Abstract:
The present invention is directed to compositions and methods for enhancing the survivability of, and/or reducing damage to, cells, tissues, organs, and organisms, particularly under ischemic or hypoxic conditions.
COMPOSITIONS AND METHODS FOR ENHANCING SURVIVABILITY AND REDUCING INJURY OF CELLS, TISSUES, ORGANS AND ORGANISMS UNDER ISCHEMIC OR HYPOXIC CONDITIONS

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates generally to the field of cell biology and physiology. More particularly, the present invention relates to compositions and methods for enhancing the survivability of, and/or reducing damage to, cells, tissues, organs, and organisms, particularly under ischemic or hypoxic conditions.

Description of the Related Art

Cells, tissues, organs, and organisms that are deprived of blood flow undergo ischemic damage due to oxidative stress and are damaged or eventually die. Traditional methods of reducing ischemic damage involve the administration of drugs and perfusing affected tissues with oxygen, but this procedure causes significant tissue damage and can result in serious lasting injury, such as brain damage during stroke or cardiac arrest.

Attempts have been made to reduce ischemic and reperfusion injury by inducing tissues and organs to enter a reduced metabolic state. In the context of living tissues being preserved for transplant or grafting, one common method for reducing their metabolic activity is by immersing tissues or organs in a physiologic fluid, such as saline, and placing them in the cold. However, such methods cannot be relied upon for extended periods, and the success of organ transplant and limb reattachments remains inversely related to the time the organ or limb is out of contact with the intact organism.

More extreme methods of reducing metabolic activity in whole organisms are known colloquially as "suspended animation." Though still considered largely within the realm of science fiction, some notoriety has been achieved when wealthy individuals have sought to be cryopreserved after death in the hopes that future medical breakthroughs will permit their revival and cure of their fatal ailments.
Allegedly, more than one hundred people have been cryopreserved since the first attempt in 1967, and more than one thousand people have made legal and financial arrangements for cryonics with one of several organizations, for example, Alcor Life Extension Foundation. Such methods involve the administration of anti-ischemic drugs, low temperature preservation, and methods to perfuse whole organisms with cryosuspension fluids. However, it has not yet been substantiated that this form of reduced metabolic activity is reversible.

On a related note, there are numerous reports of individuals who have survived apparent cessation of pulse and respiration after exposure to hypothermic conditions, usually in cold-water immersion. Though not fully understood by scientists, the ability to survive such situations likely derives from what is called the "mammalian diving reflex." This reflex is believed to stimulate the vagal nervous system, which controls the lungs, heart, larynx and esophagus, in order to protect vital organs. Presumably, cold-water stimulation of nerve receptors on the skin causes shunting of blood to the brain and to the heart, and away from the skin, the gastro-intestinal tract and extremities. At the same time, a protective reflex bradycardia, or slowing of the heart beat, conserves the dwindling oxygen supplies within the body. Unfortunately, the expression of this reflex is not the same in all people, and is believed to be a factor in only 10-20% percent of cold-water immersion cases.

While the methods described above may be useful in certain contexts for reducing ischemic reperfusion injury, dependence upon reducing temperature can be problematic, as apparatuses and agents for producing such low temperatures may not be readily available, and damage to cells and tissue may occur as a result of freeze/thaw processes. Clearly, compositions and methods that do not rely fully on hypothermia and/or oxygen would be useful in the context of organ preservation, as well as for tissue or cell preservation. Moreover, such compositions and methods would also be useful in controlling cellular and physiologic metabolism in whole organisms subjected to traumas such as severe blood loss, hypothermia, or cardiac arrest, thereby reducing ischemic and reperfusion injury. Currently, the lack of ability to control cellular and physiologic metabolism in whole organisms subjected to such traumas is a key shortcoming in the medical field.
Thus, there is a great need for improved methods for controlling metabolic processes, particularly under traumatic conditions, and pharmaceutically acceptable compositions useful in practicing these methods. The present invention fulfills these needs, and provides other related advantages.

5 BRIEF SUMMARY OF THE INVENTION

As noted previously, the present invention is directed to compositions and methods for enhancing the survivability of, and/or reducing damage to, cells, tissues, organs, and organisms, particularly under ischemic or hypoxic conditions. More particularly, the present invention is directed to the use of thiomorpholine and thiomorpholine analogs for enhancing the survivability of, and/or reducing damage to, cells, tissues, organs, and organisms, particularly under ischemic or hypoxic conditions.

Accordingly, in one aspect, the present invention provides thiomorpholine analogs having the following general structure (I):

\[ R_1 - N - \underbrace{\left( C(R_2)_{2n} \right)}_{(C(R_2)_{2m})} - A \]

or stereoisomers, prodrugs, pharmaceutically acceptable salts or solvates thereof, wherein:

- A is S or Se;
- \( n \) is 1, 2 or 3;
- \( m \) is 2, 3, 4 or 5;
- \( n + m \) is less than or equal to 6;
- \( R_1 \) is hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, arylalkyl, substituted arylalkyl, cycloalkyl, substituted cycloalkyl, cycloalkylalkyl, substituted cycloalkylalkyl, heteroaryl, substituted heteroaryl, heteroarylmethyl, substituted heteroarylmethyl, heterocyclyl, substituted heterocyclyl, heterocyclylmethyl or substituted heterocyclylmethyl; and
each R₂ is independently selected from the group consisting of hydrogen, amino, cyano, halogen, hydroxyl, imino, nitro, oxo, thioxo, alkyl, substituted alkyl, alkylamino, substituted alkylamino, alkoxy, substituted alkoxy, alkylthio, substituted alkylthio, aryl, substituted aryl, arylalkyl, substituted arylalkyl, cycloalkyl, substituted cycloalkylalkyl, substituted cycloalkylalkyl, heteroaryl, substituted heteroaryl, heteroarylalkyl, substituted heteroarylalkyl, heterocyclyl, substituted heterocyclyl, heterocyclylalkyl or substituted heterocyclylalkyl, or any two R₂ groups, taken together with the respective carbon atoms to which these groups are attached, form an unsubstituted or substituted fused cycloalkyl, aryl, heterocyclyl or heteroaryl.

In another aspect, the present invention provides methods of enhancing the survivability of a biological material exposed to ischemic or hypoxic conditions, wherein the methods comprise contacting the biological material with an effective amount of thiomorpholine or a thiomorpholine analog of structure (I).

In certain embodiments, the biological material is contacted with an effective amount of thiomorpholine. In other embodiments, the biological material is contacted with an effective amount of a thiomorpholine analog.

In certain embodiments, the biological material is contacted with the thiomorpholine or thiomorpholine analog before being exposed to the ischemic or hypoxic conditions. In other embodiments, the biological material is contacted with the thiomorpholine or thiomorpholine analog during exposure to the ischemic or hypoxic conditions. In other embodiments, the biological material is contacted with the thiomorpholine or thiomorpholine analog after being exposed to the ischemic or hypoxic conditions.

In certain embodiments, the ischemic or hypoxic conditions result from an injury to the biological material, the onset or progression of a disease that adversely affects the biological material, or hemorrhaging of the biological material. In more specific embodiments, the biological material is contacted with the thiomorpholine or thiomorpholine analog before the injury, before the onset or progression of the disease, or before hemorrhaging of the biological material. In other more specific embodiments, the biological material is not contacted with the thiomorpholine or thiomorpholine analog during or after the injury, during or after the onset or progression of the disease,
or during or after the hemorrhaging of the biological material. In other more specific embodiments, the injury is from an external physical source, such as a surgery. In other more specific embodiments, the biological material is contacted with the thiomorpholine or thiomorpholine analog in an amount and for a time that protects the biological material from damage or death resulting from the injury, the onset or progression of the disease, or the hemorrhaging in the biological material.

In certain embodiments, the biological material is selected from the group consisting of cells, tissues, organs, organisms, and animals. In more specific embodiments, the biological material is an animal, a mammal or a human. In other more specific embodiments, the biological material comprises platelets or the biological material is to be transplanted or the biological material is at risk for reperfusion injury or the biological material is at risk for hemorrhagic shock.

In certain embodiments, the biological material is contacted with the thiomorpholine or thiomorpholine analog intravenously, intradermally, intraarterially, intraperitoneally, intralesionally, intracranially, intraarticularly, intraprostatically, intrapleurally, intratracheally, intranasally, intravitreally, intravaginally, intrarectally, topically, intratumorally, intramuscularly, intraperitoneally, intraocularly, subcutaneously, subconjunctival, intravesicularly, mucosally, intrapericardially, intraumbilically, intraocularly, orally, topically, locally, by inhalation, by injection, by infusion, by continuous infusion, by absorption, by adsorption, by immersion, by localized perfusion, via a catheter, or via a lavage.

In certain embodiments, the thiomorpholine or thiomorpholine analog is provided to the biological material by infusion at a dosage in the range of 10 µg/kg/min to 500 µg/kg/min or any range derivable therein.

In certain embodiments, the method further comprises exposing the biological material to a controlled pressure environment.

In certain embodiments, the method further comprises exposing the biological material to a controlled temperature environment. In more specific embodiments, the biological material is exposed to a controlled temperature environment that is less than about 20°C. In other more specific embodiments, the biological material achieves a non-physiological core temperature.
In certain embodiments, the method farther comprises contacting the biological material with an effective amount of an additional active compound. In more specific embodiments, the biological material is contacted with the thiomorpholine or thiomorpholine analog and the additional active compound sequentially. In other more specific embodiments, the biological material is contacted with the thiomorpholine or thiomorpholine analog and the additional active compound simultaneously. In other more specific embodiments, the additional active compound is an oxygen antagonist. In other more specific embodiments, the additional active compound is a chalcogenide compound, optionally comprising sulfur (such as H₂S) or selenium (such as H₂Se). In other more specific embodiments, the additional active compound is a chalcogenide salt, optionally comprising sulfur, selected from the group consisting of Na₂S, NaHS, K₂S, KHS, Li₂S, Rb₂S, Cs₂S, (NH₄)₂S, (NH₄)HS, BeS, MgS, CaS, SrS, and BaS, or selenium, selected from the group consisting of Na₂Se, NaHSe, K₂Se, KHSe, Li₂Se, Rb₂Se, Cs₂Se, (NH₄)₂Se, (NH₄)HSe, BeSe, MgSe, CaSe, SrSe, PoSe and BaSe. In other more specific embodiments, the additional active compound is CO₂.

In certain embodiments, the thiomorpholine or thiomorpholine analog is provided to the biological material as a pharmaceutical composition.

In another aspect, the present invention provides methods for preventing or reducing damage to a biological material exposed to ischemic or hypoxic conditions, wherein the methods comprise contacting the biological material with an effective amount of thiomorpholine or a thiomorpholine analog of structure (I).

In another aspect, the present invention provides methods for reversibly inhibiting metabolism in a biological material, wherein the methods comprise contacting the biological material with an effective amount of thiomorpholine or a thiomorpholine analog of structure (I). In certain embodiments, the biological material is a mammal.

In another aspect, the present invention provides methods for enhancing the survivability of a mammal, such as a human, suffering from hemorrhagic shock or at risk of hemorrhagic shock, wherein the methods comprise administering to the mammal an effective amount of thiomorpholine or a thiomorpholine analog of structure (I). In certain embodiments, the mammal is at risk of hemorrhagic shock and suffers...
hemorrhagic shock after being provided with the thiomorpholine or thiomorpholine analog.

In another aspect, the present invention provides methods for enhancing the survivability of a mammal, such as a human, undergoing a surgery, wherein the methods comprise administering to the mammal an effective amount of thiomorpholine or a thiomorpholine analog of structure (I). In certain embodiments, the surgery is selected from elective surgery, planned surgery and emergency surgery.

In another aspect, the present invention provides methods for preserving biological material ex vivo, wherein the methods comprise contacting the biological material with an effective amount of thiomorpholine or a thiomorpholine analog of structure (I).

In certain embodiments, the biological material is stored at low temperature. In certain embodiments, the biological material is cells, tissue, or an organ. In more specific embodiments, the cells are platelets or the tissue or organ is being stored prior to transplant.

In another aspect, the present invention provides methods for preserving non-living biological material, wherein the methods comprise contacting the material with an effective amount of thiomorpholine or a thiomorpholine analog of structure (I). In certain embodiments, the non-living biological material is a dead animal or a plant or plant product, such as a food product.

In another aspect, the present invention provides methods for extending the shelf-life of a food or beverage product subject to spoilage, wherein the methods comprise contacting the food or beverage product with an effective amount of thiomorpholine or a thiomorpholine analog of structure (I). In certain embodiments, the food or beverage product is wine or beer.

In another aspect, the present invention provides methods for extending the shelf-life of a pharmaceutical, health care or cosmetic product, wherein the methods comprise contacting the pharmaceutical, health care or cosmetic product with an effective amount of thiomorpholine or a thiomorpholine analog of structure (I).
In another aspect, the present invention provides pharmaceutical compositions comprising a pharmaceutically acceptable carrier, diluent or excipient and thiomorpholine or a thiomorpholine analog of structure (I).

In another aspect, the present invention provides an article of manufacture comprising packing material and a composition comprising thiomorpholine or a thiomorpholine analog of structure (I), wherein the packing material comprises a label that indicates that the composition can be used to enhance the survivability of biological material exposed to ischemic or hypoxic conditions.

In certain embodiments, the composition comprises thiomorpholine. In certain embodiments, the article of manufacture further comprises a pharmaceutically acceptable diluent. In more specific embodiments, the composition is provided in a first sealed container and the pharmaceutically acceptable diluent is provided in a second sealed container. In certain embodiments, the article of manufacture further comprises an additional active compound. In more specific embodiments, the composition is provided in a first sealed container and the additional active compound is provided is a second sealed container.

These and other aspects of the present invention will be apparent upon reference to the following detailed description. To this end, various references are set forth herein which describe in more detail certain background information, procedures, compounds and/or compositions, and are each hereby incorporated by reference in their entirety.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a graph showing the core body temperature over time of a mouse (MJVC1 41) infused with 149 mM thiomorpholine, pH 8.0 (173 mg/kg) and exposed to hypoxic conditions of 4% O₂.

Figure 2 is a graph showing the core body temperature over time of a rat (RJVC51) infused with 149 mM thiomorpholine, pH 8.0 (99.4 mg/kg) and exposed to hypoxic conditions of 3.5% O₂.
Figure 3 is a Kaplan Meier graph comparing the survival rate measured over time of two groups of C57BL/6 mice (14 per group) that were either treated with vehicle or treated with thiomorpholine (125 mg/kg) then injected with lipopolysaccharide (LPS) (100 mg/kg) an average of 30 minutes after treatment.

5 DETAILED DESCRIPTION OF THE INVENTION

Definitions

Certain chemical groups named herein are preceded by a shorthand notation indicating the total number of carbon atoms that are to be found in the indicated chemical group. For example; C\textsubscript{7}-C\textsubscript{12}alkyl describes an alkyl group, as defined below, having a total of 7 to 12 carbon atoms. The total number of carbons in the shorthand notation does not include carbons that may exist in substituents of the group described.

Accordingly, as used in the specification and appended claims, unless specified to the contrary, the following terms have the meaning indicated:

"Alkyl" refers to a straight or branched, saturated or unsaturated, hydrocarbon chain radical consisting solely of carbon and hydrogen atoms, and having from one to twelve carbon atoms, preferably one to eight carbon atoms or one to six carbon atoms. Saturated alkyl radicals include, for example, methyl, ethyl, \textsubscript{\textit{t}}-propyl, 1-methyl ethyl (\textit{zso}-propyl), \textit{t}-butyl, \textit{t}-pentyl, 1,1-dimethyl ethyl (\textit{t}-butyl), 3-methylhexyl, 2-methylhexyl, and the like. Unsaturated alkyl radicals containing at least one double bond (also referred to as "alkenyl" radicals) include, for example, ethenyl, prop-1-enyl, but-1-enyl, pent-1-enyl, penta-1,4-dienyl, and the like. Unsaturated alkyl radicals containing at least one triple bond (also referred to as "alkynyl" radicals) include, for example, ethynyl, propynyl, butynyl, pentynyl, hexynyl, and the like.

"Alkylamino" refers to a radical, having one or two alkyl moieties attached through a nitrogen bridge, of the formula \(-\text{NHR}_a\) or \(-\text{NR}_a\text{R}_a\), e.g., methylamino, ethylamino, dimethylamino, diethylamino, and the like.
"Alkoxy" refers to a radical of the formula -OR<sub>3</sub> where R<sub>a</sub> is an alkyl radical as defined above containing one to twelve carbon atoms, e.g., methoxy, ethoxy, and the like.

"Alkylthio" refers to a radical of the formula -SR<sub>3</sub> where R<sub>a</sub> is an alkyl radical as defined above containing one to twelve carbon atoms, e.g., methylthio, ethylthio, and the like.

"Aryl" refers to aromatic monocyclic or multicyclic hydrocarbon ring systems consisting only of hydrogen and carbon and containing from 6 to 19 carbon atoms, where the ring system may be partially or fully saturated, e.g., phenyl, naphthyl, and the like.

"Arylalkyl" refers to a radical of the formula -R<sub>a</sub>R<sub>b</sub> where R<sub>a</sub> is an alkyl radical as defined above and R<sub>b</sub> is one or more aryl radicals as defined above.

"Cycloalkyl" refers to a non-aromatic monocyclic or polycyclic hydrocarbon radical consisting solely of carbon and hydrogen atoms, which may include fused or bridged ring systems, having from three to fifteen carbon atoms, preferably having from three to ten carbon atoms, and which is saturated or unsaturated. Monocyclic radicals include, for example, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, and the like. Polycyclic radicals include, for example, adamantane, norbornane, decaliny1, 7,7-dimethyl-bicyclo[2.2.1]heptanyl, and the like.

"Cycloalkylalkyl" refers to a radical of the formula -R<sub>j</sub>R<sub>d</sub> where R<sub>j</sub> is an alkyl radical as defined above and R<sub>d</sub> is one or more cycloalkyl radicals as defined above.

"Heteroaryl" or "heteroaryl ring" refers to a 5- to 18-membered aromatic ring radical which consists of three to seventeen carbon atoms and from one to ten heteroatoms selected from the group consisting of nitrogen, oxygen and sulfur. For purposes of this invention, the heteroaryl radical may be a monocyclic, bicyclic, tricyclic or tetracyclic ring system, which may include fused or bridged ring systems; and the nitrogen, carbon or sulfur atoms in the heteroaryl radical may be optionally oxidized; the nitrogen atom may be optionally quaternized. Heteroaryl radicals include, for example, azepinyl, acridinyl, benzimidazolyl, benzthiazolyl, benzindolyl,
benzodioxolyl, benzofuranyl, benzooxazolyl, benzothiazolyl, benzothiadiazolyl, benzoo[l,4]dioxepinyl, 1,4-benzodioxanyl, benzonaphthofuranyl, benzoxazolyl, benzo-1,3-dioxolyl, benzodioxinyl, benzopyranonyl, benzofuranonyl, benzothienyl (benzothiophenyl), benzotriazolyl, benzo[l,3-dioxolyl, benzodioxinyl, benzopyranyl, benzopyranonyl, benzofuranyl, benzonaphthofuranyl, 1,4-benzodioxanyl, benzo[l,4]dioxepinyl, benzo[4,6]imidazo[1,2-a]pyridinyl, carbazolyl, cinnolinyl, dibenzofuranyl, dibenzothiophenyl, furanyl, furanonyl, isothiazolyl, imidazolyl, indazolyl, indolyl, indazolyl, isoindolyl, indoliny, isoindolinyl, isoquinolyl, indoliziny, isoaxazolyl, naphthyl, naphthyridinyl, oxadiazolyl, 2-oxoazepinyl, oxazolyl, oxirany, 1-phenyl-pyrrolyl, phenazinyl, phenothiazinyl, phenoxazinyl, phthalazinyl, pteridinyl, purinyl, pyrrolyl, pyrazolyl, pyridinyl, pyrimidinyl, pyridazinyl, pyrrolyl, quinazolinyl, quinoxaliny, quinolinyl, quinuclidinyl, isoquinolinyl, tetrahydroquinolinyl, thiazolyl, thiazolidinyl, triazolyl, tetrazolyl, tetrahydroquinolinyl, thiazolyl, thiazolidinyl, tetrazolyl, triazolyl, thiophenyl (i.e., thienyl), and the like.

"Heteroarylalkyl" refers to a radical of the formula -R_aR_f where R_a is an alkyl radical as defined above and R_f is one or more heteroaryl radicals as defined above.

"Heterocyclyl" or "heterocyclyl ring" refers to a 3- to 18-membered non-aromatic ring radical which consists of two to seventeen carbon atoms and from one to ten heteroatoms selected from the group consisting of nitrogen, oxygen and sulfur. Unless stated otherwise specifically in the specification, the heterocyclyl radical may be a monocyclic, bicyclic, tricyclic or tetracyclic ring system, which may include fused or bridged ring systems; and the nitrogen, carbon or sulfur atoms in the heterocyclyl radical may be optionally oxidized; the nitrogen atom may be optionally quaternized; and the heterocyclyl radical may be partially or fully saturated.

