INHIBITORS OF P97

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None
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References Cited
U.S. PATENT DOCUMENTS

OTHER PUBLICATIONS

Keats, Jonathan J. et al.; “Promiscuous Mutations Activate the Non-Canonical NF-κB Pathway in Multiple Myeloma”; Cancer Cell; Aug. 2007; vol. 12; No. 2; pp. 131-144.

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ABSTRACT
One aspect of the invention relates to compounds that inhibit the activity of p97, such as by binding covalently to a cysteine residue in the active site. In certain embodiments, the invention relates to the treatment of disease, such as cancer, comprising administering a compound of the invention.

5 Claims, 32 Drawing Sheets
References Cited

OTHER PUBLICATIONS


Ye, Yihong. et al.; “The AAA ATPase Cdc48/p97 and its partners transport proteins from the ER into the cytosol”; Nature; vol. 414; Dec. 6, 2001; pp. 652-656.


* cited by examiner
Amino Acid Sequence Alignment of AAA ATPase in Walker A motif

Murine p97 D1 YGPPGKTL
Murine p97 D2 YGPPGCGKTL
Yeast Cdc48 D1 YGPPGKTL
Yeast Cdc48 D2 YGPPGKTL
Hamster NSF D1 YGPPGCGKTL
Hamster NSF D2 YGPPGCGKTL
Human Rpt1 YGPPGCGKTL
Human Rpt2 YGPPGCGKTL
Human Rpt3 YGPPGCGKTL
Human Rpt4 YGPPGCGKTL
Human Rpt5 YGPPGCGKTL
Human Rpt6 YGPPGCGKTL
Figure 12

One 96 well plate

0 nM Luci
1.25 nM Luci
2.5 nM Luci
5 nM Luci

0 nM p97
1.25 nM p97
2.5 nM p97
5 nM p97
Figure 13

Umbrella-GFP/HeLa p97 siRNA

Fold Accumulation vs. siRNA Concentration (nM)

0 1.25 2.5 5

24h-p97 26h-p97 45.5h-p97 48h-p97
Figure 14

Ub^-G76V-GFP/HeLa-23h transfection

Accumulation Fold

27.1
17.2
11.7
2.3
1.0
0

None
WT
E305Q
E578Q
QQ
Plasmid (pcDNA4.1, 0.05ug/well)
Accumulation of Ub-G76V-GFP in HeLa Cells

- Compound 1
- Compound 2

Fold Accumulation

Compound Concentration (uM)

Figure 15
Accumulation of Ub-\textsuperscript{G76V}-GFP in HeLa Cells

Figure 16
compound 8

Time (min)

0.16uM = -0.0324x + 3.57
R² = 0.98

0.04uM = -0.0333x + 3.4
R² = 0.99

0.01uM = -0.0141x + 3.32
R² = 0.99

0uM = -0.0382x + 3.18
R² = 0.99

ln(Fold Accumulation)
Figure 30

Fold of Accumulation

Concentration of Velcade (µM)

0 0.004 0.01 0.04 0.11 0.33 1

0 0.5 0.1 1.5 2 2.5 3
INHIBITORS OF P97

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application Nos. 61/011,436, filed Jan. 17, 2008, and 61/134, 174, filed Jul. 7, 2008. The contents of these applications are incorporated herein by reference in their entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

This invention was made with government support under Grant Numbers AI-055509, AI-074564 and MH-074404 awarded by the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND OF THE INVENTION


A prominent cellular function for p97 that has received considerable scrutiny is its role in the turnover of misfolded secretory proteins via the UPS. In this process, which is known as ERAD (endoplasmic reticulum-associated degradation), proteins that fail to fold within the ER are retro-translocated in a p97-dependent manner into the cytoplasm where they are degraded by the UPS (Ye, Y. et al. Nature (2004) 429, 841-847). In this process, p97 is thought to mediate extraction of substrates from the ER membrane. p97 is also required for the turnover of cytosolic substrates of the UPS (Janiesch, P. C. et al. Nat. Cell Biol. (2007) 9, 379-390; Cao, K. et al. Cell (2003) 115, 355-367; Fu, X. et al. J. Cell Biol. (2005) 163, 21-26), although its role in turnover of cytosolic proteins is less understood.


Thus there exists a need in the art for compounds for and methods of inhibiting the activity of p97.

SUMMARY OF THE INVENTION

One aspect of the invention relates to compounds that inhibit p97. In certain embodiments, the compounds inhibit the ATPase activity of P97. In certain embodiments, the invention relates to the treatment of a disease (e.g., cancer), comprising administering a compound of the invention.

One aspect of the invention relates to compounds having a structure of Formula I or a pharmaceutically acceptable salt thereof,

\[
\text{Ar-E} \\
\text{Ar is selected from substituted or unsubstituted aryl and heteroaryl, optionally a polycyclic aryl or heteroaryl moiety, e.g., naphthalene, anthracene, phenanthrene, quinoline, indole, etc.; and}
\]
E is an electrophilic group, such as a group capable of interacting with and forming a covalent bond or complex with a nucleophile, or a pharmaceutically acceptable salt or prodrug thereof. In certain embodiments, E is selected from

alkenyl (e.g.,

substituted acetyl (e.g.,

wherein X is a leaving group, e.g., F, Cl, Br, I, —OS(O)R², or —OS(O)R³), cyanoalkyl (e.g.,

acyl (e.g.,

aziridine (e.g.,

epoxide (e.g.,

vinyl sulfone (e.g.,

acryl (e.g.,

In certain embodiments, R¹ and R⁴, together with the atoms to which they are attached, form one or more rings.

In certain embodiments, R¹ is selected from hydrogen, haloalkyl (e.g., chloroalkyl, bromoalkyl, iodoalkyl, e.g.,

aziridine (e.g.,

epoxide (e.g.,

vinyl sulfone (e.g.,

acryl (e.g.,

In certain embodiments, R¹ has an atom susceptible to nucleophilic attack, e.g., capable of forming a covalent bond with a nucleophile. This atom is preferably within 2-6 atoms of Ar, e.g., within 2-4 atoms, most preferably 3 atoms of Ar.

In certain such embodiments, R¹ is selected from haloalkyl (e.g., chloroalkyl, bromoalkyl, iodoalkyl, e.g.,
wherein X is selected from F, Cl, Br, I, —OS(O₂)R₂, and —OS(O)R².

In certain embodiments, R² is selected from H and substituted or unsubstituted alkyl, alkenyl, alkynyl, heteroalkyl, aralkyl, cycloalkyl, heterocyclyl, aryl, heteroaryl, heteroaalkyl, cycloalkylalkyl, heterocyclylalkyl, halogen, acyl, carboxyl, ester, hydroxyl, alkoxy, alkylthio, acyloxy, amino, acylamino, carbamate, amido, amidino, cyano, sulfonyl, sulfoxido, sulfamoyl, or sulfonamido, preferably H or unsubstituted alkyl, aralkyl, heteroalkyl, halogen, acyl, carboxyl, ester, hydroxyl, alkoxy, alkylthio, acyloxy, amino, acylamino, carbamate, amido, or cyano.

In certain embodiments, R⁶ is selected from H and unsubstituted alkyl, heteroalkyl, aralkyl, cycloalkyl, heterocyclyl, aryl, heteroaryl, heteroaalkyl, cycloalkylalkyl, heterocyclylalkyl, or halogen.

In certain embodiments as discussed above, R⁶ may be substituted by one or more substituents selected from unsubstituted alkyl, alkenyl, alkynyl, heteroalkyl, cycloalkyl, heterocyclyl, aryl, heteroaryl, heteroaalkyl, cycloalkylalkyl, heterocyclylalkyl, halogen, acyl, carboxyl, ester, hydroxyl, alkoxy, alkylthio, acyloxy, amino, acylamino, carbamate, amido, amidino, cyano, sulfonyl, sulfoxido, sulfamoyl, or sulfonamido (preferably substituted or

wherein X is selected from Cl, Br, I, —OS(O₂)R₂, —OS(O)R²). In certain such embodiments, X is Cl.

