SMALL MOLECULE IRE1-ALPHA INHIBITORS

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Description

Described herein are IRE1α inhibitors, compositions containing such inhibitors, and methods of treatment that include administration of such compounds. Exemplary compounds are provided throughout the application.
FIG. 3
Compound 31
IC50 = 229 nM

Compound 36
IC50 = 657 nM

Compound 41
IC50 = 243 nM

Compound 42
IC50 = 196 nM

FIG. 4
Compound 43
IC50 = 43 nM

Compound 44
IC50 = 112 nM

FIG. 5
FIG. 6
A.

900 mg

“Building block”

FIG. 8
SMALL MOLECULE IRE1-ALPHA INHIBITORS

CROSS-REFERENCE TO RELATED APPLICATIONS


BACKGROUND

[0002] Aggressive tumors have evolved strategies that enable them to thrive under constant adverse conditions. Cancer cells respond to hypoxia, nutrient starvation, oxidative stress, and high metabolic demand by adjusting their protein folding capacity via the endoplasmic reticulum (ER) stress response pathway. Cancer patients would benefit from the development of new strategies and therapeutics.

SUMMARY

[0003] Described herein are IRE1α inhibitors, compositions containing such inhibitors, and methods of treatment that include administration of such compounds. Exemplary compounds are provided throughout the application.

[0004] The inventors have discovered that XBP1 can promote tumor progression by confounding the development of protective antitumor immunity in the ovarian cancer tumor microenvironment. Without XBP1, tumor resident dendritic cells fail to accumulate intracellular lipids, which normally disrupt effective antigen cross-presentation. This pathological lipid accumulation is fundamentally driven by reactive oxygen species-mediated lipid peroxidation, which directly destabilizes protein-folding chaperones within the endoplasmic reticulum to induce a state of ER stress and XBP1 activation. Additionally, the inventors have found that IRE1α-mediated XBP1 signaling is involved in myeloid cell production of immunosuppressive prostaglandins such as prostaglandin E2 (PGE2).

[0005] These findings have led to the development of novel small-molecule IRE1α inhibitors with the ability to induce two parallel and mutually reinforcing anti-tumor mechanisms, namely the direct inhibition of tumor growth and the simultaneous induction of robust antitumor immunity. Such a compound is highly desirable, as no effective, targeted therapies currently exist for either TNBC or ovarian cancer.

[0006] Described herein are novel IRE1α let kinase inhibitors that exhibit such immune-modulatory properties and/or that allosterically block IRE1α endonuclease function. The identified direct IRE1α inhibitors have unique chemical structures, unique binding mechanisms, inhibitory activity, and off-target effects.

[0007] One aspect of the invention is a compound of formula I:

![Chemical structure diagram]

[0008] wherein:

[0009] A and B are separately each a heterocyclic ring or a phenyl group, where the A ring has \( x \) R₁ substituents;

[0010] C is phenyl or pyridinyl;

[0011] D is heterocyclic ring;

[0012] linkage₁ is a C₃-C₄ alkylene, an alkylene, an alkenylene, an alkylamido, an acyl, or an oxo(alkyl)alkylene with a first and second terminal atom;

[0013] linkage₂ is a C₃-C₄ alkylamido, amidalkyl, amino, urea, alkylurea, or ureaalkyl with a first and second terminal atom;

[0014] \( y \) is an integer of 0-3, and when \( y = 0 \), the linkage between the rings is a single bond;

[0015] x is an integer of 0-2;

[0016] v is an integer of 0-1;

[0017] \( R_1 \) substituents on the A ring are selected from amino, C₁-C₃ alkyl, ether, alkoxy, oxo, hydroxy, —NH—SO₂—phenyl-(R₄), and cyano;

[0018] \( R_2 \) substituents on the B ring are selected from amino, and C₁-C₄ alkyl;

[0019] \( R_4 \) substituents on the C ring are selected from C₁-C₄, and C₁-C₃ alkyl; and

[0020] \( R_5 \) substituents on the D ring are selected from C₁-C₃ alkyl, C₁-C₃ alkoxy, benzyl, and benzaldehyde;

[0021] \( R_6 \) is halo; or

[0022] a pharmaceutically acceptable salt thereof.

[0023] Another aspect is a compound of formula II:

![Chemical structure diagram]

[0024] wherein:

[0025] E is phenyl;

[0026] F is phenyl, naphthalene, tetrahydroxynaphthalene, or a bicyclic heterocycle;

[0027] G is phenyl, or a heterocyclic ring; heterocyclic indene, dihydroindene, or benzodioxole;

[0028] linkage₃ is a C₁-C₃ alkyl, alkylamino, aminoalkyl, alkylaminocarboxylic, or amino;

[0029] linkage₄ is alkylamido, amidalkyl, alkylamidocarboxylic, or amino;

[0030] \( R_7 \) is amino, or C₁-C₃ alkyl;

[0031] \( R_8 \) is halo;

[0032] \( R_9 \) is C₁-C₃ alkyl, C₁-C₃ alkoxy, or hydroxy;

[0033] x is an integer of 0-2;

[0034] v is an integer of 0-1; or

[0035] a pharmaceutically acceptable salt thereof.

[0036] Another aspect of the invention is compound selected from any of the compounds in the Examples, or a pharmaceutically acceptable salt thereof.

[0037] Another aspect of the invention is a composition that includes a carrier and any of the compounds of formula I or II, pharmaceutically acceptable salts thereof, or any combination of such compounds.

[0038] Another aspect of the invention is a composition that includes a carrier and any of the compounds in the Examples, pharmaceutically acceptable salts thereof, or any combination thereof.

[0039] Another aspect of the invention is a method that includes administering one or more of such compositions to
a mammal. For example, the mammal can be in need of administration of the composition. Such a mammal can, for example, have cancer, a neurodegenerative disease, inflammation, a metabolic disorder, liver dysfunction, brain ischemia, heart ischemia, or an autoimmune disease such as systemic lupus erythematosus. In some cases, the mammal has triple negative breast cancer or ovarian cancer.

[0040] The compositions and methods described herein can include one or more agents such as vitamin E, an antioxidant, and/or a mixture of such agents can sequester lipid peroxidation byproducts, and can be effective treatments for controlling ER stress responses and sustained IRE1α/XBP1 signaling in tumor-associated dendritic cells exposed, for example, to ovarian cancer-derived ascites.

**BRIEF DESCRIPTION OF THE DRAWINGS**

[0041] The drawings illustrate generally, by way of example, but not by way of limitation, various embodiments of the present invention.

[0042] FIG. 1A is a structure of vitamin E (VitE).

[0043] FIG. 1B is the structure of hydralazine (H2z), a representative member of lipid peroxidation-sequestering hydrazines.

[0044] FIG. 1C is RT-qPCR analyses of markers of ER stress after culturing purified tumor-resident DCs in the absence (grey bars) or presence (green bars) of 25% cell-free ovarian cancer ascites supernatants for 18 hours. Data are normalized to Actb expression in each sample.

[0045] FIG. 1D is flow cytometry analysis of lipid accumulation in mouse bone marrow-derived dendritic cells exposed to the indicated treatments, measured by BODIPY 493/503 staining intensity. Both raw data and quantified geometric mean fluorescence intensity (MFI) are shown.

[0046] FIG. 2A is a cartoon of a cleavable RNA probe and IRE1α-dependent hairpin cleavage site in envelope. In FIG. 2A, the quenching dye is released, fluorescence is emitted.

[0047] FIG. 2B is a cartoon of a point mutation (G→T) in the hairpin that abrogates IRE1α activity against RNA probe, controlling for contamination by non-specific RNAs. In FIG. 2B, the quenching dye is retained, and no fluorescence is emitted.

[0048] FIG. 3 is the structure of an IRE1α inhibitor identified by computational screening and confirmed by human IRE1α FRET assay (commercially available from InterBioScreen).

[0049] FIG. 4 is the structures of novel IRE1α inhibitors and their corresponding IC50 values, according to some embodiments.

[0050] FIG. 5 is the structures of novel IRE1α inhibitors and their corresponding IC50 values, according to some embodiments.

[0051] FIG. 6 is a plot of rate as a function of compound concentration. FIG. 6 shows the inhibitory activity of some embodiments of the inventive compounds against IRE1α. All members of inhibitor series significantly block IRE1α endoribonuclease catalytic activity. Raw data analysis of IRE1α FRET assay results are shown, plotted as rate of fluorescence signal increase over time.

[0052] FIG. 7 are RT-PCR and western blot analyses of IRE1α inhibitors according to some embodiments. All members of the inhibitor series significantly block IRE1α a endoribonuclease activity and autophosphorylation in cells. Panel A in FIG. 7 illustrates an RT-PCR analysis of Xbp1 splicing in human 293FT cells. Cells were treated with the pharmacoëgial ER stressor tunicamycin in the presence or absence of indicated IRE1α inhibitors for 3 hours. The upper band corresponds to full-length, unspliced Xbp1 mRNA, while the lower band represents the shorter, spliced Xbp1 mRNA isoform generated by active IRE1α. Panel B in FIG. 7 illustrates phos-tag western blot analysis of IRE1α autophosphorylation in 293FT cells treated with tunicamycin in the presence or absence of indicated compounds. The endoribonuclease inhibitor 408C and kinase inhibitor KIRA6 are included in each experiment as controls.

[0053] FIG. 8 is a synthetic scheme to synthesize a "building block" base according to an embodiment.

[0054] FIG. 9 is a synthetic scheme to synthesize Compound 31. Alkylene head groups are generated via a TMS intermediate and then coupled with a copper coil reaction.

[0055] FIG. 10A is a plot of BODIPY 493/503 mean fluorescence staining intensity (MFI) as a function of IRE1α inhibitor concentration. Marine splenic dendritic cells that were incubated with 10% marine ovarian cancer ascites supernatants overnight in the presence or absence of indicated IRE1α inhibitors. Lipid accumulation was measured by flow cytometry as BODIPY 493/503 mean fluorescence staining intensity (MFI).