Heterocyclyl radicals include, for example, dioxolanyl, thiényl[l,3]dithianyl, decahydroisoquinolyl, imidazoliny, imidazolidiny, isothiazolidiny, isoxazolidiny, morpholiny, octahydroindolyl, octahydroisoindolyl, 2-oxopiperaziny, 2-oxopiperidiny, 2-oxopyrrolidinyl, oxazolidiny, piperidiny, piperaziny, 4-piperidonyl, pyrrolidiny, pyrazolidinyl, thiazolidinyl, tetrahydrofurul, trithianyl, tetrahydropyranyl, thiomorpholiny, thiamorpholiny, 1-oxo-thiomorpholiny, 1,1-dioxo-thiomorpholiny, and the like.
"Heterocyclylalkyl" refers to a radical of the formula \( R_a R_e \) where \( R_a \) is an alkyl radical as defined above and \( R_e \) is one or more heterocyclyl radicals as defined above, and if the heterocyclyl is a nitrogen-containing heterocyclyl, the heterocyclyl may be attached to the alkyl radical at the nitrogen atom.

The term "substituted" as used herein means any of the above groups (i.e., alkyl, alkylamino, alkoxy, alkylthio, aryl, arylalkyl, cycloalkyl, cycloalkylalkyl, heteroaryl, heteroarylalkyl, heterocyclyl and/or heterocyclylalkyl) wherein at least one hydrogen atom is replaced with a substituent. In the case of an imino ("=NH"), oxo ("=O") or thioxo ("=S") substituent, two hydrogen atoms are replaced. "Substituents" within the context of this invention include amino, cyano, halogen, hydroxyl, imino, nitro, oxo, thioxo, alkyl, alkylamino, alkoxy, alkylthio, aryl, arylalkyl, cycloalkyl, cycloalkylalkyl, heteroaryl, heteroarylalkyl, heterocyclyl and heterocyclylalkyl, as well as \( \text{-NR}_x \text{R}_y, \text{-NR}_x\text{C(=O)}\text{R}_y, \text{-NR}_x\text{C(=O)}\text{N}\text{R}_x \text{NR}_y, \text{-NR}_x\text{C(=O)}\text{OR}_y, \text{-NR}_x\text{SO}_2\text{R}_y, \text{-C(=O)}\text{R}_x \text{-C(=O)}\text{OR}_x, \text{-C(=O)}\text{NR}_x \text{R}_y, \text{-OC(=O)}\text{NR}_x \text{R}_y, \text{-OR}_x, \text{-SR}_x, \text{-SOR}_x, \text{-S(=O)}_2\text{R}_x \text{-OS(=O)}_2\text{R}_x \) and \( \text{-S(=O)}_2\text{OR}_x \) where \( \text{R}_x \) and \( \text{R}_y \) may be the same or different and independently hydrogen, amino, cyano, halogen, hydroxyl, imino, nitro, oxo, thioxo, alkyl, alkylamino, alkoxy, alkylthio, aryl, arylalkyl, cycloalkyl, cycloalkylalkyl, heteroaryl, heteroarylalkyl, heterocyclyl and heterocyclylalkyl. In addition, the above substituents may also be optionally substituted with one or more of the above substituents.

"Amino" refers to the \(-\text{NH}_2\) radical.

"Cyano" refers to the \(-\text{CN}\) radical.

"Halogen" refers to bromo, chloro, fluoro or iodo.

"Hydroxyl" refers to the \(-\text{OH}\) radical.

"Imino" refers to the \(=\text{NH}\) substituent.

"Nitro" refers to the \(-\text{NO}_2\) radical.

"Oxo" refers to the \(=\text{O}\) substituent.

"Thioxo" refers to the \(=\text{S}\) substituent.

"Fused" refers to any ring structure described herein which is fused to an existing ring structure in the compounds of the present invention. When the fused ring is a heterocyclyl ring or a heteroaryl ring, any carbon atom on the existing ring structure.
which becomes part of the fused heterocyclyl ring or the fused heteroaryl ring may be replaced with a nitrogen atom.

"Mammal" includes humans and both domestic animals such as laboratory animals and household pets, (e.g., cats, dogs, swine, cattle, sheep, goats, horses, and rabbits), and non-domestic animals such as wildlife and the like.

"Optional" or "optionally" means that the subsequently described event of circumstances may or may not occur, and that the description includes instances where said event or circumstance occurs and instances in which it does not. For example, "optionally substituted aryl" means that the aryl radical may or may not be substituted and that the description includes both substituted aryl radicals and aryl radicals having no substitution.

"Pharmaceutically acceptable carrier, diluent or excipient" includes without limitation any adjuvant, carrier, excipient, glidant, sweetening agent, diluent, preservative, dye/colorant, flavor enhancer, surfactant, wetting agent, dispersing agent, suspending agent, stabilizer, isotonic agent, solvent, or emulsifier which has been approved by the United States Food and Drug Administration as being acceptable for use in humans or domestic animals.

"Pharmaceutically acceptable salt" includes both acid and base addition salts.

"Pharmaceutically acceptable acid addition salt" refers to those salts which retain the biological effectiveness and properties of the free bases, which are not biologically or otherwise undesirable, and which are formed with inorganic acids such as, but not limited to, hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid and the like, and organic acids such as, but not limited to, acetic acid, 2,2-dichloroacetic acid, adipic acid, alginic acid, ascorbic acid, aspartic acid, benzenesulfonic acid, benzoic acid, 4-acetamidobenzoic acid, camphorcarboxylic acid, camphor-10-sulfonic acid, capric acid, caproic acid, caprylic acid, carbonic acid, cinnamic acid, citric acid, cyclamic acid, dodecylsulfuric acid, ethane-1,2-disulfonic acid, ethanesulfonic acid, 2-hydroxyethanesulfonic acid, formic acid, fumaric acid, galactaric acid, gentisic acid, glucoheptonic acid, gluconic acid, glucuronic acid, glutamic acid, glutaric acid, 2-oxo-glutaric acid, glycerophosphoric acid, glycolic acid, hippuric acid,
isobutyric acid, lactic acid, lactobionic acid, lauric acid, maleic acid, malic acid, malonic acid, mandelic acid, methanesulfonic acid, mucic acid, naphthalene-1,5-disulfonic acid, naphthalene-2-sulfonic acid, 1-hydroxy-2-naphthoic acid, nicotinic acid, oleic acid, orotic acid, oxalic acid, palmitic acid, pamoic acid, propionic acid, pyroglutamic acid, pyruvic acid, salicylic acid, 4-aminosalicylic acid, sebacic acid, stearic acid, succinic acid, tartaric acid, thiocyanic acid, toluenesulfonic acid, trifluoroacetic acid, undecylenic acid, and the like.

"Pharmaceutically acceptable base addition salt" refers to those salts which retain the biological effectiveness and properties of the free acids, which are not biologically or otherwise undesirable. These salts are prepared from addition of an inorganic base or an organic base to the free acid. Salts derived from inorganic bases include, but are not limited to, the sodium, potassium, lithium, ammonium, calcium, magnesium, iron, zinc, copper, manganese, aluminum salts and the like. Preferred inorganic salts are the ammonium, sodium, potassium, calcium, and magnesium salts. Salts derived from organic bases include, but are not limited to, salts of primary, secondary, and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines and basic ion exchange resins, such as ammonia, isopropylamine, trimethylamine, diethylamine, triethylamine, tripropylamine, diethanolamine, ethanolamine, deanol, 2-dimethylaminoethanol, 2-diethylaminoethanol, dicyclohexylamine, lysine, arginine, histidine, caffeine, procaine, hydramine, choline, betaine, benethamine, benzathine, ethylenediamine, glucosamine, methylglucamine, theobromine, triethanolamine, tromethamine, purines, piperazine, piperidine, 7V-ethylpiperidine, polyamine resins and the like. Particularly preferred organic bases are isopropylamine, diethylamine, ethanolamine, trimethylamine, dicyclohexylamine, choline and caffeine.

"Pharmaceutical composition" refers to a formulation of a compound and a medium generally accepted in the art for the delivery of the biologically active compound to mammals, e.g., humans. Such a medium includes all pharmaceutically acceptable carriers, diluents or excipients therefore.

"Prodrug" refers to a compound that may be converted under physiological conditions or by solvolysis to a biologically active compound of the
present invention. Thus, the term "prodrug" refers to a metabolic precursor of a compound of the present invention that is pharmaceutically acceptable. A prodrug may be inactive when administered to a subject in need thereof, but is converted in vivo to an active compound. Prodrugs are typically rapidly transformed in vivo to yield the active compound, for example, by hydrolysis in blood. The prodrug compound often offers advantages of solubility, tissue compatibility or delayed release in a mammalian organism (see, e.g., Bundgard, H., Design of Prodrugs (1985), pp. 7-9, 21-24 (Elsevier, Amsterdam)). A discussion of prodrugs is also provided in Higuchi, T., et al, "Pro-drugs as Novel Delivery Systems," A.C.S. Symposium Series, Vol. 14, and in Bioreversible Carriers in Drug Design, Ed. Edward B. Roche, American Pharmaceutical Association and Pergamon Press, 1987, both of which are incorporated in full by reference herein.

The term "prodrug" is also meant to include any covalently bonded carriers, which release an active compound of the present invention in vivo when such prodrug is administered to a mammalian subject. Prodrugs are generally prepared by modifying functional groups in a way such that the modification is cleaved, either by routine manipulation or in vivo, yielding the parent compound. Prodrugs include, for example, compounds of the present invention wherein hydroxy, amine or sulfhydryl groups are bonded to any group that, when administered to a mammalian subject, cleaves to form the hydroxy, amine or sulfhydryl groups. Thus, representative examples of prodrugs include (but are not limited to) acetate, formate and benzoate derivatives of alcohol and amine functional groups of the compounds of the present invention. Further, in the case of a carboxylic acid (-COOH), esters may be employed, such as methyl esters, ethyl esters, and the like.

The invention disclosed herein is also meant to encompass the in vivo metabolic products of the disclosed compounds. Such products may result from, for example, the oxidation, reduction, hydrolysis, amidation, esterification, and the like of the administered compound, primarily due to enzymatic processes. Accordingly, the invention includes compounds produced by a process comprising contacting a compound of this invention with a mammal for a period of time sufficient to yield a metabolic product thereof. Such products are typically are identified by administering a
radiolabeled compound of the invention in a detectable dose to an animal, such as rat, mouse, guinea pig, monkey, or to human, allowing sufficient time for metabolism to occur, and isolating its conversion products from the urine, blood or other biological samples.

Accordingly, the invention disclosed herein is also meant to encompass all disclosed pharmaceutically acceptable compounds being isotopically-labelled by having one or more atoms replaced by an atom having a different atomic mass or mass number. Examples of isotopes that can be incorporated into the disclosed compounds include isotopes of hydrogen, carbon, nitrogen, oxygen, fluorine, chlorine, and iodine, such as $^2$H, $^3$H, $^{11}$C, $^{13}$C, $^{14}$C, $^{15}$N, $^{15}$O, $^{17}$O, $^{18}$O, $^{35}$S, $^{18}$F, $^{36}$Cl, $^{123}$I, and $^{125}$I, respectively. These radiolabeled compounds could be useful to help determine or measure the effectiveness of the compounds, by characterizing, for example, the site or mode of action, or binding affinity to the pharmacologically important site of action. Certain isotopically-labelled compounds, for example, those incorporating a radioactive isotope, are useful in drug and/or substrate tissue distribution studies. The radioactive isotopes tritium, i.e., $^3$H, and carbon-14, i.e., $^{14}$C, are particularly useful for this purpose in view of their ease of incorporation and ready means of detection. Substitution with heavier isotopes such as deuterium, i.e., $^2$H, may afford certain therapeutic advantages resulting from greater metabolic stability, for example, increased in vivo half-life or reduced dosage requirements, and hence may be preferred in some circumstances. Substitution with positron emitting isotopes, such as $^{11}$C, $^{18}$F, $^{15}$O and $^{13}$N, can be useful in Positron Emission Topography (PET) studies for examining substrate receptor occupancy. Isotopically-labeled compounds can generally be prepared by conventional techniques known to those skilled in the art or by processes analogous to those described in the Preparations and Examples as set out below using an appropriate isotopically-labeled reagent in place of the non-labeled reagent previously employed.

"Solvate" refers to an aggregate that comprises one or more molecules of a compound of the invention with one or more molecules of solvent. The solvent may be water, in which case the solvate may be a hydrate. Alternatively, the solvent may be an organic solvent. Thus, the compounds of the present invention may exist as a hydrate, including a monohydrate, dihydrate, hemihydrate, sesquihydrate, trihydrate,
tetrahydrate and the like, as well as the corresponding solvated forms. The compound of the invention may be true solvates, while in other cases, the compound of the invention may merely retain adventitious water or be a mixture of water plus some adventitious solvent.

"Therapeutically effective amount" refers to that amount of a compound of the invention which, when administered to a mammal, preferably a human, is sufficient to effect treatment, as defined below, of a disease or condition in the mammal, preferably a human. The amount of a compound of the invention which constitutes a "therapeutically effective amount" will vary depending on the compound, the condition and its severity, the manner of administration, and the age of the mammal to be treated, but can be determined routinely by one of ordinary skill in the art having regard to his own knowledge and to this disclosure.

"Treating" or "treatment" as used herein covers the treatment of the disease or condition of interest in a mammal, preferably a human, having the disease or condition of interest, and includes: (i) preventing the disease or condition from occurring in a mammal, in particular, when such mammal is predisposed to the condition but has not yet been diagnosed as having it; (ii) inhibiting the disease or condition, i.e., arresting its development; (iii) relieving the disease or condition, i.e., causing regression of the disease or condition; or (iv) relieving the symptoms resulting from the disease or condition. As used herein, the terms "disease" and "condition" may be used interchangeably or may be different in that the particular malady or condition may not have a known causative agent (so that etiology has not yet been worked out) and it is therefore not yet recognized as a disease but only as an undesirable condition or syndrome, wherein a more or less specific set of symptoms have been identified by clinicians.

The compounds of the invention, or their pharmaceutically acceptable salts may contain one or more asymmetric centers and may thus give rise to enantiomers, diastereomers, and other stereoisomeric forms that may be defined, in terms of absolute stereochemistry, as (R)- or (S)- or, as (D)- or (L)- for amino acids. The present invention is meant to include all such possible isomers, as well as their racemic and optically pure forms. Optically active (+) and (-), (R)- and (S)-, or (D)- and
(L)-isomers may be prepared using chiral synthons or chiral reagents, or resolved using conventional techniques, for example, chromatography and fractional crystallization. Conventional techniques for the preparation/isolation of individual enantiomers include chiral synthesis from a suitable optically pure precursor or resolution of the racemate (or the racemate of a salt or derivative) using, for example, chiral high pressure liquid chromatography (HPLC). When the compounds described herein contain olefin double bonds or other centers of geometric asymmetry, and unless specified otherwise, it is intended that the compounds include both E and Z geometric isomers. Likewise, all tautomeric forms are also intended to be included. A "stereoisomer" refers to a compound made up of the same atoms bonded by the same bonds but having different three-dimensional structures, which are not interchangeable. The present invention contemplates various stereoisomers and mixtures thereof and includes "enantiomers", which refers to two stereoisomers whose molecules are nonsuperimposeable mirror images of one another.

Thiomorpholine Analog Embodiments

\[
\begin{align*}
\text{HN} & \hspace{1cm} \text{S} \\
\text{Thiomorpholine} & 
\end{align*}
\]

As noted above, the present invention is directed to the use of thiomorpholine (Compound 1 in Table I) and thiomorpholine analogs for enhancing the survivability of, and/or reducing damage to, cells, tissues, organs, and organisms, particularly under ischemic or hypoxic conditions. As further noted above, the thiomorpholine analogs have the following general structure (I):
or stereoisomers, prodrugs, pharmaceutically acceptable salts or solvates thereof, wherein A, n, m, R₁ and R₂ are as defined above.

In certain embodiments, A is S and the thiomorpholine analogs have the following structure (II):

$$\begin{align*}
\text{R₁} & \quad \text{N} & \quad (\text{C(R₂)₂}_n) & \quad \text{S} \\
& \quad (\text{C(R₂)₂}_m)
\end{align*}$$

(II)

In other embodiments, A is Se and the thiomorpholine analogs have the following structure (III):

$$\begin{align*}
\text{R₁} & \quad \text{N} & \quad (\text{C(R₂)₂}_n) & \quad \text{Se} \\
& \quad (\text{C(R₂)₂}_m)
\end{align*}$$

(III)

In more specific embodiments of the foregoing, n is 1 and m is 2, and the thiomorpholine analogs have the following structures (H-A) and (III-A):

(ii-A) (IH-A)
In other more specific embodiments of the foregoing, \( n \) is 1 and \( m \) is 3, and the thiomorpholine analogs have the following structures (II-B) and (III-B):

![II-B](image)

![III-B](image)

In other more specific embodiments of the foregoing, \( n \) is 1 and \( m \) is 4, and the thiomorpholine analogs have the following structures (H-C) and (III-C):

![H-C](image)

![III-C](image)

In other more specific embodiments of the foregoing, \( n \) is 1 and \( m \) is 5, and the thiomorpholine analogs have the following structures (H-D) and (III-D):

![H-D](image)

![III-D](image)
In other more specific embodiments of the foregoing, \( n \) and \( m \) are 2, and the thiomorpholine analogs have the following structures (II-E) and (III-E):

In other more specific embodiments of the foregoing, \( n \) is 2 and \( m \) is 3, and the thiomorpholine analogs have the following structures (II-F) and (III-F):

In other more specific embodiments of the foregoing, \( n \) is 2 and \( m \) is 4, and the thiomorpholine analogs have the following structures (II-G) and (III-G):
In other more specific embodiments of the foregoing, $n$ is 3 and $m$ is 3, and the thiomorpholine analogs have the following structures (H-H) and (III-H):

The following Table I identifies various representative thiomorpholine analogs of the present invention:

<table>
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<tr>
<th>Compound No.</th>
<th>Structure</th>
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<td>21</td>
<td><img src="" alt="Chemical Structure 21" /></td>
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</table>
Preparation of Thiomorpholine and Thiomorpholine Analogs

Thiomorpholine and thiomorpholine analogs of the present invention may be obtained from sources such as Sigma Aldrich, Lancaster Synthesis, Inc., Maybridge, Matrix Scientific, Sinova, Chemstep, MicroChemistry, ChemPacific, Fluorochem USA, TCI America, Portland, Oregon, USA, and others, or may be synthesized according to organic synthesis techniques known to those skilled in the art.

For example, thiomorpholine analogs of structures (H-E) and (H1-E) may be synthesized according to the following general Reaction Schemes A and B, respectively, wherein \( R_1 \) and \( R_2 \) are defined as in the Summary of the Invention. It is understood that one skilled in the art would be able to prepare thiomorpholine analogs of structures (H-A), (H-B),
(II-C), (II-D), (II-F), (II-G) and (H-H), as well as (III-A), (III-B), (HI-C), (HI-D), (HI-F), (III-G) and (III-H), by similar methods. It is further understood that one skilled in the art may be able to make these compounds by other methods known to one skilled in the art.

5

**Reaction Scheme A:**

Thiomorpholine analogs of structure (H-E) can be prepared by the process illustrated above (in which X is halogen (*e.g.*, chlorine), tosyl or mesyl). Diols of structure (II-E-iii) can be prepared by reacting an oxiran of structure (II-E-ii) with a hydroxyalkylamine of structure (II-E-i). Diols of structure (II-E-iii) can then be converted to compounds of structure (II-E-iv) by known methods (*e.g.*, by treatment with a hydroxyl/halogen displacement reagent such as thionyl chloride or with a tosyl or mesyl halide). Compounds of structure (II-E-iv) can then be cyclised to a thiomorpholine analog of structure (II-E) by reaction with an alkali metal sulfide (*e.g.*, sodium sulfide). Preferably, the cyclization is effected in a solvent such as
isopropanol/water or acetone/water. See, e.g., U.S. Patent 4,427,676 and UK Patent Application Publication No. 2 061 272, both of which are incorporated herein by reference in their entireties.

Similarly, thiomorpholine analogs of structure (III-E) can be prepared by the process illustrated above (in which X is halogen (e.g., chlorine), tosyl or mesyl). Diols of structure (III-E-iii) can be prepared by reacting an oxiran of structure (III-E-ii) with a hydroxyalkylamine of structure (III-E-i). Diols of structure (III-E-iii) can then be converted to compounds of structure (III-E-iv) by known methods (e.g., by treatment with a hydroxyl/halogen displacement reagent such as thionyl chloride or with a tosyl or
mesyl halide). Compounds of structure (III-E-iv) can then be cyclised to a
thiomorpholine analog of structure (III-E) by reaction with an alkali metal selenide (e.g.,
sodium selenide). Preferably, the cyclization is effected in a solvent such as
isopropanol/water or acetone/water.

It is understood that in the foregoing reaction scheme, combinations of
substituents and/or variables of the depicted formulae are permissible only if such
contributions result in stable compounds.

