CRYSTALLINE PEPTIDE EPOXYKETONE IMMUNOPROTEASOME INHIBITOR

Background of the Invention

In eukaryotes, protein degradation is predominately mediated through the ubiquitin pathway in which proteins targeted for destruction are ligated to the 76 amino acid polypeptide ubiquitin. Once targeted, ubiquitinated proteins then serve as substrates for the 26S proteasome, a multicatalytic protease, which cleaves proteins into short peptides through the action of its three major proteolytic activities. While having a general function in intracellular protein turnover, proteasome-mediated degradation also plays a key role in many processes such as major histocompatibility complex (MHC) class I presentation, apoptosis and cell viability, antigen processing, NF-κB activation, and transduction of pro-inflammatory signals.

The 20S proteasome is a 700 kDa cylindrical-shaped multicatalytic protease complex comprised of 28 subunits, classified as α- and β-type, that are arranged in 4 stacked heptameric rings. In yeast and other eukaryotes, 7 different α subunits form the outer rings and 7 different β subunits comprise the inner rings. The α subunits serve as binding sites for the 19S (PA700) and 11S (PA28) regulatory complexes, as well as a physical barrier for the inner proteolytic chamber formed by the two β subunit rings. Thus, in vivo, the proteasome is believed to exist as a 26S particle ("the 26S proteasome"). In vivo experiments have shown that inhibition of the 20S form of the proteasome can be readily correlated to inhibition of 26S proteasome.

Cleavage of amino-terminal prosequences of β subunits during particle formation expose amino-terminal threonine residues, which serve as the catalytic nucleophiles. The subunits responsible for catalytic activity in proteasome thus possess an amino terminal nucleophilic residue, and these subunits belong to the family of N-terminal nucleophile (Ntn) hydrolases (where the nucleophilic N-terminal residue is, for example, Cys, Ser, Thr, and other nucleophilic moieties). This family includes, for example, penicillin G acylase (PGA), penicillin V acylase (PVA), glutamine PRPP amidotransferase (GAT), and bacterial glycosylasparaginase. In addition to the ubiquitously expressed β subunits, higher vertebrates also possess three interferon- γ- inducible β subunits (LMP7, LMP2 and MECL1),

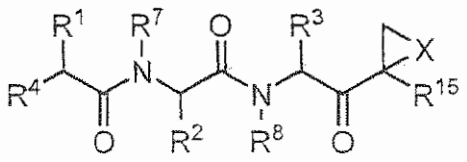
which replace their normal counterparts, β5, β1 and β2, respectively. When all three IFN-γ-inducible subunits are present, the proteasome is referred to as an "immunoproteasome". Thus, eukaryotic cells can possess two forms of proteasomes in varying ratios.

Through the use of different peptide substrates, three major proteolytic activities have been defined for the eukaryote 20S proteasomes: chymotrypsin-like activity (CT-L), which cleaves after large hydrophobic residues; trypsin-like activity (T-L), which cleaves after basic residues; and peptidylglutamyl peptide hydrolyzing activity (PGPH), which cleaves after acidic residues. Two additional less characterized activities have also been ascribed to the proteasome: BrAAP activity, which cleaves after branched-chain amino acids; and SNAAP activity, which cleaves after small neutral amino acids. Although both forms of the proteasome possess all five enzymatic activities, differences in the extent of the activities between the forms have been described based on specific substrates. For both forms of the proteasome, the major proteasome proteolytic activities appear to be contributed by different catalytic sites within the 20S core.

There are several examples of small molecules which have been used to inhibit proteasome activity; however, these compounds generally lack the specificity to delineate between the two forms of the proteasome. What is needed are improved compositions and methods for preparing and formulating proteasome inhibitor(s).

Summary

One embodiment of the disclosure relates to crystalline compounds having a structure of Formula (I) or a pharmaceutically acceptable salt thereof,



(I)

wherein

each Ar is independently an aromatic group optionally substituted with 1 to 4 substituents;

each A is independently selected from C=O, C=S, and SO_2; or

A is optionally a covalent bond when adjacent to an occurrence of Z;

B is absent or is $N(R^9)R^{10}$;

L is absent or is selected from C=O, C=S, and SO_2;

M is absent or is C_{1-12}alkyl;

Q is absent or is selected from O, NH, and N-C_{1-6}alkyl;

X is selected from O, S, NH, and N-C_{1-6}alkyl;

Y is absent or is selected from C=O and SO_2;

each Z is independently selected from O, S, NH, and N-C_{1-6}alkyl; or

Z is optionally a covalent bond when adjacent to an occurrence of A;

R^1 is selected from H, -C_{1-6}alkyl-B, C_{1-6}hydroxyalkyl, C_{1-6}alkoxyalkyl, aryl, and C_{1-6}aralkyl;

R^2 and R^3 are each independently selected from aryl, C_{1-6}aralkyl, heteroaryl, and C_{1-6}heteroaralkyl;

R^4 is $N(R^5)L\text{-}Q\text{-}R^6$;

R^5 is selected from hydrogen, OH, C_{1-6}aralkyl, and C_{1-6}alkyl;

R^6 is selected from hydrogen, C_{1-6}alkyl, C_{1-6}alkenyl, C_{1-6}alkynyl, Ar-Y-, carbocyclyl, heterocyclyl, an N-terminal protecting group, aryl, C_{1-6}aralkyl, $R^{11}ZAZ\text{-}C_{1-8}$alkyl-, $R^{14}Z\text{-}C_{1-8}$alkyl-, $(R^{11}O)(R^{12}O)P(=O)O\text{-}C_{1-8}$alkyl-ZAZ-$C_{1-8}$alkyl-, $R^{11}ZAZ\text{-}C_{1-8}$alkyl-ZAZ-C_{1-8}alkyl-, heterocyclylMZAZ-C_{1-8}alkyl-, $(R^{11}O)(R^{12}O)P(=O)O\text{-}C_{1-8}$alkyl-, $(R^{13})_2N\text{-}C_{1-12}$alkyl-, $(R^{13})_3N^{+}\text{-}C_{1-12}$alkyl-, heterocyclylM-, carbocyclylM-, $R^{14}SO_2C_{1-8}$alkyl-, and $R^{14}SO_2NH$; or

R^5 and R^6 together are C_{1-6}alkyl-Y-C_{1-6}alkyl, C_{1-6}alkyl-ZAZ-C_{1-6}alkyl, ZAZ-C_{1-6}alkyl-ZAZ-C_{1-6}alkyl, ZAZ-C_{1-6}alkyl-ZAZ, or C_{1-6}alkyl-A, thereby forming a ring;

R^7 and R^8 are independently selected from hydrogen, C_{1-6}alkyl, and C_{1-6}aralkyl;

R^9 is selected from hydrogen, OH, and C_{1-6}alkyl; and

R^{10} is an N-terminal protecting group;

R^{11} and R^{12} are independently selected from hydrogen, metal cation, C_{1-6}alkyl, C_{1-6}alkenyl, C_{1-6}alkynyl, aryl, and C_{1-6}aralkyl;

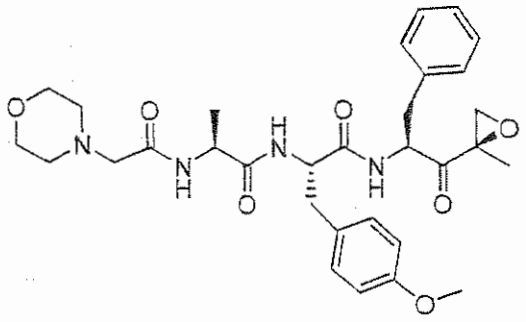
each R^{13} is independently selected from hydrogen and C_{1-6}alkyl; and

R^{14} is independently selected from hydrogen, C_{1-6}alkyl, C_{1-6}alkenyl, C_{1-6}alkynyl, carbocyclyl, heterocyclyl, aryl, and C_{1-6}aralkyl;

R^{15} is selected from C_{1-6}alkyl, C_{1-6}hydroxyalkyl, C_{1-6}alkoxy, -C(O)OC_{1-6}alkyl, -C(O)NHC_{1-6}alkyl, and C_{1-6}aralkyl;

provided that in any occurrence of the sequence ZAZ, at least one member of the sequence must be other than a covalent bond.

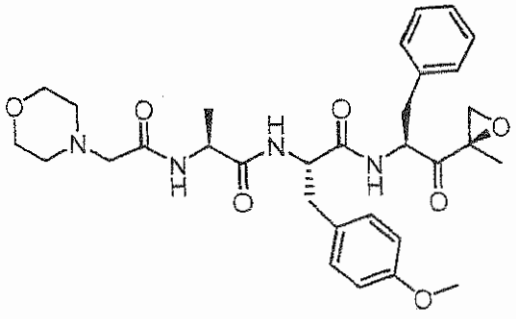
In one aspect, there is provided a crystalline compound having a structure of Formula (II)



(II)

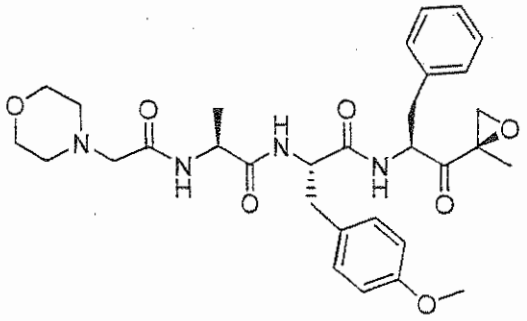
wherein the crystalline compound has 2θ values 3.62±0.20; 6.52±0.20; 7.20±0.20; 8.66±0.20; 10.04±0.20; 10.82±0.20; 12.98±0.20; 14.42±0.20; 16.46±0.20; 17.32±0.20; 17.98±0.20; 18.02±0.20; 18.88±0.20; 19.06±0.20; 19.56±0.20; 19.88±0.20; 20.40±0.20; 21.36±0.20; 22.16±0.20; 23.20±0.20; 25.40±0.20; and 28.82±0.20.

Another embodiment of the disclosure relates to a crystalline compound of Formula (II)



(II)

In one aspect, there is provided a method for preparing a crystalline compound of Formula (II)



(II)

wherein the crystalline compound has 2θ values 3.62±0.20; 6.52±0.20; 7.20±0.20; 8.66±0.20; 10.04±0.20; 10.82±0.20; 12.98±0.20; 14.42±0.20; 16.46±0.20; 17.32±0.20; 17.98±0.20; 18.02±0.20; 18.88±0.20; 19.06±0.20; 19.56±0.20; 19.88±0.20; 20.40±0.20; 21.36±0.20; 22.16±0.20; 23.20±0.20; 25.40±0.20; and 28.82±0.20,

comprising (i) preparing a solution of a compound of Formula (II) in an organic solvent;

(ii) bringing the solution to supersaturation to cause formation of crystals; and

(iii) isolating the crystals.

