

(19) DANMARK

(10) DK/EP 2640423 T3



(12)

Oversættelse af
europæisk patentskrift

Patent- og
Varemærkestyrelsen

(51) Int.Cl.: **A 61 K 47/64 (2017.01)** **C 07 D 339/04 (2006.01)** **C 07 F 9/6553 (2006.01)**
C 07 K 5/06 (2006.01) **C 07 K 5/062 (2006.01)** **C 07 K 5/065 (2006.01)**
C 07 K 5/072 (2006.01)

(45) Oversættelsen bekendtgjort den: 2017-10-02

(80) Dato for Den Europæiske Patentmyndigheds
bekendtgørelse om meddelelse af patentet: 2017-07-26

(86) Europæisk ansøgning nr.: **11793558.5**

(86) Europæisk indleveringsdag: 2011-11-10

(87) Den europæiske ansøgnings publiceringsdag: 2013-09-25

(86) International ansøgning nr.: **US2011060259**

(87) Internationalt publikationsnr.: **WO2012067947**

(30) Prioritet: **2010-11-18 US 415241 P** **2010-11-18 US 415240 P**
2011-04-22 US 201161478310 P **2011-06-24 US 201161500974 P**

(84) Designerede stater: **AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV**
MC MK MT NL NO PL PT RO RS SE SI SK SM TR

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(54) Benævnelse: **LIPOYLFORBINDELSER OG DERES ANVENDELSE TIL BEHANDLING AF ISKÆMISK SKADE**

(56) Fremdragne publikationer:

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DESCRIPTION

BACKGROUND OF THE INVENTION

[0001] Coronary heart disease is a leading cause of death and injury worldwide. Following an acute myocardial infarction (MI), early restoration of blood flow is the most effective strategy for reducing the size of the MI. Paradoxically, restoring blood flow to the area of the heart that is affected by the diminished flow can, in itself, be harmful. This is called reperfusion injury and can, by some estimates, be responsible for up to 50% of the final size of the MI (Yellon D. M., Hausenloy D. J., Myocardial reperfusion injury. New England Journal of Medicine 2007, 357:1121). The final size of the MI ultimately determines how well the heart can function after a heart attack.

[0002] Myocardial ischemia-reperfusion injury is defined as myocardial injury caused by the ischemic injury combined with injury caused by the restoration of coronary blood flow after an ischemic episode. Ischemia-reperfusion injury is mediated by an influx of calcium ions and depletion of oxygen during an ischemic event, followed by reoxygenation and generation of reactive oxygen species during reperfusion (Piper, H. M., Abdallah, C., Schafer, C., The first minutes of reperfusion: a window of opportunity for cardioprotection. Annals of Thoracic Surgery 2003, 75:644; Yellon, D. M., Hausenloy, D. J., Myocardial reperfusion injury. New England Journal of Medicine 2007, 357:1121). It is postulated that the influx of calcium and the increase in free radicals triggers cell death, or programmed cell death (Chen, X., Zhang, X., Hubo, H., et al., Ca²⁺ influx-induced sarcoplasmic reticulum Ca²⁺ overload causes mitochondrial-dependent cell death in ventricular myocytes. Circ Res 2005, 97:1009; Lopes-Neblina, F., Toledo, A.H., Toledu-Pereyra, L.H. Molecular biology of apoptosis in ischemia and reperfusion. J Invest Surg 2005, 18:335). However, treatment of patients with acute myocardial infarction with antagonists that block the influx of calcium or with scavengers of the reactive oxygen species has yielded disappointing clinical outcomes (Yellon, D. M., Hausenloy, D. J., Myocardial reperfusion injury. New England Journal of Medicine 2007, 357:1121).

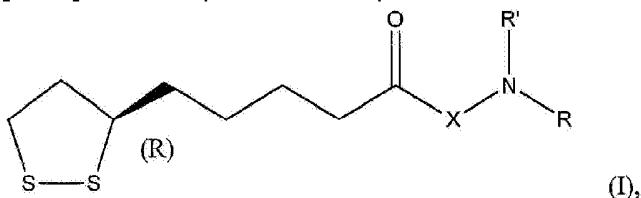
[0003] Another strategy for reducing ischemia-reperfusion injury is termed ischemic preconditioning. Short repeated bouts of ischemia followed by reperfusion will condition the myocardium to withstand a prolonged bout of ischemia (Otani, H., Ischemic preconditioning: From molecule mechanisms to therapeutic opportunities. Antioxidants & Redox Signaling, 2008,10:207). However, intentionally occluding a patient's coronary artery is associated with undue risks and is therefore undesirable.

[0004] Thus, there is a significant need for new and more effective therapies and therapeutic agents for the treatment of ischemia and ischemia-reperfusion injuries resulting from cardiovascular disease and other conditions.

SUMMARY OF THE INVENTION

[0005] The invention described herein addresses a need for treating ischemia, ischemic injury and ischemia-reperfusion injury, including myocardial ischemia. In particular, the present invention relates to compositions comprising the disclosed compounds, or pharmaceutically acceptable salts thereof, and the use of the disclosed compounds, or pharmaceutically acceptable salts thereof, to treat ischemia, ischemic injury, ischemia-reperfusion injury, and related conditions.

[0006] The compounds of the present invention are represented by Structural Formula (I):

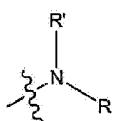


or a pharmaceutically acceptable salt thereof, wherein the diastereomeric excess of the compound or pharmaceutically acceptable salt thereof is at least 90%, wherein:

R is (C₁-C₁₈)alkyl, (C₆-C₁₈)aryl or (C₆-C₁₈)aryl(C₁-C₁₈)alkyl and is substituted with at least one acidic substituent selected from the group consisting of -CO₂H, -SO₃H, -PO₃H₂, -OSO₃H, -OPO₃H₂, -B(OH)₂ and -NHOH, wherein the aryl of the (C₆-C₁₈)aryl or (C₆-C₁₈)aryl(C₁-C₁₈)alkyl is optionally further substituted with one or more substituents selected from the group consisting of hydroxy, halo, (C₁-C₃)alkyl, halo(C₁-C₃)alkyl, cyano, nitro, (C₁-C₃)alkoxy and thio(C₁C₃)alkyl;

R' is hydrogen or (C₁-C₁₈)alkyl, wherein (C₁-C₁₈)alkyl is optionally substituted with one or more acidic substituents selected from the group consisting of -CO₂H, -SO₃H, -PO₃H₂, -OSO₃H, -OPO₃H₂, -B(OH)₂ and -NHOH; and

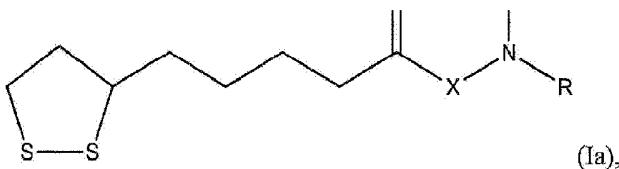
X is absent or is aspartic acid, tyrosine, glutamic acid or alanine and is an amino acid, wherein the amino acid is oriented to form an amide linkage with



provided that the compound of Structural Formula (I) is not N-(R)-lipoyl-glutamylalanine, N-(R)-lipoyl-aminoethylphosphonic acid, or (R)-5-(5-(1,2-dithiolan-3-yl)pentanamido)-2-hydroxybenzoic acid.

[0007] The present invention also provides a compound for use in a method of treating ischemic injury or ischemia-reperfusion injury in a subject in need thereof, wherein the compound is as defined in Claim 11. In one embodiment, the compound is represented by Structural Formula (Ia):



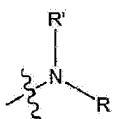


or a pharmaceutically acceptable salt thereof, wherein:

R is (C₁-C₁₈)alkyl, (C₆-C₁₈)aryl or (C₆-C₁₈)aryl(C₁-C₁₈)alkyl and is substituted with at least one acidic substituent selected from the group consisting of -CO₂H, -SO₃H, -PO₃H₂, -OSO₃H, -OPO₃H₂, -B(OH)₂ and -NHOH, wherein the aryl of the (C₆-C₁₈)aryl or (C₆-C₁₈)aryl(C₁-C₁₈)alkyl is optionally further substituted with one or more substituents selected from the group consisting of hydroxy, halo, (C₁-C₃)alkyl, halo(C₁-C₃)alkyl, cyano, nitro, (C₁-C₃)alkoxy and thio(C₁-C₃)alkyl;

R' is hydrogen or (C₁-C₁₈)alkyl, wherein (C₁-C₁₈)alkyl is optionally substituted with one or more acidic substituents selected from the group consisting of -CO₂H, -SO₃H, -PO₃H₂, -OSO₃H, -OPO₃H₂, -B(OH)₂ and -NHOH; and

X is an amino acid, wherein the amino acid is oriented to form an amide linkage with



provided that the compound of Structural Formula (Ia) is not *N*-lipoyl-glutamylalanine, *N*-lipoyl-aspartylglycine, or *N*-lipoyl-glutamylglycine.

[0008] We also disclose a method of inhibiting cell death in a subject, comprising administering to the subject an effective amount of a compound represented by Structural Formula (I) and/or (Ia), or a pharmaceutically acceptable salt or prodrug thereof.

[0009] We also disclose a method of inhibiting cell death in a cell, tissue or organ, wherein the cell, tissue or organ has experienced an ischemia or other condition or disorder that results in excessive or unwanted cell death, comprising administering to the cell, tissue or organ an effective amount of a compound represented by Structural Formula (I) and/or (Ia), or a pharmaceutically acceptable salt or prodrug thereof.

[0010] Also included in the present disclosure is the use of a compound represented by Structural Formula (I) and/or (Ia), or a pharmaceutically acceptable salt or prodrug thereof, for treating ischemic injury or ischemia-reperfusion injury in a subject.

[0011] We also disclose the use of a compound represented by Structural Formula (I) and/or (Ia), or a pharmaceutically acceptable salt or prodrug thereof, for inhibiting cell death in a subject.

[0012] The present invention also includes the use of a compound as defined in Claim 12, for the manufacture of a medicament for treating ischemic injury or ischemia-reperfusion injury in a subject.

[0013] We also disclose the use of a compound represented by Structural Formula (I) and/or (la), or a pharmaceutically acceptable salt or prodrug thereof, for the manufacture of a medicament for inhibiting cell death in a subject.

[0014] The compounds (also referred to herein as "the disclosed compounds"), compositions and uses of the present invention are efficacious for treating tissue damage resulting from ischemia and ischemic injuries, including ischemia-reperfusion injuries.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

[0015] The disclosed compounds may exist in various stereoisomeric forms unless otherwise specified. "Stereoisomers" are compounds that differ only in their spatial arrangement. "Enantiomers" are pairs of stereoisomers that are non-superimposable mirror images of one another, most commonly because they contain an asymmetrically substituted carbon atom that acts as a chiral center.

[0016] "Diastereomers" are stereoisomers that are not related as mirror images, most commonly because they contain two or more asymmetrically substituted carbon atoms. "R" and "S" represent the configuration of substituents around one or more chiral carbon atoms.

[0017] "Racemate" or "racemic mixture," as used herein, refers to a mixture containing equimolar quantities of two enantiomers of a compound. Such mixtures exhibit no optical activity (*i.e.*, they do not rotate a plane of polarized light).

[0018] Percent enantiomeric excess (ee) is defined as the absolute difference between the mole fraction of each enantiomer multiplied by 100% and can be represented by the following equation:

$$ee = \left| \frac{R-S}{R+S} \right| \times 100\%,$$

where R and S represent the respective fractions of each enantiomer in a mixture, such that R + S = 1. When a single enantiomer is named or depicted by structure, the depicted or named enantiomer is present in an ee of at least or about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, about 98%, about 99% or about 99.9%.

[0019] Percent diastereomeric excess (de) is defined as the absolute difference between the

mole fraction of each diastereomer multiplied by 100% and can be represented by the following equation:

$$de = \left| \frac{D_1 - (D_2 + D_3 + D_4 \dots)}{D_1 + (D_2 + D_3 + D_4 \dots)} \right| \times 100\%,$$

where D_1 and $(D_2 + D_3 + D_4 \dots)$ represent the respective fractions of each diastereomer in a mixture, such that $D_1 + (D_2 + D_3 + D_4 \dots) = 1$. When a single diastereomer is named or depicted by structure, the depicted or named diastereomer is present in a de of at least or about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, about 98%, about 99% or about 99.9%.

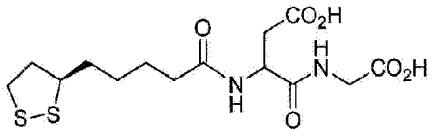
[0020] When a disclosed compound is named or depicted by structure without indicating the stereochemistry, and the compound has one chiral center, it is to be understood that the name or structure encompasses one enantiomer of the compound substantially separated from the corresponding optical isomer, a racemic mixture of the compound and mixtures enriched in one enantiomer relative to its corresponding optical isomer.

[0021] When a disclosed compound is named or depicted by structure without indicating the stereochemistry and has two or more chiral centers, it is to be understood that the name or structure encompasses a diastereomer substantially separated from other diastereomers, a pair of diastereomers substantially separated from other diastereomeric pairs, mixtures of diastereomers, mixtures of diastereomeric pairs, mixtures of diastereomers in which one diastereomer is enriched relative to the other diastereomer(s) and mixtures of diastereomeric pairs in which one diastereomeric pair is enriched relative to the other diastereomeric pair(s).

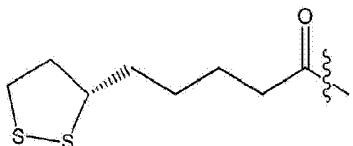
[0022] "(R)-Lipoyl" refers to a compound containing a lipoyl moiety, wherein the stereocenter in the lipoyl moiety is in the (R) configuration. An (R)-lipoyl moiety is pictured below:



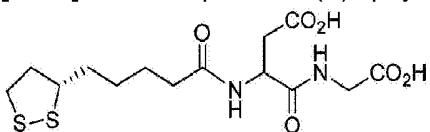
[0023] An example of an (R)-lipoyl compound is shown below:



[0024] "(S)-Lipoyl" refers to a compound containing a lipoyl moiety, wherein the stereocenter in the lipoyl moiety is in the (S) configuration. An (S)-lipoyl moiety is pictured below:



[0025] An example of an (S)-lipoyl compound is shown below:



[0026] "Alkyl" means a saturated aliphatic branched or straight-chain monovalent hydrocarbon radical having the specified number of carbon atoms. Thus, "(C₁-C₆)alkyl" means a radical having from 1-6 carbon atoms in a linear or branched arrangement. "(C₁-C₆)alkyl" includes methyl, ethyl, propyl, *i*-propyl, butyl, *i*-butyl, *t*-butyl, sec-butyl, pentyl and hexyl. Typically, alkyl has 1 to 20, 1 to 15, 1 to 10, 1 to 5 or 1 to 3 carbon atoms.

[0027] One or more hydrogen atoms of an alkyl group can be replaced with a substituent group. Suitable substituent groups include hydroxy, thio, halo, halo(C₁-C₃)alkyl, (C₁-C₃)alkoxy and thio(C₁-C₃)alkyl. Preferred alkyl substituent groups include hydroxy and halo. An alkyl can also be substituted with one or more acidic substituents selected from the group consisting of -CO₂H, -SO₃H, -PO₃H₂, -OSO₃H, -OPO₃H₂, -B(OH)₂ and -NHOH.

[0028] The term "alkoxy" means -O-alkyl, where alkyl is as defined above.

[0029] The term "halogen" means F, Cl, Br or I.

[0030] The term "aryl" means a carbocyclic aromatic ring. "(C₆-C₁₄)aryl" includes phenyl, napthyl, indenyl, and anthracenyl. Typically, aryl has 6 to 20, 6 to 14, 6 to 10, 6 to 9, or 6 carbon atoms.

[0031] As used herein, "substantially separated" or "substantially pure" means that the ee or de of the depicted or named compound is at least about 50%. For example, "substantially separated" or "substantially pure" can mean the ee or de of the depicted or named enantiomer is at least or about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, about 98%, about 99% or about 99.9%. In one example, substantially separated or substantially pure means that the ee or de of the depicted or named compound is at least or about 75%. In a specific example, substantially separated means that the ee or de of the depicted or named compound is at least or about 90%. In a more specific example, substantially separated means that the ee or de of the depicted or named compound is at least or about 95%. In yet a more specific example, substantially separated means that the ee or de of the depicted or named compound is at least or about 99%. In another specific example, substantially separated means that the ee or de of the depicted or named compound is at least or about 99.9%.

[0032] As used herein, the term "amino acid" means a molecule containing an amine group, a carboxylic acid group and a side chain which varies between different amino acids and includes both naturally-occurring amino acids and non-naturally-occurring amino acids. In one

embodiment, "amino acid" is used to refer to naturally-occurring amino acids.