It will also be appreciated by those skilled in the art that in the process
described, the functional groups of intermediate compounds may need to be protected
by suitable protecting groups. Such functional groups include hydroxy, amino,
mercapto and carboxylic acid. Suitable protecting groups for hydroxy include
trialkylsilyl or diarylalkylsilyl (e.g., ^-butyldimethylsilyl, t-butyldiphensilyl or
trimethylsilyl), tetrahydropyranyl, benzyl, and the like. Suitable protecting groups for
amino, amidino and guanpyryl include f-butoxycarbonyl, benzylxoxycarbonyl, and the
like. Suitable protecting groups for mercapto include -C(O)-R" (where R" is alkyl, aryl
or arylalkyl), f/-methoxybenzyl, trityl and the like. Suitable protecting groups for
carboxylic acid include alkyl, aryl or arylalkyl esters. Protecting groups may be added
or removed in accordance with standard techniques, which are known to one skilled in
the art and as described herein. The use of protecting groups is described in detail in
Wiley. The protecting group may also be a polymer resin such as a Wang resin or a 2-
chlorotrityl-chloride resin.

It will also be appreciated by those skilled in the art, although such
protected derivatives of compounds of this invention may not possess pharmacological
activity as such, they may be administered to a mammal and thereafter metabolized in
the body to form compounds of the invention which are pharmacologically active. Such
derivatives may therefore be described as "prodrugs". All prodrugs of compounds of
this invention are included within the scope of the invention.

With regard to stereoisomers, the thiomorpholine analogs of structure (I)
may have chiral centers and may occur as racemates, racemic mixtures and as individual
enantiomers or diastereomers. Compounds of structure (I) may also possess axial
chirality, which may result in atropisomers. All such isomeric forms are included within the present invention, including mixtures thereof. Furthermore, some of the crystalline forms of the compounds of structure (I) may exist as polymorphs, which are included in the present invention. In addition, some of the compounds of structure (I) may also form solvates with water or other organic solvents. Such solvates are similarly included within the scope of this invention.

Methods of Use

The present invention is based on the surprising discovery that thiomorpholine enhances the survivability of organisms exposed to hypoxic conditions. As described in the accompanying Examples, the present invention is the first demonstration that treatment with thiomorpholine or thiomorpholine analogs enhance the survivability of animals subjected to hypoxic conditions. This discovery provides the basis for the use of thiomorpholine and the disclosed thiomorpholine analogs to enhance the survivability of biological material exposed to ischemic or hypoxic conditions. In addition, this discovery provides the basis for the use of thiomorpholine and the disclosed thiomorpholine analogs to protect biological material from damage resulting from ischemic or hypoxic conditions, including those resulting from injury or disease.

The present invention provides a variety of methods for enhancing the survivability of, and/or reducing damage to, biological material under ischemic or hypoxic conditions, which involve contacting the biological material with thiomorpholine or a thiomorpholine analog. In various embodiments, the biological material is contacted prior to being subjected to ischemic or hypoxic conditions. In other embodiments, the biological material is contacted during all or part of the time of exposure to ischemic or hypoxic conditions. In another related embodiment, the biological material is contacted both prior to and during all or part of the time of exposure to ischemic or hypoxic conditions.

"Enhancing survivability" generally refers to either or both of (1) increasing the likelihood that a biological material will survive exposure to ischemic or hypoxic conditions and (2) extending the duration of time that a biological material
survives exposure to ischemic or hypoxic conditions. In particular embodiments, by contacting the biological material with thiomorpholine or a thiomorpholine analog, the likelihood that the biological material will survive being exposed to hypoxic or ischemic conditions is increased by at least 25%, at least 50%, at least 100%, at least 200%, at least 300%, at least 400%, at least 500%, or at least 1000%. In other embodiments, by contacting the biological material with thiomorpholine or a thiomorpholine analog, the duration of time that the biological material will survive during or after exposure to ischemic or hypoxic conditions is increase by at least 25%, at least 50%, at least 100%, at least 200%, at least 300%, at least 400%, at least 500%, or at least 1000%.

In other embodiments, the compositions and methods of the present invention may be used to induce biological material to enter a hypometabolic state wherein the biological material is alive but is characterized by one or more of: (1) at least a 50% reduction in the rate or amount of carbon dioxide production by the biological matter; and (2) at least a 50% reduction in the rate or amount of oxygen consumption by the biological matter. In another embodiment, the compositions and methods of the present invention may be used to induce biological material to enter a hypometabolic state wherein the biological material is alive but is characterized by one or more of: (1) a less than 50% reduction in the rate or amount of carbon dioxide production by the biological matter; and (2) a less than 50% reduction in the rate or amount of oxygen consumption by the biological matter. Any assay to measure oxygen consumption or carbon dioxide production may be employed, and a typical assay will involve utilizing a closed environment and measuring the difference between the oxygen put into the environment and oxygen that is left in the environment after a period of time. Typically, any reduction in the metabolic activity of a biological material is reversible.

According to various embodiments of the methods of the present invention, a hypometabolic state is induced by treating biological material with an amount of thiomorpholine or a thiomorpholine analog that induces hypometabolism directly itself or, alternatively, by treating biological material with an amount of thiomorpholine or a thiomorpholine analog that does not itself induce hypometabolism,
but instead, promotes or enhances the ability of or decreases the time required for the biological material to enter a hypometabolic state in response to another stimuli, such as, but not limited to, an injury, a disease, hypoxia, reduced temperature conditions, excessive bleeding, or treatment with one or more other active compounds (as defined herein).

It is understood that the particular applications of the methods of the present invention vary depending upon the type of biological material being treated, i.e., cells, tissues, organs, or organisms, and the particular ischemic or hypoxic conditions under which the biological material is exposed. Specific embodiments related to particular types of biological material and ischemic or hypoxic conditions are described further herein.

Ischemic and hypoxic conditions may be accidental or purposeful, and ischemic and hypoxic conditions may result from a variety of biological and environmental factors. For example, in the context of mammals, ischemic and hypoxic conditions include those resulting from injury or disease, as well as those resulting from cryopreservation techniques. In the context of tissues and organs, hypoxic and ischemic conditions may arise during procedures to preserve organs or tissues prior to transplant or grafting. Similarly, cells may be exposed to hypoxic or ischemic conditions during cryopreservation.

Specific examples of conditions leading to ischemia and hypoxia include, but are not limited to, when oxygen concentrations are reduced in the environment (hypoxia or anoxia, such as at high altitudes or under water); when biological material is incapable of receiving oxygen (such as during ischemia), which can be caused by i) reduced blood flow to organs (e.g., heart, brain, and/or kidneys) as a result of blood vessel occlusion (e.g., myocardial infarction and/or stroke), ii) extracorporeal blood shunting as occurs during heart/lung bypass surgery (e.g., "pumphead syndrome" in which heart or brain tissue is damaged as a result of cardiopulmonary bypass), or iii) blood loss due to trauma (e.g., hemorrhagic shock or surgery); hypothermia, wherein the biological material is subjected to sub-physiological temperatures, due to exposure to a cold environment or a state of low temperature of the biological material, such that it is unable to maintain adequate oxygenation;
hyperthermia, wherein the biological material is subjected to supra-physiological temperatures, due to exposure to a hot environment or a state of high temperature of the biological material such as by a malignant fever; and conditions of excess heavy metals, such as iron disorders (genetic as well as environmental) such as hemochromatosis, acquired iron overload, sickle-cell anemia, juvenile hemochromatosis African siderosis, thalassemia, porphyria cutanea tarda, sideroblastic anemia, iron-deficiency anemia and anemia of chronic disease.

It will be further appreciated that the length of time with which biological material is contacted with thiomorpholine or a thiomorpholine analog will vary depending upon the type of biological material, the desired outcome, the particular type of injury or disease, and the particular type of ischemic challenge faced by the biological material. For example, inducing a hypometabolic state with respect to a whole animal and with respect to cells or tissues may require different lengths of treatment. In addition, with respect to human subjects, e.g., subjects undergoing a surgical treatment, treatment for a hemorrhagic shock, or treatment for a hyperproliferative disorder, maintaining the subject in a hypometabolic state for 12, 18, or 24 hours is generally contemplated. With respect to non-human animal subjects, e.g. non-human animals shipped or stored for commercial purposes, maintaining the subject in a hypometabolic state for a period of 2 or 4 days, 2 or 4 weeks, or longer is contemplated.

In addition, it is also understood that the amount of thiomorpholine or a thiomorpholine analog required will vary depending upon whether the biological material is also being treated with another stimuli, i.e., an agent or conditions that induces a hypometabolic state. In such circumstances, the biological material may be contacted with thiomorpholine or a thiomorpholine analog for all or only a part of the duration of time the method is performed, in order to, e.g., enhance survivability of the biological material or protect it from ischemic damage.

Furthermore, it is understood that in all embodiments of methods of the present invention, biological matter may be contacted with one or two or more chemical entities selected from thiomorpholine and the disclosed thiomorpholine analogs. Similarly, compositions and articles of manufacture of the present invention may
comprise, in various embodiments, one or two or more of such chemical entities. In various embodiments, contact may occur simultaneously, during overlapping time period, or at different times.

The term "biological material" refers to any living biological material, including cells, tissues, organs, and/or organisms, and any combination thereof. It is contemplated that the methods of the present invention may be practiced on a part of an organism (such as in cells, in tissue, and/or in one or more organs), whether that part remains within the organism or is removed from the organism, or on the whole organism. Moreover, it is contemplated in the context of cells and tissues, both homogenous and heterogeneous cell populations may be the subject of embodiments of the invention. The term "in vivo biological matter" refers to biological matter that is in vivo, i.e., still within or attached to an organism. Moreover, the term "biological matter" will be understood as synonymous with the term "biological material." In certain embodiments, it is contemplated that one or more cells, tissues, or organs is separate from an organism. The terms "isolated" and "ex vivo" are used to describe such biological material. It is contemplated that the methods of the present invention may be practiced on in vivo and/or isolated biological material.

The cells treated according to the methods of the present invention may be eukaryotic or prokaryotic. In certain embodiments, the cells are eukaryotic. More particularly, in some embodiments, the cells are mammalian cells. Mammalian cells include, but are not limited to those from a human, monkey, mouse, rat, rabbit, hamster, goat, pig, dog, cat, ferret, cow, sheep, and horse.

Cells of the invention may be diploid but in some cases, the cells are haploid (sex cells). Additionally, cells may be polyploid, aneuploid, or anucleate. In particular embodiments, a cell is from a particular tissue or organ, such as one from the group consisting of: heart, lung, kidney, liver, bone marrow, pancreas, skin, bone, vein, artery, cornea, blood, small intestine, large intestine, brain, spinal cord, smooth muscle, skeletal muscle, ovary, testis, uterus, and umbilical cord. In certain embodiments, cells are characterized as one of the following cell types: platelet, myelocyte, erythrocyte, lymphocyte, adipocyte, fibroblast, epithelial cell, endothelial cell, smooth muscle cell, skeletal muscle cell, endocrine cell, glial cell, neuron, secretory cell, barrier function
cell, contractile cell, absorptive cell, mucosal cell, limbus cell (from cornea), stem cell (totipotent, pluripotent or multipotent), unfertilized or fertilized oocyte, or sperm.

The terms "tissue" and "organ" are used according to their ordinary and plain meanings. Though tissue is composed of cells, it will be understood that the term "tissue" refers to an aggregate of similar cells forming a definite kind of structural material. Moreover, an organ is a particular type of tissue. In certain embodiments, the tissue or organ is "isolated," meaning that it is not located within an organism.

In various embodiments, methods of the present invention are used to treat any type of organism, including but not limited to, mammals, reptiles, amphibians, birds, fish, invertebrates, fungi, plants, protests, and prokaryotes. In particular embodiments, a mammal is a marsupial, an insect, a primate, or a rodent. In other embodiments, an organism is a human or a non-human animal. In specific embodiments, an animal is a mouse, rat, cat, dog, horse, cow, rabbit, sheep, fruit fly, frog, worm, or human.

a. In vivo methods

In certain embodiments, the present invention provides methods of enhancing the survivability of biological materials, including, e.g., organisms (including mammals), that are subjected to ischemic or hypoxic conditions. In related embodiments, the present invention provides methods of preventing or reducing damage to biological materials, including, e.g., mammals, including cell or tissue injuries resulting from ischemia or hypoxia. It is understood that a whole biological material or only a portion thereof, e.g., a particular organ, may be subjected to ischemic or hypoxic conditions. However, in particular embodiments, the whole biological material may be subjected to ischemic conditions, for example, to assist in the preservation of an organism.

In particular embodiments, the ischemic or hypoxic conditions are the result of an injury or disease suffered by an organism. Accordingly, the present invention provides methods of enhancing survivability of an organism suffering from any disease or injury, including those described below, which methods comprise contacting the organism with an effective amount of thiomorpholine or a
thiomorpholine analogs. Examples of specific diseases that can induce ischemia or hypoxia include, but are not limited to, tumors, heart diseases, and neurological diseases. Examples of specific injuries that can result in ischemic or hypoxic conditions include, but are not limited to, external insults, such as burns, cutting wounds, amputations, gunshot wounds, or surgical trauma. In addition, injuries can also include internal insults, such as stroke or heart attack, which result in the acute reduction in circulation. Other injuries include reductions in circulation due to non-invasive stress, such as exposure to cold or radiation, or a planned reduction in circulation, e.g., during heart surgery. On a cellular level, such injuries often result in exposure of cells, tissues, and/or organs to hypoxic conditions, thereby resulting in induction of programmed cell death, or "apoptosis." Systemically, these injuries can lead to the induction of a series of biochemical processes, such as clotting, inflammation, hypotension, and may give rise to shock, which if it persists may lead to organ dysfunction, irreversible cell damage and death. In a specific scenario, where medical attention is not readily available, such contacting with thiomorpholine or a thiomorpholine analog, alternatively in conjunction with reduction in the temperature of the tissue, organ or organism, can "buy time" for the subject, either by bringing medical attention to the subject, or by transporting the subject to the medical attention.

The present invention also contemplates methods for inducing tissue regeneration and wound healing by prevention/delay of biological processes that may result in delayed wound healing and tissue regeneration. In this context, in scenarios in which there is a substantial wound to a limb or organism, the contacting with thiomorpholine or a thiomorpholine analog, in vivo or ex vivo, alone or in combination with another active compound or reduced oxygen conditions, alternatively in conjunction with reduction in the temperature of the tissue, organ or organism, aids in the wound healing and tissue regeneration process by managing the biological processes that inhibit healing and regeneration.

In certain embodiments, methods of the present invention can be implemented to enhance survivability and prevent ischemic injury resulting from cardiac arrest or stroke. Accordingly, in one embodiment, the present invention includes methods of enhancing survivability or reducing ischemic injury in a patient
suffering from or at risk of cardiac arrest or stroke, comprising providing an effective amount of thiomorpholine or a thiomorpholine analog to the patient before, after, or both before and after myocardial infarction, cardiac arrest or stroke.

In certain embodiments, methods of the present invention include pre-treating a biological material, e.g., a patient, prior to an ischemic or hypoxic injury or disease insult. These methods can be used when an injury or disease with the potential to cause ischemia or hypoxia is scheduled or elected in advance, or predicted in advance to likely occur. Examples of such situations include, but are not limited to, major surgery where blood loss may occur spontaneously or as a result of a procedure, cardiopulmonary bypass in which oxygenation of the blood may be compromised or in which vascular delivery of blood may be reduced (as in the setting of coronary artery bypass graft (CABG) surgery), or in the treatment of organ donors prior to removal of donor organs for transport and transplantation into a recipient in need of an organ transplant. Other examples include, but are not limited to, medical conditions in which a risk of injury or disease progression is inherent (e.g., in the context of unstable angina, following angioplasty, bleeding aneurysms, hemorrhagic strokes, following major trauma or blood loss), or in which the risk can be diagnosed using a medical diagnostic test. In one embodiment, the ischemia or hypoxia is not myocardial ischemia or hypoxia. In another embodiment, the ischemia or hypoxia is not due to myocardial infarction. In another embodiment, the biological material is not a myocyte or heart tissue.

In certain embodiments, exposure to thiomorpholine or a thiomorpholine analog enhances survivability or reduces damage when exposure occurs before the injury or disease insult. In other embodiments, exposure to thiomorpholine or a thiomorpholine analog, enhances survivability or reduces damage when exposure occurs after the onset or detection of the injurious or disease insult, and either before or after the injury or disease causes ischemia or hypoxia.

In certain embodiments, the present invention includes methods of enhancing survivability of a mammal undergoing a surgery. In a related embodiment, a method is provided for protecting a mammal from suffering ischemic injury or cellular damage resulting from a surgery. These methods comprise providing to the mammal an
effective amount of thiomorpholine or a thiomorpholine analog prior to, during, or both prior to and during the surgery. The surgery may be elective, planned, or emergency surgery, such as, e.g., cardiopulmonary surgery. The thiomorpholine or thiomorpholine analog may be administered by any means available in the art, including, e.g., intraarterially or intraperitoneally.

The invention has particular importance with respect to the risk of ischemic injury from emergency surgical procedures, such as thoractomy, laparotomy, and splenic transection. Therefore, it includes methods of enhancing survivability or reducing or preventing ischemic injury in a patient undergoing an emergency surgery, comprising providing an effective amount of thiomorpholine or a thiomorpholine analog, to the patient before surgery, after surgery, or both before and after surgery.

In another embodiment, the present invention includes a method of enhancing survivability of a mammal suffering from a disease or adverse medical condition that causes ischemia or hypoxia within a region of the mammal. A related embodiment includes a method of protecting a mammal from suffering ischemic injury or cellular damage from a disease or adverse medical condition. These methods typically comprise providing to the mammal an effective amount of thiomorpholine or a thiomorpholine analog, prior to, after, or both prior to and after, the onset of or progression of the disease or adverse medical condition. This embodiment may be used in the context of a variety of different diseases and adverse medical conditions, including, e.g., unstable angina, post-angioplasty, aneurysm, hemorrhagic stroke or shock, trauma, and blood loss.

In specific embodiments, the invention concerns methods of preventing an organism, such as a mammal, from bleeding to death or suffering irreversible tissue damage as a result of bleeding by providing to the mammal an effective amount of thiomorpholine or a thiomorpholine analog. In certain additional embodiments, the organism may go into hemorrhagic shock but not die from excessive bleeding. The terms "bleeding" and "hemorrhaging" are used interchangeably to refer to any discharge of blood from a blood vessel. It includes, but is not limited to, internal and external bleeding, bleeding from an injury (which may be from an internal source, or from an external physical source such as from a gunshot, stabbing, physical trauma, etc.).
Moreover, additional embodiments of the invention concern enhancing survivability and preventing irreversible tissue damage from blood loss or other lack of oxygenation to cells or tissue, such as from lack of an adequate blood supply. This may be the result of, for example, actual blood loss, or it may be from conditions or diseases that cause blockage of blood flow to cells or tissue, that reduce blood pressure locally or overall in an organism, that reduce the amount of oxygen is carried in the blood, or that reduces the number of oxygen carrying cells in the blood. Conditions and diseases that may be involved include, but are not limited to, blood clots and embolisms, cysts, growths, tumors, anemia (including sickle cell anemia), hemophilia, other blood clotting diseases \(e.g.,\) von Willebrand's Disease, ITP), and atherosclerosis. Such conditions and diseases also include those that create essentially hypoxic or anoxic conditions for cells or tissue in an organism because of an injury, disease, or condition.

In one embodiment, the present invention provides methods to enhance the survivability of and prevent injury or damage to biological material undergoing hemorrhagic shock, which include contacting the biological material subjected to shock with thiomorpholine or a thiomorpholine analog. In a certain embodiment, these methods are used to preserve a patient's vital organs and life. Hemorrhagic shock is a life-threatening condition in which inadequate perfusion to sustain the physiologic needs of organs or tissues occurs. The resulting inadequate oxygenation of tissues and organs can result in significant tissue and organ damage, and frequently death. Hemorrhagic shock may result from inadequate blood volume (hypovolemic shock), inadequate cardiac function (cardiogenic shock), or inadequate vasomotor tone, also referred to as distributive shock (neurogenic shock, septic shock, anaphylactic shock). Specific conditions associated with hemorrhagic shock include, \(e.g.,\) sepsis, blood loss, impaired autoregulation, and loss of autonomic tone, spontaneous hemorrhage \(e.g.,\) gastrointestinal bleeding, childbirth), surgery, and other causes. Most frequently, clinical hemorrhagic shock is caused by an acute bleeding episode with a discrete precipitating event. Less commonly, hemorrhagic shock may be seen in chronic conditions with subacute blood loss.

In certain embodiments, the present invention includes a method of contacting a patient suffering from an acute injury and at risk of or in a state of
hemorrhagic shock with an effective amount of thiomorpholine or a thiomorpholine analog, within one hour of the injury. This method allows for the patient to be transported to a controlled environment (e.g., surgery), where the initial cause of the shock can be addressed, and then the patient can be brought back to normal function in a controlled manner. For this indication, the first hour after injury, referred to as the "golden hour," is crucial to a successful outcome. Stabilizing the patient in this time period is the major goal, and transport to a critical care facility (e.g., emergency room, surgery,) where the injury can be properly addressed.