[0056] FIG. 10B is a plot of Prostaglandin E2 secretion as a function of IRE1α inhibitor concentration. Marine bone marrow-derived dendritic cells were pre-treated for 1 hour with the indicated IRE1α inhibitors, then stimulated with LPS for 6 hours. Prostaglandin E2 secretion was measured by ELISA in cell-free supernatants.

**DETAILED DESCRIPTION**

[0057] The invention relates to compounds that can modulate the activity of IRE1α. IRE1α is a type I transmembrane protein with dual enzymatic activities, including an N-terminal domain that projects into the luminal side of the endoplasmic reticulum (IRE1-LD) and a serine/threonine kinase domain plus a C-terminal endoribonuclease (RNase) domain located on the cytosolic side of the protein.

[0058] The compounds of the invention include any of the compounds described herein, in the Examples, and the figures. Embodiments of the invention include but are not limited to one or more compounds of formula I:

![Chemical Structure](image)

[0059] where,:

[0060] A and B are separately each a heterocyclic ring or a phenyl group, where the A ring has x R1 substituents;

[0061] C is phenyl or pyridinyl;

[0062] D is heterocyclic ring;

[0063] linkage1 is a C3-C5 alkylene, an alkene, an alkynylene, an alkylamido, an acyl, or an oxo(carbonyl)alkylene with a first and second terminal atom;

[0064] linkage2 is a C3-C5 alkylamido, amidoualkyl, amino, urea, alkylurea, or ureaalkyl with a first and second terminal atom;

[0065] y is an integer of 0-3, and when y is 0, the linkage between the rings is a single bond;

[0066] There is additional text not shown in the image.
[0066] x is an integer of 0-2;
[0067] v is an integer of 0-1;
[0068] R₁ substituents on the A ring are selected from amino, C₁-C₃ alkyl, ether, alkoxy, oxy, hydroxy, —NH—SO₂-phenyl-(R₂), and cyano;
[0069] R₂ substituents on the B ring are selected from amino, and C₁-C₃ alkyl;
[0070] R₃ substituents on the C ring are selected from CF₃, and C₁-C₃ alkyl; and
[0071] R₄ substituents on the D ring are selected from C₁-C₃ alkyl, C₁-C₃ alkoxy, benzyl, and benzaldehyde;
[0072] R₅ is halo; or
[0073] a pharmaceutically acceptable salt thereof.

[0074] Embodiments of the invention include but are not limited to one or more compounds of formula I:

![Chemical structure](image)

wherein:

[0075] E is phenyl;
[0076] F is phenyl, naphthalene, tetrahydroanthracene, or a bicyclic heterocycle;
[0077] G is phenyl, or a heterocyclic ring; heterocyclic indene, dihydroindene, or benzodioxole;
[0078] linkage₂ is a C₁-C₃ alkyl, alkylamino, aminoalkyl, alkylaminoalkyl, or amino;
[0079] linkage₄ is alkylamido, amidouyl, alkylamidoalkyl; or
[0080] linkage₆ is amino;
[0081] R₂ is amino, or C₁-C₃ alkyl;
[0082] R₅ is halo;
[0083] R₄ is C₁-C₃ alkyl, C₁-C₃ alkoxy, or hydroxy;
[0084] x is an integer of 0-2;
[0085] v is an integer of 0-1; or
[0086] a pharmaceutically acceptable salt thereof.

[0087] All structures encompassed within a claim are “chemically feasible”, by which is meant that the structure depicted by any combination or subcombination of optional substituents meant to be recited by the claim is physically capable of existence with at least some stability as can be determined by the laws of structural chemistry and by experimentation. Structures that are not chemically feasible are not within a claimed set of compounds.

[0088] When a substituent is specified to be an atom or atoms of specified identity, “or a bond”, a configuration is referred to when the substituent is “a bond” that the groups that are immediately adjacent to the specified substituent are directly connected to each other by a chemically feasible bonding configuration.

[0089] In generel, “substituted” and “substituent” refers to an organic group as defined herein in which one or more bonds to a hydrogen atom contained therein are replaced by one or more bonds to a non-hydrogen atom such as, but not limited to, a halogen (i.e., “halo” selected from F, Cl, Br, and I); an oxygen atom in groups such as hydroxyl groups, alkoxy groups, aryloxy groups, aralkyloxy groups, oxo (carbonyl) groups, carbonyl groups including carboxylic acids, carboxylates, and carboxylate esters; a sulfur atom in groups such as thiol groups, alkyl and aryl sulfide groups, sulfone groups, sulfone groups, sulfonyl groups, and sulfonamide groups; a nitrogen atom in groups such as amines, hydroxylamines, nitriles, nitro groups, N-oxides, hydrazides, azides, and enamines; and other heteroatoms in various other groups. Non-limiting examples of substituents that can be bonded to a substituted carbon (or other) atom include F, Cl, Br, I, OR, OC(O)N(R')₂CN, CF₃, OCF₃, R', O, S, C(O), S (O), methylmethoxy, ethylmethoxy, N(R')₂SR', SOR', SO₂R', SO₃N(R')₂, SO₃R', C(O)R', C(O)OC(O)R', C(O)CH₂C(O)R', C(S)R', C(O)OR', OC(O)R', C(O)N(R')₂, OC(O)N(R')₂, C(S)N(R')₂, (CH₂)ₙNHCOOR', (CH₂)ₙN(R')₂N(R')₂, N(R')₂N(R')₂, N(R')₂SO₂R', N(R')₂SO₃R', N(R')₂SO₃N(R')₂, N(R')₂C(O)OR', N(R')₂C(O)OR', N(R')₂C(O)OR', or C(==N)OR' wherein R' can be hydrogen or a carbon-based moiety, and wherein the carbon-based moiety can itself be further substituted. In some cases the R' group is a hydrogen, C₁-C₃ alkyl, or phenyl.

[0090] In many of the compounds described herein, the substituents are selected from amino, C₁-C₃ alkyl, ether, alkoxy, oxy, CF₃, and cyano C₁-C₃ alkoxy, benzyl, and benzaldehyde. The ether and alkoxy groups can have 1-6 carbon atoms.

[0091] Substituted alkyl, alkenyl, alkynyl, cycloalkyl, and cycloalkenyl groups as well as other substituted groups also include groups in which one or more bonds to a hydrogen atom are replaced by one or more bonds, including double or triple bonds, to a carbon atom, or to a heteroatom such as, but not limited to, oxygen in carbonyl (oxo), carboxyl ester, amide, imide, urethane, and urea groups; and nitrogen in imines, hydroxymines, oximes, hydrazones, amidines, guanidines, and nitriles.

[0092] Substituted ring groups such as substituted aryl, heterocyclic and heteroaryl groups also include rings and fused ring systems in which a bond to a hydrogen atom is replaced with a bond to a carbon atom. Therefore, substituted aryl, heterocyclic and heteroaryl groups can also be substituted with alkyl, alkenyl, cycloalkyl, aryl, heteroaryl, and alkynyl groups as defined herein, which can themselves be further substituted.

[0093] The term “heteroatoms” as used herein refers to non-carbon and non-hydrogen atoms, capable of forming covalent bonds with carbon, and is not otherwise limited. Typical heteroatoms are N, O, and S. When sulfur (S) is referred to, it is understood that the sulfur can be in any of the oxidation states in which it is found, thus including sulfides (R—S—R'), disulfides (R—S(S)—R'), thioethers (R—O—R'), thioketones (R—C(S)—R'), thiocarboxylic acids, thiocarboxylates, and thiocarboxylate esters; a sulfur atom in groups such as thiol groups, alkyl and aryl sulfide groups, sulfone groups, sulfone groups, sulfonyl groups, and sulfonamide groups; a nitrogen atom in groups such as amines, hydroxylamines, nitriles, nitro groups, N-oxides, hydrazides, azides, and enamines; and other heteroatoms in various other groups. Non-limiting examples of substituents that can be bonded to a substituted carbon (or other) atom include F, Cl, Br, I, OR, OC(O)N(R')₂CN, CF₃, OCF₃, R', O, S, C(O), S (O), methylmethoxy, ethylmethoxy, N(R')₂SR', SOR', SO₂R', SO₃N(R')₂, SO₃R', C(O)R', C(O)OC(O)R', C(O)CH₂C(O)R', C(S)R', C(O)OR', OC(O)R', C(O)N(R')₂, OC(O)N(R')₂, C(S)N(R')₂, (CH₂)ₙNHCOOR', (CH₂)ₙN(R')₂N(R')₂, N(R')₂N(R')₂, N(R')₂SO₂R', N(R')₂SO₃R', N(R')₂SO₃N(R')₂, N(R')₂C(O)OR', N(R')₂C(O)OR', N(R')₂C(O)OR', or C(==N)OR' wherein R' can be hydrogen or a carbon-based moiety, and wherein the carbon-based moiety can itself be further substituted. In some cases the R' group is a hydrogen, C₁-C₃ alkyl, or phenyl.

[0094] Alkyl groups include straight chain and branched alkyl groups and cycloalkyl groups having from 1 to about 20 carbon atoms, and typically from 1 to 12 carbons or, in some embodiments, from 1 to 8 carbon atoms. Examples of straight chain alkyl groups include those with from 1 to 8 carbon atoms such as methyl, ethyl, n-propyl, n-butyl, n-pentyl, n-hexyl, n-heptyl, and n-octyl groups. Examples of branched alkyl groups include, but are not limited to, isopropyl, isobutyl, sec-butyl, tert-butyl, neopentyl, isopentyl, and 2,2-dimethylpropyl groups. Representative substituted
alkyl groups can be substituted one or more times with any of the groups listed above, for example, amino, hydroxy, cyano, carboxy, nitro, thio, alkoxy, and halogen groups.

