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(54) Title: FAP-ACTIVATED PROTEASOME INHIBITORS FOR TREATING SOLID TUMORS

(57) Abstract: Disclosed are proteasome inhibitors, FAP-activated prodrugs of proteasome inhibitors, and pharmaceutically acceptable salts of the inhibitors and prodrugs. Also disclosed are related pharmaceutical compositions, and methods of using the inhibitors and prodrugs and compositions thereof, for example, in treating cancer or other cell proliferative diseases. In vitro and in vivo methods of quantifying the expression of FAP in a biopsy sample and a mammal, respectively, are also disclosed.

FAP-Activated Proteasome Inhibitors for Treating Solid Tumors

Related Applications

This application claims the benefit of priority to U.S. Provisional Patent Application serial number 61/528,824, filed August 30, 2011, the entirety of which is incorporated by reference.

Background of the Invention

One in four deaths in the USA is due to cancer, the second leading cause of death after heart disease. Lung cancer is the leading cause of mortality among cancers, and the majority of patients have locally advanced or metastatic non-small cell lung cancer (NSCLC) at the time of diagnosis. In women, breast cancer is the most prevalent cancer and is the second leading cause of cancer-related death.

The current standard of care for treatment of solid cancers has limited efficacy. For instance, in NSCLC survival remains poor despite improvements achieved by addition of targeted agents to first-line platinum-based chemotherapy. In metastatic breast cancer the efficacy of trastuzumab is limited by tumor resistance. When NSCLC progresses after first-line therapy, approved second-line agents only achieve modest survival rates.

More effective anticancer agents are clearly needed. Many approved cancer drugs, such as bortezomib (Velcade®), are cytotoxic agents that kill normal cells as well as tumor cells. The therapeutic benefit of these drugs depends on tumor cells being more sensitive than normal cells, thereby allowing clinical responses to be achieved at relatively safe drug doses; however, damage to normal tissues is unavoidable and often limits treatment. Following the success of bortezomib in treating multiple myeloma (MM), inhibition of the proteasome complex emerged as a promising new approach to chemotherapy. Due to its remarkable efficacy in treating multiple myeloma, bortezomib has been tested in solid cancers; unfortunately, it has generally failed to produce clinical responses.

Bortezomib inhibits an intracellular protein complex called the proteasome. The proteasome is an attractive drug target because it is involved in regulation of the cell cycle and apoptosis, processes that when dysregulated in cancer cells lead to tumor progression, drug resistance and altered immune surveillance. By inhibiting the 20S proteasome, which selectively degrades proteins involved in cellular homeostasis, bortezomib stabilizes pro-apoptotic members of the Bcl-2 family, inhibits two major pathways leading to NF-κB

activation, and causes intracellular accumulation of misfolded proteins; all of which effects contribute to killing tumor cells. Blockade of NF-κB activation increases apoptosis, reduces production of angiogenic cytokines, inhibits tumor cell adhesion to stroma, and alleviates immune suppression.

However, broader use of bortezomib to treat cancer appears to be prevented by systemic toxicity. Bortezomib distributes to healthy tissues, causing diarrhea, fatigue, fluid retention, hypokalemia, hyponatremia, hypotension, malaise, nausea, orthostasis, bortezomib-induced peripheral neuropathy (BIPN) and hematologic toxicities, of which thrombocytopenia is the most severe. At the recommended dose of bortezomib there is a therapeutic window for the treatment of MM that may be afforded by the unique sensitivity of MM cells to inhibition of nuclear factor-κB (NF-κB) and induction of the unfolded protein response. Solid cancers (e.g., prostate, pancreatic and breast cancer) appear to be less sensitive, however, and attempts to achieve efficacy by increasing bortezomib dosage have been prevented by dose-limiting toxicities (DLTs). The poor localization of bortezomib to tumors appears to contribute to its low therapeutic index (TI) in solid cancers. In mice bearing PC3 prostate tumors, healthy organ exposure to ^{14}C-bortezomib was as much as 9-fold greater than tumor exposure, and proteasome inhibition in healthy tissue appears to be greater than in solid tumors. Thus, it is necessary to design compounds that selectively target the proteasome in tumor cells to overcome the obstacle of DLTs due to proteasome inhibition in healthy tissues.

Extensive efforts over the past few decades have focused on therapies tailored to the specific patient—so-called personalized medicine. Due to advances in genetic sequencing technology it is now possible and increasingly cost-effective to genotype cancerous tissue to identify the individual genetic profile of the cancer and thus the specific mutated or dysfunctional proteins that may be responsible for tumor growth. Such "driver" proteins may be then targeted with agents that block their function and thus kill the cancer. While conceptually sound, this approach has been hampered by the unexpected genetic diversity and genomic instability of cancer. Significantly different genotypes of cancer may be present within a single tumor, making targeted therapy ineffective for many patients. Even when the majority of cancer cells in a tumor share a sufficiently similar genetic makeup that a single targeted therapy is effective, small numbers of cancer cells bearing a resistant mutation may survive the therapy, leading to relapse after an initial improvement.

Therapies selectively targeting the tumor and its microenvironment with cytotoxic agents whose effect does not depend on the genetic makeup of the cancer are needed. Such therapies remain elusive, however.

Summary of the Invention

One aspect of the present invention relates to a FAP-activated prodrug of a proteasome inhibitor represented by **A-B**, or a pharmaceutically acceptable salt thereof, wherein

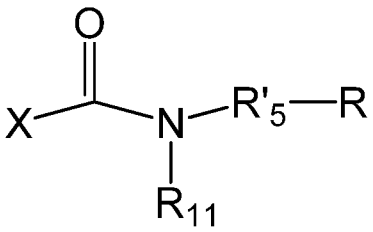
A represents a substrate for Fibroblast Activation Protein (FAP);

B represents a proteasome inhibitor moiety which, when released in a free form from the prodrug as a product of cleavage by FAP, inhibits the proteolytic activity of a proteasome with a Ki of 500 nM or less;

A and **B** being covalently linked by a bond that is enzymatically cleaved by FAP to release **B** in said free form; and

the prodrug has a k_{cat}/K_m for FAP cleavage of the bond linking **A** and **B** of at least 10 fold more than for Prolyl endopeptidase EC 3.4.14.5 (PREP).

Another aspect of the present invention relates to a FAP-activated proteasome inhibitor represented by formula I:



(I)

or a pharmaceutically acceptable salt thereof,

wherein

X-C(=O)NR_{11}-R'$_5$- represents the FAP substrate sequence, X is an N-acyl peptidyl group, -NR_{11}-R'$_5$ is an amino acid residue or analog thereof that binds the P'$_1$ specificity subsite of FAP, and the FAP substrate sequence is cleaved by FAP to release NHR_{11}-R'$_5$-R; and

NHR_{11}-R'$_5$-R is a proteasome inhibitor.

Another aspect of the present invention relates to a compound or a pharmaceutically acceptable salt thereof represented by the formula:

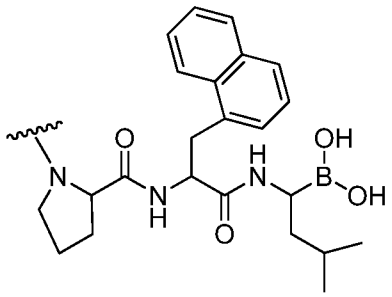
R-Xaa$_1$-Xaa$_2$-Y

wherein

R is an acyl group;

Xaa_1 is selected from the group consisting of Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, and Tyr;

Xaa_2 is selected from the group consisting of Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, and Tyr; and

Y is 

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Another aspect of the present invention relates to pharmaceutical compositions, and methods of using the compounds and compositions in, for example, treating cancer or other cell proliferative diseases.

Brief Description of the Figures

Figure 1 shows the synthesis of ARI-2727D and ARI-3996. The following reagents were used: (a) HATU/DMF/DIPEA,95%; (b) 4 M HCl in dioxane, 100%; (c) HATU/DMF/DIPEA, 90%; (d) 4M HCl in dioxane, 100%; (e) tBu-Suc-Ser(tBu)-OH, HATU/DMF/DIPEA, 90%; (f) $Pd(OH)_2$-C/H_2/methanol, 90%; (g) 2727D, HATU/DMF/DIPEA, 85%; (h) TFA/DCM, 90%; (i) $PhB(OH)_2$, pentane-water-acetonitrile, 70%.

Figure 2 shows the rate of *in vitro* cleavage of ARI-3996 by FAP and PREP as a function of the concentration of ARI-3996. Cleavage was monitored by assay of release of "warhead" ARI-2727 using LCMS.

Figure 3 shows *in vitro* cleavage of ARI-3144 ((N-Quinoline-4-carbonyl)-D-Ala-Pro-AMC) by FAP, but not by DPP IV, DPP 8, DPP 9 or PREP. Cleavage was monitored by measuring fluorescence of the AMC leaving group (excitation, 355 nm; emission, 460 nm).

Figure 4 shows the ratio of FAP proteolytic activity in humans and mice for pancreatic tumor tissue and plasma. FAP activity was assayed *ex vivo* in tumor homogenates and plasma using ARI-3144 fluorogenic substrate.

Figure 5 shows FAP proteolytic activity in xenografts of FAP-transfected HEK293 cells and HPAF-11 cells. FAP activity was assayed using the ARI-3144 assay.

Figure 6 shows a comparison of antitumor activities of ARI-3996 and bortezomib (Velcade®) at respective MTDs in SCID mice bearing established (~200 mm^3) HPAF-II carcinoma xenografts. ARI-3996 and bortezomib were administered twice weekly (day (D)1/ D4 schedule), and ARI-3996 was also given from day 1 to day 5 for 5 consecutive days (QDx5 schedule). Asterisks indicate significant ($p < 0.05$) reductions in tumor size in mice treated with ARI-3996, compared to controls.

Figure 7 shows the distribution of [^{14}C] bortezomib to tissues and tumor 1 hour after i.v. injection. The graph and data were taken from Adams *et al* (71). Data are mean dpm/100 mg tissue and mean dpm/100 μL blood.

Figure 8 shows tissue distribution of ARI-3996 and the warhead 2727D in SCID mice bearing HPAF-II s.c. tumors. Tumor-bearing mice were injected s.c. with 50 mg/kg ARI-3996. Tissues were harvested at 1 (A, B), 2 (C, D) and 3 hours ($n = 2$) after administration of ARI-3996, and drug concentrations in tissue extracts were determined by LCMS.

Figure 9 shows the antitumor effect of ARI-3996 administered by i.p. (IP) and s.c. (SQ) routes at 50 mg/kg twice daily (b.i.d) on days 1 and 4 to SCID mice bearing HPAF-II xenografts. One-way ANOVA with Dunnett's post test for test versus vehicle ($P < 0.0001$).

Figure 10 shows antitumor activity of 50 mg/kg ARI-3996 with or without 6 mg/kg gemcitabine. ARI-3996 b.i.d. s.c. and gemcitabine once per day i.p. were administered twice weekly. Mean ± SEM. The two compounds exhibit a strong synergistic effect when administered together.

Figure 11 shows a cartoon of diagnostic fluorogenic substrate ARI-3144. The FAP recognition site binds specifically to FAP and is cleaved by the enzyme to release the fluorogenic coumarin moiety.

Figure 12 shows a cartoon of ARI-3144 after binding to FAP. The FAP recognition site is cleaved to release the fluorescent coumarin moiety.

Figure 13 shows that ARI-3144 is an excellent substrate for FAP.

Figure 14 shows fluorescence measurements of the rate of ARI-3144 cleavage by PREP, DPPIV, DPP8, DPP9, and DPPII. ARI-3144 is highly selective for FAP.

Figure 15 shows a cartoon of the prodrug ARI-3996, which contains a FAP recognition site chemically bound to ARI-2727D, a proteasome inhibitor which remains inactive while bound to the FAP recognition site (top) and a cartoon of what takes place

after cleavage by FAP; the active "warhead" ARI-2727D is released from the FAP recognition site (bottom).

Figure 16 shows FAP activity in cancerous and normal mouse tissues. The much higher FAP activity in and around the tumor indicates that FAP is upregulated in that tissue.

Figure 17 shows FAP activity in human tumor cell lines and HPAF-II mouse tumor xenographs. FAP activity is generally higher in human tumor cell lines than mouse tumor xenographs. FAP activity is likely to be even higher than shown due to some deactivation of FAP during sample collection and handling.

Figure 18 shows a graph of FAP activity in several tissues. FAP transfected HEK tumor xenographs match human pancreatic tumor tissue for FAP content.

Figure 19 shows the mean tumor volume of mice treated with either a vehicle control or with ARI-3996. ARI-3996 induces tumor regression in immunocompetent mice.

Figure 20 shows the anticancer activity of ARI-3996 in FAP-transfected HEK tumor xenograft.

Figure 21 shows that FAP knockout mouse blood plasma does not activate ARI-3996 to release ARI-2727.

Figure 22 shows tissue distribution of Velcade® versus ARI-3996 in mice.

Figure 23 shows tissue distribution of Velcade® versus ARI-2727D in mice. ARI-3996 is cleaved to ARI-2727D in and around tumors, thus facilitating the buildup of ARI-2727D in tumors.

Figure 24 shows the tissue distribution of ARI-2727D 1 hour after direct administration vs. administration as the prodrug form, ARI-3996 (top); and the average ratio at which ARI-2727D accumulates in the tumor versus the liver.

Figure 25 shows the cytotoxicity of Velcade® versus ARI-2727D in multiple myeloma, normal cells, and solid tumors.

Figure 26 shows the cytotoxicity of Velcade® versus ARI-2727D versus ARI-3996 in multiple myeloma, normal cells, and solid tumors.

Figure 27 shows the FAP activity in various human cancer cell lines.

Figure 28 shows the anticancer effects of proteasome inhibitors Velcade® and ARI-3996 in U266 tumor-bearing mice.

Figure 29 shows the chemical structures and names of a number of known proteasome inhibitors.

Detailed Description of the Invention

The present invention relates to compounds designed selectively to target solid tumors with a reduced toxicity profile. Bortezomib (Velcade®) is an effective treatment for multiple myeloma, but its mechanism of action results in dose-limiting toxicities (DLTs) of peripheral neuropathy and loss of platelets, which prevent treatment of common solid cancers. The compounds of the present invention are designed to remain inactive in healthy organs and to be activated by the tumor-associated enzyme called fibroblast activation protein (FAP) to unleash a cytotoxic bortezomib-like warhead in tumors, thereby reducing the toxic side effects that prevent safe treatment of solid tumors with bortezomib.

The selective targeting and reduced toxicity of the compounds of the invention allows the treatment of solid cancers independent of their genetic makeup. Furthermore, the selective activation of the compounds in the vicinity of the tumors results in a high concentration of the cytotoxic agent in the tumor but a low concentration in the rest of the body. The high local concentration kills tumors with a lower dose of the drug than previously possible, because a drug lacking the capability to be selectively delivered circulates throughout the body, causing systemic toxicity, often at a dose that is suboptimal for treatment of the cancer.

The present invention also allows the offsetting of the immunosuppressive properties of tumors. Because solid tumors are often surrounded by cancerous stromal cells, they are protected from the immune system of the patient. This immunosuppression can be removed by killing the stromal cells, but conventional chemotherapies including Velcade® fail to do so. The present invention is capable of killing stromal cells because they overexpress FAP and thus activate the compounds of the invention to release the warhead. Thus the present invention can have multiple mechanisms of action, such as direct killing of tumors or re-activation of the patient immune response after killing of the supportive stromal tissue, resulting in killing of the tumor through a natural immune response.

The FAP address moiety, or FAP binding portion, of the invention may be chemically attached to a variety of cytotoxic warheads. Thus, any proteasome inhibitor with a validated target and mode of action would benefit from use with the claimed invention. Conjugation (chemical attachment) of a validated proteasome inhibitor possessing anticancer activity, to the FAP address moiety confers selective delivery, increased potency, and decreased off-target toxicity.

Conjugation of the FAP address moiety to a known protease inhibitor is similar to, but conceptually different from, a prodrug, because the FAP address moiety is designed to bind and be cleaved by FAP selectively over other proteases present in the body, especially DPPII, DPP8, DPP9, DPPIV, and PREP. This specificity for enzyme subtype is essential for the desired effect of delivering the released cytotoxic agent to the tumor.

Many proteasome inhibitors with anticancer activity are known in the art, and may be divided according to covalent and non-covalent inhibitors, with the covalent inhibitors further divided into aldehydes, boronates, epoxyketones, beta-lactones, vinyl sulfones, and α,β-unsaturated carbonyls, among others. Examples in the aldehyde class include MG-132, PSI, and fellutamide B. Examples in the boronate class include bortezomib (Velcade®), CEP-18770, MLN2238, and MLN9708. Examples in the epoxyketone class include epoxomicin, carfilzomib (PR-171), NC-005, YU-101, LU-005, YU-102, NC-001, LU-001, NC-022, PR-957 (LMP7), CPSI (β5), LMP2-sp-ek, BODIPY-NC-001, azido-NC-002, and ONX 0912 (opromozib). Examples in the beta-lactone class include omuralide, PS-519, marizomib, and belactosin A. Examples in the vinyl sulfone class include ^{125}I-NIP-L_3VS, NC-005-VS, and MV151. Discussion and validation of these inhibitors and others may be found, for example, in Kisselev *et al.* "Proteasome Inhibitors: An Expanding Army Attacking a Unique Target," Chemistry and Biology 19, January 27, 2012, 99-115 (incorporated by reference).

Chemical conjugation of any of these proteasome inhibitors with a FAP address moiety as described in the present invention would be expected to deliver selectively the cytotoxic agent to solid tumors and the surrounding stromal cells. Since the FAP address moiety is a selective substrate for FAP, the identity of the cytotoxic agent attached to the FAP address moiety is not important to the selective delivery. FAP will cleave the chemical bond attaching the address moiety to the warhead; such a chemical bond may be, for example, an ester or amide bond, among others.

One aspect of the present invention relates to a FAP-activated prodrug of a proteasome inhibitor represented by **A-B**, or a pharmaceutically acceptable salt thereof, wherein

A represents a substrate for Fibroblast Activation Protein (FAP);

B represents a proteasome inhibitor moiety which, when released in a free form from the prodrug as a product of cleavage by FAP, inhibits the proteolytic activity of a proteasome with a Ki of 500 nM or less;

A and **B** being covalently linked by a bond that is enzymatically cleaved by FAP to release **B** in said free form; and

the prodrug has a k_{cat}/K_m for FAP cleavage of the bond linking **A** and **B** of at least 10 fold more than for Prolyl endopeptidase EC 3.4.14.5 (PREP).

In certain embodiments, the free form of said proteasome inhibitor moiety has an IC_{50} for inhibiting proteasome activity of cells *in vitro* that is at least 10 fold less relative to said prodrug.

In certain embodiments, the free form of said proteasome inhibitor moiety has a K_i for inhibiting proteasome activity that is at least 10 fold less relative to said prodrug.

In certain embodiments, the free form of said proteasome inhibitor moiety has at least 5 fold greater cell permeability into human cells than said prodrug.

In certain embodiments, the prodrug has a therapeutic index *in vivo* at least 5 fold greater than said free form of said proteasome inhibitor moiety.

In certain embodiments, the prodrug has a therapeutic index *in vivo* of at least 10.

In certain embodiments, the prodrug has a maximum tolerated dose at least 10 times greater than [(1R)-3-methyl-1-({(2S)-3-phenyl-2-[(pyrazin-2-ylcarbonyl)amino]propanoyl}amino)butyl] boronic acid.

In certain embodiments, said free form of said proteasome inhibitor moiety is a dipeptidyl moiety, which when released from the prodrug as an open chain product of cleavage by FAP, undergoes cyclization-dependent inactivation over time.

In certain embodiments, said open chain product undergoes cyclization-dependent inactivation with a $T_{1/2}$ of 5 hours or less.

In certain embodiments, **A** represents a peptide or peptide analogue which is a substrate for FAP, which peptide or peptide analogue includes an N-terminal blocking group.

