The present invention relates to isolated deuterated compounds (e.g., compounds described by Formula (I) and pharmaceutically acceptable salts thereof. The present invention also relates to isotopically enriched compositions that include compounds according to Formula (I) and pharmaceutically acceptable salts thereof. The invention also features pharmaceutical compositions that include these compounds and their use in therapy for treating conditions in which necroptosis is likely to play a substantial role, or those conditions in which RIP1 and/or RIP3 protein is a contributing factor, (structure shown)
DEUTERATED HETEROCYCLIC INHIBITORS OF NECROPTOSIS

Cross-Reference to Related Applications
This application claims the benefit of U.S. Provisional Application No. 61/789,689, filed March 15, 2013, which is hereby incorporated by reference in its entirety.

Statement as to Federally Sponsored Research
This invention was made with government support under Grant No. R01 GM084205, awarded by the National Institutes of Health. The government has certain rights in this invention.

Background of the Invention
In many diseases, cell death is mediated through apoptotic and/or necrotic pathways. While much is known about the mechanisms of action that control apoptosis, control of necrosis is not as well understood. Understanding the mechanisms regulating both necrosis and apoptosis in cells is essential to being able to treat conditions, such as neurodegenerative diseases, stroke, coronary heart disease, kidney disease, and liver disease. A thorough understanding of necrotic and apoptotic cell death pathways is also crucial to treating AIDS and the conditions associated with AIDS, such as retinal necrosis.

Cell death has traditionally been categorized as either apoptotic or necrotic based on morphological characteristics (Wyllie et al., Int. Rev. Cytol. 68:251 (1980)). These two modes of cell death were also initially thought to occur via regulated (caspase-dependent) and non-regulated processes, respectively. Subsequent studies, however, demonstrate that the underlying cell death mechanisms resulting in these two phenotypes are much more complicated and, under some circumstances, interrelated. Furthermore, conditions that lead to necrosis can occur by either regulated caspase-independent or non-regulated processes.

One regulated caspase-independent cell death pathway with morphological features resembling necrosis, called necroptosis, has been described (Degterev et al., Nat. Chem. Biol. 1:112 (2005)). This manner of cell death can be initiated with various stimuli (e.g., TNF-a and Fas ligand) and in an array of cell types (e.g., monocytes, fibroblasts, lymphocytes, macrophages, epithelial cells and neurons). Necroptosis may represent a significant contributor to and, in some cases, predominant mode of cellular demise under pathological conditions involving excessive cell stress, rapid energy loss, and massive oxidative species generation, where the highly energy-dependent apoptosis process is not operative.

The identification and optimization of low molecular weight molecules capable of inhibiting necroptosis will assist in elucidating its role in disease pathophysiology and can provide compounds (i.e., necrostatins) for anti-necroptosis therapeutics. The discovery of compounds that prevent caspase-independent cell death (e.g., necrosis or necroptosis) would also provide useful therapeutic agents for treating or preventing conditions in which necrosis occurs. These compounds and methods would be particularly useful for the treatment of neurodegenerative diseases, ischemic brain and heart injuries, and head trauma.
Summary of the Invention

The invention features new compounds, pharmaceutical compositions, kits, and methods for treating a condition in which necrosis or necroptosis is likely to play a substantial role, or those in which RIP1 and/or RIP3 protein is a contributing factor.

In a first aspect, the invention features a composition that includes a compound of the formula

![Chemical Structure 1](image1)

(i), or any pharmaceutically acceptable salt thereof, or stereoisomer thereof, where

- $X^1$ is 0 or NR$_1^1$;
- each of $X^2$ and $X^3$ is, independently, 0 or S;
- $R^1$ is H, D, or optionally substituted C1-C6 alkyl;
- $R^2$ is H or D;
- each of $R^3^A$ and $R^3^B$ is, independently, H, D, or optionally substituted C1-C6 alkyl;
- each of $R^4$, $R^5$, and $R^6$ is, independently, H or D;
- $R^7$ is H, D, halogen, optionally substituted C1-C6 alkyl, or optionally substituted C1-C6 alkoxy;
- each of $R^8$, $R^9$, and $R^{10}$ is, independently, H, D, or optionally substituted C1-C6 alkyl; and
- where at least one of $R^1$-$R^{10}$ is D or includes a deuterium group, and
- where said composition has an isotopic enrichment factor for deuterium of at least 5.

In a second aspect, the invention features an isolated compound having a structure according to the following formula,

![Chemical Structure 2](image2)

(i), or any pharmaceutically acceptable salt thereof, or stereoisomer thereof, where

- $X^1$ is 0 or NR$_1^1$;
- each of $X^2$ and $X^3$ is, independently, 0 or S;
- $R^1$ is H, D, or optionally substituted C1-C6 alkyl;
- $R^2$ is H or D;
- each of $R^3^A$ and $R^3^B$ is, independently, H, D, or optionally substituted C1-C6 alkyl;
- each of $R^4$, $R^5$, and $R^6$ is, independently, H or D;
- $R^7$ is H, D, halogen, optionally substituted C1-C6 alkyl, or optionally substituted C1-C6 alkoxy;
- each of $R^8$, $R^9$, and $R^{10}$ is, independently, H, D, or optionally substituted C1-C6 alkyl; and
- where at least one of $R^1$-$R^{10}$ is D or includes a deuterium group, and
- where said composition has an isotopic enrichment factor for deuterium of at least 5.
In some embodiments of the compositions and compounds described herein, R\(^1\) is H. In certain embodiments of the compositions and compounds described herein, X\(^1\) is 0. In other embodiments of the compositions and compounds described herein, X\(^1\) is NR\(^5\). In still other embodiments of the compositions and compounds described herein, R\(^{10}\) is H. In some embodiments of the compositions and compounds described herein, X\(^2\) and X\(^3\) are both 0.

In other embodiments of the compositions and compounds described herein, one, two, or three of R\(^4\), R\(^5\), and R\(^6\) is D. For example, one of R\(^4\), R\(^5\), and R\(^6\) can be D, and two of R\(^4\), R\(^5\), and R\(^6\) can be H. In certain embodiments, R\(^4\) is D, R\(^5\) is D, or R\(^6\) is D. In other embodiments of the compositions and compounds described herein, R\(^2\) is H. In still other embodiments of the compositions and compounds described herein, one of R\(^3\) and R\(^8\) is D, or both R\(^3\) and R\(^8\) are D, or both R\(^3\) and R\(^8\) are H.

In some embodiments of the compositions and compounds described herein, R\(^8\) is H or D. In other embodiments of the compositions and compounds described herein, the optionally substituted C\(^1\)-C\(^6\) alkyl or said optionally substituted C\(^1\)-C\(^6\) alkoxy includes 0, 1, 2, or 3 deuterium atoms. In still other embodiments of the compositions and compounds described herein, R\(^7\) is halogen, optionally substituted C\(^1\) alkyl, or optionally substituted C\(^1\) alkoxy. In certain embodiments, the C\(^1\) alkyl or said C\(^1\) alkoxy includes 1, 2, or 3 deuterium atoms. In further embodiments, R\(^7\) is Cl, CH\(_3\), OCH\(_3\), CD\(_3\), OCD\(_3\), or CF\(_3\).

In some embodiments of the compositions and compounds described herein, R\(^9\) is optionally substituted C\(^1\) alkyl (e.g., R\(^9\) is CH\(_3\), CD\(_3\), CHD\(_2\), or CH\(_2\)D).

In certain embodiments of the compositions and compounds described herein, the compound has a structure according to one of the following formulas,
or a pharmaceutically acceptable salt thereof, or a stereoisomer thereof, and where
X 1 is NH or O;
R 7 is halogen, optionally substituted C 1-C2 alkyl, or optionally substituted C 1-C2 alkoxy; and
R 9 is optionally substituted C 1-C2 alkyl.

In some embodiments of the compositions and compounds described herein, X 1 is NH.
In certain embodiments of the compositions and compounds described herein, X 1 is O.
In other embodiments of the compositions and compounds described herein, the optionally substituted C 1-C2 alkyl or said optionally substituted C 1-C2 alkoxy includes 0, 1, 2, or 3 deuterium atoms.
In still other embodiments of the compositions and compounds described herein, R 9 is CH 3, OCH 3, CD 3, OCD 3, or CF 3.
In some embodiments of the compositions and compounds described herein, R 1-R 10 include 1, 2, 3, 4, or 5 deuterium atoms.

In certain embodiments of the compositions and compounds described herein, the isotopic enrichment factor for deuterium is at least 10, 50, 100, 500, 1000, or 3000.
In other embodiments of the compositions and compounds described herein, the carbon bearing the R 8 and X 1 groups has the (S)-configuration.
In still other embodiments of the compositions and compounds described herein, the carbon bearing the R 8 and X 1 groups has the (R)-configuration.

In a third aspect, the invention features a pharmaceutical composition that includes
(i) a pharmaceutically acceptable excipient; and
(ii) any of the compositions or compounds described herein,
In a fourth aspect, the invention features a method of treating a condition in a subject, said method including the step of contacting any of the compositions or compounds described herein, or any pharmaceutically acceptable salt thereof, or stereoisomer thereof, to the subject in a dosage sufficient to decrease necroptosis.

In another aspect, the invention features a method of treating a condition in a subject, said method comprising the step of contacting any of the compounds (e.g., a compound according to any of formulas (I)-(VI)) or compositions described herein, or any pharmaceutically acceptable salt thereof, or stereoisomer thereof, to said subject in a dosage sufficient to modulate RIP1 and/or RIP3 activity, and wherein said condition is one in which RIP1 and/or RIP3 protein is a contributing factor.

For example, the methods of the invention can include administering to a subject any of the compounds (e.g., a compound according to any of formulas (I)-(VI)) or compositions described herein, or any pharmaceutically acceptable salt thereof, or stereoisomer thereof.

In some embodiments, the condition is a neurodegenerative disease of the central or peripheral nervous system, the result of retinal neuronal cell death, the result of cell death of cardiac muscle, the result of cell death of cells of the immune system; stroke, liver disease, pancreatic disease, the result of cell death associated with renal failure; heart, mesenteric, retinal, hepatic or brain ischemic injury, ischemic injury during organ storage, head trauma, septic shock, coronary heart disease, cardiomyopathy, myocardial infarction, bone avascular necrosis, sickle cell disease, muscle wasting, gastrointestinal disease, tuberculosis, diabetes, alteration of blood vessels, muscular dystrophy, graft-versus-host disease, viral infection, Crohn's disease, ulcerative colitis, asthma, atherosclerosis, a chronic or acute inflammatory condition, pain, or any condition in which alteration in cell proliferation, differentiation or intracellular signaling is a causative factor, or any condition where RIP1 and/or RIP3 protein is a contributing factor.

In still other embodiments, the condition is a neurodegenerative disease of the central or peripheral nervous system.

In certain embodiments, the condition is hepatic or brain ischemic injury, or ischemic injury during organ storage, head trauma, septic shock, or coronary heart disease.

In some embodiments, the condition is stroke.

In other embodiments, the condition is myocardial infarction.

In some embodiments, the condition is pain (e.g., inflammatory pain, diabetic pain, pain associated with a burn, or pain associated with trauma).

In other embodiments, the condition is atherosclerosis.

In still other embodiments, the condition is a chronic or acute inflammatory condition (e.g., rheumatoid arthritis, psoriasis, or Stevens-Johnson syndrome).

In a fifth aspect, the invention features a method of decreasing necroptosis including contacting a cell with any of the compositions or compounds described herein, or any pharmaceutically acceptable salt thereof, or stereoisomer thereof.
in a sixth aspect, the invention features a kit that includes:
(a) a pharmaceutically acceptable composition that includes any of the compositions or compounds described herein, or any pharmaceutically acceptable salt thereof, or stereoisomer thereof; and
(b) instructions for the use of the pharmaceutical composition of (a) to treat a condition in a subject.

By "C1,4 alkaryl" is meant a C1-4 alkyl group having an optionally substituted aryl or an optionally substituted heteroaryl located at any position of the carbon chain. The C1,4 alkyl group may be linear or branched and may also be substituted with, for example, 1, 2, 3, 4, or 5 additional substituents as described herein.

By "C1-C6 alkoxy" is meant a group having the structure -O optionally substituted C1-C6 alkyl), where the optionally substituted C1-C6 alkyl may be branched, linear, or cyclic. The C1-C6 alkyl may be substituted or unsubstituted. A substituted C1-C6 alkyl can have, for example, 1, 2, 3, 4, 5, or 6 substituents-including deuterium-located at any position. Exemplary alkoxy groups include, but are not limited to, methoxy, ethoxy, propoxy, isopropoxy, tert-butoxy, and the like.

By "C2-C6 alkynyl" or "alkynyl" is meant an optionally substituted unsaturated C2-C6 hydrocarbon group having one or more carbon-carbon double bonds. Exemplary C2-C6 alkynyl groups include, but are not limited to -CH=CH (ethynyl), propenyl, 2-propenyl, 2-methyl-1-propenyl, 1-butenyl, 2-butenyl, and the like. A C2-C6 alkynyl may be linear or branched and may be unsubstituted or substituted. A substituted C2-C6 alkynyl may have, for example, 1, 2, 3, 4, 5, or 6 substituents located at any position.

By "C1-C6 alkyl" or "alkyl" is meant an optionally substituted C1-C6 saturated hydrocarbon group. An alkyl group may be linear, branched, or cyclic ("cycloalkyl"). Examples of alkyl radicals include, but are not limited to, methyl, ethyl, n-propyl, isopropyl, n-butyl, iso-butyl, sec-butyl, sec-pentyl, iso-pentyl, tert-butyl, n-pentyl, neopentyl, n-hexyl, sec-hexyl, n-heptyl, n-octyl, n-decyl, n-undecyl, dodecyl, and the like, which may bear one or more substituents. Substituted alkyl groups may have, for example, 1, 2, 3, 4, 5, or 6 substituents located at any position. Exemplary substituted alkyl groups include, but are not limited to, optionally substituted C1,4 alkyl groups. Substituted C1-C6 alkyl groups also encompass deuterated C1-C6 alkyl groups (e.g., C1-C6 alkyl groups that include 1, 2, 3, 4, 5, or 6 deuterium atoms).

