.2 µg/kg/min, 0.25 µg/kg/min, 0.3 µg/kg/min, 0.35 µg/kg/min, 0.4 µg/kg/min, 0.45 µg/kg/min, 0.5 µg/kg/min, 0.55 µg/kg/min, 0.6 µg/kg/min, 0.7 µg/kg/min, 0.75 µg/kg/min, 0.8 µg/kg/min, 0.85 µg/kg/min, 0.9 µg/kg/min, 0.95 µg/kg/min, 1.0 µg/kg/min, 1.1 µg/kg/min, 1.2 µg/kg/min, 1.3 µg/kg/min, 1.4 µg/kg/min, 1.5 µg/kg/min, 1.6 µg/kg/min, 1.7 µg/kg/min, 1.8 µg/kg/min, 1.9 µg/kg/min, 2.0 µg/kg/min, 2.1 µg/kg/min, 2.2 µg/kg/min, 2.3 µg/kg/min, 2.4 µg/kg/min, 2.5 µg/kg/min, 2.6 µg/kg/min, 2.7 µg/kg/min, 2.8 µg/kg/min, 2.9 µg/kg/min, or 3.0 µg/kg/min.

For instance, in some embodiments, istaroxime is administered by infusion at an effective dose from about 0.1 µg/kg/min to about 2.5 µg/kg/min, about 0.2 µg/kg/min to about 2.0 µg/kg/min,

or from about 0.5 µg/kg/min to about 1.5 µg/kg/min, or from about 1.0 µg/kg/min to about 1.5 µg/kg/min. In one particular embodiment, the istaroxime is administered by intravenous infusion at an effective dose of about 1.5 µg/kg/min. Alternatively, the effective dose of istaroxime can be initiated at about 1.0 µg/kg/min for about 1 to about 2 hours, which is then increased to 1.5 µg/kg/min for the remaining infusion time unless the individual exhibits nausea or other signs of poor or insufficient drug tolerance. In another embodiment, the effective dose of istaroxime can be initiated at about 1.5 µg/kg/min for about 1 to about 2 hours, which is then decreased to 1.0 µg/kg/min for the remaining infusion time.

In one another embodiment, an effective amount of PST 3093 or CVie 216 or equivalent of a pharmaceutically acceptable salt, solvate, or hydrate thereof, is administered to an individual with AHF symptoms and who is presenting with acute myocardial ischemia at a dosing schedule,

e.g., from about 0.1 µg/kg/min to about 3.0 µg/kg/min, *e.g.*, 0.1 µg/kg/min, 0.15 µg/kg/min, 0.2 µg/kg/min, 0.25 µg/kg/min, 0.3 µg/kg/min, 0.35 µg/kg/min, 0.4 µg/kg/min, 0.45 µg/kg/min, 0.5 µg/kg/min, 0.55 µg/kg/min, 0.6 µg/kg/min, 0.7 µg/kg/min, 0.75 µg/kg/min, 0.8 µg/kg/min, 0.85 µg/kg/min, 0.9 µg/kg/min, 0.95 µg/kg/min, 1.0 µg/kg/min, 1.1 µg/kg/min, 1.2 µg/kg/min, 1.3 µg/kg/min, 1.4 µg/kg/min, 1.5 µg/kg/min, 1.6 µg/kg/min, 1.7 µg/kg/min, 1.8 µg/kg/min, 1.9 µg/kg/min, 2.0 µg/kg/min, 2.1 µg/kg/min, 2.2 µg/kg/min, 2.3 µg/kg/min, 2.4 µg/kg/min, 2.5 µg/kg/min, 2.6 µg/kg/min, 2.7 µg/kg/min, 2.8 µg/kg/min, 2.9 µg/kg/min, or 3.0 µg/kg/min.

For instance, in some embodiments, istaroxime is administered by infusion at an effective dose from about 0.1 µg/kg/min to about 2.5 µg/kg/min, about 0.2 µg/kg/min to about 2.0 µg/kg/min,

or from about 0.5 µg/kg/min to about 1.5 µg/kg/min, or from about 1.0 µg/kg/min to about

1.5 µg/kg/min. In one particular embodiment, the PST 3093 or CVie 216 is administered by intravenous infusion at an effective dose of about 1.0 µg/kg/min or 1.5 µg/kg/min. Alternatively, the effective dose of PST 3093 or CVie 216 can be initiated at about 1.0 µg/kg/min for about 1 to about 2 hours, which is then increased to 1.5 µg/kg/min for the remaining infusion time unless
 5 the individual exhibits nausea or other signs of poor or insufficient drug tolerance. In another embodiment, the effective dose of PST 3093 or CVie 216 can be initiated at about 1.5 µg/kg/min for about 1 to about 2 hours, which is then decreased to 1.0 µg/kg/min for the remaining infusion time. In still other embodiments, a lower dose of PST 3093 is used, e.g., 0.5 µg/kg/min.

In yet another embodiment, an effective amount a compound of Formula II or Formula
 10 IV or equivalent of a pharmaceutically acceptable salt, solvate, or hydrate thereof, is administered to an individual with AHF symptoms and who is presenting with acute myocardial ischemia at a dosing schedule, e.g., from about 0.1 µg/kg/min to about 3.0 µg/kg/min, e.g., 0.1 µg/kg/min, 0.15 µg/kg/min, 0.2 µg/kg/min, 0.25 µg/kg/min, 0.3 µg/kg/min, 0.35 µg/kg/min, 0.4 µg/kg/min, 0.45 µg/kg/min, 0.5 µg/kg/min, 0.55 µg/kg/min, 0.6 µg/kg/min, 0.7 µg/kg/min,
 15 0.75 µg/kg/min, 0.8 µg/kg/min, 0.85 µg/kg/min, 0.9 µg/kg/min, 0.95 µg/kg/min, 1.0 µg/kg/min, 1.1 µg/kg/min, 1.2 µg/kg/min, 1.3 µg/kg/min, 1.4 µg/kg/min, 1.5 µg/kg/min, 1.6 µg/kg/min, 1.7 µg/kg/min, 1.8 µg/kg/min, 1.9 µg/kg/min, 2.0 µg/kg/min, 2.1 µg/kg/min, 2.2 µg/kg/min, 2.3 µg/kg/min, 2.4 µg/kg/min, 2.5 µg/kg/min, 2.6 µg/kg/min, 2.7 µg/kg/min, 2.8 µg/kg/min, 2.9 µg/kg/min, or 3.0 µg/kg/min. In a preferred embodiment, the Formula II or Formula IV
 20 compounds is selected from the group consisting of 3-beta-hydroxy-5-beta-10-beta-methyl-13-beta-methyl-14-beta-hydroxy-17-beta-(imidazol-4-yl)androstane; 3-beta-hydroxy-5-beta-10-beta-methyl-13-beta-methyl-14-beta-hydroxy-17-beta-(2-guanidino-thiazol-4-yl)androstane; 3-beta-hydroxy-5-beta-10-beta-methyl-13-beta-methyl-14-beta-hydroxy-17-beta-(pyrazol-3-yl)androstane; 3-beta-hydroxy-5-beta-10-beta-methyl-12-beta-hydroxy-13-beta-methyl-14-beta-
 25 hydroxy-17-beta-(imidazol-4-yl)androstane; 3-beta-hydroxy-5-beta-10-beta-methyl-12-beta-hydroxy-13-beta-methyl-14-beta-hydroxy-17-beta-(2-guanidino-thiazol-4-yl)androstane; 3-beta-hydroxy-5-beta-10-beta-methyl-13-beta-methyl-14-beta-hydroxy-17-beta-(N-(3-aminopropyl)-imidazol-4-yl)androstane; 3-beta-hydroxy-5-beta-10-beta-methyl-13-beta-methyl-14-beta-hydroxy-17-beta-((5-(3-aminopropyl)-isoxazol-3-yl))androstane; 3-beta-hydroxy-5-beta-10-beta-
 30 methyl-13-beta-methyl-14-beta-hydroxy-17-beta-((5-(3-aminopropyl)-isoxazol-3-yl)-

ethyl)androstane; 3-beta-hydroxy-5-beta-10-beta-methyl-13-beta-methyl-14-beta-hydroxy-17-beta-(5-(2-aminoethyl)-isoxazol-3-yl)-ethyl)androstane; 3-beta-hydroxy-5-beta-10-beta-methyl-13-beta-methyl-14-beta-hydroxy-17-beta-(5-(2-aminomethyl)-isoxazol-3-yl)-ethenyl)androstane; (E)-4-(6alpha-hydroxy-17-oxoandrostane-3-yliden)butyric acid; (Z)-4-(6alpha-hydroxy-17-oxoandrostane-3-yliden)butyric acid; (E)-4-(6beta-hydroxy-17-oxoandrostane-3-yliden)butyric acid; (Z)-4-(6beta-hydroxy-17-oxoandrostane-3-yliden)butyric acid; (E)-3-[2-(azetidin-3-yl)ethyliden]-6alpha-hydroxyandrostane-17-one; (Z)-3-[2-(azetidin-3-yl)ethyliden]-6alpha-hydroxyandrostane-17-one; (E)-3-(4-aminobutyl)-6alpha-hydroxyandrost-2-ene-17-one hydroiodide; 3-[2-(piperidin-4-yl)ethyl]-6alpha-hydroxyandrost-2-ene-17-one hydroiodide; (EZ)-3-(4-aminobutyliden)-6alpha-hydroxyandrostane-17-one; (E)-3-[2-(piperidin-4-yl)ethyliden]-6alpha-hydroxyandrostane-17-one; (Z)-3-[2-(piperidin-4-yl)ethyliden]-6alpha-hydroxyandrostane-17-one; 3 beta-[2-(piperidin-4-yl)ethyl]-6alpha-hydroxyandrostane-17-one; Ethyl (6alpha-hydroxy-17-ketoandrostane-3beta-yl) acetate; 4-(6alpha-hydroxy-17-oxoandrostane-3-yl) butyric acid; 4-(6beta-hydroxy-17-oxoandrostane-3-yl) butyric acid; 2-(6beta-hydroxy-17-oxoandrostane-3-yl) acetic acid; Ethyl 4-(6alpha-hydroxy-17-oxoandrostane-3-yl) butyrate; Ethyl 4-(6alpha-hydroxy-17-oxoandrostane-3-yl) caproate; 6-(6beta-hydroxy-17-oxoandrostane-3-yl) caproic acid; (E,Z)-3-(5-N-methylaminopentyliden)-6alpha-hydroxymethylandrostane-7,17-dione; (E,Z)-3-[2-(pyrrolidine-3yl)ethyliden]-6alpha-hydroxymethylandrostane-7,17-dione; (E,Z)-3-[2-(azetidine-3-yl)ethyliden]-6alpha-hydroxymethylandrostane-7,17-dione; (E,Z)-3-[2-(piperidin-4-yl)ethyliden]-6alpha-hydroxymethylandrostane-7,17-dione; (E,Z)-3-(5-N-methylaminopentyliden)-6alpha-hydroxymethyl-7alpha-hydroxyandrostane-17-one; 3 beta-[2-(azetidine-3-yl)ethyl]-6alpha-hydroxymethylandrostane-7,17-dione; 3 beta-[2-(azetidine-3-yl)ethyl]-6alpha-hydroxymethyl-7alpha-hydroxyandrostane-17-one; 3 beta-[2-(pyrrolidine-3yl)ethyl]-6alpha-hydroxymethylandrostane-7,17-dione; 3 beta-[2-(pyrrolidine-3yl)ethyl]6alpha-hydroxymethyl-7alpha-hydroxyandrostane-17-one; 3 beta-[2-(piperidin-4-yl)ethyl]-6alpha-hydroxymethylandrostane-7,17-dione; and 3 beta-[2-(piperidin-4-yl)ethyl]-6alpha-hydroxymethyl-7alpha-hydroxyandrostane-17-one.

In one exemplary embodiment, a pharmaceutical composition comprising istaroxime (or CVie 216) in lyophilized power form is provided. In another embodiment, the pharmaceutical composition comprises istaroxime (or CVie 216) and a bulking agent (*e.g.*, lactose) in

lyophilized powder form. For instance, the composition may be a lyophilized powder comprising about 0.1 to about 0.3 wt% istaroxime and about 0.7 to about 0.9 wt% lactose (*e.g.*, 10 mg istaroxime and 50 mg lactose). The lyophilized powder can be reconstituted with any pharmaceutically acceptable carrier, such as saline with 0.9% NaCl) prior to intravenous
5 infusion.

Methods of Treatment

Also provided herein are pharmaceutical compositions for use as a medicament in the treatment of heart failure as well as methods of treating an individual with heart failure;
10 preferably, the heart failure is acute heart failure. In some instances, the heart failure is AHF-related pre-cardiogenic shock or cardiogenic shock. In some embodiments, the individual presents with acute heart failure and acute myocardial ischemia and/or an acute myocardial infarction. Accordingly, this leads to a loss or significant reduction in blood flow to the heart requiring immediate intervention, *e.g.*, reperfusion of the heart. It is therefore an object of this
15 invention that these individuals be treated with one or more of the pharmaceutical compositions described in detail above prior to or during reperfusion. Importantly, the pharmaceutical compositions provided herein can be used to treat AHF (and related conditions, such as pre-cardiogenic shock or cardiogenic shock) while reducing the risk of or altogether preventing serious myocardial arrhythmias, such as VT or VF. Even more surprising, these pharmaceutical
20 compositions reduce the risk of myocardial arrhythmias even in the individual presenting with AHF and acute myocardial ischemia, and even during reperfusion, which is a period of blood flow oscillation that is high risk for myocardial arrhythmias.