In certain embodiments, the peptide or peptide analogue is 2-10 amino acid residues in length.

In certain embodiments, the peptide or peptide analogue is C-terminally linked to **B**.

In certain embodiments, at least one amino acid residue of the peptide or peptide analog is a non-naturally occurring amino acid analog.

In certain embodiments, the N-terminal blocking group is a moiety which, at physiological pH, reduces the cell permeability of said prodrug relative to said free form of said proteasome inhibitor.

In certain embodiments, the N-terminal blocking group includes one or more functional groups that are ionized at physiological pH.

In other embodiments, the N-terminal blocking group is a (lower alkyl)-C(=O)- substituted with one or more functional groups that are ionized at physiological pH.

In certain other embodiments, the N-terminal blocking group is represented by the formula $-C(=O)-(CH_2)_{1-10}-C(=O)-OH$.

In certain embodiments, the N-terminal blocking group includes one or more carboxyl groups. In another embodiment, the N-terminal blocking group is succinyl.

In certain embodiments, **B** is a covalent or non-covalent proteasome inhibitor.

In certain other embodiments, **B** is a covalent proteasome inhibitor.

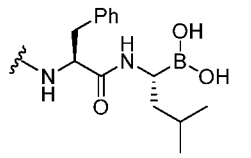
In certain embodiments, **B** is a dipeptidyl moiety having at its carboxy terminus an electrophilic functional group that can form a covalent adduct with an amino acid residue in the active site of a proteasome.

In certain embodiments, the electrophilic functional group is an aldehyde, boronic acid, boronate ester, epoxyketone, beta-lactone, vinyl sulfone, or α,β-unsaturated carbonyl.

In certain embodiments, the electrophilic functional group is an aldehyde, boronic acid, or epoxyketone.

In another embodiment, the electrophilic functional group is an epoxyketone.

In certain embodiments, **B** is selected from the group consisting of: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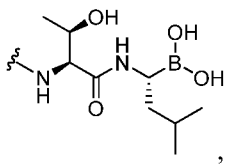
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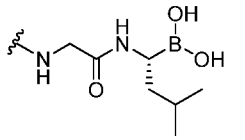
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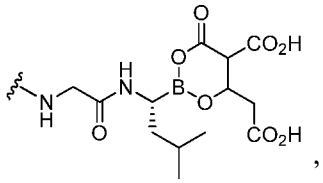
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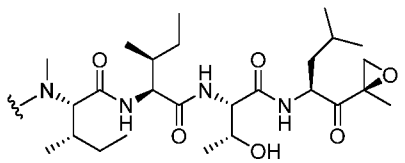
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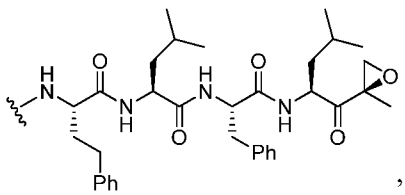
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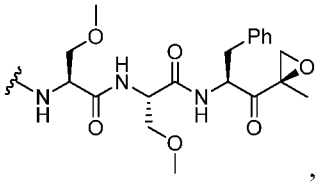
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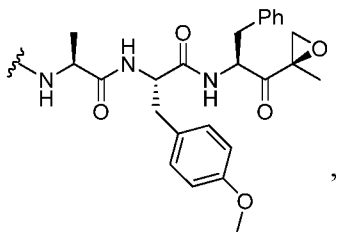
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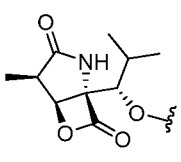
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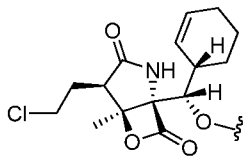
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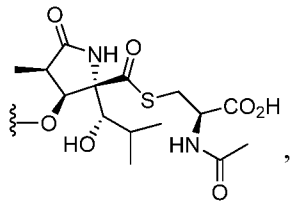
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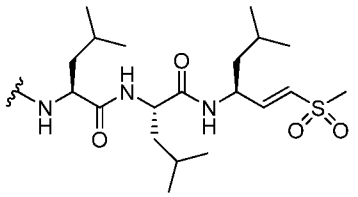
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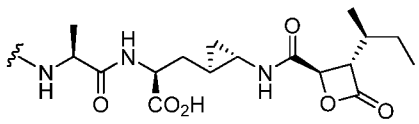
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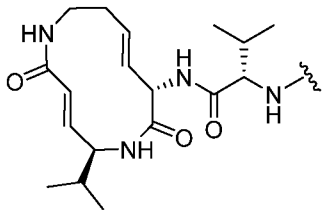
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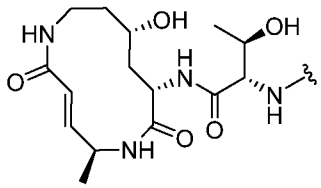
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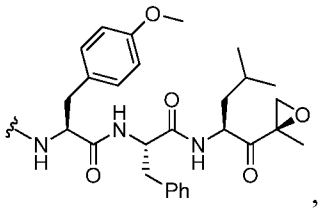
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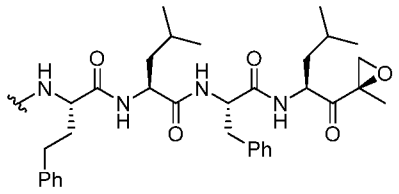
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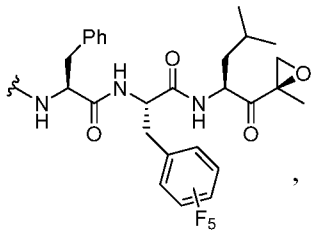
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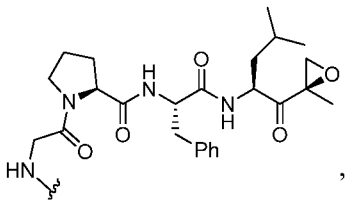
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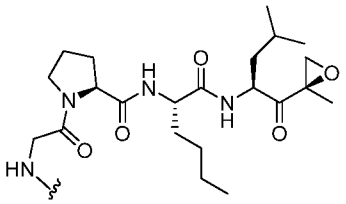
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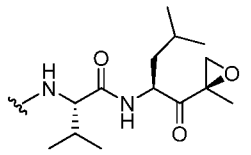
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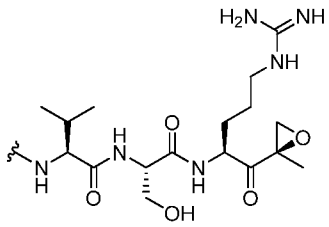
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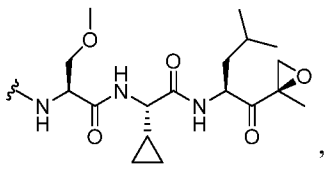
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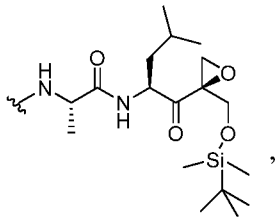
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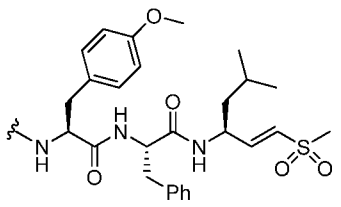
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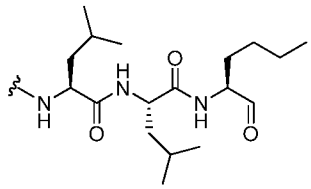
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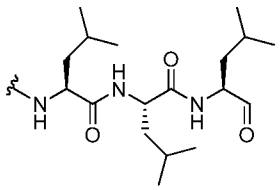
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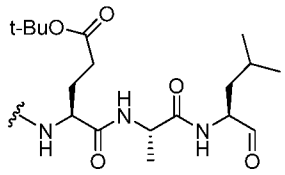
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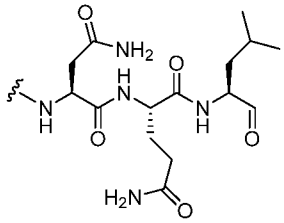
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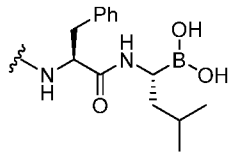
In certain embodiments, **B** is selected from the group consisting of: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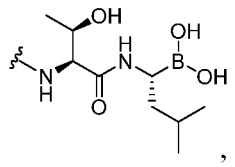
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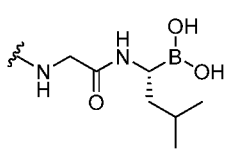
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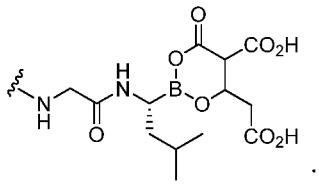
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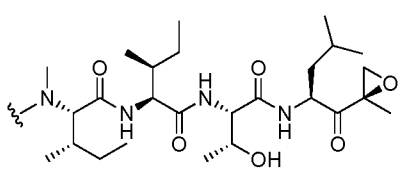
In certain other embodiments, **B** is selected from the group consisting of: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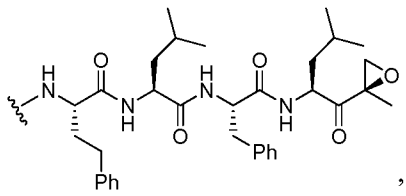
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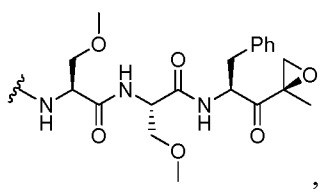
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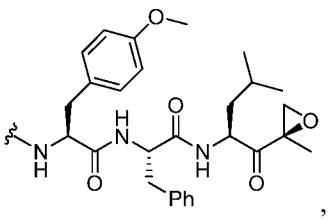
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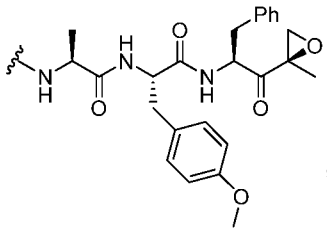
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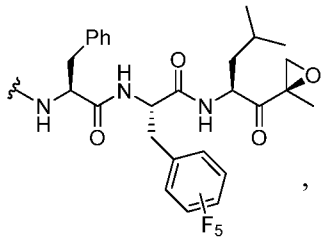
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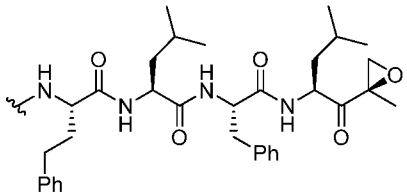
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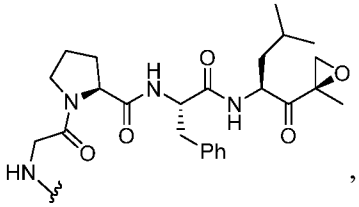
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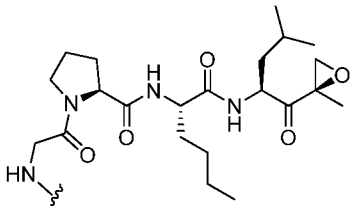
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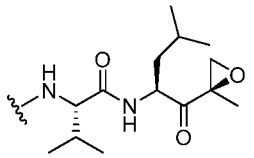
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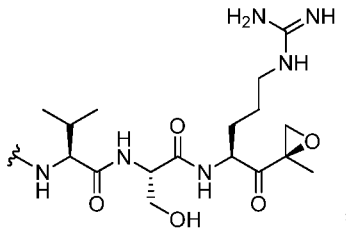
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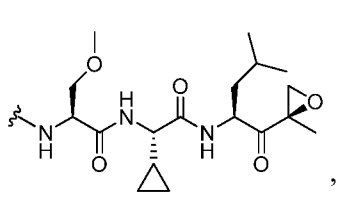
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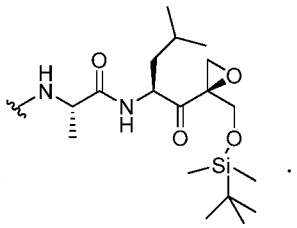

.

Another aspect of the present invention relates to the compounds described above, wherein **A** further comprises a self-eliminating linker attached to **B** by a chemical bond.

In certain embodiments, the self-eliminating linker is *p*-aminobenzyloxocarbonyl (PABC) or 2,4-bis(hydroxymethyl)aniline.

Another aspect of the present invention relates to a compound or a pharmaceutically acceptable salt thereof represented by the formula:

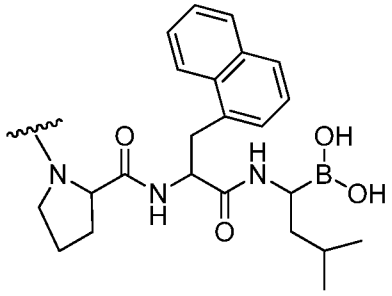
R-Xaa$_1$-Xaa$_2$-Y

wherein

R is an acyl group;

Xaa_1 is selected from the group consisting of Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, and Tyr;

Xaa_2 is selected from the group consisting of Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, and Tyr; and

Y is  .

In certain embodiments, the compound further comprises a self-eliminating linker with a chemical bond to the carboxyl terminus of Xaa_2 and a chemical bond to **Y**.

In certain embodiments, the self-eliminating linker is *p*-aminobenzyloxocarbonyl (PABC) or 2,4-bis(hydroxymethyl)aniline.

In certain embodiments, R is selected from the group consisting of formyl, acetyl, benzoyl, trifluoroacetyl, succinyl, and methoxysuccinyl.

In certain other embodiments, R is succinyl or methoxysuccinyl.

In another embodiment, R is succinyl.

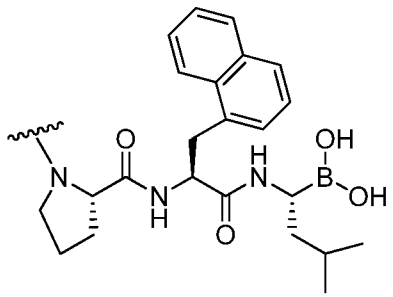
In certain embodiments, Xaa_1 is Cys, Met, Ser, or Thr.

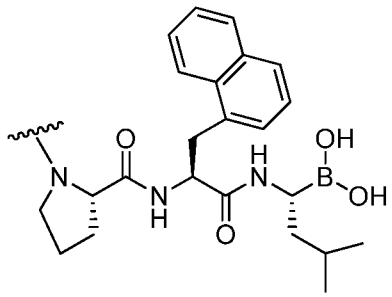
In certain other embodiments, Xaa_1 is Ser.

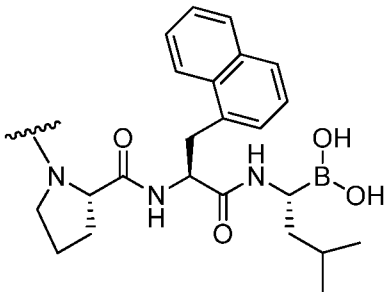
In certain embodiments, Xaa_2 is Ala, Gly, Ile, Leu, or Val.

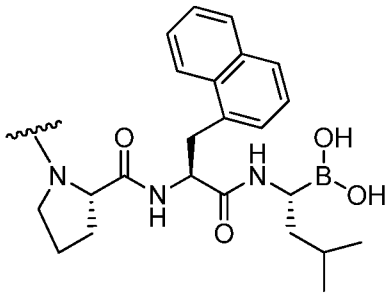
In certain embodiments, Xaa_2 is Ala.

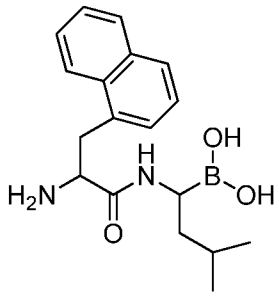
In certain other embodiments, Xaa_2 is (D)-Ala.

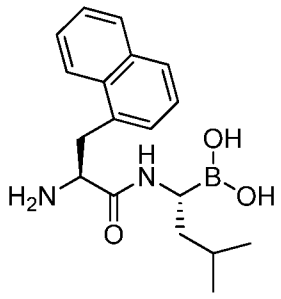
Y is  .

In certain embodiments, R is selected from the group consisting of formyl, acetyl, benzoyl, trifluoroacetyl, succinyl, and methoxysuccinyl; Xaa_1 is Cys, Met, Ser, or Thr; Xaa_2 is Ala, Gly, Ile, Leu, or Val; and Y is  .

In certain embodiments, R is succinyl or methoxysuccinyl; Xaa_1 is Ser; Xaa_2 is Ala; and Y is  .

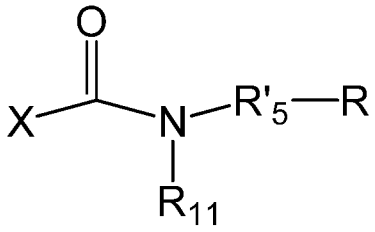
In certain embodiments, R is succinyl; Xaa_1 is Ser; Xaa_2 is (D)-Ala; and Y is  .

In certain embodiments, the present invention relates to a compound or a pharmaceutically acceptable salt thereof represented by  .



In certain embodiments, the compound is represented by .

Another aspect of the present invention relates to a FAP-activated proteasome inhibitor represented by formula I:



(I)

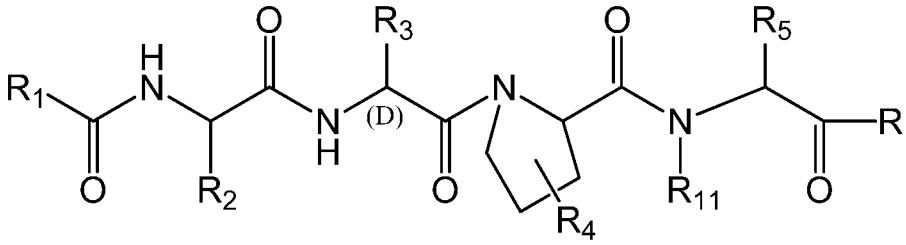
or a pharmaceutically acceptable salt thereof,

wherein

X-C(=O)NR_{11}-R'$_5$- represents the FAP substrate sequence, X is an N-acyl peptidyl group, -NR_{11}-R'$_5$ is an amino acid residue or analog thereof that binds the P'$_1$ specificity subsite of FAP, and the FAP substrate sequence is cleaved by FAP to release NHR_{11}-R'$_5$-R; R_{11} represents H or lower alkyl; and

NHR_{11}-R'$_5$-R is a proteasome inhibitor.

In certain embodiments, the present invention relates to the FAP-activated proteasome inhibitor described above, represented by formula II:



(II)

wherein

R_1-(C=O)- represents an acyl N-terminal blocking group;

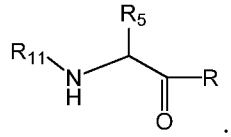
R_2 represents H, lower alkyl, or a mono- or di-hydroxy-substituted lower alkyl;

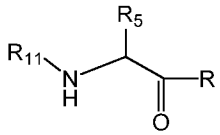
R_3 represents H, halogen, or lower alkyl;

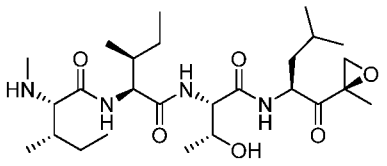
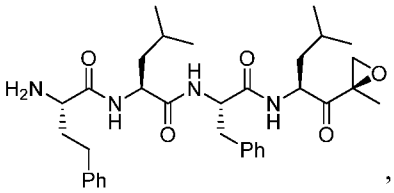
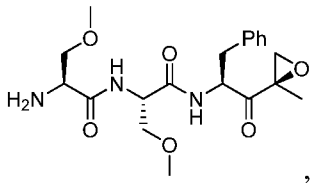
R_4 is absent or represents lower alkyl, -OH, $-NH_2$ or halogen;

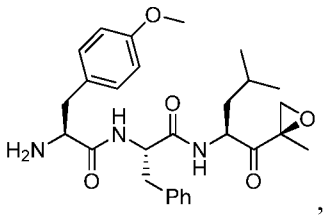
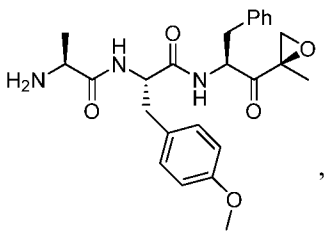
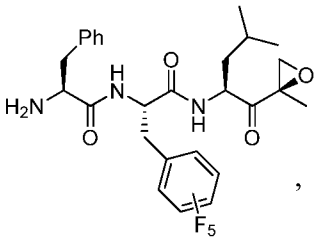
R_5 represents a large hydrophobic amino acid sidechain;

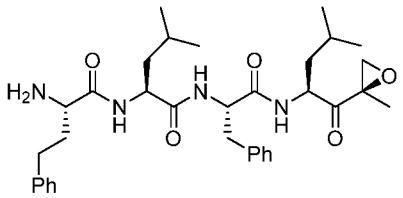
R_{11} represents H or lower alkyl; and

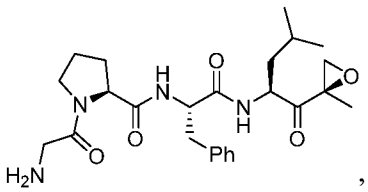
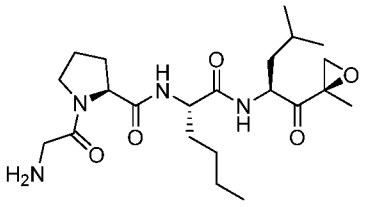
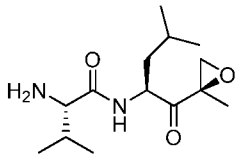
the FAP-activated proteasome inhibitor is cleaved by FAP to release a proteasome inhibitor represented by  .