[0033] As used herein, the term "naturally-occurring amino acid" means a compound represented by the formula $\text{NH}_2\text{-CHR-COOH}$, wherein R is the side chain of a naturally-occurring amino acid such as an amino acid listed or named in the Table below. "Naturally-occurring amino acid" includes both the D- and L-configuration. When an amino acid is named or depicted by structure without indicating the stereochemistry and has at least one chiral center, it is to be understood that the name or structure encompasses a single enantiomer or a diastereomer substantially separated from the other enantiomer or diastereomer, in which the one enantiomer or diastereomer is enriched relative to the other enantiomer or diastereomer(s), a racemic or diastereomeric mixture of the enantiomer or diastereomer(s) and mixtures enriched in one enantiomer or diastereomer relative to its corresponding optical isomer or other diastereomer(s).

Table of Common Naturally Occurring Amino Acids

	Amino acid	Three letter code	One letter code
Non-polar; neutral at pH 7.4	alanine	Ala	A
	isoleucine	Ile	I
	leucine	Leu	L
	methionine	Met	M
	phenylalanine	Phe	F
	proline	Pro	P
	tryptophan	Trp	W
	valine	Val	V
Polar, uncharged at pH 7.0	asparagine	Asn	N
	cysteine	Cys	C
	glycine	Gly	G
	glutamine	Gln	Q
	serine	Ser	S
	threonine	Thr	T
	tyrosine	Tyr	Y
Polar; charged at pH 7	glutamic acid	Glu	E
	arginine	Arg	R
	aspartic acid	Asp	D
	histidine	His	H
	lysine	Lys	K

[0034] "Non-natural amino acid" means an amino acid for which there is no nucleic acid codon. Examples of non-natural amino acids include natural α -amino acids with non-natural

side chains (e.g., entry 6 and 7 in Table 2); β -amino acids (e.g., β -alanine); γ -amino acids (e.g., γ -aminobutyric acid).

[0035] As used herein, an "effective amount" is an amount sufficient to achieve a desired therapeutic or prophylactic effect in a subject in need thereof under the conditions of administration, such as, for example, an amount sufficient to inhibit (e.g., prevent, delay) ischemia and ischemia-reperfusion injury in a subject (e.g., by inhibiting cell death of one or more affected cells in the subject). The effectiveness of a therapy can be determined by suitable methods known by those of skill in the art. An effective amount includes any amount of a compound (e.g., a compound of Structural Formula (I) and/or (Ia)) which prevents the onset of, alleviates the symptoms of, or stops the progression of a disorder or disease being treated (e.g., ischemia or ischemia-reperfusion injury) in a subject.

[0036] The term "treating" is defined as administering to a subject in need thereof an effective amount of a compound (e.g., of Structural Formula (I) and/or (Ia), or a pharmaceutically acceptable salt thereof) that is sufficient to prevent the onset of, alleviate the symptoms of, or stop the progression of a disorder or disease being treated.

[0037] The term "subject," as used herein, refers to a mammal. In a preferred embodiment, the subject is a human.

Compounds of the Invention

[0038] The present invention relates in one embodiment to a compound represented by Structural Formula (I) and/or (Ia).

[0039] R is $(C_1-C_{18})alkyl$, $(C_6-C_{18})aryl$ or $(C_6-C_{18})aryl(C_1-C_{18})alkyl$ and is substituted with at least one acidic substituent selected from the group consisting of $-CO_2H$, $-SO_3H$, $-PO_3H_2$, $-OSO_3H$, $-OPO_3H_2$, $-B(OH)_2$ and $-NHOH$, wherein the aryl of the $(C_6-C_{18})aryl$ or $(C_6-C_{18})aryl(C_1-C_{18})alkyl$ is optionally further substituted with one or more substituents selected from the group consisting of hydroxy, halo, $(C_1-C_3)alkyl$, halo $(C_1-C_3)alkyl$, cyano, nitro, $(C_1-C_3)alkoxy$ and thio $(C_1-C_3)alkyl$.

[0040] In one embodiment, R is $(C_1-C_{18})alkyl$. In another embodiment, R is $(C_1-C_3)alkyl$. In a further embodiment, R is $(C_3)alkyl$. In a further embodiment, R is $(C_2)alkyl$. Alternatively, R is $(C_1)alkyl$.

[0041] In another embodiment, R is $(C_6-C_{18})aryl$. In a further embodiment, R is $(C_6)aryl$.

[0042] In another embodiment, R is $(C_6-C_{18})aryl(C_1-C_{18})alkyl$. In a further embodiment, R is $(C_6)aryl(C_1-C_3)alkyl$. Alternatively, R is $(C_6)aryl(C_1-C_2)alkyl$.

[0043] In another embodiment, R is (C₆)aryl(C₂)alkyl. In a further embodiment, R is (C₆)aryl(C₁)alkyl.

[0044] The at least one acidic substituent is selected from the group consisting of -CO₂H, -SO₃H, -PO₃H₂, -OSO₃H, -OPO₃H₂, -B(OH)₂ and -NHOH. In one embodiment, the at least one acidic substituent is selected from the group consisting of -CO₂H, -SO₃H, -PO₃H₂, -OSO₃H and -OPO₃H₂.

[0045] R is substituted with at least one acidic substituent selected from the group consisting of -CO₂H, -SO₃H, -PO₃H₂, -OSO₃H, -OPO₃H₂, -B(OH)₂ and -NHOH. In one embodiment, R is substituted with one, two or three acidic substituents. In a further embodiment, R is substituted with one or two acidic substituents.

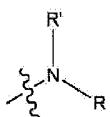
[0046] Aryl is optionally further substituted with one or more substituents selected from the group consisting of hydroxy, halo, (C₁-C₃)alkyl, halo(C₁-C₃)alkyl, cyano, nitro, (C₁-C₃)alkoxy and thio(C₁-C₃)alkyl. In one embodiment, aryl is further substituted with one, two or three substituents. In another embodiment, aryl is substituted with one substituent. Alternatively, aryl is unsubstituted. In a further embodiment, aryl is further substituted with one or more substituents selected from the group consisting of hydroxyl and halo.

[0047] R' is hydrogen or (C₁-C₁₈)alkyl, wherein said (C₁-C₁₈)alkyl is optionally substituted with one or more acidic substituents selected from the group consisting of -CO₂H, -SO₃H, -PO₃H₂, -OSO₃H, -OPO₃H₂, -B(OH)₂ and -NHOH. In one embodiment, R' is hydrogen.

[0048] In one embodiment, R' is (C₁-C₁₈)alkyl. In another embodiment, R' is (C₁-C₃)alkyl. In a further embodiment, R' is (C₃)alkyl. In a further embodiment, R' is (C₂)alkyl. Alternatively, R' is (C₁)alkyl.

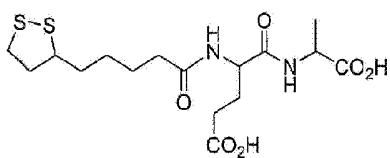
[0049] R' is substituted with at least one acidic substituent selected from the group consisting of -CO₂H, -SO₃H, -PO₃H₂, -OSO₃H, -OPO₃H₂, -B(OH)₂ and -NHOH. In one embodiment, R' is substituted with one, two or three acidic substituents. In another embodiment, R' is substituted with one or two acidic substituents. In a further embodiment, R' is substituted with one acidic substituent. Alternatively, R' is unsubstituted.

[0050] In the compounds defined in Claim 1, X is absent or an amino acid, wherein the amino acid is aspartic acid, tyrosine, glutamic acid or alanine and oriented to form an amide linkage with



For example, the moiety in *N*-lipoyl-glutamylalanine is oriented as shown in Structural Formula

below:



[0051] In one embodiment, X is absent. Alternatively, X is aspartic acid, tyrosine, glutamic acid or alanine.

[0052] The compounds of Structural Formulas (I) and/or (Ia) are not *N*-(R)-lipoyl-glutamylalanine, *N*-lipoyl-aspartylglycine, *N*-lipoyl-glutamylglycine, *N*-(R)-lipoyl-aminoethylphosphonic acid, or (R)-5-(5-(1,2-dithiolan-3-yl)pentanamido)-2-hydroxybenzoic acid.

[0053] The compounds of Structural Formulas (I) and/or (Ia) are not *N*-lipoyl-glutamylalanine, *N*-lipoyl-aspartylglycine, *N*-lipoyl-glutamylglycine, *N*-lipoyl-glutamine, *N*-lipoyl-glycine or 5-(5-(1,2-dithiolan-3-yl)pentanamido)-2-hydroxybenzoic acid.

[0054] In addition, in specific embodiments, the compound of Structural Formulas (I) and/or (Ia) is not *N*-lipoyl-aspartylalanine.

[0055] In a 1st specific embodiment, the compound is represented by Structural Formula (I) and/or (Ia), wherein the values and alternative values for the variables are as described above.

[0056] In a first aspect of the 1st specific embodiment of the present invention, the (R)-lipoyl stereoisomer of a compound represented by Structural Formulas (I) or (Ia), or a pharmaceutically acceptable salt thereof, has a diastereomeric excess of at least 90%. Values and alternative values for the remainder of the variables are as described above for Structural Formulas (I) or (Ia) or in the 1st specific embodiment.

[0057] In a second aspect of the 1st specific embodiment of the present invention, R' is H. Values and alternative values for the remainder of the variables are as described above for Structural Formulas (I) or (Ia) or in the 1st specific embodiment, or first aspect thereof.

[0058] In a third aspect of the 1st specific embodiment of the present invention, R' is H and X is a naturally-occurring amino acid. Values and alternative values for the remainder of the variables are as described above for Structural Formulas (I) or (Ia) or in the 1st specific embodiment, or first or second aspect thereof.

[0059] In a fourth aspect of the 1st specific embodiment of the present invention, R and R' are each (C₁-C₃)alkyl substituted with one or two acidic substituents each independently selected

from $-\text{CO}_2\text{H}$, $-\text{SO}_3\text{H}$, $-\text{PO}_3\text{H}_2$, $-\text{OSO}_3\text{H}$ and $-\text{OPO}_3\text{H}_2$. Values and alternative values for the remainder of the variables are as described above for Structural Formulas (I) or (Ia) or in the 1st specific embodiment, or first to third aspects thereof.

[0060] In a fifth aspect of the 1st specific embodiment of the present invention, R' is H and X is absent. Values and alternative values for the remainder of the variables are as described above for Structural Formulas (I) or (Ia) or in the 1st specific embodiment, or first to fourth aspects thereof.

[0061] In a sixth aspect of the 1st specific embodiment of the present invention, R is (C₁-C₃)alkyl substituted with one or two acidic substituents each independently selected from $-\text{CO}_2\text{H}$, $-\text{SO}_3\text{H}$, $-\text{PO}_3\text{H}_2$, $-\text{OSO}_3\text{H}$ and $-\text{OPO}_3\text{H}_2$. Values and alternative values for the remainder of the variables are as described above for Structural Formulas (I) or (Ia) or in the 1st specific embodiment, or first to fifth aspects thereof.

[0062] In a seventh aspect of the 1st specific embodiment of the present invention, R is (C₆)aryl(C₁-C₃)alkyl substituted with one or two acidic substituents each independently selected from $-\text{CO}_2\text{H}$, $-\text{SO}_3\text{H}$, $-\text{PO}_3\text{H}_2$, $-\text{OSO}_3\text{H}$ and $-\text{OPO}_3\text{H}_2$. Values and alternative values for the remainder of the variables are as described above for Structural Formulas (I) or (Ia) or in the 1st specific embodiment, or first to sixth aspects thereof.

[0063] In an eighth aspect of the 1st specific embodiment of the present invention, R is (C₂)alkyl substituted with one or two acidic substituents each independently selected from $-\text{CO}_2\text{H}$, $-\text{SO}_3\text{H}$, $-\text{PO}_3\text{H}_2$, $-\text{OSO}_3\text{H}$ and $-\text{OPO}_3\text{H}_2$. Values and alternative values for the remainder of the variables are as described above for Structural Formulas (I) or (Ia) or in the 1st specific embodiment, or first to seventh aspects thereof.

[0064] In a ninth aspect of the 1st specific embodiment of the present invention, R is (C₆)aryl substituted with one acidic substituent selected from $-\text{CO}_2\text{H}$, $-\text{SO}_3\text{H}$, $-\text{PO}_3\text{H}_2$, $-\text{OSO}_3\text{H}$ and $-\text{OPO}_3\text{H}_2$. Values and alternative values for the remainder of the variables are as described above for Structural Formulas (I) or (Ia) or in the 1st specific embodiment, or first to eighth aspects thereof.

[0065] In a tenth aspect of the 1st specific embodiment of the present invention, the compound represented by Structural Formulas (I) and/or (Ia) is not *N*-lipoyl-glutamylalanine, *N*-lipoyl-aspartylglycine, *N*-lipoyl-glutamylglycine or 5-(5-(1,2-dithiolan-3-yl)pentanamido)-2-hydroxybenzoic acid. Values and alternative values for the remainder of the variables are as described above for Structural Formulas (I) or (Ia) or in the 1st specific embodiment, or first to ninth aspects thereof.

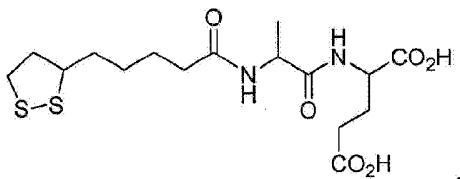
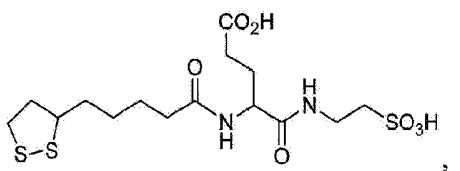
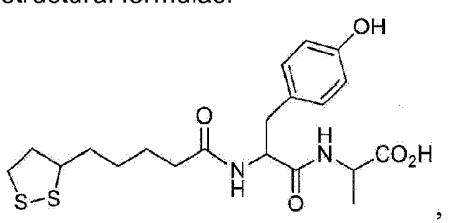
[0066] In an eleventh aspect of the 1st specific embodiment of the present invention, the compound represented by Structural Formulas (I) and/or (Ia) is not *N*-lipoyl-glutamylalanine, *N*-lipoyl-aspartylglycine, *N*-lipoyl-glutamylglycine, *N*-lipoyl-glutamic acid, *N*-lipoyl-aspartic acid, *N*-lipoyl-glycine or 5-(5-(1,2-dithiolan-3-yl)pentanamido)-2-hydroxybenzoic acid. Values and alternative values for the remainder of the variables are as described above for Structural Formulas (I) or (Ia) or in the 1st specific embodiment, or first to tenth aspects thereof.

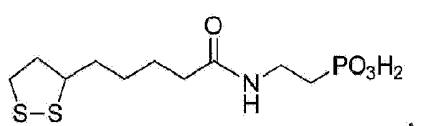
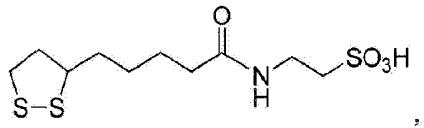
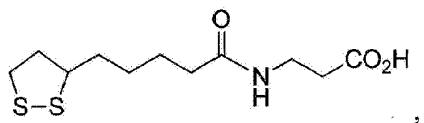
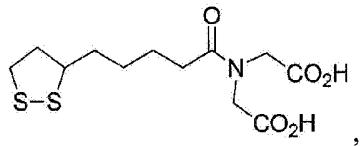
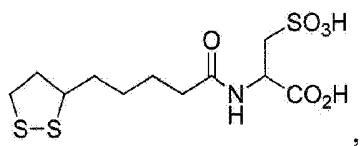
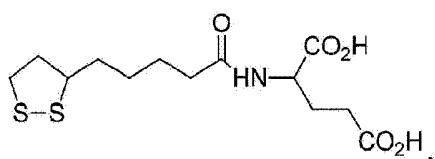
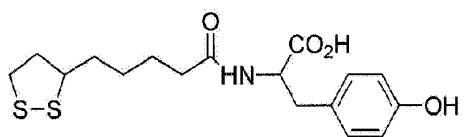
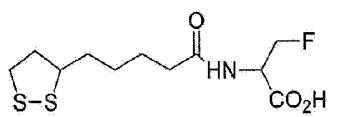
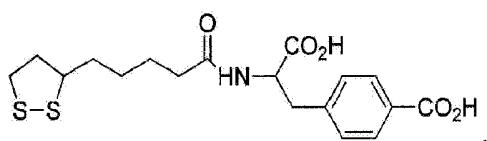
[0067] In a twelfth aspect of the 1st specific embodiment of the present invention, the compound of Structural Formula (I) is not *N*-(R)-lipoyl-glutamylalanine, *N*-(R)-lipoyl-aspartylglycine, *N*-(R)-lipoyl-aminoethylphosphonic acid, or (R)-5-(5-(1,2-dithiolan-3-yl)pentanamido)-2-hydroxybenzoic acid. Values and alternative values for the remainder of the variables are as described above for Structural Formulas (I) or (Ia) or in the 1st first specific embodiment, or first to eleventh aspects thereof.