In certain embodiments, the present invention provides methods related to treating cancer and other hyperproliferative diseases. Cancer is a leading cause of mortality in industrialized countries around the world. The most conventional approach to the treatment of cancer is by administering a cytotoxic agent or cytotoxic agents to the cancer patient (or treatment ex vivo of a tissue) such that the agent or agents have a more lethal effect on the cancer cells than normal cells. The higher the dose or the more lethal the agent, the more effective it is in killing cancer cells. However, by the same token, such agents are all that more toxic (and sometimes lethal) to normal cells. Hence, chemo- and radiotherapy are often characterized by severe side effects, some of which are life threatening, e.g., sores in the mouth, difficulty swallowing, dry mouth, nausea, diarrhea, vomiting, fatigue, bleeding, hair loss and infection, skin irritation and loss of energy (Curran, 1998; Brizel, 1998).

In one embodiment, the present invention contemplates the use of thiomorpholine or a thiomorpholine analog to protect normal tissues of a patient being treated for cancer or another hyperproliferative disease, thereby reducing the potential impact of chemo- or radiotherapy on those tissues, and enhancing survivability of the patient. These methods permit the use of higher doses of chemo- and radiotherapy, thereby increasing the anti-cancer effects of these treatments. Recent studies suggest that transient and reversible lowering of the core body temperature, or "hypothermia," may lead to improvements in the fight against cancer. Hypothermia of 28°C was recently found to reduce radiation, doxorubicin-and cisplatin-induced toxicity in mice. The cancer fighting activity of these drugs/treatments was not compromised when
administered to cooled animals; rather, it was enhanced, particularly for cisplatin (Lundgren-Eriksson et al, 2001).

Treatment of virtually any hyperproliferative disorder, including benign and malignant neoplasias, non-neoplastic hyperproliferative conditions, pre-neoplastic conditions, and precancerous lesions, is contemplated. Such disorders include restenosis, cancer, multi-drug resistant cancer, primary psoriasis and metastatic tumors, angiogenesis, rheumatoid arthritis, inflammatory bowel disease, psoriasis, eczema, and secondary cataracts, as well as oral hairy leukoplasia, bronchial dysplasia, carcinomas in situ, and intraepithelial hyperplasia. In particular, the present invention is directed at the treatment of human cancers including cancers of the prostate, lung, brain, skin, liver, breast, lymphoid system, stomach, testicles, ovaries, pancreas, bone, bone marrow, gastro intestine, head and neck, cervix, esophagus, eye, gall bladder, kidney, adrenal glands, heart, colon and blood. Cancers involving epithelial and endothelial cells are also contemplated for treatment.

In certain embodiments, thiomorpholine or a thiomorpholine analog, is provided to a patient suffering from cancer or another hyperproliferative disease or condition in combination with one or more anti-proliferative agents effective in the treatment of hyperproliferative disease. An anti-proliferative agent is an agent capable of negatively affecting cell growth in a subject, for example, by killing cancer cells, inducing apoptosis in cancer cells, reducing the growth rate of cancer cells, reducing the incidence or number of metastases, reducing tumor size, inhibiting tumor growth, reducing the blood supply to a tumor or cancer cells, promoting an immune response against cancer cells or a tumor, preventing or inhibiting the progression of cancer, or increasing the lifespan of a subject with cancer.

In specific embodiments of the present invention, a patient suffering from cancer or another hyperproliferative disease is contacted with thiomorpholine or a thiomorpholine analog, in combination with one or more anticancer agents. In particular embodiments, thiomorpholine or the thiomorpholine analog enhances survivability of the patient suffering from cancer or other hyperproliferative disease, while in other embodiments, thiomorpholine or the thiomorpholine analog protects the patient from ischemic injury caused by the one or more anticancer agents. In particular
embodiments, the use of thiomorpholine or a thiomorpholine analog allows the patient to be exposed to greater amounts of the anticancer treatment, so one embodiment includes contacting a patient with thiomorpholine or a thiomorpholine analog in combination with a higher dose of anticancer agent or a longer duration of contact with the anticancer agent as compared to the amount routinely used or considered safe in the absence of thiomorpholine or the thiomorpholine analog.

Anticancer agents include, but are not limited to, biological agents (biotherapy), chemotherapy agents, and radiotherapy agents. In one embodiment, methods of the present invention involve contacting or exposing a patient (or their cells) with thiomorpholine or a thiomorpholine analog and the anticancer agent(s) at the same time. This may be achieved by contacting the patient or cells with a single composition or pharmacological formulation that includes both agents, or by contacting or exposing the patient or cells with two distinct compositions or formulations, at the same time, wherein one composition includes thiomorpholine or a thiomorpholine analog and the other includes the anticancer agent(s). Alternatively, treatment with thiomorpholine or a thiomorpholine analog may precede or follow the anticancer agent treatment by intervals ranging from minutes to weeks. In embodiments where thiomorpholine or a thiomorpholine analog and the anticancer agent are applied separately to the patient or cells, one would generally ensure that a significant period of time did not expire between the time of each delivery, such that thiomorpholine or the thiomorpholine analog and the secondary agent would still be able to exert an advantageously combined effect on the patient or cells. In such instances, it is contemplated that one may contact the patient or cells with both modalities within about 12-24 hrs of each other and, more preferably, within about 6-12 hrs of each other. In some situations, it may be desirable to extend the time period for treatment significantly, however, where several days (2, 3, 4, 5, 6 or 7) to several weeks (1, 2, 3, 4, 5, 6, 7 or 8) lapse between the respective administrations. In some embodiments of the invention, biological matter is exposed to thiomorpholine or a thiomorpholine analog for about, at least, at least about, or at most about 30 seconds, 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 minutes, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, hours or more, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, hours or more.
4, 5, 6, 7, days, 1, 2, 3, 4, 5 weeks, and/or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 months, or any range derivable therein or combination therein.

Administration of thiomorpholine or a thiomorpholine analog and chemotherapeutics to a patient will follow general protocols for the administration of chemotherapeutics, taking into account the toxicity, if any, of the compound. It is expected that the treatment cycles would be repeated as necessary. It also is contemplated that various standard therapies, as well as surgical intervention, may be applied in combination with the above-described anti-cancer therapy. It is further contemplated that any combination treatment contemplated for use with thiomorpholine or a thiomorpholine derivative and a non-active compound (such as chemotherapy), may be applied with respect to thiomorpholine, thiomorpholine derivatives or multiple active compounds (as described below).

Chemotherapeutic agents that may be used in combination with thiomorpholine or a thiomorpholine analog according to methods of the present invention include, for example, cisplatin (CDDP), carboplatin, procarbazine, mechlorethamine, cyclophosphamide, camptothecin, ifosfamide, melphalan, chlorambucil, busulfan, nitrosourea, dactinomycin, daunorubicin, doxorubicin, bleomycin, plicomycin, mitomycin, etoposide (VP 16), tamoxifen, raloxifene, estrogen receptor binding agents, taxol, gemcitabine, navelbine, farnesyl-protein transferase inhibitors, transplatinum, 5-fluorouracil, vincristine, vinblastine and methotrexate, Temazolomide (an aqueous form of DTIC), or any analog or derivative variant of the foregoing.

The methods of the present invention may also be practiced using a combination of thiomorpholine or a thiomorpholine analog and radiotherapy. Examples of radiotherapy that have been used extensively include what are commonly known as γ-rays, X-rays, and/or the directed delivery of radioisotopes to tumor cells. Other forms of DNA damaging factors are also contemplated such as microwaves and UV-irradiation. It is most likely that all of these factors affect a broad range of damage on DNA, on the precursors of DNA, on the replication and repair of DNA, and on the assembly and maintenance of chromosomes. Dosage ranges for X-rays range from daily doses of 50 to 200 roentgens for prolonged periods of time (3 to 4 wk), to single doses
of 2000 to 6000 roentgens. Dosage ranges for radioisotopes vary widely, and depend on the half-life of the isotope, the strength and type of radiation emitted, and the uptake by the neoplastic cells.

Methods of the invention further include contacting a patient with thiomorpholine or a thiomorpholine analog in combination with an immunotherapeutic agent. Immunotherapeutics, generally, rely on the use of immune effector cells and molecules to target and destroy cancer cells. The immune effector may be, for example, an antibody specific for some marker on the surface of a tumor cell. The antibody alone may serve as an effector of therapy or it may recruit other cells to actually effect cell killing. The antibody also may be conjugated to a drug or toxin (chemotherapeutic, radionuclide, ricin A chain, cholera toxin, pertussis toxin, etc.) and serve merely as a targeting agent. Alternatively, the effector may be a lymphocyte carrying a surface molecule that interacts, either directly or indirectly, with a tumor cell target. Various effector cells include cytotoxic T cells and NK cells. In one aspect of immunotherapy, the tumor cell bears some marker that is amenable to targeting, i.e., is not present on the majority of other cells. Many tumor markers exist and any of these may be suitable for targeting in the context of the present invention. Common tumor markers include carcinoembryonic antigen, prostate specific antigen, urinary tumor associated antigen, fetal antigen, tyrosinase (p97), gp68, TAG-72, HMFG, Sialyl Lewis Antigen, MucA, MucB, PLAP, estrogen receptor, laminin receptor, erb B and p155. An alternative aspect of immunotherapy is to anticancer effects with immune stimulatory effects. Immune stimulating molecules also exist including: cytokines such as IL-2, IL-4, IL-12, GM-CSF, gamma-IFN, chemokines such as MIP-I, MCP-I, IL-8 and growth factors such as FLT3 ligand. Combining immune stimulating molecules, either as proteins or using gene delivery in combination with a tumor suppressor such as mda-7 has been shown to enhance anti-tumor effects (Ju et al., 2000)

Immunotherapies contemplated by the present invention also include, but are not limited to, immune adjuvants (e.g., Mycobacterium bovis, Plasmodium falciparum, dinitrochlorobenzene and aromatic compounds) (U.S. Patent 5,801,005; U.S. Patent 5,739,169; Hui and Hashimoto, 1998; Christodoulides et al., 1998), cytokine therapy (e.g., interferons α, β and γ; IL-1, GM-CSF and TNF) (Bukowski et
gene therapy (e.g., TNF, IL-I, IL-2, p53) (Qin et al., 1998; Austin-Ward and Villaseca, 1998; U.S. Patent 5,830,880 and U.S. Patent 5,846,945) and monoclonal antibodies (e.g., anti-ganglioside GM2, anti-HER-2, anti-pl85) (Pietras et al., 1998; Hanibuchi et al., 1998). Herceptin (trastuzumab) is a chimeric (mouse-human) monoclonal antibody that blocks the HER2-neu receptor. It possesses anti-tumor activity and has been approved for use in the treatment of malignant tumors (Dillman, 1999). Combination therapy of cancer with herceptin and chemotherapy has been shown to be more effective than the individual therapies. Thus, it is contemplated that one or more anti-cancer therapies may be employed with the anti-tumor therapies described herein.

The methods of the present invention may be used in the treatment of neurodegenerative diseases associated with ischemia or hypoxia. Neurodegenerative diseases are characterized by degeneration of neuronal tissue, and are often accompanied by loss of memory, loss of motor function, and dementia. With dementia, intellectual and higher integrative cognitive faculties become more and more impaired over time. It is estimated that approximately 15% of people 65 years or older are mildly to moderately demented. Neurodegenerative diseases include Parkinson's disease; primary neurodegenerative disease; Huntington's Chorea; stroke and other hypoxic or ischemic processes; neurotrauma; metabolically induced neurological damage; sequelae from cerebral seizures; hemorrhagic shock; secondary neurodegenerative disease (metabolic or toxic); Alzheimer's disease and other memory disorders; or vascular dementia, multi-infarct dementia, Lewy body dementia, or neurodegenerative dementia. The present invention provides methods of preventing tissue damage from neurological diseases associated with ischemia, comprising administering thiomorpholine or a thiomorpholine analog to a patient suffering from such a disease or condition.

In yet another embodiment, the methods of the present invention are used to treat a mammal with extreme hypothermia. The methods and compositions of the present invention are useful for enhancing survivability of an organism subjected to extreme hypothermia. In one embodiment, these methods include enhancing survivability of an organism by inducing mild hypothermia in the organism in combination with contacting the organism with thiomorpholine or a thiomorpholine
analog. Hypothermia can be mild, moderate or profound. Mild hypothermia comprises achievement of a core body temperature of approximately between 0.1 and 5 degrees Celsius below the normal core body temperature of the mammal. The normal core body temperature of a mammal is usually between 35 and 38 degrees Celsius. Moderate hypothermia comprises achievement of a core body temperature of approximately between 5 and 15 degrees Celsius below the normal core body temperature of the mammal. Profound hypothermia comprises achievement of a core body temperature of approximately between 15 and 37 degrees Celsius below the normal core body temperature of the mammal.

Mild hypothermia is known in the art to be therapeutically useful and effective in both non-human mammals and in humans. The therapeutic benefit of mild hypothermia has been observed in human clinical trials in the context of out-of-hospital cardiac arrest. Exposure of humans to mild hypothermia in the context of cardiac arrest results in a survival advantage and an improved neurological outcome compared to standard of care with normothermia, or absence of mild hypothermia (Bernard et al., 2002; The Hypothermia After Cardiac Arrest Study Group et al. 2002).

In one embodiment, a method of the present invention provides that patients with extreme hypothermia are administered or exposed to thiomorpholine or a thiomorpholine analog and then gradually restored to normal temperature while withdrawing, in a controlled fashion, the thiomorpholine or thiomorpholine analog. In this way, thiomorpholine or the thiomorpholine analog buffers the biological systems within the subject so that they may be initiated gradually without shock (or harm) to the subject. Ideally, the patient will be stabilized in terms of heart rate, respiration and temperature prior to effecting any change. Once stable, the ambient environmental temperature will be increased, again gradually. This may be accomplished simply by removing the subject from the hypothermic conditions. A more regulated increase in temperature may be affected by adding successive layers of clothing or blankets, by use of a thermal wrap with gradual increase in heat, or if possible, by placing the subject in chamber whose temperature may be gradually increased.

The vital signs of the subject may be monitored over the course of the temperature increase. Also, in conjunction with increasing the temperature,
thiomorpholine or the thiomorpholine analog is removed from the subject's environment. Both heat and thiomorpholine or thiomorpholine analog treatment are ceased at the appropriate endpoint, judged by the medical personnel monitoring the situation, but in any event at the time the subject's temperature and other vital signs return to a normal range. Continued monitoring following cessation of treatment is recommended for a period of at least 24 hrs.

Methods and compositions of the present invention have advantages over other methods known in the art, including, but not limited to, packing the subject in ice, or surrounding the subject with a "cooling tent" that circulates cool air or liquid, for inducing mild, moderate, or profound hypothermia in mammals or humans. In these cases, the subject resists the reduction of core body temperature below normothermia and tries to generate heat by shivering. Shivering, and the body heat engendered therein, can have a negative impact on the achievement of mild hypothermia by, for example, slowing the rate of decrease in the core body temperature that is achieved using the standard methods of hypothermia induction. Consequently, humans subjected to therapeutic levels of hypothermia are also treated with a drug that inhibits shivering (by blocking neurotransmission at the neuromuscular junctions) (Bernard et al, 2002).

In certain embodiments, methods and compositions of the present invention are combined with invasive methods or medical devices known in the art to induce therapeutic hypothermia in mammals or humans. Such invasive methods and devices include, but are not limited to, flexible probes or catheters that can be inserted into the vasculature of the subject in need of hypothermia, wherein the temperature of the catheter is adjusted to below the normal body temperature of the subject, resulting in the cooling of blood which is in contact with the catheter. The cooled blood subsequently engenders a decrease in the core body temperature of the mammal. By incorporating feedback from a thermocouple monitoring the core body temperature of the mammal, the temperature of the catheter can be modulated so as to maintain a pre-specified core body temperature. Such medical devices for achieving and maintaining mild or moderate hypothermia, referred to in the art as endovascular temperature therapy, are known in the art and are described for example on the World Wide Web at innercool.com and radiantmedical.com.
In other embodiments, the methods of the present invention are used to treat hyperthermia. Under certain conditions, which can result from genetic, infectious, drug, or environmental causes, patients can loose homeostatic temperature regulation resulting in severe uncontrollable fever (hyperthermia). This can result in mortality or long-term morbidity, especially brain damage, if it is not controlled properly. The present invention provides methods of treating hyperthermia that involve contacting the patient with thiomorpholine or a thiomorpholine analog to induce reduced metabolic activity and enhance survivability or reduce injury to potentially affected brain tissue. In particular embodiments, the patient is contacted for between about 6 and about 24 hours, during which time the source of the fever can be addressed. This treatment can be combined with whole-body temperature regulation, such as an ice bath/blanket/cooling system.

b. Ex vivo methods

In certain embodiments, the methods of the present invention are used to enhance the survivability of ex vivo biological matter subjected to hypoxic or ischemic conditions, including, e.g., isolated cells, tissues and organs. Specific examples of such ex vivo biological material include platelets and other blood products, as well as tissues and organs to be transplanted.

In one embodiment, methods of the present invention may be used to enhance survivability of biological material in the laboratory or research context, for example when cell lines or laboratory organisms are purposefully subjected to hypoxic or ischemic conditions, e.g., during cryopreservation and storage.

The present invention can be extended to protecting cells in culture, which might otherwise die or be induced into apoptosis. According to the present invention, cells are exposed to thiomorpholine or a thiomorpholine analog prior to and/or while in culture. Cells that can be cultured according to the invention include those that can eventually be placed back into a physiological context, i.e., those for subsequent transplant. Such cells include, but are not limited to, bone marrow cells, skin cells, stem cells, and epithelial cells. Also, some transplantable cells would greatly benefit from expansion in culture, thereby increasing the amount of material that can be
introduced into the host. In one particular embodiment, the methods of the present invention are applied to epithelial cells from the gastrointestinal tract.

Furthermore, the invention extends to the culture of tumor cells. Culture of tumor cells is known to result in alteration of the phenotype and, in some cases, death. This makes tissue culture experiments on tumor cells highly unpredictable.

General cell culture techniques are well known to those of skill in the art. Examples of this knowledge can be found in Shaw (1996) and Davis (1994), both of which are incorporated by reference herein. General information and modifications of traditional cell culture techniques is also found in U.S. Patent 5,580,781, which is incorporated by reference. Furthermore, techniques for culturing skin cells are described in U.S. Patent 6,057,148, which is incorporated by reference. It is contemplated that these techniques, as well as others known to those of skill in the art, will be supplemented with media containing thiomorpholine or a thiomorpholine analog.

The invention also provides methods of enhancing the survivability of, or preserving, tissues and organs for transplant, which comprise contacting the tissue or organ with thiomorpholine or a thiomorpholine analog. Initial contact can occur prior to removal from a donor or following removal from a donor. While there is a constant need for organ donors, a significant hurdle in providing those in need of an organ transplant with an organ is the limitations in current organ preservation techniques. Indeed, the primary cause of organ transplant failure for transplanted hearts in the first 30 days is ischemic-reperfusion injury. It is widely believed that a human heart must be transported within four hours for there to be any chance of the subsequent transplantation to be a success. Similarly, the maximum cold ischemic time allowed for liver is 12-24 hours, kidney is 48-72 hours, pancreas is 12-24 hours, and small intestine is 12 hours (Rager, 2004). Tissues useful for transplant include, but are not limited to, skin tissue. Organs useful for transplant include, but are not limited to, hearts, lungs, kidneys, livers, pancreas, small intestine, and cornea.

Currently, preserving solid organs depends on rapid intravascular cooling done in situ, followed by removal of the organs, storage of the organs in ice-cold preservation fluid and rapid transport to the recipients' hospitals. The cold ischemic
time is the length of time the organs are on ice, without blood flow. The maximum cold ischemic time limits the amount of time that can pass between organ recovery and the organ transplant. Between 2%-10% of matched and procured organs cannot be used due to extended ischemic time, depending on the type of organ. Similarly, approximately 10 to 20% of procured organs are not used due to poor organ function and/or infection (not including HIV/CMV/hepatitis).

Current preservation techniques involve the use of ice-cold solutions that include electrolytes, antioxidants, hydrogen ion buffers and sugars (Punch et al, 2001). Appropriate tissue matching depends on blood group matching (e.g., blood type, A, B or O) for all organs. Immunosuppressive regimens typically include three drugs: a glucocorticoid such as prednisone, an antimetabolite such as azathioprine or mycophenolate, and a calcineurin inhibitor such as cyclosporine or tacrolimus.

The two most frequently used methods for preserving/transporting hearts for transplantation are hypothermic storage and continuous perfusion. In the former method, the heart is arrested, removed from the donor, and then rapidly cooled and transported in cold storage. In the latter method, the following steps are typically employed: 1) pulsatile flow; 2) hypothermia; 3) membrane oxygenation, and 4) a perfusate containing both.