In certain embodiments, R³ is selected from any of the groups described above for R⁴, while in other embodiments R³ can be H or substituted or unsubstituted alkyl, aralkyl, cycloalkyl, heterocyclyl, aryl, heteroaryl, heteroaalkyl, cycloalkylalkyl, heterocyclylalkyl, acyl, sulfonyl, sulfamoyl, or sulfonamido.

In certain embodiments, R³ may be substituted by one or more substituents selected from substituted or unsubstituted alkyl, alkenyl, alkynyl, heteroalkyl, cycloalkyl, heterocyclyl, aryl, heteroaryl, heteroaalkyl, cycloalkylalkyl, heterocyclylalkyl, halogen, acyl, carboxyl, ester, hydroxyl, alkoxy, alkylthio, acyloxy, amino, acylamino, carbamate, amido, amidino, cyano, sulfonyl, sulfoxido, sulfamoyl, or sulfonamido (preferably substituted or unsubstituted alkyl, alkenyl, heteroalkyl, halogen, acyl, carboxyl, ester, hydroxyl, alkoxy, alkylthio, acyloxy, amino, acylamino, carbamate, amido, amidino, or cyano).
In certain embodiments, R² and R⁴, together with the atoms to which they are attached, form one or more rings.

In certain embodiments, R² is H.

In certain embodiments, R² is acryl (e.g.,)

In certain such embodiments, R⁶ is H.

In certain embodiments, Ar is selected from

In certain embodiments, independently for each occurrence, R² is selected from H and substituted or unsubstituted alkyl, alkenyl, alkynyl, heteroalkyl, aralkyl, cycloalkyl, heterocyclyl, aryl, heteroaryl, heteroaralkyl, cy cloalkylalkyl, heterocyclylalkyl, halogen, acyl, carboxyl, ester, hydroxyl, alkoxyl, alkylthio, acyloxy, amino, acylamino, carbamate, amido, amidino, cyano, sulfonyl, sulfoxido, sulfamoyl, or sulphonamido (preferably substituted or unsubstituted alkyl, alkenyl, heteroalkyl, halogen, acyl, carboxyl, ester, hydroxyl, alkoxyl, alkylthio, acyloxy, amino, acylamino, carbamate, amido, amidino, cyano, sulfonyl, sulfoxido, sulfamoyl, or sulphonamido, preferably H or substituted or unsubstituted alkyl, alkenyl, heteroalkyl, halogen, acyl, carboxyl, ester, hydroxyl, alkoxyl, alkylthio, acyloxy, amino, acylamino, carbamate, amido, amidino, cyano, sulfonyl, sulfoxido, sulfamoyl, or sulphonamido (preferably substituted or unsubstituted alkyl, alkenyl, heteroalkyl, halogen, acyl, carboxyl, ester, hydroxyl, alkoxyl, alkylthio, acyloxy, amino, acylamino, carbamate, amido, amidino, cyano, sulfonyl, sulfoxido, sulfamoyl, or sulphonamido)

In certain embodiments, independently for each occurrence, R² is selected from H and substituted or unsubstituted alkyl, alkenyl, alkynyl, heteroalkyl, aralkyl, cycloalkyl, heterocyclyl, aryl, heteroaryl, heteroaralkyl, cycloalkylalkyl, heterocyclylalkyl, halogen, acyl, carboxyl, ester, hydroxyl, alkoxyl, alkylthio, acyloxy, amino, acylamino, carbamate, amido, amidino, cyano, sulfonyl, sulfoxido, sulfamoyl, or sulphonamido (preferably H or substituted or unsubstituted alkyl, alkenyl, heteroalkyl, halogen, acyl, carboxyl, ester, hydroxyl, alkoxyl, alkylthio, acyloxy, amino, acylamino, carbamate, amido, amidino, cyano, sulfonyl, sulfoxido, sulfamoyl, or sulphonamido, preferably H or substituted or unsubstituted alkyl, alkenyl, heteroalkyl, halogen, acyl, carboxyl, ester, hydroxyl, alkoxyl, alkylthio, acyloxy, amino, acylamino, carbamate, amido, amidino, cyano, sulfonyl, sulfoxido, sulfamoyl, or sulphonamido (preferably substituted or unsubstituted alkyl, alkenyl, heteroalkyl, halogen, acyl, carboxyl, ester, hydroxyl, alkoxyl, alkylthio, acyloxy, amino, acylamino, carbamate, amido, amidino, cyano, sulfonyl, sulfoxido, sulfamoyl, or sulphonamido)

In certain embodiments, independently for each occurrence, R² is selected from H and substituted or unsubstituted alkyl, alkenyl, alkynyl, heteroalkyl, aralkyl, cycloalkyl, heterocyclyl, aryl, heteroaryl, heteroaralkyl, cycloalkylalkyl, heterocyclylalkyl, halogen, acyl, carboxyl, ester, hydroxyl, alkoxyl, alkylthio, acyloxy, amino, acylamino, carbamate, amido, amidino, cyano, sulfonyl, sulfoxido, sulfamoyl, or sulphonamido (preferably substituted or unsubstituted alkyl, alkenyl, heteroalkyl, halogen, acyl, carboxyl, ester, hydroxyl, alkoxyl, alkylthio, acyloxy, amino, acylamino, carbamate, amido, amidino, cyano, sulfonyl, sulfoxido, sulfamoyl, or sulphonamido)

In certain embodiments, R² may be substituted by one or more substituents selected from substituted or unsubstituted alkyl, alkenyl, alkynyl, heteroalkyl, aralkyl, cycloalkyl, heterocyclyl, aryl, heteroaryl, heteroaralkyl, cycloalkylalkyl, heterocyclylalkyl, halogen, acyl, carboxyl, ester, hydroxyl, alkoxyl, alkylthio, acyloxy, amino, acylamino, carbamate, amido, amidino, cyano, sulfonyl, sulfoxido, sulfamoyl, or sulphonamido (preferably substituted or unsubstituted alkyl, alkenyl, heteroalkyl, halogen, acyl, carboxyl, ester, hydroxyl, alkoxyl, alkylthio, acyloxy, amino, acylamino, carbamate, amido, amidino, cyano, sulfonyl, sulfoxido, sulfamoyl, or sulphonamido)

In certain embodiments, R² may be substituted by one or more substituents selected from substituted or unsubstituted alkyl, alkenyl, alkynyl, heteroalkyl, aralkyl, cycloalkyl, heterocyclyl, aryl, heteroaryl, heteroaralkyl, cycloalkylalkyl, heterocyclylalkyl, halogen, acyl, carboxyl, ester, hydroxyl, alkoxyl, alkylthio, acyloxy, amino, acylamino, carbamate, amido, amidino, cyano, sulfonyl, sulfoxido, sulfamoyl, or sulphonamido (preferably substituted or unsubstituted alkyl, alkenyl, heteroalkyl, halogen, acyl, carboxyl, ester, hydroxyl, alkoxyl, alkylthio, acyloxy, amino, acylamino, carbamate, amido, amidino, cyano, sulfonyl, sulfoxido, sulfamoyl, or sulphonamido)

In certain embodiments, R² is lower alkyl (e.g.,

acryl)
In certain embodiments, E is selected from

\[
\text{N}^\text{R}^1, \quad \text{O}^\text{R}^1, \quad \text{and} \quad \text{S}^\text{R}^1.
\]

In certain such embodiments, Ar is selected from

\[
\begin{align*}
\text{R}^4 \text{N} \text{R}^3 \text{R}^1, \\
\text{N} \text{R}^3 \text{R}^2, \\
\text{R}^4 \text{N} \text{R}^3 \text{R}^2, \text{ and} \\
\text{R}^4 \text{N} \text{R}^3 \text{R}^2.
\end{align*}
\]

Definitions for such embodiments may be as those that are described above, including subcombinations of embodiments of E and Ar of narrower scope as set forth above. For example, in certain embodiments, Ar is

In certain such embodiments, E is

\[
\begin{align*}
\text{N}^\text{R}^1, \\
\text{R}^1, \quad \text{and} \quad \text{R}^1.
\end{align*}
\]
Exemplary compounds of Formula I include:

and salts (including pharmaceutically acceptable salts) of the foregoing.