In an embodiment, the method of therapy includes providing or presenting the individual having heart failure or acute heart failure. In some cases, a measuring step is first carried out to
25 determine the baseline heart function of the individual. For instance, an individual with heart failure may exhibit impaired or decreased diastolic relaxation function. The measuring step may include measuring one or more parameters of heart failure, such as, but not limited to, decreased heart rate, decreased heart pressure, decreased systolic blood pressure, diastolic blood pressure, reduced left ventricular end-diastolic/systolic volume and function (LVEF), increased E/Ea ratio,
30 increased E/A ratio, reduced Ea ratio, and/or decreased stroke volume. As one having ordinary skill in the art will appreciate, any suitable measuring technique available in the art at the time of

the measuring step is suitable for use herein, and it is well within the purview of such skilled artisan to select an appropriate measuring technique corresponding to the parameter of interest. A non-limiting list of suitable measuring equipment/techniques includes ECG, echocardiogram, cardiac catheterization (for assessment of pulmonary artery pressures and filling pressures of the left side of the heart), nuclear stress test, CAT scan, radionuclide ventriculography scan, stethoscope, sphygmomanometer, and the like.

In particular embodiments, the individual exhibits AHF with acute myocardial ischemia. This ischemia can be detected and monitored using a variety of art standard techniques, including, but not limited to ECG monitoring, echocardiogram, stress test, coronary angiogram, nuclear perfusion imaging, positron emission tomography, cardiovascular magnetic resonance, a chest X-ray, heart MRI or via laboratory blood tests for proteins and enzymes that appear or increase with the heart muscle is damaged, such as elevated troponin or creatine kinase. For instance, it is well within the purview of the skilled artisan (*e.g.*, doctor or other medical professional) to interpret ECG readings (*e.g.*, ST depression equal to or greater than 1 mm) and diagnose an individual with AHF who is exhibiting acute myocardial ischemia. Accordingly, the individual is diagnosed with AHF and acute myocardial ischemia, the latter being detected at the same time as the initial diagnosis or at later time while the individual's AHF condition is being monitored.

The treatment may therefore include the step of reperfusion to reintroduce the flow of blood or increase the blood flow to the heart. Reperfusion can be performed medically by administering one or more active ingredients, such as, but not limited to recombinant tissue plasminogen activator (rTPA), reteplase, anistreplase, desmoteplase, nattokinase, lumbrokinase, serrapeptase, papain, DNase, bromelain, honioli, tenecteplase, alteplase, urokinase, streptokinase, anticoagulation with heparin, calcium channel blockers, ranolazine, beta blockers, nitrates, aspirin, or cholesterol-lowering compounds. Reperfusion can be performed mechanically using art standard techniques such as, but not limited to balloon angioplasty, stent placement, coronary artery bypass graft, enhanced external counterpulsation, and the like. The reperfusion is performed within 12 hours of the onset of ischemia symptoms; preferably, within 6 hours of the onset of symptoms; more preferably, within the first 2 hours or even 90 minutes of the onset of ischemia symptoms. The method of treatment may also involve monitoring for

incidence and duration of acute myocardial arrhythmias using, for example, a ECG with a Holter monitor.

In some cases, a measuring step is first carried out to determine the baseline heart function of the individual. For instance, an individual with heart failure may present with persistent hypotension, dyspnea, and reduced LVEF. The measuring step may include measuring one or more parameters of heart function or heart function impairment, such as, but not limited to, decreased SBP or SBP AUC, reduced left ventricular end-diastolic/systolic volume and function (LVEF), or increased E/Ea or E/A ratios, reduced Ea ratio decreased stroke volume, elevated heart rate, and others. As one having ordinary skill in the art will appreciate, any suitable measuring technique available in the art at the time of the measuring step is suitable for use herein, and it is well within the purview of such skilled artisan to select an appropriate measuring technique corresponding to the parameter of interest. A non-limiting list of suitable measuring equipment/techniques includes echocardiogram, cardiac catheterization, nuclear stress test, CAT scan, radionuclide ventriculography scan, stethoscope, sphygmomanometer, pulmonary capillary wedge pressure (PCWP), and the like. For instance, the SBP can be measured by sphygmomanometer or arterial line attached to a pressure transducer. In another embodiment, the one or more parameters of heart function are measured and selected from the group consisting of heart rate (HR), blood pressure (BP), diastolic relaxation, systolic contraction, dyspnea, chest congestion, diastolic blood pressure (DBP), systolic blood pressure (SBP), systolic blood pressure area under the curve (SBP AUC), creatine clearance, deceleration slope, mitral inflow velocity, mean arterial pressure, brain natriuretic peptide levels (BNP), NT-pro-BNP levels, troponin levels, venous lactate levels, echocardiographic measurements, left ventricle end diastolic diameter (EDD), left ventricle end systolic diameter (ESD), left ventricle end diastolic volume (EDV), left ventricle end systolic volume (ESV), left atrium diameter (LAD), left atrium area (LAA), left atrium volume (LAV), E wave, A wave, E wave deceleration time (EDT), E/A ratio, Ea, Aa, E/Ea ratio, left ventricle ejection fraction (LVEF), Sa, stroke volume (SV), cardiac output (CO), stroke volume index (SVI), cardiac index (CI), pulmonary arterial systolic pressure (PASP), tricuspid annular plane systolic excursion (TAPSE), right ventricle Sa, mitral regurgitation (MR), and inferior vena cava diameter (IVC). The measuring step may also include monitoring for myocardial arrhythmias using a ECG with Holder monitor. The measuring step may be repeated or performed for the first time following the initiation of

istaroxime infusion (or infusion with a derivative thereof) to measure the change in condition, i.e., the improvement in heart function. In some embodiments, the measuring step is done after initiation of infusion with istaroxime, a dual mechanism compound, or a pure SERCA2a activator. In other embodiments, the measuring step is done before (baseline) and after initiation of infusion. In yet others an additional measuring step is done after diagnosis of acute myocardial ischemia and/or during/after reperfusion. In summary, these measuring steps can be performed at any time during the treatment process to monitor the individual and determine whether the treatment is effective.

It may be desirable to develop a clinical study group to examine a cohort of individuals exhibiting heart function parameters within a selected range of values. For such a study, as one having ordinary skill in the art will appreciate, there are certain inclusion criteria and exclusion criteria for determine whether or not an individual will be selected for participating in the study. In one embodiment, an individual is selected for treatment having one or more inclusion criteria. In a preferred embodiment, the inclusion criteria includes one or more of clinical presentation with AHF and evidence of acute myocardial ischemia.

The methods disclosed herein also include administering to the individual a therapeutically effective amount of istaroxime or istaroxime derivative. In a preferred embodiment, the istaroxime is in a pharmaceutical composition, such as any of the combinations discussed above. The istaroxime is administered in a therapeutically effective dose. For instance, the istaroxime can be administered to the individual via intravenous infusion with a dose in the range from about 0.1 $\mu\text{g/kg/min}$ to about 3.0 $\mu\text{g/kg/min}$, *e.g.*, 0.1 $\mu\text{g/kg/min}$, 0.15 $\mu\text{g/kg/min}$, 0.2 $\mu\text{g/kg/min}$, 0.25 $\mu\text{g/kg/min}$, 0.3 $\mu\text{g/kg/min}$, 0.35 $\mu\text{g/kg/min}$, 0.4 $\mu\text{g/kg/min}$, 0.45 $\mu\text{g/kg/min}$, 0.5 $\mu\text{g/kg/min}$, 0.55 $\mu\text{g/kg/min}$, 0.6 $\mu\text{g/kg/min}$, 0.65 $\mu\text{g/kg/min}$, 0.7 $\mu\text{g/kg/min}$, 0.75 $\mu\text{g/kg/min}$, 0.8 $\mu\text{g/kg/min}$, 0.85 $\mu\text{g/kg/min}$, 0.9 $\mu\text{g/kg/min}$, 0.95 $\mu\text{g/kg/min}$, 1.0 $\mu\text{g/kg/min}$, 1.1 $\mu\text{g/kg/min}$, 1.2 $\mu\text{g/kg/min}$, 1.3 $\mu\text{g/kg/min}$, 1.4 $\mu\text{g/kg/min}$, 1.5 $\mu\text{g/kg/min}$, 1.6 $\mu\text{g/kg/min}$, 1.7 $\mu\text{g/kg/min}$, 1.8 $\mu\text{g/kg/min}$, 1.9 $\mu\text{g/kg/min}$, 2.0 $\mu\text{g/kg/min}$, 2.1 $\mu\text{g/kg/min}$, 2.2 $\mu\text{g/kg/min}$, 2.3 $\mu\text{g/kg/min}$, 2.4 $\mu\text{g/kg/min}$, 2.5 $\mu\text{g/kg/min}$, 2.6, 2.7 $\mu\text{g/kg/min}$, 2.8 $\mu\text{g/kg/min}$, 2.9 $\mu\text{g/kg/min}$, or 3.0 $\mu\text{g/kg/min}$. In a preferred embodiment, the dose of istaroxime administered to the individual is in the range from about 0.1 $\mu\text{g/kg/min}$ to about 2.5 $\mu\text{g/kg/min}$ or about 0.5 $\mu\text{g/kg/min}$ to about 3.0 $\mu\text{g/kg/min}$. In a more preferred embodiment, the dose of istaroxime administered to the

individual is in the range from about 1.0 µg/kg/min to about 2.0 µg/kg/min, or about 0.5 µg/kg/min to about 1.5 µg/kg/min, or about 1.0 µg/kg/min, or about 1.5 µg/kg/min. In one particular embodiment, the dose of istaroxime administered to the individual is about 1.0 µg/kg/min.

5 In other embodiments, the methods disclosed herein include administering to the individual a therapeutically effective amount of CVie 216. In a preferred embodiment, the CVie 216 is in a pharmaceutical composition, such as any of the combinations discussed above. The CVie 216 is administered in a therapeutically effective dose. For instance, the CVie 216 can be administered to the individual via intravenous infusion with a dose in the range from about 0.1
10 µg/kg/min to about 3.0 µg/kg/min, *e.g.*, 0.1 µg/kg/min, 0.15 µg/kg/min, 0.2 µg/kg/min, 0.25 µg/kg/min, 0.3 µg/kg/min, 0.35 µg/kg/min, 0.4 µg/kg/min, 0.45 µg/kg/min, 0.5 µg/kg/min, 0.55 µg/kg/min, 0.6 µg/kg/min, 0.65 µg/kg/min, 0.7 µg/kg/min, 0.75 µg/kg/min, 0.8 µg/kg/min, 0.85 µg/kg/min, 0.9 µg/kg/min, 0.95 µg/kg/min, 1.0 µg/kg/min, 1.1 µg/kg/min, 1.2 µg/kg/min, 1.3 µg/kg/min, 1.4 µg/kg/min, 1.5 µg/kg/min, 1.6 µg/kg/min, 1.7 µg/kg/min, 1.8 µg/kg/min,
15 1.9 µg/kg/min, 2.0 µg/kg/min, 2.1 µg/kg/min, 2.2 µg/kg/min, 2.3 µg/kg/min, 2.4 µg/kg/min, 2.5 µg/kg/min, 2.6, 2.7 µg/kg/min, 2.8 µg/kg/min, 2.9 µg/kg/min, or 3.0 µg/kg/min. In a preferred embodiment, the dose of CVie 216 administered to the individual is in the range from about 0.1 µg/kg/min to about 2.5 µg/kg/min or about 0.5 µg/kg/min to about 3.0 µg/kg/min. In a more preferred embodiment, the dose of CVie 216 administered to the individual is in the range
20 from about 1.0 µg/kg/min to about 2.0 µg/kg/min, or about 1.0 µg/kg/min to about 1.5 µg/kg/min, or about 0.5 µg/kg/min, or about 1.5 µg/kg/min.

In other embodiments, the methods disclosed herein include administering to the individual a therapeutically effective amount of a dual mechanism compound having a structure according to (i) Formula II, including, but not limited to 3-beta-hydroxy-5-beta-10-beta-methyl-
25 13-beta-methyl-14-beta-hydroxy-17-beta-(imidazol-4-yl)androstane; 3-beta-hydroxy-5-beta-10-beta-methyl-13-beta-methyl-14-beta-hydroxy-17-beta-(2-guanidino-thiazol-4-yl)androstane; 3-beta-hydroxy-5-beta-10-beta-methyl-13-beta-methyl-14-beta-hydroxy-17-beta-(pyrazol-3-yl)androstane; 3-beta-hydroxy-5-beta-10-beta-methyl-12-beta-hydroxy-13-beta-methyl-14-beta-hydroxy-17-beta-(imidazol-4-yl)androstane; 3-beta-hydroxy-5-beta-10-beta-methyl-12-beta-
30 hydroxy-13-beta-methyl-14-beta-hydroxy-17-beta-(2-guanidino-thiazol-4-yl)androstane; 3-beta-