The methods of the present invention may be used to increase the survivability of donor tissues and organs, thereby extending the time before the donor tissue must be transplanted into a recipient and blood flow restored. These methods may be combined with current preservation methods, including the use of preservation agents and oxygen perfusion. A variety of preservation solutions have been disclosed in which the organ is surrounded or perfused with the preservation solution while it is transported. One of the most commonly used solution is ViaSpan® (Belzer UW), which can be combined with cold storage. Other examples of such solutions or components of such solutions include the St. Thomas solution (Ledingham et al. 1987), Broussais solution, UW solution (Ledingham et al. 1990), Celsior solution (Menasche et al. 1994), Stanford University solution, and solution B20 (Bernard et al. 1985), as well as those described and/or claimed in U.S. Patents 6,524,785; 6,492,103; 6,365,338; 6,054,261; 5,719,174; 5,693,462; 5,599,659; 5,552,267; 5,405,742; 5,370,989;
5,066,578; 4,938,961; and, 4,798,824. In addition to solutions, other types of materials are also known for use in transporting organs and tissue. These include gelatinous or other semi-solid material, such as those described, for example, in U.S. Patent 5,736,397.

Some of the systems and solutions for organ preservation specifically involve oxygen perfusion in the solution or system to expose the organ to oxygen, because it is believed that maintaining the organ or tissue in an oxygenated environment improves viability. See Kuroda et al., (Transplantation 46(3):457-460, 1988) and U.S. Patents 6,490,880; 6,046,046; 5,476,763; 5,285,657; 3,995,444; 3,881,990; and, 3,777,507. A variety of systems and containers for transporting organs and tissues have been developed, which provide cooling and/or oxygen perfusion. These may be employed in combination with contacting the tissue or organ with thiomorpholine or a thiomorpholine analog according to the present invention. Specific apparatuses include, for example, cooling systems described in U.S. Patents 4,292,817, 4,473,637, 4,745,759, 5,434,045 and 4,723,974. Others constitute a system in which an apparatus is devised for perfusion of the organ or tissue in a preservation solution, as is described in U.S. Patents 6,490,880; 6,100,082; 6,046,046; 5,326,706; 5,285,657; 5,157,930; 4,951,482; 4,502,295; and, 4,186,565.

In certain embodiments, the present invention provides methods to enhance survivability of platelets. Platelets are small cell fragments (~1/3 size of erythrocytes) that play a vital role in the formation of blood clots at the site of bleeding. Platelet concentrates are transfused for a variety of indications, for example: 1) to prevent bleeding due to thrombocytopenia; 2) in a bleeding patient to maintain a platelet count above 50,000; 3) to address abnormal platelet function that is congenital or due to medications, sepsis, malignancy, tissue trauma, obstetrical complications, extra corporeal circulation, or organ failure such as liver or kidney disease.

Each unit of platelets contains an average of 0.8-0.85 x 10^11 platelets. Platelet concentrates also contain about 60mL of plasma (coagulation factors) and small numbers of red blood cells and leukocytes. Platelet units must be maintained at room temperature (20°C-24°C) and agitated during storage. They can be stored at the Blood Center for up to 5 days. Longer storage is not possible at present due to deterioration of
the platelets, and the risk of microbial contamination. Two sources of platelets currently exist: (1) pooled random donor platelet concentrates prepared from platelets that have been harvested by centrifuging units of whole blood; and (2) apheresis platelets, collected from a single donor, prepared in standard (equivalent to ~4 pooled units) and "large" (equivalent to ~6 pooled units) sizes.

Platelet storage poses problems that are not found with the storage of whole blood or other components. While whole blood, red and white cells may be stored at 4°C for weeks, platelets will aggregate in cold storage and when allowed to settle. Therefore, the standard method of storing platelets is at room temperature, approximately 20 to 24°C, with gentle agitation. Even under these conditions, platelets can only be stored for 5 days before they need to be discarded. This problem of outdating platelets results in approximately $500 million annually in lost revenue for US hospitals. If even a moderate increase in shelf life could be attained, approximately 90% of this loss could be avoided.

An additional problem with platelet storage is bacterial contamination. Contamination is primarily due to staphylococci from the skin during the phlebotomy, or else donor bacteremia. The bacterial contamination of platelets represents the largest infectious risk with any blood transfusion procedure.

A significant factor affecting the viability of platelets is regulation of pH. Virtually all units of platelets stored according to the currently accepted methods show a decrease in pH from their initial value of approximately 7.0. This decrease is primarily due to the production of lactic acid by platelet glycolysis and to a lesser extent to accumulation of CO₂ from oxidative phosphorylation. As the pH falls, the platelets change shape from discs to spheres. If the pH falls below 6.0, irreversible changes in platelet morphology and physiology render them non-viable after transfusion. An important goal in platelet preservation, therefore, is to prevent this decrease in pH. It was previously thought that platelets must be stored in a container permeable to oxygen since glycolysis is stimulated when oxygen availability is limited (see e.g., U.S. Patent 5,569,579). However, it has more recently been demonstrated that the viability of stored platelets can be extended by storing them in an anoxic environment.
The present invention provides methods of enhancing survivability of platelets, including, in particular embodiments, platelets stored in an anoxic environment, comprising contacting the platelets with an effective amount of thiomorpholine or a thiomorpholine analog during storage.

In various embodiments of the methods of the present invention, including those specifically exemplified above, biological material is exposed to thiomorpholine or a thiomorpholine analog once or more than one time. In certain embodiments, biological matter is exposed to thiomorpholine or a thiomorpholine analog 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more times, meaning when a biological matter is exposed multiple times that there are periods of respite (with respect to exposure to the active compound) in between.

It is also contemplated that thiomorpholine or a thiomorpholine analog may be administered before, during, after, or any combination thereof, in relation to the onset or progression of an injurious insult or disease condition. In certain embodiments, pre-treatment of biological matter with thiomorpholine or a thiomorpholine analog is sufficient to enhance survivability and/or reduce damage from an injurious or disease insult. Pre-treatment is defined as exposure of the biological matter to thiomorpholine or a thiomorpholine analog before the onset or detection of the injurious or disease insult. Pre-treatment can be followed by termination of exposure at or near the onset of the insult or continued exposure after the onset of insult.

In various embodiments, the present invention comprises contacting living biological matter with an effective amount of thiomorpholine or a thiomorpholine analog. As previously noted, the term "effective amount" means an amount that can achieve the stated result. In certain methods of the present invention, an "effective amount" is, for example, an amount that enhances the survivability of biological matter in response to ischemic or hypoxic conditions, or an amount that protects biological material from injury due to ischemic or hypoxic conditions.

In some embodiments, an effective amount is characterized as a sublethal dose. In the context of cells, tissues, or organs (not the whole organism), a "sublethal dose" means a single administration that is less than half of the amount that would cause at least a majority of cells in a biological matter to die within 24 hours of the
administration. In the context of the entire organism, a "sublethal dose" means a single administration that is less than half of the amount that would cause the organism to die within 24 hours of the administration. In other embodiments, an effective amount is characterized as a near-lethal dose. Similarly, in the context of cells, tissues, or organs (not the whole organism), a "near lethal dose" means a single administration that is within 25% of the amount that would cause at least a majority of cell(s) to die within 24 hours of the administration. If treatment of the entire organism is desired, a "near lethal dose" means a single administration that is within 25% of the amount that would cause the organism to die within 24 hours of the administration. In some embodiments a sublethal dose is administered by administering a predetermined amount of thiomorpholine or a thiomorpholine analog.

Furthermore, it is contemplated that in some embodiments an effective amount is characterized as a supralethal dose of thiomorpholine or a thiomorpholine analog. In the context of cells, tissues, or organs (not the whole organism), a "supra-lethal dose" means a single administration that is at least 1.5 times (1.5x) the amount that would cause at least a majority of cells in a biological matter to die within 24 hours of the administration. If treatment of the entire organism is desired, then a "supra-lethal dose" means a single administration that is at least 1.5 times the amount that would cause the organism to die within 24 hours of the administration. It is specifically contemplated that the supra-lethal dose can be about, at least about, or at most about 1.5x, 2x, 3x, 4x, 5x, 10x, 20x, 30x, 40x, 50x, 60x, 70x, 80x, 90x, 100x, 150x, 200x, 250x, 300x, 400x, 500x, 600x, 700x, 800x, 900x, 1000x, 1100x, 1200x, 1300x, 1400x, 1500x, 1600x, 1700x, 1800x, 1900x, 2000x, 3000x, 4000x, 5000x, 6000x, 7000x, 8000x, 9000x, 10,000x or more, or any range derivable therein, the amount that would cause at least a majority of cells in a biological matter (or the entire organism) to die within 24 hours of the administration.

The amount of thiomorpholine or a thiomorpholine analog that is provided to biological material can be about, at least, at least about, or at most about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73,
74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96,
97, 98, 99, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240,
250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410,
420, 430, 440, 441, 450, 460, 470, 480, 490, 500, 510, 520, 530, 540, 550, 560, 570,
580, 590, 600, 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740,
750, 760, 770, 780, 790, 800, 810, 820, 830, 840, 850, 860, 870, 880, 890, 900, 910,
920, 930, 940, 950, 960, 970, 980, 990, 1000 mg, mg/kg, or mg/m², or any range
derivable therein. Alternatively, the amount may be expressed as 1, 2, 3, 4, 5, 6, 7, 8, 9,
10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32,
33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55,
56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78,
79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 110,
120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280,
290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 441,
450, 460, 470, 480, 490, 500, 510, 520, 530, 540, 550, 560, 570, 580, 590, 600, 610,
620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780,
790, 800, 810, 820, 830, 840, 850, 860, 870, 880, 890, 900, 910, 920, 930, 940, 950,
960, 970, 980, 990, 1000 nM or M, or any range derivable therein.

Furthermore, when administration is intravenous, it is contemplated that

the following parameters may be applied. A flow rate of about, at least about, or at most
about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25,
26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48,
49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71,
72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94,
95, 96, 97, 98, 99, 100 gtt/min or μgtt/min, or any range derivable therein. In some
embodiments, the amount of the solution is specified by volume, depending on the
concentration of the thiomorpholine or thiomorpholine analog.

In various embodiments of the present invention, biological material is
exposed to thiomorpholine or a thiomorpholine analog for about, at least, at least about,
or at most about 30 seconds, 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 minutes,
1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 hours, 1,
2, 3, 4, 5, 6, 7 days, 1, 2, 3, 4, 5 weeks, and/or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 months, or any range derivable therein or combination therein.

Volumes of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 441, 450, 460, 470, 480, 490, 500, 510, 520, 530, 540, 550, 560, 570, 580, 590, 600, 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, 810, 820, 830, 840, 850, 860, 870, 880, 890, 900, 910, 920, 930, 940, 950, 960, 970, 980, 990, 1000 mls or liters, or any range therein, may be administered overall or in a single session.

In some embodiments, an effective amount is administered by monitoring, alone or in combination, the amount of thiomorpholine or a thiomorpholine analog administered, monitoring the duration of administration, monitoring a physiological response (e.g., pulse, respiration, pain response, movement or motility, metabolic parameters such as cellular energy production or redox state, etc?) of the biological material to the administration of the thiomorpholine or thiomorpholine analog, and reducing, interrupting or ceasing administration of the thiomorpholine or thiomorpholine analog when a predetermined floor or ceiling for a change in that response is measured. Moreover, these steps can be employed additionally in any method of the invention.

The term "expose" is used according to its ordinary meaning to indicate that biological matter is subjected to thiomorpholine or a thiomorpholine analog. In certain embodiments, this is achieved by contacting the biological matter with thiomorpholine or a thiomorpholine analog. In the case of in vivo cells, tissues, or organs, "expose" may further mean "to lay open" these materials so that it can be contacted with thiomorpholine or a thiomorpholine analog. This can be done, for example, surgically. Exposing biological matter to thiomorpholine or a thiomorpholine analog can be by incubation in or with (includes immersion), perfusion or infusion,
injection of biological matter, or applying thiomorpholine or a thiomorpholine analog to the biological matter. In addition, if treatment of an entire organism is desirable, inhalation or ingestion of thiomorpholine or a thiomorpholine analog, or any route of systemic administration is contemplated. Furthermore, the term "provide" is used according to its ordinary and plain meaning to mean "to supply." It is contemplated that thiomorpholine or a thiomorpholine analog may be provided to biological matter in one form and be converted by chemical reaction to its form as an active compound. The term "provide" encompasses the term "expose" in the context of the term "effective amount" according to the present invention.

The present invention also provides methods and compositions for preserving both non-living biological material and preserving or extending the shelf-life of non-biological material. These methods comprise contacting the non-living biological material or non-biological material with thiomorpholine or a thiomorpholine analog. In certain embodiments, the non-living biological material or non-biological material is contacted with thiomorpholine or a thiomorpholine analog prior to storage, while in other embodiments, the non-living biological material is contacted with thiomorpholine or a thiomorpholine analog during storage. In these embodiments of the present invention, an effective amount is considered an amount sufficient to preserve a non-living biological material for a duration of time at least 50% or at least 100% longer than in the absence of contacting the non-living biological material with thiomorpholine or a thiomorpholine analog. An effective amount may also be defined as an amount sufficient to reduce the degradation rate of a non-living biological material by at least 25% or 50% as compared to the rate of degradation of the biological material when not contacted with thiomorpholine or a thiomorpholine analog.

Methods of the present invention may be combined with other preservation methods available in the art, including methods such as storing a non-living biological material or non-biological material at low temperature conditions, contacting the non-living biological material or non-biological material with preservatives or embalming fluids, and contacting the non-living biological material or non-biological material with antibacterial agents.
In the context of non-living biological material, the methods of the present invention are useful in a variety of contexts. For example, they may be used to preserve a dead animal, *e.g.*, a cadaver, prior to burial, cremation, dissection, or taxidermy. The present invention, therefore, provides a method of preserving a dead animal comprising contacting the dead animal with thiomorpholine or a thiomorpholine analog. This method may be accomplished in a variety of manners, including, *e.g.*, adding thiomorpholine or a thiomorpholine analog to embalming fluid, immersing the dead animal in a solution comprising thiomorpholine or a thiomorpholine analog, or infusing the dead animal with a solution comprising thiomorpholine or a thiomorpholine analog.

These methods may also be used to preserve non-living biological material for scientific or investigative purposes, *e.g.*, when the non-living biological material is a biological sample to be tested for disease or infection or used to obtain blood type or DNA sequence information, *e.g.*, for forensic or identification purposes.

Accordingly, in one embodiment, the present invention provides a method of preserving a non-living biological sample comprising contacting the sample with or storing the sample in the presence of thiomorpholine or a thiomorpholine analog.

Compositions and method of the present invention may also be used to preserve or increase the shelf-life of other products subject to spoilage or bacterial contamination, including, *e.g.*, food products, beverage products, pharmaceutical products, health care products, and cosmetic products. These methods comprise contacting the product with thiomorpholine or a thiomorpholine analog, *e.g.*, before packaging, or formulating the product.

The amount of thiomorpholine, or thiomorpholine analog that is provided to non-living biological material or non-biological material can be about, at least, at least about, or at most about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330,
340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 441, 450, 460, 470, 480, 490, 500, 510, 520, 530, 540, 550, 560, 570, 580, 590, 600, 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, 810, 820, 830, 840, 850, 860, 870, 880, 890, 900, 910, 920, 930, 940, 950, 960, 970, 980, 990, 1000 mg, mg/kg, or mg/m², or any range derivable therein. Alternatively, the amount may be expressed as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 441, 450, 460, 470, 480, 490, 500, 510, 520, 530, 540, 550, 560, 570, 580, 590, 600, 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, 810, 820, 830, 840, 850, 860, 870, 880, 890, 900, 910, 920, 930, 940, 950, 960, 970, 980, 990, 1000 mM or M, or any range derivable therein.

In various embodiments of the present invention, non-living biological material or non-biological material is exposed for about, at least, at least about, or at most about 30 seconds, 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 minutes, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 hours, 1, 2, 3, 4, 5, 6, 7 days, 1, 2, 3, 4, 5 weeks, and/or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 months, or any range derivable therein or combination thereof.

In particular embodiments, exposure continues for the duration of the shelf-life of the material, and may be greater than one month, six months, one year, or even two years.

Combination Therapies

In certain embodiments, methods of the present invention comprise contacting biological material with a combination of thiomorpholine and a thiomorpholine analog. In other related embodiments, methods of the present invention comprise contacting biological material with thiomorpholine and/or a
thiomorpholine analog in combination with another treatment that reduces metabolic activity. Examples of such other treatments include, but are not limited to, reduced oxygen conditions and reduced temperature conditions.

In other embodiments, methods of the present invention comprise contacting biological material with a combination of thiomorpholine or thiomorpholine analogs and one or more additional active compounds. In other embodiments, methods of the present invention comprise contacting non-living biological material or non-biological material with a combination of thiomorpholine or thiomorpholine analogs and one or more additional active compounds.

As used herein, an "active compound" is a compound that can act on biological material to produce any of a number of effects, including, but not limited to, enhancing or increasing survivability under hypoxic or ischemic conditions, preventing cell or tissue damage due to ischemia or hypoxia, inducing a hypometabolic state, and/or achieving any of the therapeutic applications discussed herein. A variety of active compounds are described, e.g., in U.S. Serial Nos. 60/673,037; 60/673,295; 60/713,073; 60/731,549; 60/762,462; 60/781,036; 60/783,450; and 60/849,900.

In some embodiments, an active compound is an oxygen antagonist, which may act directly or indirectly. Oxygen metabolism is a fundamental requirement for life in aerobic metazoans. Aerobic respiration accounts for the vast majority of energy production in most animals and also serves to maintain the redox potential necessary to carry out important cellular reactions. In hypoxia, decreased oxygen availability results in inefficient transfer of electrons to molecular oxygen in the final step of the electron transport chain. This inefficiency results in both a decrease in aerobic energy production and an increase in the production of damaging free radicals, mainly due to the premature release of electrons at complex III and the formation of O$_2^-$ by cytochrome oxidase (Semenza, 1999). Limited energy supplies and free radical damage can interfere with essential cellular processes such as protein synthesis and maintenance of membrane polarities (Hochachka et ai, 1996) and ultimately lead to cell death.

In other embodiments, an active compound is a protective metabolic agent. Metabolism is generally understood as referring to chemical processes (in a cell
or organism) that are required for life; they involve a variety of reactions to sustain energy production and synthesize (anabolism) and break down (catabolism) complex molecules.

In one embodiment, an active compound is carbon monoxide (CO). CO can be toxic to organisms whose blood carries oxygen to sustain its survival. It may be poisonous by entering the lungs through normal breathing and displacing oxygen from the bloodstream. Interruption of the normal supply of oxygen jeopardizes the functions of the heart, brain and other vital functions of the body. However, the use of carbon monoxide for medical applications is being explored (Ryter et al, 2004). At amounts of 50 parts per million (ppm), carbon monoxide presents no symptoms to humans exposed to it. However, at 200 ppm, within two-three hours the carbon monoxide can cause a slight headache; at 400 ppm, within one to two hours it can cause a frontal headache that may become widespread within three hours; and, at 800 ppm it can cause dizziness, nausea, and/or convulsions within 45 minutes, and render the subject insensible within two hours. At levels of around 1000 ppm, an organism can expire after exposure for more than around 1-2 minutes.

In other embodiments, an active compound is a chalcogenide compound. Compounds containing a chalcogen element, those in Group 6 of the periodic table, but excluding oxides, are commonly termed "chalcogenides" or "chalcogenide compounds (used interchangeably herein). These elements are sulfur (S), selenium (Se), tellurium (Te), and polonium (Po). Common chalcogenide compounds contain one or more of S, Se and Te, in addition to other elements. Representative specific chalcogenide compounds and salts thereof include, but are not limited to: \( \text{H}_2\text{S}, \text{Na}_2\text{S}, \text{NaHS}, \text{K}_2\text{S}, \text{KHS}, \text{Li}_2\text{S}, \text{Rb}_2\text{S}, \text{Cs}_2\text{S}, (\text{NH}_4)_2\text{S}, (\text{NH}_4)\text{HS}, \text{BeS}, \text{MgS}, \text{CaS}, \text{SrS}, \text{BaS}, \text{H}_2\text{Se}, \text{Na}_2\text{Se}, \text{NaHSe}, \text{K}_2\text{Se}, \text{KHSe}, \text{Li}_2\text{Se}, \text{Rb}_2\text{Se}, \text{Cs}_2\text{Se}, (\text{NH}_4)_2\text{Se}, (\text{NH}_4)\text{HSe}, \text{BeSe}, \text{MgSe}, \text{CaSe}, \text{SrSe}, \text{PoSe} \) and \( \text{BaSe}. \) Chalcogenide compounds include elemental forms such as micronized and/or nanomilled particles of S and Se. Chalcogenide compounds may be provided in liquid as well as gaseous forms.

In certain embodiments, the chalcogenide compound comprises sulfur, while in others it comprises selenium, tellurium, or polonium. In certain embodiments, a chalcogenide compound contains one or more exposed sulfide groups. In particular
embodiments, it is contemplated that such a chalcogenide compound contains 1, 2, 3, 4, 5, 6 or more exposed sulfide groups, or any range derivable therein. In particular embodiments, such a sulfide-containing compound is $\text{CS}_2$ (carbon disulfide). In certain embodiments, the chalcogenide is a salt, preferably salts wherein the chalcogen is in a -2 oxidation state. As noted previously, sulfide salts encompassed by embodiments of the invention include, but are not limited to, sodium sulfide ($\text{Na}_2\text{S}$), sodium hydrogen sulfide ($\text{NaHS}$), potassium sulfide ($\text{K}_2\text{S}$), potassium hydrogen sulfide ($\text{KHS}$), lithium sulfide ($\text{Li}_2\text{S}$), rubidium sulfide ($\text{Rb}_2\text{S}$), cesium sulfide ($\text{Cs}_2\text{S}$), ammonium sulfide ((NH$_4$)$_2\text{S}$), ammonium hydrogen sulfide (NH$_4$HS), beryllium sulfide (BeS), magnesium sulfide (MgS), calcium sulfide (CaS), strontium sulfide (SrS), barium sulfide (BaS), and the like.