In one aspect, there is provided the use of a crystalline compound as defined herein in the manufacture of a medicament for treating a disease or condition selected from an immune-related disease, cancer, inflammation, infection, proliferative disease, or neurodegenerative disease.

Brief Description of the Figures

Figure 1 shows a DSC thermogram of the crystalline toluene solvate of Compound 1.

Figure 2 shows an XRPD of the crystalline toluene solvate of Compound 1.

Figure 3 shows a TG thermogram of crystalline toluene solvate of Compound 1.

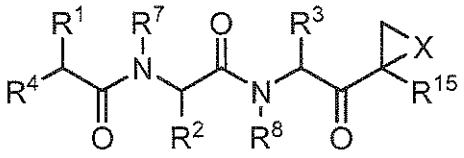
Figure 4 shows an ORTEP diagram of a single molecule of the of crystalline toluene solvate of Compound 1.

Figure 5 shows a DSC thermogram of an amorphous form of Compound 1.

Figure 6 shows an XRPD pattern of an amorphous form of Compound 1.

Detailed Description of the Invention

In certain embodiments, the invention relates to crystalline compounds having a structure of Formula (I) or a pharmaceutically acceptable salt thereof,



(I)

wherein

each Ar is independently an aromatic group optionally substituted with 1 to 4 substituents;

each A is independently selected from C=O, C=S, and SO_2; or

A is optionally a covalent bond when adjacent to an occurrence of Z;

B is absent or is $N(R^9)R^{10}$;

L is absent or is selected from C=O, C=S, and SO_2;

M is absent or is C_{1-12}alkyl;

Q is absent or is selected from O, NH, and N-C_{1-6}alkyl;

X is selected from O, S, NH, and N-C_{1-6}alkyl;

Y is absent or is selected from C=O and SO_2;

each Z is independently selected from O, S, NH, and N-C_{1-6}alkyl; or

Z is optionally a covalent bond when adjacent to an occurrence of A;

R^1 is selected from H, -C_{1-6}alkyl-B, C_{1-6}hydroxyalkyl, C_{1-6}alkoxyalkyl, aryl, and C_{1-6}aralkyl;

R^2 and R^3 are each independently selected from aryl, C_{1-6}aralkyl, heteroaryl, and C_{1-6}heteroaralkyl;

R^4 is $N(R^5)$L-Q-R^6;

R^5 is selected from hydrogen, OH, C_{1-6}aralkyl, and C_{1-6}alkyl;

R^6 is selected from hydrogen, C_{1-6}alkyl, C_{1-6}alkenyl, C_{1-6}alkynyl, Ar-Y-, carbocyclyl, heterocyclyl, an N-terminal protecting group, aryl, C_{1-6}aralkyl, $R^{11}ZAZ-C_{1-8}$alkyl-, $R^{14}Z-C_{1-8}$alkyl-, $(R^{11}O)(R^{12}O)P(=O)O-C_{1-8}$alkyl-ZAZ-$C_{1-8}$alkyl-, $R^{11}ZAZ-C_{1-8}$alkyl-ZAZ-C_{1-8}alkyl-, heterocyclylMZAZ-C_{1-8}alkyl-, $(R^{11}O)(R^{12}O)P(=O)O-C_{1-8}$alkyl-, $(R^{13})_2N-C_{1-12}$alkyl-, $(R^{13})_3N^+-C_{1-12}$alkyl-, heterocyclylM-, carbocyclylM-, $R^{14}SO_2C_{1-8}$alkyl-, and $R^{14}SO_2NH$; or

R^5 and R^6 together are C_{1-6}alkyl-Y-C_{1-6}alkyl, C_{1-6}alkyl-ZAZ-C_{1-6}alkyl, ZAZ-C_{1-6}alkyl-ZAZ-C_{1-6}alkyl, ZAZ-C_{1-6}alkyl-ZAZ, or C_{1-6}alkyl-A, thereby forming a ring;

R^7 and R^8 are independently selected from hydrogen, C_{1-6}alkyl, and C_{1-6}aralkyl;

R^9 is selected from hydrogen, OH, and C_{1-6}alkyl; and

R^{10} is an N-terminal protecting group;

R^{11} and R^{12} are independently selected from hydrogen, metal cation, C_{1-6}alkyl, C_{1-6}alkenyl, C_{1-6}alkynyl, aryl, and C_{1-6}aralkyl;

each R^{13} is independently selected from hydrogen and C_{1-6}alkyl;

R^{14} is independently selected from hydrogen, C_{1-6}alkyl, C_{1-6}alkenyl, C_{1-6}alkynyl, carbocyclyl, heterocyclyl, aryl, and C_{1-6}aralkyl; and

R^{15} is selected from C_{1-6}alkyl, C_{1-6}hydroxyalkyl, C_{1-6}alkoxy, $-C(O)OC_{1-6}$alkyl, $-C(O)NHC_{1-6}$alkyl, and C_{1-6}aralkyl;

provided that in any occurrence of the sequence ZAZ, at least one member of the sequence must be other than a covalent bond.

In certain embodiments, R^7 and R^8 are independently selected from hydrogen and C_{1-6}alkyl, preferably R^7 and R^8 are both hydrogen.

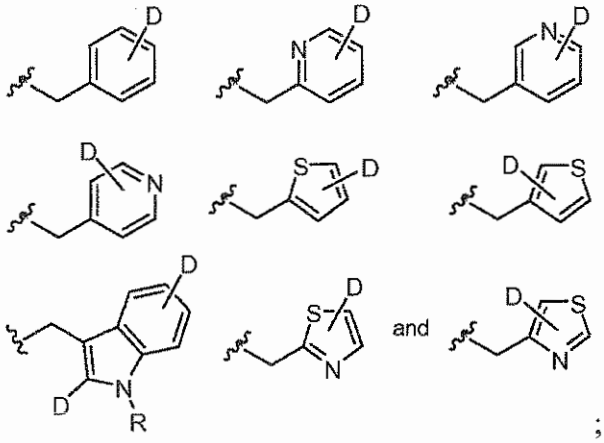
In certain embodiments, R^{15} is selected from C_{1-6}alkyl and C_{1-6}hydroxyalkyl. In certain such embodiments, R^{15} is selected from methyl, ethyl, hydroxymethyl, and 2-hydroxyethyl, preferably methyl.

In certain embodiments, R^5 is hydrogen or C_{1-6}alkyl, preferably R^5 is hydrogen.

In certain embodiments, R^1 is selected from -C_{1-6}alkylB and C_{1-6}aralkyl. In certain preferred embodiments, R^1 is selected from methyl, ethyl, isopropyl, carboxymethyl, and benzyl, preferably methyl.

In certain embodiments, R^2 is selected from C_{1-6}aralkyl and C_{1-6}heteroaralkyl. In certain such embodiments, R^2 is selected from C_{1-6}alkyl-phenyl, C_{1-6}alkyl-indolyl, C_{1-6}alkyl-thienyl, C_{1-6}alkyl-thiazolyl, and C_{1-6}alkyl-isothiazolyl, preferably C_{1-6}alkyl-phenyl.

In certain embodiments, R^2 is selected from



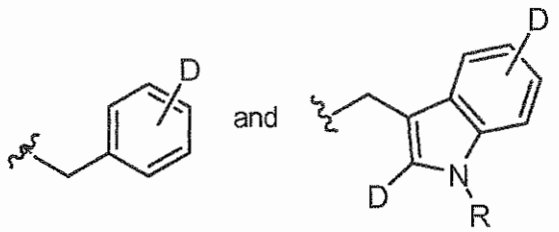
;

wherein D is selected from hydrogen, methoxy, t-butoxy, hydroxy, cyano, trifluoromethyl, and C_{1-4}alkyl; and R is hydrogen or a suitable protecting group.

In certain embodiments, R^3 is selected from C_{1-6}aralkyl and C_{1-6}heteroaralkyl.

In certain embodiments, R^3 is selected from C_{1-6}alkyl-phenyl and C_{1-6}alkyl-indolyl, preferably C_{1-6}alkyl-phenyl.

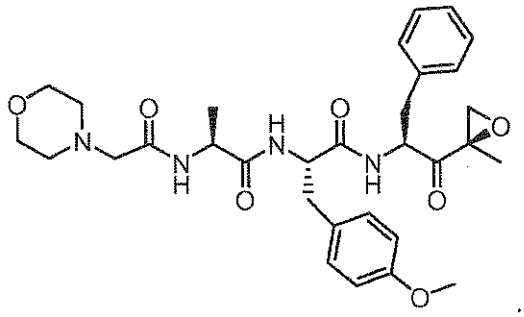
In certain embodiments, R^3 is selected from



wherein D is selected from hydrogen, methoxy, t-butoxy, hydroxy, cyano, trifluoromethyl, and C_{1-4}alkyl; and R is hydrogen or a suitable protecting group.

In certain embodiments, L is C=O, Q is absent, and R^6 is selected from heterocyclyl and heterocyclylM-, preferably heterocyclylM-. In certain such embodiments, R^6 is heterocyclylM-, where heterocyclyl is selected from morpholino, piperidino, piperazino, and pyrrolidino, preferably morpholino. In certain embodiments, R^6 is morpholinomethyl.

In certain embodiments, the invention relates to a crystalline compound of Formula (II)



(II)

In certain embodiments, the invention relates to a method for the preparation of a crystalline compound of Formula (I) or (II), comprising one or more of: (i) preparing the amorphous compound, e.g., according to U.S. Patent Application Serial No. 11/820,490; (ii) dissolving the amorphous compound in an organic solvent; (iii) bringing the solution to supersaturation to cause formation of crystals; and (iv) isolating the crystals, e.g., by filtering the crystals, by decanting fluid from the crystals, or by any other suitable separation technique. In certain embodiments, preparation further comprises inducing crystallization. In certain embodiments, preparation further comprises washing the filtered crystals, e.g., with a solvent or non-solvent fluid. In certain embodiments, preparation further comprises drying, preferably under reduced pressure, such as under vacuum pressure.