By "C2-C6 alkenyl" or "alkenyl" is meant an optionally substituted unsaturated C2-C6 hydrocarbon group having one or more carbon-carbon triple bonds. Exemplary C2-C6 alkenyl groups include, but are not limited to ethynyl, 1-propynyl, and the like.

By "amino" is meant a group having a structure - NR'R", where each R' and R" is selected independently, from H, optionally substituted C1-C6 alkyl, optionally substituted cycloalkyl, optionally substituted heterocyclyl, optionally substituted aryl, optionally substituted heteroaryl, or R' and R" combine to form an optionally substituted heterocyclyl. When R' is not H or R" is not H, R' and R" may be unsubstituted or substituted with, for example, 1, 2, 3, 4, 5, or 6 substituents.

By "aryl" is meant is an optionally substituted C6-C14 cyclic group with [4n + 2] \pi electrons in conjugation and where n is 1, 2, or 3. Non-limiting examples of aryls include heteroaryls and, for example, benzene, naphthalene, anthracene, and phenanthrene. Aryls also include bi- and tri-cyclic ring systems in which a non-aromatic saturated or partially unsaturated carbocyclic ring (e.g., a cycloalkyl or
cycloalkenyl) is fused to an aromatic ring such as benzene or naphthalene. Exemplary aryls fused to a non-aromatic ring include indanyl, tetrahydronaphthyl. Any aryls as defined herein may be unsubstituted or substituted. A substituted aryl may be optionally substituted with, for example, 1, 2, 3, 4, 5, or 6 substituents located at any position of the ring.

By "aryloxy" is meant a group having the structure -O(optimally substituted aryl), where aryl is as defined herein.

By "azido" is meant a group having the structure -N3.

By "carbamate" or "carbamoyl" is meant a group having the structure -OCONR'R" or -NR'C02R", where each R' and R" is selected, independently, from H, optionally substituted C1-C6 alkyl, optionally substituted cycloalkyl, optionally substituted heterocyclyl, optionally substituted aryl, or R' and R" combine to form an optionally substituted heterocyclyl. When R' is not H or R" is not H, R' and R" may be unsubstituted or substituted with, for example, 1, 2, 3, 4, 5, or 6 substituents.

By "carbonate" is meant a group having a the structure -OC02R", where R' is selected from H, optionally substituted C1-C6 alkyl, optionally substituted cycloalkyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl. When R' is not H, R may be unsubstituted or substituted with, for example, 1, 2, 3, 4, 5, or 6 substituents.

By "carboxamido" or "amido" is meant a group having the structure -CONR'R" or -NR'C(=0) R", where each R' and R" is selected, independently, from H, optionally substituted C1-C6 alkyl, optionally substituted cycloalkyl, optionally substituted heterocyclyl, optionally substituted aryl, or R' and R" combine to form an optionally substituted heterocyclyl. When R' is not H or R" is not H, R' and R" may be unsubstituted or substituted with, for example, 1, 2, 3, 4, 5, or 6 substituents.

By "carboxylic group" is meant a group having the structure -CO2R", where R' is selected from H, optionally substituted C1-C6 alkyl, optionally substituted cycloalkyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl. When R' is not H, R may be unsubstituted or substituted with, for example, 1, 2, 3, 4, 5, or 6 substituents.

By "cyano" is meant a group having the structure -CN.

By "C3-C10 cycloalkyl" or "cycloalkyl" is meant an optionally substituted, saturated or partially unsaturated 3- to 10-membered monocyclic or polycyclic (e.g., bicyclic, or tricyclic) hydrocarbon ring system. Where a cycloalkyl is polycyclic, the constituent cycloalkyl rings may be fused together, form a spirocyclic structure, or the polycyclic cycloalkyl may be a bridged cycloalkyl (e.g., adamantyl or norbornanyl). Exemplary cycloalkyls include cyclopentyl, cyclobutyl, cyclopentyl, cyclohexyl, and cycloheptyl. Cycloalkyls may be unsubstituted or substituted. A substituted cycloalkyl can have, for example, 1, 2, 3, 4, 5, or 6 substituents.

By "cycloalkenyl" is meant a non-aromatic, optionally substituted 3- to 10-membered monocyclic or bicyclic hydrocarbon ring system having at least one carbon-carbon double bond. For example, a cycloalkenyl may have 1 or 2 carbon-carbon double bonds. Cycloalkenyls may be unsubstituted or substituted. A substituted cycloalkenyl can have, for example, 1, 2, 3, 4, 5, or 6 substituents. Exemplary cycloalkenyls include, but are not limited to, cyclopropenyl, cyclobutenyl, cyclopentenyl, cyclopentadienyl, cyclohexenyl, 1,3-cyclohexadienyl, 1,4-cyclohexadienyl, and the like.

"D" refers to deuterium.

As used herein, when a particular position in a compound of this invention is designated as being "-D," being "deuterated," or "including deuterium" (the element deuterium is represented by the letter "D"
in chemical structures and formulas and indicated with a lower case "'" in chemical names, according to the Boughton system), it is understood that the abundance of deuterium at that position is substantially greater than the natural abundance of deuterium, which is 0.01 5%. In certain embodiments, a composition of the invention has a minimum isotopic enrichment factor of at least 5 (0.075% deuterium incorporation), e.g., at least 10 (0.15% deuterium incorporation). In other embodiments, a composition has an isotopic enrichment factor of at least 50 (0.75% deuterium incorporation), at least 100 (1.5% deuterium incorporation), at least 500 (7.5% deuterium incorporation), at least 2000 (30% deuterium incorporation), at least 3000 (45% deuterium incorporation), at least 4000 (60% deuterium incorporation), at least 4500 (67.5% deuterium incorporation), at least 5000 (75% deuterium incorporation), or at least 5500 (82.5% deuterium incorporation). Any of the chemical groups, functional groups, or substituents described herein may be deuterated if the chemical group, functional group, or substituent has H. Methods for the preparation of deuterated compounds are known in the art, and exemplary methods are described herein.

By "effective amount" or "therapeutically effective amount" of an agent, as used herein, is that amount sufficient to effect beneficial or desired results, such as clinical results, and, as such, an effective amount depends upon the context in which it is being applied. For example, in the context of contacting (e.g., administering) an agent that is an inhibitor of necroptosis, an effective amount of an agent is, for example, an amount sufficient to achieve a reduction in necroptosis as compared to the response obtained without administration of the agent.

By "ester" is meant a group having a structure selected from -OCOR', where R' is selected from H, optionally substituted C1-C6 alkyl, optionally substituted cycloalkyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl. When R' is not H, R may be unsubstituted or substituted with, for example, 1, 2, 3, 4, 5, or 6 substituents.

By "halogen" or "halo" is meant fluorine (-F), chlorine (-Cl), bromine (-Br), or iodine (-I).

By "heteroaryl" is mean an aryl group that contains 1, 2, or 3 heteroatoms in the cyclic framework. Exemplary heteroaryls include, but are not limited to, furen, thiophene, pyrrole, thiadiazole (e.g., 1,2,3-thiadiazole or 1,2,4-thiadiazole), oxadiazole (e.g., 1,2,3-oxadiazole or 1,2,5-oxadiazole), oxazole, benzoxazole, isoxazole, isothiazole, pyrazole, thiazole, benzthiazole, triazole (e.g., 1,2,4-triazole or 1,2,3-triazole), benzotriazole, pyridines, pyrimidines, pyrazines, quinoline, isoquinoline, purine, pyrazine, pteridine, triazine (e.g., 1,2,3-triazine, 1,2,4-triazine, or 1,3,5-triazine), indoles, 1,2,4,5-tetrazine, benzo[e]thiophene, benzo[c]thiophene, benzofuran, isobenzofuran, and benzimidazole. Heteroaryls may be unsubstituted or substituted. Substituted heteroaryls can have, for example, 1, 2, 3, 4, 5, or 6 substituents.

By "heterocyclic" or "heterocyclyl" is meant an optionally substituted non-aromatic, partially unsaturated or fully saturated, 3- to 10-membered ring system, which includes single rings of 3 to 8 atoms in size, and polycyclic ring systems (e.g., bi- and tri-cyclic ring systems) which may include an aryl (e.g., phenyl or naphthyl) or heteroaryl group that is fused to a non-aromatic ring (e.g., cycloalkyl, cycloalkenyl, or heterocyclyl), where the ring system contains at least one heterotom. Heterocyclic rings include those having from one to three heteroatoms independently selected from oxygen, sulfur, and nitrogen, in which the nitrogen and sulfur heteroatoms may optionally be oxidized and the nitrogen heteroatom may optionally be quaternized or substituted. In certain embodiments, the term heterocyclic
refers to a non-aromatic 5-, 6-, or 7-membered monocyclic ring wherein at least one ring atom is a heteroatom selected from O, S, and N (wherein the nitrogen and sulfur heteroatoms may be optionally oxidized), and the remaining ring atoms are carbon, the radical being joined to the rest of the molecule via any of the ring atoms. Where a heterocycle is polycyclic, the constituent rings may be fused together, form a spirocyclic structure, or the polycyclic heterocycle may be a bridged heterocycle (e.g., quinuclidyl or). Exemplary heterocyclics include, but are not limited to, aziridinyl, azetidinyl, 1,3-diazatidinyl, pyrrolidinyl, piperidinyl, piperalinyl, thiranyl, thietanyl, tetrahydrothiophenyl, dithiolanyl, tetrahydrothiopyranyl, oxiranyl, oxetanyl, tetrahydrofuranyl, tetrahydropyranyl, pyranonyl, 3,4-dihydro-2H-pyranyl, chromenyl, 2H-chromen-2-onyl, chromanyl, dioxanyl (e.g., 1,3-dioxanyl or 1,4-dioxanyl), 1,4-benzodioxanyl, oxazinyl, oxathiolanyl, morpholinyl, thiomorpholinyl, thioxanly, quinuclidinyl, and also derivatives of said exemplary heterocycles where the heterocyclic is fused to an aryl (e.g., a benzene ring) or a heteroaryl (e.g., a pyridine or pyrimidine) group. Any of the heterocyclic groups described herein may be unsubstituted or substituted. A substituted heterocycle may have, for example, 1, 2, 3, 4, 5, or 6 substituents. As used herein, the term "isotopic enrichment factor" refers to the ratio of the isotopic abundance of a composition to the natural abundance of the specified isotope. For example, deuterium has a natural abundance of 0.015%. A compound with, for example, 45% deuterium incorporation at a specified position, has an isotopic enrichment factor of 3000 at that site relative to the natural abundance of deuterium.

The term "inflammatory condition" refers to medical disorders in which inflammation is a causative factor, or in which inflammation is a result (e.g., inflammatory pain associated with rheumatoid arthritis, psoriatic arthritis, psoriatic arthropathies, lupus, or other diseases associated with tissue damage). Inflammatory conditions can be chronic or acute, and non-limiting causes of inflammatory conditions include pathogens (e.g., bacterial pathogens or viral infections), tissue injury, persistent foreign bodies, and autoimmune responses. As described herein, inflammation can be related to necrosis or necroptosis, or inflammation can be independent of necrosis or necroptosis.

By "ketone" or "acyl" is meant a group having the structure -COR', where R' is selected from H, optionally substituted C1-C6 alkyl, optionally substituted cycloalkyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl. When R' is not H, R may be unsubstituted or substituted with, for example, 1, 2, 3, 4, 5, or 6 substituents. By "nitro" is meant a group having the structure -NO2.

A "pharmaceutically acceptable excipient" as used herein refers any ingredient other than the compounds described herein (for example, a vehicle capable of suspending or dissolving the active compound) and having the properties of being nontoxic and non-inflammatory in a patient. Excipients may include, for example: antiadherents, antioxidants, binders, coatings, compression aids, disintegrants, dyes (colors), emollients, emulsifiers, fillers (diluents), film formers or coatings, flavors, fragrances, glidants (flow enhancers), lubricants, preservatives, printing inks, sorbents, suspending or dispersing agents, sweeteners, or waters of hydration. Exemplary excipients include, but are not limited to: butylated hydroxytoluene (BHT), calcium carbonate, calcium phosphate (dibasic), calcium stearate, croscarmelllose, crosslinked polyvinyl pyrrolidone, citric acid, croscevode, cysteine, ethylcellulose, gelatin, hydroxypropyl cellulose, hydroxypropyl methylcellulose, lactose, magnesium stearate, maltitol, mannitol, methionine, methylcellulose, methyl paraben, microcrystalline cellulose, polyethylene glycol, polyvinyl pyrrolidone, povidone, pregelatinized starch, propyl paraben, retinyl palm itate, shellac, silicon...
dioxide, sodium carboxymethyl cellulose, sodium citrate, sodium starch glycolate, sorbitol, starch (corn),
stearic acid, stearic acid, sucrose, talc, titanium dioxide, vitamin A, vitamin E, vitamin C, and xylitol.

The term "pharmacologically acceptable salt," as used herein, represents those salts which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and
animals without undue toxicity, irritation, allergic response and the like and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art. For example,
S. M. Berge et al. describe pharmaceutically acceptable salts in detail in J. Pharmaceutical Sciences,
1977, 66:1-19. The salts can be prepared in situ during the final isolation and purification of the
compounds of the invention or separately by reacting the free base group with a suitable organic acid.

Representative acid addition salts include acetate, adipate, alginolate, ascorbate, aspartate,
benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphersulfonate, citrate,
cyclopentanepropionate, digluconate, doceylsulfate, ethanesulfonate, fumarate, glucoheptonate,
glycerophosphate, hemisulfate, heptonate, hexanoate, hydrobromide, hydrochloride, hydroiodide, 2-
hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate,
methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate,
pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate,
sulfate, tartrate, thiodicarbonate, toluenesulfonate, undecanoate, valerate salts and the like. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium and the like,
as well as nontoxic ammonium, quaternary ammonium, and amine cations, including, but not limited to
ammonium, tetramethylammonium, tetraethylammonium, methylamine, dimethylam ine, trimethylam ine,
triethylam ine, ethylamine and the like.

