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(54) **Title**: METHODS OF TREATING DIASTOLIC DYSFUNCTION AND RELATED CONDITIONS

(57) **Abstract**: The invention provides a method of treating diastolic dysfunction, e.g., diastolic dysfunction with preserved ejection fraction, in a subject. The method comprises administering to the subject in an amount effective to treat the diastolic dysfunction a cardiac metabolic modifier comprising a structure of Formula I, as described herein. In some embodiments, the diastolic dysfunction is characterized by (i) a lack of increased late $I_{Na}$ in cardiomyocytes, (ii) an increase in myofilament calcium sensitivity, or (iii) a combination thereof. In some embodiments, the subject does not suffer from a cardiac injury or a structural heart disease, as described herein. Further provided are a method of treating heart failure with preserved ejection fraction in a subject, a method of modulating myofilament calcium sensitivity in a subject, and a method of treating a condition associated with or caused by increased myofilament calcium sensitivity.

# METHODS OF TREATING DIASTOLIC DYSFUNCTION AND RELATED CONDITIONS

## CROSS-REFERENCE TO RELATED APPLICATIONS

**[0001]** This application claims priority to U.S. Provisional Patent Application No. 61/241,585, filed on September 11, 2009, U.S. Provisional Patent Application No. 61/263,920, filed on November 24, 2009, and U.S. Provisional Patent Application No. 61/348,105, filed on May 25, 2010, each of which is incorporated by reference in their entirety.

## GRANT FUNDING

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## BACKGROUND

**[0003]** Diastolic dysfunction is characterized by prolonged relaxation of the myocardium and, if untreated, can lead to the clinical syndrome, heart failure with preserved ejection fraction (HFpEF). HFpEF is an increasingly prevalent health burden accounting for significant morbidity, mortality, and healthcare expenditures.[1-4] The underlying mechanisms in diastolic dysfunction are not clearly understood, limiting treatment options.[5] Recent large clinical trials using the standard therapies for systolic heart failure have failed to demonstrate improvement, further emphasizing differences in the underlying pathophysiology of diastolic dysfunction.[6-8]

**[0004]** There are several potential mechanisms for diastolic dysfunction. One potential mechanism for diastolic dysfunction is increased diastolic $Ca^{2+}$ resulting in a slowed ventricular relaxation and diastolic dysfunction. $Ca^{2+}$ is removed from the cytosol during diastole by the sarcoplasmic reticular $Ca^{2+}$ -ATPase (SERCA) and the $Na^{+}/Ca^{2+}$ exchanger (NCX). The NCX couples $Ca^{2+}$ extrusion to the transmembrane $Na^{+}$ gradient.[9] In the failing heart, a small number of the $Na^{+}$ channels fail to inactivate creating a late $Na^{+}$ current ($I_{Na}$).[10-13] The late $I_{Na}$ increases $Na^{+}$ entry into the cell, reducing $Ca^{2+}$ extrusion by NCX.[14] Oxidative stress[15] and myofilament $Ca^{2+}$ sensitization represent other potential mechanisms for diastolic dysfunction, and, may represent other forms or sub-types of diastolic dysfunction.

**[0005]** Because there are several potential mechanisms for diastolic dysfunction, it is possible that sub-types of this medical condition exist, such that successful treatment of a patient exhibiting diastolic dysfunction may depend on the particular sub-type of diastolic dysfunction the patient presents.

**[0006]** While studies have demonstrated an improvement of a sub-type of diastolic dysfunction in which the late $I_{Na}$ in failing hearts was increased, the relevance of this sub-type of diastolic dysfunction to the form of diastolic dysfunction presented by humans is questioned, since most of these studies were carried out in animal models in which the increased late $I_{Na}$ or diastolic dysfunction was artificially induced, e.g., toxin-induced diastolic dysfuntion, ischemia-induced diastolic dysfunction.

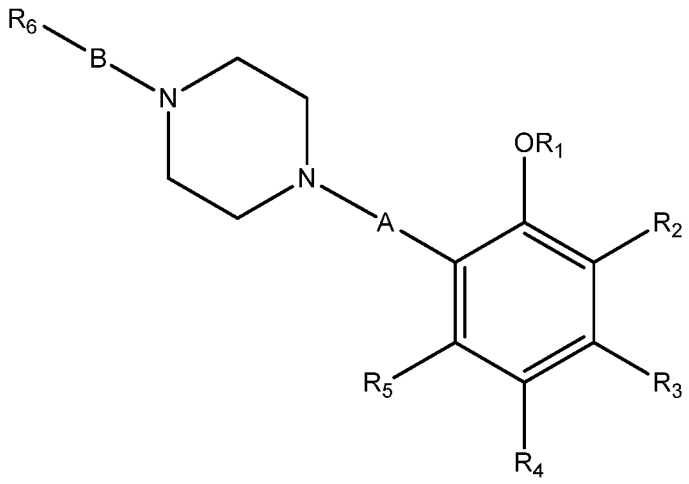
**[0007]** To date, no studies have shown evidence of the existence of a form of diastolic dysfunction in which the late $I_{Na}$ was not increased. Accordingly, no studies to date have demonstrated the treatment of diastolic dysfunction characterized by a lack of increased late $I_{Na}$.

**[0008]** Furthermore, no studies have demonstrated the existence of a form of diastolic dysfunction in which myofilament calcium sensitivity was increased. Accordingly, no studies have shown the treatment of diastolic dysfunction characterized by an increase in myofilament calcium sensitivity.

## SUMMARY OF THE INVENTION

**[0009]** Demonstrated herein for the first time are data which suggest that other sub-types of diastolic dysfunction do, in fact, exist. Also presented herein for the first time are data which demonstrate the treatment of these other diastolic dysfunction sub-types.

**[0010]** In this regard, the invention provides a method of treating diastolic dysfunction in a subject. The method comprises administering to the subject in an amount effective to treat the diastolic dysfunction a cardiac metabolic modifier comprising a structure of Formula I:




[Formula I]

wherein A comprises a main chain of 1-8 atoms, each atom of which is independently C, O, N, or S, and each atom of which is optionally bound to an additional group selected from C1-C8 alkyl, C1-C8 alkoxy, OH, NH2, NH(C1-C4 alkyl) and SH;

wherein R1 is H or a C1-C8 alkyl;

wherein each of R2, R3, R4, and R5 independently is H, a C1-C8 alkyl, or a C1-C8 alkoxy;

wherein B is H or comprises a main chain of 1-8 atoms, each atom of which is independently C, O, N, or S, and each atom of which is optionally bound to an additional group; and,

wherein $R_6$ is absent or phenyl, optionally substituted with 1 to 5 groups, each group of which is independently C1-C8 alkyl, C1-C8 alkoxy, or OH.

**[0011]** In some embodiments, the diastolic dysfunction is diastolic dysfunction with preserved ejection fraction. In some embodiments, the diastolic dysfunction, e.g., diastolic dysfunction with preserved ejection fraction, is characterized by (i) a lack of increased late $I_{Na}$ in cardiomyocytes, (ii) an increase in myofilament calcium sensitivity, or (iii) a combination thereof. Furthermore, in some embodiments, the subject does not suffer from a cardiac injury or structural heart disease, as further described herein.

**[0012]** Because diastolic dysfunction can lead to heart failure with preserved ejection fraction, the invention also provides a method of treating or preventing heart failure with preserved ejection fraction. The method comprises administering to the subject in an amount effective to

treat or prevent the heart failure with preserved ejection fraction a cardiac metabolic modifier comprising a structure of Formula I, as described herein. In specific aspects, the heart failure with preserved ejection fraction is characterized by (i) a lack of increased late $I_{Na}$ in cardiomyocytes, (ii) an increase in myofilament calcium sensitivity, or (iii) a combination thereof. In specific aspects, the subject does not suffer from a cardiac injury or structural heart disease, as further described herein.

**[0013]** The invention further provides a method of modulating (e.g., reducing) myofilament calcium sensitivity in a subject, comprising administering to the subject in an amount effective to modulate (e.g., reduce) myofilament calcium sensitivity a cardiac metabolic modifier comprising a structure of Formula I, as described herein. In some embodiments, the subject suffers from diastolic dysfunction, such as any of the sub-types of diastolic dysfunction described herein (e.g., diastolic dysfunction characterized by (i) a lack of increased late $I_{Na}$ in cardiomyocytes, (ii) an increase in myofilament calcium sensitivity, or (iii) a combination thereof). Accordingly, the invention furthermore provides a method of treating a condition associated with or caused by increased myofilament calcium sensitivity in a subject. The method comprises administering to the subject in an amount effective to treat the condition a cardiac metabolic modifier of Formula I, as described herein.

## BRIEF DESCRIPTION OF THE DRAWINGS

**[0014]** Figure 1. Representative echocardiographic assessments of LV diastolic function. Septal mitral annulus velocities interrogated with tissue Doppler imaging (TDI). The sham mouse has a larger E' (early diastolic velocity), and smaller A' (late diastolic velocity) than the hypertensive DOCA-salt mouse (upper panel). Treatment with ranolazine increased the ratio of E' to A' in the DOCA-salt mouse. Treatment of the sham mouse with ranolazine had little effect on mitral annulus velocities in the sham mouse. Sm (systolic septal mitral annulus velocity) was similar among all four groups.

**[0015]** Figure 2. Invasive hemodynamic assessment of LV diastolic dysfunction. A and B: The end-diastolic pressure-volume relation (EDPVR) slope is steeper in DOCA-salt mice as compared to sham and ranolazine treated DOCA-salt mice. In panel A, the difference in the EDPVR in three representative mice from sham, DOCA-salt, and DOCA-salt treated mice are depicted. In panel B, the mean EDPVR is significantly greater in the DOCA-salt mice compared to sham and ranolazine treated sham mice. Additionally, treatment with ranolazine reduces the

EDPVR in DOCA-salt mice to that of controls (n= 8, *p < 0.05 vs. all groups). C: Ranolazine shows a linear concentration dependent effect on the EDPVR in DOCA-salt mice with a Pearson correlation coefficient of 0.70 (n=6, p < 0.05).

**[0016]** Figure 3. Functional analysis of isolated cardiomyocytes. A: The normalized contraction of individual cardiomyocytes illustrating the difference in relaxation between a DOCA-salt mouse and a ranolazine treated DOCA-salt mouse. B: Fractional shortening of isolated cardiomyocytes paced at 0.5 Hz represented as the peak shortening divided by the baseline sarcomere length (n=10, p=NS). C: Time to 90% peak contraction in isolated cardiomyocytes (n=10, p=NS). D: Isolated cardiomyocytes from DOCA-salt mice have a prolonged relaxation constant ($\tau$) compared to control animals. The addition of ranolazine to isolated DOCA-salt cardiomyocytes normalizes relaxation kinetics (n=10, *p < 0.0001 vs. all groups).

**[0017]** Figure 4. DOCA-salt mice have no difference in intracellular calcium cycling when compared to sham mice. B: The peak Ca2+ fluorescence in isolated cardiomyocytes loaded with the ratiometric fluorescent dye, Fura-2AM, and paced at 0.5 Hz (n=10, p=NS). C: The time to 90% peak Ca2+ fluorescence representing the rate of calcium entry into the cytosol (n=10, p=NS). D: The rate of relaxation measured as the time constant $\tau$ did not differ among groups (n=10, p=NS).

**[0018]** Figure 5. DOCA-salt mice show no difference in late INa when compared to sham mice. A: Voltage-clamp studies show no increase in late INa in DOCA-salt myocytes with respect to sham (n=7, p=NS). Extracellular addition of ranolazine had little effect on late accumulated charge in both the DOCA-salt and sham groups (n=4, p=NS). Peak INa was similar among the four groups. B: Graph of the ratio of the mean accumulated late Na+ charge to the mean accumulated total Na+ charge during an activating voltage step.

**[0019]** Figure 6. Resting sarcomere length of cardiomyocytes and pCa-tension relations in skinned fiber bundles. A: The mean diastolic sarcomere length was significantly shorter in the DOCA-salt cardiomyocytes compared to the sham. The addition of ranolazine to the DOCA-salt cardiomyocytes significantly lengthened resting sarcomeres, but had no effect on sham cardiomyocytes (n=12, *p < 0.0001 vs. all groups). B: The mean steady-state isometric tension of skinned fiber bundles plotted as a function of pCa. C: pCa-tension relations normalized to maximum tension. DOCA-salt fibers (pCa50 = 6.1 +/- 0.02; Hill n = 3.42 +/- 0.30) demonstrate

a significant increase in mean Ca2+ sensitivity (n=6, $p < 0.05$) as compared to shams without DOCA treatment (pCa50 = 6.0 +/- 0.01; Hill n = 3.80 +/- 0.61) shams with DOCA treatment (pCa50 = 6.0 +/- 0.01; Hill n = 3.91 +/- 0.50) and fibers from DOCA mice and treated with ranalozine. (pCa50 = 6.0 +/- 0.03; Hill n = 3.71 +/- 1.0) D: Plot of tension normalized to maximum steady-state isometric tension as a function of pCa.

## DETAILED DESCRIPTION OF THE INVENTION

**[0020]** *Diastolic dysfunction*

**[0021]** The invention provides a method of treating diastolic dysfunction in a subject, comprising administering a cardiac metabolic modifier to the subject in an amount effective to treat the diastolic dysfunction.

**[0022]** As used herein, the term "diastolic dysfunction" refers to a condition in which abnormalities in mechanical function are present during diastole and which can occur in the presence or absence of heart failure and can co-exist with or without abnormalities in systolic function (Zile et al., *JACC* 41: 1519-1522 (2003)). Accordingly, with regard to the invention disclosed herein, the diastolic dysfunction in some embodiments is diastolic dysfunction with preserved ejection fraction, which is also known as, diastolic dysfunction with preserved systolic function, diastolic dysfunction without systolic dysfunction, and diastolic dysfunction with preserved left ventricular function. As used herein, the term "preserved ejection fraction" refers to a left ventricular ejection fraction which is greater than or about 45%, e.g., greater than or about 50%. In some aspects, the preserved ejection fraction is one which is greater than or about 50%.

**[0023]** In some embodiments, the diastolic dysfunction is characterized by measurement of left ventricle (LV) pressure, volume, wall thickness, calculations that reflect the process of active relaxation (the rate of isovolumic LV pressure and LV filling) and calculations that reflect passive stiffness (chamber compliance and myocardial viscoelastic stiffness) (Zile et al., (2010), *supra*).

**[0024]** In some embodiments, the diastolic dysfunction is evidenced via echocardiography, as described in Silberman et al., *Circulation* 121: 519-528 (2010) and as described in the EXAMPLES section set forth below. In some aspects, LV tissue Doppler and mitral valve in-flow velocity are measured by echocardiography. In some aspects, the diastolic dysfunction is

characterized by a late diastolic velocity (A') which is higher than the early diastolic velocity (E'), as shown by tissue Doppler imaging (TDI). See, for example, Figure 1 and the brief description thereof.

**[0025]** In alternative or additional embodiments, the diastolic dysfunction is characterized through magnetic resonance imaging (MRI) or by cardiac catheterization and measurement of LV end diastolic pressure and systolic function. In some aspects, the diastolic dysfunction is characterized by an end-diastolic pressure-volume relation (EDPVR) slope which is steeper than the EDPVR slope of a corresponding control (e.g., which does not suffer from diastolic dysfunction). In some aspects, the diastolic dysfunction is characterized by a slope of the line which correlates end-diastolic pressure (mm Hg) to end-diastolic volume (μL) which is steeper than the slope of the line which correlates end-diastolic pressure (mm Hg) to end-diastolic volume (μL) of a corresponding control (e.g., which does not suffer from diastolic dysfunction). See, for example Figures 2A-2C, the brief description thereof, and the EXAMPLES section set forth below. In some embodiments, the diastolic dysfunction is characterized by an EDPVR slope which is increased by about 25% or more (e.g., about 30% or more, about 35% or more about 40% or more, about 45% or more, about 50% or more), as compared to the EDPVR slope of a corresponding control (e.g., which does not suffer from diastolic dysfunction). In some embodiments, the diastolic dysfunction is characterized by an EDPVR slope which is increased by about 25% to about 60%, by about 30% to about 55%, about 35% to about 50%, or about 40% to about 45% (e.g., about 40%, about 41%, about 42%, about 43%, about 44%, about 45% ), as compared to the EDPVR slope of a corresponding control (e.g., which does not suffer from diastolic dysfunction). In exemplary embodiments, the diastolic dysfunction is characterized by an EDPVR slope which is about 0.15 or higher, e.g., about 0.16 or higher, about 0.17 or higher, about 0.19 or higher, about 0.20 or higher, about 0.21 or higher, about 0.22 or higher, about 0.23 or higher, about 0.24 or higher, about 0.25 or higher, about 0.26 or higher, about 0.27 or higher, about 0.28 or higher, about 0.29 or higher, about 0.30 or higher.

**[0026]** In some aspects, the diastolic dysfunction is characterized through one or more of the invasive and non-invasive procedures described in the EXAMPLES section set forth below.

**[0027]** In some embodiments, the diastolic dysfunction is not diastolic dysfunction which is induced by administration of a toxin or by ischemia, ischemia-reperfusion, or coronary artery-occlusion-reperfusion. In some aspects, the diastolic dysfunction is not diastolic dysfunction

which is induced by administration of sea anemone toxin ATX-II described in Sossalla et al., *J Molec Cell Cardiology*45: 32-43 (2008) or in Fraser et al., *J Molec Cell Cardiology* 41: 1031-1038 (2006). In some aspects, the diastolic dysfunction is not induced by an ischemic metabolite, e.g., palmitoyl-L-carnitine, as described in Wu et al., *J Pharmacology Expmtl Therapeutics* 330: 550-557 (2009). In some aspects, the diastolic dysfunction is not diastolic dysfunction which is induced by administration of hydrogen peroxide. In some aspects, the diastolic dysfunction is not induced by stimulating muscle at a basal stimulation frequency followed by stretching until maximum steady-state twitch force is achieved, as described in Sossalla et al. (2008), *supra*. In some aspects, the diastolic dysfunction is not induced by E-4031, 4-aminopyridine, and $BaCl_2$.

**[0028]** In some embodiments, the diastolic dysfunction co-exists with another medical condition. For example, as further discussed herein, in some aspects, the diastolic dysfunction co-exists with hypertension or a metabolic disease (e.g., diabetes, obesity).

**[0029]** In some embodiments, the diastolic dysfunction does not co-exist with a cardiac injury or structural heart disease. Cardiac injuries and structural heart diseases that do not co-exist with the diastolic dysfunction are further described herein under *Subjects*. In some aspects, the diastolic dysfunction does not co-exist with ischemic heart disease, chronic stable angina, chronic angina. In some aspects, the diastolic dysfunction does not co-exist with ischemia, ischemia-reperfusion or coronary artery occlusion-reperfusion, ischemic heart disease, myocardial injury, myocardial toxicity, myocardial infarction, congenital heart lesion, valvular stenosis or valvular regurgitation, coronary artery disease, chronic angina, chronic stable angina, arrhythmias.

**[0030]** In further aspects of the invention disclosed herein, the diastolic dysfunction is characterized by (i) a lack of increased late $I_{Na}$ in cardiomyocytes, (ii) an increase in myofilament calcium sensitivity, or (iii) a combination thereof.

**[0031]** *Late $I_{Na}$ in cardiomyocytes*

**[0032]** Late $I_{Na}$ and methods of measuring late $I_{Na}$ are described herein, as well, as in Fraser et al., *J Molecular and Cell Cardiology* 41: 1031-1038 (2006), which describes late $I_{Na}$ as a sustained/persistent influx of Na+, due to slowed or incomplete inactivation of voltage-gated sodium channels in the myocardium. In some sub-types of diastolic dysfunction, the diastolic dysfunction is hallmarked by an increase in the late $I_{Na}$. In some embodiments of the present

disclosures, this sub-type of diastolic dysfunction is different from the diastolic dysfunction treatable by the methods described herein, inasmuch as, in some embodiments, the diastolic dysfunction treatable by the methods described herein lack a substantial increase in late $I_{Na}$. In some aspects, the diastolic dysfunction is characterized by a late $I_{Na}$ which is substantially similar to the late $I_{Na}$. of subjects not suffering from diastolic dysfunction. In some aspects, the diastolic dysfunction is characterized by a ratio of the mean accumulated late Na+ charge to the mean accumulated total Na+ charge during an activating voltage step which is substantially similar to the ratio of the mean accumulated late Na+ charge to the mean accumulated total Na+ charge during an activating voltage step in a corresponding control (e.g., in a subject that does not suffer from diastolic dysfunction). In some aspects, the diastolic dysfunction is characterized by a ratio of the mean accumulated late Na+ charge to the mean accumulated total Na+ charge during an activating voltage step which differs from the ratio of the mean accumulated late Na+ charge to the mean accumulated total Na+ charge during an activating voltage step of a corresponding control (e.g., in a subject that does not suffer from diastolic dysfunction) by no more than 5% (e.g., no more than 4%, no more than 3%, no more than 2%, no more than 1%, no more than 0.75%, no more than 0.5%, no more than 0.4%, nor more than 0.3%, no more than 0.25%, no more than 0.2%, no more than 0.15%, no more than 0.1%, no more than 0.01%, no more than 0.001%). In some aspects, the late $I_{Na}$ is measured as essentially described in the EXAMPLES section set forth below. Accordingly, in some aspects, integrated late $I_{Na}$ is measured starting at 5% of peak current and ending 40 ms after depolarization.

**[0033]** In some embodiments, the late $I_{Na}$ in normal conditions (e.g., in subjects not suffering from diastolic dysfunction) constitutes only about 1% of peak, or total $I_{Na}$. See, for example, Dobesh et al., *Pharmacotherapy*, 27:1659-1675 (2007). Accordingly, in some aspects herein, a "lack of increased late $I_{Na}$" is a late $I_{Na}$ which is about 1% of peak, or total $I_{Na}$. In this regard, the diastolic dysfunction in some aspects is characterized by a late $I_{Na}$ which is about 1% of peak, or total $I_{Na}$. In some aspects, the diastolic dysfunction is characterized by a late $I_{Na}$ which is about 1.5% to about 0.5% of peak or total $I_{Na}$. In exemplary aspects, the diastolic dysfunction is characterized by a late $I_{Na}$ which is about 1.25% to about 0.75% of peak or total $I_{Na}$. In some aspects, diastolic dysfunction is characterized by a late $I_{Na}$ which is about 0.9% to about 1.1% or about 0.8% to about 1.2% of peak or total $I_{Na}$.