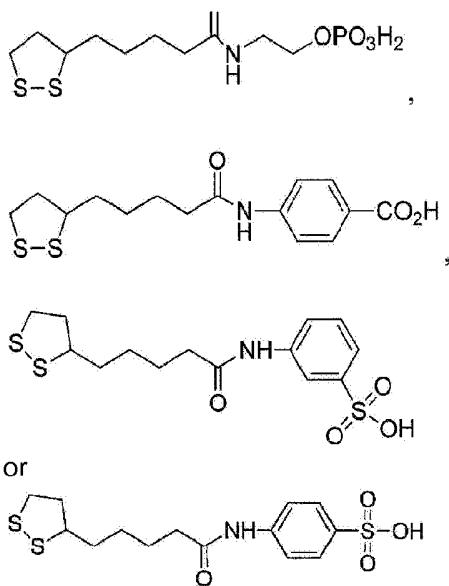
[0068] In a thirteenth aspect of the first specific embodiment, the compound is represented by Structural Formula (I), wherein the values and alternative values are as described above for Structural Formulas (I) or (Ia) or in the 1st specific embodiment, or first to twelfth aspects thereof.

[0069] In a fourteenth aspect of the 1st specific embodiment, the compound is represented by Structural Formula (Ia), wherein the values and alternative values are as described above for Structural Formulas (I) or (Ia) or in the 1st specific embodiment, or first to thirteenth aspects thereof.

[0070] In a 2nd specific embodiment, the compound is represented by one of the following structural formulas:







[0071] Values and alternative values for the remainder of the variables are as described above for Structural Formulas (I) or (Ia) or in the 1st specific embodiment, or aspects thereof.

[0072] In a first aspect of the 2nd specific embodiment of the present invention, the (R)-lipoyl stereoisomer of a compound represented by Structural Formulas (I) or (Ia), or a pharmaceutically acceptable salt thereof, has a diastereomeric excess of at least 90%. Values and alternative values for the remainder of the variables are as described above for Structural Formula (Ia), in the 1st specific embodiment, or aspects thereof, or in the 2nd specific embodiment.

[0073] The invention also relates to pharmaceutically acceptable salts of the disclosed compounds of the present invention. The term "pharmaceutically acceptable salts" embraces salts commonly used to form alkali metal salts and to form addition salts of free bases. The nature of the salt is not critical, provided that it is pharmaceutically acceptable.

[0074] The pharmaceutically acceptable salts of the compounds of the present invention include base addition salts. Suitable pharmaceutically acceptable base addition salts of compounds of the present invention include metallic salts made from aluminum, calcium, lithium, magnesium, potassium, sodium and zinc or organic salts made from *N,N'*-dibenzylethylene-diamine, chloroprocaine, choline, diethanolamine, ethylenediamine, *N*-methylglucamine, lysine and procaine. All of these salts may be prepared by conventional means from a corresponding compound of the present invention by treating, for example, a compound of Tables 1-5 with the appropriate acid or base.

[0075] In one embodiment, the pharmaceutically acceptable salt comprises a monovalent or divalent cation. As used herein, "cation" refers to an atom or molecule that has a positive charge. A cation can be, for example, a metal or an amine. In a particular embodiment, the

cation is a metal cation, such as a sodium cation.

[0076] As used herein, "amine salt" relates to a cation containing a protonated amino group. Amine salts include amino acid salts, such as lysine salts. In another embodiment, the cation is an amine and the pharmaceutically acceptable salt is an amine salt. In a particular embodiment, the pharmaceutically acceptable salt comprises lysine.

[0077] Salts can be chiral. When a disclosed salt has at least one chiral center and is named or depicted by structure without indicating the stereochemistry, it is to be understood that the name or structure encompasses one stereoisomer or enantiomer of the compound free from the corresponding stereoisomer(s) or enantiomer, a racemic mixture of the compound, or mixtures enriched in one stereoisomer or enantiomer relative to its corresponding stereoisomer(s) or enantiomer.

[0078] We also disclose pharmaceutically acceptable prodrugs of the disclosed compounds of the present invention.

[0079] In one example, the invention relates to the compounds of Structural Formulas (I) and/or (Ia), wherein the hydrogen of each acidic functionality (e.g., -COOH, -SO₃H, -OSO₃H, -PO(OH)₂, -OPO(OH)₂) is optionally and independently replaced with a hydrolyzable group. We also disclose pharmaceutically acceptable salts of the compounds including said hydrolyzable groups.

[0080] As used herein, the term "hydrolyzable group" refers to a moiety that, when present in a molecule of the invention, yields a carboxylic acid, or salt thereof, upon hydrolysis. Hydrolysis can occur, for example, spontaneously under acidic or basic conditions in a physiological environment (e.g., blood, metabolically active tissues, for example, liver, kidney, lung, brain), or can be catalyzed by an enzyme(s), (e.g., esterase, peptidases, hydrolases, oxidases, dehydrogenases, lyases or ligases). A hydrolyzable group can confer upon a compound of the invention advantageous properties *in vivo*, such as improved water solubility, improved circulating half-life in the blood, improved uptake, improved duration of action, or improved onset of action.

[0081] In one example, the hydrolyzable group does not destroy the biological activity of the compound. In an alternative example, a compound with a hydrolyzable group can be biologically inactive, but can be converted *in vivo* to a biologically active compound.

[0082] Compounds disclosed herein that include hydrolyzable groups may act as prodrugs. As used herein, the term "prodrug" means a compound that can be hydrolyzed, oxidized, metabolized or otherwise react under biological conditions to provide a compound of the invention. Prodrugs may become active upon such reaction under biological conditions, or they may have activity in their unreacted forms. A prodrug may undergo reduced metabolism under physiological conditions (e.g., due to the presence of a hydrolyzable group), thereby resulting in improved circulating half-life of the prodrug (e.g., in the blood). Prodrugs can typically be

prepared using well-known methods, such as those described by Burger's Medicinal Chemistry and Drug Discovery (1995) 172-178, 949-982 (Manfred E. Wolff ed., 5th Ed).

[0083] In one example, the hydrolyzable group is selected from the group consisting of (C₁-C₁₀)alkyl, (C₂-C₁₀)alkenyl, (C₂-C₁₀)alkynyl, (C₁-C₁₀)alkoxy(C₁-C₁₀)alkyl, (C₁-C₁₀)alkoxy(C₁-C₁₀)alkoxy(C₁-C₁₀)alkyl, aryl and aryl(C₁-C₁₀)alkyl, wherein each is optionally substituted with 1 to 3 substituents selected from the group consisting of halo, nitro, cyano, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, amino, (C₁-C₆)alkylamino, di(C₁-C₆)alkylamino, (C₁-C₆)alkyl, halo(C₁-C₆)alkyl, (C₁-C₆)alkoxy, halo(C₁-C₆)alkoxy, morpholino, phenyl, and benzyl.

[0084] In another example, the hydrolyzable group is selected from the group consisting of methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, isobutyl, tert-butyl, pentyl, hexyl, heptyl, allyl, ethoxymethyl, methoxyethyl, methoxyethoxymethyl, methoxyethoxyethyl, benzyl, pentafluorophenyl, 2-N-(morpholino)ethyl, dimethylaminoethyl and para-methoxybenzyl.

Methods

[0085] In another embodiment, the invention relates to a compound for use in a method for treating an ischemia or ischemia-reperfusion injury in a subject in need thereof, said method comprising administering to the subject an effective amount of one or more compounds of Structural Formulas (I) and/or (Ia), or a pharmaceutically acceptable salt thereof, provided that the compounds of Structural Formula (I) are not N-(R)-lipoyl-glutamylalanine, N-(R)-lipoyl-aminoethylphosphonic acid, or (R)-5-(5-(1,2-dithiolan-3-yl)pentanamido)-2-hydroxybenzoic acid and the compounds of Structural Formula (Ia) are not N-lipoyl-glutamylalanine, N-lipoyl-aspartylglycine, N-lipoyl-glutamylglycine or 5-(5-(1,2-dithiolan-3-yl)pentanamido)-2-hydroxybenzoic acid. In some embodiments, the pharmaceutically acceptable salt is a lysine salt. In one embodiment, the salt is an L-lysine salt. In a particular embodiment, the ischemia or ischemia-reperfusion injury is a myocardial ischemia or ischemia-reperfusion injury. In another embodiment, the compound is administered as a composition comprising one or more compounds of the invention.

[0086] As used herein, the "injury resulting from ischemia," "injury caused by ischemia" and "ischemic injury" refer to an injury to a cell, tissue or organ caused by ischemia, or an insufficient supply of blood (e.g., due to a blocked artery), and thus oxygen, resulting in damage or dysfunction of the tissue or organ (Piper, H. M., Abdallah, C., Schafer, C., Annals of Thoracic Surgery 2003, 75:644; Yellon, D. M., Hausenloy, D. J., New England Journal of Medicine 2007, 357:1121). Injuries that result from ischemia can affect various tissues and organs. Such injuries may be treated by the compounds and compounds for use according to the invention, including, for example, injuries caused by cardiovascular ischemia, cerebrovascular ischemia, renal ischemia, hepatic ischemia, ischemic cardiomyopathy, cutaneous ischemia, bowel ischemia, intestinal ischemia, gastric ischemia, pulmonary ischemia, pancreatic ischemia, skeletal muscle ischemia, abdominal muscle ischemia, limb

ischemia, ischemic colitis, mesenteric ischemia and silent ischemia. Thus, an injury resulting from ischemia can affect, for example, a heart, kidney, liver, brain, muscle, intestine, stomach, lung or skin.

[0087] In a particular embodiment, the injury resulting from ischemia is the result of a myocardial ischemia. An injury resulting from a myocardial ischemia can result from, for example, a myocardial infarction (e.g., an acute myocardial infarction) in a subject.

[0088] In another embodiment, the injury resulting from ischemia is an injury resulting from cerebral ischemia (e.g., a stroke) in a subject.

[0089] In another embodiment, the injury resulting from ischemia is an injury resulting from renal ischemia. An injury resulting from a renal ischemia can result from, for example, a deficiency of blood in one or both kidneys, or nephrons, usually due to functional constriction or actual obstruction of a blood vessel (e.g., an acute renal infarction) in a subject.

[0090] In another embodiment, the injury resulting from ischemia is an ischemia-reperfusion injury. As used herein, the term "ischemia-reperfusion injury" refers to an injury resulting from the restoration of blood flow to an area of a tissue or organ that had previously experienced deficient blood flow due to an ischemic event. Oxidative stresses associated with reperfusion may cause damage to the affected tissues or organs. Ischemia-reperfusion injury is characterized biochemically by a depletion of oxygen during an ischemic event followed by reoxygenation and the concomitant generation of reactive oxygen species during reperfusion (Piper, H. M., Abdallah, C., Schafer, C., Annals of Thoracic Surgery 2003, 75:644; Yellon, D. M., Hausenloy, D. J., New England Journal of Medicine 2007, 357:1121).

[0091] An ischemia-reperfusion injury can be caused, for example, by a natural event (e.g., restoration of blood flow following a myocardial infarction), a trauma, or by one or more surgical procedures or other therapeutic interventions that restore blood flow to a tissue or organ that has been subjected to a diminished supply of blood. Such surgical procedures include, for example, coronary artery bypass graft surgery, coronary angioplasty, and organ transplant surgery. In a particular embodiment, the compounds and uses of the invention are useful for treating peri-operative cardiac damage caused by an ischemia or ischemia-reperfusion injury.

[0092] For the treatment of ischemic and ischemia-reperfusion injuries caused by therapeutic interventions, such as surgical procedures, it is preferable that a compound of the invention is administered to a subject undergoing treatment prior to the therapeutic intervention (e.g., cardiac surgery, organ transplant). For example, a compound of the invention can be administered to a subject undergoing treatment, e.g., about 1 hour, about 2 hours, about 3 hours, about 4 hours, about 5 hours, about 12 hours, about 24 hours, or about 48 hours prior to the therapeutic intervention. A compound of the invention can also be administered to a subject undergoing treatment, for example, about 5 minutes, about 10 minutes, about 15 minutes, about 20 minutes, about 30 minutes or about 45 minutes prior to the therapeutic

intervention.

[0093] Alternatively, or in addition, a compound of the invention can be administered to a subject undergoing treatment at the time of, or during, the therapeutic intervention. For example, the compound can be administered one or more times during the course of a therapeutic intervention in intervals (e.g., 15 minute intervals). Alternatively, a compound can be administered continuously throughout the duration of a therapeutic intervention.

[0094] Furthermore, a compound of the invention can be administered to a subject undergoing treatment after a therapeutic intervention. For example, a compound of the invention can be administered to a subject undergoing treatment, e.g., about 1 hour, about 2 hours, about 3 hours, about 4 hours, about 5 hours, about 12 hours, about 24 hours, or about 48 hours after the therapeutic intervention. A compound of the invention can also be administered to a subject undergoing treatment, for example, about 5 minutes, about 10 minutes, about 15 minutes, about 20 minutes, about 30 minutes or about 45 minutes after the therapeutic intervention.

[0095] A compound of the invention can also be used to inhibit an ischemia or ischemia-reperfusion injury to a cell, tissue or organ, *ex vivo*, prior to a therapeutic intervention (e.g., a tissue employed in a graft procedure, an organ employed in an organ transplant surgery). For example, prior to transplant of an organ into a host individual (e.g., during storage or transport of the organ in a sterile environment), the organ can be contacted with a compound of the invention (e.g., bathed in a solution comprising a compound of the invention) to inhibit ischemia or ischemia-reperfusion injury.

[0096] As described herein, conditions resulting from ischemia, and injuries caused by ischemia or ischemia-reperfusion, can induce cell death (e.g., apoptotic cell death) in an affected cell, tissue or organ, leading to damage and dysfunction. Accordingly, the compounds of the invention also have utility in methods of inhibiting cell death in a cell, a tissue or an organ (e.g., a transplant tissue or organ or a cell, tissue or organ in a subject), wherein the cell, tissue or organ has experienced an ischemia or other condition or disorder that results in excessive or unwanted cell death. The methods comprise contacting the cells, tissue, or organ in need thereof with, or administering to a subject in need thereof, an effective amount of one or more compounds of Structural Formulas (I) and/or (Ia), or a pharmaceutically acceptable salt or prodrug thereof.

[0097] We also disclose a method of inhibiting cell death (e.g., apoptotic cell death) in a subject, comprising administering to the subject an effective amount of a compound represented by Structural Formula (I) or (Ia), or a pharmaceutically acceptable salt or prodrug thereof.

[0098] Methods of assessing cell death are well known in the art. For example, microscopic analysis (e.g., light microscopy, electron microscopy, confocal microscopy, laser-scanning microscopy) for visualizing cell death (e.g., by detecting morphological changes associated

with cell death, such as chromatin condensation and cytoplasmic shrinking) is typically employed to study cell death.

[0099] The study of DNA fragmentation in agarose gels is also considered to be indicative of apoptotic cell death. A number of techniques take advantage of DNA fragmentation for labeling the fragments and thus for quantifying the proportion of apoptotic cells. Each DNA fragment has a 3'-OH terminal portion. This terminal fragment can be labeled in various ways (for instance, with the help of a modified terminal deoxynucleotidyl transferase), so that the labeling rate is proportional to the degree of DNA fragmentation.

[0100] In particular, TdT-mediated dUTP Nick-End Labeling, or TUNEL, is a technique for detecting fragmented DNA, which occurs near the final step in the apoptotic process. Fragmented DNA of apoptotic cells can incorporate fluorescein-dUTP at 3'-OH at DNA ends using the enzyme Terminal Deoxynucleotidyl Transferase (TdT), which forms a polymeric tail using the principle of the TUNEL assay. The labeled DNA can then be visualized directly by fluorescence microscopy or quantitated by flow cytometry.

[0101] Some current techniques take advantage of the changes in membrane phospholipids that occur early in apoptotic cells. The negatively charged membrane phospholipids exposed to the external environment by the apoptotic cell are labeled with fluorochrome-conjugated molecules, and the percentage of fluorescent cells can be easily quantified.