In one aspect, the invention provides a pharmaceutical composition comprising a compound as disclosed herein and a pharmaceutically acceptable excipient or solvent.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 shows the active site of p97 bound by ADP-AlF₃.
FIG. 2 shows a different perspective from FIG. 1 of active site of p97 bound by ADP-AlF₃.
FIG. 3 shows an amino acid sequence alignment the Walker A motif in AAA ATPase and other ATPases.
FIG. 4 shows a histogram of ATPase activity upon treatment with compound 2.
FIG. 5 shows a histogram of ATPase activity.
FIG. 6 shows results from a mass spectrum.
FIG. 7 shows results from a Western blot.
FIG. 8 shows results from a Western blot.
FIG. 9 shows results from a Western blot.
FIG. 10 shows results from a Western blot.
FIG. 11 shows a histogram of proteosome activity in the presence of MG132 or compound 2.
FIG. 12 shows images of HeLa cells expressing UbG764-GFP that were treated with the indicated concentration of siRNA that target the indicated gene. Luciferase refers to Luciferase.
FIG. 13 shows a histogram of accumulation of UbG764-GFP.
FIG. 14 shows a histogram of accumulation of UbG764-GFP.
FIG. 15 shows a histogram of accumulation of UbG764-GFP.
FIG. 16 shows a histogram of accumulation of UbG764-GFP.
FIG. 17 shows a histogram of the rate of loss of accumulation of UbG764-GFP in cells treated with MG132.
FIG. 18 shows a histogram of the rate of loss of accumulation of UbG764-GFP in cells treated with compound 2.
FIG. 19 shows a histogram of accumulation of TCR-alpha-GFP.
FIG. 20 shows a histogram of accumulation of TCR-alpha-GFP.
FIG. 21 shows a histogram of survival of MCF-7 cells treated with compounds of the disclosure.
FIG. 22 shows a histogram of survival of HeLa cells treated with compounds of the disclosure.
FIG. 23 shows a histogram of the inhibition of p97 in the presence of various compounds.
FIG. 24 shows a histogram of the inhibition of C522A-p97 in the presence of various compounds.
FIG. 25 shows results from a Western blot.
FIG. 26 shows results from a Western blot.
FIG. 27 shows fluorescence images of cells with UbG764-GFP treated with DMSO, MG132, or compound 8.
FIG. 28 shows a histogram that compares fluorescence data of cells treated with various concentrations of compound 8.

FIG. 29 shows a histogram of the rate of loss of accumulation of UbGFP in cells treated with compound 8.

FIG. 30 shows a histogram that indicates accumulation of TCR-alpha-GFP in cells treated with various concentrations of Velcade.

FIG. 31 shows a histogram that indicates accumulation of TCR-alpha-GFP in cells treated with various concentrations of compound 8.

FIG. 32 shows a histogram indicating the GI_50 of cells treated with compound 2, compound 8, Velcade, or compound 2 and Velcade.

DETAILED DESCRIPTION OF THE INVENTION

Overview

The invention provides compounds that inhibit p97, as well as methods to treat or prevent a disease or condition in a subject that would benefit by inhibition of p97.

In certain embodiments, the disclosure provides methods of inhibiting p97. Preferred inhibitors for use in the methods disclosed herein bind to the active site of p97, e.g., noncovalently or covalently. In certain such embodiments, the covalent binding may be reversible or irreversible. In certain embodiments, compounds useful in the subject methods may bind reversibly or irreversibly to a cysteine residue in the active site (e.g., Cys522). Without wishing to be bound by theory, exemplary compounds disclosed herein have an acrylamide moiety that reacts with Cys522, consequently inhibiting the activity of p97.

Compounds

Compounds of the invention include compounds of Formula I as disclosed above. Such compounds are suitable for the compositions and methods disclosed herein. In other embodiments, the following compounds and their salts (including pharmaceutically acceptable salts) are compounds of the invention and are suitable for the compositions and methods disclosed herein:

DEFINITIONS

The term “acyl” is art-recognized and refers to a group represented by the general formula hydrocarby1C(O)—, preferably alkyl1C(O)—.

The term “acylamino” is art-recognized and refers to an amino group substituted with an acyl group and may be represented, for example, by the formula hydrocarby1C(O)NH—, preferably alkyl1C(O)NH—.

The term “acyloxy” is art-recognized and refers to a group represented by the general formula hydrocarby1C(O)O—, preferably alkyl1C(O)O—.

The term “aliphatic” as used herein, includes straight, chained, branched or cyclic hydrocarbons which are completely saturated or contain one or more units of unsaturation. Aliphatic groups may be substituted or unsubstituted.

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.

The term “alkoxyalkyl” refers to an alkyl group substituted with an alkoxy group and may be represented by the general formula alkyl-O-alkyl.

The term “alkenyl”, as used herein, refers to an aliphatic group containing at least one double bond and is intended to include both unsubstituted alkenyls and substituted alkenyls, the latter of which refers to alkenyl moieties having substituents replacing a hydrogen on one or more carbons of the alkenyl group. Such substituents may occur on one or more carbons that are included or not included in one or more double bonds. Moreover, such substituents include all those contemplated for alkyl groups, as discussed below, except
wherein stability is prohibitive. For example, substitution of alkenyl groups by one or more alkyl, carbocyclic, aryl, heterocyclic, or heteroaryl groups is contemplated. In preferred embodiments, a straight chain or branched chain alkyl is 1-12 carbons in its backbone, preferably 1-8 carbons in its backbone, and more preferably 1-6 carbons in its backbone. Examples of alkyl groups include alkan, propenyl, butenyl, 2-methyl-2-butyl, and the like.

The term "alkyl" refers to the radical of saturated aliphatic groups, including straight-chain alkyl groups, and branched-chain alkyl groups. In preferred embodiments, a straight chain or branched chain alkyl has 30 or fewer carbon atoms in its backbone (e.g., C_{1-5} for straight chains, C_{1-5} for branched chains), and more preferably 20 or fewer. In certain embodiments, alkyl groups are lower alkyl groups, e.g., methyl, ethyl, n-propyl, isopropyl, n-butyl and n-pentyl.

Moreover, the term "alkyl" (or "lower alkyl") as used throughout the specification, examples, and claims is intended to include both "unsubstituted alkyls" and "substituted alkyls"; the latter of which refers to alkyl moieties having substituents replacing a hydrogen on one or more carbons of the hydrocarbon backbone. In certain embodiments, a straight chain or branched chain alkyl has 30 or fewer carbon atoms in its backbone (e.g., C_{1-5} for straight chains, C_{1-5} for branched chains). In preferred embodiments, the chain has ten or fewer carbon (C_{1-10}) atoms in its backbone. In other embodiments, the chain has six or fewer carbon (C_{1-6}) atoms in its backbone.

Such substituents can include, for example, a halogen, a hydroxyl, a carbonyl (such as a carboxyl, an alkoxy carbonyl, a formyl, or an acyl), a thio carbonyl (such as a thioester, a thioacetate, or a thioformate), an alkoxyl, a phosphoryl, a phosphite, a phosphonate, a phosphinate, an amino, an amido, an amine, an imine, a cyano, a nitro, an azido, a sulfhydryl, an alkylthio, a sulinate, a sulfonate, a sulfamoyl, a sulfonyl, a heterocyclic, an aralkyl, or an aryl or heteroaryl moiety.

The term "C_{alk}" when used in conjunction with a chemical moiety, such as, acyl, acenoyl, alkenyl, alkynyl, or alkoy is meant to include groups that contain from x to y carbons in the chain. For example, the term "C_{alk}" 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_{alk} alkyl indicates a hydrogen where the group is in a terminal position, a bond if internal. The terms "C_{alk} alkynyl" and "C_{alk} 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 "alkylylamino", as used herein, refers to a amino group substituted with at least one alkyl group.