hydroxy-5-beta-10-beta-methyl-13-beta-methyl-14-beta-hydroxy-17-beta-(N-(3-aminopropyl)-imidazol-4-yl)androstane; 3-beta-hydroxy-5-beta-10-beta-methyl-13-beta-methyl-14-beta-hydroxy-17-beta-((5-(3-aminopropyl)-isoxazol-3-yl))androstane; 3-beta-hydroxy-5-beta-10-beta-methyl-13-beta-methyl-14-beta-hydroxy-17-beta-((5-(3-aminopropyl)-isoxazol-3-yl)-ethyl)androstane; 3-beta-hydroxy-5-beta-10-beta-methyl-13-beta-methyl-14-beta-hydroxy-17-beta-(5-(2-aminoethyl)-isoxazol-3-yl)-ethyl)androstane; or 3-beta-hydroxy-5-beta-10-beta-methyl-13-beta-methyl-14-beta-hydroxy-17-beta-(5-(2-aminomethyl)-isoxazol-3-yl)-ethenyl)androstane; or (ii) or pure SERCA2a activator having a structure according to Formula IV, including, but not limited to (E)-4-(6alpha-hydroxy-17-oxoandrostane-3-yliden)butyric acid; 10 (Z)-4-(6alpha-hydroxy-17-oxoandrostane-3-yliden)butyric acid; (E)-4-(6beta-hydroxy-17-oxoandrostane-3-yliden)butyric acid; (Z)-4-(6beta-hydroxy-17-oxoandrostane-3-yliden)butyric acid; (E)-3-[2-(azetidin-3-yl)ethyliden]-6alpha-hydroxyandrostane-17-one; (Z)-3-[2-(azetidin-3-yl)ethyliden]-6alpha-hydroxyandrostane-17-one; (E)-3-(4-aminobutyl)-6alpha-hydroxyandrost-2-ene-17-one hydroiodide; 3-[2-(piperidin-4-yl)ethyl]-6alpha-hydroxyandrost-2-ene-17-one 15 hydroiodide; (EZ)-3-(4-aminobutyliden)-6alpha-hydroxyandrostane-17-one; (E)-3-[2-(piperidin-4-yl)ethyliden]-6alpha-hydroxyandrostane-17-one; (Z)-3-[2-(piperidin-4-yl)ethyliden]-6alpha-hydroxyandrostane-17-one; 3 beta-[2-(piperidin-4-yl)ethyl]-6alpha-hydroxyandrostane-17-one; Ethyl (6alpha-hydroxy-17-ketoandrostane-3beta-yl) acetate; 4-(6alpha-hydroxy-17-oxoandrostane-3-yl) butyric acid; 4-(6beta-hydroxy-17-oxoandrostane-3-yl) butyric acid; 2- 20 (6beta-hydroxy-17-oxoandrostane-3-yl) acetic acid; Ethyl 4-(6alpha-hydroxy-17-oxoandrostane-3-yl) butyrate; Ethyl 4-(6alpha-hydroxy-17-oxoandrostane-3-yl) caproate; 6-(6beta-hydroxy-17-oxoandrostane-3-yl) caproic acid; (E,Z)-3-(5-N-methylaminopentyliden)-6alpha-hydroxymethylandrostane-7,17-dione; (E,Z)-3-[2-(pyrrolidine-3yl)ethyliden]-6alpha-hydroxymethylandrostane-7,17-dione; (E,Z)-3-[2-(azetidine-3-yl)ethyliden]-6alpha-hydroxymethylandrostane-7,17-dione; (E,Z)-3-[2-(piperidin-4-yl)ethyliden]-6alpha-hydroxymethylandrostane-7,17-dione; (E,Z)-3-(5-N-methylaminopentyliden)-6alpha-hydroxymethyl-7alpha-hydroxyandrostane-17-one; 3 beta-[2-(azetidine-3-yl)ethyl]-6alpha-hydroxymethylandrostane-7,17-dione; 3 beta-[2-(azetidine-3-yl)ethyl]-6alpha-hydroxymethyl-7alpha-hydroxyandrostane-17-one; 3 beta-[2-(pyrrolidine-3yl)ethyl]-6alpha-hydroxymethylandrostane-7,17-dione; 3 beta-[2-(pyrrolidine-3yl)ethyl]6alpha-hydroxymethyl-7alpha-hydroxyandrostane-17-one; 3 beta-[2-(piperidin-4-yl)ethyl]-6alpha-

hydroxymethylandrostande-7,17-dione; or 3 beta-[2-(piperidin-4-yl)ethyl]-6alpha-hydroxymethyl-7alpha-hydroxyandrostande-17-one. In a preferred embodiment, the dual mechanism compound or pure SERCA2a activator is in a pharmaceutical composition, such as any of the combinations discussed above. The compound is administered in a therapeutically effective dose. For instance, the compound can be administered to the individual via intravenous infusion with a dose in the range from about 0.1 µg/kg/min to about 3.0 µg/kg/min, *e.g.*, 0.1 µg/kg/min, 0.15 µg/kg/min, 0.2 µg/kg/min, 0.25 µg/kg/min, 0.3 µg/kg/min, 0.35 µg/kg/min, 0.4 µg/kg/min, 0.45 µg/kg/min, 0.5 µg/kg/min, 0.55 µg/kg/min, 0.6 µg/kg/min, 0.65 µg/kg/min, 0.7 µg/kg/min, 0.75 µg/kg/min, 0.8 µg/kg/min, 0.85 µg/kg/min, 0.9 µg/kg/min, 0.95 µg/kg/min, 1.0 µg/kg/min, 1.1 µg/kg/min, 1.2 µg/kg/min, 1.3 µg/kg/min, 1.4 µg/kg/min, 1.5 µg/kg/min, 1.6 µg/kg/min, 1.7 µg/kg/min, 1.8 µg/kg/min, 1.9 µg/kg/min, 2.0 µg/kg/min, 2.1 µg/kg/min, 2.2 µg/kg/min, 2.3 µg/kg/min, 2.4 µg/kg/min, 2.5 µg/kg/min, 2.6, 2.7 µg/kg/min, 2.8 µg/kg/min, 2.9 µg/kg/min, or 3.0 µg/kg/min. For instance, the compound can be administered to the individual at a dose from about 0.1 µg/kg/min to about 2.5 µg/kg/min or about 0.5 µg/kg/min to about 3.0 µg/kg/min or about 1.0 µg/kg/min to about 2.0 µg/kg/min.

The time period for the infusion may be at least about 30 minutes to about 48 hours, or more, *e.g.*, 30 m, 45 m, 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, 7 h, 8 h, 9 h, 10 h, 11 h, 12 h, 13 h, 14 h, 15 h, 16 h, 17 h, 18 h, 19 h, 20 h, 21 h, 22 h, 23 h, 24 h, 25 h, 26 h, 27 h, 28 h, 29 h, 30 h, 31 h, 32 h, 33 h, 34 h, 35 h, 36 h, 37 h, 38 h, 39 h, 40 h, 41 h, 42 h, 43 h, 44 h, 45 h, 46 h, 47 h, or 48 h.

Preferably, the infusion is carried out from about 3 hours to about 24 hours, or from about 6 hours to about 24 hours, or from about 12 hours to about 24 hours. In one particular embodiment, the individual is administered istaroxime or CVie 216 by intravenous infusion for about 24 hours. In other embodiments, the time period for infusion may be greater than 24 hours, *e.g.*, 25 h, 26 h, 27 h, 28 h, 29 h, 30 h, 31 h, 32 h, 33 h, 34 h, 35 h, 36 h, 37 h, 38 h, 39 h, 40 h, 41 h, 42 h, 43 h, 44 h, 45 h, 46 h, 47 h, 48 h, 54 h, 60 h, 66 h, 72 h, 78 h, 84 h, 90 h, 96 h, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, or longer. For instance, istaroxime or CVie 216 infusion administered by a pump can be carried out for much longer duration.

Once the individual is administered the istaroxime, CVie 216, or one of the other compounds described herein, the method can include one or more measuring steps carried out

periodically during the treatment and/or for a period of time post-treatment. The measuring steps may include measuring one or more parameters of heart function as described above prior to beginning of infusion, at the initiation of infusion administration, and/or at one or more time points during and after infusion, *e.g.*, 15 m, 30 m, 45 m, 1 h, 1.25 h, 1.5 h, 1.75 h, 2 h, 3 h, 4 h, 5 h, 6 h, 7 h, 8 h, 9 h, 10 h, 11 h, 12 h, 13 h, 14 h, 15 h, 16 h, 17 h, 18 h, 19 h, 20 h, 21 h, 22 h, 23 h, 24 h, 48 h, 72 h, 96 h, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 14 days, 21 days, 28 days, 30 days, or more following the start of infusion. For instance, for an individual administered istaroxime or CVie 216 by intravenous infusion for up to 24 hours, one or more parameters of heart function can be measured before, during, and 12 h, 24 h, 48 h, 72 h, 96 h, and 30 days following initiation of infusion. As one having ordinary skill in the art would appreciate, the one or more parameters of heart function indicate the efficacy of istaroxime treatment. Improvements in heart function include, but are not limited to, a decrease in E/e' ratio of at least -2, *e.g.*, -2, -2.2, -2.4, -2.6, -2.8, -3 over a 6 to 24 hour period after administration of 0.5 µg/kg/min or 1 µg/kg/min istaroxime (or a derivative thereof). As one having ordinary skill in the art would appreciate, the measurements indicative heart function improvement in the individual following initiation of infusion may depend on the particular diagnosis, *e.g.*, AHF with acute myocardial ischemia versus AHF-related cardiogenic shock. Other indicators of heart function improvement, include, but not limited to, increased CI, decreased left ventricular dimensions (*e.g.*, decreased left ventricular end-systolic volume and/or decreased left ventricular end-diastolic volume), and/or decreased left atrial dimensions (*e.g.*, decreased left atrial area). In other embodiments, a decrease in the level of NT-proBNP or an increase in the level of eGFR indicates treatment of pre-cardiogenic shock or prevention of cardiogenic shock.

Moreover, the absence of serious myocardial arrhythmias or the shortening in the duration of serious myocardial arrhythmias indicates a reduction in the risk of or prevention of acute myocardial arrhythmias, including, but not limited to, AF, PSVT, VT, or VF. For instance, in one embodiment, no acute myocardial arrhythmias are detected in an individual with AHF and acute myocardial ischemia after administration by infusion of istaroxime or any one of Formulas II, (*e.g.*, CVie101), III (*e.g.*, PST3093) or IV (CVie216) for at least about 3 hours after initiation of infusion; preferably, no acute myocardial arrhythmias are detected for at least about 6 hours following initiation of infusion; more preferably, no acute myocardial arrhythmias are detected for at least about 12 hours or at least about 24 hours following initiation of infusion. In other

embodiments, the incidence of myocardial arrhythmias are decreased in an individual with AHF and acute myocardial ischemia who is administered istaroxime or Formulas II, III or IV as compared to an individual or the average from among a cohort of individuals with AHF and acute myocardial ischemia who are not administered istaroxime or Formulas II, III or IV. In yet other embodiments, the incidence of myocardial arrhythmias are decreased among a cohort of individuals with AHF and acute myocardial ischemia who are administered istaroxime or Formulas II, III or IV as compared the average number of myocardial arrhythmias occurring in a cohort of individuals with AHF and acute myocardial ischemia and who are not administered istaroxime or Formulas II, III or IV. Preferably, the absence of acute myocardial arrhythmias or the reduction of incidence of acute myocardial arrhythmias following initiation of infusion of istaroxime or Formulas II, III or IV to the individual is maintained even after perfusion of the heart.

In other embodiments, the duration of the acute myocardial arrhythmia is reduced in an individual with AHF and acute myocardial ischemia who is administered istaroxime or Formulas II, III or IV as compared to the average duration of acute myocardial arrhythmias in a cohort of individuals with AHF and acute myocardial ischemia who are not administered istaroxime or Formulas II, III or IV. In some examples, the average duration of the acute myocardial arrhythmias are reduced in a cohort of individuals with AHF and acute myocardial ischemia who are administered istaroxime or Formulas II, III or IV as compared to the average duration of acute myocardial arrhythmias in a cohort of individuals with AHF and acute myocardial ischemia who are not administered istaroxime or Formulas II, III or IV.

In alternative embodiments, in evaluating the efficacy of a treatment, a treatment regimen or a particular dosage, or to determine if a treatment versus a maintenance dosage should be given, individuals, *e.g.*, patients affected by AHF, are subject to regular periodic screening for the presence and extent of organ and tissue involvement or damage, *e.g.*, heart (ventricle dilatation, third heart sound cardiac hypertrophy), fatigue, tiredness, reduced exercise tolerance, increased time to recover after exercise, kidney (renal insufficiency, oliguria), lung (orthopnea, paroxysmal nocturnal dyspnea, tachypnea), ankle swelling, elevated jugular venous pressure. A thorough physical examination should be done at a time interval chosen by those experts in the treatment of a cardiovascular disease, in particular, ADHF with pre-cardiogenic shock or cardiogenic shock which would concentrate on cardiac, pulmonary and peripheral circulation

functions. Accordingly, in alternative embodiments, therapy with istaroxime or Formulas II, III or IV or an equivalent of a pharmaceutically acceptable salt, solvate or hydrate thereof as disclosed herein, is instituted as early as possible after developing symptoms of AHF and acute myocardial ischemia to prevent worsening of symptoms the development of acute myocardial arrhythmias, and further continued after patient's discharge for years, preferably during the whole life of the patient or at least a period consistent with the way other drugs are used in heart failure.

According to the present invention, uses and methods as provided herein can further comprise co-administration with other drugs or pharmaceuticals. In fact, the present invention selectively corrects a depressed cardiac biochemical function (namely the SERCA2a activity).

This certainly contributes to relieving the existing AHF clinical symptoms, with less unwanted side effects than those of the available therapies (just because the selectivity mentioned above). However, as AHF, precardiogenic shock, cardiogenic shock, acute myocardial ischemia are complex clinical syndromes the present invention is potentially associable to existing and future drug classes and/or specific drugs such as: a) drug classes such as, ACE inhibitors, angiotensin receptor blockers (ARBs), diuretics, Ca channel blockers, beta blockers, digitalis, NO donors, vasodilators, SERCA2a stimulators, neprilysin (NEP) inhibitors, myosin filament activators, recombinant relaxin-2 mediators, recombinant NP protein, activators of the soluble Guanylate Cyclase (sGC), beta-arrestin ligand of Angiotensin II receptor; b) specific drugs: hydrochlorothiazide, furosemide, verapamil, diltiazem, carvedilol, metoprolol, hydralazine, eplerenone, spironolactone, lisinopril, ramipril, nitroglycerin, nitrates, digoxin, valsartan, olmesartan, telmisartan, candesartan, losartan, entresto, omecamtiv, sacubitril, serelaxin, ularitide, levosimendan, cinaciguat.