**[0034]** *Myofilament calcium sensitivity*

**[0035]** For contraction to occur, cardiac muscle, which is composed of alternating segments of thin and thick myofilaments, requires an inward flux of extracellular calcium ions through L-type calcium channels. In some embodiments, the diastolic dysfunction is characterized by an increased myofilament calcium sensitivity. In some aspects, the myofilament calcium sensitivity is indexed by $pCa_{50}$, which is the logarithm of the calcium concentration at which myofilaments generate 50% of the total maximum tension. In some aspects, the diastolic dysfunction is characterized by a $pCa_{50}$ which is increased by about 0.1% to about 5%, about 0.5% to about 2%, about 1% to about 1.5%, as compared to the $pCa_{50}$ of myofilaments of subjects not suffering from diastolic dysfunction. In some aspects, the diastolic dysfunction is characterized by a $pCa_{50}$ which is increased by about 0.1%, about 0.2%, about 0.3%, about 0.4%, about 0.5%, about 0.6%, about 0.7%, about 0.8%, about 0.9%, about 1%, about 1.1%, about 1.2%, about 1.3%, about 1.4%, about 1.5%, about 1.6%, about 1.7%, about 1.8%, about 1.9%, about 2%, about, 2.1%, about 2.2%, about 2.3%, about 2.4%, about 2.5%, about 2.6%, about 2.7%, about 2.8%, about 2.9%, about 3%, about 3.1%, about 3.2%, about 3.3%, about 3.4%, about 3.5%, about 3.6%, about 3.7%, about 3.8%, about 3.9%, about 4%, about 4.1%, about 4.2%, about 4.3%, about 4.4%, about 4.5%, about 4.6%, about 4.7%, about 4.8%, about 4.9%, or about 5%, as compared to the $pCa_{50}$ of myofilaments of subjects not suffering from diastolic dysfunction.

**[0036]** In some aspects, the myofilament calcium sensitivity is represented by the pCa-tension relations normalized to maximum tension, and the diastolic dysfunction is characterized by a pCa-tension relations normalized to maximum tension which is increased by about 0.1% to about 5%, about 0.5% to about 2%, about 1% to about 1.5%, as compared to the pCa-tension relations normalized to maximum tension of myofilaments of subjects not suffering from diastolic dysfunction. In some aspects, the diastolic dysfunction is characterized by a pCa-tension relations normalized to maximum tension which is increased by about 0.1%, about 0.2%, about 0.3%, about 0.4%, about 0.5%, about 0.6%, about 0.7%, about 0.8%, about 0.9%, about 1%, about 1.1%, about 1.2%, about 1.3%, about 1.4%, about 1.5%, about 1.6%, about 1.7%, about 1.8%, about 1.9%, about 2%, about, 2.1%, about 2.2%, about 2.3%, about 2.4%, about 2.5%, about 2.6%, about 2.7%, about 2.8%, about 2.9%, about 3%, about 3.1%, about 3.2%, about 3.3%, about 3.4%, about 3.5%, about 3.6%, about 3.7%, about 3.8%, about 3.9%, about 4%, about 4.1%, about 4.2%, about 4.3%, about 4.4%, about 4.5%, about 4.6%, about 4.7%, about

4.8%, about 4.9%, or about 5%, as compared to the $pCa_{50}$ of myofilaments of subjects not suffering from diastolic dysfunction.

**[0037]** Methods of measuring myofilament calcium sensitivity are known in the art (e.g., Varian et al., *Am J Physiol Heart Circ Physiol* 290:H2092-H2097 (2006)) and are described in the EXAMPLES section set forth herein.

**[0038]** *Additional characteristics*

**[0039]** In yet further aspects, the diastolic dysfunction is characterized by (i) a lack of change in calcium cycling or calcium handling in cardiomyocytes; (ii) a lack of change in calcium concentration in resting myocytes; (iii) a decrease in sarcomere length in resting myocytes; (iv) an increase in diastolic tension; or (v) a combination thereof.

**[0040]** *Intracellular calcium cycling or calcium handling and calcium concentration in resting myocytes*

**[0041]** When there is an increase in late $I_{Na}$, the influx of $Ca^{2+}$ through the reverse mode of the $Na^{+}$-$Ca^{2+}$ exchanger increases, resulting in an overload or increase in intracellular $Ca^{2+}$. In some embodiments herein, the diastolic dysfunction treated by the invention is characterized by substantially unchanged calcium cycling and/or by substantially unchanged intracellular calcium concentrations, as compared to the calcium cycling and/or intracellular calcium concentrations of myocytes of subjects not afflicted with diastolic dysfunction.

**[0042]** In some aspects, the calcium cycling or calcium flux in the myocardiocytes are similar to those found in the myocardiocytes of subjects not suffering from diastolic dysfunction. In some aspects, the baseline $Ca^{2+}$, the peak $Ca^{2+}$, the rate of $Ca^{2+}$ release, and/or the rate of intracellular $Ca^{2+}$ egress are considered normal or substantially unchanged. In some aspects, the calcium concentration of resting myocytes are substantially unchanged, as compared to the calcium concentration of resting myocytes of subjects not suffering from diastolic dysfunction. By "substantially unchanged" as used herein means that the parameter (e.g., intracellular calcium ($Ca^{2+}$) cycling, baseline $Ca^{2+}$, the peak $Ca^{2+}$, the rate of $Ca^{2+}$ release, the rate of intracellular $Ca^{2+}$ egress, calcium concentration of resting myocytes) differs by no more than about 10% (e.g., about 10% or less, about 9% or less, about 8% or less, about 7% or less, about 6% or less, about 5% or less, about 4% or less, about 3% or less, about 2% or less, about 1% or less, about 0.9% or less, about 0.8% or less, about 0.7% or less, about 0.6% or less, about 0.5% or less, about 0.4%

or less, about 0.3% or less, about 0.2% or less, about 0.1% or less), the parameter exhibited by myocytes of a subject not suffering from diastolic dysfunction.

**[0043]** Methods of measuring calcium cycling are known in the art and include, for example, the calcium experiments described in the EXAMPLES section set forth below.

**[0044]** *Unloaded sarcomere length in resting myocytes*

**[0045]** In some embodiments, the diastolic dysfunction is characterized by a decreased unloaded sarcomere length in isolated resting myocytes. In some aspects, the diastolic dysfunction is characterized by a decreased baseline sarcomere length in isolated resting myocytes or by a decreased mean diastolic sarcomere length in isolated resting myocytes. In some aspects, the decrease in unloaded sarcomere length, baseline sarcomere length, or mean diastolic sarcomere length is a decrease of about 5% (e.g., about 4.5%, about 4%, about 3.5%, about 3.0%, about 2.5%, about 2%, about 1.5%, about 1%, about 0.5%) of the unloaded sarcomere length, baseline sarcomere length, or mean diastolic sarcomere length of isolated resting myocytes of subjects not suffering from diastolic dysfunction. In some aspects, the decrease in unloaded sarcomere length, baseline sarcomere length, or mean diastolic sarcomere length in isolated resting myocytes is measured by the procedures described in the EXAMPLES section set forth below.

**[0046]** *Diastolic Tension*

**[0047]** In some embodiments, the diastolic tension is increased, as compared to the diastolic tension of subjects not suffering from diastolic dysfunction. In some aspects, the diastolic tension is increased by about 50% or less (e.g., about 25% or less, about 15% or less, about 10% or less, about 5% or less, about 4% or less, about 2.5% or less, about 1% or less, about 0.5% or less). Methods of measuring diastolic tension are known in the art (e.g., Sossalla et al., (2008), *supra* and in the EXAMPLES section set forth herein.

**[0048]** *Heart failure with preserved ejection fraction or with preserved left ventricular function*

**[0049]** Heart failure with preserved ejection fraction, which is also known as, heart failure with preserved systolic function, heart failure without systolic dysfunction, and heart failure with preserved left ventricular function, is a clinical condition in which the subject exhibits a preserved ejection fraction (e.g., an ejection fraction which is greater than or about 45%, or

greater than or about 50%) along with signs and/or symptoms of heart failure. The signs and symptoms of heart failure in some embodiments include dyspnea, fatigue, exercise intolerance, jugular venous distension, pulmonary rales, peripheral edema, pulmonary vascular redistribution, interstitial edema, pleural effusions.

**[0050]** Because diastolic dysfunction can lead to heart failure with preserved ejection fraction, the invention also provides a method of treating or preventing heart failure with preserved ejection fraction. The method comprises administering a cardiac metabolic modifier comprising a structure of Formula I, as described herein, to the subject in an amount effective to treat or prevent the heart failure with preserved ejection fraction.

**[0051]** In specific aspects, the heart failure with preserved ejection fraction is characterized by (i) a lack of increased late $I_{Na}$ in cardiomyocytes, (ii) an increase in myofilament calcium sensitivity, or (iii) a combination thereof. In further embodiments, the heart failure with preserved ejection fraction is characterized by (i) a lack of change in calcium cycling or calcium handling in cardiomyocytes; (ii) a lack of change in calcium concentration in resting myocytes; (iii) a decrease in sarcomere length in resting myocytes; (iv) an increase in diastolic tension; or (v) a combination thereof. Such characteristics are described herein and are applicable to the methods of treating or preventing heart failure with preserved ejection fraction.

**[0052]** In some embodiments, the heart failure is a Class III or Class IV heart failure, as defined by the New York Heart Association (NYHA). In some aspects, the heart failure is not a Class I or Class II heart failure, as defined by the NYHA. See, for example, The Criteria Committee of the New York Heart Association. *Nomenclature and Criteria for Diagnosis of Diseases of the Heart and Great Vessels*. 9th ed. Boston, Mass: Little, Brown & Co; 1994:253-256.. Accordingly, in some embodiments, the heart failure is heart failure in which the subject presents the symptoms of NYHA Class III or Class IV as indicated in the following table. In some embodiments, the heart failure is heart failure in which the subject does not present any of the symptoms of NYHA Class I or II as indicated in the following table.

| NYHA Class | Symptoms |
|---|---|
| I | No symptoms and no limitation in ordinary physical activity, e.g. shortness of breath when walking, climbing stairs etc. |
| II | Mild symptoms (mild shortness of breath and/or angina) and slight limitation during ordinary activity. |
| III | Marked limitation in activity due to symptoms, even during less-than-ordinary activity, e.g. walking short distances (20–100 m).<br>Comfortable only at rest. |
| IV | Severe limitations. Experiences symptoms even while **at rest**. Mostly bedbound patients. |

**[0053]** *Modulation of myofilament calcium sensitivity*

**[0054]** In addition to the treatment and prevention methods provided herein, the invention additionally provides a method of modulating myofilament calcium sensitivity in a subject. The method comprises administering a cardiac metabolic modifier comprising a structure of Formula I, as further described herein, to the subject in an amount effective to modulate myofilament calcium sensitivity. In some aspects, the method reduces the myofilament calcium sensitivity.

**[0055]** In some aspects, the myofilament calcium sensitivity is indexed by $pCa_{50}$, which is the logarithm of the calcium concentration at which myofilaments generate 50% of the total maximum tension. In some aspects, the $pCa_{50}$ is reduced by about 0.1% to about 5%, about 0.5% to about 2%, about 1% to about 1.5% upon administration of the cardiac metabolic modifier or as compared to the $pCa_{50}$ before administration of the cardiac metabolic modifier. In some aspects, the $pCa_{50}$ is reduced by about 0.1%, about 0.2%, about 0.3%, about 0.4%, about 0.5%, about 0.6%, about 0.7%, about 0.8%, about 0.9%, about 1%, about 1.1%, about 1.2%, about 1.3%, about 1.4%, about 1.5%, about 1.6%, about 1.7%, about 1.8%, about 1.9%, about 2%, about, 2.1%, about 2.2%, about 2.3%, about 2.4%, about 2.5%, about 2.6%, about 2.7%, about 2.8%, about 2.9%, about 3%, about 3.1%, about 3.2%, about 3.3%, about 3.4%, about 3.5%, about 3.6%, about 3.7%, about 3.8%, about 3.9%, about 4%, about 4.1%, about 4.2%, about 4.3%, about 4.4%, about 4.5%, about 4.6%, about 4.7%, about 4.8%, about 4.9%, or about 5% upon administration of the cardiac metabolic modifier or as compared to the $pCa_{50}$ before administration of the cardiac metabolic modifier.

**[0056]** In some aspects, the myofilament calcium sensitivity is represented by the pCa-tension relations normalized to maximum tension, and the method reduces the pCa-tension relations

normalized to maximum tension by about 0.1% to about 5%, about 0.5% to about 2%, about 1% to about 1.5% upon administration of the cardiac metabolic modifier or as compared to the pCa-tension relations normalized to maximum tension before administration of the cardiac metabolic modifier. In some aspects, the pCa-tension relations normalized to maximum tension is reduced by about 0.1%, about 0.2%, about 0.3%, about 0.4%, about 0.5%, about 0.6%, about 0.7%, about 0.8%, about 0.9%, about 1%, about 1.1%, about 1.2%, about 1.3%, about 1.4%, about 1.5%, about 1.6%, about 1.7%, about 1.8%, about 1.9%, about 2%, about, 2.1%, about 2.2%, about 2.3%, about 2.4%, about 2.5%, about 2.6%, about 2.7%, about 2.8%, about 2.9%, about 3%, about 3.1%, about 3.2%, about 3.3%, about 3.4%, about 3.5%, about 3.6%, about 3.7%, about 3.8%, about 3.9%, about 4%, about 4.1%, about 4.2%, about 4.3%, about 4.4%, about 4.5%, about 4.6%, about 4.7%, about 4.8%, about 4.9%, or about 5% upon administration of the cardiac metabolic modifier or as compared to the pCa-tension relations normalized to maximum tension before administration of the cardiac metabolic modifier.

**[0057]** In some embodiments, the subject suffers from diastolic dysfunction, such as any of the sub-types of diastolic dysfunction described herein. See, for example, the section entitled *Diastolic Dysfunction.*

**[0058]** Because increased myofilament calcium sensitivity is associated with or causes a number of medical conditions, including, for example, cardiac diseases (e.g., hypertrophic cardiomyopathy), it is postulated that reducing myofilament calcium sensitivity may effectively treat a condition associated with or caused by increased myofilament calcium sensitivity in a subject. Accordingly, the invention furthermore provides a method of treating a condition associated with or caused by increased myofilament calcium sensitivity in a subject. The method comprises administering a cardiac metabolic modifier comprising a structure of Formula I, as described herein, to the subject in an amount effective to treat the condition. In some embodiments, the subject suffers from diastolic dysfunction, such as any of the sub-types of diastolic dysfunction described herein. See, for example, the section entitled *Diastolic Dysfunction.*

**[0059]** The terms "treat," "prevent," "reduce" and "increase" as well as words stemming therefrom, as used herein, do not necessarily imply 100% or complete treatment, prevention, reduction, or increase. Rather, there are varying degrees of treatment, prevention, reduction, or increase of which one of ordinary skill in the art recognizes as having a potential benefit or

therapeutic effect. In this respect, the methods of the present disclosures can provide any amount of any level of treatment of diastolic dysfunction, e.g., diastolic dysfunction with preserved ejection fraction, treatment or prevention of heart failure with a preserved ejection fraction in a subject. Furthermore, the treatment or prevention provided by the method of the present disclosures can include treatment or prevention of one or more conditions or symptoms of the disease, e.g., diastolic dysfunction with preserved ejection fraction, heart failure with preserved ejection fraction, being treated or prevented. Also, for purposes herein, "prevention" can encompass delaying the onset of the disease, or a symptom or condition thereof.

**[0060]** *Cardiac Metabolic Modifiers*

**[0061]** As used herein, the term "cardiac metabolic modifier" refers to a compound which modulates a myocardial metabolic pathway to influence (e.g., improve, maintain) cardiac efficiency. In some embodiments, the cardiac metabolic modifier directly modulates a myocardial metabolic pathway to influence (e.g., improve, maintain) cardiac efficiency. In alternative embodiments, the cardiac metabolic modifier indirectly modulates a myocardial metabolic pathway to influence (e.g., improve, maintain) cardiac efficiency. As used herein, the term "cardiac efficiency" refers to the ratio between the work produced (blood pumped) and input of energy in order to make the heart work. More specifically, cardiac efficiency is the ratio of stroke work to oxygen consumption. Methods of measuring cardiac efficiency are known in the art, and include invasive procedures, e.g., cardiac catheterization, and non-invasive procedures, e.g., positron emission topograph (PET). See, for example, Steendijk et al., *Heart Metab* 39: 33-36(2008), Knaapen et al., *Heart Metab.* 39:14-19(2008). In some aspects, the cardiac metabolic modifier influences cardiac efficiency, such that a cardiac efficiency of about 20% to about 25% (e.g., 20%, 21%, 22%, 23%, 24%, 25%) is attained or maintained.

**[0062]** In some embodiments, the cardiac metabolic modifier modulates one or more of: fatty acid oxidation in the myocardium, carbohydrate oxidation in the myocardium, glycolysis in the myocardium, myofilament calcium sensitivity, ion channel activity in the myocardium, or sensitivity to insulin in the myocardium.

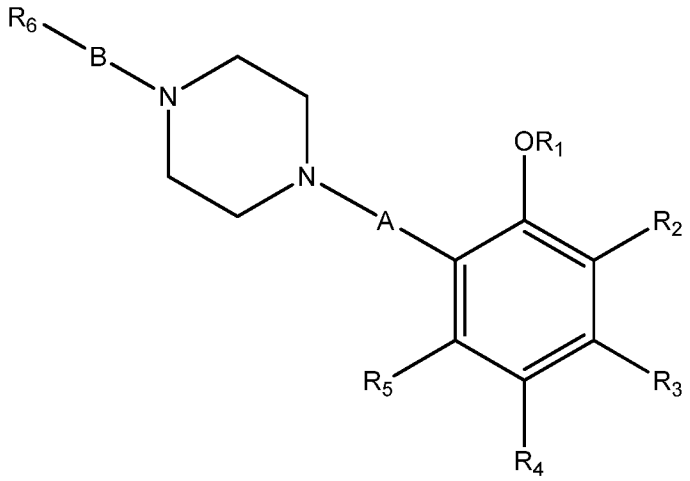
**[0063]** In some embodiments, the cardiac metabolic modifier inhibits fatty acid oxidation. Methods of assaying fatty acid oxidation are known in the art. See, for example, Wang et al., *J Pharmacology and Experimental Therapeutics* 321: 213-220 (2007).

**[0064]** In some embodiments, the cardiac metabolic modifier alters carbohydrate oxidation. Methods of assaying carbohydrate oxidation are known in the art. See, for example, Wang et al., *J Pharmacology and Experimental Therapeutics* 321: 213-220 (2007).

**[0065]** Alternatively or additionally, in some embodiments, the cardiac metabolic modifier lowers myofilament calcium sensitivity. Methods of assaying myofilament calcium sensitivity are known in the art and in the EXAMPLES section set forth below.

**[0066]** Alternatively or additionally, in some embodiments, the cardiac metabolic modifier inhibits an ion channel, such as a sodium ion channel, a calcium ion channel, a potassium channel. Methods of assaying inhibition of ion channels are known in the art and include, for example, Borgland et al., *J Physiology* 536: 35-47 (2001).

**[0067]** In some embodiments, the cardiac metabolic modifier comprises a structure of Formula I:




[Formula I]

wherein A comprises a main chain of 1-8 atoms, each atom of which is independently C, O, N, or S, and each atom or which is optionally bound to an additional group selected from C1-C8 alkyl, C1-C8 alkoxy, OH, $NH_2$, NH(C1-C4 alkyl) and SH;

wherein $R_1$ is H or a C1-C8 alkyl;

wherein each of $R_2$, $R_3$, $R_4$, and $R_5$ independently is H, a C1-C8 alkyl, or a C1-C8 alkoxy;

wherein B is H or comprises a main chain of 1-8 atoms, each atom of which is independently C, O, N, or S, and each atom of which is optionally bound to an additional group; and,

wherein $R_6$ is absent or phenyl, which phenyl is optionally substituted with 1 to 5 groups, each group of which is independently C1-C8 alkyl, C1-C8 alkoxy, or OH.

**[0068]** As used herein, "alkyl" refers to straight chained and branched saturated hydrocarbon groups, nonlimiting examples of which include methyl, ethyl, and straight and branched propyl, butyl, pentyl, hexyl, heptyl, and octyl groups containing the indicated number of carbon atoms. The term Cn means the alkyl group has "n" carbon atoms. For example, C1-C7 alkyl refers to alkyl groups having a number of carbon atoms encompassing the entire range (i.e., 1 to 7 carbon atoms), as well as all subgroups (e.g., 1-6, 2-7, 1-5, 3-6, 1, 2, 3, 4, 5, 6, and 7 carbon atoms).. Accordingly, the C1-C8 alkyl can be a methyl, ethyl, propyl, butyl, C5 alkyl, C6 alkyl, C7 alkyl, or C8 alkyl, of which the propyl, butyl, C5 alkyl, C6 alkyl, C7 alkyl, or C8 alkyl is a straight chain alkyl or branched alkyl.

**[0069]** As used herein "alkoxy" refers to –OR, wherein R is alkyl (e.g., a straight or branched chain alkyl group). Examples of alkoxy groups include, but are not limited to, methoxy, ethoxy, propoxy, isopropoxy, butoxy, n-butoxy, sec-butoxy, t-butoxy and the like. Accordingly, the C1-C8 alkoxy can be methoxy, ethoxy, C3 alkoxy, C4 alkoxy, C5 alkoxy, C6 alkoxy, C7 alkoxy, or C8 alkoxy, or which the C3 alkoxy, C4 alkoxy, C5 alkoxy, C6 alkoxy, C7 alkoxy, or C8 alkoxy is a straight chain alkoxy or branched alkoxy.

**[0070]** As used herein "NH(C1-C4 alkyl)" refers to nitrogen bound to both H and a C1-C4 alkyl.

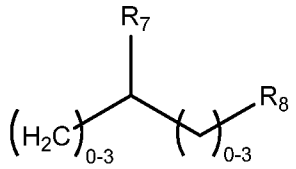
**[0071]** With regard to Formula I, A comprises a main chain of 1-8 atoms, each atom of which is independently C, O, N, or S. In some aspects, A comprises a main chain of a single atom selected from C, O, N, and S. In some aspects, A comprises a main chain of 2-8 atoms (e.g., 2, 3, 4, 5, 6, 7, or 8 atoms), each atom of which is independently C, O, N, or S.

**[0072]** Each atom of the main chain of A is optionally bound to an additional group. In some aspects, the additional group is selected from C1-C8 alkyl, C1-C8 alkoxy, OH, NH2, NH(C1-C4 alkyl) and SH. In some aspects, every atom of the main chain is bound to an additional group. In other aspects, one or more, but not all, atoms of the main chain are bound to an additional

group. In some instances, one atom of the main chain is bound to an additional group. In some instances, 2, 3, 4, 5, 6, or 7 atoms of the main chain is bound to an additional group.

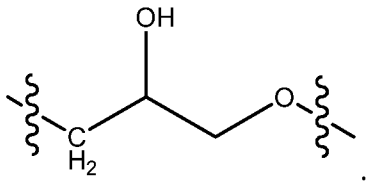
**[0073]** In some aspects, A is $(CH_2)_{1-8}$. In some aspects, A is $(CH_2)_{1-6}$. In some aspects, A is $(CH_2)_{1-4}$. In some aspects, A is $(CH_2)_1$ or $(CH_2)_2$. In some aspects, A is $(CH_2)_1$.