The term "alkylthio", as used herein, refers to a thiol group substituted with an alkyl group and may be represented by the general formulaalkylS-. The term "alkynyl", as used herein, refers to an aliphatic group containing at least one triple bond and is intended to include both "unsubstituted alkynyls" and "substituted alkynyls", the latter of which refers to alkynyl moieties having substituents replacing a hydrogen on one or more carbons of the alkynyl group. Such substituents may occur on one or more carbons that are included or not included in one or more triple bonds. Moreover, such substituents include all those contemplated for alkyl groups, as discussed above, except where stability is prohibitive. For example, substitution of alkynyl groups by one or more alkyl, carbocyclic, aryl, het-erocyclic, or heteroaryl groups is contemplated. In preferred embodiments, an alkynyl has 1-12 carbons in its backbone, preferably 1-8 carbons in its backbone, and more preferably 1-6 carbons in its backbone. Example alkynyl groups include propynyl, butynyl, 3-methylpent-1-ynyl, and the like.

The term "amine", as used herein, refers to a group wherein R^9 and R^{10} each independently represent a hydrogen or hydrocarbyl group, 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.

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

wherein R^9, R^{10}, and R^{10'} each independently represent a hydrogen or hydrocarbyl group, 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.

The term "arylamino", as used herein, refers to an alkyl group substituted with an amino group.

The term "aryl", as used herein, include substituted or unsubstituted single-ring aromatic groups in which each atom of the ring is carbon. Preferably the ring is a 5-7-membered ring, more preferably a 6-membered ring. Aaryl groups include phenyl, phenol, aniline, and the like.

The term "aryloxy", as used herein, refers to an aryl group having an oxygen attached thereto. Representative aryloxy groups include phenoxy, naphthoxy, and the like.

The term "carbamate" is art-recognized and refers to a group

wherein R^9 and R^{10} independently represent hydrogen or a hydrocarbyl group, such as an alkyl group.

The terms "carbocyclic", "carbocyclic", and "carbocyclic", as used herein, refers to a non-aromatic saturated or unsaturated ring in which each atom of the ring is carbon. Preferably a carbocyclic ring contains from 3 to 10 atoms, more preferably from 3 to 7 atoms.

The term "carbocyclylalkyl", as used herein, refers to an alkyl group substituted with a carbocyclic group.

The term "carbonate" is art-recognized and refers to a group —OCO_2—R^9, wherein R^9 represents a hydrocarbyl group, such as an alkyl group.
The term “carboxy”, as used herein, refers to a group represented by the formula -CO₂H.

The term “cycloalkyl”, as used herein, refers to the radical of a saturated aliphatic ring. In preferred embodiments, cycloalkyls have from 3-10 carbon atoms in their ring structure, and more preferably from 5-7 carbon atoms in the ring structure. Suitable cycloalkyls include cycloheptyl, cyclohexyl, cyclopentyl, cyclobutyl and cyclopropyl.

The term “ester”, as used herein, refers to a group —C(OR)₂ wherein R represents a hydrocarbonyl group, such as an alkyl group or an aralkyl group.

The term “ether”, as used herein, refers to a hydrocarbonyl group linked through an oxygen to another hydrocarbonyl group. Accordingly, an ether substituent of a hydrocarbonyl group may be hydrocarbonyl—O—. Ethers may be either symmetrical or unsymmetrical. Examples of ethers include, but are not limited to, heterocycle-O-heterocycle and aryl-O-heterocycle. Ethers include “alkoxyalkyl” groups, which may be represented by the general formula alkyl-O-alkyl.

The terms “halo” and “halogen”, as used herein, means halogen and includes chloro, fluoro, bromo, and ido.

The terms “hetaryl” and “heteroaryl”, as used herein, refers to an alkyl group substituted with a heteraryl group.

The terms “heteroaryl” and “hetaryl” include substituted or unsubstituted aromatic single ring structures, preferably 5- to 7-membered rings, more preferably 5- to 6-membered rings, whose ring structures include at least one heteroatom (e.g., O, N, or S), preferably one to four or one to 3 heteroatoms, more preferably one or two heteroatoms. When two or more heteroatoms are present in a heteroaryl ring, they may be the same or different. The terms “heteroaryl” and “hetaryl” 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 heteroaromatic, e.g., the other cyclic rings can be cycloalkyls, cycloalkenyls, cycloalkynyls, aryls, heteroaryl, and/or heterocyclics. Preferred polycyclic ring systems have two cyclic rings in which both of the rings are aromatic. Heteroaryl groups include, for example, pyrrole, furan, thiophene, imidazole, oxazole, thiazole, pyrazole, pyridine, pyrazine, pyridazine, quinoline, and pyrimidine, and the like.

The term “hetaryloxy” refers to a heteroaryl group having an oxygen attached thereto. Representative heteroaryloxy groups include pyridoxy and the like.

The term “hetaromatic”, as used herein, means an atom of any element other than carbon or hydrogen. Preferred heteroaromatics are nitrogen, oxygen, and sulfur.

The terms “hetereocyclic”, “heterecycle”, and “heterecyclic” refer to substituted or unsubstituted non-aromatic ring structures, preferably 3- to 10-membered rings, more preferably 3- to 7-membered rings, whose ring structures include at least one heteroatom, preferably one to four heteroatoms, more preferably one or two heteroatoms. Heterocyclic groups include, for example, piperidine, pyrazine, pyrrolidine, morpholine, lactones, lactams, and the like.

The terms “hetereocyclalkyl”, as used herein, refers to an alkyl group substituted with a heterocyclic group.

The term “hetercarboxylic”, as used herein, refers to a group that is bonded through a carbon atom that does not have a —O or —S substituent, and typically has at least one carbon-hydrogen bond and a primarily carbon backbone, but may optionally include heteroatoms. Thus, groups like methyl, ethoxycarbonyl, 2-pyridyl, and trifluoroethyl are considered to be hetercarboxylic for the purposes of this application, but substituents such as acetyl (which has a —O substituent on the linking carbon) and ethoxy (which is linked through oxygen, not carbon) are not. Hetercarboxyl groups include, but are not limited to, ary1, hetereocyclic, heterecyclic, alkyl, alkenyl, alkyln1, and combinations thereof.

The term “heterexyalkyl”, as used herein, refers to an alkyl group substituted with a hetereoxy group.

The term “lower” when used in conjunction with a chemical moiety, such as, acyl, acyloxy, alkyl, alkenyl, alkylnl, or alkoxy is meant to include groups where there are ten or fewer non-hydrogen atoms in the substituent, preferably six or fewer. A “lower alkyl”, for example, refers to an alkyl group that contains ten or fewer carbon atoms, preferably six or fewer. Examples of straight chain or branched chain lower alkyl include methyl, ethyl, isopropyl, propyl, butyl, tert-buty1, and the like. In certain embodiments, acyl, acyloxy, alkyl, alkenyl, alkylnl, or alkoxy substituents defined herein are respectively lower acyl, lower acyloxy, lower alkyl, lower alkenyl, lower alkynl, lower alkoxy, whether they appear alone or in combination with other groups, such as in the substitutions hetereoxyalkyl and aroyl (in which case, for example, the alkyl groups within the ary1 group are not counted when counting the carbon atoms in the alkyl substituent).

The terms “polyycylic”, “polycycle”, and “polycyclic” refer to two or more rings (e.g., cycloalkyls, cycloalkenyls, cycloalkynyls, aryls, heteroaryl, and/or heteroatoms) in which two or more atoms are common to two adjoining rings, e.g., the rings are “fused rings”. Preferred polycycles have 2-3 rings. Each of the rings of the polycycle can be substituted or unsubstituted. In certain embodiments, each ring of the polycycle contains from 3 to 10 atoms in the ring, preferably from 5 to 7.