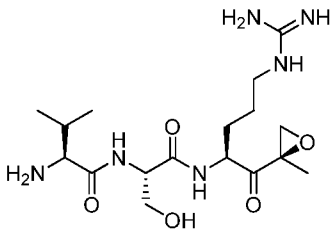
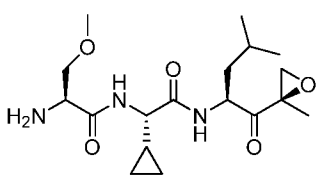
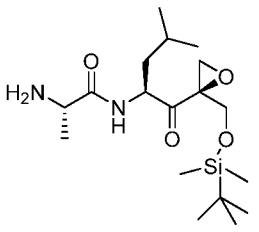
In certain embodiments of the FAP-activated proteasome inhibitor,  is selected from the group consisting of:

 ,  ,  ,

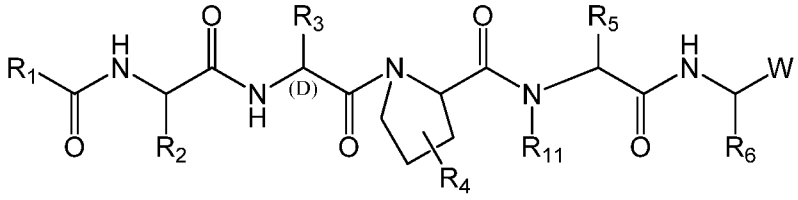
 ,  ,  ,

 ,

 ,  ,  ,

 ,  , and  .

In certain embodiments, the FAP-activated proteasome inhibitor is represented by formula III:



(III)

wherein

R_1-(C=O)- represents an acyl N-terminal blocking group;

R_2 represents H, lower alkyl, or a mono- or di-hydroxy-substituted lower alkyl;

R_3 represents H, halogen, or lower alkyl;

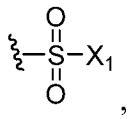
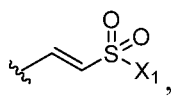
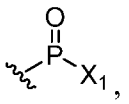
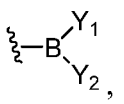
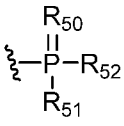
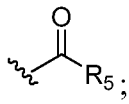
R_4 is absent or represents lower alkyl, -OH, $-NH_2$ or halogen;

R_5 represents a large hydrophobic amino acid sidechain;

R_6 represents alkyl, cycloalkyl, aryl, heterocycle or $-(CH_2)_n-R_7$;

R_7 represents aryl, aralkyl, cycloalkyl, alkoxy, alkylthio, -OH or -SH;

R_{11} represents H or lower alkyl;

W represents -CN, an epoxyketone, -CH=NR_5,  ,  ,  ,  ,  , or  ;

R_8 represents H, alkyl, alkenyl, alkynyl, $-C(X_1)(X_2)X_3$, $-(CH_2)_m-R_9$, $-(CH_2)_n-OH$, $-(CH_2)_n-O-alkyl$, $-(CH_2)_n-O-alkenyl$, $-(CH_2)_n-O-alkynyl$, $-(CH_2)_n-O-(CH_2)_m-R_9$, $-(CH_2)_n-SH$, $-(CH_2)_n-S-alkyl$, $-(CH_2)_n-S-alkenyl$, $-(CH_2)_n-S-alkynyl$, $-(CH_2)_n-S-(CH_2)_m-R_9$, $-C(=O)C(=O)NH_2$, $-C(=O)C(=O)OR_{10}$;

R_9 represents, independently for each occurrence, a substituted or unsubstituted aryl, aralkyl, cycloalkyl, cycloalkenyl, or heterocycle;

R_{10} represents, independently for each occurrence, hydrogen, or a substituted or unsubstituted alkyl, alkenyl, aryl, aralkyl, cycloalkyl, cycloalkenyl, or heterocycle;

Y_1 and Y_2 can independently or together be OH, or a group capable of being hydrolyzed to a hydroxyl group, including cyclic derivatives where Y_1 and Y_2 are connected via a ring having from 5 to 8 atoms in the ring structure;

R_{50} represents O or S;

R_{51} represents N_3, SH_2, NH_2, NO_2 or $-OR_{10}$;

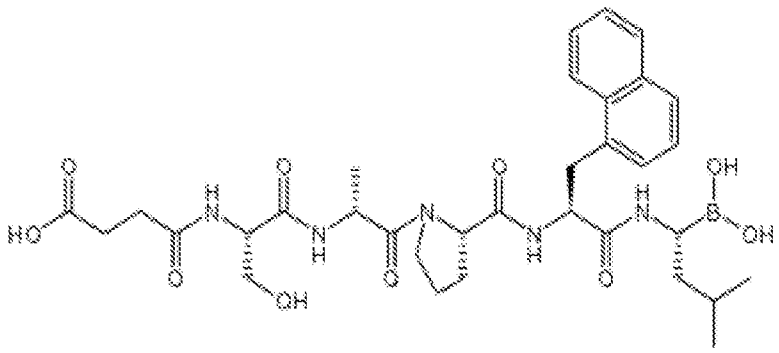
R_{52} represents hydrogen, lower alkyl, amine, $-OR_{10}$, or a pharmaceutically acceptable salt, or R_{51} and R_{52} taken together with the phosphorous atom to which they are attached complete a heterocyclic ring having from 5 to 8 atoms in the ring structure;

X_1 is halogen;

X_2 and X_3 each represent H or halogen;

m is zero or an integer in the range of 1 to 8; and n is an integer in the range of 1 to 8.

In certain embodiments, the present invention relates to the FAP-activated proteasome inhibitor described above, represented by



Another aspect of the present invention relates to a pharmaceutical composition, comprising a compound described herein; and a pharmaceutically acceptable excipient.

Another aspect of the present invention relates to a method of inhibiting proteasome function in a cell, comprising contacting the cell with an effective amount of a compound described herein.

Another aspect of the present invention relates to a method of reducing the rate of muscle protein degradation in a cell, comprising contacting the cell with an effective amount of a compound described herein.

Another aspect of the present invention relates to a method of reducing the activity of NF-κB in a cell, comprising contacting the cell with an effective amount of a compound described herein.

Another aspect of the present invention relates to a method of reducing the rate of proteasome-dependent intracellular protein breakdown, comprising contacting a cell with an effective amount of a compound described herein.

Another aspect of the present invention relates to a method of reducing the rate of degradation of p53 protein in a cell, comprising contacting the cell with an effective amount of a compound described herein.

Another aspect of the present invention relates to a method of inhibiting cyclin degradation in a cell, comprising contacting the cell with an effective amount of a compound described herein.

Another aspect of the present invention relates to a method of inhibiting antigen presentation in a cell, comprising contacting the cell with an effective amount of a compound described herein.

Another aspect of the present invention relates to a method of treating cancer, psoriasis, restenosis, or other cell proliferative disease, comprising administering to a mammal in need thereof a therapeutically effective amount of a compound described herein.

Another aspect of the present invention relates to a method of treating cancer, psoriasis, restenosis, or other cell proliferative disease, comprising co-administering to a mammal in need thereof a therapeutically effective amount of a compound described herein; and a therapeutically effective amount of a chemotherapeutic agent.

In certain embodiments, the chemotherapeutic agent is docetaxel, paclitaxel, imatinib mesylate, gemcitabine, cis-platin, carboplatin, 5-fluorouracil, pemetrexed, methotrexate, doxorubicin, lenalidomide, dexamethasone, or monomethyl auristatin.

In certain other embodiments, the chemotherapeutic agent is docetaxel, gemcitabine, carboplatin, or doxorubicin.

In yet other embodiments, the chemotherapeutic agent is MG-132, PSI, fellutamide B, bortezomib, CEP-18770, MLN-2238, MLN-9708, epoxomicin, carfilzomib (PR-171), NC-005, YU-101, LU-005, YU-102, NC-001, LU-001, NC-022, PR-957 (LMP7), CPSI (β5), LMP2-sp-ek, BODIPY-NC-001, azido-NC-002, ONX-0912, omuralide, PS-519, marizomib, belactosin A, ^{125}I-NIP-L_3VS, NC-005-VS, or MV151.

Another aspect of the present invention relates to a method of treating cancer, comprising administering to a mammal in need thereof a therapeutically effective amount of a compound described herein.

In certain embodiments, the cancer is a solid tumor.

In certain other embodiments, the method further comprises administering to a mammal in need thereof a therapeutically effective amount of a chemotherapeutic agent.

In yet other embodiments, the cancer is a solid tumor.

In still yet other embodiments, the chemotherapeutic agent is docetaxel, paclitaxel, imatinib mesylate, gemcitabine, cis-platin, carboplatin, 5-fluorouracil, pemetrexed, methotrexate, doxorubicin, lenalidomide, dexamethasone, or monomethyl auristatin.

In another embodiment, the chemotherapeutic agent is docetaxel, gemcitabine, carboplatin, or doxorubicin.

In certain embodiments, the chemotherapeutic agent is MG-132, PSI, fellutamide B, bortezomib, CEP-18770, MLN-2238, MLN-9708, epoxomicin, carfilzomib (PR-171), NC-005, YU-101, LU-005, YU-102, NC-001, LU-001, NC-022, PR-957 (LMP7), CPSI (β5), LMP2-sp-ek, BODIPY-NC-001, azido-NC-002, ONX-0912, omuralide, PS-519, marizomib, belactosin A, ^{125}I-NIP-L_3VS, NC-005-VS, or MV151.

Another aspect of the present invention relates to method of reducing the rate of loss of muscle mass in a mammal, comprising administering to a mammal in need thereof a therapeutically effective amount of a compound described herein.

Another aspect of the present invention relates to a method of reducing the activity of NF-κB in a mammal, comprising administering to a mammal in need thereof a therapeutically effective amount of a compound described herein.

Another aspect of the present invention relates to a method of reducing the rate of proteasome-dependent intracellular protein breakdown in a mammal, comprising administering to a mammal in need thereof a therapeutically effective amount of a compound described herein.

Another aspect of the present invention relates to a method of reducing the rate of degradation of p53 protein in a mammal, comprising administering to a mammal in need thereof a therapeutically effective amount of a compound described herein.

Another aspect of the present invention relates to a method of inhibiting cyclin degradation in a mammal, comprising administering to a mammal in need thereof a therapeutically effective amount of a compound described herein.

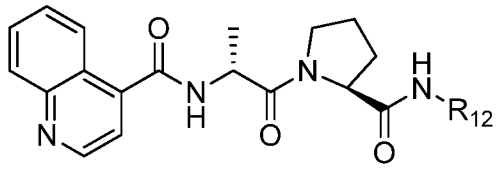
Another aspect of the present invention relates to a method of inhibiting antigen presentation in a mammal, comprising administering to a mammal in need thereof a therapeutically effective amount of a compound described herein.

Another aspect of the present invention relates to a method of inhibiting inducible NF-κB dependent cell adhesion in a mammal, comprising administering to a mammal in need thereof a therapeutically effective amount of a compound described herein.

Another aspect of the present invention relates to a method of inhibiting HIV infection in a mammal, comprising administering to a mammal in need thereof a therapeutically effective amount of a compound described herein.

Another aspect of the present invention relates to a method of quantifying the amount of FAP expressed by or in the vicinity of a tumor in a mammal, comprising the steps of:

administering to said mammal an effective amount of a compound represented by Formula IV:



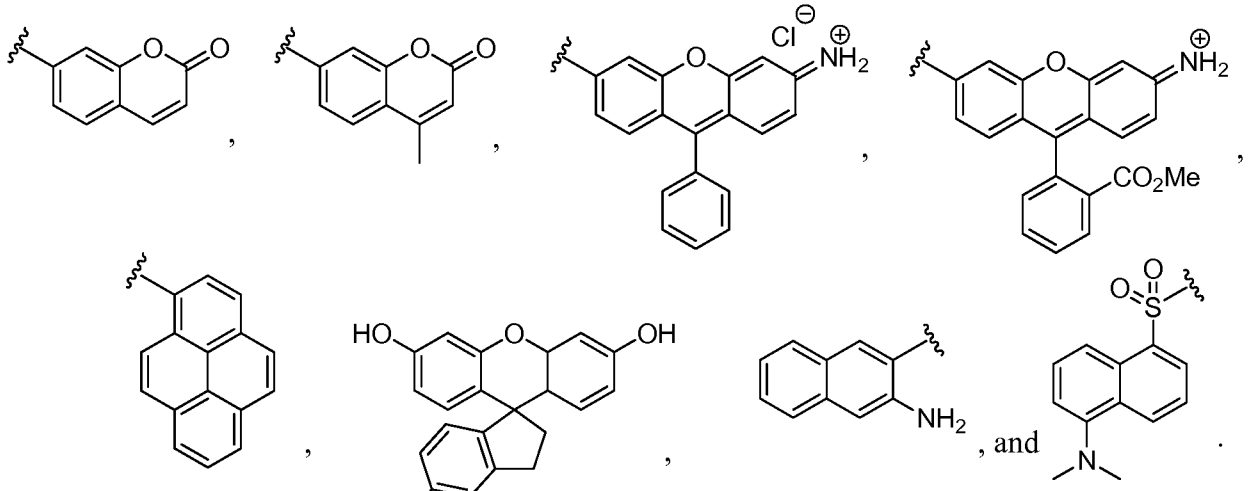
(IV)

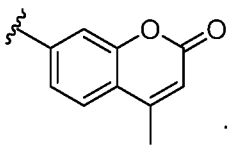
wherein R_{12} is a fluorophore or chromophore;

illuminating the mammal in the vicinity of the tumor; and

measuring the amount of fluorescence in the vicinity of the tumor.

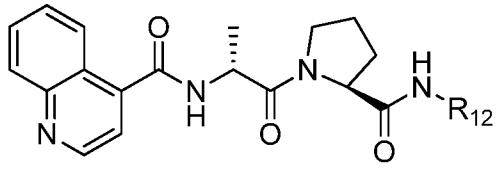
In certain embodiments, R_{12} is selected from the group consisting of:



In certain other embodiments, R_{12} is  .

Another aspect of the present invention relates to a method of quantifying the amount of FAP expressed by a tumor biopsy sample, comprising the steps of:

combining said tumor biopsy sample with an effective amount of a compound represented by Formula IV, thereby forming a mixture:



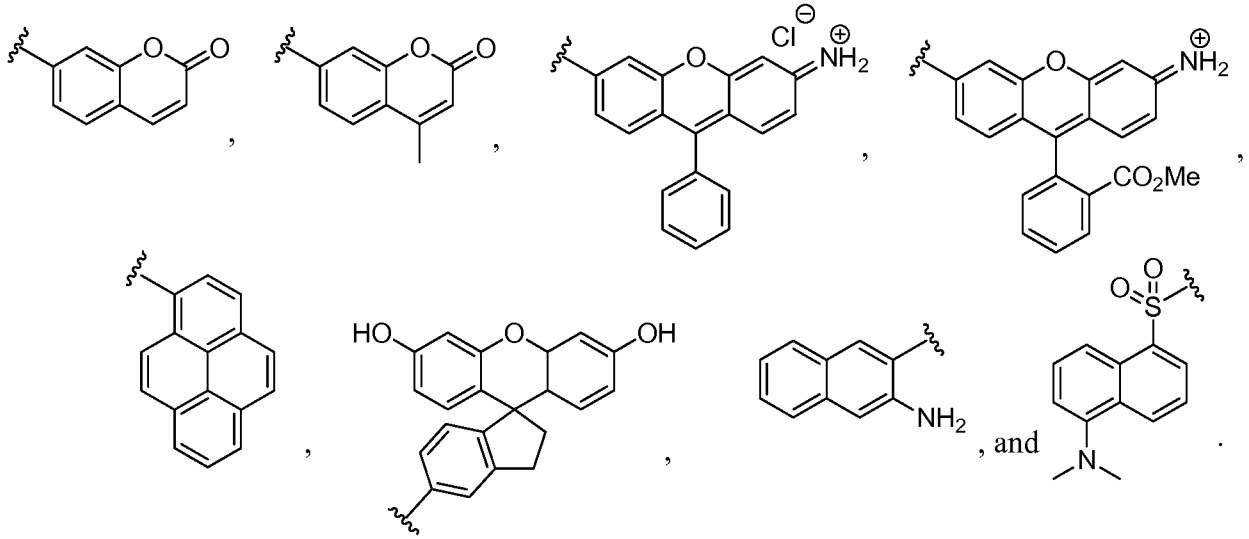
(IV)

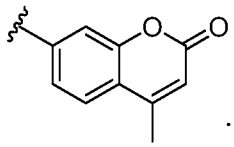
wherein R_{12} is a fluorophore or chromophore;

illuminating the mixture; and

measuring the amount of fluorescence in the mixture.

In certain embodiments, R_{12} is selected from the group consisting of:



In certain other embodiments, R_{12} is  .

Another aspect of the present invention relates to a method described herein, wherein said mammal is a primate, equine, canine, feline, or bovine.

Another aspect of the present invention relates to a method described herein, wherein said mammal is a human.

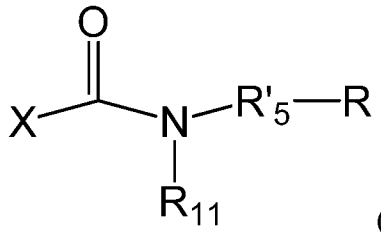
Another aspect of the present invention relates to a method described herein, wherein the compound is administered to the mammal by inhalation, orally, intravenously, sublingually, ocularly, transdermally, rectally, vaginally, topically, intramuscularly, intra-arterially, intrathecally, subcutaneously, buccally, or intranasally.

Another aspect of the present invention relates to a method described herein, wherein the compound is administered to the mammal intravenously.

Another aspect of the present invention relates to a method for reducing local immunosuppression and/or tumor supporting-activity mediated by FAP+ stromal cells, comprising administering to a patient in need thereof a therapeutically effective amount of a prodrug of an active agent, wherein the active agent is cytotoxic or inhibits protein expression or secretion to said FAP+ stromal cells, and is at least 2 fold more cytotoxic to the FAP+ stromal cells than the prodrug; and the prodrug (i) includes an FAP substrate sequence; (ii) is converted to the active agent by cleavage of the FAP substrate sequence by FAP, which substrate sequence has a k_{cat}/K_m for cleavage by FAP at least 10 fold more than for cleavage by prolyl endopeptidase EC 3.4.14.5 (PREP); and (iii) is selectively converted *in vivo* to the active agent by FAP+ stromal cells.