In certain embodiments, the invention relates to a method for the preparation of a crystalline compound of Formula (I) or (II), comprising one or more of: (i) preparing a solution of the amorphous compound, which compound may be prepared according to, for example, U.S. Patent Application Serial No. 11/820,490, in an organic solvent; (ii) bringing the solution to supersaturation to cause formation of crystals; and (iii) isolating the crystals, e.g., by filtering the crystals, by decanting fluid from the crystals, or by any other suitable

separation technique. In certain embodiments, preparation further comprises inducing crystallization. In certain embodiments, preparation further comprises washing the filtered crystals, e.g., with a solvent or non-solvent fluid. In certain embodiments, preparation further comprises drying, preferably under reduced pressure, such as under vacuum pressure.

In certain embodiments, the amorphous compound may be dissolved in an organic solvent selected from acetonitrile, acetone, diethyl ether, diisopropyl ether, ethanol, ethyl acetate, heptanes, isopropyl acetate, methanol, methyl tert-butyl ether, tetrahydrofuran, toluene, heptanes, or any combination thereof. In certain embodiments, the amorphous compound may be dissolved in an organic solvent selected from acetonitrile, acetone, diethyl ether, diisopropyl ether, ethyl acetate, heptanes, isopropyl acetate, methanol, methyl tert-butyl ether, tetrahydrofuran, toluene, heptanes, or any combination thereof. In certain embodiments, the amorphous compound may be dissolved in an organic solvent selected from diethyl ether, diisopropyl ether, methyl tert-butyl ether, tetrahydrofuran, toluene, heptanes, or any combination thereof. In certain embodiments, the amorphous compound may be dissolved in toluene, methyl tert-butyl ether, heptanes, or a combination thereof. In certain preferred embodiments, the amorphous compound may be dissolved in toluene.

In certain embodiments, bringing the solution to supersaturation comprises the addition of an anti-solvent, such as another liquid miscible with the organic solvent, allowing the solution to cool, reducing the volume of the solution, or any combination thereof. In certain embodiments, bringing the solution to supersaturation comprises adding an anti-solvent, cooling the solution to ambient temperature or lower, and reducing the volume of the solution, e.g., by evaporating solvent from the solution. In certain embodiments, allowing the solution to cool may be passive (e.g., allowing the solution to stand at ambient temperature) or active (e.g., cooling the solution in an ice bath or freezer).

In certain embodiments, the method further comprises inducing precipitation or crystallization. In certain embodiments inducing precipitation or crystallization comprises secondary nucleation, wherein nucleation occurs in the presence of seed crystals or interactions with the environment (crystallizer walls, stirring impellers, sonication, etc.).

In certain embodiments, washing the crystals comprises washing with a liquid selected from anti-solvent, acetonitrile, acetone, diethyl ether, diisopropyl ether, ethanol,

ethyl acetate, heptanes, isopropyl acetate, methanol, methyl tert-butyl ether, toluene, tetrahydrofuran, or any combination thereof. In certain embodiments, the crystals are washed with an organic solvent selected from acetonitrile, acetone, diethyl ether, diisopropyl ether, ethyl acetate, heptanes, isopropyl acetate, methanol, methyl tert-butyl ether, tetrahydrofuran, toluene, or any combination thereof. In certain embodiments, the crystals are washed with toluene, methyl tert-butyl ether, heptanes, or a combination thereof.

In certain embodiments, washing the crystals comprises washing the crystalline compound of Formula (II) with toluene or toluene in combination with methyl tert-butyl ether.

In certain embodiments, the DSC of a crystalline compound of Formula (II) has a sharp endothermic maximum at about 83 °C (onset at about 78 °C, heating rate of 10 °C/min) resulting from melting and decomposition of the crystalline form as shown in Figure 1.

In certain embodiments, the X-ray powder pattern of a crystalline compound of Formula (II) is (θ-2θ°): 3.62±0.20; 6.52±0.20; 7.20±0.20; 8.66±0.20; 10.04±0.20; 10.82±0.20; 12.98±0.20; 14.42±0.20; 16.46±0.20; 17.32±0.20; 17.98±0.20; 18.02±0.20; 18.88±0.20; 19.06±0.20; 19.56±0.20; 19.88±0.20; 20.40±0.20; 21.36±0.20; 22.16±0.20; 23.20±0.20; 25.40±0.20; 28.82±0.20 as shown in Figure 2.

In certain embodiments, the TG thermogram of a crystalline compound of Formula (II) exhibits from 5.6% weight losses up to 150 °C as shown in Figure 3.

In certain embodiments, a crystalline compound of Formula (II) is not solvated (e.g., the crystal lattice does not comprise molecules of a solvent). In certain alternative embodiments, a crystalline compound of Formula (II) is solvated. In certain preferred embodiments, a crystalline compound of Formula (II) is a toluene solvate.

In certain embodiments, the invention relates to a method for the preparation of an amorphous compound of Formula (II) comprising one or more of (i) dissolving the crystalline compound in an organic solvent; (ii) bringing the solution to supersaturation to cause formation of crystals; and (iii) isolating the crystals, e.g., by filtering the crystals, by decanting fluid from the crystals, or by any other suitable separation technique. In certain embodiments, preparation further comprises inducing precipitation. In certain embodiments,

preparation further comprises washing the amorphous compound. In certain embodiments, the method further comprises drying, preferably under reduced pressure, such as under vacuum pressure.

In certain embodiments, the invention relates to a pharmaceutical composition comprising a crystalline compound of Formula (I) or (II) and a pharmaceutically acceptable carrier. In certain embodiments, the pharmaceutical composition is selected from tablets, capsules, and injections.

Uses of Enzyme Inhibitors

The biological consequences of proteasome inhibition are numerous. Proteasome inhibition has been suggested as a prevention and/or treatment of a multitude of diseases including, but not limited to, proliferative diseases, neurotoxic/degenerative diseases, ischemic conditions, inflammation, immune-related diseases, HIV, cancers, organ graft rejection, septic shock, viral and parasitic infections, conditions associated with acidosis, macular degeneration, pulmonary conditions, muscle wasting diseases, fibrotic diseases, bone and hair growth diseases.

Proteasome inhibitors can be used to treat conditions mediated directly by the proteolytic function of the proteasome such as muscle wasting, or mediated indirectly via proteins which are processed by the proteasome such as NF-κB. The proteasome participates in the rapid elimination and post-translational processing of proteins (e.g., enzymes) involved in cellular regulation (e.g., cell cycle, gene transcription, and metabolic pathways), intercellular communication, and the immune response (e.g., antigen presentation).

At the cellular level, the accumulation of polyubiquitinated proteins, cell morphological changes, and apoptosis have been reported upon treatment of cells with various proteasome inhibitors. Yet, it should be noted that commercially available proteasome inhibitors inhibit both the constitutive and immuno forms of the proteasome. Even bortezomib, the only FDA-approved proteasome inhibitor for the treatment of relapsed multiple myeloma patients, does not distinguish between the two forms (Altun et al., Cancer Res 65:7896, 2005). Thus, what is known about therapeutic proteasome inhibition is based on work with molecules that inhibit both forms of the proteasome. Accordingly, compounds

of the invention may be beneficial for reducing the severity of side effects associated with molecules that inhibit both forms of the proteasome.

Immunoproteasome expression occurs predominantly in cells and organs that make up the lymphatic system, such as white blood cells(leukocytes), bone marrow, and the thymus, spleen and lymph nodes. Although some organs preferentially express constitutive proteasomes (e.g., heart), others such as adrenal, liver, lung and gut, appear to express both forms.

The immune system, of which leukocytes and lymphoid tissues play a major role, is responsible for protecting an organism from outside biological influences. When functioning properly, it protects the body against bacterial and viral infections. The immune system also screens for autologous cells that have undergone oncogenic transformation. Intracellular proteolysis generates small peptides for presentation to T-lymphocytes to induce MHC class I-mediated immune responses. The proteasome is the main provider of these precursor peptides; however, differences between antigenic peptides have been observed between cells with varying amounts of each proteasome form (Cascio et al., EMBO J 20:2357-2366, 2001). In certain embodiments, the invention relates to a method for inhibiting antigen presentation in a cell, including exposing the cell to a compound as described herein. In certain embodiments, the invention relates to a method for altering the repertoire of antigenic peptides produced by the proteasome or other Ntn with multicatalytic activity. For example, if the activity of the immunoproteasome proteasome is selectively inhibited, a different set of antigenic peptides may be produced by the remaining constitutive proteasome and presented in MHC molecules on the surfaces of cells than would be produced and presented without any enzyme inhibition.

Several disorders and disease states have been associated with aberrant immune system function, herein referred to as immune-related conditions. Perhaps the most common immune-related condition is the allergic disorders such as allergies, asthma and atopic dermatitis like eczema. These occur when the immune system overreacts to exposure to antigens in the environment. Thus, a further embodiment is a method for suppressing the immune system of a subject, comprising administering to the subject an effective amount of a proteasome inhibitor compound in a manner described herein.

Immunodeficiency disorders occur when a part of the immune system is not working properly or is not present. They can affect B lymophyctes, T lymphocytes, or phagocytes and be either inherited (e.g., IgA deficiency, severe combined immunodeficiency (SCID), thymic dysplasia and chronic granulomatous) or acquired (e.g., acquired immunodeficiency syndrome (AIDS), human immunodeficiency virus (HIV) and drug-induced immunodeficiencies). A dosing strategy utilizing selective proteasome inhibitors of the invention may be used to treat immune-related conditions such as immunodeficiency disorders.

In autoimmune disorders, the immune system inappropriately attacks the body's healthy organs and tissues as if they were foreign invaders. An example of an autoimmune disease is Sjogren's Syndrome, which is characterized by infiltration and focal accumulation of lymphocytes in the exocrine glands. A study examining the proteasome expression level revealed a significant up-regulation of beta5i (LMP7) exclusively in the salivary glands of SS patients (Egerer et al., Arthritis Rheum 54:1501-8, 2006). Other examples of such immune-related conditions include lupus (such as lupus nephritis and systemic lupus erythamotosus), rheumatoid arthritis (such as juvenile rheumatoid arthritis and psoriatic rheumatoid arthritis), scleroderma, ankylosing spondylitis, dermatomyositis, psoriasis, multiple sclerosis (both relapsing remitting and chronic progressive forms) and inflammatory bowel disease (such as ulcerative colitis and Crohn's disease). Tissue/organ transplant rejection occurs when the immune system mistakenly attacks the cells being introduced to the host's body. Graft versus host disease (GVHD), resulting from allogenic transplantation, arises when the T cells from the donor tissue go on the offensive and attack the host's tissues. In all three circumstances, autoimmune disease, transplant rejection and GVHD, modulating the immune system by treating the subject with a compound of the invention could be beneficial.