[0102] Apoptosis can also be detected using fluorescently-conjugated Annexin V. Annexin V is an anticoagulant protein that preferentially binds negatively charged phospholipids. An early step in the apoptotic process is disruption of membrane phospholipid asymmetry, exposing phosphatidylserine (PS) on the outer leaflet of the cytoplasmic membrane. Fluorescently conjugated Annexin V can be used to detect this externalization of phosphatidylserine on intact living cells. Propidium iodide is often combined as a second fluorochrome to detect necrotic cells. Induction of apoptosis leads to pro-caspase-3 proteolytic cleavage to generate an active 18 kDa caspase-3 fragment which then targets key modulators of the apoptotic pathway including poly-ADP-ribose polymerase and other caspases, for cleavage. Assays for detecting other active caspases in apoptotic cells are known in the art (e.g., Caspase-Glo® Assays, Promega).

[0103] Apoptotic cells can also be detected using the active 18 kDa caspase-3 fragment as a marker. Induction of apoptosis leads to pro-caspase-3 proteolytic cleavage to generate an active 18 kDa caspase-3 fragment which then targets key modulators of the apoptotic pathway, including poly-ADP-ribose polymerase and other caspases, for cleavage. Several antibodies that recognize only the active 18 kDa fragment are available from commercial suppliers (e.g., BD Biosciences, Chemicon, Cell Signaling Technology, Trevigen).

[0104] In addition, flow cytometry assays can be employed to monitor and quantify nuclear changes associated with apoptotic cells.

[0105] Conditions associated with unwanted and/or excess cell death that are treatable by the compounds and uses of the invention include neurodegenerative diseases associated with excess cell death (e.g., Parkinson's Disease, Alzheimer's Disease, amyotrophic lateral sclerosis, retinitis pigmentosa, epilepsy), haematologic diseases associated with excess cell death (e.g., aplastic anaemia, myelodysplastic syndrome, T CD4+ lymphocytopenia, G6PD deficiency), tissue damage associated with excess apoptosis (e.g., myocardial infarction, cerebrovascular accident, ischemic renal damage, polycystic kidney disease), AIDS, and preeclampsia.

[0106] The invention also relates to compositions comprising a pharmaceutical acceptable carrier or diluent and one or more of the disclosed compounds, or a pharmaceutically acceptable salt thereof. The compositions disclosed herein are prepared in accordance with standard procedures and are administered at dosages that are selected to reduce, prevent, eliminate, or to slow or halt the progression of, the condition being treated. See, e.g., Remington's Pharmaceutical Sciences, 17th ed., Remington, J. P., Easton, PA, Mack Publishing Company, 2005, and Goodman and Gilman's The Pharmaceutical Basis of Therapeutics, 12th ed., Brunton, L. et. als., eds., New York, McGraw-Hill, 2010, for a general description of the methods for administering various agents for human therapy. The compositions of the invention can be delivered using controlled or sustained-release delivery systems (e.g., capsules, bioerodible matrices). Exemplary delayed-release delivery systems for drug delivery that would be suitable for administration of the compositions of the present invention are described in U.S. Patent Nos. 5,990,092 (issued to Walsh); 5,039,660 (issued to Leonard); 4,452,775 (issued to Kent); and 3,854,480 (issued to Zaffaroni).

[0107] The compositions of the present invention comprise one or more compounds of Structural Formulas (I) and/or (Ia), or a pharmaceutically acceptable salt thereof, in association with one or more nontoxic, pharmaceutically acceptable carriers and/or diluents and/or adjuvants and/or excipients, collectively referred to herein as "carrier" materials, and optionally, other active ingredients. The compositions may contain from about 0.01% to about 99% by weight of the active ingredient, depending on the method of administration.

[0108] For preparing compositions from the compounds of the present invention, pharmaceutically acceptable carriers can either be solid or liquid. Solid form preparations include powders, tablets, pills, capsules, cachets, suppositories, and dispersible granules. For example, the compounds of the present invention may be in powder form for reconstitution at the time of delivery. A solid carrier can be one or more substances which may also act as diluents, flavoring agents, solubilizers, lubricants, suspending agents, binders, preservatives, tablet disintegrating agents, or an encapsulating material. In powders, the carrier is a finely divided solid which is in a mixture with the finely divided active ingredient.

[0109] In tablets, the active ingredient is mixed with the carrier having the necessary binding properties in suitable proportions and compacted in the shape and size desired.

[0110] The powders and tablets preferably contain from about one to about seventy percent

of the active ingredient. Suitable carriers are magnesium carbonate, magnesium stearate, talc, sugar, lactose, pectin, dextrin, starch, gelatin, tragacanth, methylcellulose, sodium carboxymethylcellulose, a low-melting wax, and cocoa butter. Tablets, powders, cachets, lozenges, fast-melt strips, capsules and pills can be used as solid dosage forms containing the active ingredient suitable for oral administration.

[0111] Liquid form preparations include solutions, suspensions, retention enemas, and emulsions, for example, water or water propylene glycol solutions. For parenteral injection, liquid preparations can be formulated in solution in aqueous polyethylene glycol.

[0112] Aqueous solutions suitable for oral administration can be prepared by dissolving the active ingredient in water and adding suitable colorants, flavors, stabilizing agents, and thickening agents as desired. Aqueous suspensions for oral administration can be prepared by dispersing the finely divided active ingredient in water with viscous material, such as natural or synthetic gums, resins, methylcellulose, sodium carboxymethylcellulose, and other well-known suspending agents.

[0113] Alternatively, the compounds or compositions of the present invention can be in powder form for reconstitution at the time of delivery.

[0114] The composition is preferably in unit dosage form. In such form, the composition is subdivided into unit doses containing appropriate quantities of the active ingredient. The unit dosage form can be a packaged preparation, the package containing discrete quantities of, for example, tablets, powders, and capsules in vials or ampules. Also, the unit dosage form can be a tablet, cachet, capsule, or lozenge itself, or it can be the appropriate amount of any of these in packaged form. The quantity of active ingredient in a unit dose preparation may be varied or adjusted from about 0.1 mg to about 1000 mg, preferably from about 0.1 mg to about 100 mg (e.g., for intravenous administration) or from about 1.0 mg to about 1000 mg (e.g., for oral administration). The dosages, however, may be varied depending upon the requirements of the subject, the severity of the condition being treated, the compound and the route of administration being employed. Determination of the proper dosage for a particular situation is within the skill in the art. In one embodiment, the dosage is from about 0.01 mg/kg to about 100 mg/kg.

[0115] In general, the methods for delivering the disclosed compounds and pharmaceutical compositions of the invention *in vivo* utilize art-recognized protocols for delivering the agent with the only substantial procedural modification being the substitution of the compounds represented by any one of the disclosed compounds for the drugs in the art-recognized protocols.

[0116] The compounds of the present invention may be administered by any route, preferably in the form of a composition adapted to such a route, and would be dependent on the condition being treated. The compounds and compositions may, for example, be administered intravascularly, intramuscularly, subcutaneously, intraperitoneally, intracardiacaally, orally or

topically. It will be obvious to those skilled in the art that the following dosage forms may comprise as the active ingredient, either compounds or a corresponding pharmaceutically acceptable salt of a compound of the present invention. Preferred methods of administration for the compounds of the invention include intravenous administration and oral administration.

[0117] For oral administration, the compositions may be in the form of, for example, a tablet, capsule, suspension or liquid. The composition is preferably made in the form of a dosage unit containing an effective amount of the active ingredient. Examples of such dosage units are tablets and capsules. For therapeutic purposes, the tablets and capsules can contain, in addition to the active ingredient, conventional carriers such as binding agents, for example, acacia gum, gelatin, polyvinylpyrrolidone, sorbitol, or tragacanth; fillers, for example, calcium phosphate, glycine, lactose, maize-starch, sorbitol, or sucrose; lubricants, for example, magnesium stearate, polyethylene glycol, silica, or talc; disintegrants, for example potato starch, flavoring or coloring agents, or acceptable wetting agents. Oral liquid preparations generally in the form of aqueous or oily solutions, suspensions, emulsions, syrups or elixirs may contain conventional additives such as suspending agents, emulsifying agents, non-aqueous agents, preservatives, coloring agents and flavoring agents. Examples of additives for liquid preparations include acacia, almond oil, ethyl alcohol, fractionated coconut oil, gelatin, glucose syrup, glycerin, hydrogenated edible fats, lecithin, methyl cellulose, methyl or propyl parahydroxybenzoate, propylene glycol, sorbitol, or sorbic acid.

[0118] The compositions may also be administered parenterally via, for example, injection. Formulations for parenteral administration can be in the form of aqueous or non-aqueous isotonic sterile injection solutions or suspensions. These solutions or suspensions can be prepared from sterile powders or granules having one or more of the carriers mentioned for use in the formulations for oral administration. The compounds can be dissolved in polyethylene glycol, propylene glycol, ethanol, corn oil, benzyl alcohol, sodium chloride, and/or various buffers.

[0119] Delivery can also be by injection into the brain or body cavity of a patient or by use of a timed release or sustained release matrix delivery systems, or by onsite delivery using micelles, gels and liposomes. Nebulizing devices, powder inhalers, and aerosolized solutions are representative of methods that may be used to administer such preparations to the respiratory tract. Delivery can be *in vitro*, *in vivo*, or *ex vivo*.

[0120] The dosage regimen for treating an ischemia, ischemic injury or ischemia-reperfusion injury with a compound and/or composition of this invention is selected in accordance with a variety of factors, including the type, age, weight, sex and medical condition of the subject, the severity of the ischemia-reperfusion injury, the route and frequency of administration, and the particular compound or composition employed. In general, dosages are determined in accordance with standard practice for optimizing the correct dosage for treating ischemia-reperfusion injury-associated disease.

[0121] The dosages of a compound of the invention provided to a subject may be varied

depending upon the requirements of the patient, the severity of the condition being treated, the route of administration and the compound being employed. Determination of the proper dosage for a particular situation is within the skill in the art. For example, suitable dosages for administration to humans can be extrapolated from data obtained in experiments performed on animal (e.g., rat) models. Guidance for extrapolating non-human animal model dosage data to human dosages can be found, for example, in *FDA Draft Guidance: Estimating the Safe Starting Dose in Clinical Trials for Therapeutics in Adult Healthy Volunteers* (2005).

[0122] For example, suitable intravenous dosages of a compound of the invention can be from about 0.001 mg/kg to about 100 mg/kg, from about 0.01 mg/kg to about 100 mg/kg, from about 0.01 mg/kg to about 10 mg/kg, from about 0.01 mg/kg to about 1 mg/kg body weight per treatment. Determining the dosage and route of administration for a particular agent, patient and ischemia or ischemia-reperfusion injury is well within the abilities of one of skill in the art. Preferably, the dosage does not cause or produces minimal adverse side effects.

[0123] An effective amount of a compound of the invention can be administered alone, or in combination with, one or more other therapeutic agents. Suitable therapeutic agents that are useful for treating ischemic injuries, which can be administered in combination with a compound of the invention, include calcium channel blockers, beta blockers, nitroglycerin, aspirin, anti-inflammatory agents, natriuretic factors, vasodilators, thrombolytic and antithrombotic agents.

[0124] Thus, a compound of the invention can be administered as part of a combination therapy (e.g., with one or more other therapeutic agents). The compound of the invention can be administered before, after or concurrently with one or more other therapeutic agents. In some embodiments, a compound of the invention and other therapeutic agent can be co-administered simultaneously (e.g., concurrently) as either separate formulations or as a joint formulation. Alternatively, the agents can be administered sequentially, as separate compositions, within an appropriate time frame, as determined by the skilled clinician (e.g., a time sufficient to allow an overlap of the pharmaceutical effects of the therapies). A compound of the invention and one or more other therapeutic agents can be administered in a single dose or in multiple doses, in an order and on a schedule suitable to achieve a desired therapeutic effect (e.g., a reduction in and/or inhibition of joint inflammation; a reduction in and/or inhibition of ischemia, a reduction in and/or inhibition of an ischemic injury; a reduction in and/or inhibition of an ischemia-reperfusion injury). Suitable dosages and regimens of administration can be determined by a clinician and are dependent on the agent(s) chosen, pharmaceutical formulation and route of administration, various patient factors and other considerations.

[0125] One of skill in the art can readily assess the efficacy of a compound for treating an ischemic injury or an ischemia-reperfusion injury by measuring biochemical or physiological parameters in the subject prior to and after treatment of the subject by using standard assays for the parameter(s) being measured. For example, the efficacy of a compound of the invention can be determined by analyzing the levels of surrogate cardiac biomarkers, including

certain cardiac enzymes (e.g., creatine kinase (CK-MB), troponin-T, troponin-I), in blood samples obtained from a subject at various time points before and after the ischemic injury or ischemia-reperfusion injury, wherein a statistically significant reduction in the levels of the enzymes is indicative of the compound having efficacy in treating the injury. In an exemplary assessment, one or more blood samples are collected from a subject prior to the injury (e.g., about 6 to about 48 hours prior to the injury) and analyzed for CK-MB and troponin-T levels. Blood samples are then obtained from the subject at various time points after the injury (e.g., at 6.0, 12.0, 18.0, and 24.0 hours after the injury) and CK-MB and troponin-T levels are analyzed in one or more of these samples.

[0126] The efficacy of a compound of the invention can also be determined by electrocardiogram (ECG) monitoring. For example, standard continuous 12-lead ECG monitoring can be performed after fitting the subject with a continuous 12-lead ECG monitoring device with electronic data storage prior to dosing (e.g., about 5 minutes prior to dosing). The ECG readings can then be obtained before and after the injury (e.g., until about 24 hours after the injury). A change from an abnormal ECG trace to a normal ECG trace (e.g., a reduction of an elevated ST segment) is indicative of the compound having efficacy in treating the injury.

[0127] In addition, the efficacy of a compound of the invention can also be assessed by determining the ratio of the myocardial infarct area (MI) to the ischemic area at risk (AR), according to methods known in the art, wherein a statistically significant reduction of MI/AR ratio is indicative of the compound having efficacy in treating the injury.

EXEMPLIFICATION

[0128] Having described the invention generally, the inventors illustrate the invention with the following examples. These examples are merely illustrative of certain embodiments of the invention, which is not limited to exemplified embodiments.

Example 1. Representative synthesis of select compounds of the present invention.

[0129] Synthesis of RLip-Tau. RLipoic Acid (RLip-OH, 10.0 g) was dissolved in acetone (100 mL, 10 mL/g). The solution was protected from direct light by covering the reaction flask with foil. *N,N*-Disuccinimidylcarbonate (15.5 g, 1.25 equivalents) and *N,N*-diisopropylethylamine (DIEA, 10.5 mL, 1.25 equivalents) were added sequentially and the reaction was stirred vented for 2 hours at room temperature to form Lip-OSu *in-situ*. Taurine (7.0 g, 1.15 equivalents) was added to the solution of Lip-OSu in acetone, followed by the addition of water (50 mL) and DIEA (19.4 mL, 2.3 equivalents). The combined solution was stirred overnight. Approximately one third of the crude reaction mixture was transferred to a rotary evaporator and reduced to approximately half volume. The remaining reaction mixture was injected multiple times directly onto a semi-preparative high-performance liquid chromatography (HPLC) system and the

product isolated on a YMC Pack Pro C18 reverse phase column using a gradient of increasing acetonitrile (0.5% acetic acid) in water (0.5% acetic acid). Product-containing fractions were identified by analytical HPLC, frozen, and lyophilized to provide 2.16 g Lip-Tau at > 95% HPLC purity (percent area at 220 nm) as a gummy solid. The product ^1H NMR was consistent with the assigned structure and the product had an observed mass of 312 (M-1), calculated 313.