The term "pharmacologically acceptable solvates," as used herein, refers to compounds that retain non-covalent associations to residual solvent molecules in the solid state. For example, solvates may be prepared by crystallization, recrystallization, or precipitation from a solution that includes organic solvents, water, or a mixture thereof. Solvates include, but are not limited to, compounds that include solvent molecules in the crystal lattice following recrystallization. The molecular stoichiometry of solvation can vary from, for example, 1:1 solvent:compound to 10:1 solvent:compound. These ratios can include a mixture of associated solvent molecules. Exemplary, non-limiting examples of solvents that can form solvates with the compounds of the invention include water (for example, mono-, di-, and tri-hydrates), N-
methylpyrrolidinone (NMP), dimethyl sulfoxide (DMSO), N,N-dimethylformamide (DMF), N,N:
dimethylacetamide (DMAC), 1,3-dimethyl-2-imidazolidinone (DMEU), 1,3-dimethyl-3,4,5,6-tetrahydro-2-
(1H)-pyrimidinone (DMPU), acetonitrile (ACN), propylene glycol, ethyl acetate, benzyl alcohol, 2-
pyrrolidone, benzyl benzoate, or any combination thereof.

By "pharmaceutical composition" is meant a composition containing a compound of the invention,
formulated with a pharmaceutically acceptable excipient, and manufactured or sold with the approval of a governmental regulatory agency as part of a therapeutic regimen for the treatment of disease in a
mammal. Excipients consisting of DMSO are specifically excluded. Pharmaceutical compositions can be formulated, for example, for oral administration in unit dosage form (e.g., a tablet, capsule, gelcap,
or syrup); for topical administration (e.g., as a cream, gel, lotion, or ointment); for intravenous
administration (e.g., as a sterile solution free of particulate emboli and in a solvent system suitable for
intravenous use); or any other formulation described herein.
By "stereoisomer" is meant a diastereomer, enantiomer, or epimer of a compound. A chiral center in a compound may have the $S$-configuration or the $R$-configuration. Enantiomers may also be described by the direction in which they rotate polarized light (i.e., (+) or (−)). Diastereomers of a compound include stereoisomers in which some, but not all, of the chiral centers have the opposite configuration as well as those compounds in which substituents are differently oriented in space (for example, trans versus cis).

Where a group is substituted, the group may be substituted with 1, 2, 3, 4, 5, or 6 substituents. Optional substituents include, but are not limited to: deuterium (-D), C1-C6 alkyl, C2-C6 alkenyl, C2-C6 alkynyl, cycloalkyl, cycloalkenyl, heterocyclyl, aryl, heteroaryl, halogen; azido(-N$_3$), nitro (-NO$_2$), cyano (-CN), acyloxy(-OC(=0) R'), alkoxy (-OR'), amido (-NR'C(=0) R'R' or -C(=0)NRR'), amino (-NRR'), carboxylic acid (-C0$_2$H), carboxylic ester (-C0$_2$ R'), carbamoyl (-OC(=0)N R'R' or -NR(=0)OR'), hydroxy (-OH), isocyno (-NC), sulfonate (-S(=O)$_2$OR), sulfonamide (-S(=O)N$_2$R'R' or -NR(=O)$_2$R'), or sulfonyl (-S(=O)$_2$R), where each R, R', or R'' is selected, independently, from H, C1-C6 alkyl, C2-C6 alkenyl, C2-C6 alkynyl, cycloalkyl, heterocyclyl, aryl, or heteroaryl. A substituted group may have, for example, 1, 2, 3, 4, 5, 6, 7, 8, or 9 substituents. In some embodiments, each hydrogen in a group may be replaced by a substituent group (e.g., perhaloalkyl groups such as -CF$_3$ or -CF$_2$CF$_3$ or perhaloaryls such as -CF$_6$F$_3$). In other embodiments, a substituent group may itself be further substituted by replacing a hydrogen of said substituent group with another substituent group such as those described herein. Substituents may be further substituted with, for example, 1, 2, 3, 4, 5, or 6 substituents as defined herein. For example, a lower C1-C6 alkyl or an aryl substituent group (e.g., heteroaryl, phenyl, or naphthyl) may be further substituted with 1, 2, 3, 4, 5, or 6 substituents (e.g., deuterium) as described herein.

**Detailed Description of the Invention**

Described herein are a series of heterocyclic derivatives that can inhibit tumor necrosis factor alpha (TNF-α)-induced necroptosis. The heterocyclic compounds of the invention are described by, e.g., any of Formulas (I)-(VI), and can inhibit TNF-α induced necroptosis in FADD-deficient variant of human Jurkat T cells. Pharmaceutical compositions including the compounds of the invention are also described. The invention also features kits and methods of treatment featuring the compounds and compositions of the invention.

The present invention features compounds, pharmaceutical compositions, kits, and methods for treating a range of conditions, e.g., those in which cell or tissue necrosis is a causative factor or result, those in which loss of proliferative capacity is a causative factor or a result, those in which cytokines of the TNF$\alpha$ family are a causative factor or a result, and those in which RIP1 and/or RIP3 protein is a contributing factor. The compounds of the present invention can be used, for example, as therapeutics to decrease necrosis in a desired cell, to increase cell proliferation, to stimulate immune response, or to modulate inflammatory conditions. In some embodiments, the compounds of the present invention can also be used, for example, to treat conditions where necroptosis is likely to play a substantial role, including, but not limited to those described herein.

Exemplary conditions in which the compounds of the invention can be useful for treatment include, but are not limited to: neurodegenerative diseases of the central or peripheral nervous system; the result of retinal neuronal cell death; the result of cell death of cardiac muscle; the result of cell death
of cells of the immune system; stroke; liver disease; pancreatic disease; the result of cell death associated with renal failure; heart, mesenteric, retinal, hepatic or brain ischemic injury; ischemic injury during organ storage; head trauma; septic shock; coronary heart disease; cardiomyopathy; bone avascular necrosis; sickle cell disease; muscle wasting; gastrointestinal disease; tuberculosis; diabetes; alteration of blood vessels; muscular dystrophy; graft-versus-host disease; viral infection; Crohn's disease; ulcerative colitis; asthma; atherosclerosis; pain (e.g., inflammatory pain, diabetic pain, or pain associated from trauma or burn); chronic or acute inflammatory conditions such as rheumatoid arthritis, psoriasis, and Stevens-Johnson syndrome; any condition in which cell or tissue necrosis is a causative factor or result; any condition in which alteration in cell proliferation, differentiation or intracellular signaling is a causative factor; and any condition in which RIP1 and/or RIP3 protein is a contributing factor. Other conditions are described herein.

The invention features isolated compounds that can be described generally by Formula (I) and isotopically enriched compositions that include compounds that can be described generally by Formula (I). Formula (I) has the following structure,

(I), or any pharmaceutically acceptable salt thereof, or stereoisomer thereof, where

X¹ is O or NR¹⁰;

each of X² and X³ is, independently, 0 or S;
R¹ is H, D, or optionally substituted C1-C6 alkyl;
R² is H or D;
each of R³⁸ and R³⁹ is, independently, H, D, or optionally substituted C1-C6 alkyl;
each of R⁴, R⁵, and R⁶ is, independently, H or D;
R⁷ is H, D, halogen, optionally substituted C1-C6 alkyl, or optionally substituted C1-C6 alkoxy;
each of R⁸, R⁹, and R¹⁰ is, independently, H, D, or optionally substituted C1-C6 alkyl; and where at least one of R¹⁻R¹⁰ is D or includes a deuterium group.

Compositions that include compounds according to formula (I) can have an isotopic enrichment factor for deuterium of at least 5 (e.g., at least 5, 10, 50, 100, 500, 1000, or 6000).
Certain embodiments of Formula (I) include compounds according to any of Formulas (II)-(VIII):

or a pharmaceutically acceptable salt thereof, or a stereoisomer thereof, and where

\[ X^1 \] is NH or O;

\[ R^7 \] is halogen, optionally substituted C1-C2 alkyl, or optionally substituted C1-C2 alkoxy; and

\[ R^9 \] is optionally substituted C1-C2 alkyl.

In some embodiments, the carbon that bears the \( X^1 \) group has the \(^\wedge\)-configuration. In other embodiments, the carbon that bears the \( X^1 \) group has the \(^/\wedge\)-configuration.

Compounds of the invention may include, for example, 1-10 deuterium atoms.

In any of the embodiments of the invention described herein, \( R^1-R^{10} \) can include, e.g., 1, 2, 3, 4, 5, or 6 deuterium groups. In compounds that feature one or more deuterated substituent groups, at least one hydrogen has been replaced with deuterium; e.g., 1, 2, 3, 4, 5, or 6 hydrogens are replaced with deuterium. In some deuterated substituents, all hydrogens have been replaced with deuterium. In some embodiments, a substituent is not further substituted. Additional optional substituents for various groups are described herein.

The deuterated compounds and compositions described herein (e.g., an isolated compound having a structure according to any of Formulas (I)-(VIII), or an isotopically enriched composition that includes a compound according to any of Formulas (I)-(VIII)) can show improved physicochemical
properties (e.g., improved metabolic stability) and can show increased potency in inhibition of necroptosis.

**Pharmaceutical Compositions**

Compounds of the invention (e.g., compounds of Formulas (I)-(VI III)) can be formulated into pharmaceutical compositions for administration to human subjects in a biologically compatible form suitable for administration in vivo. Accordingly, the present invention provides a pharmaceutical composition comprising a compound of the invention in admixture with a pharmaceutically acceptable excipient. Conventional procedures and ingredients for the selection and preparation of suitable formulations are described, for example, in Remington's Pharmaceutical Sciences (2003 - 20th edition) and in The United States Pharmacopeia: The National Formulary (USP 24 NF 19), published in 1999.

The compounds of the invention may be used in the form of the free base, in the form of salts, solvates, and as prodrugs. All forms are within the scope of the invention. In accordance with the methods of the invention, the described compounds or salts, solvates, or prodrugs thereof may be administered to or taken by a patient in a variety of forms depending on the selected route of administration, as will be understood by those skilled in the art. For example, the compounds of the invention may be administered by, e.g., oral, parenteral, buccal, sublingual, nasal, rectal, patch, pump, or transdermal administration and the pharmaceutical compositions formulated accordingly. Parenteral administration includes intravenous, intraperitoneal, subcutaneous, intramuscular, transepithelial, nasal, intrapulmonary, intrathecal, rectal, and topical modes of administration. Parenteral administration may be by continuous infusion over a selected period of time.

**Pharmaceutically Acceptable Excipients**

Pharmaceutically acceptable excipients may include, for example: antiadherents, antioxidants, binders, coatings, compression aids, disintegrants, dyes (colors), emollients, emulsifiers, fillers (diluents), film formers or coatings, flavors, fragrances, glidants (flow enhancers), lubricants, preservatives, printing inks, sorbents, suspending or dispersing agents, sweeteners, or waters of hydration. Exemplary excipients include, but are not limited to: butylated hydroxytoluene (BHT), calcium carbonate, calcium phosphate (dibasic), calcium stearate, croscarmellose, crosslinked polyvinyl pyrrolidone, citric acid, crospovidone, cysteine, ethylcellulose, gelatin, hydroxypropyl cellulose, hydroxypropyl methylcellulose, lactose, magnesium stearate, maltitol, mannitol, methionine, methylcellulose, methyl paraben, microcrystalline cellulose, polyethylene glycol, polyvinyl pyrrolidone, povidone, pregelatinized starch, propyl paraben, retinyl palmitate, shellac, silicon dioxide, sodium carboxymethyl cellulose, sodium citrate, sodium starch glycolate, sorbitol, starch (corn), stearic acid, stearic acid, sucrose, talc, titanium dioxide, vitamin A, vitamin E, vitamin C, and xylitol.

**Oral Administration**

A compound of the invention may be orally administered, for example, with an inert diluent or with an assimilable edible carrier, or it may be enclosed in hard or soft shell gelatin capsules, or it may be compressed into tablets, or it may be incorporated directly with the food of the diet. For oral therapeutic administration, a compound of the invention may be incorporated with an excipient and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like.
**Parenteral Administration**

A compound of the invention may also be administered parenterally. The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporary preparation of sterile injectable solutions or dispersions. In all cases the form must be sterile and must be fluid to the extent that may be easily administered via syringe.

**Nasal Administration**

Compositions for nasal administration may conveniently be formulated as aerosols, drops, gels, and powders. Aerosol formulations typically include a solution or fine suspension of the active substance in a physiologically acceptable aqueous or non-aqueous solvent and are usually presented in single or multidose quantities in sterile form in a sealed container, which can take the form of a cartridge or refill for use with an atomizing device. Alternatively, the sealed container may be a unitary dispensing device, such as a single dose nasal inhaler or an aerosol dispenser fitted with a metering valve which is intended for disposal after use. Where the dosage form comprises an aerosol dispenser, it will contain a propellant, which can be a compressed gas, such as compressed air or an organic propellant, such as fluorochlorohydrocarbon. The aerosol dosage forms can also take the form of a pump-atomizer.

**Buccal or Sublingual Administration**

Compositions suitable for buccal or sublingual administration include tablets, lozenges, and pastilles, where the active ingredient is formulated with a carrier, such as sugar, acacia, tragacanth, or gelatin and glycerine. Compositions for rectal administration are conveniently in the form of suppositories containing a conventional suppository base, such as cocoa butter.

The compounds of the invention may be administered to an animal alone or in combination with pharmaceutically acceptable carriers, as noted above, the proportion of which is determined by the solubility and chemical nature of the compound, chosen route of administration, and standard pharmaceutical practice.

**Dosage Amounts**

The amount of active ingredient in the compositions of the invention can be varied. One skilled in the art will appreciate that the exact individual dosages may be adjusted somewhat depending upon a variety of factors, including the protein being administered, the time of administration, the route of administration, the nature of the formulation, the rate of excretion, the nature of the subject’s conditions, and the age, weight, health, and gender of the patient. Generally, dosage levels of between 0.1 µg/kg to 100 mg/kg of body weight are administered daily as a single dose or divided into multiple doses. Desirably, the general dosage range is between 250 µg/kg to 5.0 mg/kg of body weight per day. Wide variations in the needed dosage are to be expected in view of the differing efficiencies of the various routes of administration. For instance, oral administration generally would be expected to require higher dosage levels than administration by intravenous injection. Variations in these dosage levels can be adjusted using standard empirical routines for optimization, which are well known in the art. In general, the precise therapeutically effective dosage will be determined by the attending physician in consideration of the above identified factors.
Therapeutic Uses

Cell death has traditionally been categorized as either apoptotic or necrotic based on morphological characteristics (Wyllie et al., Int. Rev. Cytol. 68: 251 (1980)). These two modes of cell death were also initially thought to occur via regulated (caspase-dependent) and non-regulated processes, respectively. Subsequent studies, however, demonstrate that the underlying cell death mechanisms resulting in these two phenotypes are much more complicated and under some circumstances interrelated. Furthermore, conditions that lead to necrosis can occur by either regulated caspase-independent or non-regulated processes.