**[0074]** In other aspects, A comprises a structure of Formula IV:




[Formula IV];

wherein $R_7$ is OH, C1-C4 alkyl, C1-C4 alkoxy, NH2, or NH(C1-C4 alkyl); and wherein $R_8$ is O or NH. In some aspects, when A comprises a structure of Formula IV, $R_7$ is OH and $R_8$ is O or NH. In some aspects, when A comprises a structure of Formula IV, $R_7$ is OH, C1-C4 alkyl, C1-C4 alkoxy, NH2, or NH(C1-C4 alkyl) and $R_8$ is O. In particular aspects, when A comprises a structure of Formula IV, $R_7$ is OH and $R_8$ is O. In some aspects, A comprises



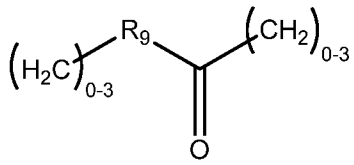

**[0075]** With regard to Formula I, $R_1$ is H or a C1-C8 alkyl. In particular aspects, $R_1$ is $CH_3$.

**[0076]** With regard to Formula I, each of $R_2$, $R_3$, $R_4$, and $R_5$ independently is H, a C1-C8 alkyl, or a C1-C8 alkoxy. In some aspects, each of $R_2$, $R_3$, $R_4$, and $R_5$ independently is H or methoxy. In alternative embodiments, each of $R_2$ and $R_3$ is a methoxy, and each of $R_4$ and $R_5$ is H.

**[0077]** With regard to Formula I, B is H or comprises a main chain of 1-8 atoms, each atom of which is independently C, O, N, or S. In some aspects, B comprises a main chain of a single atom selected from C, O, N, and S, while in other aspects, B comprises a main chain of 2-8 atoms (e.g., 2, 3, 4, 5, 6, 7, or 8 atoms), each atom of which is independently selected from C, O, N, and S.

**[0078]** Each atom of the main chain of B is optionally bound to an additional group. In some aspects, the additional group is selected from C1-C8 alkyl, C1-C8 alkoxy, OH, NH2, NH(C1-C4 alkyl) and SH. In some aspects, every atom of the main chain is bound to an additional group. In other aspects, one or more, but not all, atoms of the main chain are bound to an additional group. In some instances, one atom of the main chain is bound to an additional group. In some instances, 2, 3, 4, 5, 6, or 7 atoms of the main chain is bound to an additional group.

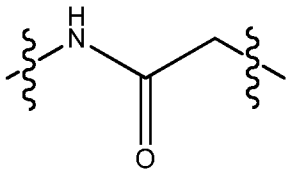
**[0079]** In some embodiments, B comprises H and $R_6$ is absent. In alternative embodiments, B comprises a structure of Formula V:



[Formula V],

wherein $R_9$ is NH or O.

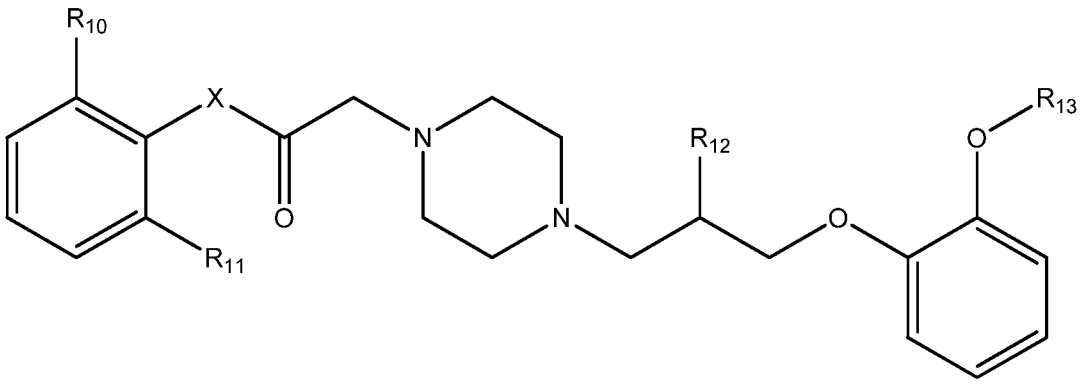
**[0080]** In some embodiments, when B comprises a structure of Formula V, R9 is NH. In further aspects, B comprises a structure of



.

**[0081]** With regard to Formula I, $R_6$ is absent or phenyl, which phenyl is optionally substituted with 1 to 5 (e.g., 1, 2, 3, 4, 5) groups, each group of which is independently selected from C1-C8 alkyl, C1-C8 alkoxy, and OH. In some aspects, $R_6$ is absent. In alternative aspects, when $R_6$ is present and comprises phenyl substituted with 1 to 5 (e.g., 1, 2, 3, 4, 5) methyl groups. In specific aspects, $R_6$ comprises phenyl substituted with two methyl groups, one at each of the ortho positions.

**[0082]** In some aspects, the cardiac metabolic modifier comprises a compound of Formula II, or a pharmaceutically acceptable salt thereof or a conjugate thereof:



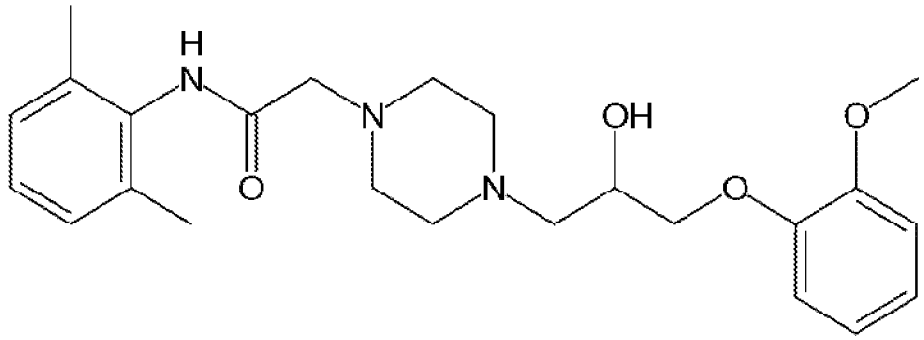

[Formula II]

wherein each of $R_{10}$, $R_{11}$, and $R_{13}$ independently is C1-C3 alkyl,

wherein X is NH or O; and

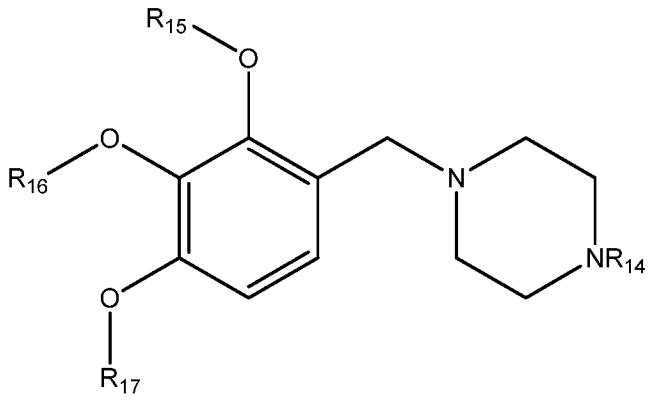
wherein $R_{12}$ is OH or C1-C3 alkyl.

**[0083]** In some embodiments, the compound of Formula II comprises the following structure:




In some aspects, the compound of Formula II is ranolazine, or a pharmaceutically acceptable salt or a conjugate of ranolazine.

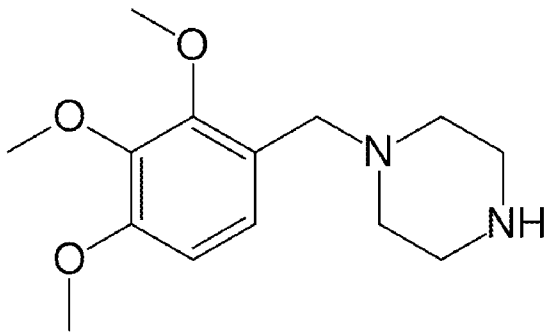
**[0084]** In some aspects, the cardiac metabolic modifier is a compound of Formula III, or a pharmaceutically acceptable salt thereof or a conjugate thereof:




[Formula III]

wherein each of $R_{14}$, $R_{15}$, $R_{16}$, and $R_{17}$ independently is H or C1-C3 alkyl.

**[0085]** In some embodiments, the compound of Formula II comprises the following structure:




**[0086]** In some aspects, the compound of Formula III is trimetazidine, or a pharmaceutically acceptable salt or a conjugate of trimetazidine.

**[0087]** *Pharmaceutically acceptable salts*

**[0088]** In some embodiments, the cardiac metabolic modifiers are in the form of a salt, e.g., a pharmaceutically acceptable salt. Such salts can be prepared in situ during the final isolation and purification of the cardiac metabolic modifier or separately prepared by reacting a free base function with a suitable acid. Examples of acids which can be employed to form pharmaceutically acceptable acid addition salts include, for example, an inorganic acid, e.g., hydrochloric acid, hydrobromic acid, sulphuric acid, and phosphoric acid, and an organic acid, e.g., oxalic acid, maleic acid, succinic acid, and citric acid.

**[0089]** Representative acid addition salts include, but are not limited to acetate, adipate, alginate, citrate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, camphorate, camphor

sulfonate, digluconate, glycerophosphate, hemisulfate, heptanoate, hexanoate, fumarate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethansulfonate (isothionate), lactate, maleate, methane sulfonate, nicotinate, 2-naphthalene sulfonate, oxalate, palmitoate, pectinate, persulfate, 3-phenylpropionate, picrate, pivalate, propionate, succinate, tartrate, thiocyanate, phosphate, glutamate, bicarbonate, p-toluenesulfonate, and undecanoate.

**[0090]** Basic addition salts also can be prepared in situ during the final isolation and purification of the cardiac metabolic modifier, or by reacting a carboxylic acid-containing moiety with a suitable base such as the hydroxide, carbonate, or bicarbonate of a pharmaceutically acceptable metal cation or with ammonia or an organic primary, secondary, or tertiary amine. Pharmaceutically acceptable salts include, but are not limited to, cations based on alkali metals or alkaline earth metals such as lithium, sodium, potassium, calcium, magnesium, and aluminum salts, and the like, and nontoxic quaternary ammonia and amine cations including ammonium, tetramethylammonium, tetraethylammonium, methylammonium, dimethylammonium, trimethylammonium, triethylammonium, diethylammonium, and ethylammonium, amongst others. Other representative organic amines useful for the formation of base addition salts include, for example, ethylenediamine, ethanolamine, diethanolamine, piperidine, piperazine, and the like.

**[0091]** Further, basic nitrogen-containing groups can be quaternized with such cardiac metabolic modifiers as lower alkyl halides such as methyl, ethyl, propyl, and butyl chlorides, bromides, and iodides; long chain halides such as decyl, lauryl, myristyl, and stearyl chlorides, bromides, and iodides; arylalkyl halides like benzyl and phenethyl bromides and others. Water or oil-soluble or dispersible products are thereby obtained.

**[0092]** *Conjugates*

**[0093]** In some embodiments, the cardiac metabolic modifier is in the form of a conjugate, e.g., is conjugated to a heterologous moiety. As used herein, the term "heterologous moiety" is synonymous with the term "conjugate moiety" and refers to any molecule (chemical or biochemical, naturally-occurring or non-coded) which is different from the cardiac metabolic modifiers described herein. Exemplary conjugate moieties that can be linked to any of the cardiac metabolic modifiers described herein include but are not limited to a heterologous peptide or polypeptide (including for example, a plasma protein), a targeting agent, an immunoglobulin or portion thereof (e.g.,variable region, CDR, or Fc region), a diagnostic label

such as a radioisotope, fluorophore or enzymatic label, a polymer including water soluble polymers, or other therapeutic or diagnostic agents. In some embodiments a conjugate is provided comprising a cardiac metabolic modifier and a plasma protein, wherein the plasma protein is selected from the group consisting of albumin, transferin, fibrinogen and globulins. In some embodiments the plasma protein moiety of the conjugate is albumin or transferin. The conjugate in some embodiments comprises one or more of the cardiac metabolic modifiers described herein and one or more of: a peptide, a polypeptide, a nucleic acid molecule, an antibody or fragment thereof, a polymer, a quantum dot, a small molecule (which is distinct from the cardiac metabolic modifiers described herein), a toxin, a diagnostic agent, a carbohydrate, an amino acid.

**[0094]** In some embodiments, the heterologous moiety is a polymer. In some embodiments, the polymer is selected from the group consisting of: polyamides, polycarbonates, polyalkylenes and derivatives thereof including, polyalkylene glycols, polyalkylene oxides, polyalkylene terepthalates, polymers of acrylic and methacrylic esters, including poly(methyl methacrylate), poly(ethyl methacrylate), poly(butylmethacrylate), poly(isobutyl methacrylate), poly(hexylmethacrylate), poly(isodecyl methacrylate), poly(lauryl methacrylate), poly(phenyl methacrylate), poly(methyl acrylate), poly(isopropyl acrylate), poly(isobutyl acrylate), and poly(octadecyl acrylate), polyvinyl polymers including polyvinyl alcohols, polyvinyl ethers, polyvinyl esters, polyvinyl halides, poly(vinyl acetate), and polyvinylpyrrolidone, polyglycolides, polysiloxanes, polyurethanes and co-polymers thereof, celluloses including alkyl cellulose, hydroxyalkyl celluloses, cellulose ethers, cellulose esters, nitro celluloses, methyl cellulose, ethyl cellulose, hydroxypropyl cellulose, hydroxy-propyl methyl cellulose, hydroxybutyl methyl cellulose, cellulose acetate, cellulose propionate, cellulose acetate butyrate, cellulose acetate phthalate, carboxylethyl cellulose, cellulose triacetate, and cellulose sulphate sodium salt, polypropylene, polyethylenes including poly(ethylene glycol), poly(ethylene oxide), and poly(ethylene terephthalate), and polystyrene.

**[0095]** In some aspects, the polymer is a biodegradable polymer, including a synthetic biodegradable polymer (e.g., polymers of lactic acid and glycolic acid, polyanhydrides, poly(ortho)esters, polyurethanes, poly(butic acid), poly(valeric acid), and poly(lactide-cocaprolactone)), and a natural biodegradable polymer (e.g., alginate and other polysaccharides including dextran and cellulose, collagen, chemical derivatives thereof (substitutions, additions of chemical groups, for example, alkyl, alkylene, hydroxylations, oxidations, and other

modifications routinely made by those skilled in the art), albumin and other hydrophilic proteins (e.g., zein and other prolamines and hydrophobic proteins)), as well as any copolymer or mixture thereof. In general, these materials degrade either by enzymatic hydrolysis or exposure to water *in vivo*, by surface or bulk erosion.

**[0096]** In some aspects, the polymer is a bioadhesive polymer, such as a bioerodible hydrogel described by H. S. Sawhney, C. P. Pathak and J. A. Hubbell in Macromolecules, 1993, 26, 581-587, the teachings of which are incorporated herein, polyhyaluronic acids, casein, gelatin, glutin, polyanhydrides, polyacrylic acid, alginate, chitosan, poly(methyl methacrylates), poly(ethyl methacrylates), poly(butylmethacrylate), poly(isobutyl methacrylate), poly(hexylmethacrylate), poly(isodecyl methacrylate), poly(lauryl methacrylate), poly(phenyl methacrylate), poly(methyl acrylate), poly(isopropyl acrylate), poly(isobutyl acrylate), and poly(octadecyl acrylate).

**[0097]** In some embodiments, the polymer is a water-soluble polymer or a hydrophilic polymer. Hydrophilic polymers are further described herein under "Hydrophilic Moieties." Suitable water-soluble polymers are known in the art and include, for example, polyvinylpyrrolidone, hydroxypropyl cellulose (HPC; Klucel), hydroxypropyl methylcellulose (HPMC; Methocel), nitrocellulose, hydroxypropyl ethylcellulose, hydroxypropyl butylcellulose, hydroxypropyl pentylcellulose, methyl cellulose, ethylcellulose (Ethocel), hydroxyethyl cellulose, various alkyl celluloses and hydroxyalkyl celluloses, various cellulose ethers, cellulose acetate, carboxymethyl cellulose, sodium carboxymethyl cellulose, calcium carboxymethyl cellulose, vinyl acetate/crotonic acid copolymers, poly-hydroxyalkyl methacrylate, hydroxymethyl methacrylate, methacrylic acid copolymers, polymethacrylic acid, polymethylmethacrylate, maleic anhydride/methyl vinyl ether copolymers, poly vinyl alcohol, sodium and calcium polyacrylic acid, polyacrylic acid, acidic carboxy polymers, carboxypolymethylene, carboxyvinyl polymers, polyoxyethylene polyoxypropylene copolymer, polymethylvinylether co-maleic anhydride, carboxymethylamide, potassium methacrylate divinylbenzene co-polymer, polyoxyethyleneglycols, polyethylene oxide, and derivatives, salts, and combinations thereof.

**[0098]** In specific embodiments, the polymer is a polyalkylene glycol, including, for example, polyethylene glycol (PEG).

**[0099]** In some embodiments, the heterologous moiety is a carbohydrate. In some embodiments, the carbohydrate is a monosaccharide (e.g., glucose, galactose, fructose), a

disaccharide (e.g., sucrose, lactose, maltose), an oligosaccharide (e.g., raffinose, stachyose), a polysaccharide (a starch, amylase, amylopectin, cellulose, chitin, callose, laminarin, xylan, mannan, fucoidan, galactomannan.

**[00100]** In some embodiments, the heterologous moiety is a lipid. The lipid, in some embodiments, is a fatty acid, eicosanoid, prostaglandin, leukotriene, thromboxane, N-acyl ethanolamine), glycerolipid (e.g., mono-, di-, tri-substituted glycerols), glycerophospholipid (e.g., phosphatidylcholine, phosphatidylinositol, phosphatidylethanolamine, phosphatidylserine), sphingolipid (e.g., sphingosine, ceramide), sterol lipid (e.g., steroid, cholesterol), prenol lipid, saccharolipid, or a polyketide, oil, wax, cholesterol, sterol, fat-soluble vitamin, monoglyceride, diglyceride, triglyceride, a phospholipid.

**[00101]** In some embodiments, the heterologous moiety is attached via non-covalent or covalent bonding to the cardiac metabolic modifier of the present disclosure. In certain aspects, the heterologous moiety is attached to the cardiac metabolic modifier of the present disclosure via a linker. Linkage can be accomplished by covalent chemical bonds, physical forces such electrostatic, hydrogen, ionic, van der Waals, or hydrophobic or hydrophilic interactions. A variety of non-covalent coupling systems may be used, including biotin-avidin, ligand/receptor, enzyme/substrate, nucleic acid/nucleic acid binding protein, lipid/lipid binding protein, cellular adhesion molecule partners; or any binding partners or fragments thereof which have affinity for each other.

**[00102]** The cardiac metabolic modifier in some embodiments is linked to conjugate moieties via direct covalent linkage. In some embodiments, reactive groups on the cardiac metabolic modifier or conjugate moiety include, e.g., an aldehyde, amino, ester, thiol, α-haloacetyl, maleimido or hydrazino group. Derivatizing agents include, for example, maleimidobenzoyl sulfosuccinimide ester, N-hydroxysuccinimide (through lysine residues), glutaraldehyde, succinic anhydride or other agents known in the art. Alternatively, the conjugate moieties can be linked to the cardiac metabolic modifier indirectly through intermediate carriers, such as polysaccharide or polypeptide carriers. Examples of polysaccharide carriers include aminodextran. Examples of suitable polypeptide carriers include polylysine, polyglutamic acid, polyaspartic acid, co-polymers thereof, and mixed polymers of these amino acids and others, e.g., serines, to confer desirable solubility properties on the resultant loaded carrier.

**[00103]** Carboxyl groups are selectively modified by reaction with carbodiimides (R-N=C=N-R'), where R and R' are different alkyl groups, such as 1-cyclohexyl-3-(2-morpholinyl-4-ethyl) carbodiimide or 1-ethyl-3-(4-azonia-4,4-dimethylpentyl) carbodiimide. Another type of covalent modification involves chemically or enzymatically coupling glycosides to the cardiac metabolic modifier. Sugar(s) may be attached to free carboxyl groups, free sulfhydryl groups, free hydroxyl groups or an amide group. These methods are described in WO87/05330 published 11 Sep. 1987, and in Aplin and Wriston, CRC Crit. Rev. Biochem., pp. 259-306 (1981).

**[00104]** In some embodiments, the conjugate comprises a linker that joins the cardiac metabolic modifier to the heterologous moiety. In some aspects, the linker comprises a chain of atoms from 1 to about 60, or 1 to 30 atoms or longer, 2 to 5 atoms, 2 to 10 atoms, 5 to 10 atoms, or 10 to 20 atoms long. In some embodiments, the chain atoms are all carbon atoms. In some embodiments, the chain atoms in the backbone of the linker are selected from the group consisting of C, O, N, and S. Chain atoms and linkers may be selected according to their expected solubility (hydrophilicity) so as to provide a more soluble conjugate. In some embodiments, the linker provides a functional group that is subject to cleavage by an enzyme or other catalyst or hydrolytic conditions found in the target tissue or organ or cell. In some embodiments, the length of the linker is long enough to reduce the potential for steric hindrance. In some embodiments, the linker is an amino acid or a peptidyl linker. Such peptidyl linkers may be any length. Exemplary linkers are from about 1 to 50 amino acids in length, 5 to 50, 3 to 5, 5 to 10, 5 to 15, or 10 to 30 amino acids in length.

**[00105]** *Conjugates: Hydrophilic moieties*

**[00106]** The cardiac metabolic modifiers described herein can be further modified to improve its solubility and stability in aqueous solutions at physiological pH, while retaining its biological activity. Hydrophilic moieties such as PEG groups can be attached to the cardiac metabolic modifiers under any suitable conditions known in the art, including, for example, via acylation, reductive alkylation, Michael addition, thiol alkylation or other chemoselective conjugation/ligation methods through a reactive group on the PEG moiety (e.g., an aldehyde, amino, ester, thiol, α-haloacetyl, maleimido or hydrazino group) to a reactive group on the target compound (e.g., an aldehyde, amino, ester, thiol, α-haloacetyl, maleimido or hydrazino group). Activating groups which can be used to link the water soluble polymer to one or more proteins include without limitation sulfone, maleimide, sulfhydryl, thiol, triflate, tresylate, azidirine,

oxirane, 5-pyridyl, and alpha-halogenated acyl group (e.g., alpha-iodo acetic acid, alpha-bromoacetic acid, alpha-chloroacetic acid). If attached to the cardiac metabolic modifier by reductive alkylation, the polymer selected should have a single reactive aldehyde so that the degree of polymerization is controlled. See, for example, Kinstler et al., *Adv. Drug. Delivery Rev.* 54: 477-485 (2002); Roberts et al., *Adv. Drug Delivery Rev.* 54: 459-476 (2002); and Zalipsky et al., *Adv. Drug Delivery Rev.* 16: 157-182 (1995).

**[00107]** Suitable hydrophilic moieties include polyethylene glycol (PEG), polypropylene glycol, polyoxyethylated polyols (e.g., POG), polyoxyethylated sorbitol, polyoxyethylated glucose, polyoxyethylated glycerol (POG), polyoxyalkylenes, polyethylene glycol propionaldehyde, copolymers of ethylene glycol/propylene glycol, monomethoxy-polyethylene glycol, mono-(C1-C10) alkoxy- or aryloxy-polyethylene glycol, carboxymethylcellulose, polyacetals, polyvinyl alcohol (PVA), polyvinyl pyrrolidone, poly-1, 3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, poly β-amino acids) (either homopolymers or random copolymers), poly(n-vinyl pyrrolidone)polyethylene glycol, propropylene glycol homopolymers (PPG) and other polyakylene oxides, polypropylene oxide/ethylene oxide copolymers, colonic acids or other polysaccharide polymers, Ficoll or dextran and mixtures thereof. Dextrans are polysaccharide polymers of glucose subunits, predominantly linked by α1-6 linkages. Dextran is available in many molecular weight ranges, e.g., about 1 kD to about 100 kD, or from about 5, 10, 15 or 20 kD to about 20, 30, 40, 50, 60, 70, 80 or 90 kD. Linear or branched polymers are contemplated. Resulting preparations of conjugates may be essentially monodisperse or polydisperse, and may have about 0.5, 0.7, 1, 1.2, 1.5 or 2 polymer moieties per compound.