Istaroxime or Formulas II, III or IV (or any of the other compounds disclosed above) as disclosed in the present invention, as used a therapeutic agent for treating AHF with acute myocardial ischemia, can be combined or co-administered with other therapeutic agents used in the treatment of the same disease and/or the underlying HF. Exemplary other therapeutic agents are diuretics, for example furosemide, bumetanide, and torasemide. Metolazone, an aldosterone antagonist, such as spironolactone or eplerenone; thiazide diuretics, such as hydrochlorothiazide, metolazone, and chlorthalidone. Other agents are ACE inhibitors, for example lisinopril and Ramipril. Also ARBs, such as valsartan, candesartan and losartan can be taken into consideration. Angiotensin receptor/neprilysin inhibitor (ARNI), sacubitril for example, are

comprised. Other agents can be selected from Beta-blockers, such as carvedilol and metoprolol for example, or Vasodilators, for example Hydralazine, optionally combined with isosorbide dinitrate, Nitrates, as nitroglycerin, amlodipine and felodipine; non-dihydropyridines such as diltiazem or verapamil. Still other exemplary other therapeutic agents may include
5 phenylephrine, norepinephrine, epinephrine, dopamine, milrinone, dobutamine, and levosimendan. The compounds of the present invention can also be combined with digoxin, if needed. Other drugs, as Ivabradine and other Anticoagulant may be considered.

The compounds of the present invention can be combined with other therapeutic agents, in particular agents useful for treating cardiovascular diseases, more in particular in the
10 combination therapy of AHF with other conditions, such as cardiogenic shock or acute myocardial ischemia. The combined active ingredients can be administered according to different protocols, decided by the medical doctor. According to an embodiment of the present invention, combination therapy can be carried out by administering istaroxime both at the same time or at different time of the further therapeutically active ingredient or ingredients. In case of
15 concomitant administration, the compound of the present invention and the further active ingredient or ingredients can be each formulated in a respective pharmaceutical composition or in the same unitary dosage form. In the former case, the present invention provides a kit, in particular for the treatment of AHF, comprising separate pharmaceutical compositions containing the compound of the present invention and the further active ingredient or ingredients,
20 respectively. In another embodiment, the present invention provides a pharmaceutical unit dosage form kit, in particular for the treatment of AHF, comprising compound of the present invention and the further active ingredient or ingredients in the same unit dosage form. Combination therapy according to the present invention provides advantageous treatment of AHF due to the inotropic-lusitropic effect of istaroxime herein disclosed in addition to or
25 synergically combined with the well-known therapeutic effect of the additional active agents herein disclosed.

Also provided are nanoparticles, nanolipoparticles, vesicles and liposomal membranes comprising compounds used to practice the uses and methods as provided herein, *e.g.*, to deliver pharmaceutically active compounds and compositions as provided herein: istaroxime or Cvie
30 216 or an equivalent of a pharmaceutically acceptable salt, solvate or hydrate thereof, optionally combined with a further therapeutically active agent as disclosed above to a subject in need

thereof. In alternative embodiments, these compositions are designed to target specific molecules, including biologic molecules, such as polypeptides, including cell surface polypeptides, *e.g.*, for targeting a desired cell type, *e.g.*, a myocyte or heart cell, an endothelial cell, and the like. A slow release of istaroxime or derivative thereof may provide a sufficient compound to selectively increase the plasma levels of the metabolite leaving the plasma levels of Istaroxime within very low ranges.

Provided are multilayered liposomes comprising compounds used to practice methods as provided herein, *e.g.*, as described in U.S. application No. 20070082042. The multilayered liposomes can be prepared using a mixture of oil-phase components comprising squalane, sterols, ceramides, neutral lipids or oils, fatty acids and lecithins, to about 200 to 5000 nm in particle size, to entrap a composition used to practice uses and methods as provided herein.

Liposomes can be made using any method, *e.g.*, as described in U.S. Patent No. 4,534,899; or U.S. application No. 20070042031, including method of producing a liposome by encapsulating an active agent according to the present invention (or a combination of active agents), the method comprising providing an aqueous solution in a first reservoir; providing an organic lipid solution in a second reservoir, and then mixing the aqueous solution with the organic lipid solution in a first mixing region to produce a liposome solution, where the organic lipid solution mixes with the aqueous solution to substantially instantaneously produce a liposome encapsulating the active agent; and immediately then mixing the liposome solution with a buffer solution to produce a diluted liposome solution.

In one embodiment, liposome compositions used to practice uses and methods as provided herein comprise a substituted ammonium and/or polyanions, *e.g.*, for targeting delivery of istaroxime or an equivalent of a pharmaceutically acceptable salt, solvate or hydrate thereof used to practice methods as provided herein to a desired cell type, as described, *e.g.*, in U.S. application No. 20070110798.

Provided are nanoparticles comprising compounds according to the present invention used to practice uses and methods as provided herein in the form of active agent-containing nanoparticles (*e.g.*, a secondary nanoparticle), as described, *e.g.*, in U.S. application No. 20070077286. In one embodiment, provided are nanoparticles comprising a fat-soluble active agent used to practice a use and method as provided herein or a fat-solubilized water-soluble active agent to act with a bivalent or trivalent metal salt.

In one embodiment, solid lipid suspensions can be used to formulate and to deliver compositions used to practice uses and methods as provided herein to mammalian cells *in vivo*, *in vitro* or *ex vivo*, as described, *e.g.*, in U.S. application No. 20050136121.

5 The compositions and formulations used to practice the uses and methods as provided herein can be delivered by the use of liposomes or nanoliposomes. By using liposomes, particularly where the liposome surface carries ligands specific for target cells, or are otherwise preferentially directed to a specific organ, one can focus the delivery of the active agent into target cells *in vivo* [*see, e.g.*, U.S. Patents Nos. 6,063,400; 6,007,839; Al-Muhammed, 1996, Microencapsul. 13:293-306; Chonn, 1995, Curr. Opin. Biotechnol. 6:698-708; Ostro, 1989, Am. 10 J. Hosp. Pharm. 46:1576-1587.] A liposome formulation of istaroxime as disclosed in Eur J Pharm Biopharm. 2011;79(2):285-93 is also provided in the present invention.

Delivery vehicles

15 In alternative embodiments, any delivery vehicle can be used to practice the uses and methods as provided herein, *e.g.*, to deliver the compounds provided herein to a subject in need thereof. For example, delivery vehicles comprising polycations, cationic polymers and/or cationic peptides, such as polyethyleneimine derivatives, can be used *e.g.* as described, *e.g.*, in U.S. application No. 20060083737.

20 In one embodiment, a dried polypeptide-surfactant complex is used to formulate a composition used to practice a use and method as provided herein, *e.g.*, as described in U.S. application No. 20040151766.

In one embodiment, a composition used to practice uses and methods as provided herein can be applied to cells using vehicles with cell membrane-permeant peptide conjugates, *e.g.*, as described in U.S. Patents No. 7,306,783; 6,589,503. In one aspect, the composition to be 25 delivered is conjugated to a cell membrane-permeant peptide. In one embodiment, the composition to be delivered and/or the delivery vehicle are conjugated to a transport-mediating

peptide, *e.g.*, as described in U.S. Patent No. 5,846,743, describing transport-mediating peptides that are highly basic and bind to poly-phosphoinositides.

In one embodiment, electro-permeabilization is used as a primary or adjunctive means to deliver the composition to a cell, *e.g.*, using any electroporation system as described *e.g.* in U.S.

5 Patents No. 7,109,034; 6,261,815; 5,874,268.

The following examples further illustrate the present invention.

Example 1: Evaluation of the effect of istaroxime and CVie 216 on reperfusion arrhythmias in isolated, Langendorff perfused rat hearts

10 Ventricular arrhythmias (VA) are serious complications of heart failure (HF). In ischemia-reperfusion (IR) cytosolic Ca²⁺ overload occurs very soon after the start of reperfusion, and excess oscillations of Ca²⁺ can contribute to reperfusion VA. Recently, we identified a new class of selective SERCA2a activators derived from the Ista metabolite PST3093 devoid of Na⁺/K⁺ ATPase inhibitory activity. We further characterized cardiac
15 performance after infusion of SERCA2a activators in a diabetic rat model with impaired SERCA2a function.

The present study assessed if SERCA2a activation could reduce IR arrhythmias both in vitro and in vivo in normal and diabetic rats. In order to evaluate the ability of istaroxime or derivatives thereof (*e.g.*, Formulas II and III) and the pure SERCA2a activator of Formula IV,
20 CVie 216, to reduce the risk of myocardial arrhythmias in ischemic hearts, isolated rat hearts from a streptozotocin (STZ) diabetic rat model were ligated to induce ischemia and then reperused to simulate a myocardial arrhythmia. The Sprague-Dawley diabetic rat model is known in the art to be especially suitable for simulating myocardial arrhythmias and was used in this study [see, for example, Hu *et al.*, 2017, *Cardiovasc. Diabetol.* 16:57].

25 Briefly, male Sprague-Dawley rats (BioLASCO Co., Taipei, Taiwan) weighing 150–170 g were given a single tail vein injection of STZ (50 mg/kg). This dose was known in the art to induce a mild form of diabetes at 8 weeks, characterized by subclinical diastolic dysfunction. STZ was dissolved in 0.9% saline containing 0.1 M sodium citrate, and the pH was adjusted to 4.5. Five to six days after STZ injection, rats that had at least a 3-fold increase in fasting plasma
30 blood glucose levels compared to pre-injection levels were classified as diabetic. Blood glucose level were determined using a digital glucometer and test strips (Ascensia ELITE®, Bayer

HealthCare AG, Leverkusen, Germany). Rats injected with comparable volumes of citrate-buffered saline served as nondiabetic controls.

Rats were then anesthetized by intraperitoneal injection of pentobarbital sodium (60 mg/kg body weight). Hearts were rapidly excised, connected immediately to an aortic cannula, and subjected to retrograde perfusion at a constant pressure (70–80 mmHg) in the Langendorff apparatus with K–H buffer (118.5 mM, NaCl; 4.7 mM, KCl; 2.5 mM, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$; 1.2 mM, MgSO_4 ; 11 mM, glucose; and 25 mM NaHCO_3). The buffer solution was saturated with a mixture of 95% O_2 /5% CO_2 at 37 °C. To measure the left ventricular pressure (LVP), a small balloon tip catheter was inserted into the left ventricle through the left auricular appendage. The isolated heart was placed in a water jacket and maintained at 37°C at all times. The balloon was inflated until the end diastolic pressure reached 6 to 10 mmHg. Special care was taken to maintain the diastolic pressure at 10 mmHg to avoid stretch-induced preconditioning. The epicardial ECG was continuously recorded from electrodes placed on the aorta and the ventricular apex. Electrical signals were amplified by bioelectric amplifiers (AB-601G, Nihon Kohden) with a time constant of 0.1 s, and recorded after digitization through a recording system (NI cDAQ-9174, National Instruments, Austin, TX, USA) at a sampling frequency of 1 kHz.

Istaroxime or CVie 216 was prepared in Krebs-Henseleit buffer. Then, isolated hearts were administered istaroxime, CVie 216, or saline using a perfusion pump (Model 5003, Precidior Infors, Basel, Switzerland). The infusion rate was set to one-hundredth of coronary flow to achieve concentrations of 10 μM or 50 μM of either istaroxime or CVie 216.

To simulate the ischemia/reperfusion environment, a 4/0 braided silk suture was passed around the left main coronary artery of the isolated rat heart, and a snare was formed by passing both ends through a piece of polyethylene tubing. Regional ischemia and reperfusion were produced by tightening and loosening the snare as described previously. Occlusion and reperfusion were confirmed by ECG changes, instantaneous changes in perfusion pressure, and the development and relief of cyanosis of the occluded zone. Coronary flow was reduced during occlusions so as to maintain the perfusion pressure at the preocclusion level, and likewise flow was increased again to upon reperfusion.

ECG readings were recorded as baseline, and istaroxime or CVie 216 was administered 5 minutes prior to ischemia. The isolated rat hearts were kept in an ischemic state for 10 minutes,

and then reperfused. ECG readings were taken during the 5 minute period following reperfusion to measure heart arrhythmias.

The mode of action of istaroxime and its derivative, CVie216, is shown in Figures 1A and 1B. As note above CVie216 behaves as a selective SERCA2a activator showing a longer
5 half-life than istaroxime and enhanced in vitro SERCA2a activity in healthy guinea pig preparations and in diseased (STZ) rat preparations. . In preparations from the control rats treated with vehicle, triggered action potentials seldom occurred, whereas triggered activity was largely increased in the vehicle-treated diabetic preparations. CVie216 and Ista administration in diabetic preparations significantly reduced the incidence of triggered activity at 2 Hz. See Figure
10 2. The action potential duration (APD) at 30% and 50% of repolarization (APD30, APD50) were prolonged significantly in the DM group (* $p < 0.05$ vs Ctr), and this phenomenon was prevented by CVie216 and Ista. # $p < 0.05$ vs DM. Representative tracings of action potential driven at 2, 3.3, and 5 Hz in preparations isolated from various group hearts exposed to high calcium (5.4 mmol/L) + isoproterenol (1 μ M). Triggered activity (spontaneous action potentials;
15 red double arrow line) could be induced in a preparation from DM+Veh group heart following 1-minute stimulation at all frequencies. Results are shown as the percentage of preparations (n) inducible into triggered activity/total of preparations.

For proarrhythmia assessment, the number and morphology of ventricular arrhythmias were recorded during the time of ischemia and reperfusion. Arrhythmias measured were
20 ventricular premature contractions (VPCs), couplets, triplets, non-sustained ventricular tachycardia (NSVT, defined as 4-6 premature beats), sustained ventricular tachycardia (VT, defined as >6 premature beats) or ventricular fibrillation (VF). VF was scored as a 6; VT was scored as a 5; NSVT was scored as a 4; triplets or couplets were scored as 3; less than 50 VPCs were assigned a 2; 5 to 50 VPCs were assigned a 1; and less than 5 VPCs or no arrhythmias were
25 assigned 0. The arrhythmia scores in the presence of 10 and 50 μ M CVie216 were averaged among the isolated rat hearts in each cohort, and the results are shown in Figures 3 and 4.

The data reveals that the incidence of VF was significantly reduced in isolated hearts administered istaroxime or CVie 216 at 50 μ M. Moreover, CVie 216 administration had a profound effect on reducing the incidence of arrhythmias, even at the lower concentration. In
30 fact, the incidence of VF was reduced to 8% in isolated hearts administered 10 μ M CVie 216.