Another aspect of the present invention relates to a method described herein, wherein the FAP substrate sequence has a k_{cat}/K_m for cleavage by FAP at least 10 fold more than for cleavage by other S9 prolyl endopeptidases.

Another aspect of the present invention relates to a method described herein, wherein the prodrug is represented by formula V:



(V)

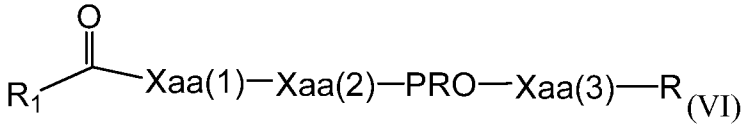
or a pharmaceutically acceptable salt thereof,

wherein

X-C(=O)NR_{11}-R'$_5$- represents the FAP substrate sequence, X is an N-acyl peptidyl group, -NR_{11}-R'$_5$ is an amino acid residue or analog thereof that binds the P'$_1$ specificity subsite of FAP, and the FAP substrate sequence is cleaved by FAP to release NHR_{11}-R'$_5$-R; R_{11} represents H or lower alkyl; and

R'$_5$ and R taken together form the cytotoxic agent, or a moiety further metabolized at the site of the FAP+ stomal cells to form the cytotoxic agent.

Another aspect of the present invention relates to a method described herein, wherein the prodrug is represented by formula VI:



(VI)

or a pharmaceutically acceptable salt thereof,

wherein

R_1-C(=O)- represents an acyl N-terminal blocking group;

Xaa(1) is an amino acid residue;

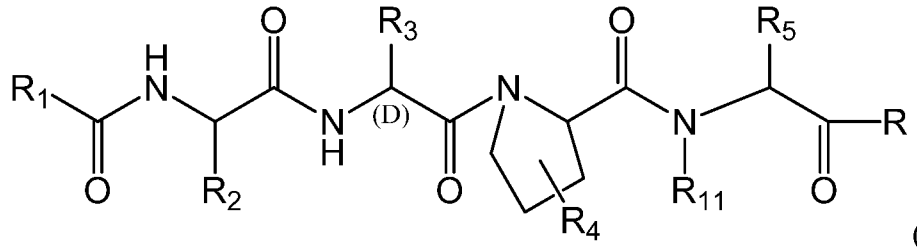
Xaa(2) is glycine, or a (D)-amino acid residue;

PRO represents a proline residue or an analog thereof;

Xaa(3) is a large hydrophobic amino acid residue; and

the prodrug is cleaved by FAP to release Xaa(3)-R, and Xaa(3)-R is the cytotoxic agent.

Another aspect of the present invention relates to a method described herein, wherein the prodrug is represented by formula VII:



(VII)

or a pharmaceutically acceptable salt thereof,

wherein

R_1-(C=O)- represents an acyl N-terminal blocking group;

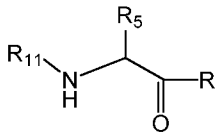
R_2 represents H, lower alkyl, or a mono- or di-hydroxy-substituted lower alkyl;

R_3 represents H, halogen, or lower alkyl;

R_4 is absent or represents lower alkyl, -OH, -NH_2 or halogen;

R_5 represents a large hydrophobic amino acid sidechain;

R_{11} represents H or lower alkyl; and

the prodrug is cleaved by FAP to release the cytotoxic agent,  .

In certain embodiments, the acyl N-terminal blocking group is a moiety which, at physiological pH, reduces the cell permeability of said prodrug relative to said cytotoxic agent.

In certain embodiments, the acyl N-terminal blocking group is selected from the group consisting of formyl, acetyl, benzoyl, trifluoroacetyl, succinyl and methoxysuccinyl.

In certain embodiments, the acyl N-terminal blocking group includes one or more functional groups that are ionized at physiological pH.

In certain embodiments, the acyl N-terminal blocking group includes one or more carboxyl groups.

In certain embodiments, the acyl N-terminal blocking group is (lower alkyl)-C(=O)- substituted with one or more functional groups that are ionized at physiological pH.

In certain embodiments, the acyl N-terminal blocking group is selected from the group consisting of aryl(C_1-C_6)acyl, and heteroaryl(C_1-C_6)acyl.

In certain embodiments, the acyl N-terminal blocking group is an aryl(C_1-C_6)acyl, wherein aryl(C_1-C_6)acyl is a (C_1-C_6)acyl substituted with an aryl selected from the group consisting of benzene, naphthalene, phenanthrene, phenol and aniline.

In certain embodiments, the acyl N-terminal blocking group is an heteroaryl(C_1-C_6)acyl, wherein heteroaryl(C_1-C_6)acyl is a (C_1-C_6)acyl substituted with a heteroaryl selected from the group consisting of pyrrole, furan, thiophene, imidazole, oxazole, thiazole, triazole, pyrazole, pyridine, pyrazine, pyridazine and pyrimidine.

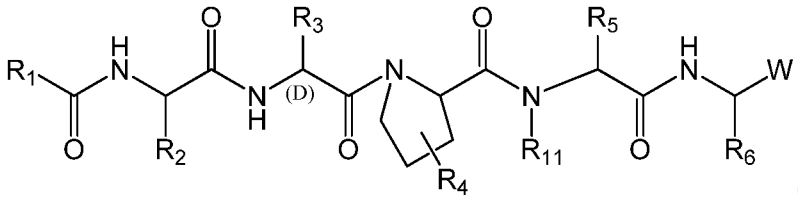
In certain embodiments, the acyl N-terminal blocking group is represented by the formula –C(=O)-$(CH_2)_{1\text{-}10}$-C(=O)-OH.

In certain embodiments, the acyl N-terminal blocking group is succinyl.

In certain embodiments, at least one of Xaa(1), Xaa(2) and Xaa(3) is a non-naturally occurring amino acid analog.

Another aspect of the invention relates to a method described herein, wherein the cytotoxic agent is a proteasome inhibitor.

Another aspect of the invention relates to a method described herein, wherein the proteasome inhibitor is represented by formula VIII:



(VIII)

or a pharmaceutically acceptable salt thereof,

wherein

R_1-(C=O)- represents an acyl N-terminal blocking group;

R_2 represents H, lower alkyl, or a mono- or di-hydroxy-substituted lower alkyl;

R_3 represents H, halogen, or lower alkyl;

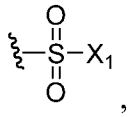
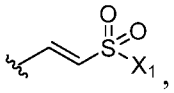
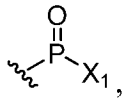
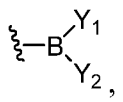
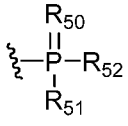
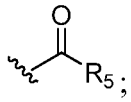
R_4 is absent or represents lower alkyl, -OH, $-NH_2$ or halogen;

R_5 represents a large hydrophobic amino acid sidechain;

R_6 is alkyl, cycloalkyl, aryl, heterocycle or $-(CH_2)_n-R_7$;

R_7 is aryl, aralkyl, cycloalkyl, alkoxy, alkylthio, -OH or -SH;

R_{11} represents H or lower alkyl;

W represents -CN, an epoxyketone, $-CH=NR_5$,  ,  ,  ,  ,  , or  ;

R_8 represents H, an alkyl, an alkenyl, an alkynyl, $-C(X_1)(X_2)X_3$, $-(CH_2)_m-R_9$, $-(CH_2)_n-OH$, $-(CH_2)_n-O$-alkyl, $-(CH_2)_n-O$-alkenyl, $-(CH_2)_n-O$-alkynyl, $-(CH_2)_n-O-(CH_2)_m-R_9$, $-(CH_2)_n-SH$, $-(CH_2)_n-S$-alkyl, $-(CH_2)_n-S$-alkenyl, $-(CH_2)_n-S$-alkynyl, $-(CH_2)_n-S-(CH_2)_m-R_9$, $-C(=O)C(=O)NH_2$, $-C(=O)C(=O)OR_{10}$;

R_9 represents, independently for each occurrence, a substituted or unsubstituted aryl, aralkyl, cycloalkyl, cycloalkenyl, or heterocycle;

R_{10} represents, independently for each occurrence, hydrogen, or a substituted or unsubstituted alkyl, alkenyl, aryl, aralkyl, cycloalkyl, cycloalkenyl, or heterocycle;

Y_1 and Y_2 can independently or together be OH, or a group capable of being hydrolyzed to a hydroxyl group, including cyclic derivatives where Y_1 and Y_2 are connected via a ring having from 5 to 8 atoms in the ring structure;

R_{50} represents O or S;

R_{51} represents N_3, SH_2, NH_2, NO_2 or $-OR_{10}$;

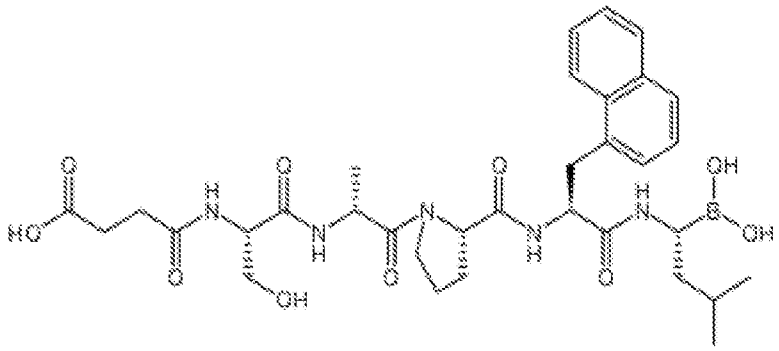
R_{52} represents hydrogen, lower alkyl, amine, $-OR_{10}$, or a pharmaceutically acceptable salt, or R_{51} and R_{52} taken together with the phosphorous atom to which they are attached complete a heterocyclic ring having from 5 to 8 atoms in the ring structure;

X_1 is halogen;

X_2 and X_3 each represent H or halogen; and

m is zero or an integer in the range of 1 to 8; and n is an integer in the range of 1 to 8.

In certain embodiments, the proteasome inhibitor is



In certain embodiments, the prodrug has a therapeutic index at least two times greater than the therapeutic index for the proteasome inhibitor when administered alone.

Another aspect of the invention relates to a method described herein, wherein the prodrug is administered as a single agent therapy.

Another aspect of the invention relates to a method described herein, wherein the prodrug is administered in a combination therapy with one or more anti-cancer agents.

Another aspect of the invention relates to a method described herein, wherein the anti-cancer agent is a covalent proteasome inhibitor.

Another aspect of the invention relates to a method described herein, wherein the anti-cancer agent is a chemotherapeutic.

Another aspect of the invention relates to a method described herein, wherein the chemotherapeutic is docetaxel, paclitaxel, imatinib mesylate, gemcitabine, cis-platin, carboplatin, 5-fluorouracil, pemetrexed, methotrexate, doxorubicin, lenalidomide, dexamethasone, or monomethyl auristatin.

Another aspect of the invention relates to a method described herein, wherein the chemotherapeutic is docetaxel, gemcitabine, carboplatin, or doxorubicin.

Another aspect of the invention relates to a method described herein, wherein the chemotherapeutic is MG-132, PSI, fellutamide B, bortezomib, CEP-18770, MLN-2238, MLN-9708, epoxomicin, carfilzomib (PR-171), NC-005, YU-101, LU-005, YU-102, NC-001, LU-001, NC-022, PR-957 (LMP7), CPSI (β5), LMP2-sp-ek, BODIPY-NC-001, azido-NC-002, ONX-0912, omuralide, PS-519, marizomib, belactosin A, ^{125}I-NIP-L_3VS, NC-005-VS, or MV151.

Another aspect of the invention relates to a method described herein, wherein the anti-cancer agent is an immunotherapeutic agent.

Another aspect of the invention relates to a method described herein, wherein the immunotherapeutic agent is an anti-tumor antibody.

Another aspect of the invention relates to a method described herein, wherein the immunotherapeutic agent is a tumor antigen vaccine or anti-tumor dendritic cell vaccine.

Another aspect of the invention relates to the use of a compound described herein in the manufacture of a medicament for the treatment of a disorder for which inhibition of proteasome activity provides therapeutic benefit.

Another aspect of the invention relates to a packaged pharmaceutical, comprising a prodrug described herein formulated in a pharmaceutically acceptable excipient, in association with instructions (written and/or pictorial) describing the recommended dosage and/or administration of the formulation to a patient.

In certain embodiments, the compounds and compositions of the invention may also be combined with chemotherapy. The efficacy of chemotherapy—a mainstay of the standard of care in carcinoma—is limited by chemoresistance due to the activation of NF-κB by chemotherapeutic agents, resulting in inhibition of the apoptotic response of tumor cells. Tumor cells also resist chemotherapy by overexpression of Bcl-2 and P-glycoprotein. Proteasome inhibitors (PIs) counter these effects by repressing activation of NF-κB, inducing cleavage of Bcl-2 into proapoptotic fragments, and preventing maturation of P-glycoprotein into the active form that removes chemotherapeutic agents from the cancer cell. Therefore, PIs could act as adjuvants to chemotherapy. Compared to bortezomib in this role, the compounds and compositions disclosed herein may reduce compounded toxicities: e.g., increased grade 3/4 hematologic toxicity associated with bortezomib plus gemcitabine, docetaxel or carboplatin. Chemotherapeutic agents are usually administered at high doses in cycles interspersed with breaks. More continuous administration of chemotherapeutic agents (metronomic chemotherapy) has recently been initiated in order to lengthen exposure of cancer cells to drug and inhibit angiogenesis. Due to reduced toxicity, the compounds and compositions disclosed herein would be ideally suited for longer periods of administration in combination with metronomic chemotherapy.

Definitions

The term "amino acid" is intended to encompass all compounds, whether natural or synthetic, which include both an amino functionality and an acid functionality, including amino acid analogues and derivatives. In certain embodiments, the amino acids contemplated in the present invention are those naturally occurring amino acids found in proteins, or the naturally occurring anabolic or catabolic products of such amino acids, which contain amino and carboxyl groups. Naturally occurring amino acids are identified

throughout by the conventional three-letter and/or one-letter abbreviations, corresponding to the trivial name of the amino acid, in accordance with the following list. All amino acids described herein are contemplated as both (D)- and (L)-isomers unless otherwise designated. The abbreviations are accepted in the peptide art and are recommended by the IUPAC-IUB commission in biochemical nomenclature.

By the term "amino acid residue" is meant an amino acid. In general the abbreviations used herein for designating the naturally occurring amino acids are based on recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature. See Biochemistry (1972) 11:1726-1732). For instance Met, Ile, Leu, Ala and Gly represent "residues" of methionine, isoleucine, leucine, alanine and glycine, respectively. By the residue is meant a radical derived from the corresponding α-amino acid by eliminating the OH portion of the carboxyl group and the H portion of the α-amino group.

The term "amino acid side chain" is that part of an amino acid residue exclusive of the backbone, as defined by K. D. Kopple, "Peptides and Amino Acids", W. A. Benjamin Inc., New York and Amsterdam, 1966, pages 2 and 33; examples of such side chains of the common amino acids are $-CH_2CH_2SCH_3$ (the side chain of methionine), $-CH_2(CH_3)-CH_2CH_3$ (the side chain of isoleucine), $-CH_2CH(CH_3)_2$ (the side chain of leucine) or H- (the side chain of glycine). These sidechains are pendant from the backbone Cα carbon.

The term "amino acid analog" refers to a compound structurally similar to a naturally occurring amino acid wherein the C-terminal carboxy group, the N-terminal amino group or side-chain functional group has been chemically modified. For example, aspartic acid-(beta-methyl ester) is an amino acid analog of aspartic acid; N-ethylglycine is an amino acid analog of glycine; or alanine carboxamide is an amino acid analog of alanine.

The phrase "protecting group" as used herein means substituents which protect the reactive functional group from undesirable chemical reactions. Examples of such protecting groups include esters of carboxylic acids and boronic acids, ethers of alcohols, and acetals and ketals of aldehydes and ketones. For instance, the phrase "N-terminal protecting group" or "amino-protecting group" as used herein refers to various amino-protecting groups which can be employed to protect the N-terminus of an amino acid or peptide against undesirable reactions during synthetic procedures. Examples of suitable groups include acyl protecting groups such as, to illustrate, formyl, dansyl, acetyl, benzoyl, trifluoroacetyl, succinyl, and methoxysuccinyl; aromatic urethane protecting groups as, for

example, benzyloxycarbonyl (Cbz); and aliphatic urethane protecting groups such as t-butoxycarbonyl (Boc) or 9-Fluorenylmethoxycarbonyl (Fmoc).

The term "amino-terminal protecting group" as used herein, refers to terminal amino protecting groups that are typically employed in organic synthesis, especially peptide synthesis. Any of the known categories of protecting groups can be employed, including acyl protecting groups, such as acetyl, and benzoyl; aromatic urethane protecting groups, such as benzyloxycarbonyl; and aliphatic urethane protecting groups, such as tert-butoxycarbonyl. See, for example, Gross and Mienhoffer, Eds., *The Peptides*, Academic Press: New York, 1981;, Vol. 3, 3-88; and Green, T. W.; Wuts, P. G. M., *Protective Groups in Organic Synthesis*, 2nd ed, Wiley: New York, 1991. Preferred protecting groups include aryl-, aralkyl-, heteroaryl- and heteroarylalkyl-carbonyl and sulfonyl moieties.

As used herein the term "physiological conditions" refers to temperature, pH, ionic strength, viscosity, and like biochemical parameters which are compatible with a viable organism, and/or which typically exist intracellularly in a viable mammalian cell

The term "prodrug" as used herein 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.

The phrase "pharmaceutically acceptable excipient" or "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, involved in carrying or transporting the subject chemical from one organ or portion of the body, to another organ or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation, not injurious to the patient, and substantially non-pyrogenic. 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 and potato starch; (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 salts" 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, and laurylsulphonate salts, and the like. See, for example, Berge et al. (1977) "Pharmaceutical Salts", *J. Pharm. Sci.* 66:1-19.

In other cases, the compounds 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*).

A "therapeutically effective amount" of a compound with respect to use in treatment, refers to an amount of the compound 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.

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 "self-eliminating linker" or "self-immolative linker" refers to a temporary extender, spacer, or placeholder unit attaching two or more molecules together by chemical bonds that are cleaved under defined conditions to release the two molecules. Examples of self-eliminating linkers include, but are not limited to, *p*-aminobenzyloxycarbonyl (PABC) and 2,4-bis(hydroxymethyl)aniline. The self-eliminating or self-immolative linker may be linear or branched, and may link two or more of the same molecules together, or may link two or more different molecules together. The self-eliminating or self-immolative linker may degrade, decompose, or fragment under, for example, physiological conditions, acidic conditions, basic conditions, or in the presence of specific chemical agents.

As noted above, certain compounds of the present invention may exist in particular geometric or stereoisomeric forms. The present invention contemplates all such compounds, including cis- and trans-isomers, *R*- and *S*-enantiomers, diastereomers, (D)-isomers, (L)-isomers, the racemic mixtures thereof, and other mixtures thereof, as falling within the scope of the invention. Additional asymmetric carbon atoms may be present in a substituent such as an alkyl group. All such isomers, as well as mixtures thereof, are intended to be included in this invention.

If, for instance, a particular enantiomer of a compound of the present invention is desired, it may be prepared by asymmetric synthesis or by derivation with a chiral auxiliary, where the resulting diastereomeric mixture is separated and the auxiliary group cleaved to provide the pure desired enantiomer. Alternatively, where the molecule contains a basic functional group, such as amino, or an acidic functional group, such as carboxyl, diastereomeric salts are formed with an appropriate optically-active acid or base, followed

by resolution of the diastereomers thus formed by fractional crystallization or chromatographic means well known in the art, and subsequent recovery of the pure enantiomer.

An aliphatic chain comprises the classes of alkyl, alkenyl and alkynyl defined below. A straight aliphatic chain is limited to unbranched carbon chain moieties. As used herein, the term "aliphatic group" refers to a straight chain, branched-chain, or cyclic aliphatic hydrocarbon group and includes saturated and unsaturated aliphatic groups, such as an alkyl group, an alkenyl group, or an alkynyl group.