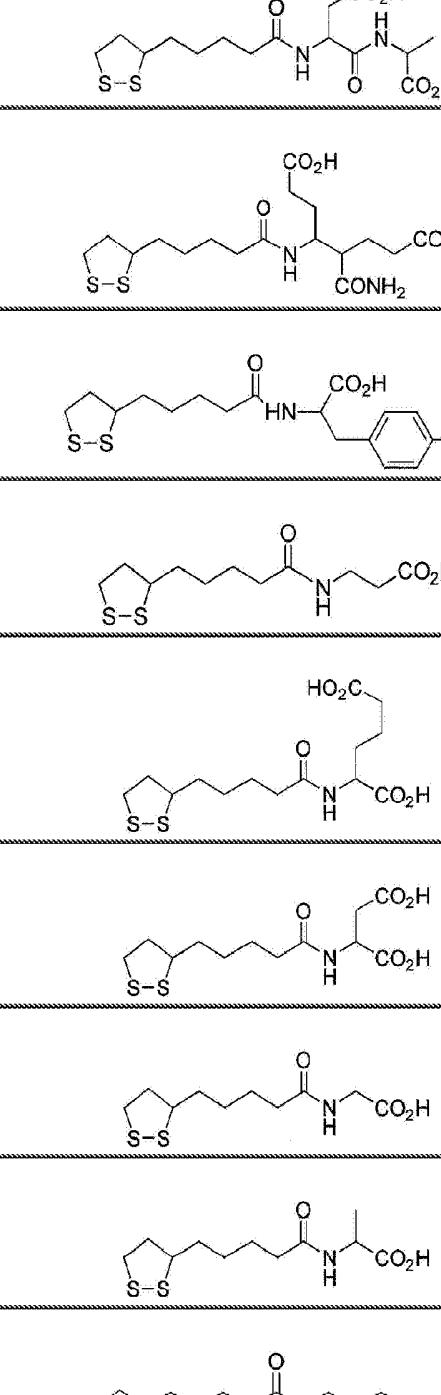
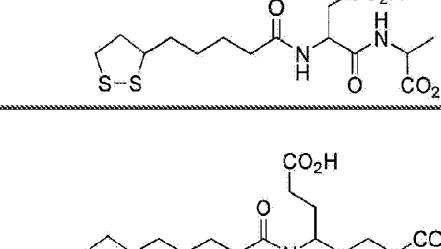
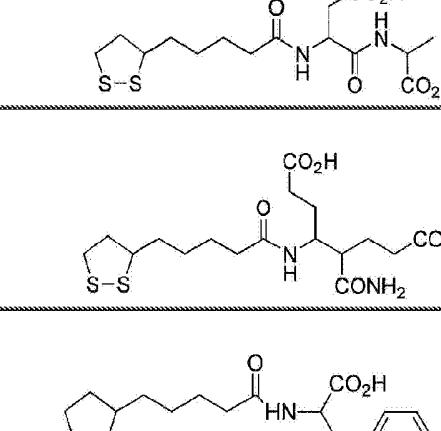
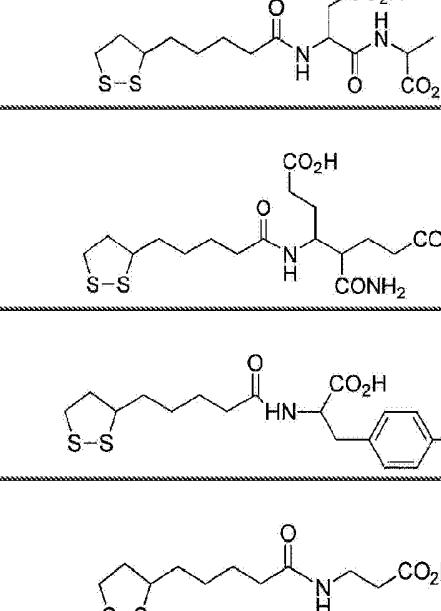
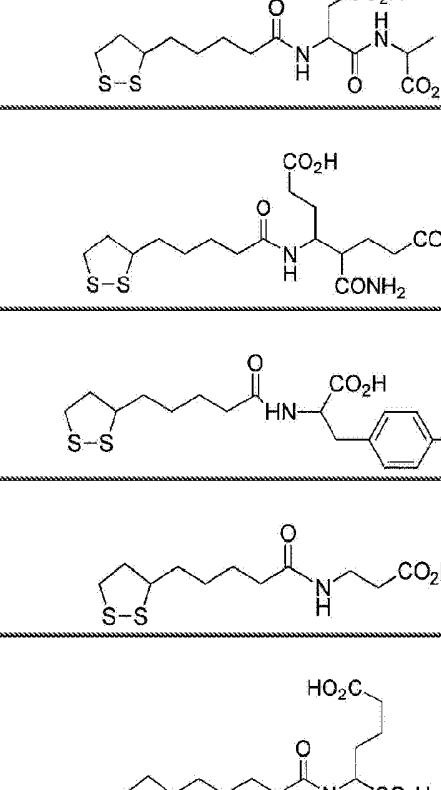
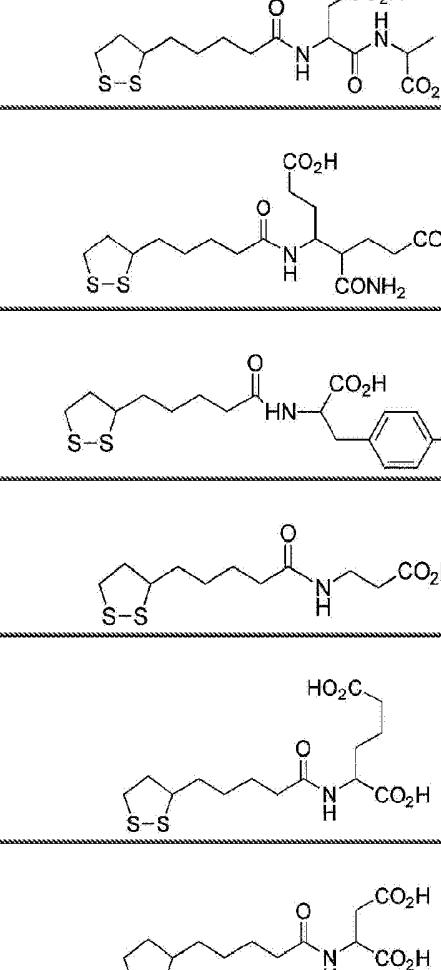
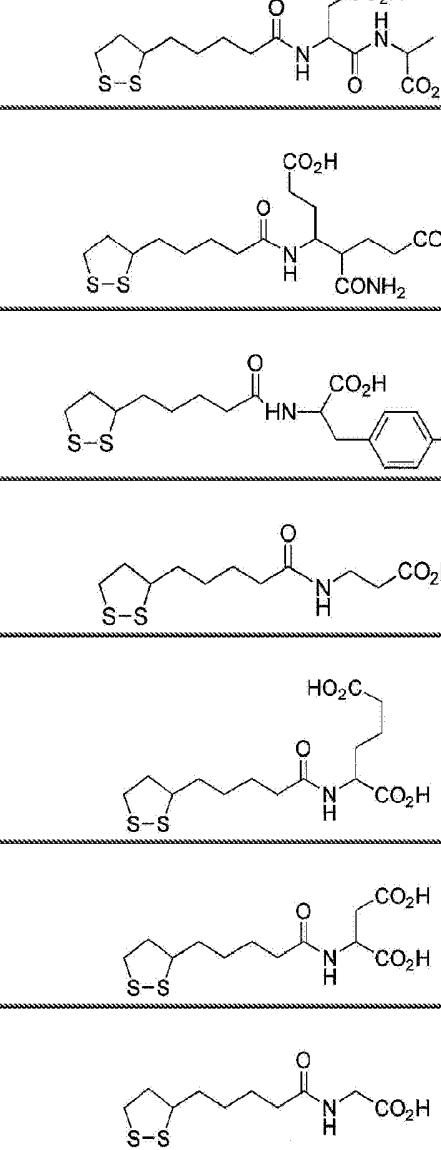
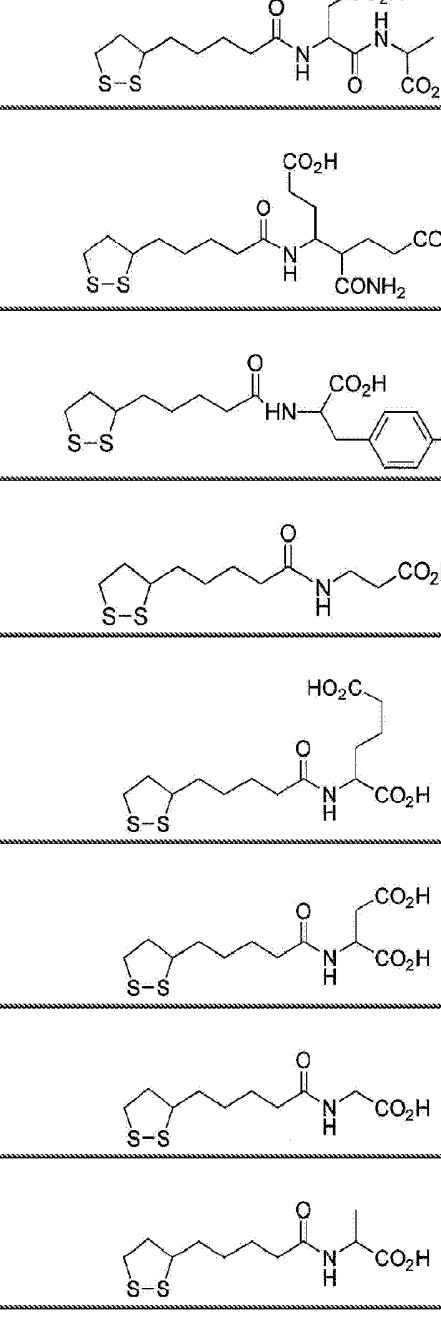
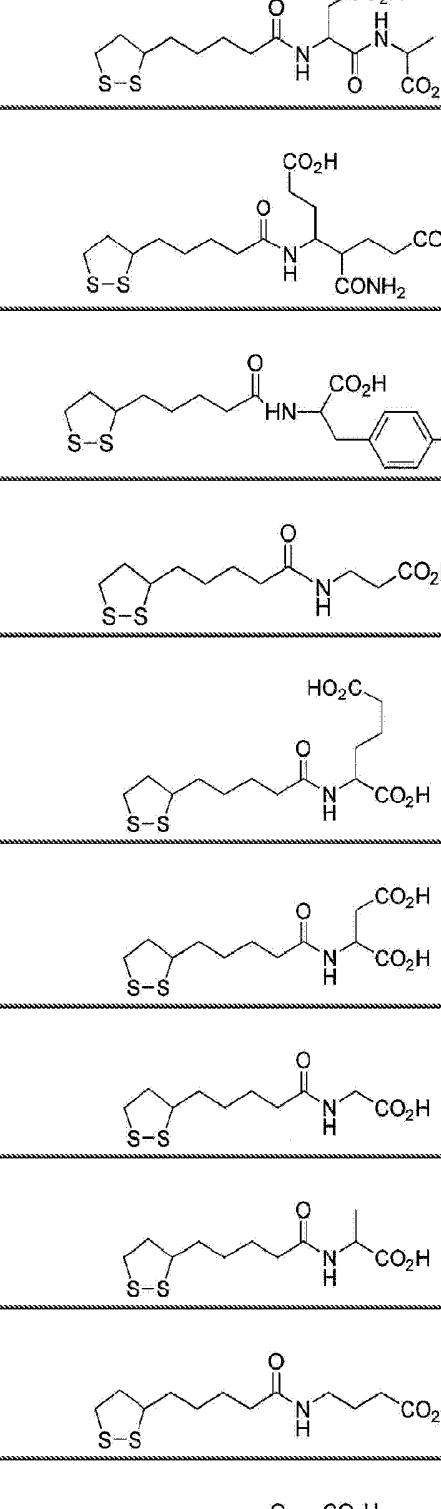
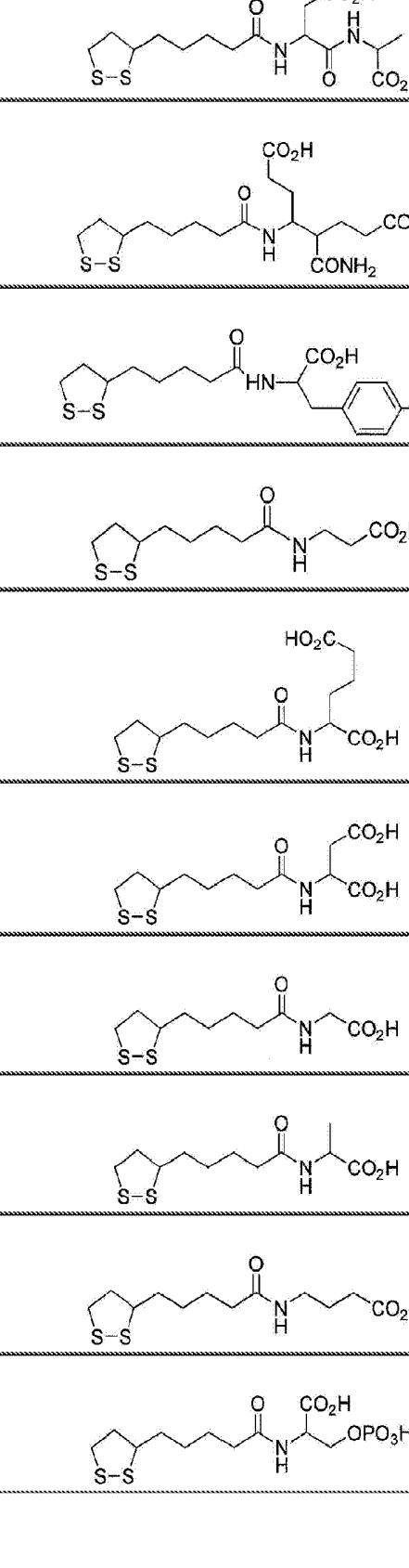
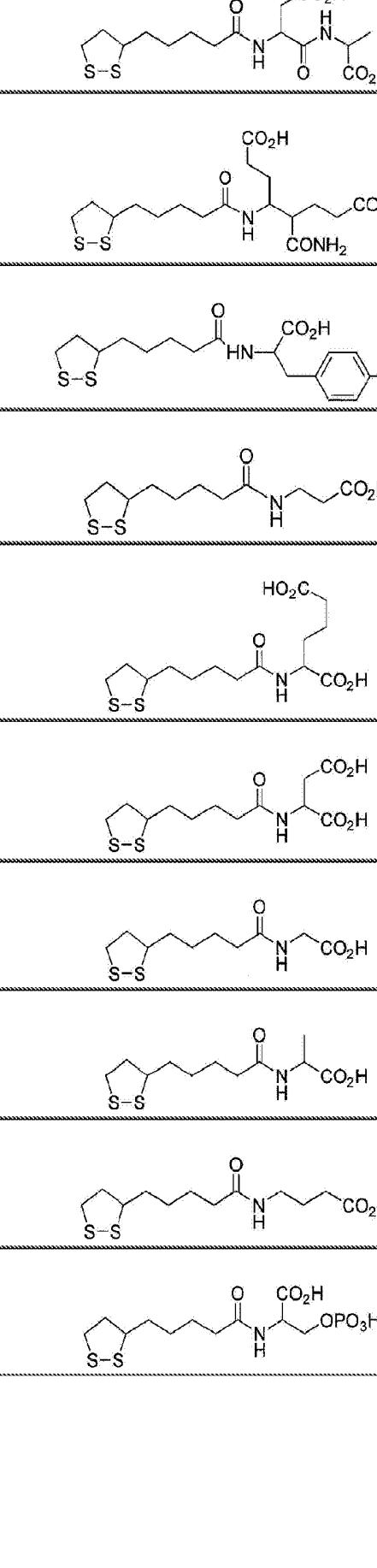
[0130] Synthesis of RLip-Tau Lysine salt. The RLip-Tau (2.16 g) isolated by semi-preparative reverse phase chromatography and lyophilized was dissolved in 70 mL ethanol. Water (3.0 mL) was added to the ethanolic solution followed by L-lysine (1.33g, 1 equivalent). The solution was shaken overnight, filtered, and rinsed 2 times with 15 mL ethanol. Isolated product was dried under vacuum to yield 3.1 g of the RLip-Tau Lysine salt with > 95% HPLC purity (percent area at 220 nm). The product ^1H NMR was consistent with the assigned structure.

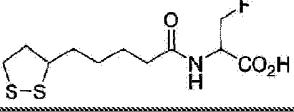
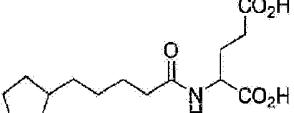
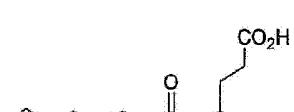
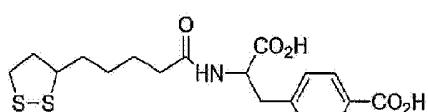
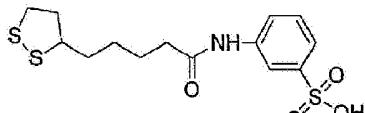
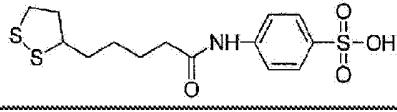
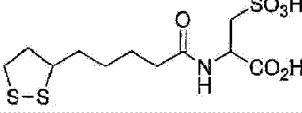
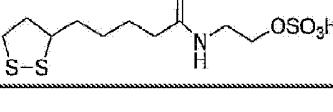
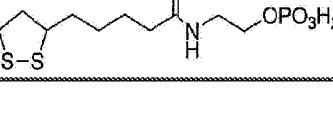
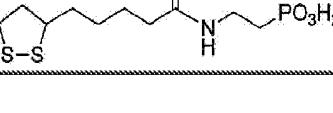
[0131] The chemical name and structure of exemplary compounds of the invention are set forth in Table A. Table B contains nuclear magnetic resonance (NMR) data, high performance liquid chromatography (HPLC) data and mass spectroscopy data for the compounds in Table A.