**[00108]** *Conjugates: Multimers*

**[00109]** In some embodiments, the conjugate comprising the cardiac metabolic modifier is in the form of a multimer or dimer, including homo- or hetero- multimers or homo- or hetero-dimers. Two or more of the cardiac metabolic modifiers can be linked together using standard linking agents and procedures known to those skilled in the art. In certain embodiments, the linker connecting the two (or more) analogs is PEG, e.g., a 5 kDa PEG, 20 kDa PEG. In some embodiments, the linker is a disulfide bond. For example, each monomer of the dimer may comprise a sulfhydryl and the sulfur atom of each participates in the formation of the disulfide bond.

**[00110]** *Conjugates: Targeted Forms*

**[00111]** One of ordinary skill in the art will readily appreciate that the cardiac metabolic modifiers of the disclosure can be modified in any number of ways, such that the therapeutic or prophylactic efficacy of the cardiac metabolic modifier of the present disclosures is increased through the modification. For instance, the cardiac metabolic modifier of the present disclosure can be conjugated either directly or indirectly through a linker to a targeting moiety. The practice of conjugating compounds to targeting moieties is known in the art. See, for instance, Wadhwa et al., *J Drug Targeting*, 3, 111-127 (1995) and U.S. Patent No. 5,087,616. The term "targeting moiety" as used herein, refers to any molecule or agent that specifically recognizes and binds to a cell-surface receptor, such that the targeting moiety directs the delivery of the cardiac metabolic modifier of the present disclosures to a population of cells on which surface the receptor is expressed. Targeting moieties include, but are not limited to, antibodies, or fragments thereof, peptides, hormones, growth factors, cytokines, and any other natural or non-natural ligands, which bind to cell surface receptors (e.g., Epithelial Growth Factor Receptor (EGFR), T-cell receptor (TCR), B-cell receptor (BCR), CD28, Platelet-derived Growth Factor Receptor (PDGF), nicotinic acetylcholine receptor (nAChR), etc.). As used herein a "linker" is a bond, molecule or group of molecules that binds two separate entities to one another. Linkers may provide for optimal spacing of the two entities or may further supply a labile linkage that allows the two entities to be separated from each other. Labile linkages include photocleavable groups, acid-labile moieties, base-labile moieties and enzyme-cleavable groups. The term "linker" in some embodiments refers to any agent or molecule that bridges the cardiac metabolic modifier of the present disclosures to the targeting moiety. One of ordinary skill in the art recognizes that sites on the cardiac metabolic modifier of the present disclosures, which are not necessary for the function of the cardiac metabolic modifier, are ideal sites for attaching a linker and/or a targeting moiety, provided that the linker and/or targeting moiety, once attached to the cardiac metabolic modifier, do(es) not interfere with the function of the cardiac metabolic modifier, i.e., the ability to treat diastolic dysfunction, as described herien.

**[00112]** *Pharmaceutical Compositions and Formulations*

**[00113]** In some embodiments, the cardiac metabolic modifier of the present disclosures, the pharmaceutically acceptable salt thereof, or the conjugate comprising the cardiac metabolic modifier, is formulated into a pharmaceutical composition comprising the cardiac metabolic

modifier, the pharmaceutically acceptable salt thereof, or the conjugate comprising the cardiac metabolic modifier, along with a pharmaceutically acceptable carrier, diluent, or excipient.

**[00114]** In some embodiments, the cardiac metabolic modifier is present in the pharmaceutical composition at a purity level suitable for administration to a patient. In some embodiments, the cardiac metabolic modifier has a purity level of at least about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98% or about 99%, and a pharmaceutically acceptable diluent, carrier or excipient. The pharmaceutical composition in some aspects comprises the cardiac metabolic modifier of the present disclosure at a concentration of at least A, wherein A is about about 0.001 mg/ml, about 0.01 mg/ml, 0 about 1 mg/ml, about 0.5 mg/ml, about 1 mg/ml, about 2 mg/ml, about 3 mg/ml, about 4 mg/ml, about 5 mg/ml, about 6 mg/ml, about 7 mg/ml, about 8 mg/ml, about 9 mg/ml, about 10 mg/ml, about 11 mg/ml, about 12 mg/ml, about 13 mg/ml, about 14 mg/ml, about 15 mg/ml, about 16 mg/ml, about 17 mg/ml, about 18 mg/ml, about 19 mg/ml, about 20 mg/ml, about 21 mg/ml, about 22 mg/ml, about 23 mg/ml, about 24 mg/ml, about 25 mg/ml or higher. In some embodiments, the pharmaceutical composition comprises the cardiac metabolic modifier at a concentration of at most B, wherein B is about 30 mg/ml, about 25 mg/ml, about 24 mg/ml, about 23, mg/ml, about 22 mg/ml, about 21 mg/ml, about 20 mg/ml, about 19 mg/ml, about 18 mg/ml, about 17 mg/ml, about 16 mg/ml, about 15 mg/ml, about 14 mg/ml, about 13 mg/ml, about 12 mg/ml, about 11 mg/ml, about 10 mg/ml, about 9 mg/ml, about 8 mg/ml, about 7 mg/ml, about 6 mg/ml, about 5 mg/ml, about 4 mg/ml, about 3 mg/ml, about 2 mg/ml, about 1 mg/ml, or about 0.1 mg/ml. In some embodiments, the compositions may contain an cardiac metabolic modifier at a concentration range of A to B mg/ml, for example, about 0.001 to about 30.0 mg/ml.

**[00115]** Depending on the route of administration, the particular cardiac metabolic modifier intended for use, as well as other factors, the pharmaceutical composition may comprise additional pharmaceutically acceptable ingredients, including, for example, acidifying agents, additives, adsorbents, aerosol propellants, air displacement agents, alkalizing agents, anticaking agents, anticoagulants, antimicrobial preservatives, antioxidants, antiseptics, bases, binders, buffering agents, chelating agents, coating agents, coloring agents, desiccants, detergents, diluents, disinfectants, disintegrants, dispersing agents, dissolution enhancing agents, dyes, emollients, emulsifying agents, emulsion stabilizers, fillers, film forming agents, flavor enhancers, flavoring agents, flow enhancers, gelling agents, granulating agents, humectants, lubricants, mucoadhesives, ointment bases, ointments, oleaginous vehicles, organic bases,

pastille bases, pigments, plasticizers, polishing agents, preservatives, sequestering agents, skin penetrants, solubilizing agents, solvents, stabilizing agents, suppository bases, surface active agents, surfactants, suspending agents, sweetening agents, therapeutic agents, thickening agents, tonicity agents, toxicity agents, viscosity-increasing agents, water-absorbing agents, water-miscible cosolvents, water softeners, or wetting agents.

**[00116]** Accordingly, in some embodiments, the pharmaceutical composition comprises any one or a combination of the following components: acacia, acesulfame potassium, acetyltributyl citrate, acetyltriethyl citrate, agar, albumin, alcohol, dehydrated alcohol, denatured alcohol, dilute alcohol, aleuritic acid, alginic acid, aliphatic polyesters, alumina, aluminum hydroxide, aluminum stearate, amylopectin, α-amylose, ascorbic acid, ascorbyl palmitate, aspartame, bacteriostatic water for injection, bentonite, bentonite magma, benzalkonium chloride, benzethonium chloride, benzoic acid, benzyl alcohol, benzyl benzoate, bronopol, butylated hydroxyanisole, butylated hydroxytoluene, butylparaben, butylparaben sodium, calcium alginate, calcium ascorbate, calcium carbonate, calcium cyclamate, dibasic anhydrous calcium phosphate, dibasic dehydrate calcium phosphate, tribasic calcium phosphate, calcium propionate, calcium silicate, calcium sorbate, calcium stearate, calcium sulfate, calcium sulfate hemihydrate, canola oil, carbomer, carbon dioxide, carboxymethyl cellulose calcium, carboxymethyl cellulose sodium, β-carotene, carrageenan, castor oil, hydrogenated castor oil, cationic emulsifying wax, cellulose acetate, cellulose acetate phthalate, ethyl cellulose, microcrystalline cellulose, powdered cellulose, silicified microcrystalline cellulose, sodium carboxymethyl cellulose, cetostearyl alcohol, cetrimide, cetyl alcohol, chlorhexidine, chlorobutanol, chlorocresol, cholesterol, chlorhexidine acetate, chlorhexidine gluconate, chlorhexidine hydrochloride, chlorodifluoroethane (HCFC), chlorodifluoromethane, chlorofluorocarbons (CFC)chlorophenoxyethanol, chloroxylenol, corn syrup solids, anhydrous citric acid, citric acid monohydrate, cocoa butter, coloring agents, corn oil, cottonseed oil, cresol, m-cresol, o-cresol, p-cresol, croscarmellose sodium, crospovidone, cyclamic acid, cyclodextrins, dextrates, dextrin, dextrose, dextrose anhydrous, diazolidinyl urea, dibutyl phthalate, dibutyl sebacate, diethanolamine, diethyl phthalate, difluoroethane (HFC), dimethyl-β-cyclodextrin, cyclodextrin-type compounds such as Captisol®, dimethyl ether, dimethyl phthalate, dipotassium edentate, disodium edentate, disodium hydrogen phosphate, docusate calcium, docusate potassium, docusate sodium, dodecyl gallate, dodecyltrimethylammonium bromide, edentate calcium disodium, edtic acid, eglumine, ethyl alcohol, ethylcellulose, ethyl gallate, ethyl laurate, ethyl

maltol, ethyl oleate, ethylparaben, ethylparaben potassium, ethylparaben sodium, ethyl vanillin, fructose, fructose liquid, fructose milled, fructose pyrogen-free, powdered fructose, fumaric acid, gelatin, glucose, liquid glucose, glyceride mixtures of saturated vegetable fatty acids, glycerin, glyceryl behenate, glyceryl monooleate, glyceryl monostearate, self-emulsifying glyceryl monostearate, glyceryl palmitostearate, glycine, glycols, glycofurol, guar gum, heptafluoropropane (HFC), hexadecyltrimethylammonium bromide, high fructose syrup, human serum albumin, hydrocarbons (HC), dilute hydrochloric acid, hydrogenated vegetable oil, type II, hydroxyethyl cellulose, 2-hydroxyethyl-β-cyclodextrin, hydroxypropyl cellulose, low-substituted hydroxypropyl cellulose, 2-hydroxypropyl-β-cyclodextrin, hydroxypropyl methylcellulose, hydroxypropyl methylcellulose phthalate, imidurea, indigo carmine, ion exchangers, iron oxides, isopropyl alcohol, isopropyl myristate, isopropyl palmitate, isotonic saline, kaolin, lactic acid, lactitol, lactose, lanolin, lanolin alcohols, anhydrous lanolin, lecithin, magnesium aluminum silicate, magnesium carbonate, normal magnesium carbonate, magnesium carbonate anhydrous, magnesium carbonate hydroxide, magnesium hydroxide, magnesium lauryl sulfate, magnesium oxide, magnesium silicate, magnesium stearate, magnesium trisilicate, magnesium trisilicate anhydrous, malic acid, malt, maltitol, maltitol solution, maltodextrin, maltol, maltose, mannitol, medium chain triglycerides, meglumine, menthol, methylcellulose, methyl methacrylate, methyl oleate, methylparaben, methylparaben potassium, methylparaben sodium, microcrystalline cellulose and carboxymethylcellulose sodium, mineral oil, light mineral oil, mineral oil and lanolin alcohols, oil, olive oil, monoethanolamine, montmorillonite, octyl gallate, oleic acid, palmitic acid, paraffin, peanut oil, petrolatum, petrolatum and lanolin alcohols, pharmaceutical glaze, phenol, liquified phenol, phenoxyethanol, phenoxypropanol, phenylethyl alcohol, phenylmercuric acetate, phenylmercuric borate, phenylmercuric nitrate, polacrilin, polacrilin potassium, poloxamer, polydextrose, polyethylene glycol, polyethylene oxide, polyacrylates, polyethylene-polyoxypropylene-block polymers, polymethacrylates, polyoxyethylene alkyl ethers, polyoxyethylene castor oil derivatives, polyoxyethylene sorbitol fatty acid esters, polyoxyethylene stearates, polyvinyl alcohol, polyvinyl pyrrolidone, potassium alginate, potassium benzoate, potassium bicarbonate, potassium bisulfite, potassium chloride, postassium citrate, potassium citrate anhydrous, potassium hydrogen phosphate, potassium metabisulfite, monobasic potassium phosphate, potassium propionate, potassium sorbate, povidone, propanol, propionic acid, propylene carbonate, propylene glycol, propylene glycol alginate, propyl gallate, propylparaben, propylparaben potassium, propylparaben sodium, protamine sulfate, rapeseed oil,

Ringer's solution, saccharin, saccharin ammonium, saccharin calcium, saccharin sodium, safflower oil, saponite, serum proteins, sesame oil, colloidal silica, colloidal silicon dioxide, sodium alginate, sodium ascorbate, sodium benzoate, sodium bicarbonate, sodium bisulfite, sodium chloride, anhydrous sodium citrate, sodium citrate dehydrate, sodium chloride, sodium cyclamate, sodium edentate, sodium dodecyl sulfate, sodium lauryl sulfate, sodium metabisulfite, sodium phosphate, dibasic, sodium phosphate, monobasic, sodium phosphate, tribasic, anhydrous sodium propionate, sodium propionate, sodium sorbate, sodium starch glycolate, sodium stearyl fumarate, sodium sulfite, sorbic acid, sorbitan esters (sorbitan fatty esters), sorbitol, sorbitol solution 70%, soybean oil, spermaceti wax, starch, corn starch, potato starch, pregelatinized starch, sterilizable maize starch, stearic acid, purified stearic acid, stearyl alcohol, sucrose, sugars, compressible sugar, confectioner's sugar, sugar spheres, invert sugar, *Sugartab*, Sunset Yellow FCF, synthetic paraffin, talc, tartaric acid, tartrazine, tetrafluoroethane (HFC), theobroma oil, thimerosal, titanium dioxide, alpha tocopherol, tocopheryl acetate, alpha tocopheryl acid succinate, beta-tocopherol, delta-tocopherol, gamma-tocopherol, tragacanth, triacetin, tributyl citrate, triethanolamine, triethyl citrate, trimethyl-β-cyclodextrin, trimethyltetradecylammonium bromide, tris buffer, trisodium edentate, vanillin, type I hydrogenated vegetable oil, water, soft water, hard water, carbon dioxide-free water, pyrogen-free water, water for injection, sterile water for inhalation, sterile water for injection, sterile water for irrigation, waxes, anionic emulsifying wax, carnauba wax, cationic emulsifying wax, cetyl ester wax, microcrystalline wax, nonionic emulsifying wax, suppository wax, white wax, yellow wax, white petrolatum, wool fat, xanthan gum, xylitol, zein, zinc propionate, zinc salts, zinc stearate, or any excipient in the *Handbook of Pharmaceutical Excipients*, Third Edition, A. H. Kibbe (Pharmaceutical Press, London, UK, 2000), which is incorporated by reference in its entirety. *Remington's Pharmaceutical Sciences*, Sixteenth Edition, E. W. Martin (Mack Publishing Co., Easton, Pa., 1980), which is incorporated by reference in its entirety, discloses various components used in formulating pharmaceutically acceptable compositions and known techniques for the preparation thereof. Except insofar as any conventional agent is incompatible with the pharmaceutical compositions, its use in pharmaceutical compositions is contemplated. Supplementary active ingredients also can be incorporated into the compositions.

**[00117]** In some embodiments, the foregoing component(s) may be present in the pharmaceutical composition at any concentration, such as, for example, at least A, wherein A is 0.0001% w/v, 0.001% w/v, 0.01% w/v, 0.1% w/v, 1% w/v, 2% w/v, 5% w/v, 10% w/v, 20% w/v,

30% w/v, 40% w/v, 50% w/v, 60% w/v, 70% w/v, 80% w/v, or 90% w/v. In some embodiments, the foregoing component(s) may be present in the pharmaceutical composition at any concentration, such as, for example, at most B, wherein B is 90% w/v, 80% w/v, 70% w/v, 60% w/v, 50% w/v, 40% w/v, 30% w/v, 20% w/v, 10% w/v, 5% w/v, 2% w/v, 1% w/v, 0.1% w/v, 0.001% w/v, or 0.0001%. In other embodiments, the foregoing component(s) may be present in the pharmaceutical composition at any concentration range, such as, for example from about A to about B. In some embodiments, A is 0.0001% and B is 90%.

**[00118]** The pharmaceutical compositions may be formulated to achieve a physiologically compatible pH. In some embodiments, the pH of the pharmaceutical composition may be at least 5, at least 5.5, at least 6, at least 6.5, at least 7, at least 7.5, at least 8, at least 8.5, at least 9, at least 9.5, at least 10, or at least 10.5 up to and including pH 11, depending on the formulation and route of administration. In certain embodiments, the pharmaceutical compositions may comprise buffering agents to achieve a physiological compatible pH. The buffering agents may include any compounds capabale of buffering at the desired pH such as, for example, phosphate buffers (e.g.,PBS), triethanolamine, Tris, bicine, TAPS, tricine, HEPES, TES, MOPS, PIPES, cacodylate, MES, and others. In certain embodiments, the strength of the buffer is at least 0.5 mM, at least 1 mM, at least 5 mM, at least 10 mM, at least 20 mM, at least 30 mM, at least 40 mM, at least 50 mM, at least 60 mM, at least 70 mM, at least 80 mM, at least 90 mM, at least 100 mM, at least 120 mM, at least 150 mM, or at least 200 mM. In some embodiments, the strength of the buffer is no more than 300 mM (e.g.,at most 200 mM, at most 100 mM, at most 90 mM, at most 80 mM, at most 70 mM, at most 60 mM, at most 50 mM, at most 40 mM, at most 30 mM, at most 20 mM, at most 10 mM, at most 5 mM, at most 1 mM).

**[00119]** *Routes of Administration*

**[00120]** With regard to the invention, the cardiac metabolic modifier, pharmaceutical composition comprising the same, conjugate comprising the same, or pharmaceutically acceptable salt thereof, may be administered to the subject by any suitable route of administration. The following discussion on routes of administration is merely provided to illustrate exemplary embodiments and should not be construed as limiting the scope in any way.

**[00121]** Formulations suitable for oral administration can consist of (a) liquid solutions, such as an effective amount of the cardiac metabolic modifier of the present disclosure dissolved in diluents, such as water, saline, or orange juice; (b) capsules, sachets, tablets, lozenges, and troches, each containing a predetermined amount of the active ingredient, as solids or granules;

(c) powders; (d) suspensions in an appropriate liquid; and (e) suitable emulsions. Liquid formulations may include diluents, such as water and alcohols, for example, ethanol, benzyl alcohol, and the polyethylene alcohols, either with or without the addition of a pharmaceutically acceptable surfactant. Capsule forms can be of the ordinary hard- or soft-shelled gelatin type containing, for example, surfactants, lubricants, and inert fillers, such as lactose, sucrose, calcium phosphate, and corn starch. Tablet forms can include one or more of lactose, sucrose, mannitol, corn starch, potato starch, alginic acid, microcrystalline cellulose, acacia, gelatin, guar gum, colloidal silicon dioxide, croscarmellose sodium, talc, magnesium stearate, calcium stearate, zinc stearate, stearic acid, and other excipients, colorants, diluents, buffering agents, disintegrating agents, moistening agents, preservatives, flavoring agents, and other pharmacologically compatible excipients. Lozenge forms can comprise the cardiac metabolic modifier of the present disclosure in a flavor, usually sucrose and acacia or tragacanth, as well as pastilles comprising the cardiac metabolic modifier of the present disclosure in an inert base, such as gelatin and glycerin, or sucrose and acacia, emulsions, gels, and the like containing, in addition to, such excipients as are known in the art.

**[00122]** The cardiac metabolic modifiers of the present disclosure, alone or in combination with other suitable components, can be delivered via pulmonary administration and can be made into aerosol formulations to be administered via inhalation. These aerosol formulations can be placed into pressurized acceptable propellants, such as dichlorodifluoromethane, propane, nitrogen, and the like. They also may be formulated as pharmaceuticals for non-pressured preparations, such as in a nebulizer or an atomizer. Such spray formulations also may be used to spray mucosa. In some embodiments, the cardiac metabolic modifier is formulated into a powder blend or into microparticles or nanoparticles. Suitable pulmonary formulations are known in the art. See, e.g., Qian et al., Int J Pharm 366: 218-220 (2009); Adjei and Garren, Pharmaceutical Research, 7(6): 565-569 (1990); Kawashima et al., J Controlled Release 62(1-2): 279-287 (1999); Liu et al., Pharm Res 10(2): 228-232 (1993); International Patent Application Publication Nos. WO 2007/133747 and WO 2007/141411.

**[00123]** Formulations suitable for parenteral administration include aqueous and non-aqueous, isotonic sterile injection solutions, which can contain anti-oxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. The term, "parenteral" means not through the alimentary

canal but by some other route such as subcutaneous, intramuscular, intraspinal, or intravenous. The cardiac metabolic modifier of the present disclosure can be administered with a physiologically acceptable diluent in a pharmaceutical carrier, such as a sterile liquid or mixture of liquids, including water, saline, aqueous dextrose and related sugar solutions, an alcohol, such as ethanol or hexadecyl alcohol, a glycol, such as propylene glycol or polyethylene glycol, dimethylsulfoxide, glycerol, ketals such as 2,2- dimethyl-l53-dioxolane-4-methanol, ethers, poly(ethyleneglycol) 400, oils, fatty acids, fatty acid esters or glycerides, or acetylated fatty acid glycerides with or without the addition of a pharmaceutically acceptable surfactant, such as a soap or a detergent, suspending agent, such as pectin, carbomers, methylcellulose, hydroxypropylmethylcellulose, or carboxymethylcellulose, or emulsifying agents and other pharmaceutical adjuvants.

**[00124]** Oils, which can be used in parenteral formulations include petroleum, animal, vegetable, or synthetic oils. Specific examples of oils include peanut, soybean, sesame, cottonseed, corn, olive, petrolatum, and mineral. Suitable fatty acids for use in parenteral formulations include oleic acid, stearic acid, and isostearic acid. Ethyl oleate and isopropyl myristate are examples of suitable fatty acid esters.

**[00125]** Suitable soaps for use in parenteral formulations include fatty alkali metal, ammonium, and triethanolamine salts, and suitable detergents include (a) cationic detergents such as, for example, dimethyl dialkyl ammonium halides, and alkyl pyridinium halides, (b) anionic detergents such as, for example, alkyl, aryl, and olefin sulfonates, alkyl, olefin, ether, and monoglyceride sulfates, and sulfosuccinates, (c) nonionic detergents such as, for example, fatty amine oxides, fatty acid alkanolamides, and polyoxyethylenepolypropylene copolymers, (d) amphoteric detergents such as, for example, alkyl-β-aminopropionates, and 2-alkyl -imidazoline quaternary ammonium salts, and (e) mixtures thereof.