Example 2: Evaluation of the effect of CVie 216 on reperfusion arrhythmias in an *in vivo* model of diabetic rats.

In order to evaluate the ability of CVie 216 to reduce the risk of myocardial arrhythmias in hearts of intact rats, STZ diabetic rats (*see* Example #1) were placed in an ischemic-reperfusion simulation and administered either saline or CVie 216 at 10 μ M or 50 μ M.

Rats were lightly anesthetized with sodium pentobarbital (75 mg/kg, intraperitoneal) prior to surgery. Once the rat was anesthetized, it was intubated and ventilated with room air (60-80 strokes/min, 10ml/kg) using small animal ventilator (model 683; Harvard, USA). The jugular vein and carotid artery were cannulated for drug administration and arterial blood pressure monitoring, respectively. The chest was opened by a left thoracotomy, followed by sectioning the fourth and fifth ribs, about 2 mm to the left of the sternum and the epicardium will be opened. Myocardial ischemia was induced by briefly exteriorizing the heart and placing a 4-0 silk slip knot around the left coronary artery, approximately 2-3 mm from its origin, effectively occluding the vessel. Successful occlusion was confirmed by the appearance of epicardium cyanosis and ST-segment elevation. Ischemia was maintained for 10 min, at which time the slip knot was released, initiating reperfusion. CVie216 (0.03-0.14 mg/kg per min) or the equivalent volume of vehicle solution was initiated by intravenous infusion 10 min before occlusion surgery and continued during the duration of the experiment. After 2 hr of reperfusion, rats were anesthetized with additional sodium pentobarbital (35 mg/kg, i.p.), and their hearts were excised and placed in ice-cold 0.9% NaCl for the subsequent experiments. The study design is summarized in FIG. 2.

For proarrhythmia assessment the number and morphology of ventricular arrhythmias were recorded during the time of ischemia and reperfusion. Arrhythmias measured were VPCs, couplets, triplets, NSVT, VT, or VF. As with Example #1. VF was scored as a 6; VT was scored as a 5; NSVT was scored as a 4; triplets or couplets were scored as 3; less than 50 VPCs were assigned a 2; 5 to 50 VPCs were assigned a 1; and less than 5 VPCs or no arrhythmias were assigned 0.

The results showed that administration of 50 μ M CVie 216 to intact diabetic rats significantly decreased the VPC duration and the arrhythmia score as compared to controls. The results are summarized in Table 3. Accordingly, these results demonstrate that the compounds provided herein surprisingly reduce the incidence of myocardial arrhythmias in ischemic hearts.

Table 3. Arrhythmia Measurements in DM Rat Hearts.

Event	DM Saline (n=10)	DM 10 μ M CVie 216 (n=5)	DM 50 μ M CVie 216 (n=11)
VF Duration	11.4 $\pm$ 29.87%	25.20 $\pm$ 41.49%	13.19 $\pm$ 29.31%
VPC Duration	28.75 $\pm$ 40.43%	5.80 $\pm$ 9.18%	4.18 $\pm$ 4.71%*
Arrhythmia Score	5.5 $\pm$ 0.85	5.6 $\pm$ 0.89	3.73 $\pm$ 2.51%*
Mortality	30%	40%	9%
MAP change	-44.61%	-21.57%	-37.26%

DM, diabetes mellitus rats; VF, ventricular fibrillation; VPC, ventricular premature contractions; MAP, mean arterial pressure; *, $P < 0.05$.

Conclusion

Our data show that the APD at 30% and 50% of repolarization (APD₃₀, APD₅₀) were significantly prolonged in the DM group ($p < 0.05$), and these phenomena were prevented by CVie216 (10 μ M) and Ista (10 μ M).

In preparations from the control rats treated with vehicle, triggered action potentials seldom occurred, whereas triggered activity was largely increased in the vehicle-treated diabetic preparations. CVie216 and Ista administration in diabetic preparations significantly reduced the incidence of triggered activity at 2 Hz.

In Langendorff-perfused control and diabetic rat hearts, the incidence of ventricular fibrillation (VF), ventricular tachycardia (VT), and arrhythmia scores were measured during early reperfusion. Administration of Ista (50 μ M) and CVie216 (50 μ M) significantly decreased both VF incidence and arrhythmia score in diabetic rat hearts ($P < 0.05$) but not in controls.

In Langendorff-perfused diabetic rat hearts, the administration of Ista (10 μ M) and CVie216 (10 μ M) showed that 10 μ M CVie216 significantly decreased both VF/VT incidence and arrhythmia score ($P < 0.05$). Finally, *in vivo* studies revealed a significant decrease in duration of premature ventricular contractions (PVC) and arrhythmia score of IR arrhythmias following CVie216 treatment.

Example 3: Safety and Efficacy of up to 60 Hours of Intravenous Istaroxime derivative CVie216 for Patients with Pre-Cardiogenic Shock.

Cardiogenic shock (CS) continues to be one of the most common fatal diseases, leading to about 50% mortality after its diagnosis. Even patients with milder forms of cardiogenic shock at presentation often rapidly deteriorate to more severe forms³. Current vasopressors and inotropes, acting through adrenergic receptor stimulation, are associated with tachycardia, arrhythmias, and, in many studies, poorer outcomes. Any therapy that would simultaneously improve systolic blood pressure (SBP) and cardiac output (CO) while unloading the LV as assessed by reduction of pulmonary capillary wedge pressure (PCWP), without causing vasodilation, arrhythmias or increased heart rate is highly desirable. Most medications that primarily increase BP (such as norepinephrine) increase arrhythmias and heart rate, while those which primarily increase CO (such as milrinone, dobutamine and levosimendan) cause arrhythmias and reduce vascular resistance leading in some cases to hypotension. Where patients require combined BP and CO increases and PCWP decrease, investigators need to resort to mechanical circulatory support (MCS) devices, most commonly Impella. This treatment was recently shown to reduce mortality in patients with CS but is available in a few specialized centers. The treatment is invasive, expensive, labor intensive and may lead to mechanical and vascular complications, along with bleeding and significant renal impairment due to hemolysis. Therefore, identification of a safe and effective inotrope that can be administered using a dosing schedule that can be adapted to any patient with CS while providing simultaneous improvements in CO, BP and reduction of PCWP without increasing heart rate or arrhythmias remains elusive and is one of the “holy grails” in cardiovascular research.

To assess the safety and efficacy of CVie216 in treating patients with pre-cardiogenic shock, patients with AHF and exhibiting pre-cardiogenic shock (pre-CS) without acute myocardial infarction can be randomized to CVie216 at the appropriate dose or placebo for 24 hours. Pre-CS was defined as defined as systolic blood pressure (SBP) <90 mmHg without hypoperfusion, venous lactate >2 mmol/L and/or mechanical or inotropic support. The inclusion criteria included AHF-related SCAI stage B pre-CS, 18-85 years of age, an ongoing hospitalization for AHF, left ventricular ejection fraction <40%, persistent hypotension (SBP between 75 and 90 mmHg), heart rate of 75-150 bpm and no need at time of screening or planned use for 6 h thereafter of mechanical support or intravenous therapy to increase BR

According to the stage B SCAI classification, patients with clinical signs of peripheral hypoperfusion, venous lactate >2 mmol/L and/or on mechanical support or treatment with intravenous vasodilators, inotropes or vasopressors were excluded. Other exclusion criteria were concomitant or planned treatment with oral digoxin (could be randomized if the plasma concentration of digoxin at screening was <0.5 ng/ml); acute coronary syndrome or stroke within the past 3 months; coronary artery bypass graft or percutaneous coronary intervention within the past month or planned in the next month; life-threatening ventricular arrhythmia or implantable cardioverter defibrillator shock within the past month; sustained ventricular tachycardia in the last 3 months or uncontrolled arrhythmia; fever $>38^{\circ}\text{C}$; estimated glomerular filtration rate (eGFR) <30 ml/min/m²; serum potassium >5.3 or <3.5 mmol/L; stroke or transient ischaemic accident within the past 3 months; and acute respiratory distress syndrome.

The patients can be randomized centrally, using an interactive response technology, to receive CVie216 or placebo at a ratio of 1:1. Study medication can be supplied in uniquely-numbered kits containing identical vials of lyophilized powder (CVie216 plus lactose), reconstituted by adding 5 ml saline to the vial. CVie216 can be administered as a continuous infusion 1.0 pg/kg/min for 24 h. The infusion rate could be decreased at the discretion of the investigator based on the development of tolerability issues (such as nausea), significant bradycardia, or greater than desired BP elevation.

The primary efficacy endpoint reflects the area under the curve representing the change in SBP from baseline, start of study drug infusion, through 6 h (SBP AUC). The secondary endpoints also include SBP AUC through 24 h; changes from baseline in SBP (particularly at 6 and 24 h), diastolic blood pressure (DBP) and mean arterial pressure (MAP); changes from baseline in heart rate (HR); treatment failure score (based on death, circulatory, respiratory, or renal mechanical support or intravenous inotrope or vasopressor treatment, and changes in SBP); treatment failure defined as death or need for circulatory, respiratory, or renal mechanical support or intravenous inotrope or vasopressor treatment; increase from baseline in SBP $>5\%$ and or >10 mmHg; changes in quality of life measured by the EuroQol 5 Dimension 5 Level (EQ-5D-5L); change from baseline to 24 h in echocardiography parameters; changes in troponin and N-terminal pro-B-type natriuretic peptide (NT-proBNP); hospital readmission for heart failure and for any cause by day 30; in-hospital worsening heart failure to day 5; and length of hospital stay. In-hospital worsening heart failure would be defined as worsening signs and/or

symptoms of heart failure since the previous assessment that required an intensification of intravenous therapy for heart failure or mechanical ventilatory, renal, or circulatory support. The safety endpoints can be assessed throughout the study and included the incidence of adverse events; changes in vital signs and in 12-lead electrocardiogram (ECG) parameters; incidence of clinically or haemodynamically significant episodes of supraventricular or ventricular arrhythmias detected by continuous ECG monitoring; standard laboratory parameters; renal function measures; cardiac troponin I or T; and mortality through day 30.

In the previously conducted SEISMiC study, administration of istaroxime was associated with increased levels of natriuretic peptides during the period of istaroxime administration. The reasons for this are not clear as the increase in NT-proBNP was not associated with worsening of congestion, and was associated with numerical decrease in weight and significant improvement in NYHA class. It is possible that some effects of istaroxime on the myocardial cells lead to direct effects on natriuretic peptide secretion. This may mirror the effects of digoxin which was previously shown to be associated with increases of natriuretic peptides⁴¹. Increase in natriuretic peptides may have contributed to some of the effects seen in the current study. The data obtained in the previous istaroxime study suggests that CVie216 will have greater efficacy for treatment of cardiogenic shock in humans.

Conclusions

Based on the results in the studies described herein CVie216 should prove efficacious as an effect IV drug that in patients with ADHF-related pre-CS providing simultaneous improvements in BP and CO and a reduction in PCWP while not increasing pulse or leading to excess arrhythmias. Accordingly, this drug has the potential of becoming the first-line therapy for patients with ADHF-CS.

While certain of the preferred embodiments of the present invention have been described and specifically exemplified above, it is not intended that the invention be limited to such embodiments. Various modifications may be made thereto without departing from the scope and spirit of the present invention, as set forth in the following claims.

Claims:

1. A method of treating pre-cardiogenic shock or cardiogenic shock with or without acute myocardial ischemia, or an acute myocardial infarction in a human subject, the method comprising: administering a pharmaceutical composition comprising administering to the human subject by intravenous infusion a pharmaceutical composition comprising istaroxime, or a pharmaceutically acceptable salt, solvate, or hydrate thereof, at a dose of about 0.1 mcg/kg/min to about 2.5 mcg/kg/min, for an infusion period of at least about 3 hours;

wherein the administering of the istaroxime results in a measurable improvement in heart function and an absence or reduction of acute myocardial arrhythmias, and wherein:

(i) the absence of acute myocardial arrhythmias continues for at least 3 hours following initiation of infusion;

(ii) the reduction of acute myocardial arrhythmias is compared to the average number of acute myocardial arrhythmias or the average duration of acute myocardial arrhythmias in a cohort of individuals not administered istaroxime.

2. The method of claim 1, wherein the infusion period is selected from:

at least about 6 hours;

at least about 12 hours;

at least about 24 hours;

at least about 48 hours; and

at least about 60 hours.

3. The method of claim 1 or claim 2, wherein the acute myocardial ischemia is detected by: (i) ECG monitoring, echocardiogram, stress test, coronary angiogram, nuclear perfusion imaging, positron emission tomography, cardiovascular magnetic resonance, a chest X-ray, or a heart MRI; (ii) or laboratory blood tests for proteins and enzymes; or (iii) both (i) and (ii).

4. The methods of claim 3, wherein the acute myocardial ischemia is detected by ECG monitoring.

5. The method of any one of claims 1-4, further comprising reperfusion following diagnoses of the acute myocardial ischemia.

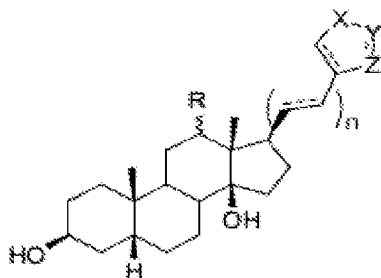
6. The method of claim 5, wherein the reperfusion is initiated within 3 hours of the diagnosis of the acute myocardial ischemia.

7. The method of claim 5 or claim 6, wherein the reperfusion comprises administration of recombinant tissue plasminogen activator (rTPA), reteplase, anistreplase, desmoteplase, nattokinase, lumbrokinase, serrapeptase, papain, DNase, bromelain, honiol, tenecteplase, alteplase, urokinase, streptokinase, anticoagulation with heparin, calcium channel blockers, ranolazine, beta blockers, nitrates, aspirin, or cholesterol-lowering compounds.

8. The method of claim 5 or claim 6, wherein the reperfusion comprises balloon angioplasty, stent placement, coronary artery bypass graft, or enhanced external counterpulsation.