Inflammation is the first response of the immune system to infection or irritation. A cellular component of inflammation involves the movement of leukocytes, which express immunoproteasome, from blood vessels into the inflamed tissue. These cells take on the important role of removing the irritant, bacteria, parasite or cell debris. Proteasome inhibitors are already known to have anti-inflammatory activity (Meng et al., PNAS 96:10403-10408, 1999). In cases of chronic inflammation, which is characterized by a

dominating presence of macrophages, the cells that originally served as defensive agents begin to release toxins and cytokines, including TNF-α, now become injurious to the body, resulting in tissue damage and loss. In certain embodiments, the invention relates to a method of treating inflammation and inflammatory diseases comprising administering a compound as described herein. Inflammatory diseases include acute (e.g., bronchitis, conjunctivitis, pancreatitis) and chronic conditions (e.g., chronic cholecstitis, bronchiectasis, aortic valve stenosis, restenosis, psoriasis and arthritis), along with conditions associated with inflammation such as fibrosis, infection and ischemia. In certain embodiments, the invention relates to methods of treating conditions whereby an organ is inflamed, such as autoimmune myocarditis and autoimmune thyroiditis.

Following tissue damage, including damage due to the inflammation process, progression of regeneration and repair begins. During the regeneration step, lost tissue is replaced by proliferation of cells of the same type, which reconstruct the normal architecture. However, improper regeneration of the tissue architecture may have severe consequences. In some cases of chronic inflammatory liver disease, the regenerated tissue forms an abnormal nodular architecture leading to cirrhosis and portal hypertension. The repair process results in lost tissue being replaced by a fibrous scar which is produced from granulation tissue. Fibrosis is the excessive and persistent formation of scar tissue resulting from the hyperproliferative growth of fibroblasts and is associated with activation of the TGF-β signaling pathway. Fibrosis involves extensive deposition of extracellular matrix and can occur within virtually any tissue or across several different tissues. Normally, the level of intracellular signaling protein (Smad) that activate transcription of target genes upon TGF-β stimulation is regulated by proteasome activity (Xu et al., 2000). However, accelerated degradation of the TGF-β signaling components has been observed in cancers and other hyperproliferative conditions. Thus, in certain embodiments, the invention relates to a method for treating a hyperproliferative condition selected from diabetes (such as type 1, type 2 and metabolic syndrome), diabetic retinopathy, macular degeneration, diabetic nephropathy, glomerulosclerosis, IgA nephropathy, cirrhosis, biliary atresia, congestive heart failure, scleroderma, radiation-induced fibrosis, and lung fibrosis (idiopathic pulmonary fibrosis, collagen vascular disease, sarcoidosis, interstitial lung diseases and extrinsic lung disorders), comprising administering a compound as described herein. The treatment of burn

victims is often hampered by fibrosis, thus, in certain embodiments, the invention relates to the topical or systemic administration of a compound as described herein to treat burns. Wound closure following surgery is often associated with disfiguring scars, which may be prevented by inhibition of fibrosis. Thus, in certain embodiments, the invention relates to a method for the prevention or reduction of scarring, comprising administering a compound as described herein.

Infection by bacteria, parasite or virus all result in initiation of the inflammatory process. When the resulting inflammation overwhelms the whole organism, systemic inflammatory response syndrome (SIRS) occurs. The term sepsis is applied when this is due to infection. Overproduction of lipopolysaccharide (LPS)-induced cytokines such as TNFα is considered to be central to the processes associated with septic shock. Not surprisingly, LPS also induces an increase in all components of the MHC-1 pathway including the immunoproteasome subunits LMP2 and LMP7 (MacAry et al., PNAS 98:3982-3987, 2001). Furthermore, it is generally accepted that the first step in the activation of cells by LPS is the binding of LPS to specific membrane receptors. The α- and β-subunits of the 20S proteasome complex have been identified as LPS-binding proteins, suggesting that the LPS-induced signal transduction may be an important therapeutic target in the treatment or prevention of sepsis (Qureshi, N. et al., J. Immun. (2003) 171: 1515-1525). Therefore, in certain embodiments, the invention relates to preventing or treating septic shock, comprising administering a compound as disclosed herein.

In another embodiment, the disclosed compositions are useful for the treatment of a parasitic infection, such as infections caused by protozoan parasites. The proteasome of these parasites is considered to be involved primarily in cell differentiation and replication activities (Paugam et al., Trends Parasitol. 2003, 19(2): 55-59). Furthermore, entamoeba species have been shown to lose encystation capacity when exposed to proteasome inhibitors (Gonzales, et al., Arch. Med. Res. 1997, 28, Spec No: 139-140). In certain such embodiments, the compounds as disclosed herein are useful for the treatment of parasitic infections in humans caused by a protozoan parasite selected from Plasmodium sps. (including P. falciparum, P. vivax, P. malariae, and P. ovale, which cause malaria), Trypanosoma sps. (including T. cruzi, which causes Chagas' disease, and T. brucei which

causes African sleeping sickness), Leishmania sps. (including L. amazonesis, L. donovani, L. infantum, L. mexicana, etc.), Pneumocystis carinii (a protozoan known to cause pneumonia in AIDS and other immunosuppressed patients), Toxoplasma gondii, Entamoeba histolytica, Entamoeba invadens, and Giardia lamblia. In certain embodiments, the disclosed compositions are useful for the treatment of parasitic infections in animals and livestock caused by a protozoan parasite selected from Plasmodium hermani, Cryptosporidium sps., Echinococcus granulosus, Eimeria tenella, Sarcocystis neurona, and Neurospora crassa. Other compounds useful as proteasome inhibitors in the treatment of parasitic diseases are described in WO 98/10779, which is incorporated herein by reference in its entirety.

In certain embodiments, compounds disclosed herein may inhibit proteasome activity in a parasite without recovery in white blood cells. In certain such embodiments, the long half-life of blood cells may provide prolonged protection with regard to therapy against recurring exposures to parasites. In certain embodiments, the compounds as described herein may provide prolonged protection with regard to chemoprophylaxis against future infection.

Viral infections contribute to the pathology of many diseases. Heart conditions such as ongoing myocarditis and dilated cardiomyopathy have been linked to the coxsackievirus B3. In a comparative whole-genome microarray analyses of infected mouse hearts, all three immunoproteasome subunits were uniformly up-regulated in hearts of mice which developed chronic myocarditis (Szalay et al., Am J Pathol 168:1542-52, 2006). Some viruses utilize the ubiquitin-proteasome system in the viral entry step where the virus is released from the endosome into the cytosol. The mouse hepatitis virus (MHV) belongs to the *Coronaviridae* family, which also includes the severe acute respiratory syndrome (SARS) coronvirus. Yu and Lai (J Virol 79:644-648, 2005) demonstrated that treatment of cells infected with MHV with a proteasome inhibitor resulted in a decrease in viral replication, correlating with reduced viral titer as compared to that of untreated cells. The human hepatitis B virus (HBV), a member of the *Hepadnaviridae* virus family, requires virally encoded envelop proteins to propagate. Inhibiting the proteasome degradation pathway causes a significant reduction in the amount of secreted envelope proteins (Simsek et al., J Virol 79:12914-12920, 2005). In addition to HBV, other hepatitis viruses (A, C, D and E) may also utilize the ubiquitin-proteasome degradation pathway for secretion, morphogenesis and

pathogenesis. Accordingly, in certain embodiments the invention relates to treating a viral infection, comprising administering a compound as disclosed herein.

The bacterium Listeria monocytogenes causes a condition known as listeriosis, the manifestations of which range from mild (nausea, vomiting and diarrhea) to severe (septicemia, meningitis, encephalitis). A quantitative analysis of changes in proteasome subunit composition revealed that infection of mice with lymphocytic choriomeningitis virus or Listeria monocytogenes lead to an almost complete replacement of constitutive proteasomes by immunoproteasomes in the liver within seven days (Khan et al., J Immunol 167:6859-6868, 2001). Prokaryotes have what is equivalent to the eukaryote 20S proteasome particle. While the subunit composition of the prokaryote 20S particle is simpler than that of eukaryotes, it does have the ability to hydrolyze peptide bonds in a similar manner. For example, the nucleophilic attack on the peptide bond occurs through the threonine residue on the N-terminus of the β-subunits. Thus, in certain embodiments, the invention relates to a method of treating prokaryotic infections, comprising administering a compound as disclosed herein. Prokaryotic infections may include diseases caused by either mycobacteria (such as tuberculosis, leprosy or Buruli Ulcer) or archaebacteria.

In certain embodiments, the invention relates to a method for treating infection (e.g., bacterial, parasitic or viral), comprising contacting a cell with a compound as disclosed herein. In certain alternative embodiments, the invention relates to a method for treating infection, comprising administering a compound as disclosed herein.

Ischemia and reperfusion injury results in hypoxia, a condition in which there is a deficiency of oxygen reaching the tissues of the body. This condition causes increased degradation of Iκ-Bα, thereby resulting in the activation of NF-κB (Koong et al., 1994). Interestingly, factors which have been identified as being able to enhance immunoproteasome expression, TNF-α and lipopolysaccharide, also stimulate NF-κB activation. It has been demonstrated that the severity of injury resulting in hypoxia can be reduced with the administration of a proteasome inhibitor (Gao et al., 2000; Bao et al., 2001; Pye et al., 2003). Therefore, in certain embodiments the invention relates to a method of treating an ischemic condition or reperfusion injury, comprising administering a compound as disclosed herein. Examples of such conditions or injuries include, but are not limited to,

acute coronary syndrome (vulnerable plaques), arterial occlusive disease (cardiac, cerebral, peripheral arterial and vascular occlusions), atherosclerosis (coronary sclerosis, coronary artery disease), infarctions, heart failure, pancreatitis, myocardial hypertrophy, stenosis, and restenosis.

Cachexia is a syndrome characterized by wasting of skeletal muscle associated with enhanced proteolysis due to the ubiquitin-proteasome pathway. Inhibiting the proteasome reduces proteolysis, thereby reducing both muscle protein loss and the nitrogenous load on kidneys or liver (Tawa et al., JCI 100:197-203, 1997). In cachexia, elevated expression of proinflammatory cytokines, TNF-α and IFN-γ, both of which stimulate expression of immunoproteasome subunits, have been reported (Acharyya et al., JCI 114:370-378, 2004). In fact, most types of muscle atrophy exhibit elevated rates of protein degradation (Lecker et al., FASEB J 18:39-51, 2004). Muscle wasting manifests itself in several life threatening diseases, including cancer, sepsis, renal failure, AIDS, fasting, denervation atrophy, acidosis, diabetes, disuse atrophy and congestive heart failure. In certain embodiments, the invention relates to the treatment of cachexia or muscle-wasting disease, comprising administering a compound as disclosed herein. Compounds of the invention may be useful for treating conditions such as cancer, chronic infectious diseases, fever, muscle disuse (atrophy) and denervation, nerve injury, fasting, renal failure associated with acidosis, and hepatic failure. See, e.g., Goldberg, U.S. Patent No. 5,340,736.