[0095] Cycloalkyl groups are alkyl groups forming a ring structure, which can be substituted or unsubstituted. Examples of cycloalkyl include, but are not limited to, cyclocpropanyl, cyclobutanyl, cyclopentanyl, cyclohexyl, cycloheptyl, and cyclooctyl groups. In some embodiments, the cycloalkyl group has 3 to 8 ring members, whereas in other embodiments the number of ring carbon atoms range from 3 to 5, 3 to 6, or 3 to 7. Cycloalkyl groups further include polycyclic cycloalkyl groups such as, but not limited to, norbornyl, adamantlyl, bornyl, camphenyl, isocamphenyl, and carenyl groups, and fused rings such as, but not limited to, decahalin, and the like. Cycloalkyl groups also include rings that are substituted with straight or branched chain alkyl groups as defined above. Representative substituted cycloalkyl groups can be mono-substituted or substituted more than once, such as, but not limited to, 2,2-, 2,3-, 2,4-, 2,5- or 2,3,6-disubstituted cyclohexyl groups or mono-, di- or tri-substituted norbornyl or cycloheptyl groups, which can be substituted with, for example, amino, hydroxy, cyano, carboxy, nitro, thio, alkoxy, and halogen groups.

[0096] The terms “carbocyclic” and “carbocycle” denote a ring structure wherein the atoms of the ring are carbon. In some embodiments, the carbocycle has 3 to 8 ring members, whereas in other embodiments the number of ring carbon atoms is 4, 5, 6, or 7. Unless specifically indicated to the contrary, the carbocyclic ring can be substituted with as many as N substituents wherein N is the size of the carbocyclic ring with, for example, amino, hydroxy, cyano, carboxy, nitro, thio, alkoxy, and halogen groups.

[0097] (Cycloalky)alkyl groups, also denoted cycloalkyalkyl, are alkyl groups as defined above in which a hydrogen or carbon bond of the alkyl group is replaced with a bond to a cycloalkyl group as defined above.

[0098] Alkenyl groups include straight and branched chain and cyclic alkyl groups as defined above, except that at least one double bond exists between two carbon atoms. Thus, alkenyl groups have from 2 to 20 carbon atoms, and typically from 2 to 12 carbons or, in some embodiments, from 2 to 8 carbon atoms. Examples include, but are not limited to —CH—CH(CH(CH)=CH2, —CH=C(CH3)2, —C(=CH2)=CH2, vinyl, cyclohexenyl, cyclopentenyl, cyclohexadienyl, butadienyl, pentadienyl, and hexadienyl among others.

[0099] The term “cycloalkenyl” alone or in combination denotes a cyclic alkenyl group wherein at least one double bond is present in the ring structure. Cycloalkenyl groups include cycloalkyl groups having at least one double bond between two adjacent carbon atoms. Thus for example, cycloalkenyl groups include but are not limited to cyclohexenyl, cyclopentenyl, and cyclohexadienyl groups.

[0100] (Cycloalkenyl)alkyl groups are alkyl groups as defined above in which a hydrogen or carbon bond of the alkyl group is replaced with a bond to a cycloalkenyl group as defined above.

[0101] Alkynyl groups include straight and branched chain alkyl groups, except that at least one triple bond exists between two carbon atoms. Thus, alkynyl groups have from 2 to 20 carbon atoms, and typically from 2 to 12 carbons or, in some embodiments, from 2 to 8 carbon atoms. Examples include, but are not limited to —C≡CH, —C≡C(CH3), —C≡C(CH2CH3), —CH2C≡CH, —CH2C≡C(CH3), and —CH2C≡C(CH2CH3), among others.

[0102] Aryl groups are cyclic aromatic hydrocarbons that do not contain heteratoms. Thus aryl groups include, but are not limited to, phenyl, azulenyl, heptalenyl, biphenyl, indacenyl, fluorenlyl, phenanthrenyl, triphenylethyl, pyrenyl, naphthacenyl, chrysenedyl, biphenylene, anthracenyl, and naphthyl groups. In some embodiments, aryl groups contain 6-14 carbons in the ring portions of the groups. The phrase “aryl groups” includes groups containing fused rings, such as fused aromatic-aliphatic ring systems (e.g., indanyl, tetrahydronaphthyl, and the like), and also includes substituted aryl groups that have other groups, including but not limited to alkyl, halo, amino, hydroxy, cyano, carboxy, nitro, thio, or alkoxy groups, bonded to one of the ring atoms. Representative substituted aryl groups can be mono-substituted or substituted more than once, such as, but not limited to, 2-, 3-, 4-, 5-, or 6-substituted phenyl or naphthyl groups, which can be substituted with groups including but not limited to those listed above.

[0103] Aralkyl groups are alkyl groups as defined above in which a hydrogen or carbon bond of an alkyl group is replaced with a bond to an aryl group as defined above. Representative aralkyl groups include benzyl and phenylethyl groups and fused (cycloalkyl)arylalkyl groups such as 4-ethyl-indanylyl. The aryl moiety or the alkyl moiety or both are optionally substituted with other groups, including but not limited to alkyl, halo, amino, hydroxy, cyano, carboxy, nitro, thio, or alkoxy groups. Aralkenyl group are aralkyl groups as defined above in which a hydrogen or carbon bond of an alkyl group is replaced with a bond to an aryl group as defined above.

[0104] Heterocyclyl groups include aromatic and nonaromatic ring compounds containing 3 or more ring members, of which one or more is a heteroatom such as, but not limited to, N, O, S, or P. Heteroaryl and heterocyclicalkyl groups are included in the definition of heterocyclyl. In some embodiments, heterocyclyl groups include 3 to 20 ring members, whereas other such groups have 3 to 15 ring members. At least one ring contains a heteroatom, but every ring in a polycyclic system need not contain a heteroatom. For example, a dioxanoyl ring and a benzoxanoyl ring system (methyleneoxyphenyl ring system) are both heterocyclyl groups within the meaning herein. A heterocyclyl group designated as a C2-heterocyclyl can be a 5-ring with two carbon atoms and three heteroatoms, a 6-ring with two carbon atoms and four heteroatoms, and so forth. Likewise a C2-heterocyclyl can be a 5-ring with one heteroatom, a 6-ring with two heteroatoms, and so forth. The number of carbon atoms plus the number of heteroatoms sums up to equal the total number of ring atoms. In some cases, the heterocyclyl is a single ring. In other cases, the heterocyclyl is a fusion of two or three rings. The phrase “heterocyclyl group” includes fused ring species including those having fused aromatic and non-aromatic groups. The phrase also includes polycyclic ring systems containing a heteroatom such as, but not limited to, quinolinyl and also includes heterocyclyl groups that have substituents, including but not limited to alkyl, halo, amino, hydroxy, cyano, carboxy, nitro, thio, or alkoxy groups, bonded to one of the ring members. A heterocyclyl group as defined herein can be a heteroaryl group or a partially or completely saturated cyclic group including at least one ring heteroatom. Heterocyclyl groups include, but are not limited to, pyrroldinyl, furanyl, tetra-
hydrofuranyl, dioxolanyl, piperdinyl, piperoxyl, morpholinyl, pyrrolidyl, pyrazolyl, triazolyl, tetrazolyl, oxazolyl, isoxazolyl, thiazolyl, pyridyl, thiophenyl, benzothiophenyl, benzofuranyl, dihydrobenzofuranyl, indolyl, dihydroindolyl, azaindoxy, indolazolyl, benzimidazolyl, azabenzenimidazolyl, benzoxazolyl, benzo[thiazolyl, benzothiazolyl, imidazopyridinyl, isoxazolopyridinyl, thiophenyl, pyridinyl, thiophenyl, benzo[b]thiophenyl, 4,5-isquinolyl, 5-isquinolyl, 6-isquinolyl, 7-isquinolyl, 8-isquinolyl, 2-(benzo[b]furanyl, 3-(benzo[b]furanyl, 4-(benzo[b]furanyl, 5-(benzo[b]furanyl, 6-(benzo[b]furanyl, 7-(benzo[b]furanyl, 2-(3,2-dihydrobenzofuran-2-yl), 4-(2,3-dihydrobenzofuran-4-yl), 6-(2,3-dihydrobenzofuran-6-yl), 7-(2,3-dihydrobenzofuran-7-yl), benzof[b]thiophenyl, 2-(benzo[b]thiophenyl, 3-(benzo[b]thiophenyl, 4-(benzo[b]thiophenyl, 5-(benzo[b]thiophenyl, 6-(benzo[b]thiophenyl, 7-(benzo[b]thiophenyl, 2-(3,2-dihydrobenzofuran-2-yl), 4-(2,3-dihydrobenzofuran-4-yl), 6-(2,3-dihydrobenzofuran-6-yl), 7-(2,3-dihydrobenzofuran-7-yl), indolyl, 1-(1-indolyl, 2-indolyl, 3-indolyl, 4-indolyl, 5-indolyl, 6-indolyl, 7-indolyl), indazole (1-indazolyl, 3-indazolyl, 4-indazolyl, 5-indazolyl, 6-indazolyl, 7-indazolyl), benzimidazolyl (1-benzimidazolyl, 2-benzimidazolyl, 4-benzimidazolyl, 5-benzimidazolyl, 6-benzimidazolyl, 7-benzimidazolyl, 8-benzimidazolyl), benzoxazolyl (1-benzoxazolyl, 2-benzoxazolyl), benzothiazolyl (1-benzothiazolyl, 2-benzothiazolyl, 4-benzothiazolyl, 5-benzothiazolyl, 6-benzothiazolyl, 7-benzothiazolyl), carbazolyl (1-carbazolyl, 2-carbazolyl, 3-carbazolyl, 4-carbazolyl), 5H-dibenzo[b,f]azepin (5H-dibenzo[b,f]azepin-1-yl, 5H-dibenzo[b,f]azepin-2-yl, 5H-dibenzo[b,f]azepin-3-yl, 5H-dibenzo[b,f]azepin-4-yl, 5H-dibenzo[b,f]azepin-5-yl), 10H-dihydro-5H-dibenzo[b,f]azepin-1-yl, 10H-dihydro-5H-dibenzo[b,f]azepin-2-yl, 10H-dihydro-5H-dibenzo[b,f]azepin-3-yl, 10H-dihydro-5H-dibenzo[b,f]azepin-4-yl, 10H-dihydro-5H-dibenzo[b,f]azepin-5-yl, and the like.