9. The method of any one of claims 1-8, wherein the absence or reduction of acute myocardial arrhythmias is measured by electrocardiogram.

10. A method of treating pre-cardiogenic shock or cardiogenic shock with or without acute myocardial ischemia, or an acute myocardial infarction in a human subject, the method comprising: administering a pharmaceutical composition comprising a dual mechanism compound of Formula (II)



(II)

wherein X, Y, Z are annular atoms comprised in a five-membered carbocyclic or heterocyclic ring, selected from the group consisting of CH, NH, N, O, S; and wherein n is 0 or 1 and said heterocyclic ring selected from the group consisting of imidazolyl, pyrazolyl, thiazolyl, isoxazolyl, and the corresponding dihydro- and tetrahydro derivatives;

R is H or OH; and

the dotted line represents an optional double bond C=C; the thick line represents a bond in the β configuration; the wavy line represents a bond both in the α and β configuration; or a pharmaceutically acceptable salt, solvate, or hydrate thereof

at a dose of about 0.1 mcg/kg/min to about 2.5 mcg/kg/min, via an intravenous infusion period over at least 3 hours to said subject; thereby treating said pre-cardiogenic shock or cardiogenic shock, improving heart function and eliminating or reducing acute myocardial arrhythmias, wherein:

- (i) the absence of acute myocardial arrhythmias continues for at least 3 hours following initiation of infusion;
- (ii) the reduction of acute myocardial arrhythmias is compared to the average number of acute myocardial arrhythmias or the average duration of acute myocardial arrhythmias in a cohort of individuals not administered Formula II.

11. The method of claim 10, wherein the dual mechanism compound is selected from the group consisting of:

3-beta-hydroxy-5-beta-10-beta-methyl-13-beta-methyl-14-beta-hydroxy-17-beta-(imidazol-4-yl)androstane;

3-beta-hydroxy-5-beta-10-beta-methyl-13-beta-methyl-14-beta-hydroxy-17-beta-(2-guanidino-thiazol-4-yl)androstane;

3-beta-hydroxy-5-beta-10-beta-methyl-13-beta-methyl-14-beta-hydroxy-17-beta-(pyrazol-3-yl)androstane;

3-beta-hydroxy-5-beta-10-beta-methyl-12-beta-hydroxy-13-beta-methyl-14-beta-hydroxy-17-beta-(imidazol-4-yl)androstane;

3-beta-hydroxy-5-beta-10-beta-methyl-12-beta-hydroxy-13-beta-methyl-14-beta-hydroxy-17-beta-(2-guanidino-thiazol-4-yl)androstane;

3-beta-hydroxy-5-beta-10-beta-methyl-13-beta-methyl-14-beta-hydroxy-17-beta-(N-(3-aminopropyl)-imidazol-4-yl)androstane;

3-beta-hydroxy-5-beta-10-beta-methyl-13-beta-methyl-14-beta-hydroxy-17-beta-((5-(3-aminopropyl)-isoxazol-3-yl))androstane;

3-beta-hydroxy-5-beta-10-beta-methyl-13-beta-methyl-14-beta-hydroxy-17-beta-((5-(3-aminopropyl)-isoxazol-3-yl)-ethyl)androstane;

3-beta-hydroxy-5-beta-10-beta-methyl-13-beta-methyl-14-beta-hydroxy-17-beta-(5-(2-aminoethyl)-isoxazol-3-yl)-ethyl)androstane; and

3-beta-hydroxy-5-beta-10-beta-methyl-13-beta-methyl-14-beta-hydroxy-17-beta-(5-(2-aminomethyl)-isoxazol-3-yl)-ethenyl)androstane.

12. The method of claim 10 or claim 11, wherein the infusion period is selected from:

at least about 6 hours;
at least about 12 hours;
at least about 24 hours;
at least about 48 hours; and
at least about 60 hours.

13. The method of claim 10, wherein acute myocardial ischemia is present and is detected by: (i) ECG monitoring, echocardiogram, stress test, coronary angiogram, nuclear perfusion imaging, positron emission tomography, cardiovascular magnetic resonance, a chest X-ray, or a heart MRI; (ii) or laboratory blood tests for proteins and enzymes; or (iii) both (i) and (ii).

14. The methods of claim 13, wherein the infusion period is between 48 and 60 hours and acute myocardial ischemia is detected by ECG monitoring.

15. The method of any one of claims 10-14, further comprising reperfusion following diagnoses of acute myocardial ischemia.

16. The method of claim 15, wherein the reperfusion is initiated within 3 hours of the diagnosis of acute myocardial ischemia or acute myocardial infarction.

17. The method of claim 12, wherein the reperfusion comprises administration of recombinant tissue plasminogen activator (rTPA), reteplase, anistreplase, desmoteplase, nattokinase, lumbrokinase, serrapeptase, papain, DNase, bromelain, honiol, tenecteplase, alteplase, urokinase, streptokinase, anticoagulation with heparin, calcium channel blockers, ranolazine, beta blockers, nitrates, aspirin, or cholesterol-lowering compounds.

18. The method of claim 12, wherein the reperfusion comprises balloon angioplasty, stent placement, coronary artery bypass graft, or enhanced external counterpulsation.

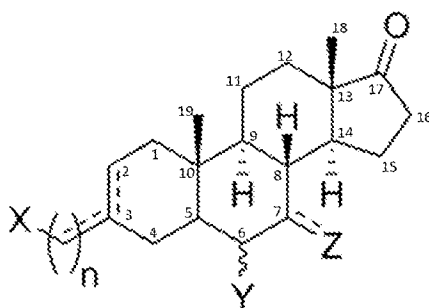
19. The method of any one of claims 10 to 18, wherein the absence or reduction of acute myocardial arrhythmias is measured by electrocardiogram.

20. The method of any one of claims 10 to 19, wherein acute myocardial arrhythmias are selected from the group consisting of ventricular tachycardia, ventricular fibrillation, and any combination thereof.

21. The method of any one of claims 10 to 20, wherein the absence or reduction of acute myocardial arrhythmias comprises a reduction in the duration of the acute myocardial arrhythmias and an improvement in renal function.

22. The method of any one of claims 10 to 21, wherein the improvement in heart function is diastolic heart function measured by echocardiography.

23. A method of treating pre-cardiogenic shock or cardiogenic shock with or without acute myocardial ischemia, or an acute myocardial infarction in a human subject, the method comprising: administering a pharmaceutical composition comprising a pure SERCA2a activator of Formula IV:



wherein X is selected from the group consisting of a carboxylic acid, carboxylic ester or a bioisoster thereof, primary alcohol, ether, and an amine group, wherein the bioisoster consists of a sulfate, sulfonic acid, phosphate, phosphonate, or a nitrogen-containing

etherocyclic ring, and wherein the amine group optionally comprises a primary amine, secondary amine, or a cyclic amine;

n is 1, 2, 3, 4, or 5;

a C3-C1' dashed line represents an optional exocyclic double bond C=C at position C3-C1';

a C2-C3 dashed line represents an optional endocyclic double bond C=C;

Y at C6 is a hydroxyl (OH) in the alpha- or beta-configuration or a hydroxymethyl (CH₂OH) in the alpha-configuration; and

Z at C7 is a -H or -OH in an alpha-configuration or a ketone, wherein a dashed line represents an optional carbonyl group (C=O) at Z,
or

a pharmaceutically acceptable salt, solvate, or hydrate thereof.

at a dose of about 0.1 mcg/kg/min to about 2.5 mcg/kg/min, via an intravenous infusion period over at least 3 hours to said subject; thereby treating said pre-cardiogenic shock or cardiogenic shock, improving heart function and eliminating or reducing ischemia associated acute myocardial arrhythmias, wherein:

- (i) the absence of acute myocardial arrhythmias continues for at least 3 hours following initiation of infusion;
- (ii) the reduction of acute myocardial arrhythmias is compared to the average number of acute myocardial arrhythmias or the average duration of acute myocardial arrhythmias in a cohort of individuals not administered Formula IV.

24. The method of claim 23, wherein the infusion period is selected from:
- at least about 6 hours;
 - at least about 12 hours;
 - at least about 24 hours;

at least about 48 hours; and
at least about 60 hours.

25. The method of claim 23 or claim 24, wherein acute myocardial ischemia is present and is detected by: (i) ECG monitoring, echocardiogram, stress test, coronary angiogram, nuclear perfusion imaging, positron emission tomography, cardiovascular magnetic resonance, a chest X-ray, or a heart MRI; (ii) or laboratory blood tests for proteins and enzymes; or (iii) both (i) and (ii).

26. The methods of claim 24, wherein the infusion period is between 48 and 60 hours and acute myocardial ischemia is detected by ECG monitoring.

27. The method of any one of claims 23 to 26, further comprising reperfusion following diagnoses of acute myocardial ischemia.

28. The method of claim 24, wherein the reperfusion is initiated within 3 hours of the diagnosis of acute myocardial ischemia.

29. The method of claim 24 or claim 25, wherein the reperfusion comprises administration of recombinant tissue plasminogen activator (rTPA), reteplase, anistreplase, desmoteplase, nattokinase, lumbrokinase, serrapeptase, papain, DNase, bromelain, honiol, tenecteplase, alteplase, urokinase, streptokinase, anticoagulation with heparin, calcium channel blockers, ranolazine, beta blockers, nitrates, aspirin, or cholesterol-lowering compounds.

30. The method of claim 24 or claim 25, wherein the reperfusion comprises balloon angioplasty, stent placement, coronary artery bypass graft, or enhanced external counterpulsation.

31. The method of any one of claims 23 to 30, wherein the absence or reduction of acute myocardial arrhythmias is measured by electrocardiogram.

32. The method of any one of claims 23 to 31, wherein acute myocardial arrhythmias are selected from the group consisting of ventricular tachycardia, ventricular fibrillation, and any combination thereof.

34. The method of any one of claims 23 to 31 wherein the absence or reduction of acute myocardial arrhythmias comprises a reduction in the duration of the acute myocardial arrhythmias and an improvement in renal function.

35. The method of any one of claims 23 to 34, wherein the improvement in heart function is diastolic heart function measured by echocardiography.

36. The method of claim 35, wherein the echocardiography comprises one or more of decreased E wave, increased E wave deceleration time, increased A wave, decreased E/A ratio, increased e', or decreased E/e' ratio.

37. The method of any one of claims 23-35, wherein the improvement in heart function comprises one or more of improved diastolic relaxation, increased diastolic blood pressure, decreased heart rate, a decrease in dyspnea, increased stroke volume, increased cardia index, increased cardia output, decreased pulmonary capillary wedge pressure, or increased stroke volume index.

38. The method of claim 37, wherein the improvement in heart function is measured by sphygmomanometer, an electrocardiogram, cardiac catheterization, a radionuclide ventriculography scan, or any combination thereof.

39. The method of any one of claims 15, wherein said subject has pre-cardiogenic shock.

40. The method of claim 23, wherein the pure SERCA2a activator is selected from the group consisting of:

(E)-4-(6alpha-hydroxy-17-oxoandrosterane-3-yliden)butyric acid;

(Z)-4-(6alpha-hydroxy-17-oxoandrostane-3-yliden)butyric acid;

(E)-4-(6beta-hydroxy-17-oxoandrostane-3-yliden)butyric acid;

(Z)-4-(6beta-hydroxy-17-oxoandrostane-3-yliden)butyric acid;

(E)-3-[2-(azetidin-3-yl)ethyliden]-6alpha-hydroxyandrostane-17-one;

(Z)-3-[2-(azetidin-3-yl)ethyliden]-6alpha-hydroxyandrostane-17-one;

(E)-3-(4-aminobutyl)-6alpha-hydroxyandrost-2-ene-17-one hydroiodide;

3-[2-(piperidin-4-yl)ethyl]-6alpha-hydroxyandrost-2-ene-17-one hydroiodide;

(EZ)-3-(4-aminobutyliden)-6alpha-hydroxyandrostane-17-one;

(E)-3-[2-(piperidin-4-yl)ethyliden]-6alpha-hydroxyandrostane-17-one;

(Z)-3-[2-(piperidin-4-yl)ethyliden]-6alpha-hydroxyandrostane-17-one;

3 beta-[2-(piperidin-4-yl)ethyl]-6alpha-hydroxyandrostane-17-one;

Ethyl (6alpha-hydroxy-17-ketoandrostane-3beta-yl) acetate;

4-(6alpha-hydroxy-17-oxoandrostane-3-yl) butyric acid;

4-(6beta-hydroxy-17-oxoandrostane-3-yl) butyric acid;

2-(6beta-hydroxy-17-oxoandrostane-3-yl) acetic acid;

Ethyl 4-(6alpha-hydroxy-17-oxoandrostane-3-yl) butyrate;

Ethyl 4-(6alpha-hydroxy-17-oxoandrostan-3-yl) caproate;

6-(6beta-hydroxy-17-oxoandrostan-3-yl) caproic acid;

(E,Z)-3-(5-N-methylaminopentyliden)-6alpha-hydroxymethylandrostan-7,17-dione;

(E,Z)-3-[2-(pyrrolidine-3-yl)ethyliden]-6alpha-hydroxymethylandrostan-7,17-dione;

(E,Z)-3-[2-(azetidine-3-yl)ethyliden]-6alpha-hydroxymethylandrostan-7,17-dione;

(E,Z)-3-[2-(piperidin-4-yl)ethyliden]-6alpha-hydroxymethylandrostan-7,17-dione;

(E,Z)-3-(5-N-methylaminopentyliden)-6alpha-hydroxymethyl-7alpha-hydroxyandrostan-17-one;

3 beta-[2-(azetidine-3-yl)ethyl]-6alpha-hydroxymethylandrostan-7,17-dione;

3 beta-[2-(azetidine-3-yl)ethyl]-6alpha-hydroxymethyl-7alpha-hydroxyandrostan-17-one;

3 beta-[2-(pyrrolidine-3-yl)ethyl]-6alpha-hydroxymethylandrostan-7,17-dione;

3 beta-[2-(pyrrolidine-3-yl)ethyl]-6alpha-hydroxymethyl-7alpha-hydroxyandrostan-17-one;

3 beta-[2-(piperidin-4-yl)ethyl]-6alpha-hydroxymethylandrostan-7,17-dione; and

3 beta-[2-(piperidin-4-yl)ethyl]-6alpha-hydroxymethyl-7alpha-hydroxyandrostan-17-one.