In particular embodiments of the present invention, an active chalcogenide compound is hydrogen sulfide. Hydrogen sulfide ($\text{H}_2\text{S}$) is a potentially toxic gas that is often associated with petrochemical and natural gas, sewage, paper pulp, leather tanning, and food processing. The primary effect, at the cellular level, appears to be inhibition of cytochrome oxidase and other oxidative enzymes, resulting in cellular hypoxia.

Typical levels of hydrogen sulfide contemplated for use in accordance with the present invention include values of about 1 to about 500 ppm, about 10 to about 400 ppm, about 50 to about 300 ppm, and about 80 to about 300 ppm, or the equivalent oral, intravenous or transdermal dosage thereof. Other relevant ranges include about 10 to about 80 ppm, about 10 to about 80 ppm, about 10 to about 70 ppm, about 20 to about 70 ppm, about 20 to about 60 ppm, and about 30 to about 60 ppm, or the equivalent oral, intravenous or transdermal thereof. It also is contemplated that, for a given animal in a given time period, the chalcogenide atmosphere should be reduced to avoid a potentially lethal build up of chalcogenide in the subject. For example, an initial environmental concentration of 80 ppm may be reduced after 30 min to 60 ppm, followed by further reductions at 1 hr (40 ppm) and 2 hrs (20 ppm). In particular embodiments, effective concentrations of hydrogen sulfide in a human are in the range of 50 ppm to 500 ppm, delivered continuously. For certain embodiments of intravenous
administration, effective concentrations are in the range of 0.5 to 50 milligrams per kilogram of body weight delivered continuously.

In one embodiment, H$_2$S is generated by the spontaneous dissociation of the chalcogenic salt and H$_2$S donor, sodium hydrosulfide (NaHS), in aqueous solution according to the equations:

$$\text{NaHS} \rightarrow \text{Na}^+ + \text{HS}^-$$

$$2\text{HS}^- \leftrightarrow \text{H}_2\text{S} + \text{S}^{2-}$$

$$\text{HS}^- + \text{H}^+ \leftrightarrow \text{H}_2\text{S}$$

In other embodiments, as active compound is a chalcogenide precursor. "Chalcogenide precursor" refers to compounds and agents that can yield a chalcogenide, e.g., hydrogen sulfide (H$_2$S), under certain conditions, such as upon exposure, or soon thereafter, to biological matter. Such precursors yield H$_2$S or another chalcogenide upon one or more enzymatic or chemical reactions. In certain embodiments, the chalcogenide precursor is dimethylsulfoxide (DMSO), dimethylsulfide (DMS), methylmercaptan (CH$_3$SH), mercaptoethanol, thiocyanate, hydrogen cyanide, methanethiol (MeSH), or carbon disulfide (CS$_2$). In certain embodiments, the chalcogenide precursor is CS$_2$, MeSH, or DMS. Compounds on the order of the size of these molecules are particularly contemplated (that is, within about 50% of their molecular weights).

Additional active compounds include, but are not limited to, the following structures, many of which are readily available and known to those of skill in the art (identified by CAS number): 104376-79-6 (Ceftriaxone Sodium Salt); 105879-42-3; 1094-08-2 (Ethopropatine HCl); 1098-60-8 (Triflupromazine HCl); 111974-72-2; 113-59-7; 113-98-4 (Penicillin G K$^+$); 115-55-9; 1179-69-7; 118292-40-3; 119478-56-7; 120138-50-3; 121123-17-9; 121249-14-7; 1229-35-2; 1240-15-9; 1257-78-9 (Prochlorperazine Edisylate Salt); 128345-62-0; 130-61-0 (Thioridazine HCl) 132-98-9 (Penicillin V K$^+$); 13412-64-1 (Dicloxacillin Na$^+$ Hydrate); 134678-17-4; 144604-00-2; 146-54-3; 146-54-5 (Fluphenazine 2HCl); 151767-02-1; 159989-65-8; 16960-16-0 (Adrenocorticotropic Hormone Fragment 1-24); 1982-37-2; 21462-39-5 (Clindamycin HCl); 22189-31-7; 22202-75-1; 23288-49-5 (Probucol); 23325-78-2; 24356-60-3 (Cephapirin); 24729-96-2 (Clindamycin); 25507-04-4; 26605-69-6; 27164-46-1
(Cefazolin Na\(^+\)); 2746-81-8; 29560-58-8; 2975-34-0; 32672-69-8 (Mesoridazine Benzene Sulfonate); 32887-01-7; 33286-22-5 ((\(+\))-cis-Diltiazem HCl); 33564-30-6 (Cefoxitin Na\(^+\)); 346-18-9; 3485-14-1; 3511-16-8; 37091-65-9 (Azlocillin Na\(^+\)); 37661-08-8; 3819-00-9; 38821-53-3 (Cephradine); 41372-02-5; 42540-40-9 (Cefamandole Nafate); 4330-99-8 (Trimeprazine hemi-(\(+\))-tartrate Salt); 440-17-5 (Trifluoperazine 2HCl); 4697-14-7 (Ticarcillin 2Na\(^+\)); 4800-94-6 (Carbenicillin 2Na\(^+\)); 50-52-2; 50-53-3; 5002-47-1; 51481-61-9 (Cimetidine); 52239-63-1 (6-propyl-2-thiouracil); 53-60-1 (Promazine HCl); 5321-32-4; 54965-21-8 (Albendazole); 5591-45-7 (Thiothixene); 56238-63-2 (Cefuroxime Na\(^+\)); 56796-39-5 (Cefmetazole Na\(^+\)); 5714-00-1; 58-33-3 (Promethazine HCl); 58-38-8; 58-39-9 (Perphenazine); 58-71-9 (Cephalothin Na\(^+\)); 59703-84-3 (Piperacillin Na\(^+\)); 60-99-1 (Methotrimeprazine Maleate Salt); 60925-61-3; 61270-78-8; 6130-64-9 (Penicillin G Procaine Salt Hydrate); 61318-91-0 Sulconazole Nitrate Salt); 61336-70-7 (Pergolide Mesylate Salt); 66104-23-2 (Ranitidine HCl); 66592-87-8 (Cloxacillin Na\(^+\)); 668401-82-1; 69-09-0 (Chlorpromazine HCl); 69-52-3 (Ampicillin Na\(^+\)); 69-53-4 (Ampicillin); 69-57-8 (Penicillin G Na\(^+\)); 70059-30-2; 70356-03-5; 7081-40-5; 7081-44-9 (Cloxacillin Na\(^+\) H\(_2\)O); 7177-50-6 (Nafcillin Na\(^+\) H\(_2\)O); 7179-49-9; 7240-38-2 (Oxacillin Na H\(_2\)O); 7246-14-2; 74356-00-6; 74431-23-5; 74849-93-7; 75738-58-8; 76824-35-6 (Famotidine); 76963-41-2; 79350-37-1; 81129-83-1; 84-02-6 (Prochlorperazine Dimaleate Salt); 87-08-1 (Phenoxymethylpenicillin Acid); 87239-81-4; 91-33-8 (Benzthiazide); 91832-40-5; 94841-17-5; 99294-94-7; 154-42-7 (6-Thioguanine); 36735-22-5; 536-33-4 (Ethionamide); 52-67-5 (D-Penicillamine); 304-55-2 (Meso-2,3-Dimercaptosuccinic Acid); 59-52-9 2,3-Dimercaptopropanol 6112-76-1 (6-mercaptopurine); 616-91-1 (N-acetyl-L-cysteine); 62571-86-2 (Captopril); 52-01-7 (spironolactone); and, 80474-14-2 (fluticasone propionate).

Other active compounds may be identified using a variety of different tests. Reduced metabolism can be measured by a number of ways, including by quantifying the amount of oxygen consumed by a biological sample, the amount of
carbon dioxide produced by the sample (indirect measurement of cellular respiration),
or characterizing motility.

It is understood according to the present invention that the dosages of thiomorpholine or a thiomorpholine analog, and duration of treatment are generally lower when using a combination of thiomorpholine, a thiomorpholine analog and another active compound or treatment as compared to using the thiomorpholine, a thiomorpholine analog, an active compound, or treatment alone. Accordingly, the combination treatments provided by the present invention may offer advantages associated with reduced side effects associated with treatment using certain active compounds or other treatments to reduce metabolic activity of prevent injury.

Methods of the present invention that involve providing to biological material thiomorpholine or a thiomorpholine analog in combination with one or more active compounds may be practiced, in various embodiments, by providing the thiomorpholine or thiomorpholine analog, and one or more additional active compounds simultaneously, contemporaneously, or at different times. For example, in one embodiment, biological material is contacted with thiomorpholine or a thiomorpholine analog, and subsequently contacted with one or more additional active compounds. Alternatively, biological material is contacted with one or more additional active compound and then subsequently contacted with thiomorpholine or a thiomorpholine analog. Contact with thiomorpholine or a thiomorpholine analog, and one or more active compounds may be discrete, wherein contact with one is terminated prior to contact with another, or it may overlap or occur concurrently.

In the context of combination methods involving contacting biological material with thiomorpholine or a thiomorpholine analog and another treatment that reduces metabolic cellular or tissue damage due to injury or disease, the biological matter may be subjected to either thiomorpholine or a thiomorpholine analog or the other treatment at the same time, one before the other, or during overlapping time periods.

In other embodiments, the methods of the present invention include combination treatment with thiomorpholine or a thiomorpholine analog and another agents effective in the treatment of the specific disease or condition being treated
(secondary therapy). For example, the treatment of stroke (antistroke treatment) typically involves an antiplatelet (aspirin, clopidogrel, dipyridamole, ticlopidine), an anticoagulant (heparin, warfarin), or a thrombolytic (tissue plasminogen activator). Any of these compounds may be used in combination with thiomorpholine or a thiomorpholine analog according to the methods of the present invention.

In other embodiments of the present invention, thiomorpholine or a thiomorpholine analog is provided to biological material in combination with an environmental condition associated with reduced metabolic activity of biological material. Such environmental conditions include low temperatures and hypoxic or anoxic conditions.

Standard methods of achieving hypoxia or anoxia are well established and include using environmental chambers that rely on chemical catalysts to remove oxygen from the chamber. Such chambers are available commercially from, for example, BD Diagnostic Systems (Sparks, MD) as GASPAK Disposable Hydrogen + Carbon Dioxide Envelopes or BIO-BAG Environmental Chambers. Alternatively, oxygen may be depleted by exchanging the air in a chamber with a non-oxygen gas, such as nitrogen. Oxygen concentration may be determined, for example using a FYRITE Oxygen Analyzer (Bacharach, Pittsburgh PA).

It is contemplated that methods of the invention can use a combination of exposure to thiomorpholine or a thiomorpholine analog, and alteration of oxygen concentrations compared to room air. Moreover, the oxygen concentration of the environment containing biological matter can be about, at least about, or at most about 1, 2, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100%, or any range derivable therein. Moreover, it is contemplated that a change in concentration can be any of the above percentages or ranges, in terms of a decrease or increase compared to room air or to a controlled environment.

In certain embodiments, reduced temperature conditions are sub-physiological temperatures with reference to the particular biological material being
treated, which are understood to differ depending upon the biological material being treated. In particular embodiments, a biological matter is contacted with thiomorpholine or a thiomorpholine analog and also subjected to reduced temperature conditions of less than 37°C, less than 30°C, less than 25°C, less than 20°C, less than 15°C, less than 10°C, less than 5°C, less than 0°C, less than -20°C, or less than -70°C.

**Pharmaceutical Compositions and Administration**

The methods of the present invention comprise contacting a biological material with thiomorpholine or a thiomorpholine analog, alone or in combination with one or more additional active compounds. The routes of administration of thiomorpholine or the thiomorpholine analog will vary, naturally, with the location and nature of the condition to be treated, and include, e.g., inhalation, intradermal, transdermal, parenteral, intravenous, intramuscular, intranasal, subcutaneous, percutaneous, intratracheal, intraperitoneal, intratumoral, perfusion, lavage, direct injection, and oral administration and formulation intravenously, intradermally, intraarterially, intraperitoneally, intralesionally, intracranially, intraarticularly, intraprostatacly, intrapleurally, intratracheally, intranasally, intrathecally, intravitreally, intravaginally, intrarectally, topically, intratumorally, intramuscularly, intraperitoneally, intraocularly, subcutaneously, subconjunctival, intravesicularly, mucosally, intrapericardially, intraumbilically, intracocularly, orally, topically, locally, by inhalation, by injection, by infusion, by continuous infusion, by localized perfusion, via a catheter, or via a lavage.

In particular embodiments, thiomorpholine or a thiomorpholine analog are administered topically, as solid dosage forms, using perfusion systems, or by catheter, as injectable liquids by intravascular, intravenous, intra-arterial, intracerebroventricular, intraperitoneal, or subcutaneous administration. In particular embodiments, thiomorpholine or a thiomorpholine analog are administered parenterally, intradermally, intramuscularly, transdermally or even intraperitoneally, as described in U.S. Patent 5,543,158; U.S. Patent 5,641,515; and U.S. Patent 5,399,363. In specific embodiments, thiomorpholine or a thiomorpholine analog is administered intraarterially, intravenously, or intraperitoneally.
The amount of thiomorpholine or a thiomorpholine analog, provided to a biological material may vary depending on the type of biological material (cell type, tissue type, organism genus and species, etc.) and/or its size (weight, surface area, etc.). It will generally be the case that the larger the organism, the larger the dose. Therefore, an effective amount for a mouse will generally be lower than an effective amount for a rat, which will generally be lower than an effective amount for a dog, which will generally be lower than an effective amount for a human.

In certain embodiments, the concentration of thiomorpholine or thiomorpholine analog administered is in a range of, about, at least about, or at most about 0.001, 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4.0, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5.0 mM or M or more or any range derivable therein. Similarly, the length of time of administration may vary depending on the type of biological material (cell type, tissue type, organism genus and species, etc.) and/or its size (weight, surface area, etc.) and will depend in part upon dosage form and route of administration. In particular embodiments, thiomorpholine or a thiomorpholine analog is provided for about or at least 30 seconds, 1 minute, 2 minutes, 3 minutes, 5 minutes, 10 minutes, 15 minutes, 30 minutes, 1 hour, 2 hours, 3 hours, four hours five hours, six hours, eight hours, twelve hours, twenty-four hours, or greater than twenty-four hours.

Thiomorpholine or a thiomorpholine analog may be administered in a single dose or multiple doses, with varying amounts of time between administered doses.

In the case of transplant, the present invention may be used pre- and or post-operatively to render host or graft materials quiescent. In a specific embodiment, a surgical site may be injected or perfused with a formulation comprising thiomorpholine or a thiomorpholine analog. The perfusion may be continued post-surgery, for example, by leaving a catheter implanted at the site of the surgery.

Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms. The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or
dispersions (U.S. Patent 5,466,468). In all cases the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (e.g., glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and/or vegetable oils. Proper fluidity may be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

For parenteral administration in an aqueous solution, for example, the solution should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous, intratumoral and intraperitoneal administration. In this connection, sterile aqueous media that can be employed will be known to those of skill in the art in light of the present disclosure. For example, one dosage may be dissolved in 1 ml of isotonic NaCl solution and either added to 1000 ml of hypodermoclysis fluid or injected at the proposed site of infusion, (see for example, "Remington's Pharmaceutical Sciences" 15th Edition, pages 1035-1038 and 1570-1580). Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject. Moreover, for human administration, preparations should meet sterility, pyrogenicity, general safety and purity standards as required by FDA Office of Biologies standards.

Sterile injectable solutions are prepared by incorporating thiomorpholine or a thiomorpholine analog in the required amount in the appropriate solvent with
various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Articles of Manufacture and Kits

The present invention further provides articles of manufacture and kits useful in practicing the methods of the present invention. In one embodiment, an article of manufacture comprises packaging material and a composition comprising thiomorpholine or a thiomorpholine analog, wherein the packaging material comprises a label that indicates that the composition can be used to enhance the survivability of biological material. The article of manufacture may further comprise a pharmaceutically acceptable diluent. In particular embodiments, the composition comprising thiomorpholine or a thiomorpholine analog is provided in a first sealed container and the pharmaceutically acceptable diluent is provided in a second sealed container.

In other related embodiments, the articles of manufacture and kits of the present invention further comprise one or more additional active compounds for use in a method of the present invention that includes combination therapy with thiomorpholine or a thiomorpholine analog. Typically, the one or more additional active compounds are each provided in separate sealed containers. Instructions for administering the combination of thiomorpholine or a thiomorpholine analog and the active compound(s) are also optionally provided.
EXAMPLES

Example 1

Liquid Pharmaceutical Composition of Thiomorpholine Increases Rodent Survival in Hypoxia

Introduction

Treatment with the chalcogenide H₂S (hydrogen sulfide) gas has been shown to enhance an animal's ability to survive under hypoxic conditions in a model of hypoxic/ischemic injury. It would be highly advantageous to be able to treat a patient with a liquid pharmaceutical composition with similar properties.

Thiomorpholine, a thiane with good solubility, was predicted, based on its structure to be a compound that is similar to H₂S. Thiomorpholine was identified as a pharmaceutical composition that may have properties that are similar to H₂S and was tested for its ability to enhance survival under hypoxic and ischemic conditions. H₂S exists at physiologic pH as both H₂S and ionized HS⁻. The activity of both ionizable and non-ionizable sulfide moieties was tested according to the general procedure described below. Thiomorpholine has a non-ionizable sulfide moiety, and contains a nitrogen that can be protonated that confers good water solubility. The molecular weight of thiomorpholine has no rotatable bonds which confers similar entropy of binding as H₂S, which has no rotatable bonds.

In these examples, thiomorpholine and thiomorpholine analogs were identified that induce stasis and these properties were verified in a series of in vivo models as a pharmaceutical candidate to induce stasis, modulate metabolism, regulate thermoregulation, and is indicated as a critical care intervention and/or treatment. In addition, the hypothesis that a liquid pharmaceutical composition of thiomorpholine or thiomorpholine analogs has protective effects similar to H₂S was tested. In a series of screening tests, it was shown that thiomorpholine and thiomorpholine analogs are effective for such pharmaceutical use and that these liquid compositions enhance survival and confer a protective effect in hypoxic and ischemic conditions.
Formulation and Administration

Thiomorpholine and thiomorpholine analog test compounds were prepared by the following general parameters. The pH was adjusted with NaOH or HCl to pH 6.0-8.0, the osmolality was adjusted with sodium chloride to 250-350 mOsmol/L and the total dose of thiomorpholine or thiomorpholine analog administered (mg) was divided by the test subject's weight (kg) and did not exceed 400% of a published (mg/kg) LD50 in a mouse or rat. In the experiments described, thiomorpholine (Sigma-Aldrich Corp. St. Louis, MO) was dissolved in water and adjusted with 8M HCl to pH 8.0. The osmolality of thiomorpholine was 292 mOsmol/L. The formulation was filtered through a two micron syringe-tip filter prior to administration into an animal. Formulation was made fresh for each experiment.

General Screening Procedure

Primary Screen

Using an infusion protocol as a primary screen, a test compound was administered to a mouse to determine whether the test compound infusion would produce a decrease in mouse subcutaneous temperature to 33°C or below. Infusion was initiated at 0.8 µL/min with body temperature measured every 3-5 minutes to observe a decrease in body temperature. The infusion rate was subsequently doubled every twenty minutes until subcutaneous temperature decreased to 33°C or below (1.6 µL/min, 3.2 µL/min, 6.3 µL/min, 12.7 µL/min and 25.4 µL/min (the maximum infusion rate).

Secondary Screen

The secondary screen determined whether the test compound caused a reversible drop in subcutaneous temperature without lethality. In the secondary screen, a mouse was infused with a test compound for 60 minutes at a rate of 50% of the effective infusion rate determined in the primary screen. When the mouse subcutaneous temperature decreased to 33°C or below, the infusion was immediately stopped, and mouse recovery was assessed by measuring subcutaneous temperature and observing mouse behavior. Observations were recorded 24 hours after treatment.
Tertiary/lethal hypoxia (3°) screen

In the tertiary screen, the mouse was infused with a test compound at the rate determined in the secondary screen. The mouse subcutaneous temperature was measured every 3-5 minutes until it decreased to 33°C, as in the secondary screen. When the mouse subcutaneous temperature registered 33°C, the infusion was stopped and the mouse was immediately transferred to a hypoxic chamber (4% O₂), together with a control mouse, infused with vehicle (saline, 148mM, osmolality 300 mOsmol/L). The closed glass chamber was perfused with air and nitrogen at a continuous flow to achieve the desired hypoxic atmosphere of 4% O₂.