Degradation of certain proteins by the proteasome effect signaling mechanisms that, in turn, effect gene transcription, cell cycle and metabolic pathways. As noted above, proteasome inhibitors block both degradation and processing of ubiquitinated NF-κB in vitro and in vivo. Proteasome inhibitors also block IκB-A degradation and NF-κB activation (Palombella et al., Cell (1994) 78:773-785; and Traenckner et al., EMBO J. (1994) 13:5433-5441). In certain embodiments, the invention relates to a method for inhibiting IκB-α degradation, comprising contacting the cell with a compound as described herein.

In certain embodiments, the invention relates to methods for affecting cyclin-dependent eukaryotic cell cycles, including exposing a cell (in vitro or in vivo) to a compound as disclosed herein. Cyclins are proteins involved in cell cycle control. The proteasome participates in the degradation of cyclins. Examples of cyclins include mitotic

cyclins, G1 cyclins, and cyclin B. Degradation of cyclins enables a cell to exit one cell cycle stage (e.g., mitosis) and enter another (e.g., division). It is believed all cyclins are associated with $p34^{cdc2}$ protein kinase or related kinases. The proteolysis targeting signal is localized to amino acids 42- 50 (destruction box). There is evidence that cyclin is converted to a form vulnerable to a ubiquitin ligase or that a cyclin-specific ligase is activated during mitosis (Ciechanover, A., Cell, (1994) 79:13-21). Inhibition of the proteasome inhibits cyclin degradation, and therefore inhibits cell proliferation, for example, in cyclin-related cancers (Kumatori et al., Proc. Natl. Acad. Sci. USA (1990) 87:7071-7075). In certain embodiments, the invention relates to a method for treating a proliferative disease in a subject (e.g., cancer, psoriasis, or restenosis), comprising administering a compound as disclosed herein. In certain embodiments, the invention also relates to a method for treating cyclin-related inflammation, comprising adminstering a compound as described herein.

In maturing reticulocytes and growing fibroblasts, or cells deprived of insulin or serum, the rate of proteolysis nearly doubles, suggesting a role for the proteasome in cellular metabolism. Accordingly, in certain embodiments, the invention relates to methods for reducing the rate of intracellular protein degradation in a cell. Each of these methods comprises contacting a cell (in vivo or in vitro) with a compound as disclosed herein.

Alzheimer's disease (AD) is a progressive neurodegenerative disease disorder associated with a loss of higher cognitive function. Pathological hallmarks of the disease include senile amyloid plaques, neurofibrillary tangles, dystrophic neuritis and significant neuronal loss in selected regions of the brain. Microglia, the resident macrophages in the brain, release numerous proinflammatory cytokines, including TNF-α, when activated by Aβ42, a peptide associated with neuritic and vascular amyloid plaques. This microglial-mediated inflammatory response contributes to significant neuronal loss. Cell-based studies demonstrated that primary cortical neurons treated with conditioned media from microglial BV2 cells stimulated either with LPS/INF-γ or sonicated Aβ42 peptides resulted in approximately a 60% decrease in cell viability (Gan et al., J. Biol. Chem. 279:5565-5572, 2004). A higher expression of immunoproteasome is found in brain tissue from AD patients than in that of non-demented elderly adults (Mishto et al., Neurobiol Aging 27:54-66, 2006).

Patients suffering from Huntington's disease (HD), another neurodegenerative disorder, display motor dysfunction and cognitive decline over a period of years until death. Upon autopsy, the presence of inclusions or intraneuronal aggregates, caused by a polyQ expansion mutation (also referred to as a CAG triplet repeat expansion), can be detected, accompanied by significant atrophy in the striatum and cortex portions of the brain. Immunohistochemistry revealed that there was a significant enhancement in immunoproteasome expression in the striatum and frontal cortex of brains from HD patients as compared to those from age-matched normal adults (Diaz-Hernandez et al., J Neurosci 23:11653-1161, 2003). Upon further analysis, it was discovered that the enhancement predominantly occurred in the degenerating neurons. Using a mouse model of HD, the researchers noted a selective increase in both chymotrypsin- and trypsin-like activities in the affected and aggregate-containing regions of the brain, primarily the cortex and striatum (Diaz-Hernandez et al, J Neurosci 23:11653-1161, 2003).

Accordingly, certain embodiments of the invention relate to a method for treating a neurodegenerative disease or condition, comprising administering a compound as disclosed herein. Neurodegenerative diseases and conditions include, but are not limited to, stroke, ischemic damage to the nervous system, neural trauma (e.g., percussive brain damage, spinal cord injury, and traumatic damage to the nervous system), multiple sclerosis and other immune-mediated neuropathies (e.g., Guillain-Barre syndrome and its variants, acute motor axonal neuropathy, acute inflammatory demyelinating polyneuropathy, and Fisher Syndrome), HIV/AIDS dementia complex, axonomy, diabetic neuropathy, Parkinson's disease, Huntington's disease, multiple sclerosis, bacterial, parasitic, fungal, and viral meningitis, encephalitis, vascular dementia, multi-infarct dementia, Lewy body dementia, frontal lobe dementia such as Pick's disease, subcortical dementias (such as Huntington or progressive supranuclear palsy), focal cortical atrophy syndromes (such as primary aphasia), metabolic-toxic dementias (such as chronic hypothyroidism or B12 deficiency), and dementias caused by infections (such as syphilis or chronic meningitis).

It has also been demonstrated that inhibitors that bind to the 20S proteasome stimulate bone formation in bone organ cultures. Furthermore, when such inhibitors have been administered systemically to mice, certain proteasome inhibitors increased bone volume

and bone formation rates over 70% (Garrett, I. R. et al., J. Clin. Invest. (2003) 111: 1771-1782), therefore suggesting that the ubiquitin-proteasome machinery regulates osteoblast differentiation and bone formation. Therefore, the disclosed proteasome inhibitor compositions may be useful in the treatment and/or prevention of diseases associated with bone loss, such as osteroporosis.

Cancer is a general term for disease characterized by uncontrolled, abnormal growth of cells. Many cancers arise via multistep pathways involving inactivation of tumor suppressor proteins and activation of oncogenic peptides. Cancer cells can spread to other parts of the body through the lymphatic system or blood stream. Usually, cancer is classified according to the type of tissue or cell most prominently involved. As noted previously, proteasome inhibition has already been validated as a therapeutic strategy for the treatment of cancer, particularly multiple myeloma. Multiple myeloma cells possess both forms of the proteasome, although the ratio can vary somewhat. Multiple myeloma is a hematologic disease characterized by an excessive number of abnormal plasma cells in the bone marrow. Plasma cells develop from B-cells, thus it is not surprising that other B-cell malignancies would also express immunoproteasome to some extent. Except for two chronic mylogenous leukemia cell lines, heme-related cancers (e.g., multiple myeloma, leukemias and lymphomas) generally appear to express immunoproteasome. Cancer cells originating from lymphoid cells express 30% or more immunoproteasome. In certain embodiments, the invention relates to a method for the treatment of cancer, comprising administering a compound as described herein. In certain preferred embodiments, the cancer is a heme-related disorder. In certain embodiments, the cancer is selected from a solid tumor, head and neck squamous cell carcinoma, cervical carcinoma and small cell lung carcinoma.

The treatment of the cells with INF-γ can induce immunoproteasome expression. Therefore, in certain embodiments, the invention relates to a method of treating cancer comprising administering a compound as disclosed herein.

Administration

Compounds prepared as described herein can be administered in various forms, depending on the disorder to be treated and the age, condition, and body weight of the patient, as is well known in the art. For example, where the compounds are to be

administered orally, they may be formulated as tablets, capsules, granules, powders, or syrups; or for parenteral administration, they may be formulated as injections (intravenous, intramuscular, or subcutaneous), drop infusion preparations, or suppositories. For application by the ophthalmic mucous membrane route, they may be formulated as eye drops or eye ointments. These formulations can be prepared by conventional means, and if desired, the active ingredient may be mixed with any conventional additive or excipient, such as a binder, a disintegrating agent, a lubricant, a corrigent, a solubilizing agent, a suspension aid, an emulsifying agent, a coating agent, a cyclodextrin, and/or a buffer. Although the dosage will vary depending on the symptoms, age and body weight of the patient, the nature and severity of the disorder to be treated or prevented, the route of administration and the form of the drug, in general, a daily dosage of from 0.01 to 2000 mg of the compound is recommended for an adult human patient, and this may be administered in a single dose or in divided doses. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will generally be that amount of the compound which produces a therapeutic effect.

The precise time of administration and/or amount of the composition that will yield the most effective results in terms of efficacy of treatment in a given patient will depend upon the activity, pharmacokinetics, and bioavailability of a particular compound, physiological condition of the patient (including age, sex, disease type and stage, general physical condition, responsiveness to a given dosage, and type of medication), route of administration, etc. However, the above guidelines can be used as the basis for fine-tuning the treatment, e.g., determining the optimum time and/or amount of administration, which will require no more than routine experimentation consisting of monitoring the subject and adjusting the dosage and/or timing.

The phrase "pharmaceutically acceptable" is employed herein to refer to those ligands, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

The phrase "pharmaceutically acceptable carrier" as used herein means a pharmaceutically acceptable material, composition, or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the patient. Some examples of materials which can serve as pharmaceutically acceptable carriers include: (1) sugars, such as lactose, glucose, and sucrose; (2) starches, such as corn starch, potato starch, and substituted or unsubstituted β-cyclodextrin; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose, and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil, and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol, and polyethylene glycol; (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16) pyrogen-free water; (17) isotonic saline; (18) Ringer's solution; (19) ethyl alcohol; (20) phosphate buffer solutions; and (21) other non-toxic compatible substances employed in pharmaceutical formulations. In certain embodiments, pharmaceutical compositions of the present invention are non-pyrogenic, i.e., do not induce significant temperature elevations when administered to a patient.

The term "pharmaceutically acceptable salt" refers to the relatively non-toxic, inorganic and organic acid addition salts of the inhibitor(s). These salts can be prepared in situ during the final isolation and purification of the inhibitor(s), or by separately reacting a purified inhibitor(s) in its free base form with a suitable organic or inorganic acid, and isolating the salt thus formed. Representative salts include the hydrobromide, hydrochloride, sulfate, bisulfate, phosphate, nitrate, acetate, valerate, oleate, palmitate, stearate, laurate, benzoate, lactate, phosphate, tosylate, citrate, maleate, fumarate, succinate, tartrate, naphthylate, mesylate, glucoheptonate, lactobionate, laurylsulphonate salts, and amino acid salts, and the like. (See, for example, Berge et al. (1977) "Pharmaceutical Salts", J. Pharm. Sci. 66: 1-19.)