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 the 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 any substituents described herein, for example, a halogen, a hydroxyl, a carbonyl (such as a carboxyl, an alkoxyalkynyl, or an acyl), a thioalky1 (such as a thioether, a thioacetate, or a thioformate), an alkoxyl, a phosphory1, a phospate, a phosphonate, a phosphinate, an amino, an amid0, an amide, an imine, a cyano, a nitro, an azido, a sulfonyl, an alkylthio, a sulfite, a sulfnate, a sulfamoy1, a sulfonylamido, a sulfonyl, a heterocyclic, an aralkyl, or an aromatic or heterocyclic moiety.

Unless specifically stated as “unsubstituted,” references to chemical moieties herein are understood to include substituted variants. For example, reference to an “ary1” group or moiety implicitly includes both substituted and unsubstituted variants.

The term “sulfate” is art-recognized and refers to the group —OSO₃H, or a pharmaceutically acceptable salt or ester thereof.
The term “sulfonamide” is art-recognized and refers to the group represented by the general formulae

wherein \( R^2 \) and \( R^{10} \) independently represents hydrogen or hydrocarbaryl, such as alkyl.

The term “sulfoxide” is art-recognized and refers to the group \(-\text{S(O)}-R^2\), wherein \( R^2 \) represents a hydrocarbaryl, such as alkyl, aryl, or heterocararyl.

The term “sulfonate” is art-recognized and refers to the group \(-\text{SO}_2\) \( R^2 \), or a pharmaceutically acceptable salt or ester thereof.

The term “sulfone” is art-recognized and refers to the group \(-\text{S(O)}_2-R^2\), wherein \( R^2 \) represents a hydrocarbaryl, such as alkyl, aryl, or heterocararyl.

The term “thioalkyl”, as used herein, refers to an alkyl group substituted with a thiol group.

The term “thioester”, as used herein, refers to a group \(-\text{C(O)S}R^2\) or \(-\text{SC(O)R}^2\) wherein \( R^2 \) represents a hydrocarbaryl, such as alkyl.

The term “thioether”, as used herein, is equivalent to an ether, wherein the oxygen is replaced with a sulfur.

The term “urea” is art-recognized and may be represented by the general formula

wherein \( R^2 \) and \( R^{10} \) independently represent hydrogen or a hydrocarbaryl, such as alkyl.

At various places in the present specification substituents of compounds of the invention are disclosed in groups or in ranges. It is specifically intended that the invention include each and every individual subcombination of the members of such groups and ranges. For example, the term “C1–C6 alkyl” is specifically intended to individually disclose methyl, ethyl, propyl, isopropyl, n-butyl, sec-butyl, isobutyl, etc. For a number qualified by the term “about”, a variance of 2%, 5%, 10% or even 20% is within the ambit of the qualified number.

As used herein, the term “inhibitor” is meant to describe a compound that blocks or reduces an activity of an enzyme (for example, inhibition of various activities of p97). An inhibitor can act with competitive, uncompetitive, or non-competitive inhibition. An inhibitor can bind reversibly or irreversibly, and therefore the term includes compounds that are suicide substrates of an enzyme. An inhibitor can modify one or more sites on or near the active site of the enzyme, or it can cause a conformational change elsewhere on the enzyme.

As used herein, a therapeutic that “prevents” a disorder or condition refers to a compound that, in a statistical sample, reduces the occurrence of the disorder or condition in the treated sample relative to an untreated control sample, or delays the onset or reduces the severity of one or more symptoms of the disorder or condition relative to the untreated control sample.

The term “preventing” is art-recognized, and when used in relation to a condition, such as a local recurrence (e.g., pain), a disease such as cancer, a syndrome complex such as heart failure or any other medical condition, is well understood in the art, and includes administration of a composition which reduces the frequency of, or delays the onset of, symptoms of a medical condition in a subject relative to a subject which does not receive the composition. Thus, prevention of cancer includes, for example, reducing the number of detectable cancerous growths in a population of patients receiving a prophylactic treatment relative to an untreated control population, and/or delaying the appearance of detectable cancerous growths in a treated population versus an untreated control population, e.g., by a statistically and/or clinically significant amount. Prevention of an infection includes, for example, reducing the number of diagnoses of the infection in a treated population versus an untreated control population, and/or delaying the onset of symptoms of the infection in a treated population versus an untreated control population.

Prevention of pain includes, for example, reducing the magnitude of, or alternatively delaying, pain sensations experienced by subjects in a treated population versus an untreated control population.

The term “prodrug” encompasses compounds that, under physiological conditions, are converted into therapeutically active agents. A common method for making a prodrug is to include selected moieties that are hydrolyzed under physiological conditions to reveal the desired molecule. In other embodiments, the prodrug is converted by an enzymatic activity of the host animal. In certain embodiments, a prodrug of a compound is an ester or amide of the compound. For example, a carboxylic acid residue of the compound can be converted to an amide or ester by techniques well known in the art, or, similarly, a hydroxyl or amine residue of the compound can be acylated.

The term “prophylactic or therapeutic” treatment is art-recognized and includes administration to the host of one or more of the subject compositions. If it is administered prior to clinical manifestation of the unwanted condition (e.g., disease or other unwanted state of the host animal) then the treatment is prophylactic, (i.e., it protects the host against developing the unwanted condition), whereas if it is administered after manifestation of the unwanted condition, the treatment is therapeutic, (i.e., it is intended to diminish, ameliorate, or stabilize the existing unwanted condition or side effects thereof).

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 alkoxy-
carbonyl, a formyl, or an acyl), a thiacarbonyl (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, an azido, a sulfhydryl, an alkylthio, a sulfite, a sulfonate, a sulfamoyl, a sulfonamido, a sulfonyl, a heterocyclic, 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.

The term "treating" includes prophylactic and/or therapeutic treatments. The term "prophylactic or therapeutic" treatment is art-recognized and includes administration to the host of one or more of the subject compositions. If it is administered prior to clinical manifestation of the unwanted condition (e.g., disease or other unwanted state of the host animal) then the treatment is prophylactic, (i.e., it protects the host against developing the unwanted condition), whereas if it is administered after the manifestation of the unwanted condition, the treatment is therapeutic, (i.e., it is intended to diminish, ameliorate, or stabilize the existing unwanted condition or side effects thereof). 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.

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.

Synthetic Preparation

The novel compounds of the present invention can be prepared in a variety of ways known to one skilled in the art of organic synthesis. The compounds of the present invention can be synthesized using the methods as hereinbefore described below, together with synthetic methods known in the art of synthetic organic chemistry or variations thereon as appreciated by those skilled in the art.

Preparation of Compounds can involve the Protection and Deprotection of Various chemical groups. The need for protection and deprotection, and the selection of appropriate protecting groups can be readily determined by one skilled in the art. The chemistry of protecting groups can be found, for example, in Greene and Wuts, Protective Groups in Organic Synthesis. 4th Ed., Wiley & Sons, 2006, which is incorporated herein by reference in its entirety.

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.
phosphate, tosylate, citrate, maleate, fumarate, succinate, tartrate, naphthylate, mesylate, glucoheptonate, laetobionate, 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 ethylenimine, diethylenimine, ethanediamine, 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 bisulfite, sodium metabsulfite, 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 glycercin, 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, algic 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 as 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 by wetting 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.

Tables, 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, tetrahydrofurfuryl 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.

Solutions, 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 metaphosphate, bento- nite, 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 tem-
perature and, therefore, will melt in the rectum or vaginal cavity and release the active agent.

Formulations which are suitable for vaginal administration also include pastes, 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 unsubstututed 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 (Iwets, Pluronic, sorbitan esters, lecithin, Crosporphors), pharmaceutically acceptable co-solvents such as polyethylene glycol, innocuous proteins such as 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 materi-
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 concomitant therapy wherein one or more other therapeutic agents are administered with the inhibitor. Such concomitant 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 concomitantly administered with one or more proteasome inhibitor(s).