41. The method of claim 40, wherein the pure SERCA2a activator is 2-(6beta-hydroxy-17-oxoandrostan-3-yl) acetic acid.
42. The method of claim 40 or claim 41, wherein the infusion period is selected from:
at least about 6 hours;
at least about 12 hours;
at least about 24 hours;
at least about 48 hours; and
at least about 60 hours.
43. The method of any one of claims 40 to 42, wherein the acute myocardial ischemia is detected by: (i) ECG monitoring, echocardiogram, stress test, coronary angiogram, nuclear perfusion imaging, positron emission tomography, cardiovascular magnetic resonance, a chest X-ray, or a heart MRI; (ii) or laboratory blood tests for proteins and enzymes; or (iii) both (i) and (ii).
44. The methods of claim 43, wherein the acute myocardial ischemia is detected by ECG monitoring, said method, further comprising reperfusion following diagnoses of the acute myocardial ischemia.
45. The method of claim 44, wherein the reperfusion comprises administration of recombinant tissue plasminogen activator (rTPA), reteplase, anistreplase, desmoteplase, nattokinase, lumbrokinase, serrapeptase, papain, DNase, bromelain, honiol, tenecteplase, alteplase, urokinase, streptokinase, anticoagulation with heparin, calcium channel blockers, ranolazine, beta blockers, nitrates, aspirin, or cholesterol-lowering compounds.
46. The method of claim 43, wherein the absence or reduction of acute myocardial arrhythmias is measured by electrocardiogram.

47. The method of claim 46, wherein acute myocardial arrhythmias are selected from the group consisting of ventricular tachycardia, ventricular fibrillation, atrial fibrillation, and any combination thereof.

48. The method of claim 47, wherein the absence or reduction of acute myocardial arrhythmias comprises a reduction in the duration of the acute myocardial arrhythmias.

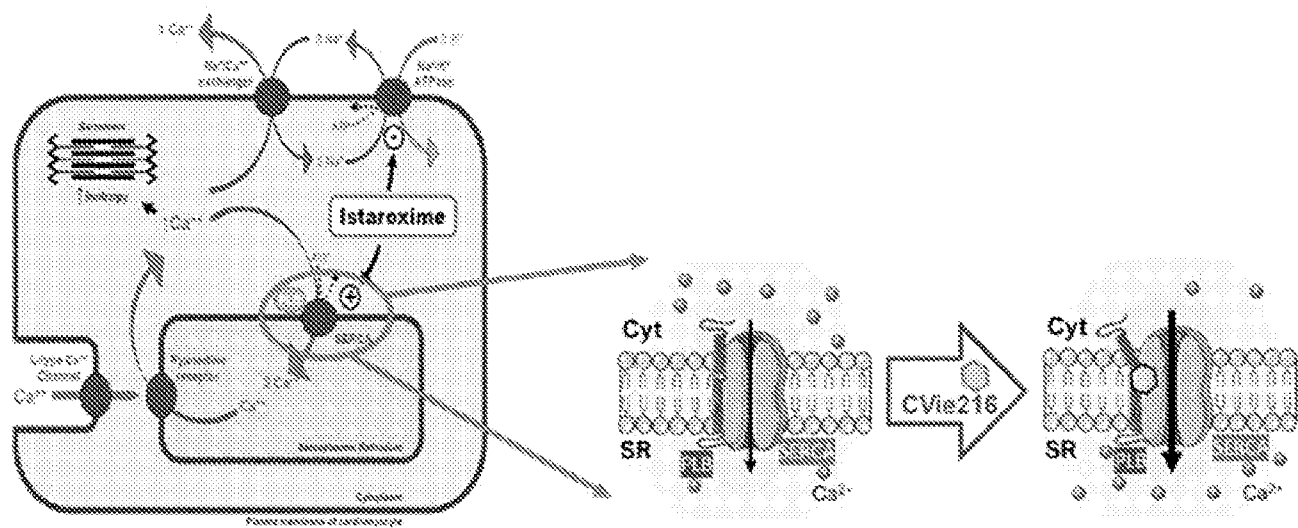


FIG. 1A

FIG. 1B

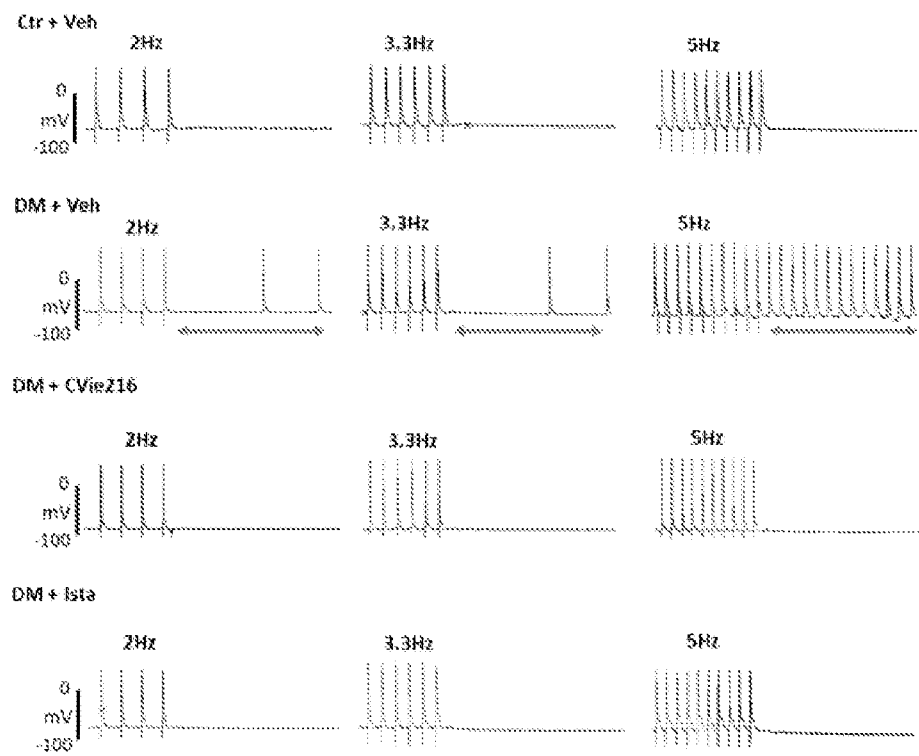


FIG. 2A

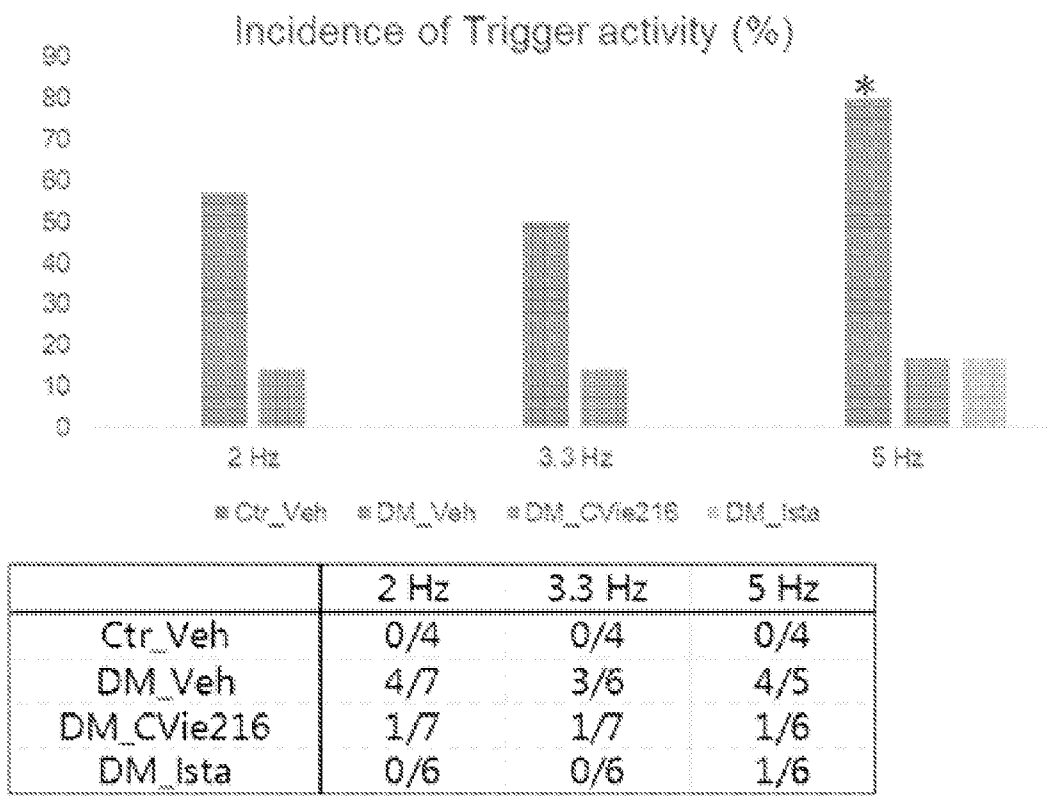


FIG. 2B

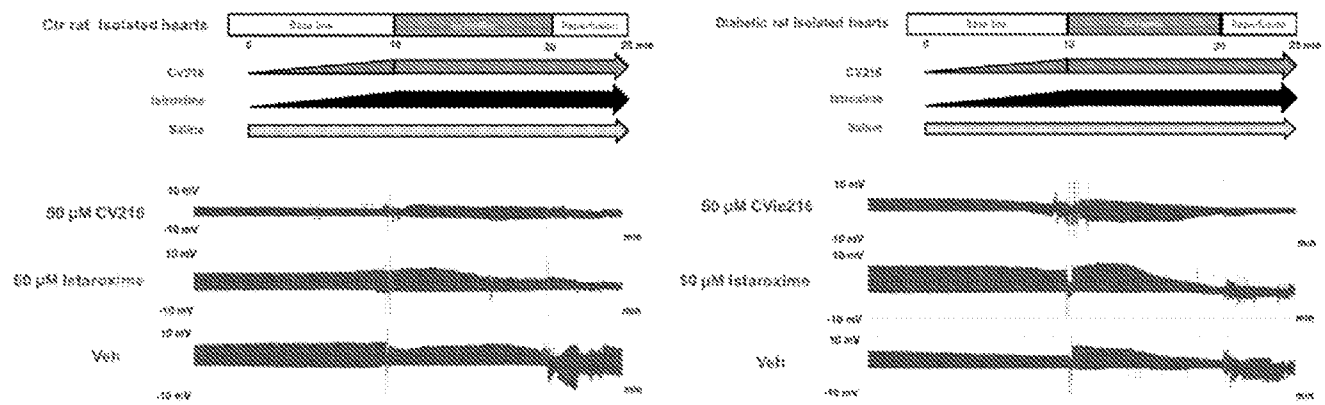


FIG. 3A

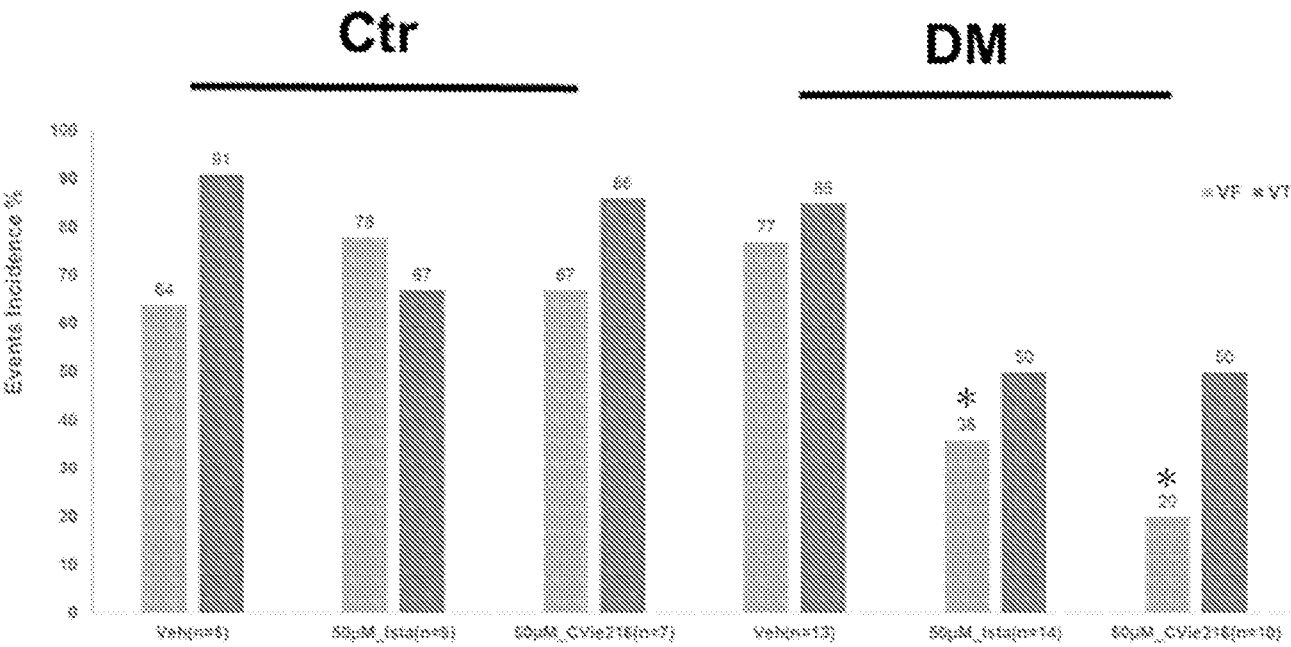


FIG. 3B

Score	Evants
6	VF
5	VT
4	NSVT
3	triplets or couplets
2	>50 PVC
1	5~50 PVC
0	<5 PVC