In other cases, the inhibitors useful in the methods of the present invention may contain one or more acidic functional groups and, thus, are capable of forming

pharmaceutically acceptable salts with pharmaceutically acceptable bases. The term "pharmaceutically acceptable salts" in these instances refers to the relatively non-toxic inorganic and organic base addition salts of an inhibitor(s). These salts can likewise be prepared in situ during the final isolation and purification of the inhibitor(s), or by separately reacting the purified inhibitor(s) in its free acid form with a suitable base, such as the hydroxide, carbonate, or bicarbonate of a pharmaceutically acceptable metal cation, with ammonia, or with a pharmaceutically acceptable organic primary, secondary, or tertiary amine. Representative alkali or alkaline earth salts include the lithium, sodium, potassium, calcium, magnesium, and aluminum salts, and the like. Representative organic amines useful for the formation of base addition salts include ethylamine, diethylamine, ethylenediamine, ethanolamine, diethanolamine, piperazine, and the like (see, for example, Berge et al., supra).

Wetting agents, emulsifiers, and lubricants, such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, release agents, coating agents, sweetening, flavoring, and perfuming agents, preservatives and antioxidants can also be present in the compositions.

Examples of pharmaceutically acceptable antioxidants include: (1) water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite, and the like; (2) oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and (3) metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

Formulations suitable for oral administration may be in the form of capsules, cachets, pills, tablets, lozenges (using a flavored basis, usually sucrose and acacia or tragacanth), powders, granules, or as a solution or a suspension in an aqueous or non-aqueous liquid, or as an oil-in-water or water-in-oil liquid emulsion, or as an elixir or syrup, or as pastilles (using an inert matrix, such as gelatin and glycerin, or sucrose and acacia) and/or as mouthwashes, and the like, each containing a predetermined amount of an inhibitor(s) as an active ingredient. A composition may also be administered as a bolus, electuary, or paste.

In solid dosage forms for oral administration (capsules, tablets, pills, dragees, powders, granules, and the like), the active ingredient is mixed with one or more pharmaceutically acceptable carriers, such as sodium citrate or dicalcium phosphate, and/or any of the following: (1) fillers or extenders, such as starches, cyclodextrins, lactose, sucrose, glucose, mannitol, and/or silicic acid; (2) binders, such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinyl pyrrolidone, sucrose, and/or acacia; (3) humectants, such as glycerol; (4) disintegrating agents, such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate; (5) solution retarding agents, such as paraffin; (6) absorption accelerators, such as quaternary ammonium compounds; (7) wetting agents, such as, for example, acetyl alcohol and glycerol monostearate; (8) absorbents, such as kaolin and bentonite clay; (9) lubricants, such a talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof; and (10) coloring agents. In the case of capsules, tablets, and pills, the pharmaceutical compositions may also comprise buffering agents. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugars, as well as high molecular weight polyethylene glycols, and the like.

A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared using binder (for example, gelatin or hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (for example, sodium starch glycolate or cross-linked sodium carboxymethyl cellulose), surface-active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered inhibitor(s) moistened with an inert liquid diluent.

Tablets, and other solid dosage forms, such as dragees, capsules, pills, and granules, may optionally be scored or prepared with coatings and shells, such as enteric coatings and other coatings well known in the pharmaceutical-formulating art. They may also be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile, other polymer matrices, liposomes, and/or microspheres. They may be sterilized by, for example, filtration through a bacteria-retaining filter, or by incorporating

sterilizing agents in the form of sterile solid compositions which can be dissolved in sterile water, or some other sterile injectable medium immediately before use. These compositions may also optionally contain opacifying agents and may be of a composition that they release the active ingredient(s) only, or preferentially, in a certain portion of the gastrointestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes. The active ingredient can also be in micro-encapsulated form, if appropriate, with one or more of the above-described excipients.

Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups, and elixirs. In addition to the active ingredient, the liquid dosage forms may contain inert diluents commonly used in the art, such as, for example, water or other solvents, solubilizing agents, and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofuryl alcohol, polyethylene glycols, and fatty acid esters of sorbitan, and mixtures thereof.

Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, coloring, perfuming, and preservative agents.

Suspensions, in addition to the active inhibitor(s) may contain suspending agents as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, and mixtures thereof.

Formulations for rectal or vaginal administration may be presented as a suppository, which may be prepared by mixing one or more inhibitor(s) with one or more suitable nonirritating excipients or carriers comprising, for example, cocoa butter, polyethylene glycol, a suppository wax or a salicylate, which is solid at room temperature, but liquid at body temperature and, therefore, will melt in the rectum or vaginal cavity and release the active agent.

Formulations which are suitable for vaginal administration also include pessaries, tampons, creams, gels, pastes, foams, or spray formulations containing such carriers as are known in the art to be appropriate.

Dosage forms for the topical or transdermal administration of an inhibitor(s) include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches, and inhalants. The active component may be mixed under sterile conditions with a pharmaceutically acceptable carrier, and with any preservatives, buffers, or propellants which may be required.

The ointments, pastes, creams, and gels may contain, in addition to inhibitor(s), excipients, such as animal and vegetable fats, oils, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc, and zinc oxide, or mixtures thereof.

Powders and sprays can contain, in addition to an inhibitor(s), excipients such as lactose, talc, silicic acid, aluminum hydroxide, calcium silicates, and polyamide powder, or mixtures of these substances. Sprays can additionally contain customary propellants, such as chlorofluorohydrocarbons and volatile unsubstituted hydrocarbons, such as butane and propane.

The inhibitor(s) can be alternatively administered by aerosol. This is accomplished by preparing an aqueous aerosol, liposomal preparation, or solid particles containing the composition. A nonaqueous (e.g., fluorocarbon propellant) suspension could be used. Sonic nebulizers are preferred because they minimize exposing the agent to shear, which can result in degradation of the compound.

Ordinarily, an aqueous aerosol is made by formulating an aqueous solution or suspension of the agent together with conventional pharmaceutically acceptable carriers and stabilizers. The carriers and stabilizers vary with the requirements of the particular composition, but typically include nonionic surfactants (Tweens, Pluronics, sorbitan esters, lecithin, Cremophors), pharmaceutically acceptable co-solvents such as polyethylene glycol, innocuous proteins like serum albumin, oleic acid, amino acids such as glycine, buffers, salts, sugars, or sugar alcohols. Aerosols generally are prepared from isotonic solutions.

Transdermal patches have the added advantage of providing controlled delivery of an inhibitor(s) to the body. Such dosage forms can be made by dissolving or dispersing the agent in the proper medium. Absorption enhancers can also be used to increase the flux of the inhibitor(s) across the skin. The rate of such flux can be controlled by either providing a rate controlling membrane or dispersing the inhibitor(s) in a polymer matrix or gel.

Pharmaceutical compositions of this invention suitable for parenteral administration comprise one or more inhibitors(s) in combination with one or more pharmaceutically acceptable sterile aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use, which may contain antioxidants, buffers, bacteriostats, solutes which render the formulation isotonic with the blood of the intended recipient or suspending or thickening agents.

Examples of suitable aqueous and nonaqueous carriers which may be employed in the pharmaceutical compositions of the invention include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

These compositions may also contain adjuvants such as preservatives, wetting agents, emulsifying agents, and dispersing agents. Prevention of the action of microorganisms may be ensured by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include tonicity-adjusting agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

In some cases, in order to prolong the effect of a drug, it is desirable to slow the absorption of the drug from subcutaneous or intramuscular injection. For example, delayed

absorption of a parenterally administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle.

Injectable depot forms are made by forming microcapsule matrices of inhibitor(s) in biodegradable polymers such as polylactide-polyglycolide. Depending on the ratio of drug to polymer, and the nature of the particular polymer employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissue.

The preparations of agents may be given orally, parenterally, topically, or rectally. They are, of course, given by forms suitable for each administration route. For example, they are administered in tablets or capsule form, by injection, inhalation, eye lotion, ointment, suppository, infusion; topically by lotion or ointment; and rectally by suppositories. Oral administration is preferred.

The phrases "parenteral administration" and "administered parenterally" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal and intrasternal injection, and infusion.

The phrases "systemic administration," "administered systemically," "peripheral administration" and "administered peripherally" as used herein mean the administration of a ligand, drug, or other material other than directly into the central nervous system, such that it enters the patient's system and thus, is subject to metabolism and other like processes, for example, subcutaneous administration.

These inhibitors(s) may be administered to humans and other animals for therapy by any suitable route of administration, including orally, nasally, as by, for example, a spray, rectally, intravaginally, parenterally, intracisternally, and topically, as by powders, ointments or drops, including buccally and sublingually.

Regardless of the route of administration selected, the inhibitor(s), which may be used in a suitable hydrated form, and/or the pharmaceutical compositions of the present invention, are formulated into pharmaceutically acceptable dosage forms by conventional methods known to those of skill in the art.

Actual dosage levels of the active ingredients in the pharmaceutical compositions of this invention may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient.

The concentration of a disclosed compound in a pharmaceutically acceptable mixture will vary depending on several factors, including the dosage of the compound to be administered, the pharmacokinetic characteristics of the compound(s) employed, and the route of administration. In general, the compositions of this invention may be provided in an aqueous solution containing about 0.1-10% w/v of a compound disclosed herein, among other substances, for parenteral administration. Typical dose ranges are from about 0.01 to about 50 mg/kg of body weight per day, given in 1-4 divided doses. Each divided dose may contain the same or different compounds of the invention. The dosage will be an effective amount depending on several factors including the overall health of a patient, and the formulation and route of administration of the selected compound(s).

Another aspect of the invention provides a conjoint therapy wherein one or more other therapeutic agents are administered with the proteasome inhibitor. Such conjoint treatment may be achieved by way of the simultaneous, sequential, or separate dosing of the individual components of the treatment.

In certain embodiments, a compound of the invention is conjointly administered with one or more other proteasome inhibitor(s).