In certain embodiments, a compound of the invention is concomitantly administered with a chemotherapeutic. Suitable chemotherapeutics may include, natural products such as vinca alkaloids (i.e. vinblastine, vincristine, and vinorelbine), paclitaxel, epidodophyllotoxins (i.e. etoposide, teniposide), antibiotics (actinomycin (actinomycin D) daunorubicin, doxorubicin and idarubicin), anthracyclines, mitoxantrone, bleomycins, plicamycin (mithramycin) and mitomycin, enzymes (L-asparaginase which systematically metabolizes L-asparagine and depletes cells which do not have the capacity to synthesize their own asparagine), anti-platelet agents; antiproliferative/antimitotic alkylating agents such as nitrogen mustards (melphalan and cyclophosphamide and analogs, melphalan, chlorambucil), ethylenimines and methylmelamines (hexamethylmelamine and thiopeta), alkyl sulfonates (busulfan), nitrosoureas (carmustine (BCNU) and analogs, streptozocin), taxanes—dactarbazine (DTIC); antiproliferative/antimitotic antimalbolites such as folic acid analogs (methotrexate), pyrimidine analogs (5-flourouracil, 5-flouridine, 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 (i.e. estragon) and hormone agonists such as leutinizating hormone releasing hormone (LH-RH) agonists (goserelin, leuprolide and triptorelin). Other chemotherapeutic agents may include melpholamine, camptothecin, ifosfamide, tamoxifen, raloxifene, gemcitabine, navelbine, or any analog or derivative variant of the foregoing.

In certain embodiments, a compound of the invention is concomitantly administered with a steroid. Suitable steroids may include, but are not limited to, 21-acetoxyproglandonolone, alcolmetasone, algestone, amcinonide, beclometasone, betamethasone, budesonide, chloroprednisone, clobetasol, clocurtolone, clobrednol, corticosterone, cortisol, cortiva- zol, deflazacort, desonide, desoximetasone, dexamethasone, difloracone, diflucortolone, difuprednate, enoxolone, fluza- cort, flucloronide, flumethasone, flunisolide, flunisolone acetonide, flunisolone, fluocoric butyl, fluocortolone, fluo-
example, a purine scaffold was derivatized with various electrophilic groups were attached to the C6 position as shown below, including Michael acceptors (compounds 2, 3, 6 and 7), and a chloracetamide (compound 4). As negative controls, compounds were prepared that retained an amino group attached at C6 (compounds 1 and 5).

Compounds were tested for their ability to inhibit the ATPase activity of purified wild type murine p97 and T532C-Cdc48, a mutant of yeast Cdc48 in which the residue analogous to cysteine 522, threonine 532, was converted to cysteine. Compounds were individually preincubated at various concentrations with 200 nM p97 for 20 min at 23°C., after which ATP was added and ATPase activity was measured using the malachite green assay method (Lanzetta, P. A. et al. Anal. Biochem. (1979) 100, 95-97) to determine the amount of compound required to achieve 50% inhibition (IC_{50}). All compounds demonstrated inhibitory activity in this assay, except for the control compounds 1 and 5 (see Table 1). The most potent inhibitor was compound 2, which contains a phenyl substituent at C7 and a single Michael acceptor at C6 (see FIG. 4).

<table>
<thead>
<tr>
<th>TABLE 1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>In vitro ATP Hydrolysis Activity</strong></td>
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<tr>
<td></td>
</tr>
<tr>
<td><strong>Compound</strong></td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
</tbody>
</table>
TABLE 1-continued

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC_{50} (µM) of Enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT-mp97</td>
<td>4.5 ± 3.5</td>
</tr>
<tr>
<td>T532C-Cdc48</td>
<td>35 ± 8</td>
</tr>
<tr>
<td>100</td>
<td>3 ± 1</td>
</tr>
<tr>
<td>24 ± 7</td>
<td>1.3 ± 0.7</td>
</tr>
</tbody>
</table>

To determine whether cysteine 522 is necessary for inhibition of p97 by compound 2, a mutant of p97 was constructed in which cysteine 522 was converted to either alanine (CS22A-mpp97) or threonine (CS22T-mpp97). The IC_{50} values of compound 2 were measured against various enzymes during a 20-minute preincubation (see Table 2). Mutation of cysteine 522 in p97 to either alanine or threonine decreased sensitivity to compound 2 by greater than 100-fold. On the other hand, yeast Cdc48, which normally is quite resistant to compound 2, became nearly 10-fold more sensitive to the compound upon introduction of a cysteine in place of threonine 532. Together, these data indicate that a cysteine at position 522 is both necessary and sufficient to confer sensitivity to compound 2.

TABLE 2

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>IC_{50} (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT-mp97</td>
<td>0.62 ± 0.25</td>
</tr>
<tr>
<td>CS22A-mpp97</td>
<td>110 ± 33</td>
</tr>
<tr>
<td>CS22T-mpp97</td>
<td>82 ± 45</td>
</tr>
<tr>
<td>Yeast Cdc48</td>
<td>376 ± 95</td>
</tr>
<tr>
<td>T532C-yeast Cdc48</td>
<td>14 ± 5</td>
</tr>
<tr>
<td>Hamster NSF</td>
<td>105 ± 31</td>
</tr>
<tr>
<td>Human 19S ATPase</td>
<td>75 ± 19</td>
</tr>
</tbody>
</table>

*Human Rpt3 contains a cysteine in Walker A motif.

To further address the specificity of inhibition by compound 2, its activity against two other AAA ATPases, NSF and the 26S proteasome, was assessed. Although NSF is known to be sensitive to thiol-reactive agents, it was more than 100-fold less sensitive to compound 2 than p97 (Table 2). Likewise, inhibition of ATP hydrolysis by human 19S ATPase of the 26S proteasome was observed only at high concentrations of compound 2 (IC_{50} > 75 µM, Table 2). The results with NSF and the 26S proteasome are noteworthy because both the active D1 domain of NSF and the Rpt3 subunit of the 26S proteasome have a cysteine in the position analogous to cysteine 522 of p97 (Fig. 3).

To evaluate whether compound 2 can target p97 activity in cells, we sought to determine whether this compound induces accumulation of high molecular weight ubiquitin conjugates, since it is known that RNAi-mediated knockdown of p97 in mammalian cells has this effect (Wojcik, C. et al. J. Cell Sci. (2004) 117, 281-292). As a positive control for this experiment, the proteasome inhibitor MG132 was used. HeLa or RPMI18226 cells were treated for 60 minutes at 37°C with 10, 20, or 50 µM of MG132, compound 2, or the inactive compound 1 that lacks the Michael acceptor. The cells were then harvested, lysed, and centrifuged to separate soluble and insoluble fractions. These fractions were then immunoblotted with anti-ubiquitin antibodies to detect accumulation of high molecular weight conjugates. Blots were also probed with anti-PTSAIRE antibodies as a loading control. The data shown in Fig. 7 (HeLa cells) and Fig. 8 (RPMI18226 cells) reveal that compound 2, but not its inactive analog compound 1, caused accumulation of high molecular weight ubiquitin conjugates. This effect is particularly pronounced in the insoluble fraction. Moreover, co-treatment of compound 2 with MG132 in the presence of a protein synthesis inhibitor, cycloheximide, caused more accumulation of ubiquitin conjugates in HeLa and PC3 (Fig. 9) and DU145 and LNCaP (Fig. 10) cell lines.