One regulated caspase-independent cell death pathway with morphological features resembling necrosis, called necroptosis, has been described (Degterev et al., Nat. Chem. Biol. 1:112 (2005)). This manner of cell death can be initiated with various stimuli (e.g., TNF-a and Fas ligand) and in an array of cell types (e.g., monocytes, fibroblasts, lymphocytes, macrophages, epithelial cells and neurons). Necroptosis may represent a significant contributor to and in some cases predominant mode of cellular demise under pathological conditions involving excessive cell stress, rapid energy loss and massive oxidative species generation, where the highly energy-dependent apoptosis process is not operative.

The identification and optimization of low molecular weight molecules capable of inhibiting necroptosis will assist in elucidating its role in disease patho-physiology and can provide compounds (i.e., necrostatins) for anti-necroptosis therapeutics. The discovery of compounds that prevent caspase-independent cell death (e.g., necrosis or necroptosis) would also provide useful therapeutic agents for treating or preventing conditions in which necrosis occurs. For example, necrostatins can suppress necroptosis by specifically inhibiting receptor interacting protein 1 (RIP1) activity (e.g., Xie et al., Structure, 21(3):493-499, 2013). RIP3, which is a RIP1 family member, has also been implicated in necroptosis (see, e.g., Christofferson et al., Curr. Opin. Cell Biol. 22(2):263-268, 2010). Accordingly, methods by which RIP1 and/or RIP3 activity can be modulated can also be useful for the treatment of conditions in which RIP1 and/or RIP3 protein is a contributing factor.

These compounds and methods would be particularly useful for the treatment of neurodegenerative diseases, ischemic brain and heart injuries, and head trauma. Exemplary assays for identifying inhibitors of necrosis and necroptosis are described herein in the Examples.

Accordingly, the compounds and compositions disclosed herein can be used to treat disorders where necroptosis is likely to play a substantial role or where RIP1 and/or RIP3 protein is a contributing factor. Exemplary conditions that can be treated using the methods described herein include: cerebral ischemia, traumatic brain injury (Gennarelli et al. In Textbook of Traumatic Brain Injury; Silver et al., Eds.; American Psychiatric Publishing Inc.: Washington DC, 2005; p 37), a neurodegenerative disease of the central or peripheral nervous system (Martin et al. Brain Res. Bull. 1998, 46, 281), the result of retinal neuronal cell death, the result of cell death of cardiac muscle, the result of cell death of cells of the immune system; organ ischemia such as stroke (Lo et al. Nat. Rev. Neurosci. 2003, 4, 399.), myocardial infarction (McCully et al. Am. J. Physiol. Heart Circ. Physiol. 2004, 286, H1923), or retinal ischemia (Osborne et al. Prog. Retin. Eye Res. 2004, 23, 91); liver disease (Kaplowitz, J. Hepatol. 2000, 32 (1 Suppl.), 39; Malhi et al. Hepatology 2006, 43 (2 Suppl. 1), S31; and Ferrell et al. In Pathology of the Liver, 4th Edition; MacSween et al., Eds.; Churchill Livingstone: London, 2002; p 314), pancreatic disease, the result of cell death associated with renal failure; heart, mesenteric, retinal, hepatic or brain ischemic...
injury, ischemic injury during organ storage, head trauma, septic shock, coronary heart disease, cardiomyopathy, myocardial infarction, bone avascular necrosis, sickle cell disease, muscle wasting, gastrointestinal disease, tuberculosis, diabetes, alteration of blood vessels, muscular dystrophy, graft-versus-host disease, viral infection, Crohn's disease, ulcerative colitis, asthma, or any condition in which alteration in cell proliferation, differentiation or intracellular signaling is a causative factor; cancer chemo/radiation therapy-induced necrosis (Giglio et al. Neurologist 2003, 9, 180; Ramesh et al. Am. J. Physiol Renal Physiol. 2003, 285, F610; and Miyaguchi et al. J. Laryngol. Otol. 1997, 111, 763); acute necrotizing pancreatitis (Rosai. In Rosai and Ackerman's Surgical Pathology, 11th Edition; Mosby: New York, 2004; Vol. 1, p 1063; Wrobleski et al. J. AACN Clin. Issues 1999, 10, 464; and Mareninova et al., J Biol Chem. 2006, 257, 3370); atherosclerosis (e.g., Lin et al., Cell Reports, 3:200-210, 2013); and inflammatory conditions (e.g., Wallach et al., Trends in Immunology, 32(11):505-509, 2011; Kang et al., Immunity, 38:27-40, 2013; and Chan, Cold Spring Harb. Perspect. Biol., 1:12, 2012). Compounds of the invention can also be used in screening methods to identify targets of necroptosis and to identify additional inhibitors of necroptosis, as well as in assay development.

The compounds and compositions disclosed herein can be evaluated for their pharmacological properties in animal models of disease. The compounds identified to decrease necrosis or necroptosis may be structurally modified and subsequently used to decrease necrosis or necroptosis, or to treat a subject with a condition in which necrosis or necroptosis occurs. The methods used to generate structural derivatives of the small molecules that decrease necrosis or necroptosis are readily known to those skilled in the fields of organic and medicinal chemistry.

Therapy according to the invention may be performed alone or in conjunction with another therapy, for example in combination with apoptosis inhibitors, and may be provided at home, the doctor's office, a clinic, a hospital's outpatient department, or a hospital. Treatment generally begins at a hospital so that the doctor can observe the therapy's effects closely and make any adjustments that are needed. The duration of the therapy depends on the age and condition of the patient, as well as how the patient responds to the treatment. Additionally, a person having a greater risk of developing a condition may receive prophylactic treatment to inhibit or delay symptoms of the disease.

In some embodiments, the compounds and compositions described herein can be used to treat any of the following disorders where necroptosis is likely to play a substantial role: a neurodegenerative disease of the central or peripheral nervous system, the result of retinal neuronal cell death, the result of cell death of cardiac muscle, the result of cell death of cells of the immune system; stroke, liver disease, pancreatic disease, the result of cell death associated with renal failure; heart, mesenteric, retinal, hepatic or brain ischemic injury, ischemic injury during organ storage, head trauma, septic shock, coronary heart disease, cardiomyopathy, myocardial infarction, bone avascular necrosis, sickle cell disease, muscle wasting, gastrointestinal disease, tuberculosis, diabetes, alteration of blood vessels, muscular dystrophy, graft-versus-host disease, viral infection, bacterial infection, Crohn's disease, ulcerative colitis, asthma, and any condition in which alteration in cell proliferation, differentiation or intracellular signaling is a causative factor.
Conditions Caused by Alteration in Cell Proliferation, Differentiation, or Intracellular Signaling

Conditions in which alteration in cell proliferation, differentiation or intracellular signaling is a causative factor include cancer and infection, e.g., by viruses (e.g., acute, latent and persistent), bacteria, fungi, or other microbes.

Exemplary viruses are human immunodeficiency virus (HIV), Epstein-Barr virus (EBV), cytomegalovirus (CMV), human herpesviruses (HHV), herpes simplex viruses (HSV), human T-Cell leukemia viruses (HTLV), Varicella-Zoster virus (VZV), measles virus, papovaviruses (JC and BK), hepatitis viruses, adenovirus, parvoviruses, and human papillomaviruses. Exemplary diseases caused by viral infection include, but are not limited to, chicken pox, Cytomegalovirus infections, genital herpes, Hepatitis B and C, influenza, and shingles.

Exemplary bacteria include, but are not limited to Campylobacter jejuni, Enterobacter species, Enterococcus faecium, Enterococcus faecalis, Escherichia coli (e.g., E. coli 0157:H7), Group A streptococci, Haemophilus influenzae, Helicobacter pylori, listeria, Mycobacterium tuberculosis, Pseudomonas aeruginosa, S. pneumoniae, Salmonella, Shigella, Staphylococcus aureus, and Staphylococcus epidermidis. Exemplary diseases caused by bacterial infection include, but are not limited to, anthrax, cholera, diphtheria, foodborne illnesses, leprosy, meningitis, peptic ulcer disease, pneumonia, sepsis, tetanus, tuberculosis, typhoid fever, and urinary tract infection.

Neurodegenerative Diseases

Exemplary neurodegenerative diseases are Alzheimer's disease, Huntington's disease, Parkinson's disease, amyotrophic lateral sclerosis, HIV-associated dementia, cerebral ischemia, amyotrophic lateral sclerosis, multiple sclerosis, Lewy body disease, Menke's disease, Wilson's disease, Creutzfeldt-Jakob disease, Fahr disease, and progressive supranuclear palsy. Exemplary muscular dystrophies or related diseases are Becker's muscular dystrophy, Duchenne muscular dystrophy, myotonic dystrophy, limb-girdle muscular dystrophy, Landouzy-Dejerine muscular dystrophy, facioscapulohumeral muscular dystrophy (Steinert's disease), myotonia congenita, Thomsen's disease, and Pompe's disease. Muscle wasting can be associated with cancer, AIDS, congestive heart failure, and chronic obstructive pulmonary disease, as well as include necrotizing myopathy of intensive care.


Exemplary muscular dystrophies or related diseases are Becker's muscular dystrophy, Duchenne muscular dystrophy, myotonic dystrophy, limb-girdle muscular dystrophy, Landouzy-Dejerine muscular dystrophy, facioscapulohumeral muscular dystrophy (Steinert's disease), myotonia congenita, Thomsen's disease, and Pompe's disease.

Muscle wasting can be associated with cancer, AIDS, congestive heart failure, and chronic obstructive pulmonary disease, as well as include necrotizing myopathy of intensive care.

The compounds and compositions described herein can additionally be used to boost the immune system, whether or not the patient being treated has an immunocompromising condition. For example, the compounds described herein can be used in a method to strengthen the immune system during immunization, e.g., by functioning as an adjuvant, or by being combined with an adjuvant.
The compounds and compositions described herein can also be used to treat inflammatory conditions, which may be, e.g., chronic or acute. Exemplary inflammatory conditions include: alkylating spondylitis, arthritis (e.g., osteoarthritis, rheumatoid arthritis (RA), and psoriatic arthritis), asthma, atherosclerosis, Crohn's disease, colitis, dermatitis, diverticulitis, fibromyalgia, hepatitis, irritable bowel syndrome (IBS), psoriasis, Stevens-Johnson syndrome, systemic lupus erythematosus (SLE), nephritis, and ulcerative colitis. Still other inflammatory conditions include: immunoinflammatory disorders such as acne vulgaris; acute respiratory distress syndrome; Addison's disease; allergic rhinitis; allergic intraocular inflammatory diseases, ANCA-associated small-vessel vasculitis; ankylosing spondylitis; arthritis, asthma; atherosclerosis; atopic dermatitis; autoimmune hemolytic anemia; autoimmune hepatitis; Behcet's disease; Bell's palsy; bullous pemphigoid; cerebral ischemia; chronic obstructive pulmonary disease; cirrhosis; Cogan's syndrome; contact dermatitis; COPD; Crohn's disease; Cushing's syndrome; dermatomyositis; diabetes mellitus; discoid lupus erythematosus; eosinophilic fasciitis; erythema nodosum; exfoliative dermatitis; fibromyalgia; focal glomerulosclerosis; giant cell arteritis; gout; glycyrrhiza; graft-versus-host disease; hand eczema; Henoch-Schonlein purpura; herpes gestationis; hirsutism; idiopathic cerato-scleritis; idiopathic pulmonary fibrosis; idiopathic thrombocytopenic purpura; inflammatory bowel or gastrointestinal disorders, inflammatory dermatoses; lichen planus; lupus nephritis; lymphomatous tracheobronchitis; macular edema; multiple sclerosis; myasthenia gravis; myositis; osteoarthritis; pancreatitis; pemphigoid gestationis; pemphigus vulgaris; polyarteritis nodosa; polymyalgia rheumatica; pruritus scroti; pruritus/inflammation, psoriasis; psoriatic arthritis; rheumatoid arthritis; relapsing polychondritis; rosacea caused by sarcoidosis; rosacea caused by scleroderma; rosacea caused by Sweet's syndrome; rosacea caused by systemic lupus erythematosus; rosacea caused by urticaria; rosacea caused by zoster-associated pain; sarcoidosis; scleroderma; segmental glomerulosclerosis; septic shock syndrome; shoulder tendinitis or bursitis; Sjogren's syndrome; Still's disease; stroke-induced brain cell death; Sweet's disease; systemic lupus erythematosus; systemic sclerosis; Takayasu's arteritis; temporal arteritis; toxic epidermal necrolysis; tuberculosis; type-1 diabetes; ulcerative colitis; uveitis; vasculitis; and Wegener's granulomatosis.

Further, the compounds and compositions described herein can also be used in the treatment or prevention of pain, including nociceptive pain, inflammatory pain, functional pain and neuropathic pain, all of which may be acute or chronic. For example, the subject (e.g., a human) being treated may be diagnosed as having peripheral diabetic neuropathy, compression neuropathy, post herpetic neuralgia, trigeminal or glossopharyngeal neuralgia, post traumatic or post surgical nerve damage, lumbar or cervical radiculopathy, AIDS neuropathy, metabolic neuropathy, drug induced neuropathy, complex regional pain syndrome, arachnoiditis, spinal cord injury, bone or joint injury, tissue injury, psoriasis, scleroderma, pruritus, cancer (e.g., prostate, colon, breast, skin, hepatic, or kidney), cardiovascular disease (e.g., myocardial infarction, angina, ischemic or thrombotic cardiovascular disease, peripheral vascular occlusive disease, or peripheral arterial occlusive disease), sickle cell anemia, migraine cluster or tension-type headaches, inflammatory conditions of the skin, muscle, or joints, fibromyalgia, irritable bowel syndrome, non cardiac chest pain, cystitis, pancreatitis, or pelvic pain. Alternatively, the pain may be the result of or associated with trauma (e.g., a traumatic injury), diabetes, surgery, burn of the cutaneous tissue (caused by a thermal, chemical, or radiation stimulus), or a sunburn.