Table A. Chemical Name and Structure of Exemplary Compounds of the Invention and Reference Compound AA (denoted with *)

Entry	Chemical Name	Structure
A	RLip-Taurine-OH	
B	RLip-Idaa	
C	RLip-E-OH	
D	RLip-EG-OH	
E	(R/S)Lip-DG-OH	

Entry	Chemical Name	Structure
F	(R/S)Lip-QG-OH	
G	RLip-EE-OH	
H	RLip-ES-OH	
I	(R/S)Lip-HA-OH	
J	RLip-YA-OH	
K	RLip-AE-OH	
L	RLip-Glu-Sar-OH	
M	RLip-Glu-βAla-OH	
N	RLip-Glu-Tau-OH	

Entry	Chemical Name	Structure
		
O	RLip-DA-OH	
P	RLip-EE-NH2	
Q	RLip-Y-OH	
R	RLip-βAla-OH	
S	N-(RLip)-Aminoadipic-OH RLip-Aad	
T	RLip-D-OH	
U	RLip-G-OH	
V	RLip-A-OH	
W	RLip-γ-aminobutyric acid	
X	RLip-Phospho-Ser-OH RLip-S(O3PH2)-OH	

Entry	Chemical Name	Structure
Y	RLip- β -Fluoro-Ala-OH	
Z	RLip-DGlu-OH RLip-e-OH	
AA *	SLip-DGlu-OH SLip-e-OH	
AB	<i>N</i> -(RLip)-4-CarboxyPhe-OH	
AC	<i>N</i> -(RLip)-3-aminobenzene-sulfonic acid RLip-ABS *Lys	
AD	<i>N</i> -(RLip)-Sulfanilic-OH RLip-Sulf	
AE	RLip-Cysteic-OH RLip-Cya *2Lys	
AF	<i>N</i> -(RLip)-2-aminoethyl hydrogen sulfate RLip-AEHS *Lys	
AG	<i>N</i> -(RLip)-O-Phosphoryl-ethanolamine RLip-PEA *2Lys	
AH	<i>N</i> -(RLip)aminoethyl-phosphonic acid RLip-AEP *2Lys	

Entry	Chemical Name	Structure
A1	<i>N</i> -(RLip)-4-aminobenzoic acid RLip-PABA	

Table B. NMR Data, HPLC Data, and Mass Spectroscopy Data of the Compounds of Table A

Entry	¹ H NMR	HPLC Retention Time (min)/Purity (%)	Mass
A	Dithiolane -CH-S- m, 1H, δ 3.12 Taurinyl -CH ₂ -m, 2H, δ 3.65, t, 2H, δ 2.98	8.29 (100)	Calc: 313 Found (M-1): 312
B	Dithiolane -CH-S- m, 1H, δ 3.12 Iminodiacetic -CH ₂ - d, 4H, δ 4.20	11.4 (90)	Calc: 321 Found (M-1): 320
C	Dithiolane -CH-S- m, 1H, δ 3.05 Glutamyl αC-H m, 1H, δ 4.37	11.7 (78)	Calc: 335 Found (M-1): 334
D	Dithiolane-CH-S- m, 1H, δ 3.11 Glutamyl αC-H m, 1H, δ 4.5 Glycyl -CH ₂ - d, 2H, δ 3.9	8.9 (98)	Calc: 392 Found (M-1): 391
E	Dithiolane -CH-S- m, 1H, δ 3.10 Glutamyl αC-H m, 1H, δ 4.85 Glycyl -CH ₂ - d, 2H, δ 3.9	10.0 (100)	Calc: 378 Found (M+1): 379
F	NA	9.19 (99.9)	Calc: 391 Found (M-1): 392
G	Dithiolane -CH-S- m, 1H, δ 3.10 Glutamyl, Glutamyl αC-H m, 2H, δ 4.40	11.0 (97)	Calc: 464 Found (M+1): 463
H	Dithiolane -CH-S- m, 1H, δ 3.10 Glutamyl αC-H m, 1H, δ 4.45 Serinyl αC-H m, 1H, δ 3.90	10.3 (86)	Calc: 422 Found (M-1): 421
I	NA	9.16/9.29 (99)	Calc: 414 Found (M+1): 415
J	Dithiolane -CH-S- m, 1H, δ 3.10 Alaninyl, Tyrosinyl αC-H m, 1H, δ 4.63 m, 1H, δ 4.40	13.1 (98)	Calc: 440 Found (M-1): 439
K	NA	11.25 (98)	Calc: 406 Found (M-1): 405
L	Dithiolane -CH-S- m, 1H, δ 3.10 Sarcosine -CH ₂ - m, 2H, δ 4.48	11.4 (89)	Calc: 406 Found (M-1): 405

Entry	¹ H NMR	HPLC Retention Time (min)/Purity (%)	Mass
M	Dithiolane -CH-S- m, 1H, δ 3.10 Glutamyl αC-H m, 1H, δ 4.40	10.9 (100)	Calc: 406 Found (M-1): 405
N	Dithiolane -CH-S- m, 1H, δ 3.18 Glutamyl αC-H m, 1H, δ 4.2 Taurinyl -CH2-m, 2H, δ 3.36, t, 2H, δ 2.85	9.05 (100)	Calc: 442 Found (M-1): 441
O	Dithiolane -CH-S- m, 1H, δ 3.05 Aspartyl, Alaninyl αC-H m, 1H, δ 4.70 m, 1H, δ 4.28	15.3 (98)	Calc: 392 Found (M-1): 391
P	Dithiolane -CH-S- m, 1H, δ 3.05 Glutamyl, Glutamyl αC-H m, 2H, δ 4.25	10.8 (100)	Calc: 463 Found (M-1): 462
Q	NA	12.98 (95)	Calc: 369 Found (M+1): 370
R	NA	10.95 (100)	Calc: 277 Found (M+1): 278
S	NA	11.23 (96)	Calc: 349 Found (M+1): 350
T	NA	10.4 (98)	Calc: 321 Found (M+1): 322
U	NA	10.7 (100)	Calc: 263 Found (M+1): 264
V	NA	11.76 (100)	Calc: 277 Found (M+1): 278
W	NA	11.55 (98)	Calc: 291 Found (M+1): 292
X	NA	8.19 (96)	Calc: 373 Found (M+1): 374
Y	NA	12.49 (100)	Calc: 295 Found (M+1): 296
Z	NA	11.6 (99)	Calc: 335 Found (M+1): 336

Entry	¹ H NMR	HPLC Retention Time (min)/Purity (%)	Mass
AA *	NA	11.6 (97)	Calc: 335 Found (M+1): 336
AB	NA	12.69 (99)	Calc: 397 Found (M+1): 398
AC	NA	12.50 (95)	Calc: 361 Found (M-1): 360
AD	NA	12.37 (92)	Calc: 361 Found (M-1): 360
AE	Dithiolane -CH-S- m, 1H, δ 3.75 Lys αC-H m, 1H, δ 3.75 Cysteic αC-H m, 1H, δ 4.55	7.61 (97)	Calc: 359 Found (M+1): 360
AF	Dithiolane -CH-S- m, 1H, δ 3.65 Lys αC-H t, 1H, δ 3.75 AEHS -CH2- t, 2H, δ 4.16	9.05 (100)	Calc: 329 Found (M-1): 328
AG	Dithiolane -CH-S- m, 1H, δ 3.65 Lys αC-H t, 1H, δ 3.75 PEA -CH2- q, 2H, δ 3.84	8.32 (89)	Calc: 329 Found (M-1): 328
AH	Dithiolane -CH-S- m, 1H, δ 3.25 Lys αC-H t, 1H, δ 3.77 Aminoethyl -CH2- m, 2H, δ 2.05	8.63 (98)	Calc: 313 Found (M-1): 312
AI	NA	15.25 (99)	Calc: 325 Found (M+1): 326

Example 2. Efficacy of select lipoyl compounds of the invention in a rat model of MI/AR injury.

Materials and Methods

[0132] A rat model of MI/AR injury was used as an *in vivo* screen to determine if the compounds of Tables 1-5 were cardio-protective (e.g., against myocardial ischemia-reperfusion injury). This model is analogous to the ischemia-reperfusion injury observed in cardiac patients following coronary occlusions and cardiac surgery procedures, such as

coronary artery bypass grafting (CABG) (Matsui, T., Tao, J., del Monte, F., Lee, K.-H. et al., Akt Activation Preserves Cardiac Function and Prevents Injury After Transient Cardiac Ischemia In vivo, *Circulation* 2001, 104:330).

General Procedure

[0133] The circumflex branch of the left coronary artery (LCA) was ligated temporarily to induce regional ischemia in the left ventricular mass, followed by the injection of fluorescent microspheres to delineate the ischemic region. 15 minutes prior to ligation (pre-occlusion, pre-ischemic episode), a compound from Tables 1-5 was administered to the animals. Doses of the compounds listed in Tables 1-5 ranged from 1-20 mg/kg. The animals were sacrificed about 24 hours after reperfusion and the hearts were excised, sectioned and stained with triphenyltetrazolium. The direct impact of the pharmacologic intervention was determined by measuring the myocardial infarct area (MI), the ischemic area at risk (AR) and the left ventricular area (LV). The reduction of MI over the AR (MI/AR ratio) was used as the primary measure of drug efficacy relative to vehicle controls. The results are reported in Tables 1-5.

Table 1. Di-amino Acids Containing an Acidic Functionality

Entry	Name	Structure	MI/AR Reduction (%)
1	<i>N</i> -(R)-lipoyl-L-glutamyl-L-alanine (RLip-EA-OH)		31
2	<i>N</i> -lipoyl-L-aspartylglycine (RLip-DG-OH)		28
3	<i>N</i> -(R)-lipoyl-L-tyrosinyl-L-alanine (RLip-YA-OH)		31
4	<i>N</i> -(R)-lipoyl-L-glutamyltaurine (RLip-E-Tau-OH)		33

Entry	Name	Structure	MI/AR Reduction (%)
5	<i>N</i> -(R)-lipoyl-L-alanyl-L-glutamic acid (RLip-AE-OH)		24

Table 2. Single Amino Acid Containing Acidic Functionality

Entry	Name	Structure	MI/AR Reduction (%)
6	<i>N</i> -(R)-lipoyl-L-4-carboxyphenylalanine (RLip-(4-carboxy)Phe-OH)		26
7	<i>N</i> -(R)-lipoyl-fluoroalanine RLip-fluoroalanine		10
8	<i>N</i> -(R)-lipoyl-L-tyrosine (RLip-Y-OH)		29
9	<i>N</i> -(R)-lipoyl-L-glutamic acid (RLip-E-OH)		33
10	<i>N</i> -(R)-lipoyl-L-cysteic acid RLip-Cya-OH		30
11	<i>N</i> -(R)-lipoyliminodi-acetic acid RLip-Idaa		21

Table 3. Alkyl Acids

Entry	Name	Structure	MI/AR Reduction (%)
12	<i>N</i> -(R)-lipoyl-β-alanine RLip-βAla-OH		39

Entry	Name	Structure	MI/AR Reduction (%)
13	<i>N</i> -(R)-lipoyl-taurine RLip-Tau-OH		45 to 52
14	<i>N</i> -(R)-lipoyl-aminoethyl hydrogensulfate		30
15	<i>N</i> -(R)-lipoyl-aminoethylphosphonic acid		53
16	<i>N</i> -(R)-lipoyl-aminoethyl dihydrogen-phosphate <i>N</i> -(R)-lipoyl-O-phosphoryl-ethanolamine		47

Table 4. Alkyl Bis-Acids

Entry	Name	Structure	MI/AR Reduction (%)
9	<i>N</i> -(R)-lipoyl-L-glutamic acid (RLip-E-OH)		33
10	<i>N</i> -(R)-lipoyl-L-cysteic acid RLip-Cya-OH		30
11	<i>N</i> -(R)-lipoyliminodi-acetic acid RLip-Idaa		21

Table 5. Aromatic Acids

Entry	Name	Structure	MI/AR Reduction (%)
17	<i>N</i> -(R)-lipoyl-para-aminobenzoic acid (RLip-PABA)		22
	<i>N</i> -(R)-lipoyl-L- 4-carboxy-		0

Entry	Name	Structure	MI/AR Reduction (%)
6	phenylalanine (R)Lip-(4-carboxy)F-OH)		26
18	<i>N</i> -(R)-lipoyl- <i>meta</i> -aminobenzene sulfonic acid		33
19	<i>N</i> -(R)-lipoyl- <i>para</i> -aminobenzene sulfonic acid <i>N</i> -(R)-lipoyl-sulfanilic acid		44
8	<i>N</i> -(R)-lipoyl-L-tyrosine (RLip-Y-OH)		29

Detailed procedure

[0134] Male Sprague-Dawley rats between 300 and 350 g were used for these experiments. Anesthesia was induced with 3-4% isoflurane in an induction chamber. After induction, anesthesia was maintained at a surgical plane with 1.5 - 2.0% isoflurane, administered by a Rodent Ventilator through a 16-gauge angiocatheter introduced orally into the trachea. The ventilator was set at 2.5 cc at a rate of 60-65 breaths per minute to maintain ventilation during surgery. The core temperature of the animal was monitored and maintained at 37 °C using a rectal probe and a heating lamp attached to a temperature controller.

[0135] A left anterior thoracotomy was performed and the heart was exposed using a vertical pericardotomy. The circumflex branch of the left coronary artery (LCx) was ligated approximately 4 mm from the aorta using a cardiovascular 7.0 monofilament suture on an 11 mm needle to induce ischemia in the left ventricle.

[0136] Fluorescent microspheres (300 µL) were injected into the left ventricular cavity 10-20 minutes after the ligation to delineate the ischemic area. The suture was removed 30 minutes after ligation to reperfuse the ischemic area and the ischemic area was checked for reperfusion.

[0137] The chest was then closed in layers using absorbable suture (Dexon 5-0) for the muscle layers and monofilament Nylon 5-0 suture was used to close the cutaneous layer. The animals were allowed to recover, then were returned to the colony.

[0138] Twenty-four hours after reperfusion, anesthesia was induced using ketamine hydrochloride and the chest was opened. The animals were sacrificed with 15% potassium chloride aqueous solution (w/v) injected into the LV cavity to arrest the heart in diastole. The heart was excised distal to the aortic valve and washed with saline to remove the blood. Sagittal slices of the heart were obtained between the base of the ventricle and the apex. Five slices of heart tissue were obtained, each 2 mm thick. The slices were immersed in a 1% 2,3,5-triphenyl-2*H*-tetrazolium chloride (TTC) in saline solution and then stored in the dark for 30 minutes to stain.

[0139] Images of the slices were obtained under bright field (to observe the TTC staining) and under fluorescence (to observe the microspheres). The area at risk was determined by the absence of microspheres and the infarct area was determined by the absence of TTC staining.

Results

[0140] The compounds of Tables 1-5, administered as an intravenous injection, effectively reduced the myocardial infarct (MI) size relative to the area at risk (AR). A significant reduction in the area of cardiac damage was observed in myocardial tissue sections following treatment with a compound of Tables 1-5.

Example 3. Efficacy of select lipoyl compounds of the invention in a PAC rat model of ischemia-induced renal injury.

Materials and Methods

[0141] A partial aortic clamping (PAC) rat model of ischemia-induced renal injury was used as an *in vivo* screen to determine if RLip-EA-OH (Entry 1), RLip-Cya-OH (Entry 10), RLip-Tau (Entry 13), and RLip-aminoethylphosphonic acid (Entry 15) were renal-protective (e.g., against renal ischemia-reperfusion injury). This model simulates ischemia-reperfusion injury observed in renal patients following ischemia-induced renal failure (Molitoris, B.A., Dagher, P.C., Sandoval, R.M., Campos, S.B., Ashush, H., Fridman, E., Brafman, A. Faerman, A., Atkinson, S.J., Thompson, J.D., Kalinski, H., Skaliter, R., Erlich, S., Feinstein, E. "siRNA Targeted to p53 Attenuates Ischemic and Cisplatin-Induced Acute Kidney Injury." *J Am Soc Nephrol*, 2009, vol. 20, 1754-1764). Serum creatinine concentrations (SCr) typically rise due to renal ischemia and effective treatment should show a reduction in serum creatinine concentrations. Reduction in serum creatinine concentrations after renal ischemia induction indicates that the administered compound has a protective effect and is effective in reducing ischemia-induced kidney injury.