The effects of compound 2 on ubiquitin conjugate accumulation are not due to inhibition of the proteasome, because it was shown that compound 2 synergizes with a proteasome inhibitor and compound 2 does not inhibit hydrolysis of a proteasome substrate, LLVY-AMC, in vitro (Fig. 11). Human 26S proteasome complex was affinity purified from HEK293 cells that stably express tagged human Rpn 11 as described (Wang et al. Biochemistry (2007) 46, 3553-3565). Purified human 26S proteasome (19 nM) was incubated with either DMSO, MG132 or compound 2 for 30 min at room temperature and fluorogenic proteasome substrate LLVY-AMC (60 µM) was added to initiate the reaction. Fluorescence intensity was monitored every 3 min over 30 min. As a second test of whether compound 2 can inhibit p97 in vivo, it was evaluated whether 2 can cause accumulation of the normally unstable protein Ub_{765}-GFP, which has been shown to accumulate in cells in which p97 has been depleted by siRNA (see Fig. 12 and Fig. 13; see Wojcik, C. et al. Mol. Biol. Cell (2006) 17, 4666-4681) or in cells that express a dominant-negative version of p97 (Fig. 14). A HeLa cell line that stably expresses Ub_{765}-GFP was transfected with either wild type (WT), D1 mutant (E305Q), D2 mutant (E578Q) or E305Q, E578Q double mutant (QQ) murine 97-pcDNA4.1 plasmid (0.05 µg/100 µl) in a 96-well plate. After 23 h, the intensity of Ub_{765}-GFP signal was measured on a Molecular Devices ImageXpress automated fluorescence microscope. As a control, the same cells were treated with the proteasome inhibitor MG132. In the latter set of experiments, a HeLa cell line that stably expresses Ub_{765}-GFP was transfected with either wild type (WT), D1 mutant (E305Q), D2 mutant (E578Q) or E305Q, E578Q double mutant (QQ) murine 97-pcDNA4.1 plasmid (0.05 µg/100 µl) in a 96-well plate. After 23 h, the intensity of Ub_{765}-GFP signal was measured on a Molecular Devices ImageXpress automated fluorescence microscope. These results provide evidence that p97 ATPase activity is required for the proper turnover of Ub_{765}-GFP in HeLa cells. Both MG132 (see Fig. 15) and compound 2, but not compound 1 (see Fig. 16), caused accumulation of Ub_{765}-GFP. The direct accumulation of Ub_{765}-GFP caused by compound 2 at 25 µM is 4-fold less than that caused by MG132 at 2 µM. This is likely due to the fact that only fully folded Ub_{765}-GFP should require p97 for degradation by the proteasome.

To evaluate this possibility, an assay was developed to determine the rate of degradation of pre-accumulated Ub_{765}-GFP molecules. Ub_{765}-GFP/HeLa cells were treated with MG132 (2 µM) for 1 h, during which time Ub_{765}-GFP is synthesized and may fold into its active (fluorescent) conformation. Cells were then washed with PBS three times remove the MG132. Cells were then
refreshed by adding DMEM containing cycloheximide (50 
μg/mL; to block production of more Ub-G765-GFP) and test 
compounds (0–10 μM) were added into cells. Eight 96-well 
plates were prepared and one of the plates was imaged at 25, 
50, 70, 100, 110, 125, 145, or 170 min after washing with PBS 
three times. After washing out MG132, degradation of pre-
accumulated Ub-G765-GFP appears to follow pseudo-first 
order reaction. Rate constant (K; 1/min) can be determined 
from the slope of plotting Ln (Fold Accumulation) versus 
Time (min) (see FIG. 17 and FIG. 18). Half-lives of pre-
accumulated Ub-G765-GFP can be calculated by Ln2/K. IC50 
values of MG132 and compound 2 on the decay of Ub-G765-
GFP measured by this method were calculated to be 
0.24 ± 0.04 μM (Table 3) and 1.25 ± 0.47 μM (Table 4), 
respectively. Compound 1 showed no effect up to 10 μM.

### Table 3

<table>
<thead>
<tr>
<th>Conc. MG132 (μM)</th>
<th>K (1/min)</th>
<th>t1/2 (min)</th>
<th>Ln(2)/K</th>
<th>% K</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.0382</td>
<td>18</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>0.01</td>
<td>0.0336</td>
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<td>0.04</td>
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<td>27</td>
<td>68</td>
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<tr>
<td>0.63</td>
<td>0.0095</td>
<td>73</td>
<td>25</td>
<td></td>
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<td>2.5</td>
<td>0.0005</td>
<td>1386</td>
<td>1</td>
<td></td>
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<tr>
<td>10</td>
<td>0.0009</td>
<td>770</td>
<td>2</td>
<td></td>
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</tbody>
</table>

IC50 = 0.24 ± 0.04 μM

### Table 4

<table>
<thead>
<tr>
<th>Conc. Compd. 1 (μM)</th>
<th>K (1/min)</th>
<th>t1/2 (min)</th>
<th>Ln(2)/K</th>
<th>% K</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.0388</td>
<td>18</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>0.01</td>
<td>0.0318</td>
<td>22</td>
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<tr>
<td>0.04</td>
<td>0.0306</td>
<td>23</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>0.16</td>
<td>0.0292</td>
<td>24</td>
<td>76</td>
<td></td>
</tr>
<tr>
<td>0.63</td>
<td>0.0237</td>
<td>29</td>
<td>62</td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td>0.0180</td>
<td>37</td>
<td>49</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0.0151</td>
<td>462</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

IC50 = 1.25 ± 0.47 μM

Since p97 has a well-established role in ERAD, it was also 
determined whether compound 2 affects the accumulation 
of an ERAD-reporter, TCR-alpha-GFP. HEK293 cells that sta-
bly expressed TCR-GFP were treated with MG132 (see FIG. 
19), compound 1 or compound 2 (FIG. 20) at the indicated 
fumarate concentrations for 2 h at 37°C. Prior to being imaged 
by fluorescence microscopy to determine TCR-GFP accumula-
tion. Compound 2 clearly provoked substantial TCR-alpha-
GFP accumulation (FIG. 20). It was thus concluded that 
compound 2 inhibits p97 in cells, resulting in the accumula-
tion of both soluble and insoluble high molecular weight 
ubiquitin conjugates and UPS substrates whose degradation 
depends upon p97.

To probe further the effect of compound 2 and related 
compounds 1, 3, 5, 6 and 7, various concentrations of these 
compounds were added to 13 different cell lines and in-
cubated for 48 or 72 h. An MT1 assay was then performed to 
evaluate cell growth and survival. IC50 values were deter-
mined and are shown in Tables 5-7.

### Table 5

<table>
<thead>
<tr>
<th>Compound</th>
<th>Cell line inhibition ofgrowth G10 (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HeLa</td>
</tr>
<tr>
<td>1</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>2</td>
<td>0.4 ± 0.3</td>
</tr>
<tr>
<td>3</td>
<td>5.3 ± 2.5</td>
</tr>
<tr>
<td>5</td>
<td>1.0 ± 0.3</td>
</tr>
<tr>
<td>6</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>7</td>
<td>1.8 ± 0.6</td>
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</table>

### Table 6

<table>
<thead>
<tr>
<th>Compound</th>
<th>Cell line inhibition ofgrowth G10 (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RPMI826</td>
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<tr>
<td>1</td>
<td>1.1 ± 0.7</td>
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<tr>
<td>2</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>3</td>
<td>1.2 ± 0.6</td>
</tr>
<tr>
<td>5</td>
<td>9.8 ± 1.8</td>
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<tr>
<td>6</td>
<td>1.2 ± 0.3</td>
</tr>
<tr>
<td>7</td>
<td>3.4 ± 1.7</td>
</tr>
</tbody>
</table>

### Table 7

<table>
<thead>
<tr>
<th>Compound</th>
<th>Cell line inhibition ofgrowth G10 (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HTCC116 WT</td>
</tr>
<tr>
<td>1</td>
<td>10 ± 2</td>
</tr>
<tr>
<td>2</td>
<td>2.4 ± 1.2</td>
</tr>
<tr>
<td>3</td>
<td>5.1 ± 2</td>
</tr>
<tr>
<td>4</td>
<td>19 ± 6</td>
</tr>
<tr>
<td>5</td>
<td>4.3 ± 1.8</td>
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<td>6</td>
<td>2.8 ± 1.6</td>
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<tr>
<td>7</td>
<td>5.5 ± 1.4</td>
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</table>

Data for the assay with MCF-7 cells and HeLa cells is shown 
in FIG. 21 and FIG. 22, respectively. Of all the compounds 
tested, compound 2 appears to be the most potent, 
yielding 50% inhibition of growth (G100) at concentrations 
of compound 2 ranging from 0.4–5.1 μM across the 13 different 
cell lines. By contrast, the GI50 for compound 1 was 3 to 
18-fold higher across the same cell lines. Thus, compound 2 
blocks p97-dependent processes in cells and also blocks the 
proliferation of cancer cell lines, illustrating its potential 
utility as an anti-proliferative drug to treat cancer.