"Alkyl" refers to a fully saturated cyclic or acyclic, branched or unbranched carbon chain moiety having the number of carbon atoms specified, or up to 30 carbon atoms if no specification is made. For example, alkyl of 1 to 8 carbon atoms refers to moieties such as methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, and octyl, and those moieties which are positional isomers of these moieties. Alkyl of 10 to 30 carbon atoms includes decyl, undecyl, dodecyl, tridecyl, tetradecyl, pentadecyl, hexadecyl, heptadecyl, octadecyl, nonadecyl, eicosyl, heneicosyl, docosyl, tricosyl and tetracosyl. In certain embodiments, a straight chain or branched chain alkyl has 30 or fewer carbon atoms in its backbone (e.g., C_1-C_{30} for straight chains, C_3-C_{30} for branched chains), and more preferably 20 or fewer.

"Cycloalkyl" means mono- or bicyclic or bridged saturated carbocyclic rings, each having from 3 to 12 carbon atoms. Likewise, preferred cycloalkyls have from 5-12 carbon atoms in their ring structure, and more preferably have 6-10 carbons in the ring structure.

Unless the number of carbons is otherwise specified, "lower alkyl," as used herein, means an alkyl group, as defined above, but having from one to ten carbons, more preferably from one to six carbon atoms in its backbone structure such as methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, and tert-butyl. Likewise, "lower alkenyl" and "lower alkynyl" have similar chain lengths. Throughout the application, preferred alkyl groups are lower alkyls. In certain embodiments, a substituent designated herein as alkyl is a lower alkyl.

"Alkenyl" refers to any cyclic or acyclic, branched or unbranched unsaturated carbon chain moiety having the number of carbon atoms specified, or up to 26 carbon atoms if no limitation on the number of carbon atoms is specified; and having one or more double bonds in the moiety. Alkenyl of 6 to 26 carbon atoms is exemplified by hexenyl, heptenyl, octenyl, nonenyl, decenyl, undecenyl, dodenyl, tridecenyl, tetradecenyl, pentadecenyl, hexadecenyl, heptadecenyl, octadecenyl, nonadecenyl, eicosenyl,

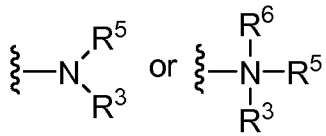
heneicosoenyl, docosenyl, tricosenyl, and tetracosenyl, in their various isomeric forms, where the unsaturated bond(s) can be located anywherein the moiety and can have either the (Z) or the (E) configuration about the double bond(s).

"Alkynyl" refers to hydrocarbyl moieties of the scope of alkenyl, but having one or more triple bonds in the moiety.

The term "alkylthio" refers to an alkyl group, as defined above, having a sulfur moiety attached thereto. In certain embodiments, the "alkylthio" moiety is represented by one of -(S)-alkyl, -(S)-alkenyl, -(S)-alkynyl, and -(S)-$(CH_2)_m$-R^1, wherein m and R^1 are defined below. Representative alkylthio groups include methylthio, ethylthio, and the like.

The terms "alkoxyl" or "alkoxy" as used herein refers to an alkyl group, as defined below, having an oxygen moiety attached thereto. Representative alkoxyl 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 alkoxyl, such as can be represented by one of -O-alkyl, -O-alkenyl, -O-alkynyl, -O-$(CH_2)_m$-R^1, where m and R_1 are described below.

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

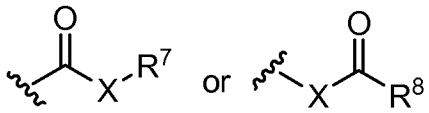


wherein R^3, R^5 and R^6 each independently represent a hydrogen, an alkyl, an alkenyl, -$(CH_2)_m$-R^1, or R^3 and R^5 taken together with the N atom to which they are attached complete a heterocycle having from 4 to 8 atoms in the ring structure; R^1 represents an alkenyl, aryl, cycloalkyl, a cycloalkenyl, a heterocyclyl, or a polycyclyl; and m is zero or an integer in the range of 1 to 8. In certain embodiments, only one of R^3 or R^5 can be a carbonyl, e.g., R^3, R^5, and the nitrogen together do not form an imide. In even more certain embodiments, R^3 and R^5 (and optionally R^6) each independently represent a hydrogen, an alkyl, an alkenyl, or -$(CH_2)_m$-R^1. Thus, the term "alkylamine" as used herein means an amine group, as defined above, having a substituted or unsubstituted alkyl attached thereto, i.e., at least one of R_3 and R_5 is an alkyl group. In certain embodiments, an amino group or an alkylamine is basic, meaning it has a conjugate acid with a $pK_a \geq 7.00$, i.e., the protonated forms of these functional groups have pK_as relative to water above about 7.00.

The term "aryl" as used herein includes 3- to 12-membered substituted or unsubstituted single-ring aromatic groups in which each atom of the ring is carbon (i.e., carbocyclic aryl) or where one or more atoms are heteroatoms (i.e., heteroaryl). Preferably, aryl groups include 5- to 12-membered rings, more preferably 6- to 10-membered rings 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. Carboycyclic aryl groups include benzene, naphthalene, phenanthrene, phenol, aniline, and the like. Heteroaryl groups include substituted or unsubstituted aromatic 3- to 12-membered ring structures, more preferably 5- to 12-membered rings, more preferably 6- to 10-membered rings, whose ring structures include one to four heteroatoms. Heteroaryl groups include, for example, pyrrole, furan, thiophene, imidazole, oxazole, thiazole, triazole, pyrazole, pyridine, pyrazine, pyridazine and pyrimidine, and the like.

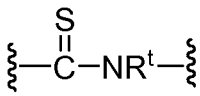
The terms "heterocyclyl" or "heterocyclic group" refer to 3- to 12-membered ring structures, more preferably 5- to 12-membered rings, more preferably 6- to 10-membered rings, whose ring structures include one to four heteroatoms. Heterocycles can also be polycycles. Heterocyclyl groups include, for example, thiophene, thianthrene, furan, pyran, isobenzofuran, chromene, xanthene, phenoxathiin, pyrrole, imidazole, pyrazole, isothiazole, isoxazole, pyridine, pyrazine, pyrimidine, pyridazine, indolizine, isoindole, indole, indazole, purine, quinolizine, isoquinoline, quinoline, phthalazine, naphthyridine, quinoxaline, quinazoline, cinnoline, pteridine, carbazole, carboline, phenanthridine, acridine, pyrimidine, phenanthroline, phenazine, phenarsazine, phenothiazine, furazan, phenoxazine, pyrrolidine, oxolane, thiolane, oxazole, piperidine, piperazine, morpholine, lactones, lactams such as azetidinones and pyrrolidinones, sultams, sultones, and the like. The heterocyclic ring can be substituted at one or more positions with such substituents as described above, as for example, halogen, alkyl, aralkyl, alkenyl, alkynyl, cycloalkyl, hydroxyl, amino, nitro, sulfhydryl, imino, amido, phosphate, phosphonate, phosphinate, carbonyl, carboxyl, silyl, sulfamoyl, sulfinyl, ether, alkylthio, sulfonyl, ketone, aldehyde, ester, a heterocyclyl, an aromatic or heteroaromatic moiety, $-CF_3$, -CN, and the like.

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



wherein X is a bond or represents an oxygen or a sulfur, and R^7 represents a hydrogen, an alkyl, an alkenyl, $-(CH_2)_m-R^1$ or a pharmaceutically acceptable salt, R^8 represents a hydrogen, an alkyl, an alkenyl or $-(CH_2)_m-R^1$, where m and R^1 are as defined above. Where X is an oxygen and R^7 or R^8 is not hydrogen, the formula represents an "ester." Where X is an oxygen, and R^7 is as defined above, the moiety is referred to herein as a carboxyl group, and particularly when R^7 is a hydrogen, the formula represents a "carboxylic acid". Where X is an oxygen, and R^8 is a hydrogen, the formula represents a "formate." In general, where the oxygen atom of the above formula is replaced by a sulfur, the formula represents a "thiocarbonyl" group. Where X is a sulfur and R^7 or R^8 is not hydrogen, the formula represents a "thioester" group. Where X is a sulfur and R^7 is a hydrogen, the formula represents a "thiocarboxylic acid" group. Where X is a sulfur and R^8 is a hydrogen, the formula represents a "thioformate" group. On the other hand, where X is a bond, and R^7 is not hydrogen, the above formula represents a "ketone" group. Where X is a bond, and R^7 is a hydrogen, the above formula represents an "aldehyde" group.

The term "thioxamide," as used herein, refers to a moiety that can be represented by the formula:



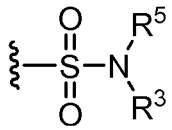
in which R^t is selected from the group consisting of the group consisting of hydrogen, alkyl, cycloalkyl, aralkyl, or aryl, preferably hydrogen or alkyl. Moreover, "thioxamide-derived" compounds or "thioxamide analogues" refer to compounds in which one or more amide groups have been replaced by one or more corresponding thioxamide groups. Thioxamides are also referred to in the art as "thioamides."

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 nonaromatic substituents of organic compounds. Illustrative substituents include, for example, those described herein above. 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. This invention is not intended to be limited in any manner by the permissible substituents of organic compounds. 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.

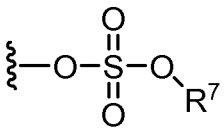
As used herein, the term "nitro" means $-NO_2$; the term "halogen" designates -F, -Cl, -Br, or -I; the term "sulfhydryl" means -SH; the term "hydroxyl" means -OH; the term "sulfonyl" means $-SO_2-$; the term "azido" means $-N_3$; the term "cyano" means –CN; the term "isocyanato" means –NCO; the term "thiocyanato" means –SCN; the term "isothiocyanato" means –NCS; and the term "cyanato" means –OCN.

The term "sulfamoyl" is art-recognized and includes a moiety that can be represented by the formula:



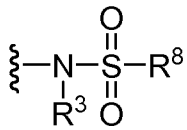
in which R^3 and R^5 are as defined above.

The term "sulfate" is art recognized and includes a moiety that can be represented by the formula:



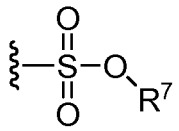
in which R^7 is as defined above.

The term "sulfonamide" is art recognized and includes a moiety that can be represented by the formula:



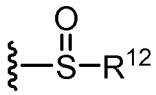
in which R^3 and R^8 are as defined above.

The term "sulfonate" is art-recognized and includes a moiety that can be represented by the formula:



in which R^7 is an electron pair, hydrogen, alkyl, cycloalkyl, or aryl.

The terms "sulfoxido" or "sulfinyl", as used herein, refers to a moiety that can be represented by the formula:



in which R^{12} is selected from the group consisting of the group consisting of hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, heterocyclyl, aralkyl, or aryl.

As used herein, the definition of each expression, e.g., alkyl, m, n, etc., when it occurs more than once in any structure, is intended to be independent of its definition elsewhere in the same structure.

For purposes of this invention, the chemical elements are identified in accordance with the Periodic Table of the Elements, CAS version, *Handbook of Chemistry and Physics*, 67th ed., 1986-87, inside cover.

Exemplification

The invention now being generally described, it will be more readily understood by reference to the following examples, which are included merely for purposes of illustration of certain aspects and embodiments of the present invention, and are not intended to limit the invention.

Example 1. ARI-3996 and Proteasome Inhibitors of the Invention

ARI-3996 and its PI warhead ARI-2727D were synthesized as shown in Figure 1, using synthetic and analytical methods previously described for obtaining peptide boronic acids. Bortezomib was purchased from Selleck Chemicals or ChemieTek. Each batch of ARI-3996 was validated for selective cleavage by FAP versus PREP as described herein.

The following reagents were used: (a) HATU/DMF/DIPEA, 95% yield; (b) 4 M HCl in dioxane, 100% yield; (c) HATU/DMF/DIPEA, 90% yield; (d) 4M HCl in dioxane, 100% yield; (e) tBu-Suc-Ser(tBu)-OH, HATU/DMF/DIPEA, 90% yield; (f) $Pd(OH)_2$-C/H_2/methanol, 90% yield; (g) 2727D, HATU/DMF/DIPEA, 85% yield; (h) TFA/DCM, 90% yield; (i) $PhB(OH)_2$, pentane-water-acetonitrile, 70% yield. The chemical synthesis of peptides, particularly short peptides such as di- and tripeptides such as those described herein, is well-known in the art and sufficiently predictable due to its modular nature.

Therefore the synthetic methods of Table 1 or standard solid-phase synthetic methods as applied to peptides would be successful in delivering any compound of formula I.

ARI-3996 is a pro-drug version of a bortezomib-like cytotoxic agent designed to more selectively target the proteasome in solid tumors (Fig. 15). ARI-3996 was designed to reduce the mechanism-based DLTs associated with proteasome inhibition by remaining inactive until it is cleaved by fibroblast activation protein (FAP) on the surface of reactive stromal fibroblasts of epithelial tumors. Because FAP is produced in epithelial tumors but not usually in healthy tissues (FAP is expressed in the stroma of many common epithelial tumors—lung, colon, breast and pancreatic cancer), ARI-3996 should not be activated in nervous tissue or in bone marrow where platelets are generated. ARI-3996 is relatively non-toxic to all cells and cannot kill tumor cells until it is activated by FAP. Therefore, ARI-3996 should kill FAP-producing tumors with less severe PN and thrombocytopenia than that associated with bortezomib.

Fibroblast activation protein (FAP) is a post-prolyl cleaving serine protease belonging to the (dipeptidyl peptidase) DPP-IV-like subfamily in which FAP and prolyl endopeptidase (PREP) are the only mammalian proteases that can cleave on the C-terminal side of internal proline. Unlike FAP, PREP is constitutively and ubiquitously expressed. We have solved the specificity problem of FAP versus PREP cleavage required to make a pro-drug selectively activated by FAP. FAP's P_4-P_1 cleavage specificity requires proline at P_1, glycine or D-amino acids at P_2, prefers small uncharged amino acids at P_3, and tolerates most amino acids at P_4. We have discovered that D-alanine at P_2 allows cleavage by FAP as expected but prevents cleavage by PREP. Thus, linkage of the tripeptide Suc-Ser-D-Ala-Pro by a C-terminal peptide bond to the bortezomib-like aminoboronate dipeptide Ala(1-Nal)-boroLeu (ARI-2727D) produces the pro-drug ARI-3996 in which the proteasome inhibitory activity is unleashed selectively by FAP cleavage of the Pro-Ala(1-Nal) bond. *In vitro*, ARI-3996 is cleaved by FAP, but to a far lesser extent by PREP, to yield the cytotoxic "warhead" ARI-2727D as demonstrated by mass spectrometry (Fig. 2) and by assay of tumor-cell killing *in vitro* by fluorescent cell-titer blue (Promega) (Table 1).

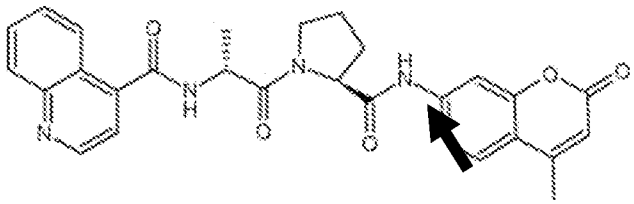
Table 1. FAP specifically activates ARI-3996 to kill tumor cells *in vitro*

Cell line	Cytotoxic EC_{50}[a] (μM) of ARI-3996 incubated with[b]:		
	None	FAP	PREP
RPMI 8226	5.8	0.18	3.1
KG-1	22.0	0.30	13.0
RPMI 1788	6.2	0.13	3.6
BxPC-3	34.0	0.80	21.0

[a]Drug concentration that kills 50% of cells.

[b]24 hours at 37°C, except BxPC-3, 48 hours

Example 2. Use of ARI-3144 as a Diagnostic/Patient Stratification Tool



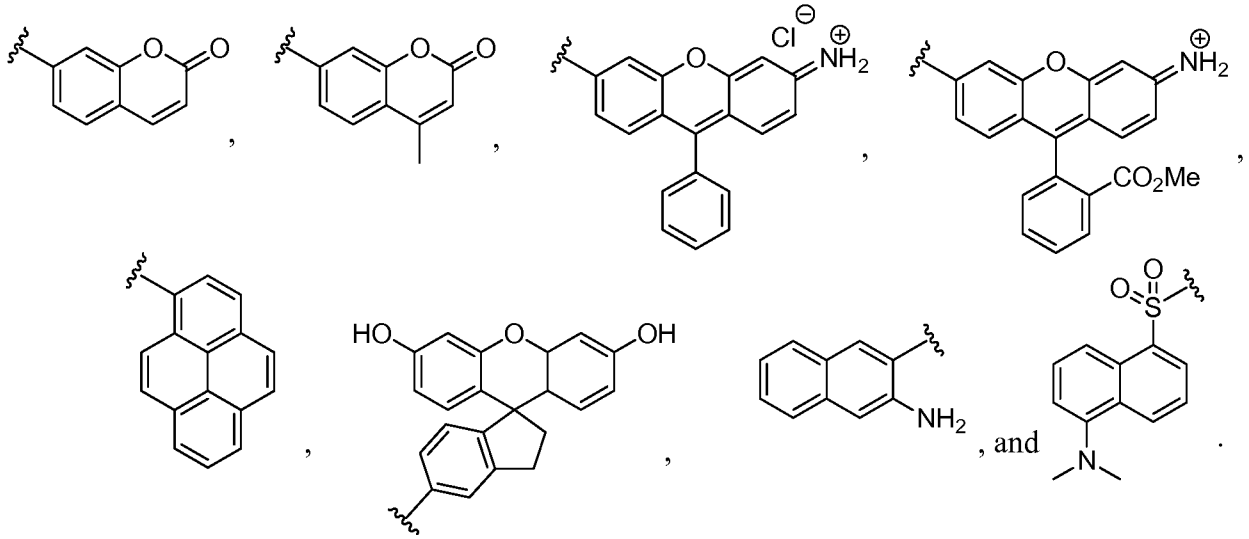
ARI-3144

The structure of ARI-3144 is shown above and includes an arrow indicating the chemical bond that is cleaved by FAP. Figure 11 shows a cartoon of the concept underlying the diagnostic use of this compound. The FAP recognition site is chemically attached to the fluorogenic coumarin (AMC) moiety. After binding of ARI-3144 to FAP, chemical bond cleavage takes place (Fig. 12) to release the FAP recognition site from the AMC. Once released, AMC is now fluorescent and its concentration can be quantified by measuring its spectroscopic properties.

The D-alanine at P_2 of the fluorogenic substrate, N-(Quinoline-4-carbonyl)-D-Ala-Pro-AMC [AMC = 7-amino-4-methylcoumarin] (ARI-3144), confers selectivity for FAP so that it is cleaved to release fluorescent AMC by recombinant FAP but not by other DPP-IV-like proteases (Fig. 3). As shown in Figure 13, ARI-3144 is an excellent substrate for FAP, and no cleavage by PREP, DPPIV, DPP8, DPP9, or DPPII was detected (Fig. 14). FAP is reportedly not expressed constitutively in healthy tissue with the exception of endometrium, although FAP proteolytic activity is detectable in plasma. Ovarian and prostate tumors excepted, induction of FAP expression in stromal fibroblasts of common epithelial tumors (lung, colon, breast and pancreas) has been demonstrated by immunohistochemistry and mRNA analysis. The ability to measure FAP proteolytic activity in tumors is required to

evaluate the usefulness of ARI-3996 as a chemotherapeutic agent. Hitherto, FAP activity has not been quantified in human or mouse tumors. We have used the ARI-3144 assay *ex vivo* to demonstrate increased FAP proteolytic activity in human pancreatic carcinoma relative to healthy tissues and plasma. We will use the assay to select a mouse tumor model for investigation of ARI-3996's activity in which the increase in tumor-associated FAP activity relative to plasma is equivalent to that in human epithelial cancer.