In certain embodiments, a compound of the invention is conjointly administered with a chemotherapeutic. Suitable chemotherapeutics may include, natural products such as vinca alkaloids (e.g., vinblastine, vincristine, and vinorelbine); paclitaxel; epidipodophyllotoxins (e.g., etoposide, teniposide); antibiotics (e.g., dactinomycin (actinomycin D) daunorubicin, doxorubicin and idarubicin); anthracyclines; mitoxantrone; bleomycins; plicamycin (mithramycin) and mitomycin; enzymes (L-asparaginase which systemically metabolizes L-

asparagine and deprives cells which do not have the capacity to synthesize their own asparagine); antiplatelet agents; antiproliferative/antimitotic alkylating agents such as nitrogen mustards (mechlorethamine, cyclophosphamide and analogs, melphalan, chlorambucil), ethylenimines and methylmelamines (hexamethylmelamine and thiotepa); alkyl sulfonates (busulfan); nitrosoureas (carmustine (BCNU) and analogs, streptozocin); trazenes (e.g.,dacarbazine (DTIC)); antiproliferative/antimitotic antimetabolites such as folic acid analogs (methotrexate), pyrimidine analogs (fluorouracil, floxuridine, and cytarabine), purine analogs and related inhibitors (mercaptopurine, thioguanine, pentostatin and 2-chlorodeoxyadenosine); aromatase inhibitors (anastrozole, exemestane, and letrozole), and platinum coordination complexes (cisplatin, carboplatin); procarbazine; hydroxyurea; mitotane;aminoglutethimide; hormones (e.g., estrogen); and hormone agonists such as leutinizing hormone releasing hormone (LHRH) agonists (e.g., goserelin, leuprolide and triptorelin).

In certain embodiments, the other therapeutic agent is an HDAC inhibitor (e.g., Trichostatin A, depsipeptide, apicidin, A-161906, scriptaid, PXD-101, CHAP, butyric acid, depudecin, oxamflatin, phenylbutyrate, valproic acid, SAHA (Vorinostat), MS275 (N-(2-Aminophenyl)-4-[N-(pyridine-3-ylmethoxy-carbonyl)aminomethyl]benzamide), LAQ824/LBH589, CI994, or MGCD0103). In certain such embodiments, the other agent is SAHA (suberoylanilide hydroxamic acid).

In certain embodiments, the other therapeutic agent is a protein kinase inhibitor (e.g., sorafenib, imatinib, dasatinib, sunitinib, pazopanib, and nilotinib). In certain such embodiments, the protein kinase inhibitor is sorafenib.

In certain embodiements, the other chemotherapeutic agent is selected from mechlorethamine, camptothecin, ifosfamide, tamoxifen, raloxifene, gemcitabine, navelbine, or any analog or derivative variant of the foregoing.

In certain embodiments, a compound of the invention is conjointly administered with a steroid. Suitable steroids may include, but are not limited to, 21-acetoxypregnenolone, alclometasone, algestone, amcinonide, beclomethasone, betamethasone, budesonide, chloroprednisone, clobetasol, clocortolone, cloprednol, corticosterone, cortisone, cortivazol, deflazacort, desonide, desoximetasone, dexamethasone, diflorasone, diflucortolone,

difuprednate, enoxolone, fluazacort, flucloronide, flumethasone, flunisolide, fluocinolone acetonide, fluocinonide, fluocortin butyl, fluocortolone, fluorometholone, fluperolone acetate, fluprednidene acetate, fluprednisolone, flurandrenolide, fluticasone propionate, formocortal, halcinonide, halobetasol propionate, halometasone, hydrocortisone, loteprednol etabonate, mazipredone, medrysone, meprednisone, methylprednisolone, mometasone furoate, paramethasone, prednicarbate, prednisolone, prednisolone 25-diethylaminoacetate, prednisolone sodium phosphate, prednisone, prednival, prednylidene, rimexolone, tixocortol, triamcinolone, triamcinolone acetonide, triamcinolone benetonide, or triamcinolone hexacetonide, or a salt and/or derivative thereof.

In certain embodiments, a compound of the invention is conjointly administered with an immunotherapeutic agent. Suitable immunotherapeutic agents may include, but are not limited to, cyclosporine, thalidomide (or thalidomide analogs such as lenalidomide), or monoclonal antibodies. The monoclonal antibodies can be either naked or conjugated such as rituximab, tositumomab, alemtuzumab, epratuzumab, ibritumomab tiuxetan, gemtuzumab ozogamicin, bevacizumab, cetuximab, erlotinib or trastuzumab.

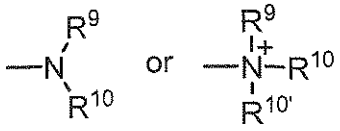
Definitions

The term "C_{x-y}alkyl" refers to substituted or unsubstituted saturated hydrocarbon groups, including straight-chain alkyl and branched-chain alkyl groups that contain from x to y carbons in the chain, including haloalkyl groups such as trifluoromethyl and 2,2,2-trifluoroethyl, etc. C_0alkyl indicates a hydrogen where the group is in a terminal position, a bond if internal. The terms "C_{2-y}alkenyl" and "C_{2-y}alkynyl" refer to substituted or unsubstituted unsaturated aliphatic groups analogous in length and possible substitution to the alkyls described above, but that contain at least one double or triple bond respectively.

The term "alkoxy" refers to an alkyl group having an oxygen attached thereto. Representative alkoxy groups include methoxy, ethoxy, propoxy, tert-butoxy and the like. An "ether" is two hydrocarbons covalently linked by an oxygen. Accordingly, the substituent of an alkyl that renders that alkyl an ether is or resembles an alkoxy.

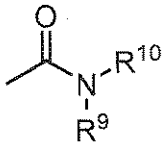
The term "C_{1-6}aralkyl", as used herein, refers to a C_{1-6}alkyl group substituted with an aryl group.

The terms "amine" and "amino" are art-recognized and refer to both unsubstituted and substituted amines and salts thereof, e.g., a moiety that can be represented by the general formulae:



wherein R^9, R^{10} and $R^{10'}$ each independently represent a hydrogen, an alkyl, an alkenyl, $-(CH_2)_m-R^8$, or R^9 and R^{10} taken together with the N atom to which they are attached complete a heterocycle having from 4 to 8 atoms in the ring structure; R^8 represents an aryl, a cycloalkyl, a cycloalkenyl, a heterocyclyl or a polycyclyl; and m is zero or an integer from 1 to 8. In preferred embodiments, only one of R^9 or R^{10} can be a carbonyl, e.g., R^9, R^{10}, and the nitrogen together do not form an imide. In even more preferred embodiments, R^9 and R^{10} (and optionally $R^{10'}$) each independently represent a hydrogen, an alkyl, an alkenyl, or $-(CH_2)_m-R^8$. In certain embodiments, the amino group is basic, meaning the protonated form has a $pK_a \geq 7.00$.

The terms "amide" and "amido" are art-recognized as an amino-substituted carbonyl and includes a moiety that can be represented by the general formula:

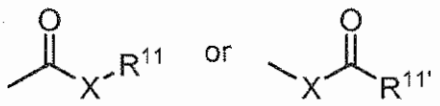


wherein R^9, R^{10} are as defined above. Preferred embodiments of the amide will not include imides which may be unstable.

The term "aryl" as used herein includes 5-, 6-, and 7-membered substituted or unsubstituted single-ring aromatic groups in which each atom of the ring is carbon. The term "aryl" also includes polycyclic ring systems having two or more cyclic rings in which two or more carbons are common to two adjoining rings wherein at least one of the rings is aromatic, e.g., the other cyclic rings can be cycloalkyls, cycloalkenyls, cycloalkynyls, aryls, heteroaryls, and/or heterocyclyls. Aryl groups include benzene, naphthalene, phenanthrene, phenol, aniline, and the like.

The terms "carbocycle" and "carbocyclyl", as used herein, refer to a non-aromatic substituted or unsubstituted ring in which each atom of the ring is carbon. The terms "carbocycle" and "carbocyclyl" also include polycyclic ring systems having two or more cyclic rings in which two or more carbons are common to two adjoining rings wherein at least one of the rings is carbocyclic, e.g., the other cyclic rings can be cycloalkyls, cycloalkenyls, cycloalkynyls, aryls, heteroaryls, and/or heterocyclyls.

The term "carbonyl" is art-recognized and includes such moieties as can be represented by the general formula:



wherein X is a bond or represents an oxygen or a sulfur, and R^{11} represents a hydrogen, an alkyl, an alkenyl, $-(CH_2)_m-R^8$ or a pharmaceutically acceptable salt, $R^{11'}$ represents a hydrogen, an alkyl, an alkenyl or $-(CH_2)_m-R^8$, where m and R^8 are as defined above. Where X is an oxygen and R^{11} or $R^{11'}$ is not hydrogen, the formula represents an "ester". Where X is an oxygen, and R^{11} is a hydrogen, the formula represents a "carboxylic acid".

The terms "heteroaryl" includes substituted or unsubstituted aromatic 5- to 7-membered ring structures, more preferably 5- to 6-membered rings, whose ring structures include one to four heteroatoms. The term "heteroaryl" also includes polycyclic ring systems having two or more cyclic rings in which two or more carbons are common to two adjoining rings wherein at least one of the rings is heteroaromatic, e.g., the other cyclic rings can be cycloalkyls, cycloalkenyls, cycloalkynyls, aryls, heteroaryls, and/or heterocyclyls. Heteroaryl groups include, for example, pyrrole, furan, thiophene, imidazole, oxazole, thiazole, triazole, pyrazole, pyridine, pyrazine, pyridazine and pyrimidine, and the like.

The term "heteroatom" as used herein means an atom of any element other than carbon or hydrogen. Preferred heteroatoms are nitrogen, oxygen, phosphorus, and sulfur.

The terms "heterocyclyl" or "heterocyclic group" refer to substituted or unsubstituted non-aromatic 3- to 10-membered ring structures, more preferably 3- to 7-membered rings, whose ring structures include one to four heteroatoms. The term terms "heterocyclyl" or "heterocyclic group" also include polycyclic ring systems having two or more cyclic rings in

which two or more carbons are common to two adjoining rings wherein at least one of the rings is heterocyclic, e.g., the other cyclic rings can be cycloalkyls, cycloalkenyls, cycloalkynyls, aryls, heteroaryls, and/or heterocyclyls. Heterocyclyl groups include, for example, piperidine, piperazine, pyrrolidine, morpholine, lactones, lactams, and the like.

The term "C_{1-6}hydroxyalkyl" refers to a C_{1-6}alkyl group substituted with a hydroxy group.

The terms "polycyclyl" or "polycyclic" refer to two or more rings (e.g., cycloalkyls, cycloalkenyls, cycloalkynyls, aryls, heteroaryls, and/or heterocyclyls) in which two or more carbons are common to two adjoining rings, e.g., the rings are "fused rings". Each of the rings of the polycycle can be substituted or unsubstituted.