**[00126]** The parenteral formulations in some embodiments contain from about 0.5% to about 25% by weight of the cardiac metabolic modifier of the present disclosure in solution. Preservatives and buffers may be used. In order to minimize or eliminate irritation at the site of injection, such compositions may contain one or more nonionic surfactants having a hydrophile-lipophile balance (HLB) of from about 12 to about 17. The quantity of surfactant in such formulations will typically range from about 5% to about 15% by weight. Suitable surfactants include polyethylene glycol sorbitan fatty acid esters, such as sorbitan monooleate and the high

molecular weight adducts of ethylene oxide with a hydrophobic base, formed by the condensation of propylene oxide with propylene glycol. The parenteral formulations in some aspects are presented in unit-dose or multi-dose sealed containers, such as ampoules and vials, and can be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid excipient, for example, water, for injections, immediately prior to use. Extemporaneous injection solutions and suspensions in some aspects are prepared from sterile powders, granules, and tablets of the kind previously described.

**[00127]** Injectable formulations are in accordance with the invention. The requirements for effective pharmaceutical carriers for injectable compositions are well-known to those of ordinary skill in the art (see, e.g., *Pharmaceutics and Pharmacy Practice*, J. B. Lippincott Company, Philadelphia, PA, Banker and Chalmers, eds., pages 238-250 (1982), and *ASHP Handbook on Injectable Drugs*, Toissel, 4th ed., pages 622-630 (1986)).

**[00128]** Additionally, the cardiac metabolic modifier of the present disclosures can be made into suppositories for rectal administration by mixing with a variety of bases, such as emulsifying bases or water-soluble bases. Formulations suitable for vaginal administration can be presented as pessaries, tampons, creams, gels, pastes, foams, or spray formulas containing, in addition to the active ingredient, such carriers as are known in the art to be appropriate.

**[00129]** It will be appreciated by one of skill in the art that, in addition to the above- described pharmaceutical compositions, the cardiac metabolic modifier of the disclosure can be formulated as inclusion complexes, such as cyclodextrin inclusion complexes, or liposomes.

**[00130]** *Dosages*

**[00131]** The cardiac metabolic modifiers of the disclosure are believed to be useful in methods of treating a diastolic dysfunction, as well as related conditions, as described herein. For purposes of the disclosure, the amount or dose of the cardiac metabolic modifier administered should be sufficient to effect, e.g., a therapeutic or prophylactic response, in the subject or animal over a reasonable time frame. For example, the dose of the cardiac metabolic modifier of the present disclosure should be sufficient to treat diastolic dysfunction as described herein in a period of from about 1 to 4 minutes, 1 to 4 hours or 1 to 4 weeks or longer, e.g., 5 to 20 or more weeks, from the time of administration. In certain embodiments, the time period could be even longer. The dose will be determined by the efficacy of the particular cardiac metabolic modifier

and the condition of the animal (e.g., human), as well as the body weight of the animal (e.g., human) to be treated.

**[00132]** Many assays for determining an administered dose are known in the art. For purposes herein, an assay, which comprises comparing the extent to which diastolic dysfunction is treated upon administration of a given dose of the cardiac metabolic modifier of the present disclosure to a mammal among a set of mammals, each set of which is given a different dose of the cardiac metabolic modifier, could be used to determine a starting dose to be administered to a mammal. The extent to which diastolic dysfunction is treated upon administration of a certain dose can be assayed by methods known in the art, including, for instance, the methods described in the Examples set forth below.

**[00133]** The dose of the cardiac metabolic modifier of the present disclosure also will be determined by the existence, nature and extent of any adverse side effects that might accompany the administration of a particular cardiac metabolic modifier of the present disclosure. Typically, the attending physician will decide the dosage of the cardiac metabolic modifier of the present disclosure with which to treat each individual patient, taking into consideration a variety of factors, such as age, body weight, general health, diet, sex, cardiac metabolic modifier of the present disclosure to be administered, route of administration, and the severity of the condition being treated. By way of example and not intending to limit the invention, the dose of the cardiac metabolic modifier of the present disclosure can be about 0.0001 to about 1 g/kg body weight of the subject being treated/day, from about 0.0001 to about 0.001 g/kg body weight/day, or about 0.01 mg to about 1 g/kg body weight/day.

**[00134]** In some embodiments, the cardiac metabolic modifier is formulated for injection, is a compound of Formula II, e.g., ranolazine, and is administered to the subject at a dose between about 1 and about 20 mg/kg body weight of the subject for an injection, (e.g., between about 5 and about 15 mg/kg, between about 10 to about 12 mg/kg, about 9 mg/kg, about 10 mg/kg, about 11 mg/kg, about 12 mg/kg). In some embodiments, the cardiac metabolic modifier is formulated for infusion (e.g., intravenous infusion), is a compound of Formula II, e.g., ranolazine, and is administered at a dose between about 1 mg/kg/h to about 20 mg/kg/h (e.g., about 1 mg/kg/h, about 2 mg/kg/h, about 3 mg/kg/h, about 4 mg/kg/h, about 5 mg/kg/h, about 6 mg/kg/h, about 7 mg/kg/h, about 8 mg/kg/h, about 9 mg/kg/h, about 10 mg/kg/h, about 11 mg/kg/h, about 12

mg/kg/h, about 13 mg/kg/h, about 14 mg/kg/h, about 15 mg/kg/h, about 16 mg/kg/h, about 17 mg/kg/h, about 18 mg/kg/h, about 19 mg/kg/h, about 20 mg/kg/h)

**[00135]** In some embodiments, wherein the cardiac metabolic modifier is formulated for oral administration and is a compound of Formula II, e.g., ranolazine, the dose administered to the subject is between about 100 and about 2000 mg (e.g., about 100 mg, about 200 mg, about 250 mg, about 300 mg, about 400 mg, about 500 mg, about 600 mg, about 700 mg, about 750 mg, about 800 mg, about 900 mg, about 1000 mg, about 1100 mg, about 1200 mg, about 1300 mg, about 1400 mg, about 1500 mg, about 1600 mg, about 1700 mg, about 1800 mg, about 1900 mg, about 2000 mg). In some aspects, the oral dosage is administered once daily, twice daily, three times daily, or four times daily.

**[00136]** In some embodiments, the dosage of the cardiac metabolic modifier is any of the above dosages, but the cardiac metabolic modifier is other than a compound of Formula II (e.g., ranolazine).

**[00137]** In some embodiments, the administered dose of the cardiac metabolic modifier (e.g., any of the doses described above), provides the subject with a plasma concentration of the cardiac metabolic modifier of at least or about 500 nM. In some aspects, the administered dose of the cardiac metabolic modifier provides the subject with a plasma concentration of the cardiac metabolic modifier within a range of about 500 nM to about 2500 nM (e.g., about 750 nM to about 2000 nM, about 1000 nM to about 1500 nM). In some aspects, the dose of the cardiac metabolic modifier provides the subject with a plasma concentration of the cardiac metabolic modifier which is below 100 μmol/L, e.g., below 50 μmol/L, below 25 μmol/L, below 10 μmol/L.

**[00138]** *Controlled Release Formulations*

**[00139]** In some embodiments, the cardiac metabolic modifiers described herein can be modified into a depot form, such that the manner in which the cardiac metabolic modifier of the present disclosures is released into the body to which it is administered is controlled with respect to time and location within the body (see, for example, U.S. Patent No. 4,450,150). Depot forms of cardiac metabolic modifiers of the present disclosures can be, for example, an implantable composition comprising the cardiac metabolic modifiers and a porous or non-porous material, such as a polymer, wherein the cardiac metabolic modifiers is encapsulated by or diffused throughout the material and/or degradation of the non-porous material. The depot is then

implanted into the desired location within the body of the subject and the cardiac metabolic modifiers is released from the implant at a predetermined rate.

**[00140]** The pharmaceutical composition comprising the cardiac metabolic modifier in certain aspects is modified to have any type of *in vivo* release profile. In some aspects, the pharmaceutical composition is an immediate release, controlled release, sustained release, extended release, delayed release, or bi-phasic release formulation. Methods of formulating peptides for controlled release are known in the art. See, for example, Qian et al., *J Pharm* 374: 46-52 (2009) and International Patent Application Publication Nos. WO 2008/130158, WO2004/033036; WO2000/032218; and WO 1999/040942.

**[00141]** The instant compositions may further comprise, for example, micelles or liposomes, or some other encapsulated form, or may be administered in an extended release form to provide a prolonged storage and/or delivery effect. The disclosed pharmaceutical formulations may be administered according to any regime including, for example, daily (1 time per day, 2 times per day, 3 times per day, 4 times per day, 5 times per day, 6 times per day), every two days, every three days, every four days, every five days, every six days, weekly, bi-weekly, every three weeks, monthly, or bi-monthly.

**[00142]** *Combinations*

**[00143]** In some embodiments, the cardiac metabolic modifiers described herein are administered alone, and in alternative embodiments, the cardiac metabolic modifiers described herein are administered in combination with another therapeutic agent which aims to treat or prevent any of the diseases or medical conditions described herein, e.g., diastolic dysfunction. In exemplary embodiments, a cardiac metabolic modifier of a first structure is co-administered with (simultaneously or sequentially) another cardiac metabolic modifier of different structure. In alternative or additional embodiments, the cardiac metabolic modifiers described herein may be co-administered with (simultaneously or sequentially) a therapeutic agent for the treatment of hypertension, including, for example, a thiazide diuretic (e.g., chlorothiazine, hydrochlorothiazide, metolazone), a beta blocker (a.k.a, beta-adrenergic blocking agent (e.g., acebutolol, atenolol, bisoprolol, carvedilol, metoprolol, nadolol, nebivolol, penbutolol, propranolol)), an angiotensin-convertine enzyme (ACE) inhibitor (e.g., benazepril, captopril, enalapril, fosinopril, lisinopril, moexipril, perindopril, quinapril, ramipril, trandolarpil), an angiotensin II receptor blocker (a.k.a., ARBs (e.g., candesartan, eprosartan, irbesartan, losartan,

olmesartan, telmisartan, valsartan)), a calcium channel blocker (a.k.a., calcium antagonist, (e.g., amlodipine, diltiazem, felodipine, isradipine, nicardipine, nifedipine, nisoldipine, verapramil)), a rennin inhibitor (e.g., Aliskiren), an alpha blocker (a.k.a, an alpha-adrenergic antagonist, alpha-adrenergic blocking agent, adrenergic blocking agent, alpha-blocking agent, (e.g., doxazosin, prazosin, terazosin, tamsulosin, alfuzosin)), an alpha-beta blocker (a.k.a, alpha-beta adrenergic blocker (e.g., carvedilol, labetalol), , a central-acting agent (a.k.a., central adrenergic inhibitor, central alpha agonist, central agonist, (e.g., clonidine, guanfacine, methyldopa)), a vasodilator (e.g., hydralazine, minoxidil).

**[00144]** In alternative or additional embodiments, the cardiac metabolic modifier is co-administered with (simultaneously or sequentially) a therapeutic agent for the treatment of diabetes or obesity. Anti-diabetic agents known in the art or under investigation include insulin, leptin, Peptide YY (PYY), Pancreatic Peptide (PP), fibroblast growth factor 21 (FGF21), Y2Y4 receptor agonists, sulfonylureas, such as tolbutamide (Orinase), acetohexamide (Dymelor), tolazamide (Tolinase), chlorpropamide (Diabinese), glipizide (Glucotrol), glyburide (Diabeta, Micronase, Glynase), glimepiride (Amaryl), or gliclazide (Diamicron); meglitinides, such as repaglinide (Prandin) or nateglinide (Starlix); biguanides such as metformin (Glucophage) or phenformin; thiazolidinediones such as rosiglitazone (Avandia), pioglitazone (Actos), or troglitazone (Rezulin), or other PPARγ inhibitors; alpha glucosidase inhibitors that inhibit carbohydrate digestion, such as miglitol (Glyset), acarbose (Precose/Glucobay); exenatide (Byetta) or pramlintide; Dipeptidyl peptidase-4 (DPP-4) inhibitors such as vildagliptin or sitagliptin; SGLT (sodium-dependent glucose transporter 1) inhibitors; glucokinase activators (GKA); glucagon receptor antagonists (GRA); or FBPase (fructose 1,6-bisphosphatase) inhibitors, GLP-1 agonists.

**[00145]** Anti-obesity agents known in the art or under investigation include appetite suppressants, including phenethylamine type stimulants, phentermine (optionally with fenfluramine or dexfenfluramine), diethylpropion (Tenuate®), phendimetrazine (Prelu-2®, Bontril®), benzphetamine (Didrex®), sibutramine (Meridia®, Reductil®); rimonabant (Acomplia®), other cannabinoid receptor antagonists; oxyntomodulin; fluoxetine hydrochloride (Prozac); Qnexa (topiramate and phentermine), Excalia (bupropion and zonisamide) or Contrave (bupropion and naltrexone); or lipase inhibitors, similar to XENICAL (Orlistat) or Cetilistat (also known as ATL-962), or GT 389-255.

**[00146]** In some embodiments, the cardiac metabolic modifier is administered in combination with aspirin, or other therapeutic agent which promotes cardiac efficiency. In some aspects, the cardiac metabolic modifier is administered in combination with tetahydrobiopterin ($BH_4$) or an analog thereof.

**[00147]** In view of the foregoing, the invention further provides pharmaceutical compositions and kits additionally comprising one of these other therapeutic agents in combination with the cardiac metabolic modifier. The additional therapeutic agent may be administered simultaneously or sequentially with the cardiac metabolic modifier of the present disclosure. In some aspects, the cardiac metabolic modifier is administered before the additional therapeutic agent, while in other aspects, the cardiac metabolic modifier is administered after the additional therapeutic agent.

**[00148]** *Subjects*

**[00149]** In some embodiments, the subject is a mammal, including, but not limited to, mammals of the order Rodentia, such as mice and hamsters, and mammals of the order Logomorpha, such as rabbits, mammals from the order Carnivora, including Felines (cats) and Canines (dogs), mammals from the order Artiodactyla, including Bovines (cows) and Swines (pigs) or of the order Perssodactyla, including Equines (horses). In some aspects, the mammals are of the order Primates, Ceboids, or Simoids (monkeys) or of the order Anthropoids (humans and apes). In some aspects, the mammal is a human.

**[00150]** In some embodiments, the subject is in need of treatment for diastolic dysfunction, e.g., any of the diastolic dysfunctions described herein (e.g., diastolic dysfunction with preserved ejection fraction, diastolic dysfunction with preserved left ventricular function, diastolic dysfunction with preserved systolic function or diastolic dysfunction without systolic dysfunction, diastolic dysfunction characterized by (i) a lack of increased late $I_{Na}$ in cardiomyocytes, (ii) an increase in myofilament calcium sensitivity, or (iii) a combination thereof). In exemplary embodiments, the subject exhibits an ejection fraction which is greater than or about 45%, e.g., greater than or about 50%.

**[00151]** In some embodiments, the subject suffers from a form of diastolic dysfunction epidemiologically associated with hypertension, also known as, high blood pressure. In this regard, the subject in some embodiments, suffers from hypertension as well as diastolic dysfunction with preserved ejection fraction. Hypertension is a chronic medical condition in

which the systemic arterial blood pressure is elevated. The hypertension in some embodiments is classified as a primary hypertension for which no medical cause is found. In some embodiments, the hypertension is a secondary hypertension caused by another condition that affects the kidneys, arteries, heart, or endocrine system.

**[00152]** A systolic or the diastolic blood pressure measurement higher than the accepted normal values for the age of the individual is classified as prehypertension or hypertension.

| Classification | Systolic pressure | | Diastolic pressure | |
|---|---|---|---|---|
| | mmHg | kPa | mmHg | kPa |
| Normal | 90–119 | 12–15.9 | 60–79 | 8.0–10.5 |
| Prehypertension | 120–139 | 16.0–18.5 | 80–89 | 10.7–11.9 |
| Stage 1 | 140–159 | 18.7–21.2 | 90–99 | 12.0–13.2 |
| Stage 2 | ≥160 | ≥21.3 | ≥100 | ≥13.3 |
| Isolated systolic hypertension | ≥140 | ≥18.7 | <90 | <12.0 |
| *Source*: Chobanian et al. (2003) | | | | |

**[00153]** Hypertension has several sub-classifications including, hypertension stage I, hypertension stage II, and isolated systolic hypertension. Isolated systolic hypertension refers to elevated systolic pressure with normal diastolic pressure and is common in the elderly. These classifications are made after averaging a patient's resting blood pressure readings taken on two or more office visits. Individuals older than 50 years are classified as having hypertension if their blood pressure is consistently at least 140 mmHg systolic or 90 mmHg diastolic. Patients with blood pressures higher than 130/80 mmHg with concomitant presence of diabetes mellitus or kidney disease require further treatment (Chobanian *et al.* (December 2003). "Seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure". *Hypertension* 42(6): 1206–52.)

**[00154]** In some aspects, the subject suffers from diastolic dysfunction which is epidemiologically associated with a metabolic disease or metabolic syndrome. In this regard, the subject in some embodiments suffers from diastolic dysfunction and a metabolic disease or metabolic syndrome. Metabolic Syndrome, also known as metabolic syndrome X, insulin resistance syndrome or Reaven's syndrome, is a disorder that affects over 50 million Americans. Metabolic Syndrome is typically characterized by a clustering of at least three or more of the following risk factors: (1) abdominal obesity (excessive fat tissue in and around the abdomen), (2) atherogenic dyslipidemia (blood fat disorders including high triglycerides, low HDL

cholesterol and high LDL cholesterol that enhance the accumulation of plaque in the artery walls), (3) elevated blood pressure, (4) insulin resistance or glucose intolerance, (5) prothrombotic state (e.g.,high fibrinogen or plasminogen activator inhibitor-1 in blood), and (6) pro-inflammatory state (e.g.,elevated C-reactive protein in blood). Other risk factors may include aging, hormonal imbalance and genetic predisposition.

**[00155]** Metabolic Syndrome is associated with an increased the risk of coronary heart disease and other disorders related to the accumulation of vascular plaque, such as stroke and peripheral vascular disease, referred to as atherosclerotic cardiovascular disease (ASCVD). Patients with Metabolic Syndrome may progress from an insulin resistant state in its early stages to full blown type II diabetes with further increasing risk of ASCVD. Without intending to be bound by any particular theory, the relationship between insulin resistance, Metabolic Syndrome and vascular disease may involve one or more concurrent pathogenic mechanisms including impaired insulin-stimulated vasodilation, insulin resistance-associated reduction in NO availability due to enhanced oxidative stress, and abnormalities in adipocyte-derived hormones such as adiponectin (Lteif and Mather, Can. J. Cardiol. 20 (suppl. B):66B-76B (2004)).

**[00156]** According to the 2001 National Cholesterol Education Program Adult Treatment Panel (ATP III), any three of the following traits in the same individual meet the criteria for Metabolic Syndrome: (a) abdominal obesity (a waist circumference over 102 cm in men and over 88 cm in women); (b) serum triglycerides (150 mg/dl or above); (c) HDL cholesterol (40 mg/dl or lower in men and 50 mg/dl or lower in women); (d) blood pressure (130/85 or more); and (e) fasting blood glucose (110 mg/dl or above). According to the World Health Organization (WHO), an individual having high insulin levels (an elevated fasting blood glucose or an elevated post meal glucose alone) with at least two of the following criteria meets the criteria for Metabolic Syndrome: (a) abdominal obesity (waist to hip ratio of greater than 0.9, a body mass index of at least 30 kg/m2, or a waist measurement over 37 inches); (b) cholesterol panel showing a triglyceride level of at least 150 mg/dl or an HDL cholesterol lower than 35 mg/dl; (c) blood pressure of 140/90 or more, or on treatment for high blood pressure). (Mathur, Ruchi, "Metabolic Syndrome," ed. Shiel, Jr., William C., MedicineNet.com, May 11, 2009).

**[00157]** For purposes herein, if an individual meets the criteria of either or both of the criteria set forth by the 2001 National Cholesterol Education Program Adult Treatment Panel or the WHO, that individual is considered as afflicted with Metabolic Syndrome.

**[00158]** With regard to the methods of the invention, in some embodiments, the subject suffers from diabetes or obesity or suffers from both diabetes and obesity, in addition to diastolic dysfunction.

**[00159]** In some embodiments, the subject does not suffer from a cardiac injury or a structural heart disease other than the diastolic dysfunction or heart failure being treated or prevented by the inventive method. By "cardiac injury" is meant a disruption of normal cardiac myocyte membrane integrity resulting in the loss into the extracellular space or intracellular constituents including detectable levels of biologically active cytosolic and structure proteins (e.g., troponin, creatine kinase, myoglobin, heart-type fatty acid binding protein, lactate dehydrogenase). By "structural heart disease" is meant any disease that affects the heart muscle or changes the architecture of the heart. In some aspects, the subject does not suffer from ischemic heart disease, chronic stable angina, chronic angina. In some aspects, the subject does not suffer from ischemia, ischemia-reperfusion or coronary artery occlusion-reperfusion, ischemic heart disease, myocardial injury, myocardial toxicity, myocardial infarction, congenital heart lesion, valvular stenosis or valvular regurgitation, coronary artery disease, chronic angina, chronic stable angina, arrhythmias. In some aspects, the subject does not suffer from a myocardial trauma, a myocardial toxicity, a viral infection, a deficiency in nutrients. In some aspects, the subject does not suffer from myocarditis.

**[00160]** In some aspects, the subject does not suffer from the cardiac injury or structural heart disease at the time of administration of the cardiac metabolic modifier. In some aspects, the subject has never experienced the cardiac injury or structural heart disease, or experienced the cardiac injury or structural heart disease at least one year prior to administration of the cardiac metabolic modifier. In some aspects, the subject experienced the cardiac injury or structural heart disease at least two, three, four or five years prior to administration of the cardiac metabolic modifier. In some aspects, the subject experienced the cardiac injury or structural heart disease more than five years (e.g., more than 10 years) prior to administration of the cardiac metabolic modifier.

**[00161]** In some embodiments, the subject suffers from diastolic dysfunction but demonstrates signs of having a structurally normal heart. In some aspects, the heart appears to be structurally normal. In exemplary embodiments, the subject exhibits neither cardiac wall thinning nor a regional wall motion abnormality.

**[00162]** In some aspects, the subject is female, e.g., a female human. In some aspects, the subject is greater than 40 years old, e.g., greater than 45 years old, greater than 50 years old, greater than 55 years old, greater than 60 years old, greater than 65 years old, greater than 70 years old, greater than 75 years old, greater than 80 years old, greater than 85 years old, greater than 90 years old, greater then 95 years old.

**[00163]** The following examples are given merely to illustrate the present invention and not in any way to limit its scope.

## EXAMPLES

### EXAMPLE 1

**[00164]** The following methods were executed during the studies described below:

**[00165]** *Generation of DOCA-salt mouse model:* Previously, it was shown that this model leads to mild hypertension, myocardial oxidative stress, and diastolic dysfunction.[20] A gradual and mild elevation in blood pressure was induced by unilateral nephrectomy, subcutaneous implantation of a controlled release deoxycorticosterone acetate (DOCA) pellet (0.7 mg/d; Innovative Research of America, Sarasota, FL), and substituting drinking water with 1.05% saline. Control animals underwent a sham operation, had placebo pellet implantation, and received water without salt.