Besides coumarin-based chromophores, other widely-used chromophores would work just as well as long as an amide linkage could be used for attachment (i.e., the chromophore has a primary amino group available for attachment to the proline of the FAP recognition site. Such commonly-used chromophores are, for example:



Example 3. Mouse Model of Epithelial Cancer in Which the FAP Proteolytic Activity of Tumor Tissue is Equivalent to That in Human Cancer Patients.

The FAP-specific fluorogenic substrate ARI-3144 was used to measure FAP proteolytic activity in tissue homogenates and plasma in a standard continuous fluorometric assay as previously described.

In tissue specimens from 14 pancreatic cancer patients at the Fox Chase Cancer Center, we determined mean (± SE) FAP activity in cancerous tissue of 903.7 ± 161.4 expressed as change in fluorescent units (ΔFU)/min/mg protein. FAP activity varied between patients with 4 high-expressers that were in the range of 1,200 to 3,000. In contrast, HPAF-II pancreatic adenocarcinoma xenografts in *scid* mice exhibited mean activity of only 200 ± 12.5. In mice, we have found that circulating levels of plasma FAP

activity are approximately 6-fold higher than in humans regardless of whether either species bears a tumor. Thus, the tumor:plasma ratio of FAP activity is at least 100:1 in humans; but only 3:1 in the HPAFII xenografted mice (Fig. 4 and 16).

The TI of a FAP-activated pro-drug such as ARI-3996 is expected to depend on the difference between the systemic level of FAP proteolytic activity and the level in tumor tissue. ARI-3996 has exhibited significant antitumor activity in HPAF-II mice (see below). However, the HPAF-II model does not accurately reflect the tumor:systemic ratio of FAP activity in human pancreatic carcinoma patients (Fig. 16 and 17). In order to test the feasibility of ARI-3996 as a safer and more effective PI than bortezomib in solid cancer, a carcinoma model in which tumor FAP activity is ~35-fold higher than in HPAF-II xenografts is needed. Because FAP is induced in reactive stromal fibroblasts during tumorigenesis, the level of tumor FAP activity should be higher in mouse models that recapitulate the pattern of stromal development in human cancer than in xenografts of cell lines. Two different models appear promising. In the Cre-recombinase inducible lung adenocarcinoma model in *Lox-Stop-Lox (LSL)-K-ras*^{G12D} mice, endogenous tumor development induces a FAP^{+} stroma that closely resembles that in human carcinoma histologically. An alternative model is provided by patient tumors directly transplanted into immunodeficient mice. The transplanted human tumors are reported to maintain the stromal organization and vasculature of the original tumor. FAP activity will be assayed in samples of xenograft transplants of human epithelial carcinomas with well-developed stroma provided by Oncotest http://www.oncotest.de/for-pharma/index.php.

As shown in Figure 5, the FAP transfected HEK293 xenograft model can be used to model human tumors overexpressing FAP to a similar degree as in tumors found in human cancer patients. A FAP-transfected variant of the HEK293 cell line forms tumors of FAP^{+} epithelial cells in *scid* mice (69). We have demonstrated FAP activity levels of 6,000 to 12,500 ΔFU/min/mg in FAP-HEK293 tumors *in vivo* (Fig. 5). The FAP-HEK293 model is, therefore, suitable for investigation of ARI-3996's TI. However, unlike the *K-ras*^{G12D}-driven lung tumor and direct patient transplant models, the HEK293 model does not mimic the stromal expression of FAP in human carcinoma.

Furthermore, as shown in Figure 18, FAP transfected HEK tumor xenografts have FAP activity matching human pancreatic cancer tumors. A battery of human pancreatic tumor samples had FAP activity levels ranging from negligible to over 250 (tumor

FAP/plasma FAP). The right hand side of Figure 18 shows that HEK mouse xenograft samples display ratios of tumor FAP/plasma FAP from 150-270.

Example 4. Validation of the HEK Tumor Xenograft Mouse Model

Having determined the levels of FAP expression in the HEK xenograft model, the anticancer activity of ARI-3996 was next evaluated in a 40-day study. Figure 20 shows the results. Impressively, while Velcade® hardly slowed the growth of the tumors, both ARI-2727D and ARI-3996 exhibited potent tumor inhibition. ARI-2727D is expected to be less potent than ARI-3996 because it lacks the FAP recognition site, or address moiety. It also suffers conformation-dependent inactivation over time. Nonetheless, ARI-2727 showed a markedly greater inhibitory effect than Velcade®.

At a dose of 25 mg/kg, ARI-3996 showed nearly complete inhibition of tumor growth. Even in an HEK-mock model (Fig. 20 bottom) at a dose of 50 mg/kg ARI-3996 was well tolerated and showed an inhibitory effect over the control.

As a further test of its efficacy, ARI-3996 was administered to immunocompetent WT BALB/c mice. As Figure 19 shows, tumor regression was observed over the course of a 30-day experiment.

Example 5. Maximum Tolerated Doses (MTD) and Minimum Effective Doses (MED) of ARI-3996, ARI-2727D, and Bortezomib in the FAP^+ Cancer Model.

ARI-3996 administered (i.p.) to mice xenografted with the HPAF-II cell line significantly reduced tumor growth at its MTD of 100 mg/kg (Fig. 6). The antitumor effect of ARI-3996 was confirmed both as a single agent and in combination with gemcitabine (Figs. 9 & 10). In particular, highly significant antitumor effect was observed when ARI-3996 was administered s.c. instead of i.p. (Fig. 9). In contrast, HPAF-II tumors were refractory to bortezomib at its MTD of 1 mg/kg (Fig. 6). ARI-3996, therefore, appears to be 100-fold safer than bortezomib based on MTD and to outperform bortezomib in a model of epithelial cancer. However, the antitumor effect of ARI-3996 was likely limited by the relatively low level of FAP activity, which is required to activate the prodrug, in HPAF-II tumors. As described above, the FAP tumor:plasma ration is ≥100:1 for human pancreatic cancer versus 3:1 in HPAF-II xenografted mice (Fig. 4). Therefore, in order to better judge ARI-3996's potential for producing antitumor effects in carcinoma patients at tolerated dose levels, MTDs and MEDs of ARI-3996, bortezomib and ARI-2727D will be compared

in the mouse model selected for tumor-associated FAP activity equivalent to that in human cancer.

MTDs will be determined by administering (i.p.) escalating doses of compounds twice weekly (days 1 and 4) to groups of normal and tumor-bearing mice (n = 2 female + 2 male). Comparison of toxicity in tumor-bearing versus non-tumor-bearing mice will determine whether activation of ARI-3996 by tumor FAP contributes to systemic toxicity. Health of mice will be monitored daily, and mice will be weighed twice weekly. At sublethal dose levels, the highest dose that causes no ill health and no greater than 10% weight loss will be defined as the MTD. MEDs will be determined from dose responses of antitumor effects in tumor-bearing mice (n = 5-7 per treatment group) administered compounds twice weekly. The MED will be defined as the smallest dose that produces a significant reduction in tumor growth as determined by unpaired, two-tailed Student's t test for comparison of tumor sizes between test and control mice. Experimental details will depend on the model chosen in Experiment 1. Study design will be similar to that previously described for the demonstration of the antitumor effect of the FAP-targeting antitumor agent, Glu-boroPro. TIs for ARI-3996, bortezomib and the 'warhead', ARI-2727D, will be calculated by the formula: TI = MTD ÷ MED. If the availability of *LSL-K-ras*^{G12D} or Oncotest mice is limiting, toxicity and MTD can be investigated in FAP-HEK293 xenografted *scid* mice (Fig. 5).

Example 6. Characterization of the Mechanism of the Antitumor Effect of ARI-3996 by Investigating Inhibition of the 20S Proteasome, Induction of Apoptosis, and Reduction of Angiogenesis in FAP$^+$ tumors.

One hour after final drug administration at termination of Example 5, peripheral blood, tumor, spleen and liver will be collected. Tissue lysates will be prepared from snap-frozen samples for assay of proteasome inhibition. Histological tissue specimens will be fixed in formalin and embedded in paraffin under conditions suited to immunostaining and apoptosis assay and sectioned.

Chymotryptic subunit activity of the 20S proteasome will be determined using the fluorogenic substrate succ-Leu-Leu-Val-Tyr-AMC (Enzo Life Science). Bortezomib and ARI-2727D will distribute to all tissues and are expected to inhibit proteasome activity in all tissues, uniformly, in a dose-dependent manner. The assay will test whether ARI-3996 targets tumor proteasome activity more selectively using a paired, two-tailed Student's t test

for comparisons between paired samples of tumor and non-tumor tissue (e.g., spleen) in each animal. Histological sections of tumors that responded optimally to drug treatments will be compared to controls for microvessel density (MVD) by immunostaining with mouse CD34-specific antibody (BD-Pharmingen). Apoptosis will be quantified by terminal deoxynucleotidyl transferase-mediated nick end labeling (TUNEL) using the ApopTag peroxidase *in situ* apoptosis detection kit (Millipore). Events will be counted microscopically in a blinded manner, and significance of differences between vehicle- and drug-treated tumors will be determined by unpaired, two-tailed Student's *t* test for at least 5 mice per treatment group. Tissues will also be stained with H&E for investigation of systemic toxicity.

We have found that FAP proteolytic activity of blood plasma appears to be approximately 6-fold higher in mice (~60 ΔFU/min) than in humans (~10 ΔFU/min) regardless of tumor status. Mouse models may over-report systemic toxicity of ARI-3996 compared with that possible in cancer patients. This was further verified by treatment of FAP knockout mice with ARI-3996. In knockout mice (Fig. 21), no activation of ARI-3996 to release ARI-2727 took place, whereas in FAP+ mice a concentration of the released warhead ARI-2727 of 150 ng/mL was reached.

Greater pro-drug activation in the peripheral blood of mice could result in greater systemic exposure to the ARI-2727D "warhead" than in humans. If mouse toxicity prevents achievement of the targeted 10-fold greater TI for ARI-3996 versus bortezomib, we will investigate toxicity in mice that are genetically deficient in FAP ($Fap^{LacZ/LacZ}$) (70). We have demonstrated that FAP-deficient mice have no significant proteolytic activity detectable with the FAP-specific substrate ARI-3144 (Fig. 1). Therefore, comparison of the MTD of ARI-3996 in FAP-sufficient versus FAP-deficient mice will determine how plasma FAP activity affects toxicity. If we find that ARI-3996 has highly significant preclinical antitumor activity, but the TI is compromised due to the basal level of plasma FAP activity in mice, we would consider the Test of Feasibility to be met.

Example 7. Evidence for Tumor Delivery of ARI-2727D by the FAP Activated Prodrug ARI-3996.

Dose-limiting toxicity (DLT) prevents administration of high-enough doses of bortezomib to produce tumor responses in solid cancer. Preclinical results in mice xenografted s.c. with the human PC-3 prostate tumor suggest that DLT is due to the low

exposure of solid tumors to bortezomib relative to the exposure of non-cancerous tissue (Fig. 7). ARI-3996 is a pro-drug designed to release a bortezomib-like PI, ARI-2727D, at the tumor site upon cleavage by proteolytic activity of fibroblast activation protein (FAP). Because FAP is predominantly expressed in stroma of human epithelial cancer, ARI-3996 should increase tumor exposure to the PI and reduce exposure in healthy tissues relative to bortezomib.

In SCID mice xenografted with the human HPAF-II pancreatic adenocarcinoma, we have compared the tissue distribution of ARI-3996 and ARI-2727D in liver, peripheral blood cells (PBC) and tumor following a single s.c. injection of ARI-3996 at a dose of 50 mg/kg. At 1, 3 and 6 hours after administration, as for bortezomib (Fig. 7), liver exposure to intact ARI-3996 is greater than tumor exposure (Fig. 8 A, C, E), and exposure of PBC and tumor to pro-drug is similar at 3 hours. However, at all time points, tumor exposure to the active "warhead", ARI-3996 exceeded either liver or PBC exposures (Fig. 8 B, D, F).

Further evaluation (Figures 22 and 23) of the tissue distribution of Velcade® vs. ARI-3996 in mice showed that Velcade® reached much higher concentrations in the heart, lung, kidney, liver, spleen, and lung than in the tumor, suggesting that the drug's ineffectiveness against solid tumors results from its low concentration in the tumor. Velcade®'s high toxicity would also result from the accumulation of the drug in the organs at the expense of accumulation in the tumor. In contrast, ARI-3996 accumulates primarily in the liver initially; FAP activation/cleavage to form ARI-2727D results in ARI-2727D forming much higher relative concentrations in the tumor with lesser amounts in the lungs and plasma. Thus ARI-2727D is being selectively delivered to solid tumors via FAP activation of ARI-3996.

Finally, FAP activation was verified as the mode by which ARI-2727D tumor accumulation was taking place. In Figure 24, ARI-2727D concentrations at 1 hour post-injection were compared with ARI-2727D concentrations 1 hour after injection of the prodrug form (ARI-3996). Direct injection of ARI-2727D resulted in the highest concentration of the drug accumulating in the kidneys and lung; when ARI-3996 was injected the highest concentration of ARI-2727D was found in the tumor, followed by lungs and plasma.

Remarkably, the results suggest that ARI-3996 increases tumor exposure to the active PI while sparing non-tumor tissue. Interestingly, in the HPAF-II tumor model, we have found that bortezomib lacks significant antitumor activity at the maximum tolerated

dose of 1 mg/kg in mice (Fig. 6), whereas ARI-3996 is well tolerated and produces significant reductions in tumor size at 50 mg/kg (Figs. 9 and 10). The HPAF-II tumor response to ARI-3996 strengthens our hypothesis that solid tumors can respond to proteasome inhibition. The tumor:plasma ratio of FAP activity is only 3:1 in HPAF-II mice, whereas in pancreatic cancer patients the ratio is 100:1 or greater. Therefore, we anticipate significantly greater activation of ARI-3996 and, consequently, further improvements in tumor responses in the mouse model with a higher tumor:plasma FAP will be identified in further studies.

Example 8. Cytotoxicity of Velcade® versus ARI-2727D and ARI-3996 in Multiple Myeloma, Normal Cells, and Solid Tumors

Although Velcade® has robust clinical activity in MM patients, drug resistance develops in all patients who initially respond to treatment. Stromal fibroblasts in epithelial tumors promote tumor progression and metastasis through the remodeling of the extracellular matrix and as a source of paracrine growth factors such as fibroblast growth factor, epidermal growth factor and transforming growth factor-β. By targeting proteasome inhibition to the tumor microenvironment, ARI-3996 may kill stromal fibroblasts as well as malignant epithelial cells. This would provide the opportunity to attack the tumor by killing a cell type that is less likely than the tumor cell itself to develop drug-resistance.

Figure 25 and 26 demonstrate further biological evaluation of ARI-2727D and ARI-3996 vs. Velcade® for cytotoxicity toward Multiple Myeloma (MM), normal cells, and various solid tumors. Figure 25 shows that both Velcade® and ARI-2727D have extremely high potency against various MM cell lines and slightly lower toxicity against normal cells. Their toxicity is much lower against solid tumors. In Figure 26 ARI-3996 is compared with ARI-2727D and Velcade®. Its cytotoxicity is much lower across the board, particularly in solid tumors.

These results underscore the importance of selective delivery in solving the ongoing challenges in conventional cancer chemotherapy. Without selective delivery of cytotoxic agents to cancer cells, they often display equal toxicity to normal and cancerous cells alike.

Example 9. FAP Activity of Human Cancers

One important aspect of determining which cancers will benefit from treatment with the compounds of the invention. As mentioned above, FAP has very low expression in

normal human tissues. A large number of tissue samples from tumors were collected and their FAP activity—not expression levels—measured. As Figure 27 shows, virtually all the samples show a much higher level of FAP activity in the tumor vs. the serum. Thus, most solid tumors susceptible to proteasome inhibitors are expected to respond to treatment with FAP-activated prodrugs of the invention. As shown in Figure 4, human tumors have, on average, 100:1 the FAP activity levels of normal human tissue.

Example 10. Anticancer Effects of Proteasome Inhibitors in U266 Tumor-bearing Mice

ARI-3996 consistently outperformed Velcade® in a mouse MM model. Mice bearing U266 tumor xenografts (2 female, 2 male) were treated with either the vehicle, ARI-3996, or Velcade® twice a week (day 1 and day 4) for 2 weeks. As shown in Figure 28, ARI-3996 was dosed at 50 mg/kg (½ the MTD) and Velcade® at 0.5 mg/kg (also ½ the MTD). The inhibition of the tumor was evaluated using ELISA (μg/mL) and bioluminescence. ARI-3996 showed a marked advantage over Velcade®. While 1 death took place in the Velcade® group, all mice in the ARI-3996 group survived with improved outcomes vs. the Velcade® group.

Example 11. Conjugation of FAP Recognition Site to Known Proteasome Inhibitors

Since the above Examples demonstrate that the FAP recognition site (the short peptide chain conferring FAP specificity) when attached to ARI-2727D confers selective delivery of the warhead to tumors and the surrounding stromal cells, it is reasonable to conclude that the same FAP recognition sequence could be attached to other proteasome inhibitors to yield the same effect. Many short peptide and peptide analogue sequences are known to inhibit the proteasome. Attachment of these sequences to the FAP recognition site by the N-terminal amide of the inhibitor/warhead will form prodrug of similar potency, specificity, and (low) toxicity as ARI-3996.

Many of the most potent proteasome inhibitors contain 2-4 peptides or peptide analogues with an electrophilic moiety replacing or appended to the carboxyl terminus. This electrophilic moiety is a reactive species that covalently modifies a nucleophilic residue of the proteasome, destroying its catalytic activity. Such a method of inactivating an enzyme is commonly referred to as "suicide inhibition" in the literature. Examples of successfully validated electrophilic moieties include boronates, epoxyketones, aldehydes, cyanates, vinyl sulfones, α,β-unsaturated carbonyls, and ketoaldehydes.

The structures of a number of clinically relevant or otherwise validated proteasome inhibitors are shown in Figure 29. Careful consideration of the chemical structures reveals certain similarities. Most are di-, tri-, or tetrapeptides with an electrophilic moiety attached to the carboxyl terminus. At the amino terminus is generally an acyl or aracyl group (bortezomib, CEP-18770, MLN2238, MLN9708, MG-132, PSI, ^{125}I-NIP-L_3VS, carfilzomib, oprozomib, epoximicin, PR-957, NC-005, NC-005-VS, YU-101, LU-005, YU-102, NC-001, NC-022, CPSI, and IPSI-001). These acyl or aracyl groups are present to increase the resistance of the proteasome inhibitor against nonspecific proteases that might otherwise degrade short peptides.

If these N-terminal acyl or aracyl groups attached to the various proteasome inhibitors shown in Figure 29 are removed and replaced with the FAP recognition site described herein, the result would be novel FAP-activated proteasome inhibitors whose specificity and toxicity would be greatly improved over their parent molecules.

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Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

Incorporation by Reference

All of the U.S. patents and U.S. patent application publications cited herein are hereby incorporated by reference.

We claim:

1. A FAP-activated prodrug of a proteasome inhibitor represented by **A-B**, or a pharmaceutically acceptable salt thereof, wherein

A represents a substrate for Fibroblast Activation Protein (FAP);

B represents a proteasome inhibitor moiety which, when released in a free form from the prodrug as a product of cleavage by FAP, inhibits the proteolytic activity of a proteasome with a Ki of 500 nM or less;

A and **B** being covalently linked by a bond that is enzymatically cleaved by FAP to release **B** in said free form; and

the prodrug has a k_{cat}/K_m for FAP cleavage of the bond linking **A** and **B** of at least 10 fold more than for Prolyl endopeptidase EC 3.4.14.5 (PREP).

2. The prodrug of claim 1, wherein the free form of said proteasome inhibitor moiety has an IC_{50} for inhibiting proteasome activity of cells *in vitro* that is at least 10 fold less relative to said prodrug.