Additional conditions that can be treated using the compounds provided herein include those described in, e.g.: U.S. Patent Nos. 6,756,394; 7,253,201; 7,491,743; 8,143,300; 8,278,344; and...

Combination therapy

If desired, treatment with the compounds and compositions described herein can be combined with therapies for the treatment of any of the conditions described herein. Such treatments include surgery, radiotherapy, chemotherapy, or the administration of one or more additional compounds.

Exemplary compounds suitable for combination therapy with Nec compounds are described below.

The compounds and compositions described herein can be administered in combination with compounds that are apoptosis inhibitors, i.e., compounds that inhibit apoptosis, including but not limited to reversible and irreversible caspase inhibitors. An example of an apoptosis inhibitor includes zVAD, IETD, YVAD, DEVD, and LEHD.

In some instances, the compounds of the invention are administered in combination with PARP poly(ADP-ribose) polymerase inhibitors. Non-limiting examples of PARP inhibitors include 6(5H)-phenantrindione, 4-Amino-1,8-naphthalimide, 1,5-isoquinoledioli, and 3-Am inobenzamide.

Compounds of the invention can also be administered in combination with Src inhibitors. Src proteins are mammalian cytoplasmic tyrosine kinases that play an extensive role in cell transduction.

Examples of Src inhibitors include but are not limited to: PP1 (1-(1,1-dimethylethyl)-1 -(4-methylphenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-am ine), PP2 (3-(4-chlorophenyl)-1-(1,1-dimethylethyl)-1H-pyrazolo[3,4-d]pyrim idin-4-amine), damnacanthal (3-hydroxy-1-methoxy-2-anthra-quinonecarboxaldehyde), and SU-5565.

The methods of the invention involve, in some aspects, combinations of compounds that are inhibitors of cellular necrosis (e.g., heterocyclic thiohydantoin, hydantoin, oxazolidinone, thiooxazolidinone, pyrimidinone, or oxazinanone compounds, or combinations thereof) with agents for the treatment of cardiovascular disorders. Such agents include anti-inflammatory agents, anti-thrombotic agents, anti-platelet agents, fibrinolytic agents, lipid reducing agents, direct thrombin inhibitors, glycoprotein IIb/IIIa receptor inhibitors, agents that bind to cellular adhesion molecules and inhibit the ability of white blood cells to attach to such molecules (e.g. anti-cellular adhesion molecule antibodies), calcium channel blockers, beta-adrenergic receptor blockers, cyclooxygenase-2 inhibitors, angiotensin system inhibitors, and any combinations thereof. One preferred agent is aspirin.

Anti-inflammatory agents include alclofenac; alclometasone dipropionate; algestone acetoniode; alpha amylase; amcinafal; amcinafide; amfenac sodium; amprilone hydrochloride; anakinra; anilorac; anitrazafen; apazone; balsalazine disodium; bendazac; benoxaprofen; benzylamine hydrochloride; bromelains; bronperamole; budesonide; carprofen; ciclopuren; cipentazine; ciplolen; clobetasol propionate; clobetasone butyrate; clopirac; cloticasone propionate; cornethasone acetate; cortodoxone; deflazacort; desonide; desoximetasone; dexamethasone dipropionate; diclofenac potassium; diclofenac sodium; diffloran sodium; diacetate; diffumide sodium; diffuralis; difluprednate; diftalone; dimethyl sulfoxide; drocinonide; endrysone; enlimomab; enolicam sodium; epirimole; etodolac; etofenamate; felbinac; fenamole; fenbufen; fenclonafen; fenclorac; fendosal; fenpipalone; fentiazac; flazalone; fluazacort; flufenamic acid; flumizole; flunisolide acetate; flunixin; flunixin meglumine; flucortin butyl; fluoromethalone acetate; fluquazone; flurbiprofen; fluretofen; fluticasone propionate; furaprofen;
furobufen; halcinonide; halobetasol propionate; halopredone acetate; ibufenac; ibuprofen; ibuprofen aluminum; ibuprofen piconol; ilonidap; indomethacin; indomethacin sodium; indopropen; indoxole; intrazole; isofluropredone acetate; isoxepac; isoxicam; ketoprofen; lofenazol hydrochloride; lomoxicam; lotepredon etabonate; meclofenamate sodium; meclofenamic acid; meclorinone dibutyrate; mefenamic acid; mesalamine; meseclazone; methylprednisolone sulfate; morniflumate; nabumetone; naproxen; naproxen sodium; naproxol; nimazine; olsalazine sodium; orgotein; orpanoxin; oxaprozin; oxyphenbutazone; paranyline hydrochloride; pentosan polysulfate sodium; phenbutazone sodium glycerate; pirfenidone; piroxicam; piroxicam cinnamate; piroxicam olamine; pirprofen; prednizaol; prifelone; prodolic acid; proquazone; prazoxide; prazoxol citrate; rimexolone; romazarit; salclex; salnacedin; salglate; salycilates; sanguinarium chloride; seclazone; sermetacil; sudoxicam; sulindac; suprofen; talmetacin; talniflumate; talosalate; tebufelone; tenidap; tenidap sodium; tenoxicam; tesamople; tesimide; tetrydamine; tiopinac; trifoxcord pivalate; tolmetin; tolmetin sodium; triclonide; triflum idate; zidometacin; glucoelectrodes; and zomepiric acid.

Anti-thrombotic and fibrinolytic agents include plasminogen (to plasmin via interactions of prekallikrein, kininogens, factors X I; X Ila, plasminogen proactivator, and tissue plasminogen activator (TPA)) streptokinase; urokinase; anisoylated plasminogen-streptokinase activator complex; pro-urokinase (pro-U K); rTPA (alteplase or activase); rPro-U K; abbokinase; eminase; streptase anagrelide hydrochloride; bivalirudin; dalteparin sodium; danaparoid sodium; dazoxiben hydrochloride; efgatran sulfate; enoxaparin sodium; ifetroban; ifetroban sodium; tinzaparin sodium; retaplase; trifinagrel; warfarin; and dextrins.

Anti-platelet agents include clopidogrel; sulfinpyrazone; aspirin; dipiridamole; clofibrate; pyridinol carbamate; PG E; glucagon; antiserotonin drugs; caffeine; theophyllin; pentoxifyllin; ticlopidine; and anagrelide.

Lipid reducing agents include gemfibrozol, cholestyramine, colestipol, nicotinic acid, probucol, lovastatin, fluvasatin, simvastatin, atorvastatin, pravastatin, and cirivastatin.

Direct thrombin inhibitors include hirudin, hirugen, hirulog, agatroban, PPACK, and thrombin aptamers.

Glycoprotein lbll lb receptor inhibitors include both antibodies and non-antibodies, and include but are not limited to ReoPro (abcixamab), lamifiban, and tiopiban.

Calcium channel blockers are a chemically diverse class of compounds having important therapeutic value in the control of a variety of diseases including several cardiovascular disorders, such as hypertension, angina, and cardiac arrhythmias (Fleckenstein, Curr. Res. 52:13-1 6 (1983); Fleckenstein, Experimental Facts and Therapeutic Prospects, John Wiley, New York (1983); McCall, D., Curr. Pract. Cardiol. 10:1 - 11 (1985)). Calcium channel blockers are a heterogenous group of drugs that prevent or slow the entry of calcium into cells by regulating cellular calcium channels. Remington, The Science and Practice of Pharmacy, Nineteenth Edition, Mack Publishing Company, Eaton, Pa., p. 963 (1995)). Most of the currently available calcium channel blockers, and useful according to the present invention, belong to one of three major chemical groups of drugs, the dihydropyridines, such as nifedipine, the phenyl alkyl amines, such as verapam II, and the benzothiazepines, such as diltilazem. Other calcium channel blockers useful according to the invention, include, but are not limited to, amrinone, amlopidine, bencyclane, felodipine, fendiline, flunarizine, isradipine, nicardipine, nimodipine, perhexylene, gallopamil,
tiapamil and tiapamil analogues (such as 1993RO-1 2933), phenyloin, barbiturates, and the peptides dynorphin, omega-conotoxin, and omega-agatoxin, and pharmaceutically acceptable salts thereof.

Beta-adrenergic receptor blocking agents are a class of drugs that antagonize the cardiovascular effects of catecholamines in angina pectoris, hypertension, and cardiac arrhythmias. Beta-adrenergic receptor blockers include, but are not limited to, atenolol, acebutolol, alpenolol, bufunolol, betaxolol, bunitrolol, carteolol, celeprolol, hydroxalol, indenolol, labelatol, levobunolol, meziprindol, metindol, metoprolol, metrizanol, oxrenolol, pindolol, propranolol, practolol, practolol, sotalol, nadolol, tiprenolol, tomadolol, timolol, bunaprolol, pethanolol, trimeparanol, 2-(3-(1,1-dimethyllethyl)-amino-2-hydroxypropoxy)-3-pyrindencarbonitrile HCl, 1-butylamino-3-(2,5-dichlorophenoxyc)-2-propanol, 1-isopropylamino-3-(4-(2-cyclopropylmethoxyethyl)phenoxy)-2-propanol, 3-isopropylamino-1-(7-methylindan-4-yloxy)-2-butanol, 2-(3-t-butylamino-2-hydroxy-propythio)-4-(5-carbamoyl-2-thienyl)thiazol-7(2-hydroxy-3-t-butylaminopropoxy)phthalide. These compounds can be used as isomeric mixtures, or in their respective levorotating or dextrorotating form.

Cyclooxygenase-2 (COX-2) is an enzyme complex present in most tissues that produces various prostaglandins and thromboxanes from arachidonic acid. A number of selective COX-2 inhibitors are known in the art. These include, but are not limited to, those described in U.S. Patent Nos. 5,474,995, 5,521,213, 5,536,752, 5,550,402, 5,552,422, 5,604,253, 5,604,260, 5,639,780, 5,677,318, 5,691,374, 5,698,584, 5,710,140, 5,733,909, 5,789,413, 5,815,700, 5,849,943, 5,861,419, 5,922,742, 5,925,631, and 5,643,933. A number of the above-identified COX-2 inhibitors are prodrugs of selective COX-2 inhibitors and exert their action by conversion in vivo to the active and selective COX-2 inhibitors. The active and selective COX-2 inhibitors formed from the above-identified COX-2 inhibitor prodrugs are described in detail in PCT/WO95/00501, PCT/WO95/18799, and U.S. Patent No. 5,474,995. Given the teachings of U.S. Patent No. 5,543,297, a person of ordinary skill in the art would be able to determine whether an agent is a selective COX-2 inhibitor or a precursor of a COX-2 inhibitor.

Angiotensin system inhibitors are capable of interfering with the function, synthesis or catabolism of angiotensin II. These agents include, but are not limited to, angiotensin-converting enzyme (ACE) inhibitors, angiotensin II antagonists, angiotensin II receptor antagonists, agents that activate the catabolism of angiotensin II, and agents that prevent the synthesis of angiotensin II from which angiotensin II is ultimately derived. The renin-angiotensin system is involved in the regulation of hemodynamics and water and electrolyte balance. Factors that lower blood volume, renal perfusion pressure, or the concentration of Na⁺ in plasma tend to activate the system, while factors that increase these parameters tend to suppress its function.

Angiotensin I and angiotensin II are synthesized by the enzymatic renin-angiotensin pathway. The synthetic process is initiated when the enzyme renin acts on angiotensinogen, pseudoglobulin in blood plasma, to produce the decapeptide angiotensin I. Angiotensin I is converted by angiotensin converting enzyme (ACE) to angiotensin II (angiotensin-[1-8] octapeptide). The latter is an active pressor substance which has been implicated as a causative agent in several forms of hypertension in various mammalian species, e.g., humans.

Angiotensin (renin-angiotensin) system inhibitors are compounds that act to interfere with the production of angiotensin II from angiotensinogen or angiotensin I or interfere with the activity of angiotensin II. Such inhibitors are well known to those of ordinary skill in the art and include compounds that act to inhibit the enzymes involved in the ultimate production of angiotensin II, including renin and
ACE. They also include compounds that interfere with the activity of angiotensin II once produced. Examples of classes of such compounds include antibodies (e.g., to renin), amino acids and analogs thereof (including those conjugated to larger molecules), peptides (including peptide analogs of angiotensin and angiotensin II, pro-renin related analogs, etc. Among the most potent and useful renin-angiotensin system inhibitors are renin inhibitors, ACE inhibitors, and angiotensin II antagonists. In a preferred embodiment of the invention, the renin-angiotensin system inhibitors are renin inhibitors, ACE inhibitors, and angiotensin II antagonists.

Angiotensin II antagonists are compounds which interfere with the activity of angiotensin II by binding to angiotensin II receptors and interfering with its activity. Angiotensin II antagonists are well known and include peptide compounds and non-peptide compounds. Most angiotensin II antagonists are slightly modified congeners in which agonist activity is attenuated by replacement of phenylalanine in position 8 with some other amino acid; stability can be enhanced by other replacements that slow degeneration in vivo. Examples of angiotensin II antagonists include: peptideic compounds (e.g., saralasin, [San''](Val)5[Ala]8) angiotensin-(1-8) octapeptide and related analogs; N-substituted imidazole-2-one (U.S. Patent No. 5,087,634); imidazole acetate derivatives including 2-N-butyl-4-chloro-1-((2-chlorobenzyl) imidazole-5-acetic acid (see Long et al., J. Pharmacol. Exp. Ther. 247(1), 1-7 (1988)); 4, 5, 6, 7-tetrahydro-1H-imidazo[4,5-c]pyridine-6-carboxylic acid and analog derivatives (U.S. Patent No. 4,816,463); N2-tetrazole beta-glucuronide analogs (U.S. Patent No. 5,085,992); substituted pyrroles, pyrazoles, and triazoles (U.S. Patent No. 5,081,27); phenyl and heterocyclic derivatives such as 1,3-imidazoles (U.S. Patent No. 5,073,566); imidazo-fused 7-member ring heterocycles (U.S. Patent No. 5,064,825); peptides (e.g., U.S. Patent No. 4,772,684); antibodies to angiotensin II (e.g., U.S. Patent No. 4,302,386); and aralkyl imidazole compounds such as biphenyl-methyl substituted imidazoles (e.g., EP Number 253,310, Jan. 20, 1988); ES8891 (N-morpholinoacetyl-(1-naphthyl)-L-alanyl-(4-thiazoyl)-L-alanyl (35, 45)-4-amin-3-hydroxy-5-cyclo-hexapentanoyl-N-hexylamide, Sankyo Company, Ltd., Tokyo, Japan); SKF 108566 (E-alpha-2-[2-butyl-1-carboxy phenyl] methyl] H-imidazole-5-yl[methylene]-2-thiophene propanoic acid, Smith Kline Beecham Pharmaceuticals, PA); Losartan (DPU753/MK954, DuPont Merck Pharmaceutical Company); Remivirin (R042-5892, F. Hoffman LaRoche AG); A2 agonists (Marion Merril Dow) and certain non-peptide heterocycles (G.D.Searle and Company).