Hydrocyclalkyl groups are cyclic alkyl groups as defined above in which a hydrogen or carbon bond of an alkyl group is replaced with a bond to a heterocyclic group as defined above. Representative heterocyclalkyl groups include, but are not limited to, furan-2-yl methyl, furan-3-yl methyl, pyridine-2-yl methyl (pyridyl), pyridine-3-yl methyl (pyrindyl), pyridine-4-yl methyl (pyridyl), tetrahydrofuran-2-yl ethyl, and indol-2-ylpropyl. Heterocyclalkyl groups can be substituted on the heterocyclic moiety, the alkyl moiety, or both.

Heterocyclalkyl groups are alkyl groups as defined above in which a hydrogen or carbon bond of an alkyl group is replaced with a bond to a heterocyclic group as defined above. Heterocyclalkyl groups can be substituted on the heterocyclic moiety, the alkyl moiety, or both.

By a “ring system” or “ring,” as the term is used herein, is meant a moiety comprising one, two, three or more rings, which can be substituted with non-ring groups or with other ring systems, or both, which can be fully saturated, partially unsaturated, fully unsaturated, or aromatic, and when the ring system includes more than a single ring, the rings can be fused, bridged, or spirocyclic. By “spirocyclic” is meant the class of structures wherein two rings are fused at a single tetrahedral carbon atom, as is well known in the art.

A “monocyclic, bicyclic or polycyclic, aromatic or partially aromatic ring” as the term is used herein refers to a ring system including an unsaturated ring possessing 4n+2 pi electrons, or a partially reduced (hydrogenated) form thereof. The aromatic or partially aromatic ring can include additional fused, bridged, or spiro rings that are not them-
selves aromatic or partially aromatic. For example, naphthalene and tetralin, naphthalene and tetralin are both a “monocyclic, bicyclic or polycyclic, aromatic or partially aromatic ring” within the meaning herein. Also, for example, a benz-2.2.2-bicyclooctane is also a “monocyclic, bicyclic or polycyclic, aromatic or partially aromatic ring” within the meaning herein, containing a phenyl ring fused to a bridged bicyclic system. A fully saturated ring has no double bonds therein, and is carbocyclic or heterocyclic depending on the presence of heteroatoms within the meaning herein.

[0111] The term “alkoxy” refers to an oxygen atom connected to an alkyl group, including a cycloalkyl group, as are defined above. Examples of linear alkoxy groups include but are not limited to methoxy, ethoxy, n-propoxy, n-butoxy, n-pentyloxy, n-hexyloxy, and the like. Examples of branched alkoxy include but are not limited to isopropoxy, sec-butoxy, tert-butoxy, isopentyloxy, isohexyloxy, and the like. Examples of cyclic alkoxy include but are not limited to cyclopropyloxy, cyclobutyloxy, cyclopentyloxy, cyclohexyloxy, and the like.

[0112] The terms “aryloxy” and “arylalkoxy” refer to, respectively, an aryl group bonded to an oxygen atom and an arylalkyl group bonded to the oxygen atom at the alkyl moiety. Examples include but are not limited to phenox, naphthoxy, and benzoxyl.

[0113] An “acyl” group as the term is used herein refers to a group containing a carbonyl moiety wherein the group is bonded via the carbonyl carbon atom. The carbonyl carbon atom is also bonded to another carbon atom, which can be part of an alkyl, aryl, cycloalkyl, acylcycloalkyl, heterocyclic, heterocyclycalkyl, heteroaryl, heteroaryalkyl group or the like. In the special case wherein the carbonyl carbon atom is bonded to a hydrogen, the group is a “formyl” group, an acyl group as the term is defined herein. An acyl group can include 0 to about 12-20 additional carbon atoms bonded to the carbonyl group. An acyl group can include double or triple bonds within the meaning herein. An acryloyl group is an example of an acyl group. An acyl group can also include heteroatoms within the meaning herein. A nicotinoyl group (pyridyl-3-carbonyl) group is an example of an acyl group within the meaning herein. Other examples include acetyl, benzoyl, phenacyl, pyridyl-acetyl, cinnamoyl, and acryloyl groups and the like. When the group containing the carbon atom that is bonded to the carbonyl carbon atom contains a halogen, the group is termed a “haloacyl” group. An example is 2-trifluoracetyl group.

[0114] The term “amine” or “amino” includes primary, secondary, and tertiary amines having, e.g., the formula N(group), wherein each group can independently be H or non-H, such as alkyl, aryl, and the like. Amines include but are not limited to R—NH₂, for example, alkylamines, arylamines, alkylarylamines; R₂NH wherein each R is independently selected, such as dialkylamines, diarylamines, aralkylamines, heterocyclicamines and the like; and R₃N wherein each R is independently selected, such as trialkylamines, dialkylarylamines, aralkylarylamines, triarylamines, and the like. The term “amine” also includes ammnonium ions as used herein.

[0115] An “aminos” group is a substituent of the form —NH₂, —NHR, —N(R)₂, —N(R)₃⁺, wherein each R is independently selected, and protonated forms of each. Accordingly, any compound substituted with an amino group can be viewed as an amine.

[0116] An “ammonium” ion includes the unsubstituted ammonium ion NH₄⁺, but unless otherwise specified, it also includes any protonated or quaternized forms of amines. Thus, trimethylammonium hydrochloride and tetramethylammonium chloride are both ammonium ions, and amines, within the meaning herein.

[0117] The term “amide” (or “amidic”) includes C- and N-amide groups, i.e., —C(O)NH(R)₂, and —NRC(O)R₂ groups, respectively. Amide groups therefore include but are not limited to carbamoyl groups (—C(O)NH₂) and formamide groups (—NH₂CO). A “carboxamido” group is a group of the formula C(O)N(R)₂, wherein R can be H, alkyl, aryl, etc.

[0118] The term “urethane” (or “carbonyl”) includes N- and O-urethane groups, i.e., —NRC(O)OR and —OC(O)N(R)₂ groups, respectively.

[0119] The term “sulfonamide” (or “sulfonamido”) includes S- and N-sulfonamide groups, i.e., —SO₂NR₂ and —NRSO₂R₂ groups, respectively. Sulfonamide groups therefore include but are not limited to sulfonyl groups (—SO₂NH₂).

[0120] The term “amidine” or “amidino” includes groups of the formula —C(NR)N(R)₂. Typically, an amidino group is —C(NH)NH₂.

[0121] The term “guanidine” or “guanidino” includes groups of the formula —NRC(NR)N(R)₂. Typically, a guanidine group is —NHC(NH)NH₂.

[0122] “Hal,” “halogen,” and “halide” include fluorine, chlorine, bromine and iodine.

[0123] The terms “comprising,” “including,” “having,” “composed of,” are open-ended terms used herein, and do not preclude the existence of additional elements or components. In a claim element, use of the forms “comprising,” “including,” “having,” or “composed of,” means that whatever element is comprised, had, included, or composes is not necessarily the only element encompassed by the subject of the claim that contains that word.

[0124] A “salt” as is well known in the art includes an organic compound such as a carboxylic acid, a sulfonic acid, or an amine, in ionic form, in combination with a counterion. For example, acids in their anionic form can form salts with cations such as metal cations, for example sodium, potassium, and the like; with ammonium salts such as NH₄⁺ or the cations of various amines, including tetraalkyl ammonium salts such as tetrabutylammonium, or other cations such as trimethylsulfonium, and the like. A “pharmacologically acceptable” or “pharmacologically acceptable” salt is a salt formed from an ion that has been approved for human consumption and is generally non-toxic, such as a chloride salt or a sodium salt. A “zwitterion” is an internal salt such as can be formed in a molecule that has at least two ionizable groups, one forming an anion and the other a cation, which serve to balance each other. For example, amino acids such as glycine can exist in a zwitterionic form. A “zwitterion” is a salt within the meaning herein. The compounds of the present invention may take the form of salts. The term “salts” embraces additional salts of free acids or free bases which are compounds of the invention. Salts can be “pharmacologically acceptable salts.” The term “pharmacologically acceptable salt” refers to salts which possess toxicity profiles within a range that affords utility in pharmaceutical applications. Pharmacologically unacceptable salts may nonetheless possess properties such as high crystallinity, which have utility in the practice of the present invention, such as for
example utility in process of synthesis, purification or formulation of compounds of the invention.

Suitable pharmaceutically acceptable acid addition salts may be prepared from an inorganic acid or from an organic acid. Examples of inorganic acids include hydrochloric, hydrobromic, hydroiodic, nitric, carbonic, sulfuric, and phosphoric acids. Appropriate organic acids may be selected from aliphatic, cycloaliphatic, aromatic, aliphatic, heterocyclic, carboxylic and sulfonic classes of organic acids, examples of which include formic, acetic, propionic, succinic, glycolic, gluconic, lactic, malic, tartaric, citric, ascorbic, glucuronic, maleic, fumaric, pyruvic, aspartic, glutamic, benzoic, anthranilic, 4-hydroxybenzoic, phenylacetic, mandelic, embonic (pamoic), methanesulfonylic, ethanesulfonylic, benzenesulfonylic, pantothenic, trifluoromethanesulfonylic, 2-hydroxyethanesulfonylic, p-toluenesulfonylic, sulfanilic, cyclohexylaminosulfonylic, stearic, alginic, β-hydroxybutyric, salicylic, galactaric and galacturonic acid.

Examples of pharmaceutically unacceptable acid addition salts include, for example, perchlorates and tetrafluoroborates.

Suitable pharmaceutically acceptable base addition salts of compounds of the invention include, for example, metal salts including alkaline earth metal and transition metal salts such as, for example, calcium, magnesium, potassium, sodium and zinc salts. Pharmaceutically acceptable base addition salts also include organic salts made from basic amines such as, for example, N,N'-dibenzylethylenediamine, chloroprocaine, chloral, diethanolamine, ethylenediamine, meglumine (N-methylglucamine) and procaine. Examples of pharmaceutically unacceptable base addition salts include lithium salts and cyanate salts. Although pharmaceutically unacceptable salts are generally useful as medicaments, such salts may be useful, for example as intermediates in the synthesis of Formula I or II compounds, for example in their purification by recrystallization. All of these salts may be prepared by conventional means from the corresponding compound. According to Formula I or II by reacting, for example, the appropriate acid or base with the compound according to Formula I or II. The term “pharmaceutically acceptable salts” refers to nontoxic inorganic or organic acid and/or base addition salts, see, for example, Lii et al. Salt Selection for Basic Drugs (1986), Int J. Pharm., 33, 201-217, incorporated by reference herein.