General procedure

[0142] The abdominal aorta just below the renal arteries was isolated and temporarily ligated to induce regional ischemia using an aortic clamp. An initial blood sample was drawn at study initiation for baseline creatinine measurement and at 24 hours post-surgery for functional evaluation of the severity of kidney injury. The animals were sacrificed about 24 hours after reperfusion.

Detailed procedure

[0143] Male Sprague-Dawley rats between 200 to 250 g were used for these experiments. Anesthesia was induced with 5% halothane and maintained with 1-1.5% halothane in oxygen-enriched air via a face mask. The rats were maintained on a warming blanket throughout the procedure to maintain body temperature at 37°C. After shaving the abdomen of the rat, a midline incision was made through the skin and musculature to expose the abdominal cavity to quantify the aortic blood flow (ABF).

[0144] The abdominal aorta just below the renal arteries was isolated through blunt dissection from the inferior vena cava, and an ultrasonic probe (2.0-mm diameter, Transit Time Perivascular Flowmeter TS420) was placed and secured. The upper abdominal aorta was then isolated through blunt dissection and freed from the surrounding structures to expose the aorta between the celiac artery and superior mesenteric artery.

[0145] Aortic clamps comprised of two 4-mm in length polyethylene tubes (PE-100, 0.86-mm diameter) were then placed around the aorta to induce renal ischemia. One clamp was placed around the aorta and the other was placed to exert variable tension via a 10-in. 3.0 silk suture. The silk thread was then tied and the tension on the two ends of the thread increased until there was a 90% reduction of initial (ABF) rate measured on the ultrasonic probe reader. A 10% baseline blood flow was maintained for a period of 30 minutes.

[0146] RLip-EA-OH (Entry 1) at 10 mg/kg; RLip-Cya-OH (Entry 10) at 3 mg/kg; RLip-Tau (Entry 13) at 3 mg/kg; and RLip-aminoethylphosphonic acid at 10 mg/kg, 3mg/kg, and 1 mg/kg were administered intravenously via the femoral vein as a bolus dose 15 minutes prior to ischemia. A 0.15-mL venous blood sample was drawn at study initiation for baseline creatinine measurement and at 24 hours postsurgery for functional evaluation of the severity of kidney injury. Serum creatinine concentrations were then measured on a Creatinine Analyzer 2. The efficacy of RLip-EA-OH and RLip-Tau were measured against the results of vehicle-treated animals in the blinded studies. A 2-tail t-test was used to determine differences between treatments.

Results

[0147] RLip-EA-OH (Entry 1), RLip-Cya-OH (Entry 10), RLip-Tau (Entry 13), and RLip-aminoethylphosphonic acid (Entry 15), administered intravenously, effectively reduced serum creatinine concentrations relative to the control animals, as shown in Table 6. This reduction in SCr following treatment indicated that RLip-EA-OH, RLip-Cya-OH, RLip-Tau, and RLip-aminoethylphosphonic acid had protective effects and minimized renal ischemic injury. The data for reduction in cardiac injury reported in the last column in Table 6 were obtained using the procedure described in Example 2.

Table 6. Effects of compounds on renal ischemia reperfusion injury in PAC rat model and heart ischemia reperfusion injury in LCA rat model.

Dose Group	Reduction of SCr vs. control	Reduction in Cardiac injury
Vehicle (Meta)	-	-
Lip-EA 10 mg/kg	39.5%	33%
Lip-Tau 3 mg/kg	39.5%	50%
Lip-Cya-OH 3 mg/kg	39.5%	30%
Lip-aminoethyl phosphonic acid 10 mg/kg	76.0%	32%
Lip-aminoethyl phosphonic acid 3 mg/kg	28.6%	50%
Lip-aminoethyl phosphonic acid 1 mg/kg	28.6%	30%

REFERENCES CITED IN THE DESCRIPTION

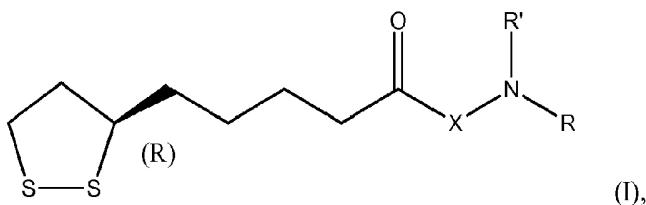
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- [US5039660A \[0106\]](#)
- [US4452775A \[0106\]](#)
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- **MOLITORIS, B.A.DAGHER, P.C.SANDOVAL, R.M.CAMPOS, S.B.ASHUSH, H.FRIDMAN, E.BRAFMAN, A.FAERMAN, A.ATKINSON, S.J.THOMPSON, J.D.** siRNA Targeted to p53 Attenuates Ischemic and Cisplatin-Induced Acute Kidney Injury *J Am Soc Nephrol*, 2009, vol. 20, 1754-1764 [0141]

Patentkrav**1. Forbindelse vist med strukturformel (I):**

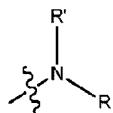
eller et farmaceutisk acceptabelt salt deraf, hvor:

5 det enantiomere overskud eller diastereomere overskud af forbindelsen
eller farmaceutisk acceptabelt salt deraf er mindst 90%;

10 R er (C_1 - C_{18})alkyl, (C_6 - C_{18})aryl eller (C_6 - C_{18})aryl(C_1 - C_{18})alkyl og er
substitueret med mindst én syreholdig substituent valgt fra gruppen
bestående
af $-CO_2H$, $-SO_3H$, $-PO_3H_2$, $-OSO_3H$, $-OPO_3H_2$, $-B(OH)_2$ og $-NHOH$, hvor
arylen af (C_6 - C_{18})aryl eller (C_6 - C_{18})aryl(C_1 - C_{18})alkyl eventuelt er yderligere
substitueret med én eller flere substituenter valgt fra gruppen bestående
af hydroxy, halo, (C_1 - C_3)alkyl, halo(C_1 - C_3)alkyl, cyano, nitro, (C_1 - C_3)alkoxy
og thio(C_1 - C_3)alkyl;

15 R' er hydrogen eller (C_1 - C_{18})alkyl, hvor (C_1 - C_{18})alkyl eventuelt er
substitueret med én eller flere syreholdige substituenter valgt fra gruppen
bestående af $-CO_2H$, $-SO_3H$, $-PO_3H_2$, $-OSO_3H$, $-OPO_3H_2$, $-B(OH)_2$ og $-NHOH$;
og

20 X er fraværende eller er en aminosyre, hvor aminosyren er asparaginsyre,
tyrosin, glutaminsyre eller alanin og er orienteret til at danne en
amidbinding med



25 , forudsat at forbindelsen med strukturformel (I) ikke er N -(R)-lipoyl-
glutamylalanin, N -(R)-lipoyl-aminoethylphosphonsyre, eller (R)-5-(5-(1,2-
dithiolan-3-yl)pentanamido)-2-hydroxybenzoesyre.

2. Forbindelsen ifølge krav 1, hvor X er fraværende.

3. Forbindelsen ifølge krav 1, hvor X er en aminosyre og R' er hydrogen.

5 **4.** Forbindelsen ifølge krav 1, hvor R is:

(C₁-C₃)alkyl substitueret med mindst én syreholdig substituent valgt fra gruppen bestående af -CO₂H, -SO₃H, -PO₃H₂, -OSO₃H, -OPO₃H₂, -B(OH)₂ og -NHOH; eller

10 (C₆)aryl substitueret med mindst én syreholdig substituent valgt fra gruppen bestående af -CO₂H, -SO₃H, -PO₃H₂, -OSO₃H, -OPO₃H₂, -B(OH)₂ og -NHOH, hvor aryl af (C₆)aryl eventuelt er yderligere substitueret med én eller flere substituenter valgt fra gruppen bestående af hydroxy, halo, (C₁-C₃)alkyl, halo(C₁-C₃)alkyl, cyano, nitro, (C₁-C₃)alkoxy og thio(C₁-C₃)alkyl; eller

15 (C₆)aryl(C₁-C₃)alkyl substitueret med mindst én syreholdig substituent valgt fra gruppen bestående af -CO₂H, -SO₃H, -PO₃H₂, -OSO₃H, -OPO₃H₂, -B(OH)₂ og -NHOH, hvor aryl af (C₆)aryl(C₁-C₃)alkyl eventuelt er yderligere substitueret med én eller flere substituenter valgt fra gruppen bestående af hydroxy, halo, (C₁-C₃)alkyl, halo(C₁-C₃)alkyl, cyano, nitro, (C₁-C₃)alkoxy og thio(C₁-C₃)alkyl; eller

20 (C₁-C₃)alkyl substitueret med en eller to syreholdige substituenter hver uafhængigt valgt fra -CO₂H, -SO₃H, -PO₃H₂, -OSO₃H og -OPO₃H₂.

5. Forbindelsen ifølge krav 1, hvor X er fraværende og R' er hydrogen.

25

6. Forbindelsen ifølge krav 5, hvor R er:

(C₁-C₃)alkyl substitueret med en eller to syreholdige substituenter hver uafhængigt valgt fra -CO₂H, -SO₃H, -PO₃H₂, -OSO₃H og -OPO₃H₂; eller

30 (C₆)aryl(C₁-C₃)alkyl substitueret med en eller to syreholdige substituenter hver uafhængigt valgt fra -CO₂H, -SO₃H, -PO₃H₂, -OSO₃H og -OPO₃H₂, og hvor aryl

eventuelt er substitueret med halo og hydroxy; eller

(C₂)alkyl substitueret med en eller to syreholdige substituenter hver uafhængigt valgt fra -CO₂H, -SO₃H, -PO₃H₂, -OSO₃H og -OPO₃H₂; eller (C₆)aryl substitueret med en syreholdig substituent valgt fra -CO₂H, -SO₃H, -PO₃H₂, -OSO₃H og

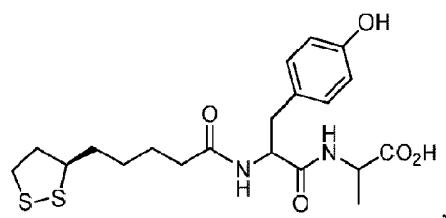
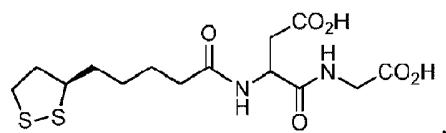
5 -OPO₃H₂.

7. Forbindelsen ifølge krav 1, hvor R' er (C₁-C₃)alkyl substitueret med mindst én syreholdig substituent valgt fra gruppen bestående af -CO₂H, -SO₃H, -PO₃H₂, -OSO₃H, -OPO₃H₂, -B(OH)₂ og -NHOH.

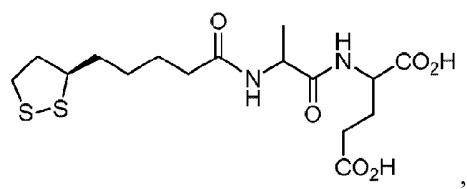
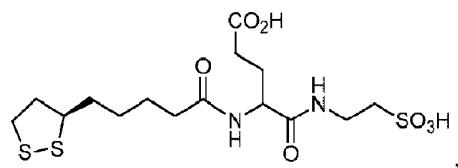
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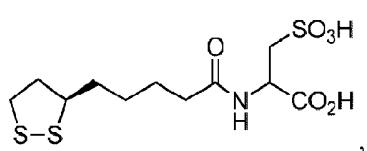
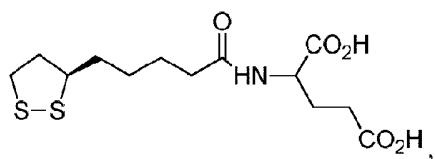
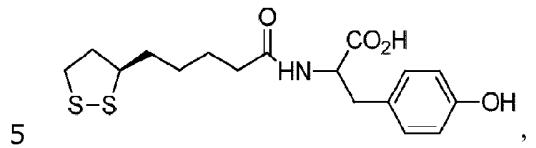
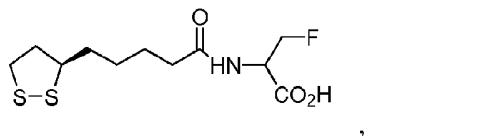
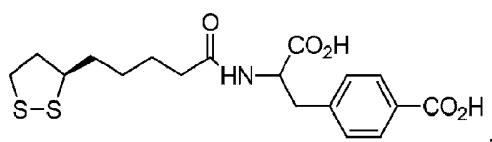
8. Forbindelsen ifølge krav 1 eller krav 7, hvor X er fraværende og R og R' hver er (C₁-C₃)alkyl substitueret med en syreholdig substituent valgt fra -CO₂H, -SO₃H, -PO₃H₂, -OSO₃H og -OPO₃H₂.

15 **9.** Forbindelsen ifølge krav 1, hvor forbindelsen er vist med én af følgende strukturformler:

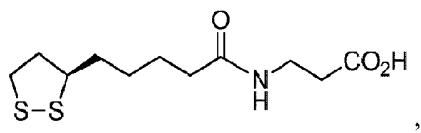
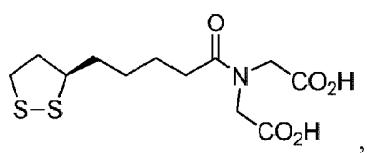


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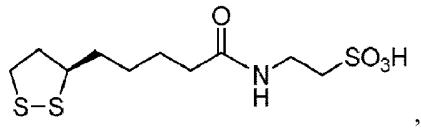


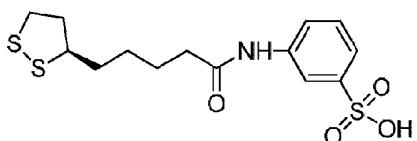
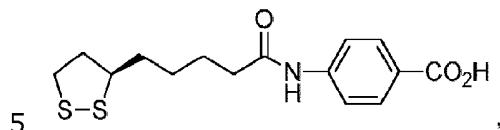
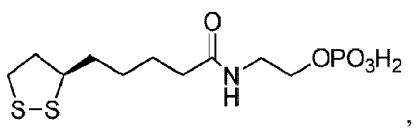
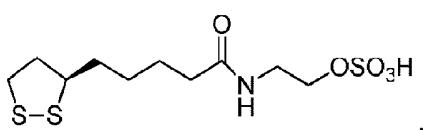


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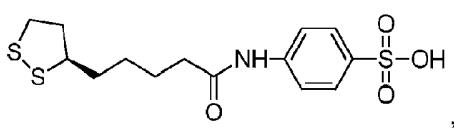


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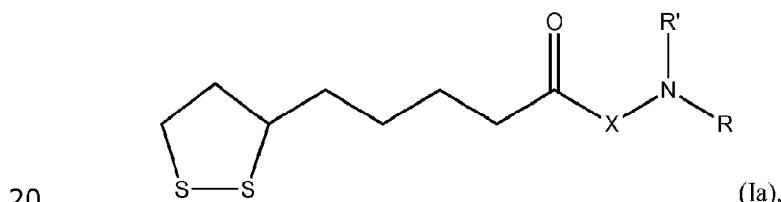
10 eller et farmaceutisk acceptabelt salt deraf.

10. Sammensætning, omfattende en farmaceutisk acceptabel bærer eller fortyndingsmiddel og én eller flere forbindelser ifølge et af kravene 1 til 9, eller et farmaceutisk acceptabelt salt deraf.