3. The prodrug of claim 1, wherein the free form of said proteasome inhibitor moiety has a K_i for inhibiting proteasome activity that is at least 10 fold less relative to said prodrug.

4. The prodrug of claim 1, wherein the free form of said proteasome inhibitor moiety has at least 5 fold greater cell permeability into human cells than said prodrug.

5. The prodrug of claim 1, wherein the prodrug has a therapeutic index *in vivo* at least 5 fold greater than said free form of said proteasome inhibitor moiety.

6. The prodrug of claim 1, wherein the prodrug has a therapeutic index *in vivo* of at least 10.

7. The prodrug of claim 1, wherein the prodrug has a maximum tolerated dose at least 10 times greater than [(1R)-3-methyl-1-({(2S)-3-phenyl-2-[(pyrazin-2-ylcarbonyl)amino]propanoyl}amino)butyl] boronic acid.

8. The prodrug of claim 1, wherein said free form of said proteasome inhibitor moiety is a dipeptidyl moiety, which when released from the prodrug as an open chain product of cleavage by FAP, undergoes cyclization-dependent inactivation over time.

9. The prodrug of claim 8, wherein said open chain product undergoes cyclization-dependent inactivation with a $T_{1/2}$ of 5 hours or less.

10. The prodrug of claim 1, wherein **A** represents a peptide or peptide analog which is a substrate for FAP, which peptide or peptide analog includes an N-terminal blocking group.

11. The prodrug of claim 10, wherein the peptide or peptide analog is 2-10 amino acid residues in length.

12. The prodrug of claim 10, wherein the peptide or peptide analog is C-terminally linked to **B**.

13. The prodrug of claim 10, wherein at least one amino acid residue of the peptide or peptide analog is a non-naturally occurring amino acid analog.

14. The prodrug of claim 10, wherein the N-terminal blocking group is a moiety which, at physiological pH, reduces the cell permeability of said prodrug relative to said free form of said proteasome inhibitor.

15. The prodrug of claim 14, wherein the N-terminal blocking group includes one or more functional groups that are ionized at physiological pH.

16. The prodrug of claim 14, wherein the N-terminal blocking group is a (lower alkyl)-C(=O)- substituted with one or more functional groups that are ionized at physiological pH.

17. The prodrug of claim 14, wherein the N-terminal blocking group is represented by the formula $–C(=O)-(CH_2)_{1-10}-C(=O)-OH$.

18. The prodrug of claim 14, wherein the N-terminal blocking group includes one or more carboxyl groups.

19. The prodrug of claim 14, wherein the N-terminal blocking group is succinyl.

20. The prodrug of claim 1, wherein **B** is a covalent or non-covalent proteasome inhibitor.

21. The prodrug of claim 1, wherein **B** is a covalent proteasome inhibitor.

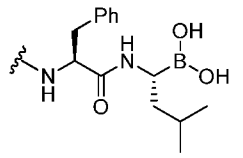
22. The prodrug of claim 21, wherein **B** is a dipeptidyl moiety having at its carboxy terminus an electrophilic functional group that can form a covalent adduct with an amino acid residue in the active site of a proteasome.

23. The prodrug of claim 22, wherein the electrophilic functional group is an aldehyde, boronic acid, boronate ester, epoxyketone, beta-lactone, vinyl sulfone, or α,β-unsaturated carbonyl.

24. The prodrug of claim 22, wherein the electrophilic functional group is an aldehyde, boronic acid, or epoxyketone.

25. The prodrug of claim 22, wherein the electrophilic functional group is an epoxyketone.

26. The prodrug of claim 20, wherein **B** is selected from the group consisting of: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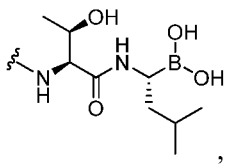
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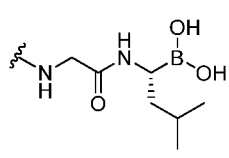
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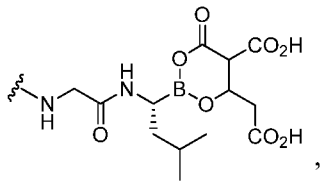
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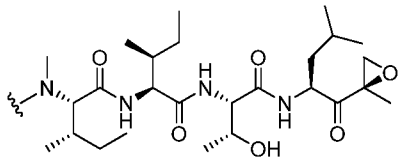
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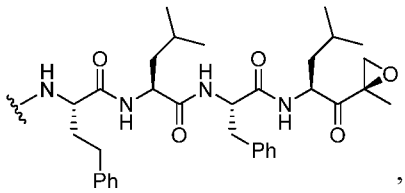
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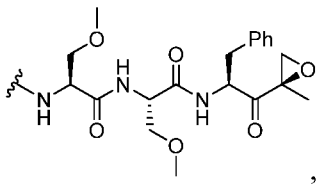
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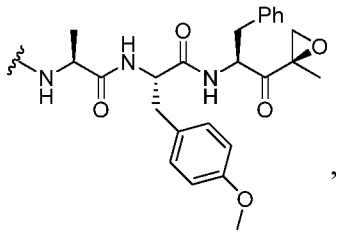
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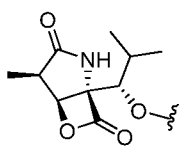
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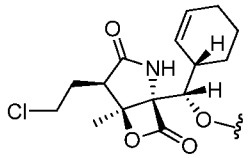
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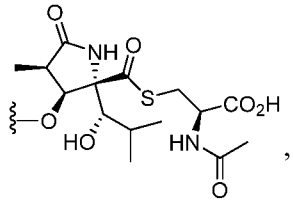
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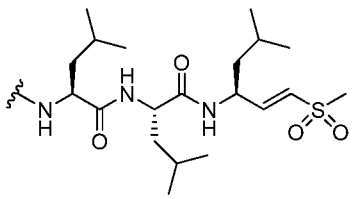
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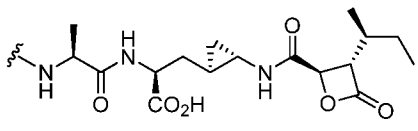
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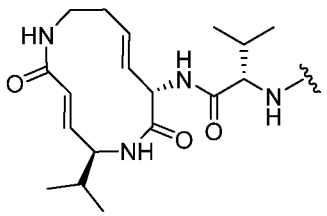
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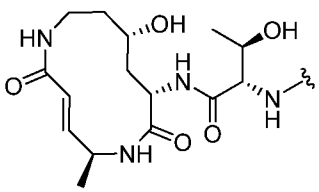
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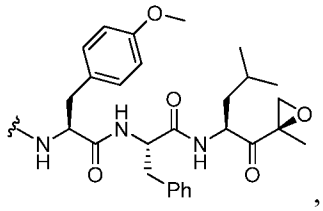
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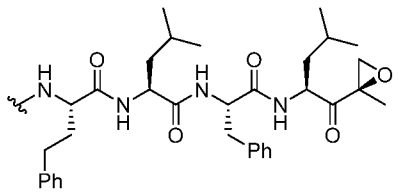
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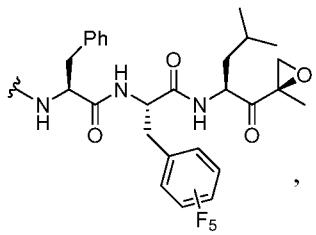
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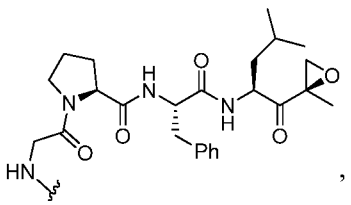
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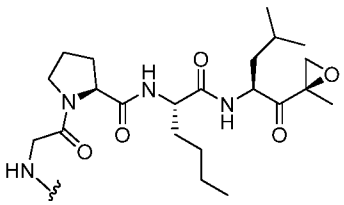
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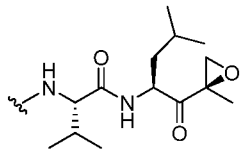
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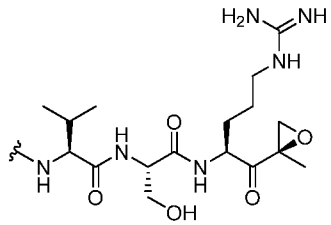
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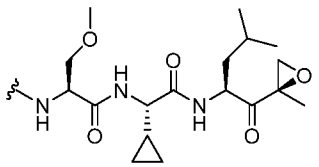
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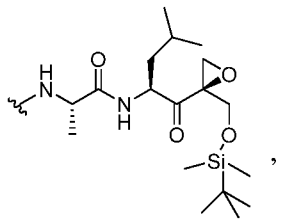
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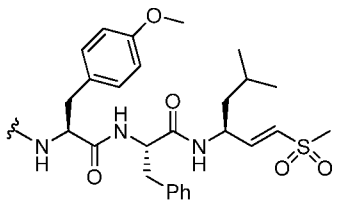
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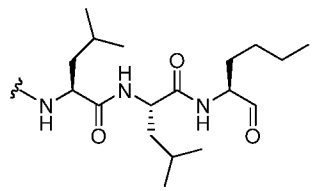
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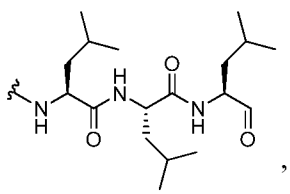
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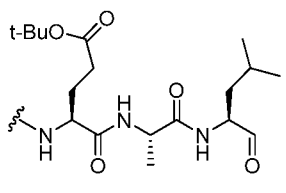
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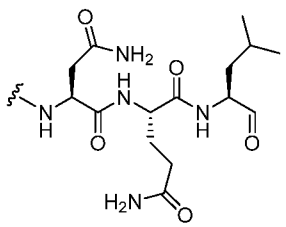
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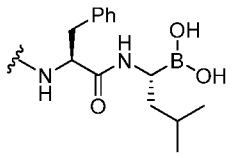
5 27. The prodrug of claim 20, wherein **B** is selected from the group consisting of: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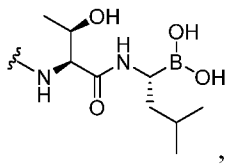
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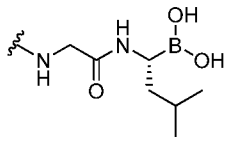
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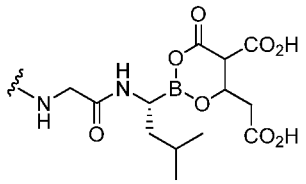
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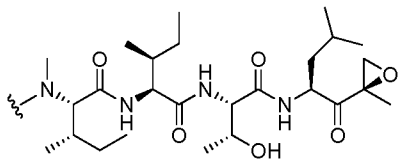
28. The prodrug of claim 20, wherein **B** is selected from the group consisting of: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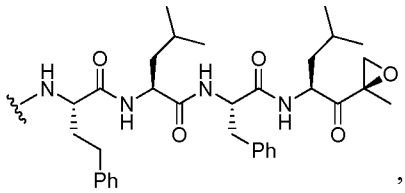
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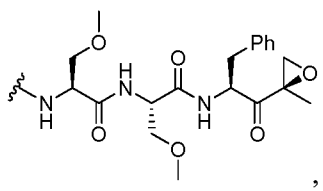
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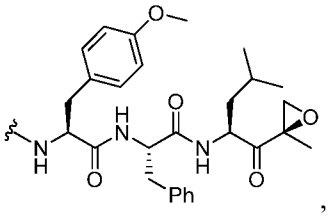
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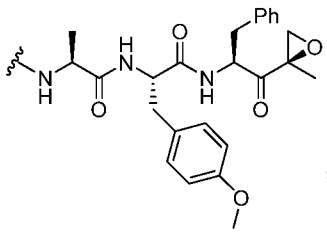
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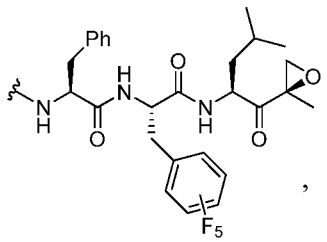
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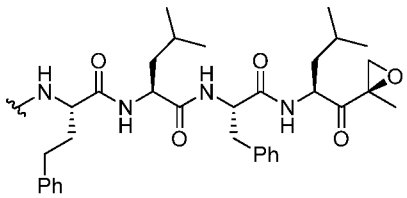
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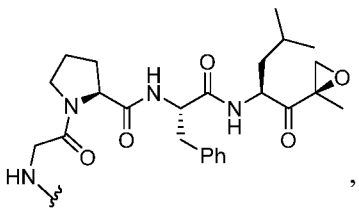
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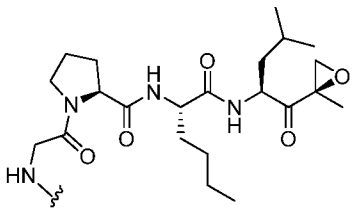
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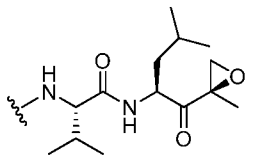
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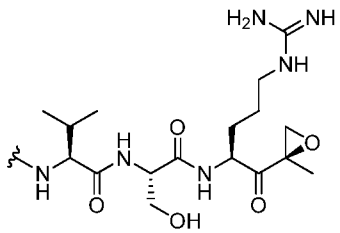
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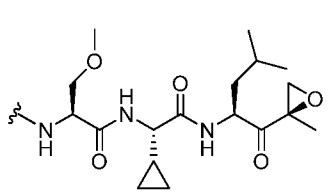
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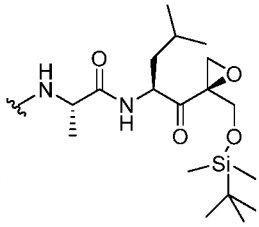
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29. The FAP-activated prodrug of any of claims 1-28, wherein **A** further comprises a self-eliminating linker attached to **B** by a chemical bond.

30. The FAP-activated prodrug of claim 29, wherein the self-eliminating linker is *p*-aminobenzyloxocarbonyl (PABC) or 2,4-bis(hydroxymethyl)aniline.

31. A compound or a pharmaceutically acceptable salt thereof represented by the formula:

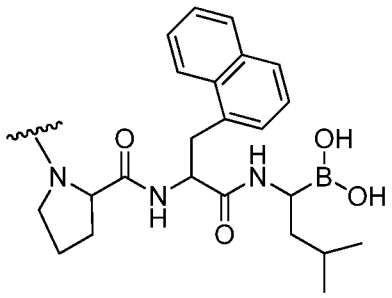
R-Xaa$_1$-Xaa$_2$-Y

wherein

R is an acyl group;

Xaa$_1$ is selected from the group consisting of Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, and Tyr;

Xaa$_2$ is selected from the group consisting of Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, and Tyr; and

Y is 

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32. The FAP-activated prodrug of claim 31, wherein the compound further comprises a self-eliminating linker with a chemical bond to the carboxyl terminus of Xaa$_2$ and a chemical bond to **Y**.

33. The FAP-activated prodrug of claim 31, wherein the self-eliminating linker is *p*-aminobenzyloxocarbonyl (PABC) or 2,4-bis(hydroxymethyl)aniline.

34. The compound of claim 31, wherein R is selected from the group consisting of formyl, acetyl, benzoyl, trifluoroacetyl, succinyl, and methoxysuccinyl.

35. The compound of claim 31, wherein R is succinyl or methoxysuccinyl.

36. The compound of claim 31, wherein R is succinyl.

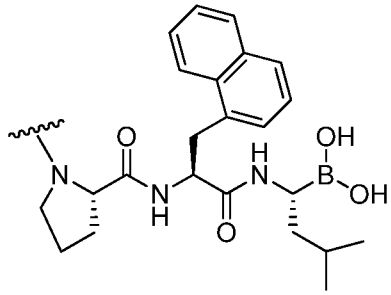
37. The compound of claim 31, wherein Xaa$_1$ is Cys, Met, Ser, or Thr.

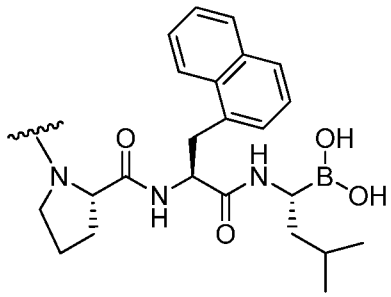
38. The compound of claim 31, wherein Xaa$_1$ is Ser.

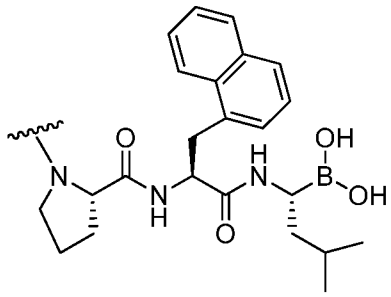
39. The compound of claim 31, wherein Xaa$_2$ is Ala, Gly, Ile, Leu, or Val.

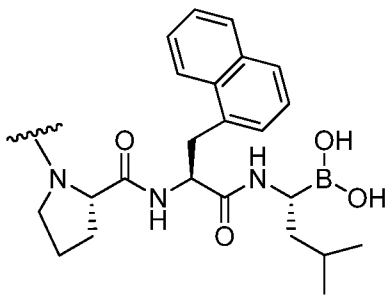
40. The compound of claim 31, wherein Xaa_2 is Ala.

41. The compound of claim 31, wherein Xaa_2 is (D)-Ala.

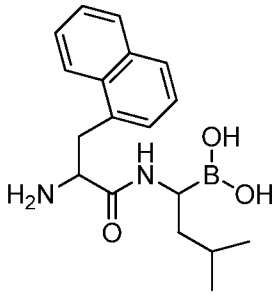
42. The compound of claim 31, wherein Y is  .

43. The compound of claim 31, wherein R is selected from the group consisting of formyl, acetyl, benzoyl, trifluoroacetyl, succinyl, and methoxysuccinyl; Xaa_1 is Cys, Met, Ser, or Thr; Xaa_2 is Ala, Gly, Ile, Leu, or Val; and Y is  .

44. The compound of claim 31, wherein R is succinyl or methoxysuccinyl; Xaa_1 is Ser; Xaa_2 is Ala; and Y is  .

45. The compound of claim 31, wherein R is succinyl; Xaa_1 is Ser; Xaa_2 is (D)-Ala; and Y is  .

46. A compound or a pharmaceutically acceptable salt thereof represented by



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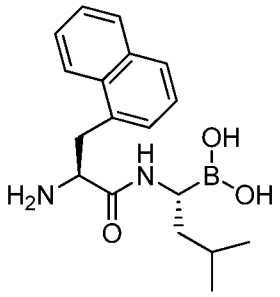
47. The compound of claim 46, wherein the compound is represented by



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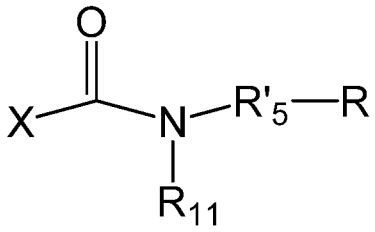
48. A FAP-activated proteasome inhibitor represented by formula I:



(I)

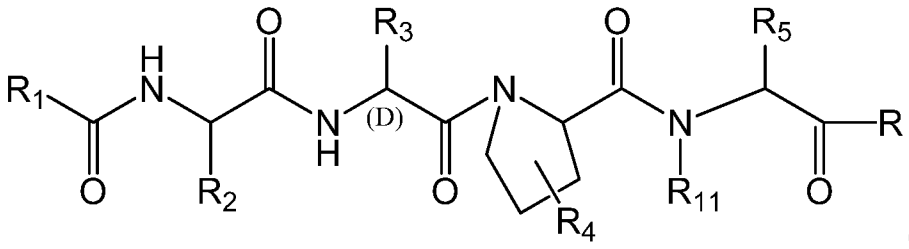
or a pharmaceutically acceptable salt thereof,

wherein

X-C(=O)NR_{11}-R'_5- represents the FAP substrate sequence, X is an N-acyl peptidyl group, -NR_{11}-R'_5 is an amino acid residue or analog thereof that binds the P'_1 specificity subsite of FAP, and the FAP substrate sequence is cleaved by FAP to release NHR_{11}-R'_5-R; R_{11} represents H or lower alkyl; and

NHR_{11}-R'_5-R is a proteasome inhibitor.