A “hydrate” is a compound that exists in a composition with water molecules. The composition can include water in stoichiometric quantities, such as a monohydrate or a dihydrate, or can include water in random amounts. As the term is used herein a “hydrate” refers to a solid form, i.e., a compound in water solution, while it may be hydrated, is not a hydrate as the term is used herein.

A “solvate” is a similar composition except that a solvent other that water replaces the water. For example, methanol or ethanol can form an “alcoholate”, which can again be stoichiometric or non-stoichiometric. As the term is used herein a “solvate” refers to a solid form, i.e., a compound in solution in a solvent, while it may be solvated, is not a solvate as the term is used herein.

A “prodrug” as is well known in the art is a substance that can be administered to a patient where the substance is converted in vivo by the action of biochemicals within a mammal’s body (e.g., in a patient’s body), such as enzymes, to the active pharmaceutical ingredient. Examples of prodrugs include esters of carboxylic acid groups, which can be hydrolyzed by endogenous esterases as are found in the bloodstream of humans and other mammals.

In addition, where features or aspects of the invention are described in terms of Markush groups, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group. For example, if X is described as selected from the group consisting of bromine, chlorine, and iodine, claims for X being bromine and claims for X being bromine and chlorine are fully described. Moreover, where features or aspects of the invention are described in terms of Markush groups, those skilled in the art will recognize that the invention is also thereby described in terms of any combination of individual members or subgroups of members of Markush groups. Thus, for example, if X is described as selected from the group consisting of bromine, chlorine, and iodine, and Y is described as selected from the group consisting of methyl, ethyl, and propyl, claims for X being bromine and Y being methyl are fully described.

In various embodiments, the compound or set of compounds, either per se or as are used in practice of embodiments of the inventive methods, can be any one of any of the combinations and/or sub-combinations of the various embodiments recited.

Provisos may apply to any of the disclosed categories or embodiments wherein any one or more of the other above disclosed embodiments or species may be excluded from such categories or embodiments.

Compounds

The compounds of the invention include any of those described herein, including compounds shown in the Examples. In some instances, the compounds are embraced by formula I:
wherein a hydrogen atom on Ring A is replaced by the first terminal atom of linkage, and a hydrogen atom on Ring is replaced by the second terminal atom of linkage.

[0138] In some cases the C ring can be a phenyl group, and in other cases, a pyridinyl group. For example, the R₃ substituent on the C ring is CF₃.

[0139] The linkage₂ group can, for example, be selected from any of the following:

wherein a hydrogen atom on Ring B is replaced by the first terminal atom of linkage₂ and a hydrogen atom on Ring C is replaced by the second terminal atom of linkage₂.

[0140] The D ring can, for example, be selected from any of the following:

[0141] The R₄ substituents on the D ring can in some cases be selected from CH₃, CH₃CHCH₃, CH₃CH(CH₂CH₃), and CH₃CH₂CH₂OH.

[0142] Embodiments of the invention include but are not limited to one or more compounds of formula II:
The F ring can, for example, be phenyl, naphthalene, tetrahydro-naphthalene, or a bicyclic heterocycle. Such an F bicyclic heterocycle can be a spirodecaene where one or two of the ring carbons is nitrogen rather than carbon. For example, an F bicyclic heterocycle can have any of the following structures:

-[continued]

The G ring can be phenyl, a heterocycle indene, a dihydro-indene, or benzodioxole. In some instances, the compounds are the compounds as shown in Table 1 and Table 2.

TABLE 1

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<td>-------------</td>
<td>-----------</td>
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TABLE 2-continued

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<td>![Structure Image]</td>
</tr>
<tr>
<td>BS6</td>
<td>![Structure Image]</td>
</tr>
</tbody>
</table>

[0146] More specifically, the inventive compound can be any of the specific examples shown herein as exemplary compounds of the invention.

Methods of Use

[0147] The inventors and coworkers have recently demonstrated XBP1 sustains dendritic cell immunosuppressive activity within the tumor microenvironment by directly upregulating enzymes involved in triglyceride biosynthesis (Cubillos-Ruiz, et al., Cell 161(7): 1527-38 (2015)). XBP1, also known as X-box binding protein 1, is a transcription factor that regulates the expression of genes involved in the proper functioning of the immune system and in the cellular stress response. The inventors demonstrated that IRE1α-mediated XBP1 activation was fueled by the induction of reactive oxygen species and subsequent formation of peroxized lipids.

[0148] The most conserved arm of the endoplasmic reticulum (ER) stress response is the dual enzyme, IRE1α. Activated during periods of ER stress, the IRE1α endoribonuclease domain excises a short nucleotide fragment from Xbp1 mRNA to generate the functional transcription factor, XBP1. This potent, multitasking protein promotes cell survival by upregulating expression of a broad range of critical genes involved in protein folding and quality control. XBP1 drives the pathogenesis of triple negative breast cancer (TNBC) by promoting tumor cell survival and metastatic capacity under hypoxic conditions. Silencing of XBP1 in TNBC leads to suppression of tumor initiation, progression, and recurrence.

[0149] Unexpectedly, the inventors have identified a second mechanism by which XBP1 promotes tumor progression: by confounding the development of protective antitumor immunity in the ovarian cancer tumor microenvironment. Without XBP1, tumor resident dendritic cells failed to accumulate intracellular lipids, which normally disrupt effective antigen cross-presentation. This pathological lipid accumulation is fundamentally driven by reactive oxygen species-mediated lipid peroxidation, which directly destabilizes protein-folding chaperones within the endoplasmic reticulum to induce a state of ER stress and XBP1 activation. Additionally, it is believed that IRE1α-mediated XBP1 signaling is also critical for myeloid cell production of immunosuppressive prostaglandins such as prostaglandin E2 (PGE2).

[0150] Novel small-molecule IRE1α inhibitors with the ability to induce two parallel and mutually reinforcing anti-tumor mechanisms, namely the direct inhibition of tumor growth and the simultaneous induction of robust anti-tumor immunity, are highly desirable, as no effective, targeted therapies currently exist for either TNBC or ovarian cancer. The compositions and methods described herein are novel IRE1α kinase inhibitors that exhibit immune-modulatory properties. No currently existing compounds possess activity in the presence of human or mouse ovarian cancer ascites, a critical requirement for IRE1α inhibitor usage clinically.

[0151] Novel direct and indirect small molecule IRE1α inhibitors can prevent lipid accumulation in myeloid cells exposed to ovarian cancer-derived ascites supernatants. Furthermore, the identified direct IRE1α inhibitors have unique chemical structures compared to currently available compounds, and therefore can have unique binding mechanisms, inhibitory activity, and off-target effects. Additionally, the inventors have demonstrated that these compounds block myeloid cell immunosuppression mediated by tumor-associated factors. The invention also includes novel uses for
vitamin E and hydralazine derivatives, which indirectly prevent IRE1α activation and thereby suppress cancer cell-induced lipid accumulation in myeloid dendritic cells.

The IRE1α-XBP1 pathway is therefore involved in a variety of pathological conditions, including neurodegenerative diseases, inflammation, metabolic disorders, liver dysfunction, brain ischemia, heart ischemia, autoimmune diseases, and cancer. Hence, modulation of this pathway provides therapeutic methods useful for treatment of such diseases. The identified small molecule compounds can, for example, be employed as therapeutic compounds that enhance dendritic cell and T cell anti-tumor activity in mammals. For example, the compounds disclosed herein can be used to treat murine and human ovarian cancers.

Hence, a method is described herein that includes administering any of the compounds or the composition described herein. The mammal can be in need of administration of the composition. For example, the mammal can have cancer, a neurodegenerative disease, inflammation, a metabolic disorder, liver dysfunction, brain ischemia, heart ischemia, or an autoimmune disease. In some cases, the mammal has triple negative breast cancer or ovarian cancer.

Composition and Combination Treatments

The IRE1α inhibitor compounds, their pharmaceutically acceptable salts or hydroxyethyl esters of the present invention may be combined with a pharmaceutically acceptable carrier to provide pharmaceutical compositions useful for treating the biological conditions or disorders noted herein in mammalian species, and more preferably, in humans. The particular carrier employed in these pharmaceutical compositions may vary depending upon the type of administration desired (e.g. intravenous, oral, topical, suppository, or parenteral).

In preparing the compositions in oral liquid dosage forms (e.g. suspensions, elixirs and solutions), typical pharmaceutical media, such as water, glycols, oils, alcohols, flavoring agents, preservatives, coloring agents and the like can be employed. Similarly, when preparing oral solid dosage forms (e.g. powders, tablets and capsules), carriers such as starches, sugars, diluents, granulating agents, lubricants, binders, disintegrating agents and the like can be employed.

Another aspect of an embodiment of the invention provides compositions of the compounds of the invention, alone or in combination with another IRE1α inhibitor or another type of therapeutic agent, or both. For example, the compositions and methods described herein can include one or more agents such as vitamin E, an antioxidant, and/or hydralazine. Such agents can sequester lipid peroxidation byproducts, and can be effective treatments for controlling ER stress responses and sustained IRE1α/XBP1 signaling in tumor-associated dendritic cells exposed, for example, to ovarian cancer-derived ascites.

As set forth herein, compounds of the invention include stereoisomers, tautomers, solvates, hydrates, salts including pharmaceutically acceptable salts, and mixtures thereof. Compositions containing a compound of the invention can be prepared by conventional techniques, e.g. as described in Remington: The Science and Practice of Pharmacy, 19th Ed., 1995, incorporated by reference herein. The compositions can appear in conventional forms, for example capsules, tablets, aerosols, solutions, suspensions or topical applications.