15

11. Forbindelse til anvendelse i behandling af iskæmisk traume/skade eller iskæmi-reperfusionsskade, hvor:

forbindelsen er vist med strukturformel (Ia):

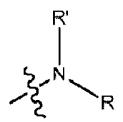


eller et farmaceutisk acceptabelt salt deraf, hvor:

R er (C₁-C₁₈)alkyl, (C₆-C₁₈)aryl eller (C₆-C₁₈)aryl(C₁-C₁₈)alkyl og er substitueret med mindst én syreholdig substituent valgt fra gruppen bestående af -CO₂H, -SO₃H, -PO₃H₂, -OSO₃H, -OPO₃H₂, -B(OH)₂ og -NHOH, hvor aryen af (C₆-C₁₈)aryl eller (C₆-C₁₈)aryl(C₁-C₁₈)alkyl eventuelt er yderligere substitueret med én eller flere substituenter valgt fra gruppen bestående af hydroxy, halo, (C₁-C₃)alkyl, halo(C₁-C₃)alkyl, cyano, nitro, (C₁-C₃)alkoxy og thio(C₁-C₃)alkyl;

R' er hydrogen eller (C₁-C₁₈)alkyl, hvor (C₁-C₁₈)alkyl eventuelt er substitueret med én eller flere syreholdige substituenter valgt fra gruppen bestående af -CO₂H, -SO₃H, -PO₃H₂, -OSO₃H, -OPO₃H₂, -B(OH)₂ og -NHOH; og

X er en aminosyre, hvor aminosyren er orienteret til at danne en amidbinding med



forudsat at forbindelsen med strukturformel (Ia) ikke er *N*-lipoyl-glutamylalanin, *N*-lipoyl-aspartylglycin, eller *N*-lipoyl-glutamylglycin; eller forbindelsen er en (R)-lipoyl stereoisomer af en forbindelse vist med strukturformel (Ia), eller et farmaceutisk acceptabelt salt deraf og det enantiomere overskud eller diastereomere overskud af forbindelsen eller farmaceutisk acceptabelt salt deraf er mindst 90%; og

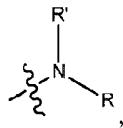
R er (C₁-C₁₈)alkyl, (C₆-C₁₈)aryl eller (C₆-C₁₈)aryl(C₁-C₁₈)alkyl og er substitueret med mindst én syreholdig substituent valgt fra gruppen bestående af -CO₂H, -SO₃H, -PO₃H₂, -OSO₃H, -OPO₃H₂, -B(OH)₂ og -NHOH, hvor aryl af (C₆-C₁₈)aryl eller (C₆-C₁₈)aryl(C₁-C₁₈)alkyl eventuelt er yderligere substitueret med én eller flere substituenter valgt fra gruppen bestående af hydroxy, halo, (C₁-C₃)alkyl, halo(C₁-C₃)alkyl, cyano, nitro, (C₁-C₃)alkoxy og thio(C₁-C₃)alkyl;

R' er hydrogen eller (C₁-C₁₈)alkyl, hvor (C₁-C₁₈)alkyl eventuelt er substitueret med én eller flere syreholdige substituenter valgt fra gruppen

bestående af $-\text{CO}_2\text{H}$, $-\text{SO}_3\text{H}$, $-\text{PO}_3\text{H}_2$, $-\text{OSO}_3\text{H}$, $-\text{OPO}_3\text{H}_2$, $-\text{B}(\text{OH})_2$ og $-\text{NHOH}$;
og

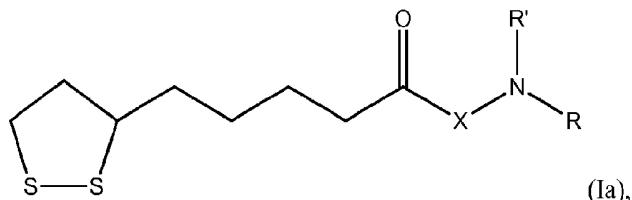
X er fraværende eller er en aminosyre, hvor aminosyren er orienteret til at danne en amidbinding med

5



forudsat at forbindelsen med strukturformel (Ia) ikke er *N*-lipoyl-glutamylalanin, *N*-lipoyl-aspartylglycin, *N*-lipoyl-glutamylglycin eller 5-(5-(1,2-dithiolan-3-yl)pentanamido)-2-hydroxybenzoesyre.

10 **12.** Anvendelse af en forbindelse i fremstillingen af et medikament til behandling af iskæmisk skade eller iskæmi-reperfusionsskade, hvor forbindelsen er vist med strukturformel (Ia):

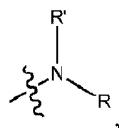


eller et farmaceutisk acceptabelt salt deraf, hvor:

15 R er $(\text{C}_1\text{-C}_{18})\text{alkyl}$, $(\text{C}_6\text{-C}_{18})\text{aryl}$ eller $(\text{C}_6\text{-C}_{18})\text{aryl}(\text{C}_1\text{-C}_{18})\text{alkyl}$ og er substitueret med mindst én syreholdig substituent valgt fra gruppen bestående af $-\text{CO}_2\text{H}$, $-\text{SO}_3\text{H}$, $-\text{PO}_3\text{H}_2$, $-\text{OSO}_3\text{H}$, $-\text{OPO}_3\text{H}_2$, $-\text{B}(\text{OH})_2$ og $-\text{NHOH}$, hvor aryl af $(\text{C}_6\text{-C}_{18})\text{aryl}$ eller $(\text{C}_6\text{-C}_{18})\text{aryl}(\text{C}_1\text{-C}_{18})\text{alkyl}$ eventuelt er yderligere substitueret med én eller flere substituenter valgt fra gruppen bestående af hydroxy, halo, $(\text{C}_1\text{-C}_3)\text{alkyl}$, halo $(\text{C}_1\text{-C}_3)\text{alkyl}$, cyano, nitro, $(\text{C}_1\text{-C}_3)\text{alkoxy}$ og thio $(\text{C}_1\text{-C}_3)\text{alkyl}$;

20 25 R' er hydrogen eller $(\text{C}_1\text{-C}_{18})\text{alkyl}$, hvor $(\text{C}_1\text{-C}_{18})\text{alkyl}$ eventuelt er substitueret med én eller flere syreholdige substituenter valgt fra gruppen bestående af $-\text{CO}_2\text{H}$, $-\text{SO}_3\text{H}$, $-\text{PO}_3\text{H}_2$, $-\text{OSO}_3\text{H}$, $-\text{OPO}_3\text{H}_2$, $-\text{B}(\text{OH})_2$ og $-\text{NHOH}$;
og

X er en aminosyre, hvor aminosyren er orienteret til at danne en amidbinding med



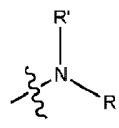
forudsat at forbindelsen med strukturformel (Ia) ikke er *N*-lipoyl-glutamylalanin,

5 *N*-lipoyl-aspartylglycin, eller *N*-lipoyl-glutamylglycin; eller
 forbindelsen er en (R)-lipoylstereoisomer af forbindelse vist med strukturformel
 (Ia), eller et farmaceutisk acceptabelt salt deraf og det enantiomere overskud
 eller diastereomere overskud af forbindelsen eller farmaceutisk acceptabelt salt
 deraf er mindst 90%; og

10 R er (C₁-C₁₈)alkyl, (C₆-C₁₈)aryl eller (C₆-C₁₈)aryl(C₁-C₁₈)alkyl og er
 substitueret med mindst én syreholdig substituent valgt fra gruppen
 bestående af -CO₂H, -SO₃H, -PO₃H₂, -OSO₃H, -OPO₃H₂, -B(OH)₂ og -NHOH,
 hvor aryl af (C₆-C₁₈)aryl eller (C₆-C₁₈)aryl(C₁-C₁₈)alkyl eventuelt er
 yderligere substitueret med én eller flere substituenter valgt fra gruppen
 bestående af hydroxy, halo, (C₁-C₃)alkyl, halo(C₁-C₃)alkyl, cyano, nitro,
 (C₁-C₃)alkoxy og thio(C₁-C₃)alkyl;

15 R' er hydrogen eller (C₁-C₁₈)alkyl, hvor (C₁-C₁₈)alkyl eventuelt er
 substitueret med én eller flere syreholdige substituenter valgt fra gruppen
 bestående af -CO₂H, -SO₃H, -PO₃H₂, -OSO₃H, -OPO₃H₂, -B(OH)₂ og -NHOH;
 20 og

X er fraværende eller er en aminosyre, hvor aminosyren er orienteret til at
 danne en amidbinding med



forudsat at forbindelsen med strukturformel (Ia) ikke er *N*-lipoyl-glutamylalanin,

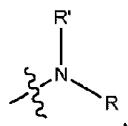
25 *N*-lipoyl-aspartylglycin, *N*-lipoyl-glutamylglycin eller 5-(5-(1,2-dithiolan-3-
 yl)pentanamido)-2-hydroxybenzoesyre.

13. Forbindelsen til anvendelse ifølge krav 11 eller anvendelse ifølge krav 12, hvor:

R er (C_1 - C_{18})alkyl, (C_6 - C_{18})aryl eller (C_6 - C_{18})aryl(C_1 - C_{18})alkyl og er substitueret med mindst én syreholdig substituent valgt fra gruppen bestående af $-CO_2H$, $-SO_3H$, $-PO_3H_2$, $-OSO_3H$, $-OPO_3H_2$, $-B(OH)_2$ og $-NHOH$, hvor arylen af (C_6 - C_{18})aryl eller (C_6 - C_{18})aryl(C_1 - C_{18})alkyl eventuelt er yderligere substitueret med én eller flere substituenter valgt fra gruppen bestående af hydroxy, halo, (C_1 - C_3)alkyl, halo(C_1 - C_3)alkyl, cyano, nitro, (C_1 - C_3)alkoxy og thio(C_1 - C_3)alkyl;

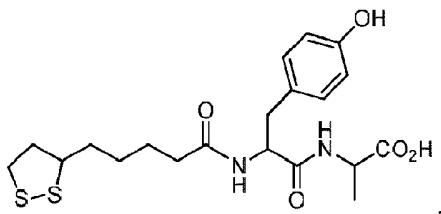
10 R' er hydrogen eller (C_1 - C_{18})alkyl, hvor (C_1 - C_{18})alkyl eventuelt er substitueret med én eller flere syreholdige substituenter valgt fra gruppen bestående af $-CO_2H$, $-SO_3H$, $-PO_3H_2$, $-OSO_3H$, $-OPO_3H_2$, $-B(OH)_2$ og $-NHOH$; og

15 X er en aminosyre, hvor aminosyren er orienteret til at danne en amidbinding med

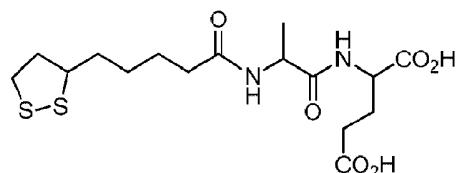
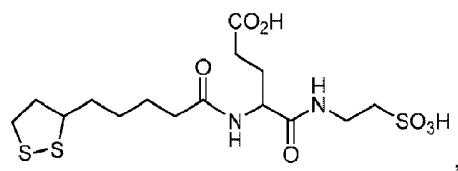


forudsat at forbindelsen med strukturformel (Ia) ikke er *N*-lipoyl-glutamylalanin, *N*-lipoyl-aspartylglycin, eller *N*-lipoyl-glutamylglycin; eventuelt hvor forbindelsen er en (R)-lipoylstereoisomer af forbindelse vist med strukturformel (Ia), eller et farmaceutisk acceptabelt salt deraf, og det enantiomere overskud eller diastereomere overskud af forbindelsen eller farmaceutisk acceptabelt salt deraf er mindst 90%.

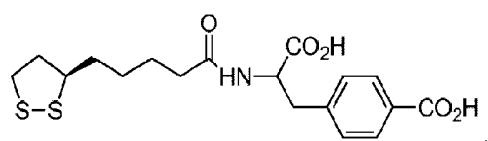
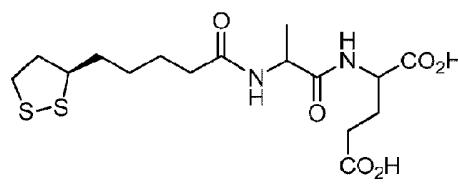
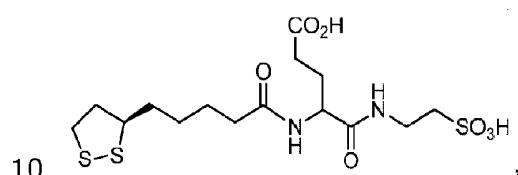
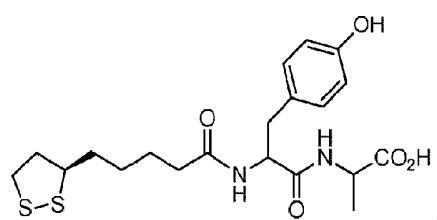
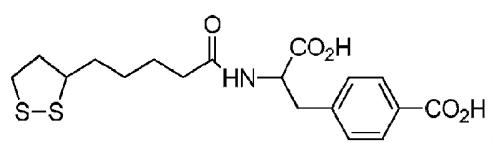
14. Forbindelsen til anvendelse eller anvendelse ifølge et af kravene 11 til 13, hvor forbindelsen er vist med én af følgende strukturformler:



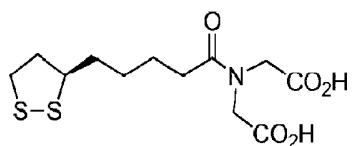
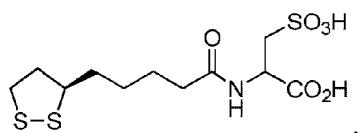
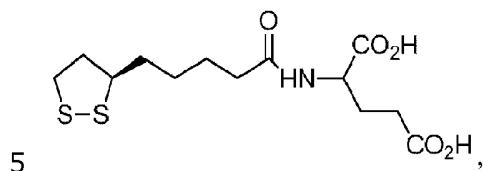
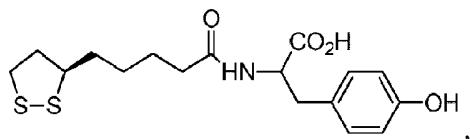
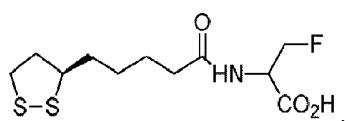
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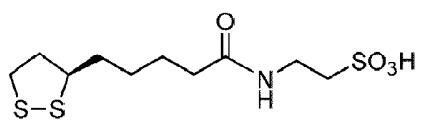
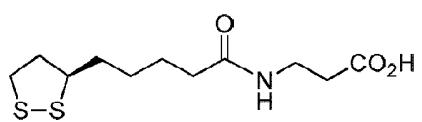
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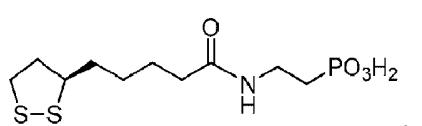
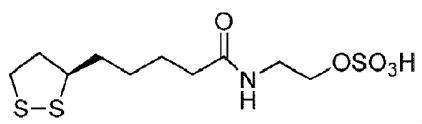
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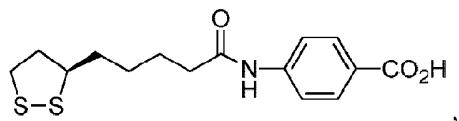
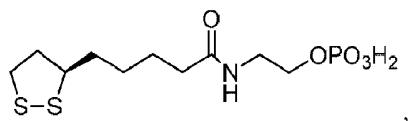


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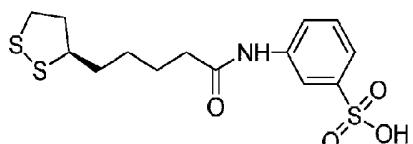


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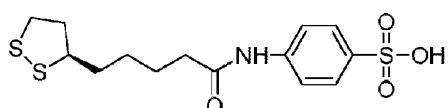




5



or



eller et farmaceutisk acceptabelt salt deraf.

10

15. Forbindelsen til anvendelse eller anvendelse ifølge et af kravene 11 til 14, hvor:

15 iskæmi-reperfusionsskaden er valgt fra gruppen bestående af en cerebrovaskulær iskæmi-reperfusionsskade, en renal iskæmi-reperfusionsskade, en hepatisk iskæmi-reperfusionsskade, iskæmi-reperfusionscardiomyopati, kutan iskæmi-reperfusionsskade, tarm-iskæmi-reperfusionsskade, tarm-iskæmi-reperfusionsskade, gastrisk iskæmi-reperfusionsskade, pulmonær iskæmi-reperfusionsskade, bugspytkirtel-iskæmi-reperfusionsskade, skelettal muskel-iskæmi-reperfusionsskade, abdominal muskel-iskæmi-reperfusionsskade, lem-iskæmi-reperfusionsskade, iskæmi-reperfusionscolitis, mesenterisk iskæmi-reperfusionsskade og en stille iskæmi-reperfusionsskade; eller

20 iskæmiskaden er en myokardial iskæmiskade; eller

25 iskæmiskaden er deraf følgende iskæmi valgt fra gruppen bestående af kardiovaskulær iskæmi, cerebrovaskulær iskæmi, renal iskæmi, hepatisk

iskæmi, iskæmi-reperfusionskardiomyopati, kutan iskæmi, tarmiskæmi, intestinal iskæmi, gastrisk iskæmi, pulmonær iskæmi, bugspytkirtel-iskæmi, skeletal muskeliskæmi, abdominal muskeliskæmi, lem-iskæmi, iskæmi-reperfusionscolitis, mesenterisk iskæmi og en stille iskæmi; eller

5 iskæmi-reperfusionsskaden er a myokardial iskæmi-reperfusionsskade; eller

iskæmiskaden eller iskæmi-reperfusionsskaden omfatter perioperativ hjerteskade; eller

iskæmi-reperfusionsskaden er en renal iskæmi-reperfusionsskade; eller

10 iskæmiskaden eller iskæmi-reperfusionsskaden er deraf følgende terapeutisk intervention, såsom en terapeutisk intervention valgt fra gruppen bestående af hjertearterie-bypass-transplantationskirurgi, hjerte-angioplastikirurgi, transplantationskirurgi og kardiopulmonær bypass-kirurgi.