Thiomorpholine Administration - Mouse

In one experiment thiomorpholine (149 mM) was tested in a male mouse C57BL/6, jugular vein catheterized (JVC), 5-6 weeks old (Taconic), by infusing the mouse with thiomorpholine using either a 1 mL or 5 mL Luer-Lok syringe (Becton Dickison). An IPTT-300 transponder from Bio Medic Data Systems (BMDS) was used to monitor body temperature. The transponder was injected subcutaneous (S.C.) into the back of the animal at least 24 hours prior to the experiment. A DAS-6008 data acquisition module from BMDS recorded body temperature of the mouse via the transponder, and data was input into a computer spreadsheet and plotted against time. Each mouse was dosed with thiomorpholine through the in-dwelling catheter using an infusion pump (Harvard Apparatus). The mouse was infused until the temperature chip implanted in the skin registered a body temperature of 33°C. In this experiment, if the mouse showed signs of distress before the temperature dropped to 33°C, then the infusion was stopped for 10 minutes and restarted at a rate lower than the previous rate. Once the animal's temperature dropped to 33°C or below, the infusion was stopped and the mouse was transferred into a hypoxic atmosphere (4.0% O₂).

These experiments showed that a thiomorpholine infusion (3.2 µL/min, 150 µM total dose administered or a dose of 7.33 µM/kg) produced a decrease in subcutaneous temperature below 33°C with a total decrease in body temperature of 10.3°C. Mice infused with thiomorpholine survived exposure to lethal hypoxia (4% O₂) for 60 minutes and were observed for 24 hours after removal from the hypoxic
chamber. These survival rates were compared to the survival rate of a control mouse exposed to lethal hypoxia, which typically died within 6-7 minutes in 4% O₂.

In another experiment, a mouse (ID: MJVC141) was infused with thiomorpholine (149 mM, pH 8.0, 297 mOsmol/L) and exposed to hypoxic conditions of 4.0% O₂. In this experiment, the mouse was infused with a total dose of 34.9 µmols of thiomorpholine (total dose 173.3 mg/kg). The body temperature was 32.5 °C at the beginning of hypoxia and 25.6 °C at the end of hypoxia. The treated mouse survived for 60 minutes in hypoxic conditions (4.0% O₂). A control mouse treated with vehicle died in hypoxia at 6.7 minutes.

These studies demonstrate that pretreatment with thiomorpholine provided protection in a model of lethal hypoxia. In addition, the results of these studies further demonstrate that thiomorpholine may be used to protect patients from injury resulting from hypoxic and ischemic injury.

15 **Thiomorpholine Administration - Rats**

The rats used in these studies were obtained from certified vendors (Taconic) and allowed 24 hours acclimation period after arrival. Cannulated rats were housed individually. Animals used for dosing were surgically implanted with an indwelling vascular catheter and were examined for signs of stress and disease prior to any procedure. Animals were weighed prior to procedures and weights were noted on the cage card. Rats were implanted dorsally with a subcutaneous RFID temperature sensor (IPTT-300, Bio Medic Data Systems, Inc. (BMDS)). Rats were allowed to recover at least 24 hours following implantation of the sensor. A DAS-6008 data acquisition module from BMDS recorded subcutaneous temperature of the rodent via the transponder, and this data was input into a computer spreadsheet and plotted against time.

In this experiment, the secondary screen to determine the infusion rate was initiated at a starting infusion rate of 7.9 µL/min and was doubled every 20 minutes until the temperature chip implanted in the skin decreased to 33.0°C. When the rat subcutaneous temperature decreased to 33.0°C or below, the infusion was immediately stopped and the rat recovery was assessed by measuring subcutaneous temperature and
observing the rat behavior. The rat temperature and behavior were observed and recorded 24 hours after treatment. The result of the secondary screen determined that the test compound, thiomorpholine, caused a reversible drop in subcutaneous temperature without lethality.

A test rat (RJVC51) was then infused with the test compound, thiomorpholine, at the rate determined in the secondary screen (63.5 µL/min). The rodent subcutaneous temperature was measured every 3-5 minutes until it decreased to 33.0°C. The infusion was stopped and the rodent was immediately transferred to a hypoxic chamber (3.5% O₂). The chamber was perfused with air and nitrogen at a continuous flow to achieve the desired hypoxic atmosphere.

A rat infused with thiomorpholine in the model described survived 60 minutes in the hypoxia (3.5%). Following exposure to hypoxia, it was transferred back to room air, and its recovery was monitored for 24 hours by recording subcutaneous temperatures and by behavioral observation. After the rat that received thiomorpholine survived hypoxia, a control rat (treated with vehicle) was placed inside the hypoxic chamber to verify lethality of the environment. The control rat died in 32 minutes.

These results confirm that thiomorpholine protects animals from lethal hypoxia, either by preventing death or extending survival. These results further indicate that thiomorpholine would be useful to treat patients who have may undergo, are undergoing, have undergone, or who are susceptible to injury, trauma or critical care treatment.

Example 2

Protection from Lethal Hypoxia Using Thiomorpholine

Thiomorpholine was tested according to the foregoing three step screening procedure. Table II show the dose response data for mice infused with thiomorpholine (149 mM, pH 8.0 (adjusted with 8M HCl), osmolality 297 mOsmol/L) by the specified rates (mg/kg min⁻¹) until a 5°C temperature drop was detected, and monitored until achieving a net temperature drop of 2.4°C.
As shown by the results in Figures 1 and 2 (for mice and rats, respectively), rodents infused with thiomorpholine survived exposure to lethal hypoxia for 60 minutes. The times at which infusion was started and stopped and the times at which exposure to hypoxic conditions was started and stopped are indicated. The infusion rates at varying rates are indicated. These survival rates were compared to the survival rate of control mice, which typically died within 6-7 minutes in the hypoxic environment, and control rats, which typically died within 32-33 minutes in the hypoxic environment.

These results establish that thiomorpholine may be used to protect animals from lethal hypoxia, either by preventing death or extending survival. In addition, these results support the conclusion that thiomorpholine is useful in treating diseases and conditions caused by, or resulting in, ischemia and hypoxia, such as, e.g., myocardial infarction, stroke, hemorrhagic shock, traumatic brain injury, kidney ischemia, tissue hypoperfusion and trauma.
Example 3

Pretreatment with Thiomorpholine Provides a Protective Benefit to Endotoxin-Induced Death in Mice in a Model of Fatal Septic Shock

5 Background

Sepsis or septic syndrome is the severe systemic inflammatory response to infection, is a major cause of morbidity and is a leading cause of death (see Okada, K. and Ogata H., Shock-From Molecular and Cellular Level to Whole Body (1996), Elsevier Science B.V.). Sepsis is a frequent and deadly syndrome characterized by the response of the body to bacteria, particularly gram-negative bacteria. Severe sepsis leads to multiple organ failure and systemic hypotension followed by circulatory and cardiac dysfunction and/or cardiac failure (see Soriano, F., et al, (2006); Crit Care Med, 34:1073-79).

In septic syndrome, pro-inflammatory mediators in the body launch a massive defense to the endotoxin produced by gram-negative bacteria. The body's substantial activation of the innate response to bacterial infection produces tissue and organ destruction that is characteristic of the multiple organ failure observed in sepsis.

The biologically active component of endotoxin is bacterial lipopolysaccharide (LPS), a major component of the outer membrane of gram-negative bacteria. When LPS is released from gram-negative bacteria, the body responds with an activation of a cascade of inflammatory mediators that act as the protective response to gram-negative sepsis. The release of LPS in vivo from the infecting bacteria produces many systemic effects including fever, increased white cell response, coagulation disorders and shock. The systemic response to LPS includes the production of the pro-inflammatory cytokines and particularly IL-1, TNFα, and IL-6 in macrophages, lymphocytes, and endothelial cells, as well as prostaglandins, and leukotrienes.

At the cellular level, sepsis is known to induce the formation of reactive oxygen species (free oxygen radical superoxide (O₂⁻) which is a source for another reactive species H₂O₂) and lipid peroxidation products that produce damage to target organs that include the liver and heart. Additionally, oxidative stress produces the hydroxyl radical (HO⁻). The elevated production of superoxide and NO can result in...
rapid formation of peroxynitrite (ONOO-), which is a reactive cytotoxic oxidant species. Oxidative stress can lead to the overactivation of PARP (Poly(adenosine 5'-diphosphate-ribose) polymerase) a nuclear enzyme of eukaryotic cells that is involved in the response to DNA injury (strand breaks). PARP activates a metabolic cycle that leads to the depletion of NAD+ and ATP pools in the cell which slows glycolysis and mitochondrial respiration, leading to cellular dysfunction and cell death. PARP activation is a key mechanism for the tissue damage that results in the organ damage associated with oxidant stress (i.e., ischemia, myocardial reperfusion injury, stroke, shock, and inflammation) (see Soriano, F., et al, (2006); Crit Care Med, 34:1073-79; Soriano, F., et al (2001); Nature Medicine, 7:108-113).

Thiomorpholine Formulation

Because thiomorpholine showed protective effects in a rodent model of hypoxia and ischemia (Examples 1 and 2), the hypothesis was tested that pre-treatment of a septic shock (endotoxemic) mouse model with thiomorpholine would provide therapeutic and protective effects from the innate inflammatory response to endotoxemia. In the following study, we determined that pretreatment with thiomorpholine has a significant protective effect enhancing survival in a rodent model of septic shock (see Figure 3).

Animal Model

The test model comprised male C57BL/6 mice, 5-6 weeks old (Taconic, NY). Animals were acclimated in a temperature and humidity controlled environment for 3-6 days prior to the commencement of experimental procedures. Food and water were provided ad libitum. An IPTT-300 transponder from Bio Medic Data Systems (BMDS) was used to monitor body temperature. The transponder was injected subcutaneous (S.C.) into the back of the animals at least 24 hours prior to the experiment. A DAS-6008 data acquisition module from BMDS recorded body temperature of the mouse via the transponder, and data was input into a computer spreadsheet and plotted against time.
**Experimental Procedure**

Lipopolysaccharide (Escherichia coli, Serotype 0127:B8) was purchased from Sigma-Aldrich CoΦ (St. Louis, MO) and dissolved in saline. Thiomorpholine (Sigma-Aldrich CoΦ, St. Louis, MO) was dissolved in water and adjusted with 8M HCl, pH 8.0, osmolality, 292 mOsmol/L. The formulation was filtered through a two micron syringe-tip filter. Formulation was made fresh for each experiment. LPS (100 mg/kg) was administered to mice by intraperitoneal (IP) injection thirty minutes after administration of thiomorpholine (test article) or vehicle.

Two cohorts of 14 mice were dosed with vehicle (125 mg/kg) IP or thiomorpholine (125 mg/kg). At times 27 - 33 minutes (average 30 minutes), a dose of LPS (100 mg/kg) was administered IP to both cohorts of mice. Mice were returned to their cages and monitored for lethality by observation over the next 80 hours.

**Results**

This study showed that within 24 hours after treatment with LPS, one hundred percent (100%) of the vehicle treated mice had died. In contrast, 50% of the mice pretreated with thiomorpholine prior to injection with LPS were alive at 24 hours. The remaining mice treated with thiomorpholine showed enhanced survival (see Figure 3). These data demonstrate that administration of thiomorpholine in a rodent model of septic shock provides significant protective benefits and significantly enhanced survival (pO.0001).

**Example 4**

**Thiazolidine Enhanced Survival in a Rodent under Lethal Hypoxia**

Treatment with thiomorpholine has been shown to enhance an animal’s ability to survive under hypoxic conditions in a model of hypoxic/ischemic injury (see Example 1). Thiazolidine, a thiomorpholine analog, was identified and, as set forth below, was shown to confer a protective effect and enhanced survival in hypoxic and ischemic conditions.
In one set of experiments, thiazolidine was tested in a male C57BL/6 jugular vein catheterized (JVC) mouse, 5-6 weeks old (Taconic), by infusing the animal with thiazolidine using a 1 mL or 5 mL Luer-Lok syringe (Becton Dickision) according to the foregoing three step screening procedure.

In one experiment, a mouse (ID: MJVC147) was infused with a thiazolidine solution, with an effective dose of 3.9 mM/kg over a period of 60 minutes at an initial infusion rate of 3.2 µL/min and a final infusion rate of 6.4 µL/min until the temperature chip implanted in the skin registered a body temperature of 33°C. The infusion was then stopped, and the animal was placed into a hypoxic atmosphere (4.0% O₂) within one minute. The mouse infused with thiazolidine survived 19 minutes in hypoxia. A control mouse survived 4.9 minutes in hypoxia. This experiment demonstrated that pretreatment with thiazolidine provides significantly enhanced survival in hypoxia and is evidence that thiazolidine has protective effects in ischemic and hypoxic injury.

It will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without departing from the spirit and scope of the invention. Accordingly, the invention is not limited except as by the appended claims.

All of the above U.S. patents, U.S. patent application publications, U.S. patent applications, foreign patents, foreign patent applications and non-patent publications referred to in this specification and/or listed in the Application Data Sheet are incorporated herein by reference, in their entirety. In addition, the following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference:

U.S. Patent 3,777,507
U.S. Patent 3,881,990
U.S. Patent 3,989,816
U.S. Patent 3,995,444
U.S. Patent 4,034,753
<table>
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<th>Patent Number</th>
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<tr>
<td>U.S. Patent 4,186,565</td>
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<td>U.S. Patent 4,266,573</td>
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<td>U.S. Patent 4,292,817</td>
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<td>U.S. Patent 4,442,856</td>
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<td>U.S. Patent 4,473,637</td>
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<td>U.S. Patent 4,502,295</td>
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<td>U.S. Patent 4,559,258</td>
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<td>U.S. Patent 4,745,759</td>
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<td>U.S. Patent 4,798,824</td>
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<td>U.S. Patent 4,828,976</td>
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<td>U.S. Patent 4,938,961</td>
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<td>U.S. Patent 4,951,482</td>
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<td>U.S. Patent 5,066,578</td>
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<td>U.S. Patent 5,217,860</td>
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<td>U.S. Patent 5,231,025</td>
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<td>U.S. Patent 5,285,657</td>
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<td>U.S. Patent 5,326,706</td>
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<td>U.S. Patent 5,370,989</td>
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<td>U.S. Patent 5,395,314</td>
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<td>U.S. Patent 5,399,363</td>
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<td>U.S. Patent 5,405,742</td>
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<td>U.S. Patent 5,434,045</td>
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<td>U.S. Patent 5,466,468</td>
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<td>U.S. Patent 5,470,738</td>
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<td>U.S. Patent 5,476,763</td>
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<tr>
<td>U.S. Patent 5,543,158</td>
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<tr>
<td>U.S. Patent 5,552,267</td>
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</table>


Chapter IV; Chapter VI; Chapter VII; Chapter VIII; Chapter IX of Klayman, D. L.; Gunther, W. H. H. Eds, Wiley Interscience, New York, 1973.


CUT (Chemical Industry Institute of Toxicology), In: *90 day vapor inhalation toxicity study of hydrogen sulfide*, Toxigenics, 420-0710, 1983.


PCT Appln. WO 94/17178

Rogers et al, Genome, 8:71 1-713, 1997.


The Hypothermia After Cardiac Arrest Study Group et al, 2002.


CLAIMS

What is claimed is:

1. A method of enhancing the survivability of a biological material exposed to ischemic or hypoxic conditions, wherein the method comprises contacting the biological material with an effective amount of thiomorpholine or a thiomorpholine analog having the following general structure (I):

\[
\begin{align*}
R_1 & \quad \text{N} \quad (C(R_2)_2)_n \quad \text{A} \\
& \quad \text{N} \quad (C(R_2)_2)_m
\end{align*}
\]

(I)

or stereoisomer, prodrug, pharmaceutically acceptable salt or solvate thereof,

wherein:

- \( A \) is \( S \) or \( Se \);
- \( n \) is 1, 2 or 3;
- \( m \) is 2, 3, 4 or 5;
- \( n + m \) is less than or equal to 6;
- \( R_1 \) is hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, arylalkyl, substituted arylalkyl, cycloalkyl, substituted cycloalkyl, cycloalkylalkyl, substituted cycloalkylalkyl, heteroaryl, substituted heteroaryl, heteroaryalkyl, substituted heteroaryalkyl, hererocyclyl, substituted heterocyclyl, heterocyclylalkyl or substituted heterocyclylalkyl; and

\( R_2 \) is independently selected from the group consisting of hydrogen, amino, cyano, halogen, hydroxyl, imino, nitro, oxo, thio, alkyl, substituted alkyl, alkylamino, substituted alkylamino, alkoxy, substituted alkoxy, alkylthio, substituted alkylthio, aryl, substituted aryl, arylalkyl, substituted arylalkyl, cycloalkyl, substituted cycloalkyl, cycloalkylalkyl, substituted cycloalkylalkyl, heteroaryl, substituted heteroaryl, heteroaryalkyl, substituted heteroaryalkyl, hererocyclyl, substituted heterocyclyl, heterocyclylalkyl or substituted heterocyclylalkyl, or any two \( R_2 \) groups, taken together with the respective carbon atoms to which these groups are attached, form an unsubstituted or substituted fused cycloalkyl, aryl, heterocyclyl or heteroaryl.
2. The method of claim 1, wherein the biological material is contacted with an effective amount of thiomorpholine.

3. The method of claim 1, wherein the biological material is contacted with an effective amount of a thiomorpholine analog.

4. The method of claim 3, wherein the thiomorpholine analog is thiazolidine.

5. The method of claim 1, wherein the biological material is contacted with the thiomorpholine or thiomorpholine analog before being exposed to the ischemic or hypoxic conditions.

6. The method of claim 1, wherein the biological material is contacted with the thiomorpholine or thiomorpholine analog during exposure to the ischemic or hypoxic conditions.

7. The method of claim 1, wherein the biological material is contacted with the thiomorpholine or thiomorpholine analog after being exposed to the ischemic or hypoxic conditions.

8. The method of claim 1, wherein the ischemic or hypoxic conditions result from an injury to the biological material, the onset or progression of a disease that adversely affects the biological material, or hemorrhaging of the biological material.

9. The method of claim 8, wherein the biological material is contacted with the thiomorpholine or thiomorpholine analog before the injury, before the onset or progression of the disease, or before hemorrhaging of the biological material.
10. The method of claim 8, wherein the biological material is not contacted with the thiomorpholine or thiomorpholine analog during or after the injury, during or after the onset or progression of the disease, or during or after the hemorrhaging of the biological material.

11. The method of claim 8, wherein the injury is from an external physical source.

12. The method of claim 11, wherein the injury is a surgery.

13. The method of claim 8, wherein the biological material is contacted with the thiomorpholine or thiomorpholine analog in an amount and for a time that protects the biological material from damage or death resulting from the injury, the onset or progression of the disease, or the hemorrhaging in the biological material.

14. The method of claim 1, wherein the biological material is selected from the group consisting of cells, tissues, organs, organisms, and animals.

15. The method of claim 14, wherein the biological material is an animal.

16. The method of claim 15, wherein the animal is a mammal.

17. The method of claim 16, wherein the mammal is a human.

18. The method of claim 14, wherein the biological material comprises platelets.

19. The method of claim 14, wherein the biological material is to be transplanted.
20. The method of claim 1, wherein the biological material is at risk for reperfusion injury.

21. The method of claim 1, wherein the biological material is at risk for hemorrhagic shock.

22. The method of claim 1, wherein the biological material is contacted with the thiomorpholine or thiomorpholine analog intravenously, intradermally, intraarterially, intraperitoneally, intralesionally, intracranially, intraarticularly, intraprostatically, intrapleurally, intratracheally, intranasally, intravitreally, intravaginally, intrarectally, topically, intratumorally, intramuscularly, intraperitoneally, intraocularly, subcutaneously, subconjunctival, intravesicularly, mucosally, intrapericardially, intraumbilically, intraocularly, orally, topically, locally, by inhalation, by injection, by infusion, by continuous infusion, by absorption, by adsorption, by immersion, by localized perfusion, via a catheter, or via a lavage.

23. The method of claim 1, wherein the thiomorpholine or thiomorpholine analog is provided to the biological material by infusion at a dosage in the range of 0.01 mg/kg to 400 mg/kg.

24. The method of claim 1, further comprising exposing the biological material to a controlled pressure environment.

25. The method of claim 1, further comprising exposing the biological material to a controlled temperature environment.

26. The method of claim 25, wherein the biological material is exposed to a controlled temperature environment that is less than about 20°C.

27. The method of claim 26, wherein the biological material achieves a non-physiological core temperature.
28. The method of claim 1, further comprising contacting the biological material with an effective amount of an additional active compound.

29. The method of claim 28, wherein the biological material is contacted with the thiomorpholine or thiomorpholine analog and the additional active compound sequentially.

30. The method of claim 28, wherein the biological material is contacted with the thiomorpholine or thiomorpholine analog and the additional active compound simultaneously.