Typical compositions include one or more compounds of the invention and a pharmaceutically acceptable excipient which can be a carrier or a diluent. For example, the active compound will usually be mixed with a carrier, or diluted by a carrier, or enclosed within a carrier which can be in the form of an ampoule, capsule, sachet, paper, or other container. When the active compound is mixed with a carrier, or when the carrier serves as a diluent, it can be solid, semi-solid, or liquid material that acts as a vehicle, excipient, or medium for the active compound. The active compound can be adsorbed on a granular solid carrier, for example contained in a sachet. Some examples of suitable carriers are water, salt solutions, alcohols, polyethylene glycols, polyhydroxyethoxylated castor oil, peanut oil, olive oil, gelatin, lactose, terra alba, sucrose, dextrin, magnesium carbonate, sugar, cyclodextrin, amylose, magnesium stearate, talc, gelatin, agar, pectin, acacia, stearic acid or lower alkyl ethers of cellulose, silicic acid, fatty acids, fatty acid amines, fatty acid monoglycerides and diglycerides, pentenylthanol fatty acid esters, polyoxyethylene, hydroxymethylcellulose and polyvinylpyrrolidone. Similarly, the carrier or diluent can include any sustained release material known in the art, such as glyceryl monostearate or glyceryl distearate, alone or mixed with a wax.

The formulations can be mixed with auxiliary agents which do not deleteriously react with the active compounds. Such additives can include wetting agents, emulsifying and suspending agents, salt for influencing osmotic pressure, buffers and/or coloring substances preserving agents, sweetening agents or flavoring agents. The compositions can also be sterilized if desired.

The route of administration can be any route which effectively transports the active compound of the invention which inhibits the activity of the IRE1α to the appropriate or desired site of action, such as oral, nasal, pulmonary, buccal, subdermal, intradermal, transdermal or parenteral, e.g. rectal, depot, subcutaneous, intravenous, intraurethral, intramuscular, intranasal, ophthalmic solution or an ointment, the oral route being preferred.

For parenteral administration, the carrier will typically comprise sterile water, although other ingredients that aid solubility or serve as preservatives can also be included. Furthermore, injectable suspensions can also be prepared, in which case appropriate liquid carriers, suspending agents and the like can be employed.

For topical administration, the compositions of the invention can be formulated using bland, moisturizing bases such as ointments or creams.

If a solid carrier is used for oral administration, the preparation can be tableted, placed in a hard gelatin capsule in powder or pellet form or it can be in the form of a troche or lozenge. If a liquid carrier is used, the preparation can be in the form of a syrup, emulsion, soft gelatin capsule or sterile injectable liquid such as an aqueous or non-aqueous liquid suspension or solution.

Injectable dosage forms generally include aqueous suspensions or oil suspensions which can be prepared using a suitable dispersant or wetting agent and a suspending agent. Injectable forms can be in solution phase or in the form of a suspension, which is prepared with a solvent or diluent. Acceptable solvents or vehicles include sterilized water, Ringer’s solution, or an isotonic aqueous saline solution. Alternatively, sterile oils can be employed as solvents or
suspending agents. Preferably, the oil or fatty acid is non-volatile, including natural or synthetic oils, fatty acids, mono-, di- or tri-glycerides.

[0165] For injection, the formulation can also be a powder suitable for reconstitution with an appropriate solution as described above. Examples of these include, but are not limited to, freeze dried, rotary dried or spray dried powders, amorphous powders, granules, precipitates, or particulates. For injection, the formulations can optionally contain stabilizers, pH modifiers, surfactants, bioavailability modifiers and combinations of these. The compounds can be formulated for parenteral administration by injection such as by bolus injection or continuous infusion. A unit dosage form for injection can be in ampoules or in multi-dose containers.

[0166] The formulations of the invention can be designed to provide quick, sustained, or delayed release of the active ingredient after administration to the patient by employing procedures well known in the art. Thus, the formulations can also be formulated for controlled release or for slow release.

[0167] Compositions contemplated by the present invention can include, for example, micelles or liposomes, or some other encapsulated form, or can be administered in an extended release form to provide a prolonged storage and/or delivery effect. Therefore, the formulations can be compressed into pellets or cylinders and implanted intramuscularly or subcutaneously as depot injections. Such implants can employ known inert materials such as silicones and biodegradable polymers, e.g., poly(lactide-co-glycolide). Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides).

[0168] For nasal administration, the preparation can contain a compound of the invention which inhibits the enzymatic activity of the focal adhesion kinase, dissolved or suspended in a liquid carrier, preferably an aqueous carrier, for aerosol application. The carrier can contain additives such as solubilizing agents, e.g., propylene glycol, surfactants, absorption enhancers such as lecithin (phosphatidylcholine) or cyclodextrin, or preservatives such as parabens.

[0169] For parenteral application, particularly suitable are injectable solutions or suspensions, preferably aqueous solutions with the active compound dissolved in polyhydroxyalkyl castor oil.

[0170] Tablets, dragees, or capsules having talc and/or a carbohydrate carrier or binder or the like are particularly suitable for oral application. Preferable carriers for tablets, dragees, or capsules include lactose, corn starch, and/or potato starch. A syrup or elixir can be used in cases where a sweetened vehicle can be employed.

[0171] A typical tablet that can be prepared by conventional tableting techniques can contain:

<table>
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<tbody>
<tr>
<td>Active compound (as free compound or salt thereof)</td>
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<tr>
<td>Colloidal silicon dioxide (Aerosil) ®</td>
</tr>
<tr>
<td>Cellulose, microcryst. (Avicel) ®</td>
</tr>
<tr>
<td>Modified cellulose gum (Ac-Di-Sol) ®</td>
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<tr>
<td>Magnesium stearate</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Coating:</th>
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<tr>
<td>HPMC approx.</td>
</tr>
<tr>
<td>*Mycelac 9–40 T approx.</td>
</tr>
</tbody>
</table>

*Acetylated monoglyceride used as plasticizer for film coating.

[0172] A typical capsule for oral administration contains compounds of the invention (250 mg), lactose (75 mg) and magnesium stearate (15 mg). The mixture is passed through a 60 mesh sieve and packed into a No. 1 gelatin capsule. A typical injectable preparation is produced by aseptically placing 250 mg of compounds of the invention into a vial, aseptically freeze-drying and sealing. For use, the contents of the vial are mixed with 2 mL of sterile physiological saline, to produce an injectable preparation.

[0173] The compounds of the invention can be administered to a human in need of such treatment, prevention, elimination, alleviation or amelioration of a malcondition that is mediated through the action of IRE1α, for example, cancer, neurodegenerative diseases, inflammation, metabolic disorders, liver dysfunction, brain ischemia, or heart ischemia.

[0174] The pharmaceutical compositions and compounds of the present invention can generally be administered in the form of a dosage unit (e.g., tablet, capsule, etc.) in an amount from about 1 ng/kg of body weight to about 0.5 g/kg of body weight, or from about 1 μg/kg of body weight to about 500 mg/kg of body weight, or from about 10 μg/kg of body weight to about 250 mg/kg of body weight, most preferably from about 20 μg/kg of body weight to about 100 mg/kg of body weight. Those skilled in the art will recognize that the particular quantity of pharmaceutical composition and/or compounds of the present invention administered to an individual will depend upon a number of factors including, without limitation, the biological effect desired, the condition of the individual and the individual’s tolerance for the compound.

[0175] The compounds of the invention are effective over a wide dosage range. For example, in the treatment of adult humans, dosages from about 0.05 to about 5000 mg, preferably from about 1 to about 2000 mg, and more preferably between about 2 and about 2000 mg per day can be used. A typical dosage is about 10 mg to about 1000 mg per day. In choosing a regimen for patients it can frequently be necessary to begin with a higher dosage and when the condition is under control to reduce the dosage. The exact dosage will depend upon the activity of the compound, mode of administration, on the therapy desired, form in which administered, the subject to be treated and the body weight of the subject to be treated, and the preference and experience of the physician or veterinarian in charge.

[0176] Generally, the compounds of the invention are dispensed in unit dosage form including from about 0.05 mg to about 1000 mg of active ingredient together with a pharmaceutically acceptable carrier per unit dosage.

[0177] Usually, dosage forms suitable for oral, nasal, pulmonary or transdermal administration include from about 125 μg to about 1250 μg, preferably from about 250 μg to about 500 μg, and more preferably from about 2.5 mg to about 250 mg, of the compounds admixed with a pharmaceutically acceptable carrier or diluent.

[0178] Dosage forms can be administered daily, or more than once a day, such as twice or thrice daily. Alternatively
dosage forms can be administered less frequently than daily, such as every other day, or weekly, if found to be advisable by a prescribing physician.

[0179] An embodiment of the invention also encompasses prodrugs of a compound of the invention which on administration undergo chemical conversion by metabolic or other physiological processes before becoming active pharmacological substances. Conversion by metabolic or other physiological processes includes without limitation enzymatic (e.g., specific enzymatically catalyzed) and non-enzymatic (e.g., general or specific acid or base induced) chemical transformation of the prodrug into the active pharmacological substance. In general, such prodrugs will be functional derivatives of a compound of the invention which are readily convertible in vivo into a compound of the invention. Conventional procedures for the selection and preparation of suitable prodrug derivatives are described, for example, in Design of Prodrugs, ed. H. Bundgaard, Elsevier, 1985.

[0180] In another embodiment, there are provided methods of making a composition of a compound described herein including formulating a compound of the invention with a pharmaceutically acceptable carrier or diluent. In some embodiments, the pharmaceutically acceptable carrier or diluent is suitable for oral administration. In some such embodiments, the methods can further include the step of formulating the composition into a tablet or capsule. In other embodiments, the pharmaceutically acceptable carrier or diluent is suitable for parenteral administration. In some such embodiments, the methods further include the step of lyophilizing the composition to form a lyophilized preparation.

[0181] The compounds of the invention can be used therapeutically in combination with i) one or more other IRE1α inhibitors and/or ii) one or more other types of protein kinase inhibitors and/or one or more other types of therapeutic agents which can be administered orally in the same dosage form, in a separate oral dosage form (e.g., sequentially or non-sequentially) or by injection together or separately (e.g., sequentially or non-sequentially).

[0182] Accordingly, in another embodiment the invention provides combinations, comprising:

[0183] a) a compound of the invention as described herein; and

[0184] b) one or more compounds comprising:

[0185] i) other compounds of the present invention,

[0186] ii) other agents or medicaments adapted for treatment of a disease or malcondition for which inhibition of IRE1α is medically indicated, for example, vitamin E, an antioxidant, hydralazine, or any combination thereof. Such compounds, agents or medicaments can be medically indicated for treatment of cancers such as TNBC or ovarian cancer, neurodegenerative diseases, inflammation, metabolic disorders, liver dysfunction, autoimmune diseases, brain ischemia, or heart ischemia.