**[00166]** Invasive hemodynamic studies, noninvasive echocardiography, and myocyte isolation were done on postoperative day 14-18 for DOCA-salt and control mice. All experiments were approved by the University of Illinois at Chicago Animal Care and Use Committee.

**[00167]** *Noninvasive assessment of diastolic dysfunction:* Mice were anesthetized, maintained at 37°C, and studied by echocardiography (Vevo 770, VisualSonics Inc, Toronto, Canada). M-mode images in the parasternal long axis and the left ventricle (LV) short-axis views at the mid-papillary level. Measurements were averaged from three consecutive beats during expiration. LV inflow velocities (E and A waves) were interrogated by conventional pulsed-wave Doppler from the apical four-chamber view. The mitral annulus longitudinal velocities (Sm, E', and A') were determined by pulsed-wave tissue Doppler from the apical four-chamber view. Interpretation was done by two investigators blinded to the treatment groups. First, baseline

images were acquired. Subsequently, the mice were injected with 30 mg/kg ranolazine by intraperitoneal route, followed by a second echocardiogram 30 min later.

**[00168]** *Invasive assessment of diastolic dysfunction:* Mice were anesthetized with 1-1.5% isoflurane and maintained at 37°C. The pressure-volume (PV) catheter was inserted into the right common carotid artery and advanced into the LV. Inferior vena cava occlusion was performed via a midline abdominal incision. Volume and parallel conductance calibration were performed as previously described.[20] Baseline hemodynamic measurements were obtained, and subsequently, the mice received an intravenous injection of ranolazine (5 mg/kg) followed by an infusion at 4.8 mg/kg/h, while additional hemodynamic measurements were recorded. Blood samples were obtained during the last five minutes of the procedure to determine the plasma ranolazine concentration.

**[00169]** *Myocyte isolation:* Cardiac ventricular myocytes were isolated from the hearts of DOCA-salt or age matched controls mice 14-18 d post-operatively using a modified enzymatic digestion protocol from the Alliance for Cellular Signaling as previously described.[20]

**[00170]** *Cell shortening and calcium transient measurements:* The mechanical properties of the cardiomyocytes were assessed using an IonOptix Myocam System (Ionoptix Inc., Milton, MA). Unloaded cardiomyocytes were placed on a glass slide and allowed to adhere for 10 min. Cardiomyocytes were then imaged with an inverted microscope and perfused with a Tyrode's buffer containing 1.2 mmol/L calcium at room temperature. Cardiomyocytes were paced at 0.5 Hz for 10 ms duration, and sarcomere shortening and relengthening were assessed using the following indices: peak fractional shortening (FS), time to 90% peak shortening, and $\tau$, the relaxation time constant ($a_0+a_1e^{t/\tau}$, t = time). Cardiomyocytes were treated with 10 μmol/L ranolazine for 10 min prior to evaluation. For calcium transient measurements, cardiomyocytes were loaded with 1 μmol/L Fura 2-AM for 10 min at room temperature, and fluorescence measurements were recorded with a dual-excitation fluorescence photomultiplier system (IonOptix). After loading, the cells were washed and resuspended in normal Tyrode solution. The cardiomyocytes were placed then in the cell chamber, stimulated at 0.5 Hz for 10 ms duration, and imaged through a Fluor x 40 objective lens. Cells were exposed to light emitted by a 75-W Xenon lamp and passed though either a 340- or 380-nm wavelength filter. The emitted fluorescence was detected at 510 nm. To take into account any inference from background

fluorescence, the background fluorescence for each cardiomyocyte was determined by moving the cardiomyocyte out of the view and recording the fluorescence from the bath solution alone.

**[00171]** *Electrophysiological determination of sodium current:* Voltage-clamp experiments were performed on isolated murine ventricular myocytes with an Axopatch 200B amplifier (Molecular Devices, Sunnyvale, CA) in whole cell configuration. Data acquisition was performed at a sampling rate of 20 kHz and filtered at 10 kHz. Data recording and analysis were done with the pClamp8 software suite (Molecular Devices) and OriginPro 8 (Originlab, Northampton, MA). All experiments were carried out at room temperature. Myocytes were plated on glass cover slips and were perfused with a low-sodium Tyrode solution containing the following (in mM): N-methyl-D-glucamine 100 (titrated to pH 7.4 with HCl), NaCl 15, tetramethylammonium chloride 20, CsCl 5, $MgCl_2$ 1, glucose 10, 4-aminopyridine 3, $MnCl_2$ 2, HEPES 10, and $CaCl_2$ 1 (final pH 7.4, CsOH). Patch electrodes were pulled from capillaries purchased from Harvard Apparatus (Holliston, MA) using a model P-97 puller from Sutter Instruments (Novato, CA). Electrodes were filled with an electrode solution containing (in mM): CsCl 20, tetraethylammonium chloride 20, glutamic acid 80, NaCl 10, $MgCl_2$ 1, MgATP 5, $Li_2GTP$ 0.3, HEPES 10, EGTA 10, $CaCl_2$ 0.13 (corresponding to $[Ca^{2+}]_{free}$ of < 10 nM).[23] Electrode solution pH was adjusted to 7.2 with CsOH. Electrodes used for these experiments had access resistances between 1.0 and 1.5 MΩ.

**[00172]** Ranolazine was provided as a crystalline solid by Gilead Sciences. Prior to experiments, a DMSO stock solution was prepared and diluted (minimum 100:1) directly into the Tyrode solution used for perfusion. Cells that were treated with ranolazine were exposed to the drug for 15 min prior to beginning voltage-clamp experiments.

**[00173]** *$Ca^{2+}$-sensitivity of tension in skinned fibers:* Myofilament response to $Ca^{2+}$ was measured as essentially described in Reference 24. Left ventricular papillary muscles were dissected from mouse hearts and placed in 4°C high relaxing solution (HR), which contained 10.00 mM EGTA, 41.89 mM K-Prop, 6.57 mM $MgCl_2$, 100.00 mM 2-[bis(2-hydroxyethyl)amino]ethanesulfonic acid (BES), 6.22 mM Na-ATP, 10.00 mM CrP, 5.00 mM Na-azide, 2.5 µg/mL Pepstatin, 1 µg/mL Leupeptin, and 50 µM PMSF, pH 7.0. The muscles were then dissected further under the microscope into uniform thin bundles of about 3-5 mm in length and 150-250 µm in diameter. The fiber bundles were placed then into a skinning solution of HR that contained 1% triton for 1-2 h at 4° C. The skinned fiber bundles were mounted with

cellulose acetate glue in a force measuring apparatus and sarcomere length was adjusted to 2.2 μm using a laser diffraction pattern and width and thickness were determined for calculation of cross-sectional area. The fiber bundles were incubated in ranolazine or vehicle for 15 minutes, and then were activated at 22°C over a series of pCa (-log of the M $Ca^{2+}$ concentration) values between pCa 8.00 and pCa 4.5. Activating solutions were prepared by mixing HR with a pCa 4.50 solution of 10.00 mM EGTA, 9.99 mM $CaCl_2$, 22.16 mM K-Prop (K-Prop stock had 1.00 M propionic acid and 1.00 M KOH), 6.20 mM $MgCl_2$, 100.00 mM BES, 6.29 mM Na-ATP, 10.00 mM creatine phosphate (CrP), 5.00 mM Na-azide, 2.5 μg/mL pepstatin, 1 μg/mL leupeptin, and 50 μM PMSF, pH 7.0. HR and pCa 8.00-pCa 4.50 solutions had one unit of creatine phosphokinase per 200 μL of solution. Tension generated was computed from the force/cross-sectional area and data were analyzed in the Graphpad Prism software and fit into a sigmoidal modified Hill equation in order to generate tension-pCa curves, Hill coefficients, and $pCa_{50}$ (pCa value at half-maximum tension).

**[00174]** *Statistical analysis:* Each value is expressed as mean ± SE. A two-way ANOVA was used to test for mean differences in invasive and noninvasive parameters. A one-way ANOVA was used to test for mean differences in all other experiments. Where appropriate, post hoc ANOVA testing (Tukey's) was used to assess mean differences between groups at a given time point. A p value $< 0.05$ was considered significant.

EXAMPLE 2

**[00175]** *Ranolazine attenuated diastolic dysfunction in vivo.* As previously described, DOCA-salt mice had evidence of diastolic dysfunction with preserved systolic function by transthoracic echocardiography at postoperative days 14-18 (Table 1).[20] Intraperitoneal injection of ranolazine improved diastolic dysfunction without affecting systolic function. DOCA-salt mice had significant reductions in tissue mitral annulus early longitudinal (E') velocities and the ratio of early annulus to late annulus (E'/A') velocities, which improved to sham levels with ranolazine treatment (Figure 1). The ratio of early diastolic filling velocity to the early diastolic mitral annulus velocity (E/E') has been reported to have the highest correlation with invasive hemodynamic measures of diastolic dysfunction. [20, 25] Hypertensive mice had a higher E/E' compared to controls, and ranolazine returned this ratio toward normal in hypertensive mice. The mitral inflow velocities, E and A, were similar among the groups, a pseudonormal pattern, as reported before.[20] The changes in relaxation parameters occurred in the absence of valvular

regurgitation, LV wall motion abnormalities, or hypertrophy. Systolic function including LVEF (%), fractional shortening (FS, %), and septal annulus systolic velocity (Sm) were statistically indistinguishable among the groups (Table 1).

**Table 1. Effect of ranolazine on echocardiographic parameters**

| | Sham | Sham + ranolazine | DOCA-salt | DOCA-salt + ranolazine |
|---|---|---|---|---|
| FS (%) | 52.6 ± 1.5 | 52.8 ± 0.8 | 49.6 ± 1.3 | 46.9 ± 1.6 |
| Sm (cm/s) | 2.45 ± 0.08 | 2.31 ± 0.09 | 2.18 ± 0.15 | 2.15 ± 0.14 |
| E/A | 1.47 ± 0.05 | 1.63 ± 0.07* | 1.21 ± 0.10 | 1.62 ± 0.11* |
| E' (cm/s) | 2.77 ± 0.15* | 2.67 ± 0.11* | 2.15 ± 0.11 | 2.47 ± 0.18 |
| E'/A' | 1.22 ± 0.06* | 1.16 ± 0.02* | 0.82 ± 0.06 | 1.31 ± 0.11* |
| E/E' | 31.9 ± 2.8* | 30.2 ± 1.9* | 41.8 ± 2.6 | 31.9 ± 2.6* |

Data are means ± SEM. FS, fractional shortening; Sm, systolic septal mitral annulus velocity measured by tissue doppler imaging (TDI); E, early diastolic filling velocity and A, late diastolic filling velocity measured by conventional doppler; E', early septal mitral annulus velocity (TDI); A', late diastolic septal mitral annulus velocity (TDI). n=8-10, *$p < 0.05$ vs. DOCA.

**[00176]** Invasive hemodynamic evaluation confirmed the echocardiographic findings (Table 2). As expected, systolic blood pressure (SBP), diastolic blood pressure (DBP), and left ventricle (LV) end-systolic pressure were mildly elevated in DOCA-salt mice compared with sham and sham treated mice, although DOCA-salt mice treated with ranolazine did not differ significantly from DOCA-salt mice in any of these parameters. As described before, the best fit for the end-diastolic pressure-volume relation (EDPVR) was by the following linear function: $\text{pressure}_{\text{end diastole}} = \text{EDPVR} \times \text{volume}_{\text{end diastole}} + \text{intercept}$ (Table 2, Figure 2A).[20] Hypertensive DOCA-salt mice had a steeper EDPVR compared with DOCA-salt treated and control groups ($p< 0.005$; Figure 2A and 2B). The slopes were 0.23 ± 0.026, 0.17 ± 0.01, 0.16 ± 0.01, and 0.18 ± 0.01 mm Hg/μL; for DOCA-salt, DOCA-salt + ranolazine, sham, and sham + ranolazine, respectively. Additionally, the EDPVR in DOCA-salt mice demonstrated a linear response to serum ranolazine levels (correlation coefficient = 0.70, $p < 0.05$; Figure 2C).

**Table 2. Effect of ranolazine on hemodynamics**

| | Sham | Sham + ranolazine | DOCA-salt | DOCA-salt + ranolazine |
|---|---|---|---|---|
| SBP (mm Hg) | 88 ± 3* | 93 ± 3* | 109 ± 6 | 102 ± 3 |
| DBP (mm Hg) | 52 ± 5* | 58 ± 2* | 78 ± 4 | 73 ± 2 |
| HR (bpm) | 618 ± 24 | 620 ± 5 | 588 ± 16 | 588 ± 16 |
| LVESP (mm Hg) | 83 ± 2* | 84 ± 3* | 107 ± 7 | 98 ± 3 |
| LVEDP (mm Hg) | 3.2 ± 0.4 | 4.2 ± 0.5 | 5.1 ± 0.9 | 3.6 ± 0.4 |
| EF (%) | 71 ± 5 | 73 ± 5 | 67 ± 6 | 67 ± 4 |
| EDPVR(mm Hg/μL) | 0.16 ± 0.01* | 0.18 ± 0.01* | 0.23 ± 0.026 | 0.17 ± 0.01* |

Data are means ± SEM. SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; LVESP, left ventricular end systolic pressure; LVEDP, left ventricular end diastolic pressure; EF, ejection fraction; EDPVR, end diastolic pressure volume relationship. n=8 , *p < 0.05 vs. DOCA-salt.

EXAMPLE 3

**[00177]** *Ranolazine improved relaxation in DOCA-salt cardiomyocytes.* Silberman et al. showed that impaired relaxation of cardiomyocytes was the main contributor to diastolic dysfunction in the DOCA-salt hypertensive model, finding no increase in cardiac fibrosis or inflammation.[20] To confirm that ranolazine was working directly on DOCA-salt cardiomyocytes to improve relaxation, freshly isolated ventricular cardiomyocytes were studied. DOCA-salt cardiomyocytes had preserved contractile function, as previously reported (Figures 3A, 3B, and 3C).[20] Additionally, treatment with ranolazine did not affect contraction in sham and DOCA-salt mice (Figures 3A, 3B, and 3C). In contrast, relaxation $\tau$ was significantly impaired in DOCA-salt mice and improved to normal levels with ranolazine treatment (DOCA-salt 0.15 ± 0.03, DOCA-salt + ranolazine 0.08 ± 0.01, sham 0.08 ± 0.01, sham + ranolazine 0.07 ± 0.01 seconds, $p < 0.0001$; figure 3A and 3D).

EXAMPLE 4

**[00178]** *Diastolic dysfunction was independent of intracellular calcium cycling.* To elucidate the mechanism underlying the impaired diastolic function in hypertensive mice, $Ca^{2+}$ transients were measured in freshly isolated ventricular myocytes. $Ca^{2+}$ transients did not differ significantly between sham and DOCA-salt mice, and the addition of 10 μM ranolazine did not affect either group (Figure 4). Baseline intracellular $Ca^{2+}$ was similar in all groups (Figure 4A). Additionally, peak intracellular $Ca^{2+}$ and the rate of $Ca^{2+}$ release were similar between all four

groups (Figure 4B and 4C). Surprisingly, there was no difference among the rates of intracellular $Ca^{2+}$ egress among the four groups measured as the time constant $\tau$ (Figure 4D).

EXAMPLE 5

**[00179]** *Late $I_{Na}$ was not elevated in DOCA-salt mice.* Oxidative stress is known to induce late $I_{Na}$ that can be blocked by the anti-anginal drug, ranolazine, and the DOCA-salt model is associated with increased cardiac oxidative stress.[20, 26] Nevertheless, voltage-clamp studies show no increase in late $I_{Na}$ in DOCA-salt cardiomyocytes compared to sham (sham 0.10532 ± 0.00999, sham + ranolazine 0.11257 ± 0.00406, DOCA-salt 0.10088 ± 0.00792, DOCA-salt + ranolazine 0.11422 ± 0.01272, p=NS) (Figure 5A). Integrated late $I_{Na}$ was measured starting at 5% of peak current and ending 40 ms after depolarization. DOCA-salt myocytes accumulated a similar amount of charge as sham. Extracellular addition of 10 μmol/L ranolazine did not affect the late accumulated charge in DOCA-salt cardiomyocytes, which was similar to that seen for sham and sham treated myocytes (Figure 5B). Peak $I_{Na}$ was similar among all four groups.

EXAMPLE 6

**[00180]** *DOCA-salt cardiomyocytes demonstrated increased myofilament $Ca^{2+}$ sensitivity.* The baseline sarcomere length was significantly reduced in DOCA-salt mice compared to sham mice (Figure 6A). Treatment with ranolazine improved resting sarcomere length in the DOCA-salt mice to levels similar to sham, and ranolazine did not affect sham sarcomere length (DOCA-salt 1.75 ± 0.01, DOCA-salt + ranolazine 1.81 ± 0.01, sham 1.82 ± 0.01, sham + ranolazine 1.82 ± 0.01 μm, $p< 0.0001$). In the absence of changes in resting $Ca^{2+}$, this suggested that myofilament $Ca^{2+}$ sensitivity may be altered in diastolic dysfunction. To examine directly myofilament function, the calcium sensitivity of steady-state isometric force development was measured in skinned sham and DOCA-salt cardiomyocytes. The mean steady-state isometric tension and normalized tension plotted as a function of calcium in skinned ventricular myofibers showed the DOCA-salt curve shifted to the left representing an increase in $Ca^{2+}$ sensitivity (Figure 6B and 6D). Myofilament $Ca^{2+}$ sensitivity, indexed by $pCa_{50}$, was significantly ($p< 0.02$) greater in DOCA-salt than in sham cardiomyocytes (Figure 6C). Ranolazine treatment returned DOCA-salt myofilament sensitivity to levels similar to sham myocytes with little effect on sham myocytes.

## DISCUSSION OF THE FOREGOING EXAMPLES

**[00181]** The effects of ranolazine, a late $I_{Na}$ inhibitor, on the mechanical derangements induced in the DOCA-salt hypertensive model of diastolic dysfunction[26, 27] were investigated. Mild hypertension in this model resulted in impaired relaxation that improved acutely after ranolazine treatment. Without changes in heart rate or blood pressure, ranolazine rapidly improved relaxation when measured both noninvasively and invasively. EDPVR, the most widely accepted measurement of relaxation, significantly improved in the DOCA-salt mice confirming our noninvasive echocardiographic results. Additionally, ranolazine did not significantly affect hemodynamics in the sham mouse. At the cellular level, isolated DOCA-salt cardiomyocytes demonstrated impaired relaxation that improved with ranolazine.

**[00182]** Despite the efficacy of ranolazine in the relief of diastolic dysfunction, no increase in late $I_{Na}$ in the DOCA-salt cardiomyocytes was noted nor were there changes in calcium cycling to indicate significant alterations in $Ca^{2+}$ handling in this form of diastolic dysfunction. On the other hand, at rest, the DOCA-salt myocytes had a significant decrease in sarcomere length in the absence of changes in $Ca^{2+}$ concentration, suggesting increased diastolic tension compared to sham mice. Myofilaments from DOCA-salt mice had increased sensitivity to $Ca^{2+}$ compared to sham mice that normalized with ranolazine treatment. Taken together, these results suggest ranolazine improved diastolic function at the cardiomyocyte level through the modulation of myofilament sensitivity to $Ca^{2+}$.

**[00183]** Silberman et al. reported the DOCA-salt model of diastolic dysfunction was associated with cardiac oxidative stress and targeting of reactive oxygen species production improved diastolic function.[20] The downstream mediators of the increased oxidant load were investigated to better elucidate the mechanisms regulating diastolic function. Oxidative stress is known to modulate a number of proteins important in cardiac function including: the SR $Ca^{2+}$ release channel, SR $Ca^{2+}$ pump, the sarcolemmal L-type $Ca^{2+}$ channel, the sodium-calcium exchanger, phospholamban, myofilaments, and the late $I_{Na}$.[15, 28-32] Largely based on analogy to systolic dysfunction, it was expected to find increased diastolic $Ca^{2+}$ resulting in slowed myocyte relaxation and diastolic dysfunction, but surprisingly and unexpectedly, no changes in $Ca^{2+}$ cycling between DOCA-salt and sham mice were found. Instead, changes in myofilament sensitivity in the absence of changes in $Ca^{2+}$ handling were noted. This is consistent with other models of diastolic dysfunction including a model of diabetic cardiomyopathy in which similar

changes in sarcomere length were reported with no correlation with changes in $Ca^{2+}$ cycling.[16-18] Previously, it was reported that myocyte relaxation can be dissociated from the decline of intracellular $Ca^{2+}$,[33] and myofilament $Ca^{2+}$ sensitivity is a consistent functional abnormality seen in dilated cardiomyopathy.[34] These results suggest that although oxidative stress is associated with both systolic and diastolic dysfunction, mediators of this dysfunction appear to differ. This helps explain the unimpressive results treating diastolic dysfunction when using drugs proven to be beneficial in systolic heart failure.[6-8]

**[00184]** In vitro studies demonstrating the effectiveness of ranolazine to treat impaired relaxation have used isolated muscle strips, isolated cardiomyocytes, and working heart preparations.[35, 36] Previous studies in both rabbit and rat models have shown that ranolazine attenuates diastolic dysfunction in ischemia/reperfusion.[37, 38] Additionally, in a dog model of chronic heart failure, ranolazine reduces LVEDP.[39-41] Finally, trials in humans with ischemic heart disease and type-3 long-QT syndrome have supported a role for ranolazine in the treatment of diastolic dysfunction.[42, 43] The mechanism for this effect was consistent with a reduction in late $I_{Na}$ with a subsequent reduction in diastolic $Ca^{2+}$. Nevertheless, we did not find any changes in $Ca^{2+}$ handling with acute ranolazine treatment, despite the drug's efficacy to improve diastolic dysfunction parameters. Instead, it appears that ranolazine may work by altering myofilament $Ca^{2+}$ sensitivity. At this point, it is unclear if this is a direct or indirect effect, but the changes in myofilament $Ca^{2+}$ sensitivity are consistent with a potential antiarrhythmic effect of this drug.[44]

**[00185]** In addition to being a potent inhibitor of late $I_{Na}$, ranolazine has also been shown to inhibit fatty acid oxidation and the potassium channel, $I_{Kr}$.[45, 46] Interestingly, in a diabetic cardiomyopathy model in which transgenic mice overexpressing the fatty acid transport protein (FATP) have increased fatty acid uptake, the animals develop diastolic dysfunction with similar changes in sarcomere length and myofilament sensitivity.[16] It is possible that ranolazine can inhibit fatty acid oxidation, limiting toxic metabolites, and preserving diastolic function. Clinical studies have shown ranolazine to have a positive effect of glycemic control providing evidence of a metabolic target in vivo.[44, 47]

**[00186]** In conclusion, the present study demonstrates that ranolazine treatment improves diastolic function through modulation of myofilament sensitivity to calcium. These results suggest that ranolazine may be of value in the treatment of diastolic dysfunction in the absence of systolic dysfunction.