The term "proteasome" as used herein is meant to include immuno- and constitutive proteasomes.

The term "substantially pure" as used herein, refers to a crystalline polymorph that is greater than 90% pure, meaning that contains less than 10% of any other compound, including the corresponding amorphous compound. Preferably, the crystalline polymorph is greater than 95% pure, or even greater than 98% pure.

The term "substituted" refers to moieties having substituents replacing a hydrogen on one or more carbons of the backbone. It will be understood that "substitution" or "substituted with" includes the implicit proviso that such substitution is in accordance with permitted valence of the substituted atom and the substituent, and that the substitution results in a stable compound, e.g., which does not spontaneously undergo transformation such as by rearrangement, cyclization, elimination, etc. As used herein, the term "substituted" is contemplated to include all permissible substituents of organic compounds. In a broad aspect, the permissible substituents include acyclic and cyclic, branched and unbranched, carbocyclic and heterocyclic, aromatic and non-aromatic substituents of organic compounds. The permissible substituents can be one or more and the same or different for appropriate organic compounds. For purposes of this invention, the heteroatoms such as nitrogen may have hydrogen substituents and/or any permissible substituents of organic compounds described herein which satisfy the valences of the heteroatoms. Substituents can include, for example, a halogen, a hydroxyl, a carbonyl (such as a carboxyl, an alkoxycarbonyl, a formyl,

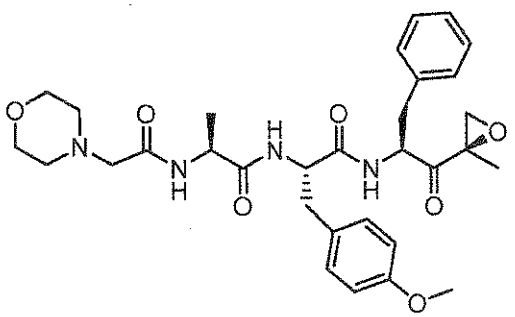
or an acyl), a thiocarbonyl (such as a thioester, a thioacetate, or a thioformate), an alkoxyl, a phosphoryl, a phosphate, a phosphonate, a phosphinate, an amino, an amido, an amidine, an imine, a cyano, a nitro, an azido, a sulfhydryl, an alkylthio, a sulfate, a sulfonate, a sulfamoyl, a sulfonamido, a sulfonyl, a heterocyclyl, an aralkyl, or an aromatic or heteroaromatic moiety. It will be understood by those skilled in the art that the moieties substituted on the hydrocarbon chain can themselves be substituted, if appropriate.

A "therapeutically effective amount" of a compound with respect to the subject method of treatment, refers to an amount of the compound(s) in a preparation which, when administered as part of a desired dosage regimen (to a mammal, preferably a human) alleviates a symptom, ameliorates a condition, or slows the onset of disease conditions according to clinically acceptable standards for the disorder or condition to be treated or the cosmetic purpose, e.g., at a reasonable benefit/risk ratio applicable to any medical treatment.

As used herein, the term "treating" or "treatment" includes reversing, reducing, or arresting the symptoms, clinical signs, and underlying pathology of a condition in manner to improve or stabilize a subject's condition.

Exemplification

Example 1



Compound 1

Amorphous Compound 1 (90.8% purity by HPLC, 6.56 g) prepared according to U.S. Patent Application Serial No. 11/820,490, was dissolved in toluene (129 mL) in a oil bath at 60 °C, the solution was cooled to ambient temperature, and the mixture was stirred for three hours. The resutling crystals were filtered, washed with toluene and dried under vacuum for

12 hours at 22 °C to provide a toluene solvate of Compound 1 (99.4% purity by HPLC, 4.63 g).

The characteristic DSC curve of the sample as shown in Figure 1 was recorded on a TA Instruments Differential Scanning Calorimeter 2920 at a heating rate of 10 °C/minute.

The characteristic X-ray powder pattern as shown in Figure 2 was recorded on a Shimadzu XRD-6000 under Cu Kα radiation [voltage and current set at 40 kV and 40 mA; divergence and scattering slits set at 1° and receiving slit set at 0.15 mm; NaI scintillation detector used for diffracted radiation; a θ-2θ continuous scan at 3°/min (0.4 sec/0.02° step) from 2.5 to 40° 2θ was used; samples were placed in an aluminum holder with silicon insert; and data collected and analyzed with XRD-6100/7000 v.5.0].

Thermogravimetric analyses were performed with a TA Instruments 2950 at a heating rate of 10 °C /minute.

Example 2

Amorphous Compound 1 (221 g) prepared according to U.S. Patent Application Serial No. 11/820,490, was dissolved in 1:1 toluene-methyl tert-butyl ether (4.2 L), seeded with a crystalline toluene solvate of Compound 1, and allowed to crystallize at 15 °C to 25 °C over 24 hours. The crystals were filtered, washed with heptanes and dried under vacuum for 24 hours at 40 °C to provide a crystalline toluene solvate of Compound 1 (97.0% purity by HPLC, 115 g).

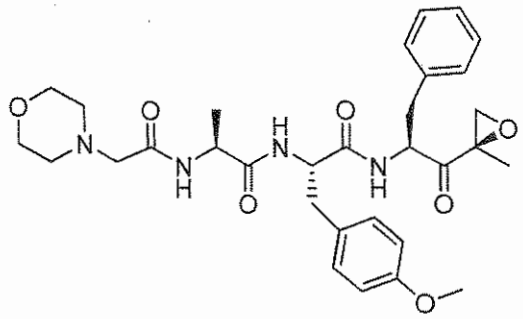
Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, numerous equivalents to the compounds and methods of use thereof described herein. Such equivalents are considered to be within the scope of this invention and are covered by the following claims.

All of the above-cited references and publications are hereby incorporated by reference.

We Claim:

1. A method for preparing a crystalline compound of Formula (II)



(II)

wherein the crystalline compound has 2θ values 3.62±0.20; 6.52±0.20; 7.20±0.20; 8.66±0.20; 10.04±0.20; 10.82±0.20; 12.98±0.20; 14.42±0.20; 16.46±0.20; 17.32±0.20; 17.98±0.20; 18.02±0.20; 18.88±0.20; 19.06±0.20; 19.56±0.20; 19.88±0.20; 20.40±0.20; 21.36±0.20; 22.16±0.20; 23.20±0.20; 25.40±0.20; and 28.82±0.20,

comprising (i) preparing a solution of a compound of Formula (II) in an organic solvent;

(ii) bringing the solution to supersaturation to cause formation of crystals; and

(iii) isolating the crystals.

2. A method of claim 1, wherein the organic solvent is selected from acetonitrile, acetone, diethyl ether, diisopropyl ether, ethyl acetate, heptanes, isopropyl acetate, methanol, methyl tert-butyl ether, tetrahydrofuran, toluene, or any combination thereof.

3. A method of claim 2, wherein the organic solvent is selected from toluene, methyl tert-butyl ether, heptanes, or a combination thereof.

4. A method of claim 1, wherein bringing the solution to supersaturation comprises addition of an anti-solvent, allowing the solution to cool, reducing the volume of the solution, or any combination thereof.

5. A method of claim 4, wherein bringing the solution to supersaturation comprises cooling the solution to ambient temperature and reducing the volume of the solution.

6. A method of claim 1, further comprising washing the crystals.

7. A method of claim 6, wherein the washing comprises washing with a liquid selected from anti-solvent, acetonitrile, acetone, diethyl ether, diisopropyl ether, ethanol, ethyl acetate, heptanes, isopropyl acetate, methanol, methyl tert-butyl ether, tetrahydrofuran, toluene, or any combination thereof, or a combination thereof.

8. A method of claim 7, wherein washing comprises washing with a combination of toluene and methyl tert-butyl ether.

9. A method of claim 7, wherein washing comprises washing with toluene.

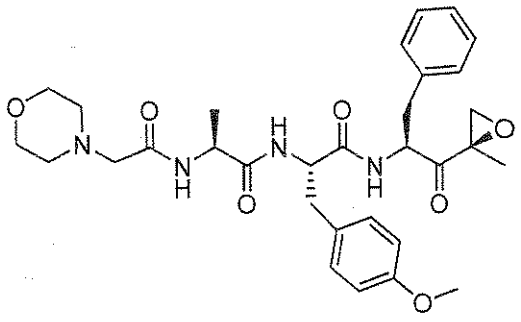
10. A method of claim 1, wherein isolating the crystals comprises filtering the crystals.

11. A method of claim 1, further comprising drying the crystals under reduced pressure.

12. A method of claim 1, wherein the crystalline compound of Formula (II) is a solvate.

13. A method of claim 12, wherein the crystalline compound of Formula (II) is a toluene solvate.

14. A crystalline compound having a structure of Formula (II)



(II)

wherein the crystalline compound has 2θ values 3.62±0.20; 6.52±0.20; 7.20±0.20; 8.66±0.20; 10.04±0.20; 10.82±0.20; 12.98±0.20; 14.42±0.20; 16.46±0.20; 17.32±0.20; 17.98±0.20; 18.02±0.20; 18.88±0.20; 19.06±0.20; 19.56±0.20; 19.88±0.20; 20.40±0.20; 21.36±0.20; 22.16±0.20; 23.20±0.20; 25.40±0.20; and 28.82±0.20.

15. A crystalline compound of claim 14, having a DSC thermogram substantially as shown in Figure 1.

16. A crystalline compound of claim 14, having an XRPD pattern substantially as shown in Figure 2.

17. A crystalline compound of claim 14, which is a solvate.

18. A crystalline compound of claim 17, which is a toluene solvate.

19. Use of a crystalline compound of claim 14 in the manufacture of a medicament for treating a disease or condition selected from an immune-related disease, cancer, inflammation, infection, proliferative disease, or neurodegenerative disease.

20. The use of claim 19, wherein the disease or condition is an immune-related disease.

21. The use of claim 20, wherein the immune-related disease is selected from autoimmune disease, Crohn's disease, diabetes, lupus, multiple sclerosis, myositis, psoriasis, rheumatoid arthritis and ulcerative colitis.

22. The use of claim 19, wherein the disease or condition is cancer.

23. The use of claim 22, wherein the cancer is selected from leukemia, lymphoma and multiple myeloma.

24. The use of claim 19, wherein the disease or condition is inflammation.

25. The use of claim 19, wherein the disease or condition is an infection.

26. The use of claim 19, wherein the disease or condition is a proliferative disease.

27. The use of claim 19, wherein the disease or condition is a neurodegenerative disease.