Angiotensin converting enzyme (ACE) is an enzyme which catalyzes the conversion of angiotensin I to angiotensin II. ACE inhibitors include amino acids and derivatives thereof, peptides, including di and tri peptides and antibodies to ACE which intervene in the renin-angiotensin system by inhibiting the activity of ACE thereby reducing or eliminating the formation of pressor substance angiotensin II. ACE inhibitors have been used medically to treat hypertension, congestive heart failure, myocardial infarction and renal disease. Classes of compounds known to be useful as ACE inhibitors include acylmercapto and mercaptoalkanoyl prolines such as captopril (U.S. Patent No 4,105,776) and zofenopril (U.S. Patent No. 4,316,906), carboxyalkyl dipeptides such as enalapril (U.S. Patent No. 4,374,829), lisinopril (U.S. Patent No. 4,374,829), quinapril (U.S. Patent No. 4,344,949), ramipril (U.S. Patent No. 4,587,258), and perindopril (U.S. Patent No. 4,508,729), carboxyalkyl dipeptide mimics such as cilazapril (U.S. Patent No. 4,512,924) and benazapril (U.S. Patent No. 4,410,520), phosphonyllalkanoyl prolines such as fosinopril (U.S. Patent No. 4,337,201) and trandolapril.

Renin inhibitors are compounds which interfere with the activity of renin. Renin inhibitors include amino acids and derivatives thereof, peptides and derivatives thereof, and antibodies to renin. Examples
of renin inhibitors that are the subject of United States patents are as follows: urea derivatives of peptides (U.S. Patent No. 5,116,835); amino acids connected by nonpeptide bonds (U.S. Patent No. 5,114,937); di and tri peptide derivatives (U.S. Patent No. 5,106,835); amino acids and derivatives thereof (U.S. Patent Nos. 5,104,869 and 5,095,119); diol sulfonamides and sulfonils (U.S. Patent No. 5,098,924); modified peptides (U.S. Patent No. 5,095,006); peptidyl beta-aminoacyl aminodiol carbamates (U.S. Patent No. 5,089,471); pyrrolid idazolones (U.S. Patent No. 5,075,451); fluorine and chlorine statine or statone containing peptides (U.S. Patent No. 5,066,643); peptidyl amino diols (U.S. Patent Nos. 5,063,208 and 4,845,079); N-morpholino derivatives (U.S. Patent No. 5,055,466); pepstatin derivatives (U.S. Patent No. 4,980,283); N-heterocyclic alcohols (U.S. Patent No. 4,885,292); monoclonal antibodies to renin (U.S. Patent No. 4,780,401); and a variety of other peptides and analogs thereof (U.S. Patent Nos. 5,071,837, 5,064,965, 5,063,207, 5,036,054, 5,036,053, 5,034,512, and 4,894,437).

Agents that bind to cellular adhesion molecules and inhibit the ability of white blood cells to attach to such molecules include polypeptide agents. Such polypeptides include polyclonal and monoclonal antibodies, prepared according to conventional methodology. Such antibodies already are known in the art and include anti-ICAM 1 antibodies as well as other such antibodies. Significantly, as is well-known in the art, only a small portion of an antibody molecule, the paratope, is involved in the binding of the antibody to its epitope (see, in general, Clark, W. R. (1986) The Experimental Foundations of Modern Immunology, Wiley & Sons, Inc., New York; Roitt, I. (1991) Essential Immunology, 7th Ed., Blackwell Scientific Publications, Oxford). The pFc' and Fc regions, for example, are effectors of the complement cascade but are not involved in antigen binding. An antibody from which the pFc' region has been enzymatically cleaved, or which has been produced without the pFc' region, designated an F(ab')2 fragment, retains both of the antigen binding sites of an intact antibody. Similarly, an antibody from which the Fc region has been enzymatically cleaved, or which has been produced without the Fc region, designated an Fab fragment, retains one of the antigen binding sites of an intact antibody molecule.

Proceeding further, Fab fragments consist of a covalently bound antibody light chain and a portion of the antibody heavy chain denoted Fd. The Fd fragments are the major determinant of antibody specificity (a single Fd Fragment may be associated with up to ten different light chains without altering antibody specificity) and Fd fragments retain epitope-binding ability in isolation.

Within the antigen-binding portion of an antibody, as is well-known in the art, there are complementarity determining regions (CDRs), which directly interact with the epitope of the antigen, and framework regions (FRs), which maintain the tertiary structure of the paratope (see, in general, Clar, 1986; Roitt, 1991). In both the heavy chain Fd fragment and the light chain of IgG immunoglobulins, there are four framework regions (FR1 through FR4) separated respectively by three complementarity determining regions (CDR1 through CDR3). The CDRs, and in particular the CDR3 regions, and more particularly the heavy chain CDR3, are largely responsible for antibody specificity.

It is now well-established in the art that the non-CDR regions of a mammalian antibody may be replaced with similar regions of conspecific or heterospecific antibodies while retaining the epitopic specificity of the original antibody. This is most clearly manifested in the development and use of "humanized" antibodies in which non-human CDRs are covalently joined to human FR and/or Fc/pFc' regions to produce a functional antibody. Thus, for example, PCT International Publication Number WO 92/04381 teaches the production and use of humanized murine RSV antibodies in which at least a portion
of the murine FR regions have been replaced by FR regions of human origin. Such antibodies, including fragments of intact antibodies with antigen-binding ability, are often referred to as "chimeric" antibodies.

Thus, as will be apparent to one of ordinary skill in the art, the present invention also provides for F(\(ab')_2\), Fab, Fv and Fd fragments; chimeric antibodies in which the Fc and/or Fr and/or CDR1 and/or CDR2 and/or light chain CDR3 regions have been replaced by homologous human or non-human sequences; chimeric F(\(ab')_2\)fragment antibodies in which the FR and/or CDR1 and/or CDR2 and/or light chain CDR3 regions have been replaced by homologous human or non-human sequences; chimeric Fab fragment antibodies in which the FR and/or CDR1 and/or CDR2 and/or light chain CDR3 regions have been replaced by homologous human or non-human sequences; and chimeric Fd fragment antibodies in which the FR and/or CDR1 and/or CDR2 regions have been replaced by homologous human or nonhuman sequences. The present invention also includes so-called single chain antibodies.

Thus, the invention involves polypeptides of numerous size and type that bind specifically to cellular adhesion molecules. These polypeptides may be derived also from sources other than antibody technology. For example, such polypeptide binding agents can be provided by degenerate peptide libraries which can be readily prepared in solution, in immobilized form or as phage display libraries. Combinatorial libraries also can be synthesized of peptides containing one or more amino acids. Libraries further can be synthesized of peptoids and non-peptide synthetic moieties.

Phage display can be particularly effective in identifying binding peptides useful according to the invention. Briefly, one prepares a phage library (using, e.g., m13, fd, or lambda phage), displaying inserts from 4 to about 80 amino acid residues using conventional procedures. The inserts may represent, for example, a completely degenerate or biased array. One then can select phage-bearing inserts which bind to the cellular adhesion molecule. This process can be repeated through several cycles of reselection of phage that bind to the cellular adhesion molecule. Repeated rounds lead to enrichment of phage bearing particular sequences. DNA sequences analysis can be conducted to identify the sequences of the expressed polypeptides. The minimal linear portion of the sequence that binds to the cellular adhesion molecule can be determined. One can repeat the procedure using a biased library containing inserts containing part of all of the minimal linear portion plus one or more additional degenerate residues upstream or downstream thereof. Yeast two-hybrid screening methods also may be used to identify polypeptides that bind to the cellular adhesion molecules. Thus, cellular adhesion molecules, or a fragment thereof, can be used to screen peptide libraries, including phage display libraries, to identify and select peptide binding partners of the cellular adhesion molecules.

**Kits**

Any of the compounds or pharmaceutical compositions of the invention (e.g., compounds of Formulas (I)-(VI)) can be used together with a set of instructions, i.e., to form a kit. The kit may include instructions for use of the compounds of the invention in a screening method or as a therapy as described herein.

The following non-limiting examples are illustrative of the present invention.
EXAMPLES

Synthesis of Compounds

The compounds described herein can be prepared according to methods known in the art. For example, deuterated indole compounds can be prepared according to methods described in, e.g.: Vining et al., “Deuterium exchange labelling of biologically important phenols, indoles and steroids,” Journal of Labelled Compounds and Radiopharmaceuticals, 18(1):683-1692, 1981; Magnus et al., “Synthesis of 4,5,6,7 and 2,4,5,6,7 deuterium-labeled indole-3-acetic acid for use in mass spectrometric assays,” Plant Physiology, 66(4):775-781, 1980; and Kiado et al., “Synthesis of L-tryptophan labeled with hydrogen isotopes in the indole ring,” Journal of Radioanalytical and Nuclear Chemistry, 279(2):675-678, 2009.

Scheme 1 provides an exemplary method for the synthesis of the compounds of the invention.

Scheme 1

As shown in Scheme 1, a starting material such as toluene-<sup>6</sup>D<sub>4</sub> (compound A) can be nitrated in order to afford compound B as a mixture of isomers. Indole formation can be accomplished by treating compound B with, e.g., vinyl magnesium bromide to afford compound C. Formylation under Vilsmeier-Haack conditions followed by cyanide addition can afford the C3-aminonitrile compound D. Partial reduction of the nitrile followed by cyclization then affords the desired deuterated compound E.

Compounds of the invention where R<sup>9</sup> includes a deuterium group can also be prepared according to the following method shown in Scheme 2.

Scheme 2

Compounds such as Compound G can be prepared according to methods in the art (see, e.g.: U.S. Patent Nos. 7,491,743, 8,143,300, and 8,324,262; U.S. Patent Application Publication No. 2012/0149702; and U.S. Patent Application No. 13/665,263, each of which is hereby incorporated by
reference in its entirety. Treatment of the starting material with a deuterated alkylating agent such as CD$_3$I can afford the desired product H. Still further deuterated compounds can be prepared by modifying known synthetic methods for the preparation of compounds such as Compound G by incorporating other deuterated reagents.

**Scheme 3** provides an exemplary synthetic scheme for compounds of the invention where, e.g., R$^{2A}$ and R$^{2B}$ are both deuterium, and R$^{3}$ is a deuterated alkyl group.

For the preparation of Compound O as described herein, $^1$H NMR spectra were recorded on Bruker Avance II 400 MHz, Bruker Fourier 300 MHz and Varian Mercury plus 300 MHz, and trimethylsilane was used as an internal standard. LCMS was taken on a quadrupole Mass Spectrometer on Agilent LC/MSD 1200 Series (Column : ODS 2000 (50 x 4.6 mm, 5 µm)) operating in ES (+) or (-) ionization mode; T = 30 °C; flow rate = 1.5 mL/min; detected wavelength : 214 nm.

**Synthesis of Compound J**

To a mixture of Compound I (1.0 g, 100 mmol), CD$_3$OD (5.4 g, 150 mmol) and PPh$_3$ (39 g, 150 mmol) in THF (500 mL) at 0 °C was added drop wise DIAD (29.5 mL, 150 mmol). The solution was stirred for two hours at room temperature and partially concentrated. Water (500 mL) and CH$_2$Cl$_2$ (250 mL) were added. The organic phase was washed with water (3 x 150 mL). The aqueous phase was washed with CH$_2$Cl$_2$ (3 x 50 mL) and concentrated to give Compound J (8.0 g, 68%) as a white solid. $^1$H NMR (300 MHz, DMSO-d$_6$): $\delta$ 8.00 (1H, br s), 3.88 (2H, s). MS Calcd.: 117; MS Found: 116 ([M-1]).

**Synthesis of Compound L**

To DMF-d$_7$ (1.9 g, 25.0 mmol) in toluene (60 mL) at 0 °C was added dropwise phosphoryl chloride (2.15 mL, 23.0 mmol). After 15 minutes at room temperature, Compound K (2.5 g, 16.5 mmol)
was added. The solution was stirred for two hours at room temperature. The reaction was poured into ice and saturated NaHCO₃ and extracted with ethyl acetate. The organic solution was washed with brine, dried over Na₂SO₄, filtered and concentrated to give Compound L (2.5 g, 85%) as a white solid. ¹H NMR (300 MHz, DMSO-δ6): 8 12.55 (1H, br s), 8.39 (1H, d, J = 3.3 Hz), 8.06 (1H, d, J = 7.5 Hz), 7.36 (1H, d, J = 7.8 Hz), 7.25 (1H, t, J = 7.8 Hz). MS Calcd.: 180; MS Found: 181 ([M+1]+).

Synthesis of Compound N

Compound J (0.5 g, 4.2 mmol), ethanolamine (Compound M; 0.1 mL) in EtOH (3 mL), and H₂O (3 mL) were warmed to 90 °C for 30 minutes. Compound K (0.5 g, 2.8 mmol) was added. The solution was refluxed for five hours and partially concentrated. The crude solid was washed with water and MeOH to give Compound N (0.52 g, 67%) as a yellow solid. ¹H NMR (300 MHz, DMSO-d6): 8 12.17 (1H, br s), 10.45 (1H, br s), 8.23 (1H, s), 7.78 (1H, d, J = 8.1 Hz), 7.27 (1H, d, J = 7.7 Hz), 7.12 (1H, t, J = 7.8 Hz). MS Calcd.: 279; MS Found: 280 ([M+1]+).