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47. Timmis AD, Chaitman BR, Crager M. Effects of ranolazine on exercise tolerance and $Hb_{A1c}$ in patients with chronic angina and diabetes. *Eur Heart J.* 2006;27(1):42-48.

**[00188]** All references, including publications, patent applications, and patents, cited herein are hereby incorporated by reference to the same extent as if each reference were individually and specifically indicated to be incorporated by reference and were set forth in its entirety herein.

**[00189]** The use of the terms "a" and "an" and "the" and similar referents in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The terms "comprising," "having," "including," and "containing" are to be construed as open-ended terms (i.e., meaning "including, but not limited to,") unless otherwise noted.

**[00190]** Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range and each endpoint, unless otherwise indicated herein, and each separate value and endpoint is incorporated into the specification as if it were individually recited herein.

**[00191]** All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., "such as") provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention.

**[00192]** Preferred embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Variations of those preferred embodiments may become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventors expect skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

**WHAT IS CLAIMED:**

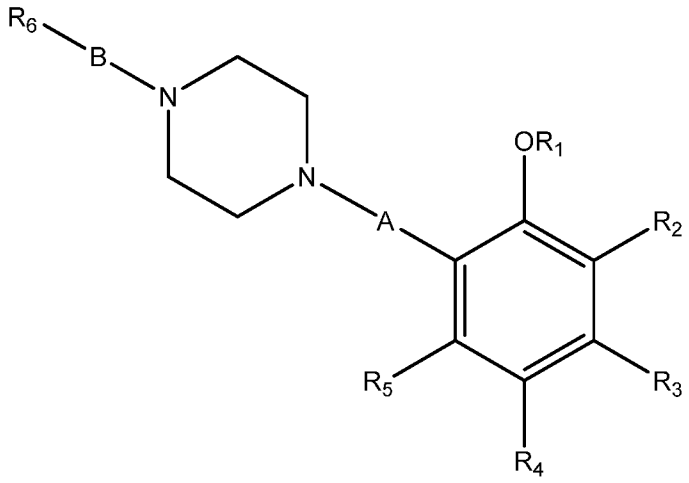
1. A method of treating diastolic dysfunction with preserved ejection fraction in a subject, wherein the subject does not suffer from a cardiac injury or structural heart disease, wherein the diastolic dysfunction with preserved ejection fraction is characterized by:

(i) a lack of increased late $I_{Na}$ in cardiomyocytes,

(ii) an increase in myofilament calcium sensitivity, or

(iii) a combination thereof,

the method comprising administering to the subject in an amount effective to treat the diastolic dysfunction with preserved ejection fraction a cardiac metabolic modifier comprising a structure of Formula I:




[Formula I]

wherein A comprises a main chain of 1-8 atoms, each atom of which is independently C, O, N, or S, and each atom of which is optionally bound to an additional group selected from C1-C8 alkyl, C1-C8 alkoxy, OH, NH2, NH(C1-C4 alkyl) and SH;

wherein $R_1$ is H or a C1-C8 alkyl;

wherein each of $R_2$, $R_3$, $R_4$, and $R_5$ independently is H, a C1-C8 alkyl, or a C1-C8 alkoxy;

wherein B is H or comprises a main chain of 1-8 atoms, each atom of which is independently C, O, N, or S, and each atom of which is optionally bound to an additional group; and,

wherein $R_6$ is absent or phenyl, optionally substituted with 1 to 5 groups, each group of which is independently C1-C8 alkyl, C1-C8 alkoxy, or OH.

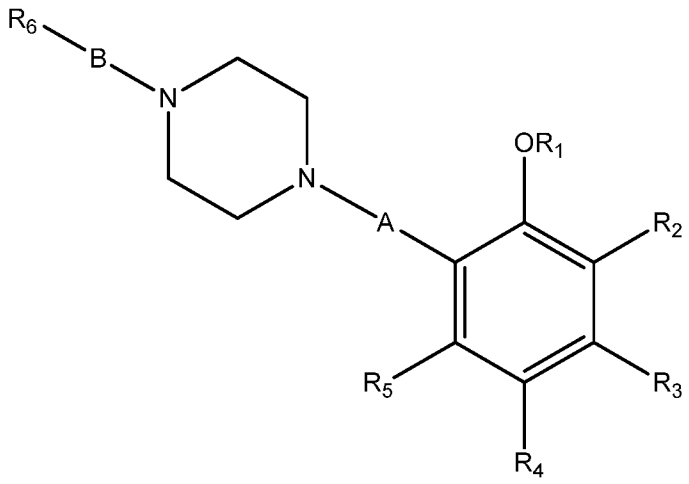
2. The method of claim 1, wherein the diastolic dysfunction with preserved ejection fraction is characterized by:

(i) a lack of change in calcium cycling or calcium handling in cardiomyocytes;
(ii) a lack of change in calcium concentration in resting myocytes;
(iii) a decrease in sarcomere length in resting myocytes;
(iv) an increase in diastolic tension; or
(v) a combination thereof.

3. A method of treating or preventing heart failure with preserved ejection fraction in a subject, wherein the subject does not suffer from a cardiac injury or structural heart disease, wherein the heart failure with preserved ejection fraction is characterized by:

(i) a lack of increased late $I_{Na}$ in cardiomyocytes,
(ii) an increase in myofilament calcium sensitivity, or
(iii) a combination thereof,

the method comprising administering to the subject in an amount effect to treat or prevent heart failure with preserved ejection fraction a cardiac metabolic modifier comprising a structure of Formula I:




[Formula I]

wherein A comprises a main chain of 1-8 atoms, each atom of which is independently C, O, N, or S, and each atom of which is optionally bound to an additional group selected from C1-C8 alkyl, C1-C8 alkoxy, OH, NH2, NH(C1-C4 alkyl) and SH;

wherein $R_1$ is H or a C1-C8 alkyl;

wherein each of $R_2$, $R_3$, $R_4$, and $R_5$ independently is H, a C1-C8 alkyl, or a C1-C8 alkoxy;

wherein B is H or comprises a main chain of 1-8 atoms, each atom of which is independently C, O, N, or S, and each atom of which is optionally bound to an additional group; and,

wherein $R_6$ is absent or phenyl, optionally substituted with 1 to 5 groups, each group of which is independently C1-C8 alkyl, C1-C8 alkoxy, or OH.

4. The method of claim 3, wherein the heart failure with preserved ejection fraction is characterized by:

(i) a lack of change in calcium cycling or calcium handling in cardiomyocytes;
(ii) a lack of change in calcium concentration in resting myocytes;
(iii) a decrease in sarcomere length in resting myocytes;
(iv) an increase in diastolic tension; or
(v) a combination thereof.

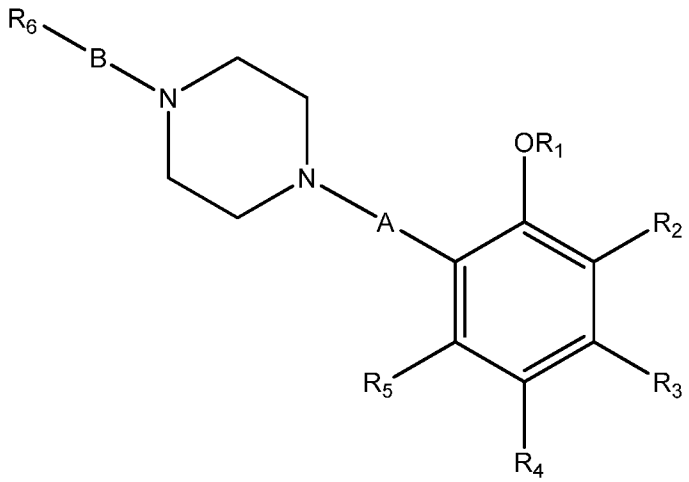
5. The method of any of claims 1 to 4, wherein the cardiac injury or structural heart disease is selected from the group consisting of: ischemia, ischemia-reperfusion or artery occlusion-reperfusion, myocardial injury, myocardial toxicity, myocardial infarction, congenital heart lesion, valvular stenosis or valvular regurgitation, coronary artery disease, chronic angina, chronic stable angina, arrhythmias.

6. The method of any of claims 1 to 5, wherein the subject suffers from neither a New York Heart Association (NYHA) Class I heart failure nor a NYHA Class II heart failure.

7. The method of any of claims 3 to 6, wherein the subject suffers from a NYHA Class III or IV heart failure.

8. The method of any of claims 1 to 7, wherein the subject does not present signs of myocardial wall thinning or regional wall motion abnormalities.

9. A method of modulating myofilament calcium sensitivity in a subject, comprising administering to the subject in an amount effective to modulate myofilament calcium sensitivity a cardiac metabolic modifier comprising a structure of Formula I:




[Formula I]

wherein A comprises a main chain of 1-8 atoms, each atom of which is independently C, O, N, or S, and each atom of which is optionally bound to an additional group selected from C1-C8 alkyl, C1-C8 alkoxy, OH, NH2, NH(C1-C4 alkyl) and SH;

wherein $R_1$ is H or a C1-C8 alkyl;

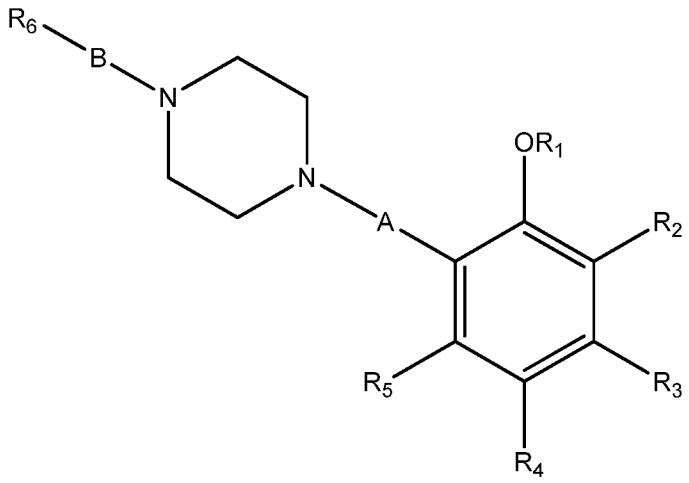
wherein each of $R_2$, $R_3$, $R_4$, and $R_5$ independently is H, a C1-C8 alkyl, or a C1-C8 alkoxy;

wherein B is H or comprises a main chain of 1-8 atoms, each atom of which is independently C, O, N, or S, and each atom of which is optionally bound to an additional group; and,

wherein $R_6$ is absent or phenyl, optionally substituted with 1 to 5 groups, each group of which is independently C1-C8 alkyl, C1-C8 alkoxy, or OH.

10. The method of claim 9, wherein the subject suffers from a diastolic dysfunction.

11. A method of treating a condition associated with or caused by increased myofilament calcium sensitivity in a subject, comprising administering to the subject in an amount effective to treat the condition a cardiac metabolic modifier comprising a structure of Formula I:




[Formula I]

wherein A comprises a main chain of 1-8 atoms, each atom of which is independently C, O, N, or S, and each atom of which is optionally bound to an additional group selected from C1-C8 alkyl, C1-C8 alkoxy, OH, NH2, NH(C1-C4 alkyl) and SH;

wherein $R_1$ is H or a C1-C8 alkyl;

wherein each of $R_2$, $R_3$, $R_4$, and $R_5$ independently is H, a C1-C8 alkyl, or a C1-C8 alkoxy;

wherein B is H or comprises a main chain of 1-8 atoms, each atom of which is independently C, O, N, or S, and each atom of which is optionally bound to an additional group; and,

wherein $R_6$ is absent or phenyl, optionally substituted with 1 to 5 groups, each group of which is independently C1-C8 alkyl, C1-C8 alkoxy, or OH..

12. The method of claim 11, wherein the condition is a diastolic dysfunction.

13. The method of claim 10 or 12, wherein the diastolic dysfunction is diastolic dysfunction with preserved ejection fraction.

14. The method of claim 10 or 12, wherein the diastolic dysfunction is diastolic dysfunction with preserved systolic function or diastolic dysfunction without systolic dysfunction.

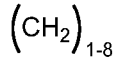
15. The method of any of claims 10 to 14, wherein the condition or diastolic dysfunction is characterized by one or more of the following:

(i) a lack of increased late $I_{Na}$ in cardiomyocytes,
(ii) an increase in myofilament calcium sensitivity, or
(iii) a combination thereof.

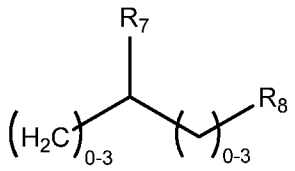
16. The method of claim 15, wherein the diastolic dysfunction with preserved ejection fraction is characterized by:

(i) a lack of change in calcium cycling or calcium handling in cardiomyocytes;
(ii) a lack of change in calcium concentration in resting myocytes;
(iii) a decrease in sarcomere length in resting myocytes;
(iv) an increase in diastolic tension; or
(v) a combination thereof.

17. The method of any of claims 1 to 16, wherein A is



or comprises a structure of Formula IV:



[Formula IV]

wherein $R_7$ is OH, C1-C4 alkyl, C1-C4 alkoxy, NH2, or NH(C1-C4 alkyl); and wherein $R_8$ is O or NH.

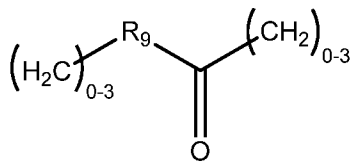
18. The method of any of claims 1 to 17, wherein $R_1$ is CH3.

19. The method of any of claims 1 to 18, wherein each of $R_2$, $R_3$, $R_4$, and $R_5$ independently is H or methoxy.

20. The method of claim 19, wherein each of $R_2$ and $R_3$ is a methoxy, and each of $R_4$ and $R_5$ is H.

21. The method of any of claims 1 to 20, wherein B comprises H and $R_6$ is absent.

22. The method of any of claims 1 to 20, wherein B comprises a structure of Formula V:



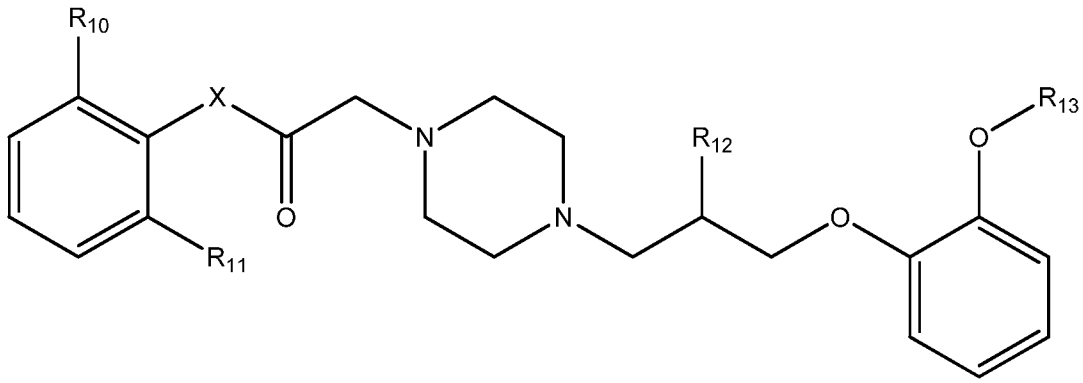
[Formula V]

wherein $R_9$ is NH or O.

23. The method of any of claims 1 to 20 and 22, wherein $R_6$ comprises phenyl substituted with 1 to 5 methyl groups.

24. The method of claim 23, wherein $R_6$ comprises phenyl substituted with methyl at each of the ortho positions.

25. The method of any of claims 1 to 18, wherein the cardiac metabolic modifier comprises a compound of Formula II, or a pharmaceutically acceptable salt thereof or a conjugate thereof:

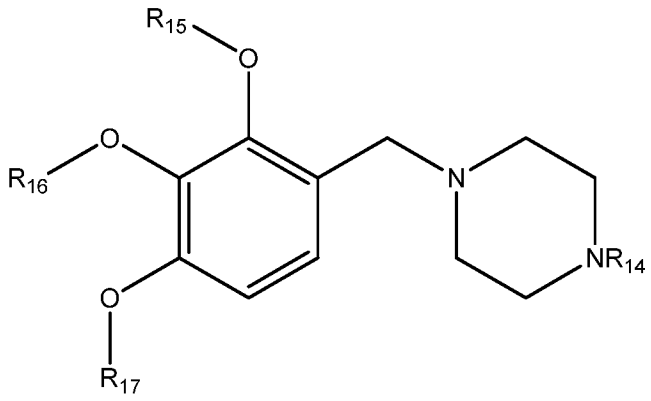



[Formula II]

wherein each of $R_{10}$, $R_{11}$, and $R_{13}$ independently is C1-C3 alkyl,
wherein X is NH or O; and
wherein $R_{12}$ is OH or C1-C3 alkyl.

26. The method of claim 25, wherein the compound of Formula II is ranolazine, or a pharmaceutically acceptable salt thereof or a conjugate thereof.

27. The method of any of claims 1 to 18, wherein the cardiac metabolic modifier is a compound of Formula III, or a pharmaceutically acceptable salt thereof or a conjugate thereof:




[Formula III]

wherein each of $R_{14}$, $R_{15}$, $R_{16}$, and $R_{17}$ independent is H or C1-C3 alkyl.

28. The method of claim 27, wherein the compound of Formula III is trimetazidine, or a pharmaceutically acceptable salt thereof or a conjugate thereof.

29. The method of any of the preceding claims, wherein the cardiac metabolic modifier (i) inhibits fatty acid oxidation in cardiomyocytes, (ii) lowers myofilament calcium sensitivity, (iii) inhibits an ion channel, or (iv) a combination thereof.

30. The method of any of the preceding claims, wherein the cardiac metabolic modifier is administered orally or parenterally.

31. The method of claim 30, wherein the cardiac metabolic modifier is administered intraperitoneally or intravenously.

32. The method of any of the preceding claims, wherein the cardiac metabolic modifier is administered in an amount which provides the subject with a plasma concentration of the cardiac metabolic modifier of at least 1000 nM after administration.

33. The method of claim 32, wherein the cardiac metabolic modifier is administered in an amount which provides the subject with a plasma concentration of the cardiac metabolic modifier which is at least about 1000 nM and less than 2000 nM after administration.

34. The method of any of the preceding claims, wherein the subject is a mammal.

35. The method of claim 34, wherein the mammal is a human.

36. The method of any of the preceding claims, wherein the subject suffers from hypertension, obesity, diabetes, or a combination thereof.

37. The method of any of claims 9 to 36, wherein the subject does not suffer from a cardiac injury or structural heart disease.

38. The method of claim 37, wherein the cardiac injury or structural heart disease is selected from the group consisting of: ischemia, ischemia-reperfusion or artery occlusion-reperfusion, myocardial injury, myocardial toxicity, myocardial infarction, congenital heart lesion, valvular stenosis or valvular regurgitation, coronary artery disease, chronic angina, chronic stable angina, arrhythmias.

39. The method of any of the preceding claims, wherein the subject does not present signs of myocardial wall thinning or regional wall motion abnormalities.

40. The method of any of claims 35 to 39, wherein the human is 40 years old or greater.

41. The method of any of claims 35 to 40, wherein the human is a female human.

*FIG. 1*

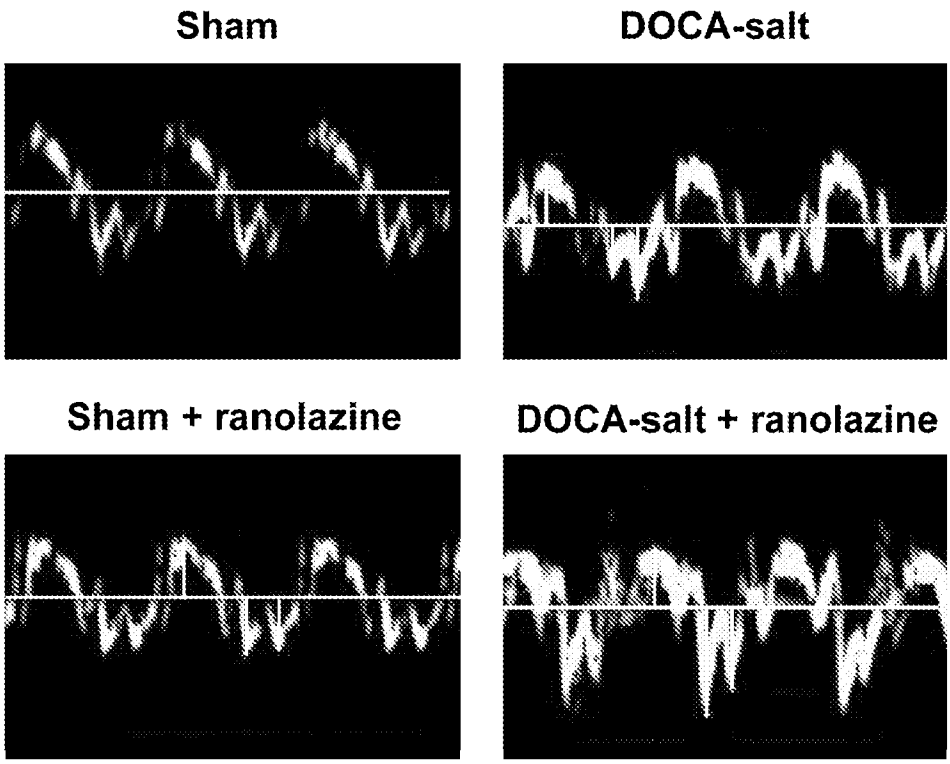
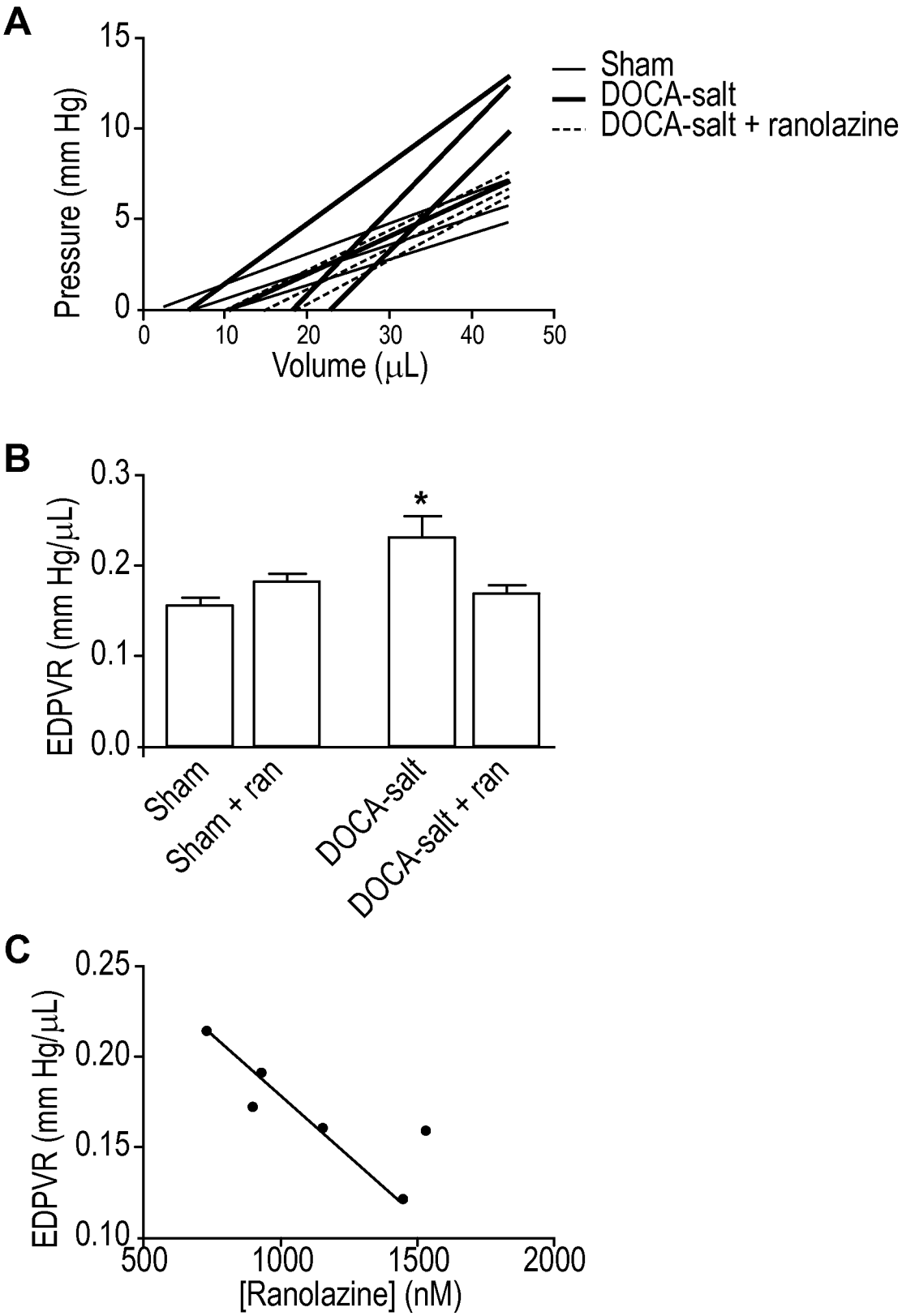