31. The method of claim 28, wherein the additional active compound is an oxygen antagonist.

32. The method of claim 28, wherein the additional active compound is a chalcogenide compound or chalcogenide salt.

33. The method of claim 32, wherein the additional active compound comprises sulfur.

34. The method of claim 33, wherein the additional active compound is H₂S.

35. The method of claim 32, wherein the active compound comprises selenium.

36. The method of claim 35, wherein the active compound is H₂Se.

37. The method of claim 32, wherein the additional active compound is a chalcogenide salt.
38. The method of claim 37, wherein the chalcogenide salt is selected from the group consisting of Na$_2$S, NaHS, K$_2$S, KHS, Li$_2$S, Rb$_2$S, Cs$_2$S, (NH$_4$)$_2$S, (NH$_4$)HS, BeS, MgS, CaS, SrS, and BaS.

39. The method of claim 37, wherein the chalcogenide salt is selected from the group consisting of Na$_2$Se, NaHSe, K$_2$Se, KHSe, Li$_2$Se, Rb$_2$Se, Cs$_2$Se, (NH$_4$)$_2$Se, (NH$_4$)HSe, BeSe, MgSe, CaSe, SrSe, PoSe and BaSe.

40. The method of claim 31, wherein the additional active compound is CO$_2$.

41. The method of claim 1, wherein the thiomorpholine or thiomorpholine analog is provided to the biological material as a pharmaceutical composition.

42. A method for preventing or reducing damage to a biological material exposed to ischemic or hypoxic conditions, wherein the method comprises contacting the biological material with an effective amount of thiomorpholine or a thiomorpholine analog having the following general structure (I):

\[
R_1 - N - \frac{(C(R_2)_2)_n}{(C(R_2)_2)_m} - A
\]

(I)

or stereoisomer, prodrug, pharmaceutically acceptable salt or solvate thereof,

wherein:

- A is S or Se;
- \(n\) is 1, 2 or 3;
- \(m\) is 2, 3, 4 or 5;
- \(n + m\) is less than or equal to 6;
$R_1$ is hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, arylalkyl, substituted arylalkyl, cycloalkyl, substituted cycloalkyl, cycloalkylalkyl, substituted cycloalkylalkyl, heteroaryl, substituted heteroaryl, heteroarylalkyl, substituted heteroarylalkyl, hererocyclyl, substituted heterocyclyl, heterocyclylalkyl or substituted heterocyclylalkyl; and

each $R_2$ is independently selected from the group consisting of hydrogen, amino, cyano, halogen, hydroxyl, imino, nitro, oxo, thioxo, alkyl, substituted alkyl, alkylamino, substituted alkylamino, alkoxy, substituted alkoxy, alkylthio, substituted alkylthio, aryl, substituted aryl, arylalkyl, substituted arylalkyl, cycloalkyl, substituted cycloalkyl, cycloalkylalkyl, substituted cycloalkylalkyl, heteroaryl, substituted heteroaryl, heteroarylalkyl, substituted heteroarylalkyl, hererocyclyl, substituted heterocyclyl, heterocyclylalkyl or substituted heterocyclylalkyl, or any two $R_2$ groups, taken together with the respective carbon atoms to which these groups are attached, form an unsubstituted or substituted fused cycloalkyl, aryl, heterocyclyl or heteroaryl.

43. A method for reversibly inhibiting metabolism in a biological material, wherein the method comprises contacting the biological material with an effective amount of thiomorpholine or a thiomorpholine analog having the following general structure (I):

\[
\begin{align*}
R_1 & \quad -N- \quad (C(R_2)_2)_n \quad A \\
& \quad (C(R_2)_2)_m
\end{align*}
\]

(I)

or stereoisomer, prodrug, pharmaceutically acceptable salt or solvate thereof,

wherein:

A is S or Se;

$n$ is 1, 2 or 3;

$m$ is 2, 3, 4 or 5;

$n + m$ is less than or equal to 6;
R\text{1} \text{ is } \text{hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, arylalkyl, substituted arylalkyl, cycloalkyl, substituted cycloalkyl, cycloalkylalkyl, substituted cycloalkylalkyl, heteroaryl, substituted heteroaryl, heteroarylalkyl, substituted heteroarylalkyl, hererocycl, substituted heterocycl, heterocyclylalkyl or substituted heterocyclylalkyl; and}

each R\text{2} \text{ is independently selected from the group consisting of hydrogen, amino, cyano, halogen, hydroxyl, imino, nitro, oxo, thixo, alkyl, substituted alkyl, alkylamino, substituted alkylamino, alkoxy, substituted alkoxy, alkylthio, substituted alkylthio, aryl, substituted aryl, arylalkyl, substituted arylalkyl, cycloalkyl, substituted cycloalkyl, cycloalkylalkyl, substituted cycloalkylalkyl, heteroaryl, substituted heteroaryl, heteroarylalkyl, substituted heteroarylalkyl, hererocycl, substituted heterocycl, heterocyclylalkyl or substituted heterocyclylalkyl, or any two R\text{2} \text{ groups, taken together with the respective carbon atoms to which these groups are attached, form an unsubstituted or substituted fused cycloalkyl, aryl, heterocycl or heteroaryl.}

44. The method of claim 43, wherein the biological material is a mammal.

45. A method for enhancing the survivability of a mammal, such as a human, suffering from hemorrhagic shock or at risk of hemorrhagic shock, wherein the method comprises administering to the mammal an effective amount of thiomorpholine or a thiomorpholine analog having the following general structure (I):

$$\text{R}_1 - N - (\text{C(R}_2\text{)_2})_n - \text{A}$$

(I)

or stereoisomer, prodrug, pharmaceutically acceptable salt or solvate thereof,

wherein:

A is S or Se;

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

n + m is less than or equal to 6;

R₁ is hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, arylalkyl, substituted arylalkyl, cycloalkyl, substituted cycloalkyl, cycloalkylalkyl, substituted cycloalkylalkyl, heteroaryl, substituted heteroaryl, heteroarylalkyl, substituted heteroarylalkyl, hererocyclyl, substituted heterocyclyl, heterocyclylalkyl or substituted heterocyclylalkyl; and

each R₂ is independently selected from the group consisting of hydrogen, amino, cyano, halogen, hydroxyl, imino, nitro, oxo, thio, alkyl, substituted alkyl, alkylamino, substituted alkylamino, alkoxy, substituted alkoxy, alkylthio, substituted alkylthio, aryl, substituted aryl, arylalkyl, substituted arylalkyl, cycloalkyl, substituted cycloalkyl, cycloalkylalkyl, substituted cycloalkylalkyl, heteroaryl, substituted heteroaryl, heteroarylalkyl, substituted heteroarylalkyl, hererocyclyl, substituted heterocyclyl, heterocyclylalkyl or substituted heterocyclylalkyl, or any two R₂ groups, taken together with the respective carbon atoms to which these groups are attached, form an unsubstituted or substituted fused cycloalkyl, aryl, heterocyclyl or heteroaryl.

46. The method of claim 45, wherein the mammal is at risk of hemorrhagic shock and suffers hemorrhagic shock after being provided with the thiomorpholine or thiomorpholine analog.

47. A method for enhancing the survivability of a mammal, such as a human, undergoing a surgery, wherein the method comprises administering to the mammal an effective amount of thiomorpholine or a thiomorpholine analog having the following general structure (I):

\[
\begin{align*}
R_1 & \quad \text{N} \quad (C(R_2)_2)_n \quad \text{A} \\
(C(R_2)_2)_m &
\end{align*}
\]

(I)

or stereoisomer, prodrug, pharmaceutically acceptable salt or solvate thereof,
wherein:

A is S or Se;

\( n \) is 1, 2 or 3;

\( m \) is 2, 3, 4 or 5;

\( n + m \) is less than or equal to 6;

\( R_i \) is hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, arylalkyl, substituted arylalkyl, cycloalkyl, substituted cycloalkyl, cycloalkylalkyl, substituted cycloalkylalkyl, heteroaryl, substituted heteroaryl, heteroarylalkyl, substituted heteroarylalkyl, hererocyclyl, substituted heterocyclyl, heterocyclylalkyl or substituted heterocyclylalkyl; and

each \( R_2 \) is independently selected from the group consisting of hydrogen, amino, cyano, halogen, hydroxyl, imino, nitro, oxo, thiooxo, alkyl, substituted alkyl, alkylamino, substituted alkylamino, alkoxy, substituted alkoxy, alkylthio, substituted alkylthio, aryl, substituted aryl, arylalkyl, substituted arylalkyl, cycloalkyl, substituted cycloalkyl, cycloalkylalkyl, substituted cycloalkylalkyl, heteroaryl, substituted heteroaryl, heteroarylalkyl, substituted heteroarylalkyl, hererocyclyl, substituted heterocyclyl, heterocyclylalkyl or substituted heterocyclylalkyl, or any two \( R_2 \) groups, taken together with the respective carbon atoms to which these groups are attached, form an unsubstituted or substituted fused cycloalkyl, aryl, heterocyclyl or heteroaryl.

48. The method of claim 47, wherein the surgery is selected from elective surgery, planned surgery and emergency surgery.
49. A method for preserving biological material *ex vivo*, wherein the method comprises contacting the biological material with an effective amount of thiomorpholine or a thiomorpholine analog having the following general structure (I):

\[
\begin{align*}
R_1 & - N - \left( \begin{array}{c} 
\text{(C(R₂)₂)}_n \\
\text{(C(R₂)₂)}_m 
\end{array} \right) - A \\
\end{align*}
\]

(I)

or stereoisomer, prodrug, pharmaceutically acceptable salt or solvate thereof,

wherein:

- A is S or Se;
- \(n\) is 1, 2 or 3;
- \(m\) is 2, 3, 4 or 5;
- \(n + m\) is less than or equal to 6;
- \(R₁\) is hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, arylalkyl, substituted arylalkyl, cycloalkyl, substituted cycloalkyl, cycloalkylalkyl, substituted cycloalkylalkyl, heteroaryl, substituted heteroaryl, heteroarylalkyl, substituted heteroarylalkyl, heterocyclyl, substituted heterocyclyl, heterocyclylalkyl or substituted heterocyclylalkyl; and

- each \(R₂\) is independently selected from the group consisting of hydrogen, amino, cyano, halogen, hydroxyl, imino, nitro, oxo, thioxo, alkyl, substituted alkyl, alkylamino, substituted alkylamino, alkoxy, substituted alkoxy, alkylthio, substituted alkylthio, aryl, substituted aryl, arylalkyl, substituted arylalkyl, cycloalkyl, substituted cycloalkyl, cycloalkylalkyl, substituted cycloalkylalkyl, heteroaryl, substituted heteroaryl, heteroarylalkyl, substituted heteroarylalkyl, heterocyclyl, substituted heterocyclyl, heterocyclylalkyl or substituted heterocyclylalkyl, or any two \(R₂\) groups, taken together with the respective carbon atoms to which these groups are attached, form an unsubstituted or substituted fused cycloalkyl, aryl, heterocyclyl or heteroaryl.
50. The method of claim 49, wherein the biological material is stored at low temperature.

51. The method of claim 49, wherein the biological material is cells, tissue, or an organ.

52. The method of claim 51, wherein the cells are platelets.

53. The method of claim 51, wherein the tissue or organ is being stored prior to transplant.

54. A method for preserving non-living biological material, wherein the method comprises contacting the material with an effective amount of thiomorpholine or a thiomorpholine analog having the following general structure (I):

\[
\begin{array}{c}
R_1 \quad \text{N} \quad (C(R_2)_2)_n \quad \text{A} \\
\quad \quad \quad (C(R_2)_2)_m
\end{array}
\]

(I)

or stereoisomer, prodrug, pharmaceutically acceptable salt or solvate thereof, wherein:

- A is S or Se;
- \( n \) is 1, 2 or 3;
- \( m \) is 2, 3, 4 or 5;
- \( n + m \) is less than or equal to 6;
- \( R_i \) is hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, arylalkyl, substituted arylalkyl, cycloalkyl, substituted cycloalkyl, cycloalkylalkyl, substituted cycloalkylalkyl, heteroaryl, substituted heteroaryl, heteroaryalkyl, substituted heteroaryalkyl, heterocyclyl, substituted heterocyclyl, heterocyclylalkyl or substituted heterocyclylalkyl; and
each R₂ is independently selected from the group consisting of hydrogen, amino, cyano, halogen, hydroxyl, imino, nitro, oxo, thio, alkyl, substituted alkyl, alkylamino, substituted alkylamino, alkoxy, substituted alkoxy, alkylthio, substituted alkylthio, aryl, substituted aryl, arylalkyl, substituted arylalkyl, cycloalkyl, substituted cycloalkyl, cycloalkylalkyl, substituted cycloalkylalkyl, heteroaryl, substituted heteroaryl, heteroarylalkyl, substituted heteroarylalkyl, hererocyclyl, substituted heterocyclyl, heterocyclylalkyl or substituted heterocyclylalkyl, or any two R₂ groups, taken together with the respective carbon atoms to which these groups are attached, form an unsubstituted or substituted fused cycloalkyl, aryl, heterocyclyl or heteroaryl.

55. The method of claim 54, wherein the non-living biological material is a dead animal.

56. The method of claim 54, wherein the non-living biological material is a plant or plant product.

57. The method of claim 56, wherein the plant or plant product is a food product.

58. A method for extending the shelf-life of a food or beverage product subject to spoilage, wherein the method comprises contacting the food or beverage product with an effective amount of thiomorpholine or a thiomorpholine analog having the following general structure (I):

\[
\begin{align*}
\text{R}_1 & \quad \text{N} \quad (\text{C(R}_2\text{)}_2)_n \quad \text{A} \\
\text{R}_1 & \quad \text{N} \quad (\text{C(R}_2\text{)}_2)_m \quad \text{A}
\end{align*}
\]

(I)

or stereoisomer, prodrug, pharmaceutically acceptable salt or solvate thereof,

wherein:

A is S or Se;
\[ n \text{ is } 1, 2 \text{ or } 3; \]
\[ m \text{ is } 2, 3, 4 \text{ or } 5; \]
\[ n + m \text{ is less than or equal to } 6; \]

\( R_i \) is hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, arylalkyl, substituted arylalkyl, cycloalkyl, substituted cycloalkyl, cycloalkylalkyl, substituted cycloalkylalkyl, heteroaryl, substituted heteroaryl, heteroarylalkyl, substituted heteroarylalkyl, herocyclyl, substituted heterocyclyl, heterocyclylalkyl or substituted heterocyclylalkyl; and

each \( R_2 \) is independently selected from the group consisting of hydrogen, amino, cyano, halogen, hydroxyl, imino, nitro, oxo, thio, alkyl, substituted alkyl, alkylamino, substituted alkylamino, alkoxy, substituted alkoxy, alkylthio, substituted alkylthio, aryl, substituted aryl, arylalkyl, substituted arylalkyl, cycloalkyl, substituted cycloalkyl, cycloalkylalkyl, substituted cycloalkylalkyl, heteroaryl, substituted heteroaryl, heteroarylalkyl, substituted heteroarylalkyl, herocyclyl, substituted heterocyclyl, heterocyclylalkyl or substituted heterocyclylalkyl, or any two \( R_2 \) groups, taken together with the respective carbon atoms to which these groups are attached, form an unsubstituted or substituted fused cycloalkyl, aryl, heterocyclyl or heteroaryl.

59. The method of claim 58, wherein the food or beverage product is wine or beer.

60. A method for extending the shelf-life of a pharmaceutical, health care or cosmetic product, wherein the method comprises contacting the pharmaceutical, health care or cosmetic product with an effective amount of thiomorpholine or a thiomorpholine analog having the following general structure (I):

\[
\begin{align*}
\text{R}_1 - \text{N} & \quad \text{(C(R_2)2)}_n - \text{A} \\
\text{(C(R_2)2)}_m &
\end{align*}
\]

(I)

or stereoisomer, prodrug, pharmaceutically acceptable salt or solvate thereof,
wherein:

A is S or Se;

\( n \) is 1, 2 or 3;

\( m \) is 2, 3, 4 or 5;

\( n + m \) is less than or equal to 6;

\( R_1 \) is hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, arylalkyl, substituted arylalkyl, cycloalkyl, substituted cycloalkyl, cycloalkylalkyl, substituted cycloalkylalkyl, heteroaryl, substituted heteroaryl, heteroarylalkyl, substituted heteroarylalkyl, hererocycl, substituted heterocycl, heterocyclalkyl or substituted heterocyclalkyl; and each \( R_2 \) is independently selected from the group consisting of hydrogen, amino, cyano, halogen, hydroxyl, imino, nitro, oxo, thioxo, alkyl, substituted alkyl, alkylamino, substituted alkylamino, alkoxy, substituted alkoxy, alkylthio, substituted alkylthio, aryl, substituted aryl, arylalkyl, substituted arylalkyl, cycoalkyl, substituted cycoalkyl, cycoalkylalkyl, substituted cycoalkylalkyl, heteroaryl, substituted heteroaryl, heteroarylalkyl, substituted heteroarylalkyl, heterocycl, substituted heterocycl, heterocyclalkyl or substituted heterocyclalkyl, or any two \( R_2 \) groups, taken together with the respective carbon atoms to which these groups are attached, form an unsubstituted or substituted fused cycloalkyl, aryl, heterocycl or heteroaryl.

61. A pharmaceutical composition comprising a pharmaceutically acceptable carrier, diluent or excipient and thiomorpholine or a thiomorpholine analog having the following general structure (I):

\[
\begin{align*}
R_1 & \quad N \quad ((C(R_2)_2)_n \quad A \\
\quad & \quad (C(R_2)_2)_m
\end{align*}
\]

(I)

or stereoisomer, prodrug, pharmaceutically acceptable salt or solvate thereof, wherein:

A is S or Se;
$n$ is 1, 2 or 3;
$m$ is 2, 3, 4 or 5;
$n + m$ is less than or equal to 6;

$R_i$ is hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, arylalkyl, substituted arylalkyl, cycloalkyl, substituted cycloalkyl, cycloalkylalkyl, substituted cycloalkylalkyl, heteroaryl, substituted heteroaryl, heteroarylalkyl, substituted heteroarylalkyl, heterocyclyl, substituted heterocyclyl, heterocyclylalkyl or substituted heterocyclylalkyl; and

each $R_2$ is independently selected from the group consisting of hydrogen, amino, cyano, halogen, hydroxyl, imino, nitro, oxo, thiooxo, alkyl, substituted alkyl, alkylamino, substituted alkylamino, alkoxy, substituted alkoxy, alkylthio, substituted alkylthio, aryl, substituted aryl, arylalkyl, substituted arylalkyl, cycloalkyl, substituted cycloalkyl, cycloalkylalkyl, substituted cycloalkylalkyl, heteroaryl, substituted heteroaryl, heteroarylalkyl, substituted heteroarylalkyl, heterocyclyl, substituted heterocyclyl, heterocyclylalkyl or substituted heterocyclylalkyl, or any two $R_2$ groups, taken together with the respective carbon atoms to which these groups are attached, form an unsubstituted or substituted fused cycloalkyl, aryl, heterocyclyl or heteroaryl.

62. An article of manufacture comprising packing material and a composition comprising thiomorpholine or a thiomorpholine analog having the following general structure (I):

$$\begin{align*}
R_1 - N & \quad \left\langle \left( C(R_2)_{2} \right)_n \quad \right\rangle \\
\quad \left\langle \left( C(R_2)_{2} \right)_m \quad \right\rangle \quad A
\end{align*}$$

(I)

or stereoisomer, prodrug, pharmaceutically acceptable salt or solvate thereof,

wherein:

$A$ is S or Se;

$n$ is 1, 2 or 3;

$m$ is 2, 3, 4 or 5;
wherein the packing material comprises a label that indicates that the composition can be used to enhance the survivability of biological material exposed to ischemic or hypoxic conditions.

63. The article of manufacture of claim 62, wherein the composition comprises thiomorpholine.

64. The article of manufacture of claim 62, further comprising a pharmaceutically acceptable diluent.

65. The article of manufacture of claim 64, wherein the composition is provided in a first sealed container and the pharmaceutically acceptable diluent is provided in a second sealed container.

66. The article of manufacture of claim 62, further comprising an additional active compound.
67. The article of manufacture of claim 66, wherein the composition is provided in a first sealed container and the additional active compound is provided is a second sealed container.
Fig. 1
Fig. 3
# A. Classification of Subject Matter

INV. A61K31/54 A61K31/425 A61P9/10 A61P39/00

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

# B. Fields Searched

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

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

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

# C. Documents Considered TO BE RELEVANT

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<td>ALEXANDER KELSEY ET AL: &quot;The modulation of metabolism by hydrogen sulfide (H2S)&quot; ARCTIC SCIENCE CONFERENCE ABSTRACTS, October 2006 (2006-10), page 9, XP001538068 &amp; ARCTIC SCIENCE CONFERENCE 2006; FAIRBANKS, AK, USA; OCTOBER 02-04, 2006 the whole document</td>
<td>1-67</td>
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Further documents are listed in the continuation of Box C.

See patent family annex.

Date of the actual completion of the international search

12 March 2008

Date of mailing of the international search report

26/03/2008

Name and mailing address of the ISA/
European Patent Office, P B. 5818 Patentlaan 2 NL-2280 HV Rijswijk
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Authorized officer
Giacobbe, Simone
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<td>WO 2006/113914 A (HUTCHINSON FRED CANCER RES [US]; ROTH MARK B [US]; MORRISON MIKE; GOLD) 26 October 2006 (2006-10-26) the whole document</td>
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