Synthesis of Compound O

To a mixture of compound N (140 mg, 0.5 mmol) and CoCl₂ (0.25 g, 4 mmol) in MeOH (15 mL) and THF (15 mL) at 0 °C was added portionwise NaBD₄ (0.45 g, 11 mmol). After 1 hour, concentrated HCl was added until bubbling ceased. The solution was then partially concentrated, and saturated aqueous Na₂CO₃ solution was added until the pH was about 7. The mixture was extracted with ethyl acetate. The organic phase was concentrated, filtered over a small pad of silica gel (ethyl acetate/methanol = 95:5), and recrystallized with MeOH to give Compound O (60 mg, 43%) as a white solid. ¹H NMR (300 MHz, CDCl₃): 8 8.37 (1H, br s), 7.49 (1H, d, J = 7.8 Hz), 7.23 (1H, d, J = 7.2 Hz), 7.13 (1H, d, J = 1.8 Hz), 7.06 (1H, t, J = 7.8 Hz), 5.53 (1H, br s), 4.27 (1H, s). MS Calcd.: 283 ([M+1]+).

Assays for Identifying Inhibitors of Necrosis and Necroptosis

Evaluation of necroptosis inhibitory activity can be performed using a FADD-deficient variant of human Jurkat T cells treated with TNF-α as previously described (Degterev et al., Nat. Chem. Biol. 1:112 (2005) and Jagtap et al., J. Med. Chem. 50: 1886 (2007)). For EC₅₀ value determinations, cells can be treated with 10 ng/mL of human TNF-α in the presence of increasing concentration of test compounds for 24 hours followed by ATP-based viability assessment.

ATP-based viability assessment: Briefly, necroptosis activity can be performed using a FADD-deficient variant of human Jurkat T cells treated with TNF-α. For EC₅₀ value determinations, cells (500,000 cells/mL, 100 μL per well in a 96-well plate) can be treated with 10 ng/mL of human TNF-α in the presence of increasing concentration of test compounds for 24 hours at 37 °C in a humidified incubator with 5% CO₂ followed by ATP-based viability assessment. Stock solutions (30 mM) in DMSO can be prepared and then diluted with DMSO to give testing solutions, which were added to each test well. The final DMSO concentration can be 0.5%. Eleven compound test concentrations (0.030 - 100 μM) can be used, and each concentration can be done in duplicate.

Cell viability assessments can be performed using a commercial luminescent ATP-based assay kit (CellTiter-Glo, Promega, Madison, WI) according to the manufacturer’s instructions. Briefly, 40 μL of the cell lysis/ATP detection reagent can be added to each well. Plates can be incubated on a rocking
platform for 10 minutes at room temperature and luminescence was measured using a Wallac Victor 3 plate-reader (Perkin Elmer, Wellesley, MA). Cell viability can be expressed as a ratio of the signal in the well treated with TNF-a and compound to the signal in the well treated with compound alone in order to account for nonspecific toxicity. EC_{50} values can be calculated using nonlinear regression analysis of sigmoid dose-response (variable slope) curves from plots of log([I]) verses viability values.

Activity may be also demonstrated using still other procedures known in the art (see, for example, Teng et al., Bioorg. Med. Chem. Lett., 15:5039 (2005) and Jagtap et al., J. Med. Chem. 50: 1886(2007)).

The following protocols are exemplary methods by which necroptosis inhibition can be assayed.

**General**

Cells are seeded at the density of 50,000 cell/well in 100 μL of RPMI media supplemented with 10% Fetal Bovine Serum into 96-well plates and treated with a range of necrostatin concentrations (30 nM to 100 μM; 11 dose points) in the presence and absence of 10 ng/ml human TNFα for 24 hours. For these and all other cellular assays, compound stocks (in DMSO) are diluted to appropriate concentrations in DMSO before addition to the cells to maintain final concentration of DMSO for all samples at 0.5%. Cell viability is determined using CellTiter-Glo luminescent cell viability assay (Promega). The ratio of luminescence in compound and TNF-treated wells to compound-treated, TNF-un-treated wells was calculated (viability, %) and used to calculate EC_{50} by nonlinear regression in GraphPad Prizm.

(A) **In Vitro Kinase Assay of RIP1**

**Protein Expression and purification in Sf9 cells**

3. **Sf9 cell transfection and baculovirus propagation**

1. Sf9 cells are grown in a T175 flask at 27°C in SF-900 II SFM media. Cells are passaged (1:3 or 1:5) when they approach confluence or every 3 to 4 days.

2. To generate the baculovirus, Transfection Buffer A & B Set according to the manufacturer’s protocol (BD Biosciences) is used. After 4 days, media from the 60 mm dish is transferred to a 15 ml conical tube and centrifuged at 1200 rpm for 5 minutes to pellet any cells. The supernatant is transferred to a new conical tube. This is passage 1 (P1) baculovirus.

3. Half of the P1 baculovirus is added to Sf9 cells in a 15 cm dish in 16 ml of SF-900 II SFM medium (Gibco/Invitrogen). Cells are incubated at 27 °C for 4 days, and media is collected from the dishes and centrifuged as described above. This step is repeated two more times to generate P4 GST-RIP1 8-327 baculovirus.

**Recombinant RIP1 protein purification**

1. 500 ml of Sf9 cells are infected in a 2L flask at a density of 3 x 10^6 cells/ml in ESF921 Protein Free medium (Expression Systems) with 1.5 ml of P4 GST-RIP1 8-327 baculovirus. Cells are incubated at 27°C, shaking at 150 rpm for 4 days.

2. The cells are pelleted in a refrigerated centrifuge at 5,000 rpm for 15 minutes.

3. The cells are resuspended in 20 ml of cell lysis buffer and incubated on ice for 20 minutes. Cell lysis buffer: 40 mM HEPES pH 7.3, 150 mM NaCl, 5 mM EDTA, 0.5 mM NaF, 0.2 mM NaVO₃, 10 mM...
sodium pyrophosphate, 17.5 mM β-glycerolphosphate, 1 pg/ml aprotonin, 1 µg/ml leupeptin, 1 µg/ml pepstatin, and 50 µg/ml PMSF.

4. The cell lysate is sonicated in Sonifier Cell Disruptor 200 (Branson) using an output of 7 and 40% duty cycle for 30 seconds followed by 2 minute incubation on ice, repeated 3 times.

5. The cell lysate is centrifuged at 4 °C for 60 minutes at 12,000 rpm.

6. The filtered supernatant is loaded onto a 5 ml glutathione 4B sepharose column equilibrated with cell lysis buffer. Using the ATKA purifier, column is washed with at 6 column volumes (CVs) of GST wash buffer. GST wash buffer: 50 mM Tris pH 8.0, 150 mM NaCl. 9. GST-RIP1 8-327 protein is eluted with 6 CVs of GST elution buffer collecting 2 ml fractions using the ATKA purifier. GST elution buffer: 50 mM Tris pH 8.0, 150 mM NaCl, 20 mM reduced glutathione, SEC buffer: 50 mM Tris pH 8.0, 150 mM NaCl, 2 mM β-mercaptoethanol.

7. Fractions are run on 10% SDS-PAGE and stained with BioSafe Coomassie to determine the GST-RIP1 8-327 protein (about 66 kD) containing fractions. Fractions and concentrated using a Vivaspin 15 concentrator to ~1-2 ml.

8. Using the ATKA purifier, concentrated GST-RIP1 8-327 protein is injected onto a Superdex 200 10/300 GL column equilibrated with 2 CVs of SEC Buffer. Protein is eluted with an isocratic gradient of SEC Buffer with 1.5 CVs collecting 1 ml fractions. SEC buffer: 50 mM Tris pH 8.0, 150 mM NaCl, 2 mM β-mercaptoethanol.

9. Fractions are again analyzed on 10% SDS-PAGE and fractions containing GAST-RI protein are pooled and concentrated to ~200 µl.

In-vitro HTRF KinEASE Assay

1. Following conditions are used for GST-RIP1 8-327: 100 nM protein, 2% DMSO, 50 µM ATP, 1 µM substrate 3 from HTRF KinEASE kit (Cisbio), three hour room temperature reaction, duplicate reactions.

2. Following is prepared on ice: 500 nM GST-RIP1 8-327, compounds in DMSO (<5% DMSO final), 5 µM substrate 3 and 250 µM ATP all diluted in 1X kinase buffer.

3. The following is added to the black Corning 384 well plate: 2 µl of 500 nM GST-RIP1 8-327 and 4 µl of inhibitor. Plate is sealed with sealing tape and incubated at room temperature for 10 minutes.

4. 2 µl of 5 µM substrate 3 and 2 µl of 250 µM ATP are added to the wells. Plated is sealed with sealing tape and incubated at room temperature for three hours.

7. 250 nM streptavidin-XL665 is prepared in HTRF detection buffer.

8. Kinase reactions are quenched by adding 5 µl of XL-665 followed by 5 µl of cryptate. Plated is sealed with sealing tape and incubated at room temperature for one hour.

9. Fluorescence is measured at 620 nm (cryptate) and 665 nm (XL-665).

10. Data is analyzed by calculating the ratio for each reaction \((\text{Ratio} = (665 \text{ nm} / 620 \text{ nm}) \times 10^4)\). Next, specific signal for each reaction is determined as \((\text{Ratio (sample)} - \text{Ratio (1X kinase buffer)})\). Mean ratio (average) of the duplicate GST-RIP1 8-327/DMSO sample is determined. Specific signal from compound-treated well is converted to percent inhibition as

\[
\frac{((\text{mean ratio (DMSO sample)} - \text{specific signal (sample)})/\text{mean ratio (DMSO sample)}) \times 100)}{100}.
\]
(B) Fluorescence Thermal Shift Analysis of compound/RIP1 binding

The thermal shift of recombinant RIP1 is measured in clear Light Cycler 480 multiwell plate 96 (Roche) with a final volume of 20 μl. All components are diluted in thermal shift buffer (50 mM Tris pH 8.0, 150 mM NaCl, 1 mM MgCl₂) with final concentrations of RIP1 9 μM, necrostatins 180 μM, Sypro Orange (Life Technologies) 5X, and DMSO 3%. In duplicate wells, RIP1 and the compounds are added to the microplate and incubated at room temperature for 5 minutes. Sypro Orange dye is added and the plate is measured using a LightCycler 480 instrument (Roche) with excitation at 465 nm and emission at 580 nm. The protein denaturation fluorescence data is collected using melting curves analysis mode from 25 to 85 °C in continuous acquisition mode with 10 acquisitions per °C and a ramp rate of 0.06 °C/s. The data is analyzed to calculate Tₘ using nonlinear regression with Boltzmann sigmoidal equation in GraphPad 5 using approximately two degrees of pre- and post-transition baseline data.

Liver Microsome Stability Assays

Microsome stability can be determined in pooled mouse liver microsomes. A test compound (3 DM final concentration) along with 0.5 mg/mL microsome protein and 1 mM NADPH can be incubated for 0, 5, 15, 30 and 60 minutes. Incubation of test compound and microsomes in the absence of NADPH can serve as a negative control. The samples can be quenched with methanol and centrifuged for 20 minutes at 2500 rpm to precipitate proteins. Sample supernatants can be analyzed (N=3) by LC/MS. The in peak area ratio (compound peak area/ internal standard peak area) can be plotted against time and the slope of the line determined to give the elimination rate constant \( k = \frac{\text{slope}}{1} \). The half life (\( t_{1/2} \) in minutes), and the in vitro intrinsic clearance \( CL_{int} \) in \( \text{L/min/mg protein} \) can be calculated according to the following equations, where \( V \) = incubation volume in \( \text{L/mL} \):

\[
t_{1/2} = \frac{0.693}{k}; CL_{int} = \frac{V(0.693)}{t_{1/2}}.
\]

Example 1

The metabolic stability of deuterated compound H was measured and compared to the metabolic stability of compound P (Table 2) using a mouse liver microsomal assay. Data relating to inhibition of necroptosis are shown in Table 3. As shown in the table, the deuterated compound H showed both improved metabolic stability and improved potency as a necroptosis inhibitor.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Metabolic Stability ( t_{1/2} ) in liver microsome assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>94 minutes</td>
</tr>
</tbody>
</table>
Accordingly, the deuterated compounds and compositions described herein (e.g., an isolated compound having a structure according to any of Formulas (I)-(VII), or an isotopically enriched composition that includes a compound according to any of Formulas (I)-(VII)) can show improved

<table>
<thead>
<tr>
<th>Compound</th>
<th>EC$_{50}$ (TNF-α in FADD deficient cells)</th>
<th>EC$_{50}$ (RIP1 HTRF KinEASE assay)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>100.2 nM</td>
<td>--</td>
</tr>
<tr>
<td>(R)-H</td>
<td>27.9 nM</td>
<td>300 nM</td>
</tr>
<tr>
<td>O</td>
<td>81.9 nM</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>222.1 nM</td>
<td>910 nM</td>
</tr>
<tr>
<td>(R)-P</td>
<td>45.5 nM</td>
<td>540 nM</td>
</tr>
</tbody>
</table>
physicochemical properties (e.g., improved metabolic stability) and can show increased potency in inhibition of necroptosis.

All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each independent publication or patent application was specifically and individually indicated to be incorporated by reference.

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure that come within known or customary practice within the art to which the invention pertains and may be applied to the essential features hereinbefore set forth, and follows in the scope of the claims.

Other embodiments are within the claims.

What is claimed is:
1. A composition comprising a compound of the formula

\[
\text{(I)} ,
\]

or any pharmaceutically acceptable salt thereof, or stereoisomer thereof, wherein

- \( X^1 \) is 0 or \( NR^1 \);
- each of \( X^2 \) and \( X^3 \) is, independently, O or S;
- \( R^1 \) is H, D, or optionally substituted C1-C6 alkyl;
- \( R^2 \) is H or D;
- each of \( R^3 \) and \( R^8 \) is, independently, H, D, or optionally substituted C1-C6 alkyl;
- each of \( R^4, R^5, \) and \( R^6 \) is, independently, H or D;
- \( R^7 \) is H, D, halogen, optionally substituted C1-C6 alkyl, or optionally substituted C1-C6 alkoxy;
- each of \( R^9, R^{10}, \) and \( R^{10} \) is, independently, H, D, or optionally substituted C1-C6 alkyl; and
- wherein at least one of \( R^1-R^{10} \) is D or comprises a deuterium group, and wherein said composition has an isotopic enrichment factor for deuterium of at least 5.