[0187] Combinations of the invention include mixtures of compounds from (a) and (b) in a single formulation and compounds from (a) and (b) as separate formulations. Some combinations of the invention can be packaged as separate formulations in a kit. In some embodiments, two or more compounds from (b) are formulated together while a compound of the invention is formulated separately.

[0188] The dosages and formulations for the other agents to be employed, where applicable, will be as set out in the latest edition of the Physicians’ Desk Reference, incorporated herein by reference.

[0189] The Examples illustrate some of experimental work performed in the development of the invention.

**EXAMPLE 1**

Vitamin E and Hydralazine Suppress Lipid Accumulation in Myeloid Dendritic Cells

[0190] Consistently, both vitamin E and hydralazine suppressed pathological intracellular lipid accumulation in myeloid dendritic cells exposed to ovarian cancer ascites supernatants (FIG. 1). Based on the strong evidence linking aberrant lipid accumulation myeloid cell immunosuppression, these agents can be used to restore the function of antigen presenting cells in the tumor microenvironment.

**EXAMPLE 2**

FRET Assay

[0191] In addition to the indirect inhibitors vitamin E and hydralazine, the compositions and methods described herein can include one or more direct, small molecule IRE1α inhibitors.

[0192] IRE1α is a dual enzyme, containing a kinase and endoribonuclease domain. Phosphorylation of the kinase domain during times of ER stress leads to activation of the endoribonuclease domain and subsequent Xbp1 splicing, indicating that small molecules designed to block either the kinase domain or the endoribonuclease domain are feasible inhibitory strategies.

[0193] To evaluate potential small molecule IRE1 inhibitors, a Förster resonance energy transfer (FRET)-based small molecule IRE1 screening system was used. In brief, a small XBP1-mimetic RNA hairpin containing sequence features required for IRE1α-mediated splicing has been synthesized with the fluorophore 6FAM attached to the 5' end and the quenching dye Black Hole Quencher 1 (BHQ1) attached to the 3' end. When the hairpin is intact, 6FAM fluorescence is completely absorbed by BHQ1; however, IRE1α-mediated cleavage of the RNA hairpin leads to an increase in the fluorescence signal. The inventors also incorporated a point mutant version of this same RNA hairpin that is resistant to IRE1α-mediated cleavage to control for non-specific RNAse contamination (FIG. 2).

**EXAMPLE 3**

Potent IRE1α Inhibitors Identified by FRET Assay

[0194] Approximately 170 compounds were obtained and evaluated using the FRET assay, and at least one active compound was identified from this screen (IC50: 26 μM, FIG. 3). Compound activity was evaluated at the biotechnology company Cyclophilin guided entirely by the inventors.

[0195] A small panel of published type II kinase inhibitors structurally related to the FDA-approved kinase inhibitor Ponatinib (Desai et al., Med Chem 56(7): 3033-47 (2013)) were separately tested (FIG. 4 and FIG. 5). Several of these analogues displayed potent IRE1α inhibitory activity first
confirmed by FRET (FIG. 6) and later validated with several cellular assays that measure XBP1/IRE1α function (FIG. 7).

**EXAMPLE 4**

**Synthesis of IRE1α Inhibitors**

The synthetic strategy used to generate many of the IRE1α inhibitors is shown in FIG. 8 and FIG. 9.

**EXAMPLE 5**

**Potent IRE1α Inhibitor Reduces Immunosuppressive Lipid Accumulation in Dendritic Cells and Inhibits Myeloid Cell Prostaglandin E2 Production**

The inventors have developed and tested dozens of structural analogues based around the Ponatinib-like compound series. The most potent of these compounds can reduce immunosuppressive lipid accumulation in dendritic cells and inhibit myeloid cell prostaglandin E2 production in vitro (FIG. 10).

**EXAMPLE 6**

**Computational Screening**

After establishing the FRET system, computational models of IRE1α based on published crystal structures were used to dock over 7 million compounds commercially available from the company eMolecules. Docking was performed using the Schrodinger software suite.

The cytoplasmic domain of human IRE1α (approximately residues 465-977) has been crystallized five separate times (PDB 4PL3, 4U6R, 4PL4, 4PL5, and 3P23) in different states of phosphorylation and activation, as well as with both endoribonuclease inhibitors and kinase inhibitors (Sanchez et al., Nature Communications 5:4202 (2014); Harrington et al., ACS Med Chem Lett 6:68-72 (2015); Ali et al., The EMBO J 30:894-905 (2011)). These studies and others (see, e.g., Wang et al., Nature Chem Biol 8:982-9 (2012)) provide substantial evidence that IRE1α kinase inhibitors can either inhibit or activate the IRE1α endoribonuclease domain depending on their binding mode.

**EXAMPLE 7**

**Design of Additional Compounds**

After grafting the SRC DFG-loop onto the IRE1α crystal structure, the SRC inhibitor was docked into the hybrid model for quality control.

**EXAMPLE 8**

The SRC inhibitor exhibited a good docking score using unconstrained rigid receptor docking, as its urea group made hydrogen bonds with both a serine residue N-terminal to the DFG loop and a lysine-aspartate salt bridge in the kinase active site. With the initial model validated, the set of all compounds commercially available from the company eMolecules (over 7 million in sum) was used in the screen collection. From the full list of 7 million compounds, only urea and amide-containing structures were computationally docked (~800,000 compounds), as these motifs form key hydrogen bond interactions with kinase DFG loops. Top scoring compounds were clustered by structural similarity and filtered for those with desirable properties, and the top scoring compounds from each of 49 clusters were ordered and tested by FRET assay.

**EXAMPLE 9**

Initial results indicated that one of the 49 compounds exhibited some activity against IRE1. The structure-activity relationships of an additional 62 compounds were examined. However, secondary screening after FRET assay optimization, performed at the CRO Cyclofluidic, revealed that none of these 111 compounds exerted any activity. Cyclofluidic had published a small set of approximately 50 compounds from their internal work on derivatives of the BCR-ABL kinase inhibitor Ponatinib, and the inventors also screened these published compounds for IRE1α inhibitory activity.

**EXAMPLE 10**

Serendipitously, the inventors identified the compounds shown in FIG. 4 and FIG. 5 from this additional effort.

**EXAMPLE 11**

New crystal structures of IRE1α in complex with Type II, DFG-out-inducing kinase inhibitors became available (Concha et al., Molecular Pharmacol 88:1011-2 (2015)), which was used to develop a second model of IRE1α.

First, the ponatinib-like compounds were docked into this new model, allowing some structural flexibility, to control for model quality. Subsequently, a second computational screen was performed with the updated model, and compounds for follow up were selected as follows:

1. Docked into the ponatinib-like compound induced structure.
2. H-bond with hinge NH
3. Strained compounds removed
4. Any compound with similarity to any of the known compounds tested thus far removed
5. Clustering
6. Selecting the highest docking score per cluster
7. The top 63 compounds based on this strategy were then tested, and only one compound showed weak activity by FRET assay (FIG. 3).

**EXAMPLE 12**

**Design of Additional Compounds**

New small molecule compounds were designed to inhibit the human IRE1alpha kinase domain. Multiple variants of the original compound series were designed and ranked by computational docking score using the software LiveDesign. High scoring compounds were synthesized and evaluated biochemically by the IRE1α FRET assay.
EXAMPLE 8

In Vitro FRET Assay Protocol

[0216] In vitro FRET assay was performed to evaluate the ability of select compounds to inhibit IRE1, the results of which are summarized in the following table.

[0217] To perform the in vitro FRET assay, 1x complete assay buffer (CAB; 1M DTT, 50 mM sodium citrate pH 7.15, 1 mM magnesium acetate, 0.02% tween 20) was used to dilute SignalChem IRE1αr protein to a final concentration of 2 nM. Selected compounds were serially diluted with DMSO in a non-binding black 384-well plate for a total of 15 µl in each well. 2 µl of the serially diluted compound or DMSO control were then added to new wells containing 98 µl of 1xCAB, for a total volume of 100 µl. 10 µl of which were then transferred to wells of a new plate. 5 µl of the diluted IRE1α was then added to each well. 5 µl of a 400 mM XBPI RNA probe was then added to each well. Fluorescence was then read over 30 minutes in kinetic mode (485/515 nm).

[0218] Two RNA probes were used, XBPI wildtype (CAUUGUCGCAGCAUAG; SEQ_ID NO: 1) which is able to be spliced by active IRE1α or XBPI mutant (CAUGUGCCCGACGCAUG; SEQ_ID NO: 2) which is unable to be spliced. Each probe contained a 3'-6-FAM modification and a 3'-Iowa Black FQ modification.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Ref. No.</th>
<th>Mean EC&lt;sub&gt;50&lt;/sub&gt; (nM)</th>
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<tbody>
<tr>
<td>A25</td>
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<tr>
<td>A24</td>
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</table>

Note: Biochemical assay Mean EC<sub>50</sub> data are designated within the following ranges:
A: &gt;5000 nM
B: &gt;5000 nM to &lt;5000 nM
C: &gt;5000 nM to &lt;50000 nM
D: &gt;100000 nM

REFERENCES


**REα Endonuclease Activity.** Molecular pharmacology. 2015:88:1011-23.

[0236] All patents and publications referenced herein are hereby specifically incorporated by reference to the same extent as if it had been incorporated by reference in its entirety individually or set forth herein in its entirety. Applicants reserve the right to physically incorporate into this specification any and all materials and information from any such cited patents or publications.