**Example 2**

**High-Throughput Screening to Identify Inhibitors of p97**

An assay that measures the ATPase activity of p97 and that is 
amenable to high-throughput screening (HTS) was developed 
based on the commercially available “Kinase-Glo” assay format, 
wherein the ATP hydrolytic activity of p97 is
monitored by using luciferase to measure the level of residual ATP in a sample following incubation with p97. Using this assay, circa 16,000 compounds were screened to evaluate their effect on the ATP hydrolysis activity of p97. This effort yielded several candidates that inhibit p97 activity. Among the compounds that were identified, one that was of particular interest was compound 8 (9-(2-nitrovinyl) anthracene shown below:

![Chemical Structure]

It was shown that a key feature of compound 8 is that its mode of inhibition is dependent upon the presence of a cysteine at position 522 in the D2 domain of p97. Compound 8 inhibited activity of wild type p97 (see FIG. 23) to a significantly greater degree than did the C522A-p97 mutant as determined by the ATP Kinase glo assay (see FIG. 24).

To evaluate whether compound 8 can target p97 activity in cells, it was determined whether this compound induces accumulation of high molecular weight ubiquitin conjugates, since it is known that RNAi-mediated knockdown of p97 in mammalian cells has this effect (Wojcik, C. et al. J. Cell Sci. (2004) 117, 281-292). The proteasome inhibitor bortezomib (velcade) was employed as a positive control for this experiment. HeLa (FIG. 25) or RPMI8226 (FIG. 26) cells were treated with VelaCe, compound 8, or a combination thereof for 1 h at 37°C. The cells were then harvested and cell lysates were immunoblotted with anti-ubiquitin antibodies to detect accumulation of high molecular weight conjugates. Blots were also probed with anti-PSTARE antibodies as a loading control. The data revealed that compound 8 caused accumulation of high molecular weight ubiquitin conjugates.

As a second test of whether compound 8 can inhibit p97 in vivo, it was evaluated whether it can cause accumulation of the UbG756-GFP reporter. A HeLa cell line that stably expresses UbG756-GFP was treated with compound 8 for 1 h at 37°C, and was then evaluated by fluorescence microscopy. As a control, the same cells were treated with the proteasome inhibitor MG132. Both MG132 and compound 8 caused detectable accumulation of UbG756-GFP (FIG. 27), although the reporter exhibited a more punctuate pattern of accumulation in cells treated with compound 8. The level of GFP fluorescence was quantified in cells treated with compound 8 at different doses and for different periods of time (FIG. 28).

To determine whether compound 8 has an effect on turnover of accumulated reporter, the same cycloheximide chase experiment that was described in Example 1 was performed. UbG756-GFP/HeLa cells were treated with MG132 (2 µM) for 1 h and washed with PBS three times. DMEM containing cycloheximide (50 µg/mL) and compound 8 (0-10 µM) was added into cells. Eight 96-well plates were prepared and one of the plates was imaged at 25, 50, 70, 100, 110, 125, 145, or 170 min after washing with PBS three times. Compound 8 inhibited turnover of accumulated UbG756-GFP with an IC50 value of 10±4 µM (see FIG. 29 and Table 8).

<table>
<thead>
<tr>
<th>Conc. Compd. 8 (µM)</th>
<th>K (1/min)</th>
<th>1t1/2 (min) = Ln(2)/k</th>
<th>% K</th>
</tr>
</thead>
<tbody>
<tr>
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<td>100</td>
</tr>
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<td>89</td>
</tr>
<tr>
<td>0.04</td>
<td>0.033</td>
<td>20.8</td>
<td>87</td>
</tr>
<tr>
<td>0.16</td>
<td>0.032</td>
<td>21.4</td>
<td>85</td>
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<td>0.63</td>
<td>0.031</td>
<td>22.5</td>
<td>81</td>
</tr>
<tr>
<td>2.5</td>
<td>0.027</td>
<td>25.4</td>
<td>71</td>
</tr>
<tr>
<td>10</td>
<td>0.023</td>
<td>30.1</td>
<td>60</td>
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IC50 = 10±4 µM

Like compound 2, compound 8 also induced accumulation of the ERAD reporter, TCR-alpha-GFP. HEK293 cells that stably expressed TCR-GFP were treated with the VelaCe (FIG. 30) or compound 8 (FIG. 31) at the indicated concentrations for 2 h at 37°C. Prior to being imaged by fluorescence microscopy to quantify TCR-GFP accumulation. These data show that compound 8 inhibits p97 in cells, resulting in the accumulation of high molecular weight ubiquitin conjugates and UPS substrates whose degradation depends upon p97.

To compare the ability of compound 2 and compound 8 to selectively kill transformed cells, the concentration of compound required to inhibit growth of cells by 50% (GI50) in cell culture assays was measured. Genetically matched parental MRC5 cells (WT) or cells engineered to express SV40 were treated with various compounds for 48 h at 37°C. GI50 values were determined by CellTiter-Glo assay (see FIG. 32 and).

<table>
<thead>
<tr>
<th>Compound</th>
<th>MRC5-WT (µM)</th>
<th>MRC5-SV40 (µM)</th>
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</thead>
<tbody>
<tr>
<td>2</td>
<td>17 ± 10</td>
<td>7.3 ± 5.5</td>
</tr>
<tr>
<td>8</td>
<td>17 ± 9</td>
<td>5 ± 3</td>
</tr>
<tr>
<td>VelaCe</td>
<td>0.2 ± 0.07</td>
<td>0.1 ± 0.01</td>
</tr>
<tr>
<td>2 + VelaCe</td>
<td>0.1 ± 0.01</td>
<td>0.01 ± 0.0006</td>
</tr>
</tbody>
</table>

The SV40-transformed cells were 2-fold and 3.4-fold more sensitive to the growth inhibitory effects of Compound 2 and Compound 8, respectively. VelaCe also showed 2-fold greater potency towards the transformed cell line, whereas a combination of Compound 2 and VelaCe blocked cell growth of the SV40-transformed cell line at a GI50 10-fold lower than that of the WT cell line. (FIG. 20)

**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.
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<213> ORGANISM: Unknown
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1  5  10

<210> SEQ ID NO 4
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1  5  10

<210> SEQ ID NO 7
We claim:

1. A method for treating cancer in a patient in need thereof, comprising inhibiting the activity of AAA p97 having the descriptive name Valosin-containing protein, by contacting a cell with a therapeutically effective amount of a compound that binds to and inhibits p97, wherein the compound has a structure of formula (I):

![Chemical Structure](image)

wherein

- $R^1$ is $-\text{C}(\text{O})\text{--CH}--\text{CHR}^6$ or $-\text{C}(\text{O})\text{--CH}_2--X$, $X$ is chloro, $R^2$ is H or $-\text{C}(\text{O})\text{--CH}--\text{CH}_2$, $R^3$ is hydrogen, $R^4$ is aryl, and $R^5$ is lower alkyl,
- or pharmaceutically acceptable salts thereof.

2. The method of claim 1, wherein the compound covalently binds to an active site of AAA p97 having the descriptive name Valosin-containing protein.

3. The method of claim 2, wherein the compound forms a covalent bond with a cysteine residue of the active site.

4. The method of claim 3, wherein the cysteine residue is Cys522.

5. The method according to claim 1 wherein $R^1$ has an atom susceptible to nucleophilic attack.

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* * * * *