2. The composition of claim 1, wherein \( R^1 \) is H.

3. The composition of claim 1 or 2, wherein \( X^1 \) is 0.

4. The composition of claim 1 or 2, wherein \( X^1 \) is \( NR^1 \).

5. The composition of claim 4, wherein \( R^{10} \) is H.

6. The composition of any of claims 1-5, wherein \( X^2 \) and \( X^3 \) are both O.

7. The composition of any of claims 1-6, wherein one, two, or three of \( R^4, R^5, \) and \( R^6 \) is D.

8. The composition of claim 7, wherein one of \( R^4, R^5, \) and \( R^6 \) is D, and two of \( R^4, R^5, \) and \( R^6 \) are H.

9. The composition of claim 8, wherein \( R^4 \) is D, \( R^5 \) is D, or \( R^6 \) is D.

10. The composition of any of claims 1-9, wherein \( R^2 \) is D.

11. The composition of any of claims 1-9, wherein \( R^2 \) is H.
12. The composition of any of claims 1-11, wherein one of \( R^{3A} \) and \( R^{3B} \) is D, or both \( R^{3A} \) and \( R^{3B} \) are D, or both \( R^{4A} \) and \( R^{4B} \) are H.

13. The composition of any of claims 1-12, wherein \( R^8 \) is H or D.

14. The composition of any of claims 1-13, wherein said optionally substituted \( C_1-C_6 \) alkyl or said optionally substituted \( C_1-C_6 \) alkoxy comprises 0, 1, 2, or 3 deuterium atoms.

15. The composition of any of claims 1-14, wherein \( R^7 \) is halogen, optionally substituted \( C_1 \) alkyl, or optionally substituted \( C_1 \) alkoxy.

16. The composition of claim 15, wherein said \( C_1 \) alkyl or said \( C_1 \) alkoxy comprises 1, 2, or 3 deuterium atoms.

17. The composition of claim 15, wherein \( R^7 \) is Cl, \( CH_3 \), \( OCH_3 \), \( CD_3 \), \( OCD_3 \), or \( CF_3 \).

18. The composition of any of claims 1-17, wherein \( R^9 \) is optionally substituted \( C_1 \) alkyl.

19. The composition of claim 18, wherein \( R^9 \) is \( CH_3 \), \( CD_3 \), \( CHD_2 \), or \( CH_2D \).

20. The composition of claim 1, wherein said compound has a structure according to one of the following formulas,
or a pharmaceutically acceptable salt thereof, or a stereoisomer thereof, and wherein
X\(^1\) is NH or O;
R\(^7\) is halogen, optionally substituted C\(_1\)-C\(_2\) alkyl, or optionally substituted C\(_1\)-C\(_2\) alkoxy; and
R\(^8\) is optionally substituted C\(_1\)-C\(_2\) alkyl.

21. The composition of claim 18, wherein X\(^1\) is NH.

22. The composition of claim 18, wherein X\(^1\) is O.

23. The composition of any of claims 20-22, wherein said optionally substituted C\(_1\)-C\(_2\) alkyl or said optionally substituted C\(_1\)-C\(_2\) alkoxy comprises 0, 1, 2, or 3 deuterium atoms.

24. The composition of any of claims 18-23, wherein R\(^9\) is CH\(_3\).

25. The composition of any of claims 18-23, wherein R\(^9\) is CD\(_3\).

26. The composition of any of claims 18-25, wherein R\(^7\) is Cl, CH\(_3\), OCH\(_3\), GD\(_3\), OCD\(_3\), or CF\(_3\).

27. The composition of any of claims 1-26, wherein R\(^1\)-R\(^{10}\) comprise 1, 2, or 3 deuterium atoms.

28. The composition of any of claims 1-26, wherein R\(^1\)-R\(^{10}\) comprise 4 or 5 deuterium atoms.

29. The composition of any of claims 1-28, wherein said isotopic enrichment factor for deuterium is at least 10.

30. The composition of any of claims 1-28, wherein said isotopic enrichment factor for deuterium is at least 50.

31. The composition of any of claims 1-28, wherein said isotopic enrichment factor for deuterium is at least 100.

32. The composition of any of claims 1-28, wherein said isotopic enrichment factor for deuterium is at least 500.

33. The composition of any of claims 1-28, wherein said isotopic enrichment factor for deuterium is at least 1000.
34. The composition of any of claims 1-28, wherein said isotopic enrichment factor for deuterium is at least 3000.

35. The composition of any of claims 1-34, wherein the carbon bearing the \( R^8 \) and \( X^1 \) groups has the (S)-configuration.

36. The composition of any of claims 1-34, wherein the carbon bearing the \( R^8 \) and \( X^1 \) groups has the (S)-configuration.

37. An isolated compound having a structure according to the following formula

\[
\begin{align*}
\text{Formula (I)}, \text{ or any pharmaceutically acceptable salt thereof, or}
\end{align*}
\]

stereoisomer thereof, wherein

- \( X^1 \) is 0 or NR\(^{10} \);
- each of \( X^2 \) and \( X^3 \) is, independently, O or S;
- \( R^1 \) is H, D, or optionally substituted C\(_1\)-C\(_6\) alkyl;
- \( R^2 \) is H or D;
- each of \( R^{3A} \) and \( R^{3B} \) is, independently, H, D, or optionally substituted C\(_1\)-C\(_6\) alkyl;
- each of \( R^4 \), \( R^5 \), and \( R^6 \) is, independently, H or D;
- \( R^7 \) is H, D, halogen, optionally substituted C\(_1\)-C\(_6\) alkyl, or optionally substituted C\(_1\)-C\(_6\) alkoxy;
- each of \( R^8 \), \( R^9 \), and \( R^{10} \) is, independently, H, D, or optionally substituted C\(_1\)-C\(_6\) alkyl; and

wherein at least one of \( R^1 \cdot R^{10} \) is D or comprises a deuterium group.

38. The compound of claim 37, wherein \( R^1 \) is H.

39. The compound of claim 37 or 38, wherein \( X^1 \) is 0.

40. The compound of claim 37 or 38, wherein \( X^1 \) is NR\(^{10} \).

41. The compound of claim 40, wherein \( R^{10} \) is H.

42. The compound of any of claims 37-41, wherein \( X^2 \) and \( X^3 \) are both O.

43. The compound of any of claims 37-42, wherein one, two, or three of \( R^4 \), \( R^5 \), and \( R^6 \) is D.

44. The compound of claim 43, wherein one of \( R^4 \), \( R^5 \), and \( R^6 \) is D, and two of \( R^4 \), \( R^5 \), and \( R^6 \) are H.
45. The compound of claim 44, wherein R⁴ is D, R⁵ is D, or R⁶ is D.

46. The compound of any of claims 37-45, wherein R² is D.

47. The compound of any of claims 37-46, wherein one of R²ᴬ and R³ᴮ is D, or both R²ᴬ and R³ᴮ are D, or both R²ᴬ and R³ᴮ are H.

48. The compound of any of claims 37-47, wherein R⁰ is H or D.

49. The composition of any of claims 37-48, wherein said optionally substituted C₁-C₆ alkyl or said optionally substituted C₁-C₆ alkoxy comprises 0, 1, 2, or 3 deuterium atoms.

50. The compound of any of claims 37-49, wherein R⁷ is halogen, optionally substituted C₁ alkyl, or optionally substituted C₁ alkoxy.

51. The compound of claim 50, wherein said C₁ alkyl or said C₁ alkoxy comprises 1, 2, or 3 deuterium atoms.

52. The compound of claim 51, wherein R⁷ is Cl, CH₃, OCH₃, CD₃, OCD₃, or CF₃.

53. The compound of any of claims 37-52, wherein R⁰ is optionally substituted C₁ alkyl.

54. The compound of claim 53, wherein R⁰ is CH₃, CD₃, CHD₂, or CH₂D.

55. The compound of claim 37, wherein said compound has a structure according to one of the following formulas,

(I), (II), (III), (IV), (V).
or a pharmaceutically acceptable salt thereof, or a stereoisomer thereof, wherein
X₁ is NH or O;
R⁷ is halogen, optionally substituted C₁-C₂ alkyl, or optionally substituted C₁-C₂ alkoxy; and
R⁹ is optionally substituted C₁-C₂ alkyl.

56. The compound of claim 55, wherein X₁ is NH.

57. The compound of claim 55, wherein X₁ is O.

58. The compound of any of claims 55-57, wherein said optionally substituted C₁-C₂ alkyl or said optionally substituted C₁-C₂ alkoxy comprises 0, 1, 2, or 3 deuterium atoms.

59. The compound of any of claims 55-58, wherein R⁹ is CH₃.

60. The compound of any of claims 55-58, wherein R⁹ is CD₃.

61. The compound of any of claims 55-60, wherein R⁷ is Cl, CH₃, OCH₃, CD₃, OCD₃, or CF₃.

62. The compound of claim 61, wherein R⁹ is CH₃.

63. The composition of any of claims 37-62, wherein R¹⁻Rⁿ⁺⁺ comprise 1, 2, or 3 deuterium atoms.

64. The composition of any of claims 37-62, wherein R¹⁻Rⁿ⁺⁺ comprise 4 or 5 deuterium atoms.

65. A composition comprising the compound of any of claims 37-64, wherein said isotopic enrichment factor for deuterium is at least 10.

66. The composition of claim 65, wherein said isotopic enrichment factor for deuterium is at least 50.
67. The composition of claim 65, wherein said isotopic enrichment factor for deuterium is at least 100.

68. The composition of claim 65, wherein said isotopic enrichment factor for deuterium is at least 500.

69. The composition of claim 65, wherein said isotopic enrichment factor for deuterium is at least 1000.

70. The composition of claim 65, wherein said isotopic enrichment factor for deuterium is at least 3000.

71. A pharmaceutical composition comprising
   (i) a pharmaceutically acceptable excipient; and
   (ii) the composition of any of claims 1-36,
       or any pharmaceutically acceptable salt thereof, or stereoisomer thereof.

72. A pharmaceutical composition comprising
   (i) a pharmaceutically acceptable excipient; and
   (ii) the compound of any of claims 37-70, or any pharmaceutically acceptable salt thereof, or
        stereoisomer thereof.

73. A method of treating a condition in a subject, said method comprising the step of contacting the
    compound or composition of any of claims 1-72, or any pharmaceutically acceptable salt thereof, or
    stereoisomer thereof, to said subject in a dosage sufficient to decrease necroptosis,
    and wherein said condition is one in which necroptosis is likely to play a substantial role.

74. A method of treating a condition in a subject, said method comprising the step of contacting the
    compound or composition of any of claims 1-72, or any pharmaceutically acceptable salt thereof, or
    stereoisomer thereof, to said subject in a dosage sufficient to modulate RIP1 and/or RIP3 activity,
    and wherein said condition is one in which RIP1 and/or RIP3 protein is a contributing factor.

75. The method of claim 73 or 74, wherein said condition is a neurodegenerative disease of the central
    or peripheral nervous system, the result of retinal neuronal cell death, the result of cell death of cardiac
    muscle, the result of cell death of cells of the immune system; stroke, liver disease, pancreatic disease,
    the result of cell death associated with renal failure; heart, mesenteric, retinal, hepatic or brain ischemic
    injury, ischemic injury during organ storage, head trauma, septic shock, coronary heart disease,
    cardiomyopathy, myocardial infarction, bone avascular necrosis, sickle cell disease, muscle wasting,
    gastrointestinal disease, tuberculosis, diabetes, alteration of blood vessels, muscular dystrophy, graft-
    versus-host disease, viral infection, Crohn's disease, ulcerative colitis, asthma, atherosclerosis, a chronic
    or acute inflammatory condition, pain, or any condition in which alteration in cell proliferation,
    differentiation or intracellular signaling is a causative factor, or any condition where RIP1 and/or RIP3
    protein is a contributing factor.
76. The method of claim 75, wherein said condition is a neurodegenerative disease of the central or peripheral nervous system.

77. The method of claim 75, wherein said condition is hepatic or brain ischemic injury, or ischemic injury during organ storage, head trauma, septic shock, or coronary heart disease.

78. The method of claim 75, wherein said condition is stroke.

79. The method of claim 75, wherein said condition is myocardial infarction.

80. The method of claim 75, wherein said condition is pain.

81. The method of claim 80, wherein said pain is inflammatory pain, diabetic pain, pain associated with a burn, or pain associated with trauma.

82. The method of claim 75, wherein said condition is atherosclerosis.

83. The method of claim 75, wherein said condition is a chronic or acute inflammatory condition.

84. The method of claim 83, wherein said chronic or acute inflammatory condition is rheumatoid arthritis, psoriasis, or Stevens-Johnson syndrome.

85. A method of decreasing necroptosis comprising contacting a cell with the composition or compound of any of claims 1-72, or any pharmaceutically acceptable salt thereof, or stereoisomer thereof.

86. A kit comprising
   (a) a pharmaceutically acceptable composition comprising the composition or compound of any of claims 1-72, or any pharmaceutically acceptable salt thereof, or stereoisomer thereof; and
   (b) instructions for the use of the pharmaceutical composition of (a) to treat a condition in a subject.
INTERNATIONAL SEARCH REPORT

PCT/US 14/27045

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) : A01N 43/38; A61K 31/44, 31/405 (2014.01)
USPC : 514/415

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)

IPC(8): A01N 43/38; A61K 31/44, 31/405 (2014.01)
USPC: 514/340, 342, 415

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 practicable, search terms used)

search terms: necroptosis; deuterium; isotopic enrichment factor; oxazole; indole;

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category*</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
</table>

Further documents are listed in the continuation of Box C.

Date of the actual completion of the international search: 06 June 2014 (06.06.2014)

Date of mailing of the international search report: 07 JUL 2014

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450
Facsimile No. 571-273-3201

Authorized officer: Shane Thomas

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-1774

Form PCT/ISA 210 (second sheet) (July 2009)
INTERNATIONAL SEARCH REPORT

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

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

1. Claims Nos.:

   because they relate to subject matter not required to be searched by this Authority, namely:

2. Claims Nos.:

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

3. □ Claims Nos.: 6-19, 21-36, 42-54 and 59-86

   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

1. □ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. □ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. □ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. □ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest  □ The additional search fees were accompanied by the applicant’s protest and, where applicable, the payment of a protest fee.

□ The additional search fees were accompanied by the applicant’s protest but the applicable protest fee was not paid within the time limit specified in the invitation.

□ No protest accompanied the payment of additional search fees.

Form PCT/ISA/2 10 (continuation of first sheet (2)) (July 2009)