FIG. 3C

	Ctr			DM		
	Veh (n=5)	50_Ista (n=9)	50_CVie216 (n=7)	Veh (n=13)	50uM Ista (n=14)	50uM CVie216 (n=10)
Arrhythmia Score	5.80±0.45	4.78±2.44	5.29±1.11	5.46±1.05	4.43±1.28 *	3.50±1.90 **

FIG. 3D

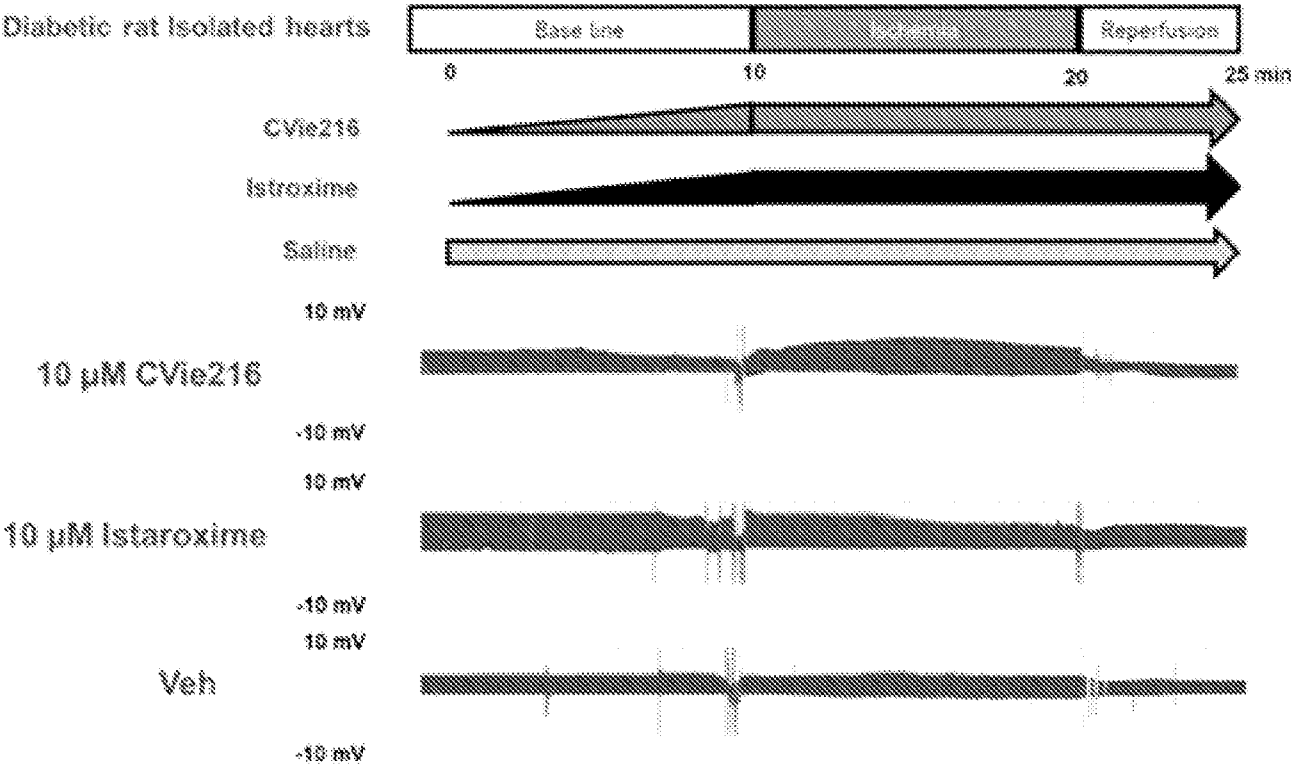
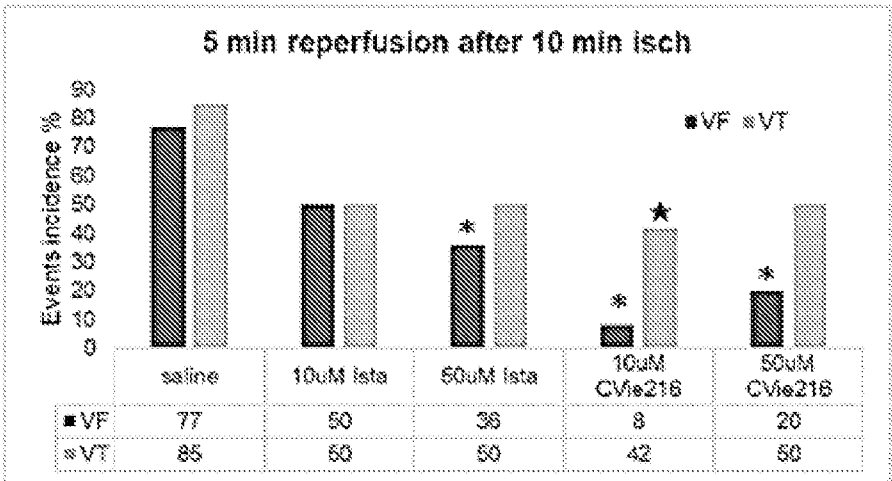


FIG. 4A



* $p < 0.05$, compare to saline group (ANOVA)
★ $p < 0.05$, compare to saline group (Dunnett t, one side)

FIG. 4B

	Veh (n=13)	10µM Ista (n=10)	10µM CVie216 (n=12)
Arrhythmia Score	5.46±1.05	4.40±1.71	2.83±1.75 *

FIG. 4C

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2024/058923

A. CLASSIFICATION OF SUBJECT MATTERIPC: **A61K 31/566** (2025.01); **A61K 31/58** (2025.01); **A61K 45/06** (2025.01); **A61P 9/00** (2025.01)CPC: **A61K 31/566**; **A61K 31/58**; **A61K 45/06**; **A61P 9/00**; A61K 2300/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History Document

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

See Search History Document

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

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2023/205183 A1 (WINDTREE THERAPEUTICS INC) 26 October 2023 (26.10.2023)	1, 2
Y	entire document	3, 4
Y	WO 2022/159678 A1 (WINDTREE THERAPEUTICS INC) 28 July 2022 (28.07.2022)	3, 4
Y	entire document	3, 4
A	US 2023/0009312 A1 (WINDTREE THERAPEUTICS INC) 12 January 2023 (12.01.2023)	1-4
A	entire document	1-4



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

“A” document defining the general state of the art which is not considered to be of particular relevance

“D” document cited by the applicant in the international application

“E” earlier application or patent but published on or after the international filing date

“L” document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

“O” document referring to an oral disclosure, use, exhibition or other means

“P” document published prior to the international filing date but later than the priority date claimed

“T” later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

“X” document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

“Y” document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

“&” document member of the same patent family

Date of the actual completion of the international search

22 January 2025 (22.01.2025)

Date of mailing of the international search report

24 March 2025 (24.03.2025)

Name and mailing address of the ISA/US

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

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

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

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

3. ☒ Claims Nos.: **5-9, 15, 16, 19-22, 27, 29-32, 34-39, 43-48**
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

Claim 33 is objected to under PCT Rule 66.2(a)(v) as lacking clarity under PCT Article 6 because claim 33 is indefinite for the following reason:

Claim 33 is not present in the claims. Therefore, claim 33 not included in the Written Opinion.

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees need to be paid.

Group I: Claims 1-4 are drawn to methods of treating including istaroxime.

Group II+: Claims 10-14, 17, and 18 are drawn to methods of treating including a dual mechanism compound of Formula (II).

Group III+: Claims 23-26, 28, and 40-42 are drawn to methods of treating including a pure SERCA2a activator of Formula IV.

An exemplary election of Group II+ is a method of treating pre-cardiogenic shock or cardiogenic shock with or without acute myocardial ischemia, or an acute myocardial infarction in a human subject, the method comprising: administering a pharmaceutical composition comprising a dual mechanism compound of Formula (II), wherein the dual mechanism compound is 3-beta-hydroxy-5-beta-10-beta-methyl-13-beta-methyl-14-beta-hydroxy-17-beta-(imidazol-4-yl)androstane, at a dose of about 0.1 mcg/kg/min to about 2.5 mcg/kg/min, via an intravenous infusion period over at least 3 hours to said subject; thereby treating said pre-cardiogenic shock or cardiogenic shock, improving heart function and eliminating or reducing acute myocardial arrhythmias, wherein: (i) the absence of acute myocardial arrhythmias continues for at least 3 hours following initiation of infusion; (ii) the reduction of acute myocardial arrhythmias is compared to the average number of acute myocardial arrhythmias or the average duration of acute myocardial arrhythmias in a cohort of individuals not administered Formula II. The exemplary election has been selected based on the guidance set forth in section 10.54 of the PCT International Search and Preliminary Examination Guidelines. Specifically, the exemplary election was selected based on the first listed compound species of Formula (II) presented in the claims (see claim 11).

An exemplary election of Group III+ is a method of treating pre-cardiogenic shock or cardiogenic shock with or without acute myocardial ischemia, or an acute myocardial infarction in a human subject, the method

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

comprising: administering a pharmaceutical composition comprising a pure SERCA2a activator of Formula IV: wherein the pure SERCA2a activator is (E)-4-(6 α -hydroxy-17-oxoandrostane-3-yliden)butyric acid, at a dose of about 0.1 mcg/kg/min to about 2.5 mcg/kg/min, via an intravenous infusion period over at least 3 hours to said subject; thereby treating said pre-cardiogenic shock or cardiogenic shock, improving heart function and eliminating or reducing ischemia associated acute myocardial arrhythmias, wherein: (i) the absence of acute myocardial arrhythmias continues for at least 3 hours following initiation of infusion; (ii) the reduction of acute myocardial arrhythmias is compared to the average number of acute myocardial arrhythmias or the average duration of acute myocardial arrhythmias in a cohort of individuals not administered Formula IV.

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

The special technical features of Group I, methods of treating including istaroxime, are not present in Groups II+ and III+; the special technical features of Group II+, methods of treating including a dual mechanism compound of Formula (II), are not present in Groups I and III+; and the special technical features of Group III+, methods of treating including a pure SERCA2a activator of Formula IV, are not present in Groups I and II+.

The Groups II+ and III+ formulae do not share a significant structural element requiring the selection of alternatives for the compound variables R, X, Y, Z, and n, and accordingly these groups lack unity a priori.

Additionally, even if Groups I, II+, and III+ were considered to share the technical features of a method of treating pre-cardiogenic shock or cardiogenic shock with or without acute myocardial ischemia, or an acute myocardial infarction in a human subject, the method comprising: administering a pharmaceutical composition comprising a dual mechanism compound of core Formula (II), at a dose of about 0.1 mcg/kg/min to about 2.5 mcg/kg/min, via an intravenous infusion period over at least 3 hours to said subject; thereby treating said pre-cardiogenic shock or cardiogenic shock, improving heart function and eliminating or reducing acute myocardial arrhythmias, wherein: (i) the absence of acute myocardial arrhythmias continues for at least 3 hours following initiation of infusion; (ii) the reduction of acute myocardial arrhythmias is compared to the average number of acute myocardial arrhythmias or the average duration of acute myocardial arrhythmias in a cohort of individuals not administered Formula II; and a pure SERCA2a activator of core Formula IV, these shared technical features do not represent a contribution over the prior art as taught by WO 2023/205183 A1 to Windtree Therapeutics, Inc. (hereinafter, "Windtree '183"), and US 2023/0009312 A1 to Windtree Therapeutics, Inc. (hereinafter, "Windtree '312").

Windtree '183 teaches a method of treating pre-cardiogenic shock or cardiogenic shock with or without acute myocardial ischemia, or an acute myocardial infarction in a human subject, the method comprising: administering a pharmaceutical composition comprising a dual mechanism compound of core Formula (II) at a dose of about 0.1 mcg/kg/min to about 2.5 mcg/kg/min, via an intravenous infusion period over at least 3 hours to said subject; thereby treating said pre-cardiogenic shock or cardiogenic shock, improving heart function and eliminating or reducing acute myocardial arrhythmias, wherein: (i) the absence of acute myocardial arrhythmias continues for at least 3 hours following initiation of infusion; (ii) the reduction of acute myocardial arrhythmias is compared to the average number of acute myocardial arrhythmias or the average duration of acute myocardial arrhythmias in a cohort of individuals not administered (Claim 28, method of treating or preventing early cardiogenic shock or cardiogenic shock in an individual, the method comprising: ... administering to the individual a therapeutically effective amount of a pharmaceutical composition comprising: (i) pharmaceutically acceptable carrier; and (ii) istaroxime or a pharmaceutically acceptable salt, solvate, or hydrate thereof, wherein the istaroxime or pharmaceutically acceptable salt, solvate, or hydrate thereof is administered at a dose of about 0.2 mcg/kg/min to about 2.0 mcg/kg/min, and wherein the administering comprises intravenous infusion for a second period of time of at least about 3 hours; Pg. 3, last paragraph, dual mechanisms; because the patient populations are the same and because the method steps for administering are the same, at least some subjects from each group will inherently result wherein: (i) the absence of acute myocardial arrhythmias continues for at least 3 hours following initiation of infusion; (ii) the reduction of acute myocardial arrhythmias is compared to the average number of acute myocardial arrhythmias or the average duration of acute myocardial arrhythmias in a cohort of individuals not administered Formula II); but fails to explicitly disclose a pure SERCA2a activator of core Formula IV.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2024/058923

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

Windtree '312 teaches a pure SERCA2a activator of core Formula IV (Para. [0067], CVie201, see shown structure; Para. [0038], "pure" SERCA2a stimulators). It would have been obvious to one of ordinary skill in the art to modify Windtree '183 with the teachings of Windtree '312 for the purposes of reducing arrhythmogenic risk (Para. [0003]).

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

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: **1-4**

Remark on Protest

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