**FIG. 2**


A
Pressure (mm Hg)
Volume (μL)
Sham
DOCA-salt
DOCA-salt + ranolazine
B
EDPVR (mm Hg/μL)
Sham
Sham + ran
DOCA-salt
DOCA-salt + ran
C
EDPVR (mm Hg/μL)
[Ranolazine] (nM)

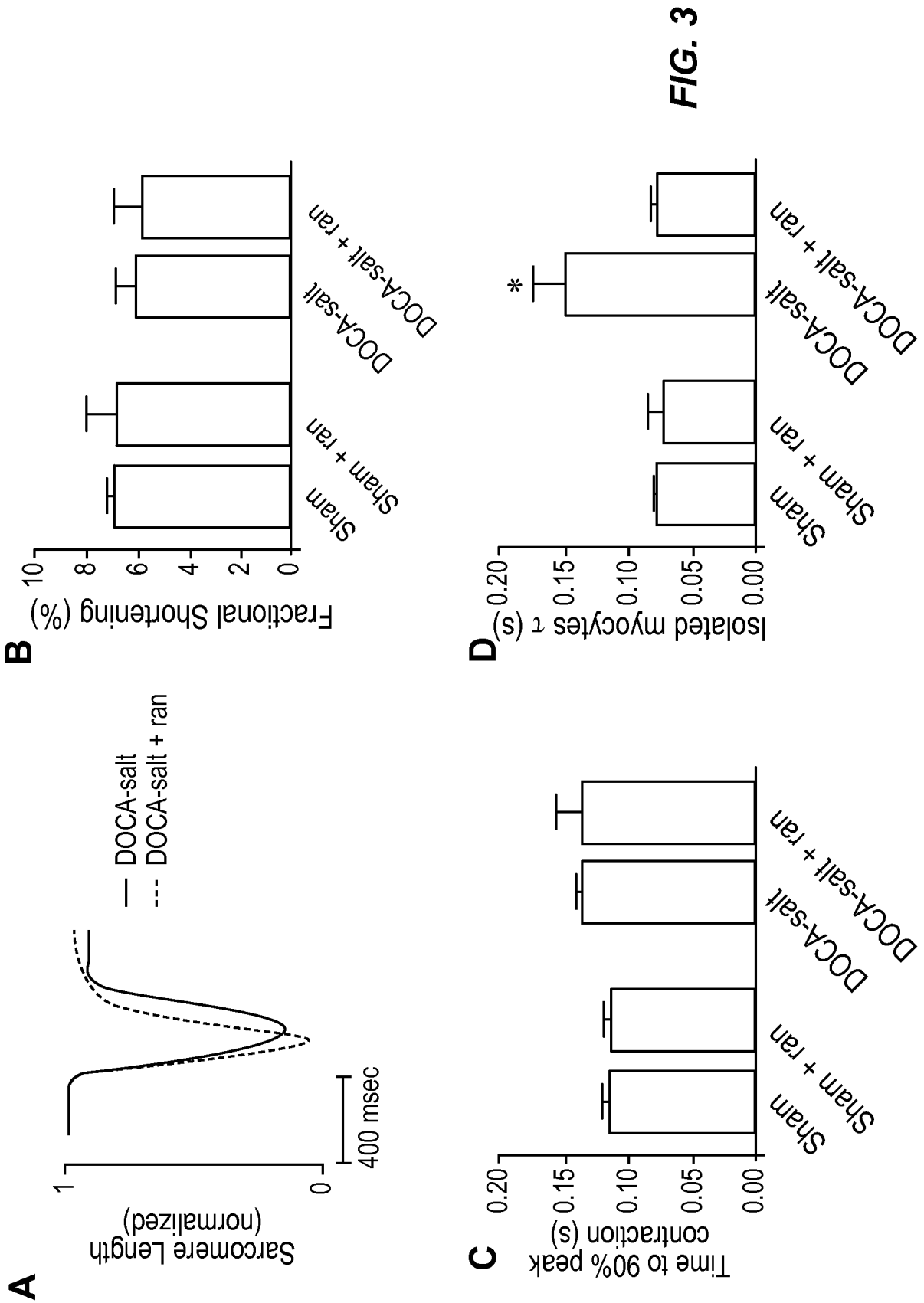

A
Sarcomere Length (normalized)
1
0
400 msec
— DOCA-salt
--- DOCA-salt + ran
B
Fractional Shortening (%)
10
8
6
4
2
0
Sham
Sham + ran
DOCA-salt
DOCA-salt + ran
C
Time to 90% peak contraction (s)
0.20
0.15
0.10
0.05
0.00
Sham
Sham + ran
DOCA-salt
DOCA-salt + ran
D
Isolated myocytes τ (s)
0.20
0.15
0.10
0.05
0.00
*
Sham
Sham + ran
DOCA-salt
DOCA-salt + ran

FIG. 3

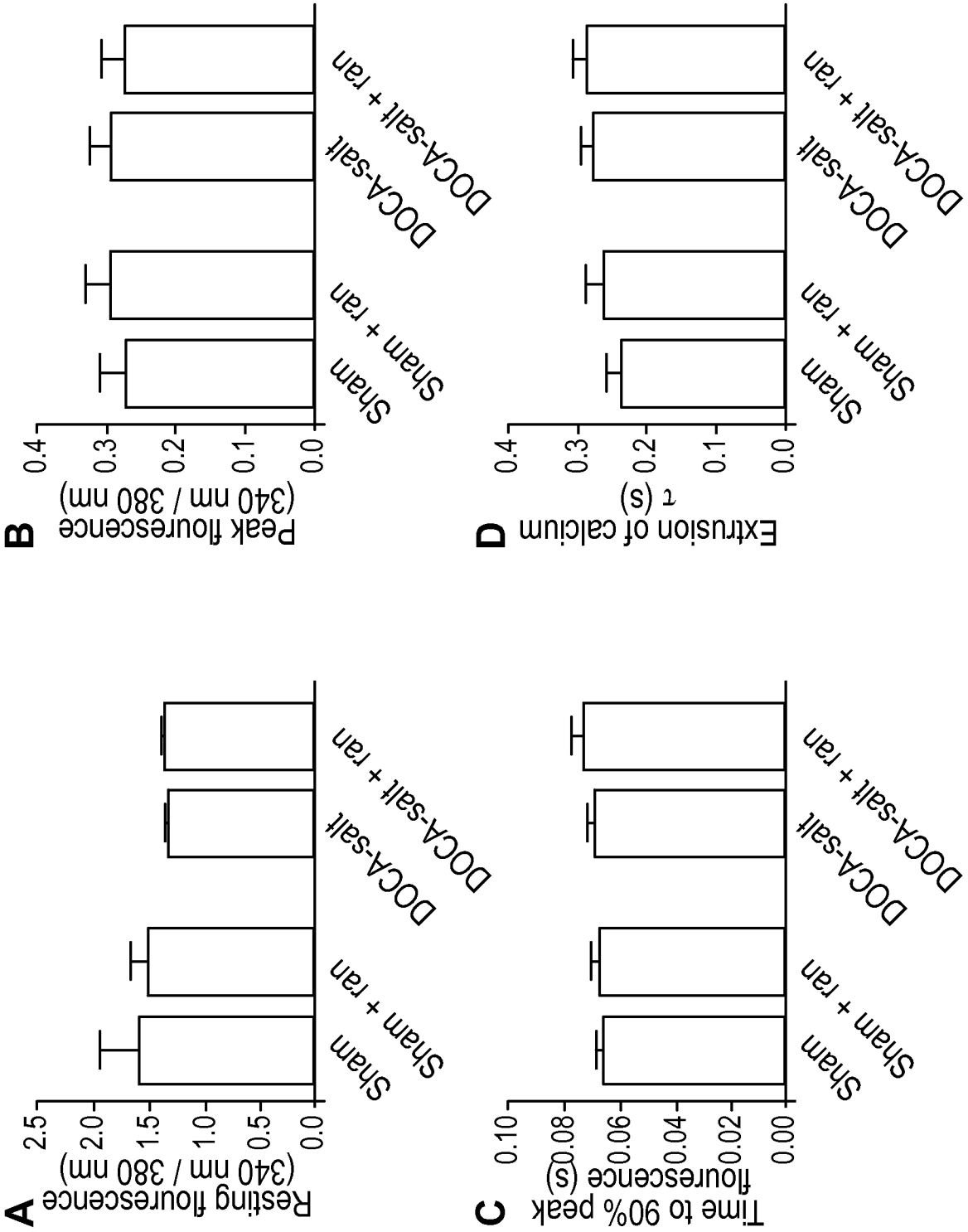

A
Resting fluorescence (340 nm / 380 nm)
2.5
2.0
1.5
1.0
0.5
0.0
Sham
Sham + ran
DOCA-salt
DOCA-salt + ran
B
Peak fluorescence (340 nm / 380 nm)
0.4
0.3
0.2
0.1
0.0
C
Time to 90% peak fluorescence (s)
0.10
0.08
0.06
0.04
0.02
0.00
D
Extrusion of calcium
τ (s)

FIG. 4

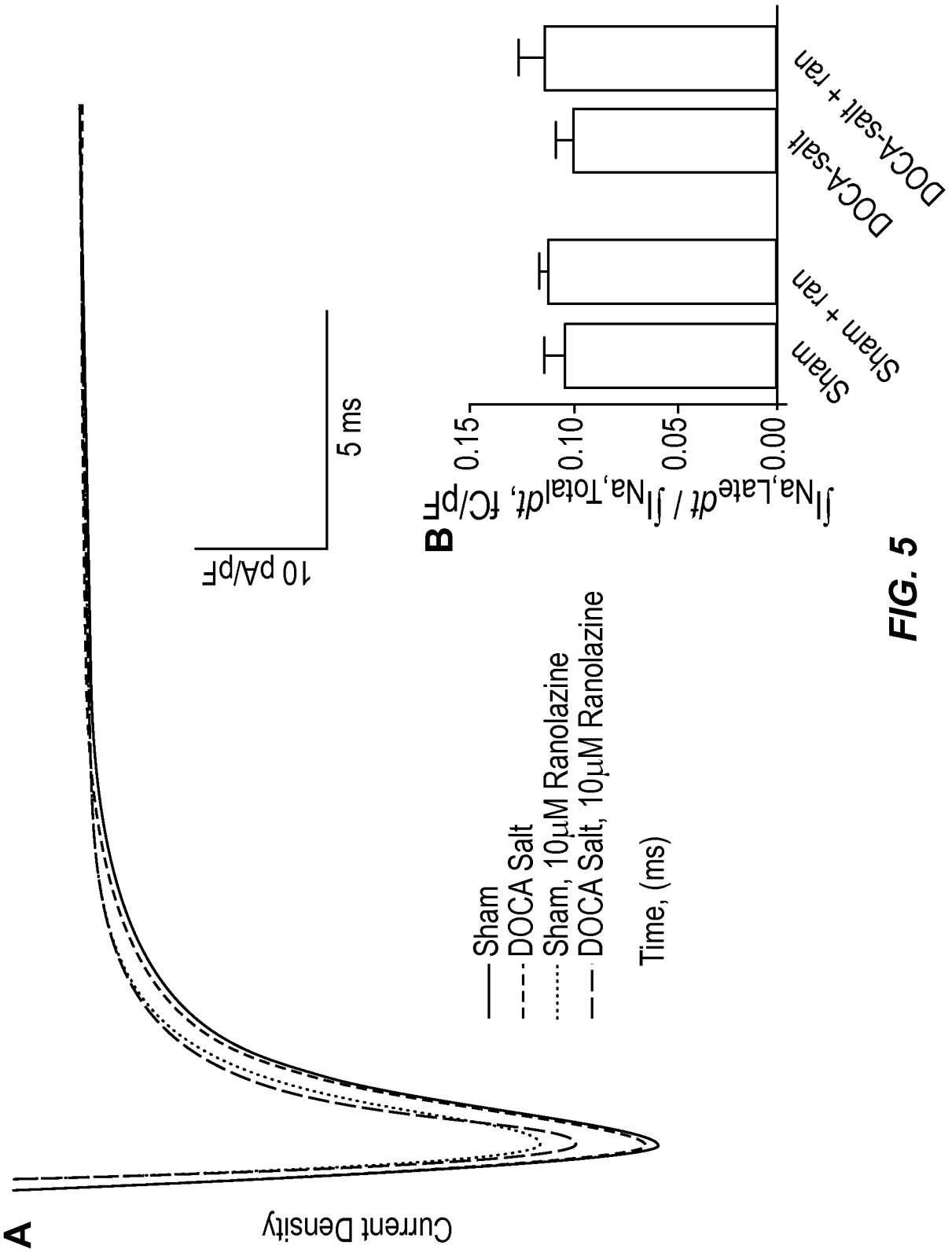

A
Current Density
Time, (ms)
Sham
DOCA Salt
Sham, 10μM Ranolazine
DOCA Salt, 10μM Ranolazine
10 pA/pF
5 ms
B
∫INa,Late dt / ∫INa,Total dt, fC/pF
0.15
0.10
0.05
0.00
Sham
Sham + ran
DOCA-salt
DOCA-salt + ran

FIG. 5

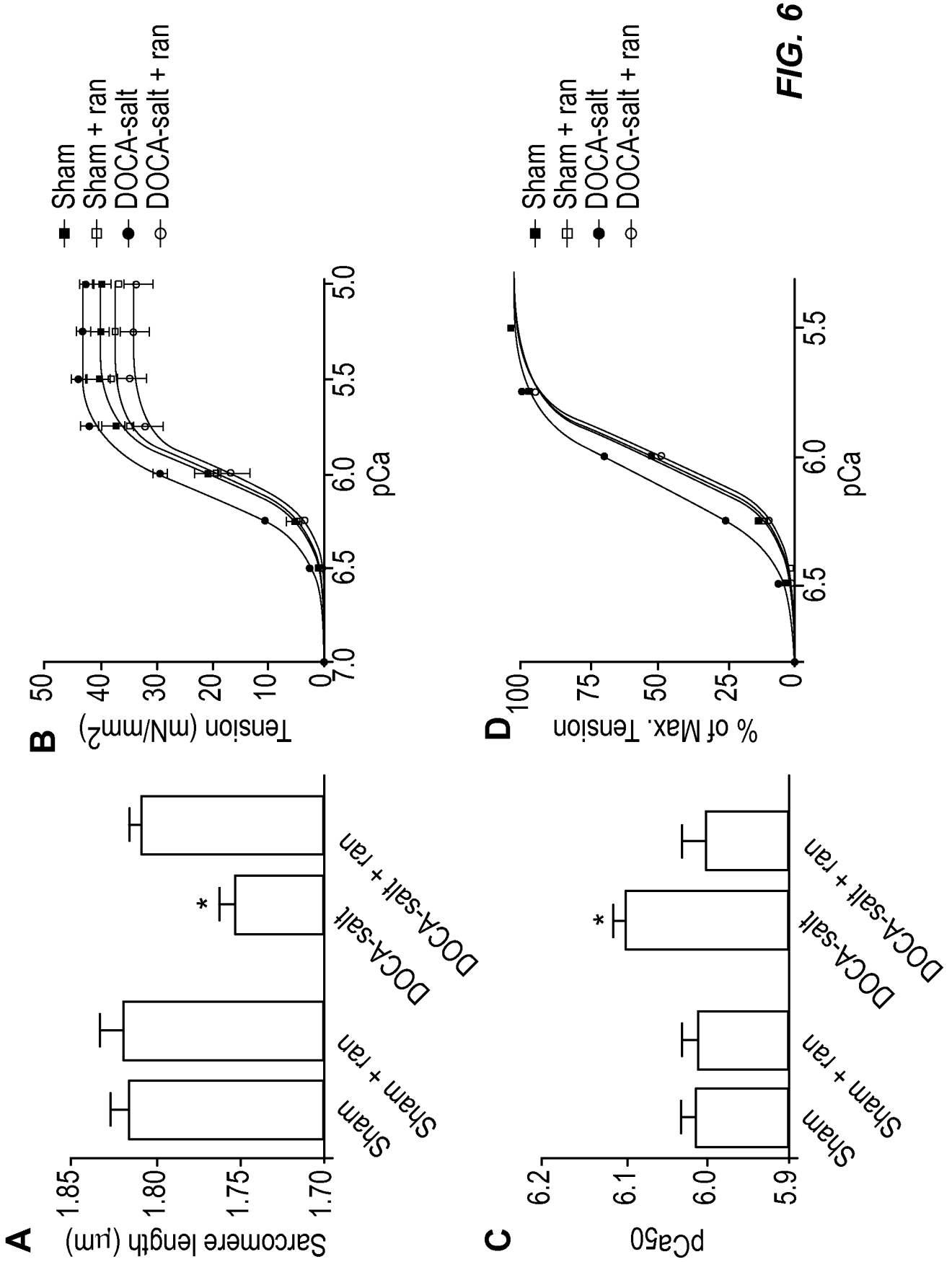

A
Sarcomere length (μm)
1.85
1.80
1.75
1.70
Sham
Sham + ran
DOCA-salt
DOCA-salt + ran
*
B
Tension (mN/mm2)
50
40
30
20
10
0
7.0
6.5
6.0
5.5
5.0
pCa
Sham
Sham + ran
DOCA-salt
DOCA-salt + ran
C
pCa50
6.2
6.1
6.0
5.9
Sham
Sham + ran
DOCA-salt
DOCA-salt + ran
*
D
% of Max. Tension
100
75
50
25
0
6.5
6.0
5.5
pCa
Sham
Sham + ran
DOCA-salt
DOCA-salt + ran

FIG. 6

# INTERNATIONAL SEARCH REPORT

International application No
PCT/US2010/048650

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/495 A61P9/00
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

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

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | TRET'IAKOV S V ET AL: "[Aspects of diastolic function of the heart in vibration disease]" TERAPEVTICHESKI § ARKHIV 2001 LNKD-PUBMED:11494444, vol. 73, no. 4, 2001, pages 34-37, XP009141016 ISSN: 0040-3660 * abstract page 35, column 1, paragraph 3 ----- -/-- | 1-18,27, 28,30, 34,35, 37-40 |

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents :

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

"E" earlier document but published on or after the international filing date

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

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

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

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

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

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

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 12 November 2010 | 24/11/2010 |

| Name and mailing address of the ISA/ | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040,<br>Fax: (+31-70) 340-3016 | Uryga-Polowy, V |

# INTERNATIONAL SEARCH REPORT

International application No
PCT/US2010/048650

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | WIERZBICKI P ET AL: "[Influence of trimetazidine on echocardiography parameters and free radical stress index in coronary artery disease and end-stage renal failure patients treated by hemodialysis: preliminary communication]" POLSKIE ARCHIWUM MEDYCYNY WEWN TRZNEJ JUL 1999 LNKD- PUBMED:10835920, vol. 102, no. 1, July 1999 (1999-07), pages 589-594, XP009140960 ISSN: 0032-3772 page 590, column 2, paragraph 3 page 591, column 1, paragraph 2 | 9-12,17, 18,27,28 |
| X | SOSSALLA S ET AL: "Ranolazine improves diastolic dysfunction in isolated myocardium from failing human hearts - Role of late sodium current and intracellular ion accumulation" JOURNAL OF MOLECULAR AND CELLULAR CARDIOLOGY, ACADEMIC PRESS, GB LNKD- DOI:10.1016/J.YJMCC.2008.03.006, vol. 45, no. 1, 1 July 2008 (2008-07-01), pages 32-43, XP022764730 ISSN: 0022-2828 [retrieved on 2008-03-14] the whole document | 1-25, 27-41 |
| A | STONE PETER H: "Ranolazine: new paradigm for management of myocardial ischemia, myocardial dysfunction, and arrhythmias" CARDIOLOGY CLINICS, W.B. SAUNDERS COMPANY, PHILADELPHIA, US, vol. 26, no. 4, 1 January 2008 (2008-01-01), pages 603-614, XP009140939 ISSN: 0733-8651 the whole document | 1-41 |
| A | WU Y ET AL: "The late Na<+> current (INa) inhibitor ranolazine attenuates effects of palmitoyl-L-carnitine to increase late INa and cause ventricular diastolic dysfunction" JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS 2009 AMERICAN SOCIETY FOR PHARMACOLOGY AND EXPERIMENTAL THERAPY USA LNKD- DOI:10.1124/JPET.109.151936, vol. 330, no. 2, August 2009 (2009-08), pages 550-557, XP002609273 the whole document | 1-41 |

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# INTERNATIONAL SEARCH REPORT

International application No
PCT/US2010/048650

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | KINDERMANN M ET AL: "Heart Failure With Normal Left Ventricular Ejection Fraction: What is the Evidence?" TRENDS IN CARDIOVASCULAR MEDICINE, ELSEVIER SCIENCE, NEW YORK, NY, US LNKD-DOI:10.1016/J.TCM.2008.12.003, vol. 18, no. 8, 1 November 2008 (2008-11-01), pages 280-292, XP026061081 ISSN: 1050-1738 [retrieved on 2009-04-02] the whole document | 1-41 |
| A | YIP G W-K ET AL: "Heart failure with a normal ejection fraction: New developments" HEART, BMJ, LONDON, GB LNKD-DOI:10.1136/HRT.2009.176222, vol. 95, no. 19, 29 July 2009 (2009-07-29) , pages 1549-1552, XP009140922 online ISSN: 1355-6037 page 1551, column 3, paragraph 2 - page 1552, column 1, paragraph 1 | 1-41 |