Additional Embodiments

[0237] The following exemplary embodiments are provided, the numbering of which is not to be construed as designating levels of importance:

[0238] Embodiment 1 provides a compound of formula I:

![Chemical Structure](image)

[0239] wherein:

[0240] A and B are separately each a heterocyclic ring or a phenyl group, where the A ring has x R₁ substituents;

[0241] C is phenyl or pyridinyl;

[0242] D is heterocyclic ring;

[0243] linkage₁ is a C₁-C₃ alkenylene, an alkynylene, an alkylalkynylene, an alkylamido, an acyl, or an oxo(carbonyl)alkylene with a first and second terminal atom;

[0244] linkage₂ is a C₁-C₃ alkylamido, amidocarbalkyl, amino, urea, alkyurea, or ureaalkyl with a first and second terminal atom;

[0245] y is an integer of 0-3, and when y is 0, the linkage between the rings is a single bond;

[0246] x is an integer of 0-2;

[0247] v is an integer of 0-1;

[0248] R₁ substituents on the A ring are selected from amino, C₁-C₃ alkyl, ether, alkoxy, oxy, hydroxy, —NH—SO₂—phenyl-(R₂), and cyano;

[0249] R₂ substituents on the B ring are selected from amino, and C₁-C₃ alkyl;

[0250] R₃ substituents on the C ring are selected from CF₃, and C₁-C₃ alkyl; and R₄ substituents on the D ring are selected from C₁-C₃ alkyl, C₁-C₃ alkoxy, benzyl, and benzaldehyde;

[0251] R₅ is halo; or

[0252] a pharmaceutically acceptable salt thereof.

[0253] Embodiment 2 provides the compound of embodiment 1, where the A ring is heteroaromatic.

[0254] Embodiment 3 provides a compound of any one of embodiments 1 or 2, where the A ring is a fusion of two rings.

[0255] Embodiment 4 provides a compound of any one of embodiments 1-3, where the A ring is indazole, imidazo[1,2-a]pyridine, imidazopyrazine, imidazopyridazine, pyrrolopyridine, hexahydro[1,4]thienopyrimidine, imidazole, pyrazole, pyrazine, pyridine, pyrimidine, phenylpyrimidimidamine, quinolyl, isoquinolyl, and tetrahydroquinolyl.

[0256] Embodiment 5 provides a compound of any one of embodiments 1-4, where the A ring is selected from:

![Chemical Structures](images)

[0257] Embodiment 6 provides a compound of any one of embodiments 1-5, where the B ring is a single, non-fused ring.

[0258] Embodiment 7 provides a compound of any one of embodiments 1-5, where the B ring is a fusion of two rings.

[0259] Embodiment 8 provides a compound of any one of embodiments 1-7, where the B ring is selected from:

![Chemical Structures](images)

[0260] Embodiment 9 provides a compound of any one of embodiments 1-8, wherein linkage₁ is selected from:

![Chemical Structures](images)
wherein a hydrogen atom on Ring A is replaced by the first terminal atom of linkage 1 and a hydrogen atom on Ring B is replaced by the second terminal atom of linkage 1.

[0261] Embodiment 10 provides a compound of any one of embodiments 1-9, wherein the C ring is phenyl.

[0262] Embodiment 11 provides a compound of any one of embodiments 1-10, wherein the linkage 2 is selected from:

wherein a hydrogen atom on Ring B replaced by the first terminal atom of linkage 2 and a hydrogen atom on Ring C is replaced by the second terminal atom of linkage 2.

[0263] Embodiment 12 provides a compound of any one of embodiments 1-11, wherein D ring is selected from:

[0264] Embodiment 13 provides a compound of any one of embodiments 1-12, where the R1 substituents on the A ring are selected from amino and C1-C3 alkyl.

[0265] Embodiment 14 provides a compound of any one of embodiments 1-13, where the R1 substituents on the A ring are selected from −NH2 and CH3.

[0266] Embodiment 15 provides a compound of any one of embodiments 1-14, with x=0.

[0267] Embodiment 16 provides a compound of any one of embodiments 1-14, with x=1.

[0268] Embodiment 17 provides a compound of any one of embodiments 1-15, with x=0 when the A ring is a fusion of two rings.

[0269] Embodiment 18 provides a compound of any one of embodiments 1-14, with x=1 or 2 when the A ring is a single, nonfused ring.

[0270] Embodiment 19 provides a compound of any one of embodiments 1-18, wherein the R3 substituent on the C ring is CF3.

[0271] Embodiment 20 provides a compound of any one of embodiments 1-18, wherein the R4 substituents on the D ring are selected from CH3, CH2CH(CH3)2, CH2CH(CH2)2 CH3, and CH2CH2CH2OH.

[0272] Embodiment 21 provides a compound of formula II:

wherein:
E is phenyl;
F is phenyl, naphthalene, tetrahydro-naphthalene, or a bicyclic heterocycle;
G is phenyl, or a heterocyclic ring; heterocycle indene, dihydronaphthene, or benzodioxole;
linkage 2 is a C1-C3 alkyl, alkylamino, aminoalkyl, alkylaminoalkyl, or amino; linkage 3 is alkylamido, amidoalkyl, alkylamidoalkyl; R1 is amino, or C1-C3 alkyl; R2 is halo; R3 is C1-C3 alkyl, C1-C3 alkoxy, or hydroxy; x is an integer of 0-2; v is an integer of 0-1; or a pharmaceutically acceptable salt thereof.

[0273] Embodiment 22 provides a compound from any of the compounds in the Examples, or pharmaceutically acceptable salts thereof.

[0274] Embodiment 23 provides a compound of any one of embodiments 1-22, pharmaceutically acceptable salts thereof, or any combination of such compounds or salts.

[0275] Embodiment 24 provides a compound of embodiment 23, further comprising vitamin E, an antioxidant, hyalurazine, or any combination thereof.

[0276] Embodiment 25 provides a method comprising administering the composition of any one of embodiments 23 or 24 to a mammal.

[0277] Embodiment 26 provides a method of embodiment 25, wherein the mammal is in need of administration of the composition.

[0278] Embodiment 26 provides a method of any one of embodiments 25 or 26, wherein the mammal has cancer, a neurodegenerative disease, inflammation, a metabolic disorder, liver dysfunction, an autoimmune disease, brain ischemia, or heart ischemia.

[0279] Embodiment 27 provides a method of any one of embodiments 25-27, wherein the mammal has triple negative breast cancer or ovarian cancer.

[0280] Embodiment 28 provides a compound of any one of embodiments 1-22 for use in treating cancer.

[0281] Embodiment 29 provides a use of a compound of any one of embodiments 1-22 for treating cancer.

[0282] The specific compositions and methods described herein are representative, exemplary and not intended as limitations on the scope of the invention. Other objects, aspects, and embodiments will occur to those skilled in the art upon consideration of this specification, and are encompassed within the spirit of the invention as defined by the
The invention has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the invention. This includes the generic description of the invention with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein. In addition, where features or aspects of the invention are described in terms of Markush groups, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group.

[0287] The Abstract is provided to comply with 37 C.F.R. §1.72(b) to allow the reader to quickly ascertain the nature and gist of the technical disclosure. The Abstract is submitted with the understanding that it will not be used to interpret or limit the scope or meaning of the claims.

[0288] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

[0289] Additional embodiments of the invention are found in the following listing of compounds:

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What is claimed is:

1. A method of inhibiting IREx1 comprising:
contacting IREx1 with a compound of formula (I)

\[
\begin{align*}
& \text{A} \quad \text{linkage} \quad \text{B} \quad \text{linkage} \quad \text{C} \quad (\text{CH}_2)_n \quad \text{D} \\
& \quad (\text{R}_1)_y \\
& \quad (\text{R}_2)_y \\
& \quad (\text{R}_3)_y \\
& \quad (\text{R}_4)_y
\end{align*}
\]

wherein:
A and B are separately each a heterocyclcyl ring or a phenyl group, where the A ring has x R\(_1\) substituents; 
C is phenyl or pyridinyl; 
D is heterocyclcyl ring; 
linkage is a C\(_{1-0}\) alkylene, an alkenylene, an alkinylene, an alkylamido, an acyl, or an oxo(carbonyl)alkylene with a first and second terminal atom; 
linkage\(_2\) is a C\(_{1-0}\) alkylamido, amidoalkyl, amino, urea, alkylurea, or ureaalkyl with a first and second terminal atom; 
y is an integer of 0-3, and when y is 0, the linkage between the rings is a single bond; 
x is an integer of 0-2; 
v is an integer of 0-1; 
R\(_1\) substituents on the A ring are selected from amino, C\(_{1-3}\) alkyl, ether, alkoxy, oxy, hydroxy, —NH—SO\(_2\)-phenyl-(R\(_3\)), and cyano; 
R\(_2\) substituents on the B ring are selected from amino, and C\(_{1-3}\) alkyl; 
R\(_3\) substituents on the C ring are selected from CF\(_3\), and C\(_{1-3}\) alkyl; and 
R\(_4\) substituents on the D ring are selected from C\(_{1-3}\) alkyl, C\(_{1-3}\) alkoxy, benzyl, and benzaldehyde; 
R\(_5\) is halo; or a pharmaceutically acceptable salt thereof.

2. The method of claim 2, further comprising vitamin E, an antioxidant, a hydralazine, or any combination thereof.

3. The method of claim 1, further comprising a pharmaceutical composition comprising at least one compound of formula (I), a pharmaceutically acceptable carrier, and at least one pharmaceutically acceptable excipient.

4. The method of claim 3, further comprising administering the composition to a mammal in need thereof.

5. The method of claim 3, wherein the composition is suitable for oral, nasal, pulmonary, buccal, subdermal, intradermal, transdermal or parenteral, e.g., rectal, depot, subcutaneous, intravenous, intrarethral, intramuscular, intranasal, ophthalmic solution or topical administration.

6. The method of claim 1, further comprising treating a condition selected from the group consisting of neurodegenerative diseases, inflammation, metabolic disorders, liver dysfunction, brain ischemia, heart ischemia, autoimmune diseases, and cancer.

7. The method of claim 6, wherein the cancer is ovarian cancer or triple negative breast cancer.

8. The method of claim 3, wherein the composition enhances dendritic cell and T cell anti-tumor activity in mammals.


10. The method of claim 1, wherein the compound is selected from the group consisting of Compound 31, Compound 36, Compound 41, Compound 42, Compound 43, and Compound 44.

11. The method of claim 1, wherein the compound is selected from any of the compounds in the Examples, and any combinations thereof.

* * * * *