49. The FAP-activated proteasome inhibitor of claim 48, represented by formula II:



(II)

wherein

R_1-(C=O)- represents an acyl N-terminal blocking group;

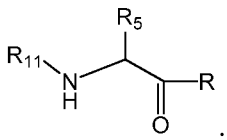
R_2 represents H, lower alkyl, or a mono- or di-hydroxy-substituted lower alkyl;

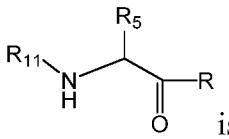
R_3 represents H, halogen, or lower alkyl;

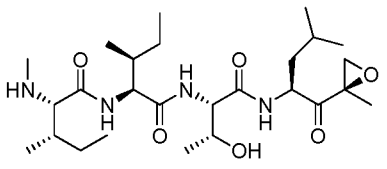
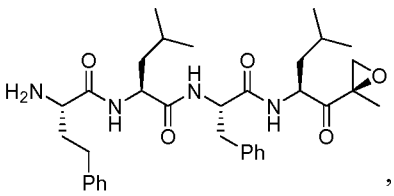
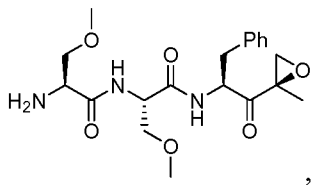
R_4 is absent or represents lower alkyl, -OH, $-NH_2$ or halogen;

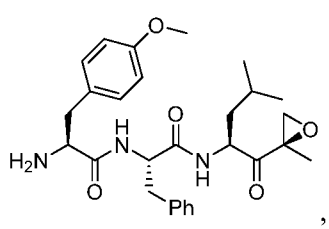
R_5 represents a large hydrophobic amino acid sidechain;

R_{11} represents H or lower alkyl; and

the FAP-activated proteasome inhibitor is cleaved by FAP to release a proteasome inhibitor represented by  .

50. The FAP-activated proteasome inhibitor of claim 49, wherein  is selected from the group consisting of:

 ,  ,  ,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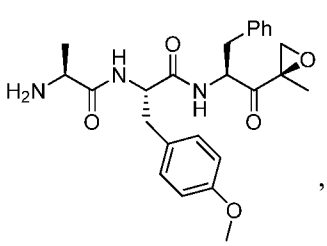
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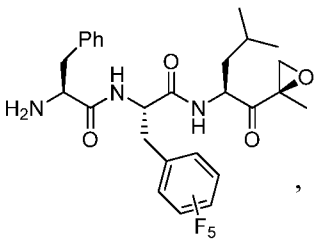
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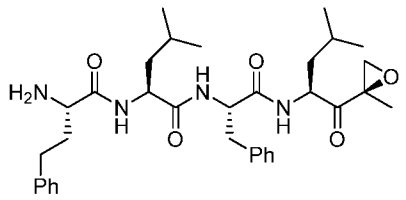
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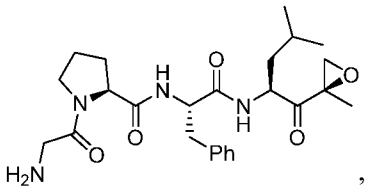
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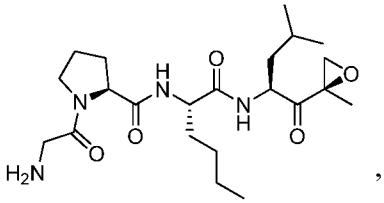
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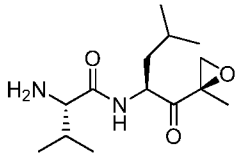
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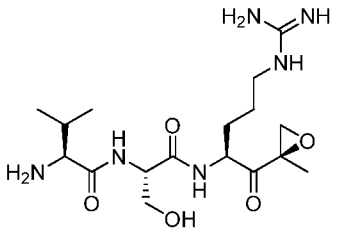
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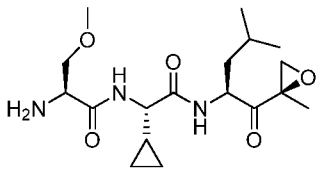
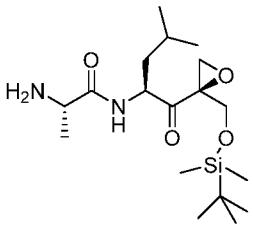

, and

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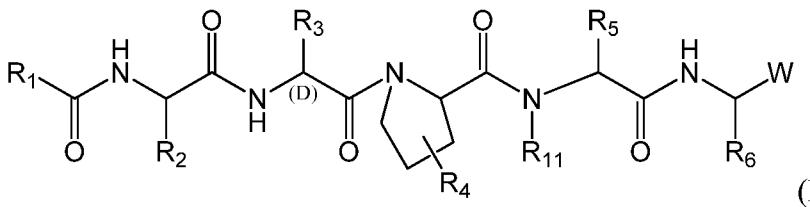
51. The FAP-activated proteasome inhibitor of claim 49, represented by formula III:


(III)

wherein

R_1-(C=O)- represents an acyl N-terminal blocking group;

R_2 represents H, lower alkyl, or a mono- or di-hydroxy-substituted lower alkyl;

R_3 represents H, halogen, or lower alkyl;

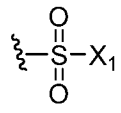
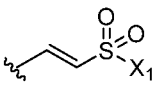
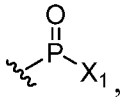
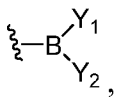
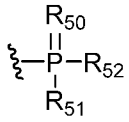
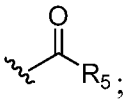
R_4 is absent or represents lower alkyl, -OH, -NH_2 or halogen;

R_5 represents a large hydrophobic amino acid sidechain;

R_6 represents alkyl, cycloalkyl, aryl, heterocycle or $-(CH_2)_n-R_7$;

R_7 represents aryl, aralkyl, cycloalkyl, alkoxy, alkylthio, -OH or -SH;

R_{11} represents H or lower alkyl;

W represents -CN, an epoxyketone, -CH=NR$_5$,  ,  ,  ,  ,  , or  ;

R_8 represents H, alkyl, alkenyl, alkynyl, $-C(X_1)(X_2)X_3$, $-(CH_2)_m-R_9$, $-(CH_2)_n-OH$, $-(CH_2)_n-O-alkyl$, $-(CH_2)_n-O-alkenyl$, $-(CH_2)_n-O-alkynyl$, $-(CH_2)_n-O-(CH_2)_m-R_9$, $-(CH_2)_n-SH$, $-(CH_2)_n-S-alkyl$, $-(CH_2)_n-S-alkenyl$, $-(CH_2)_n-S-alkynyl$, $-(CH_2)_n-S-(CH_2)_m-R_9$, $-C(=O)C(=O)NH_2$, $-C(=O)C(=O)OR_{10}$;

R_9 represents, independently for each occurrence, a substituted or unsubstituted aryl, aralkyl, cycloalkyl, cycloalkenyl, or heterocycle;

R_{10} represents, independently for each occurrence, hydrogen, or a substituted or unsubstituted alkyl, alkenyl, aryl, aralkyl, cycloalkyl, cycloalkenyl, or heterocycle;

Y_1 and Y_2 can independently or together be OH, or a group capable of being hydrolyzed to a hydroxyl group, including cyclic derivatives where Y_1 and Y_2 are connected via a ring having from 5 to 8 atoms in the ring structure;

R_{50} represents O or S;

R_{51} represents N_3, SH_2, NH_2, NO_2 or $-OR_{10}$;

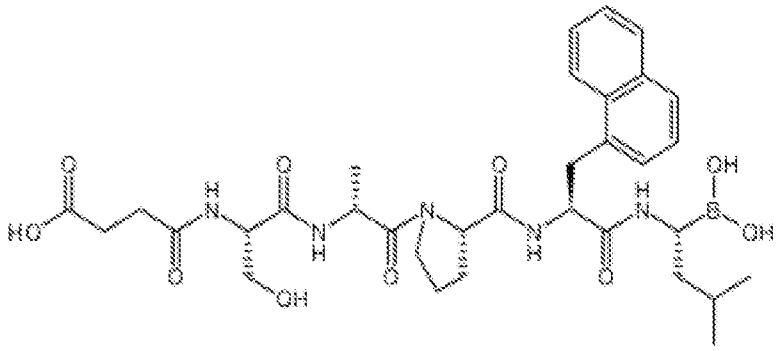
R_{52} represents hydrogen, lower alkyl, amine, $-OR_{10}$, or a pharmaceutically acceptable salt, or R_{51} and R_{52} taken together with the phosphorous atom to which they are attached complete a heterocyclic ring having from 5 to 8 atoms in the ring structure;

X_1 is halogen;

X_2 and X_3 each represent H or halogen;

m is zero or an integer in the range of 1 to 8; and n is an integer in the range of 1 to 8.

52. The FAP-activated proteasome inhibitor of claim 51, represented by



53. A pharmaceutical composition, comprising a compound of any one of claims 1-52; and a pharmaceutically acceptable excipient.

54. A method of inhibiting proteasome function in a cell, comprising contacting the cell with an effective amount of a compound of any one of claims 1-52.

55. A method of reducing the rate of muscle protein degradation in a cell, comprising contacting the cell with an effective amount of a compound of any one of claims 1-52.

56. A method of reducing the activity of NF-κB in a cell, comprising contacting the cell with an effective amount of a compound of any one of claims 1-52.

57. A method of reducing the rate of proteasome-dependent intracellular protein breakdown, comprising contacting a cell with an effective amount of a compound of any one of claims 1-52.

58. A method of reducing the rate of degradation of p53 protein in a cell, comprising contacting the cell with an effective amount of a compound of any one of claims 1-52.

59. A method of inhibiting cyclin degradation in a cell, comprising contacting the cell with an effective amount of a compound of any one of claims 1-52.

60. A method of inhibiting antigen presentation in a cell, comprising contacting the cell with an effective amount of a compound of any one of claims 1-52.

61. A method of treating cancer, psoriasis, restenosis, or other cell proliferative disease, comprising administering to a mammal in need thereof a therapeutically effective amount of a compound of any one of claims 1-52.

62. A method of treating cancer, psoriasis, restenosis, or other cell proliferative disease, comprising co-administering to a mammal in need thereof a therapeutically effective amount of a compound of any one of claims 1-52; and a therapeutically effective amount of a chemotherapeutic agent.

63. The method of claim 62, wherein the chemotherapeutic agent is docetaxel, paclitaxel, imatinib mesylate, gemcitabine, cis-platin, carboplatin, 5-fluorouracil, pemetrexed, methotrexate, doxorubicin, lenalidomide, dexamethasone, or monomethyl auristatin.

64. The method of claim 62, wherein the chemotherapeutic agent is docetaxel, gemcitabine, carboplatin, or doxorubicin.

65. The method of claim 62, wherein the chemotherapeutic agent is MG-132, PSI, fellutamide B, bortezomib, CEP-18770, MLN-2238, MLN-9708, epoxomicin, carfilzomib (PR-171), NC-005, YU-101, LU-005, YU-102, NC-001, LU-001, NC-022, PR-957 (LMP7), CPSI (β5), LMP2-sp-ek, BODIPY-NC-001, azido-NC-002, ONX-0912, omuralide, PS-519, marizomib, belactosin A, ^{125}I-NIP-L_3VS, NC-005-VS, or MV151.

66. A method of treating cancer, comprising administering to a mammal in need thereof a therapeutically effective amount of a compound of any one of claims 1-52.

67. The method of claim 66, wherein the cancer is a solid tumor.

68. The method of claim 66, further comprising administering to a mammal in need thereof a therapeutically effective amount of a chemotherapeutic agent.

69. The method of claim 68, wherein the cancer is a solid tumor.

70. The method of claim 69, wherein the chemotherapeutic agent is docetaxel, paclitaxel, imatinib mesylate, gemcitabine, cis-platin, carboplatin, 5-fluorouracil, pemetrexed, methotrexate, doxorubicin, lenalidomide, dexamethasone, or monomethyl auristatin.

71. The method of claim 70, wherein the chemotherapeutic agent is docetaxel, gemcitabine, carboplatin, or doxorubicin.

72. The method of claim 69, wherein the chemotherapeutic agent is MG-132, PSI, fellutamide B, bortezomib, CEP-18770, MLN-2238, MLN-9708, epoxomicin, carfilzomib (PR-171), NC-005, YU-101, LU-005, YU-102, NC-001, LU-001, NC-022, PR-957

(LMP7), CPSI (β5), LMP2-sp-ek, BODIPY-NC-001, azido-NC-002, ONX-0912, omuralide, PS-519, marizomib, belactosin A, ^{125}I-NIP-L_3VS, NC-005-VS, or MV151.

73. A method of reducing the rate of loss of muscle mass in a mammal, comprising administering to a mammal in need thereof a therapeutically effective amount of a compound of any one of claims 1-52.

74. A method of reducing the activity of NF-κB in a mammal, comprising administering to a mammal in need thereof a therapeutically effective amount of a compound of any one of claims 1-52.

75. A method of reducing the rate of proteasome-dependent intracellular protein breakdown in a mammal, comprising administering to a mammal in need thereof a therapeutically effective amount of a compound of any one of claims 1-52.

76. A method of reducing the rate of degradation of p53 protein in a mammal, comprising administering to a mammal in need thereof a therapeutically effective amount of a compound of any one of claims 1-52.

77. A method of inhibiting cyclin degradation in a mammal, comprising administering to a mammal in need thereof a therapeutically effective amount of a compound of any one of claims 1-52.

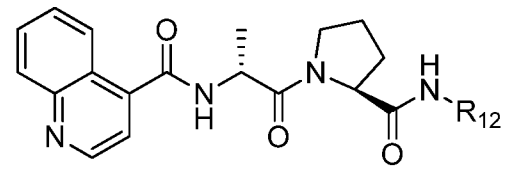
78. A method of inhibiting antigen presentation in a mammal, comprising administering to a mammal in need thereof a therapeutically effective amount of a compound of any one of claims 1-52.

79. A method of inhibiting inducible NF-κB dependent cell adhesion in a mammal, comprising administering to a mammal in need thereof a therapeutically effective amount of a compound of any one of claims 1-52.

80. A method of inhibiting HIV infection in a mammal, comprising administering to a mammal in need thereof a therapeutically effective amount of a compound of any one of claims 1-52.

81. A method of quantifying the amount of FAP expressed by or in the vicinity of a tumor in a mammal, comprising the steps of:

administering to said mammal an effective amount of a compound represented by Formula IV:



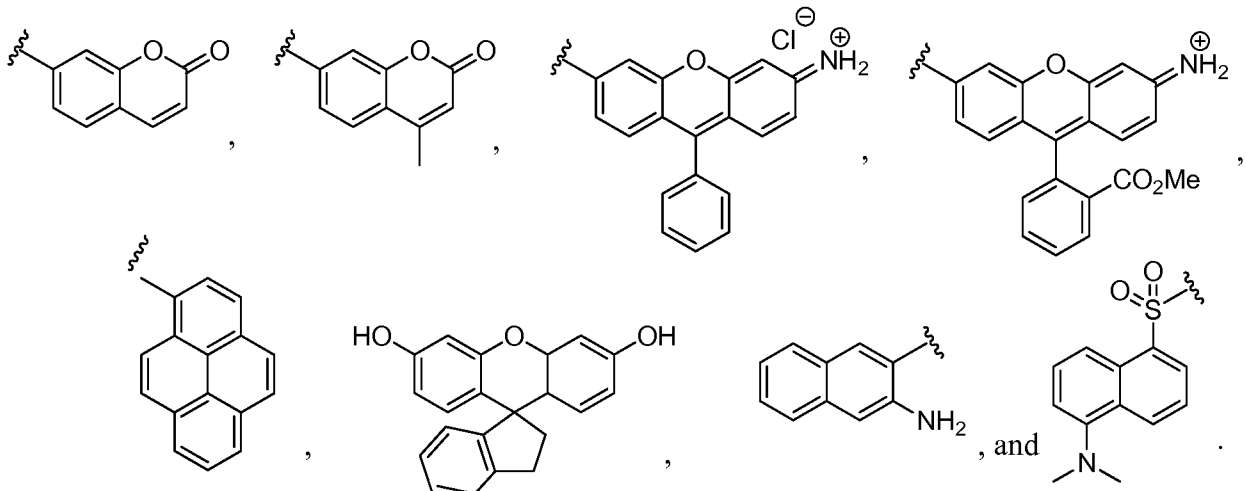
(IV)

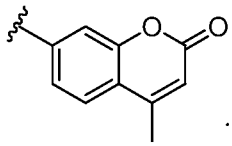
wherein R_{12} is a fluorophore or chromophore;

illuminating the mammal in the vicinity of the tumor; and

measuring the amount of fluorescence in the vicinity of the tumor.

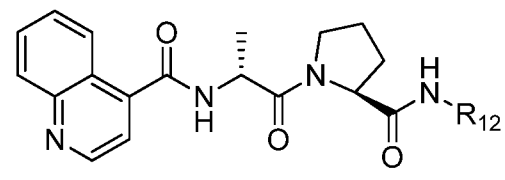
82. The method of claim 81, wherein R_{12} is selected from the group consisting of:



83. The method of claim 82, wherein R_{12} is  .

84. A method of quantifying the amount of FAP expressed by a tumor biopsy sample, comprising the steps of:

combining said tumor biopsy sample with an effective amount of a compound represented by Formula IV, thereby forming a mixture:



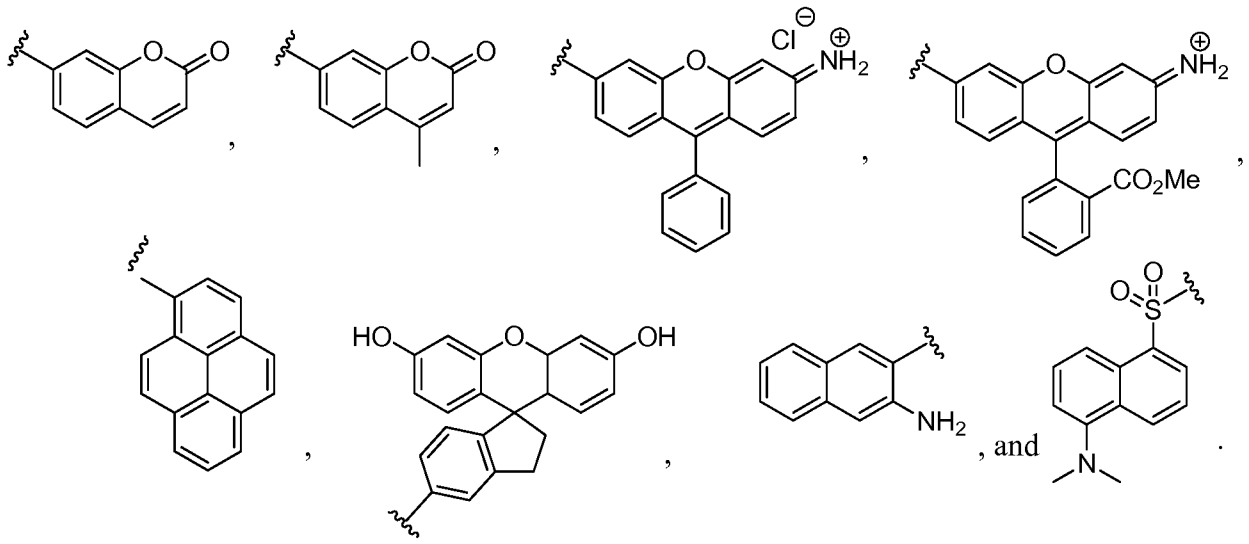
(IV)

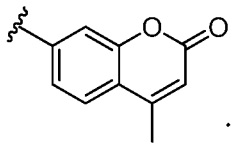
wherein R_{12} is a fluorophore or chromophore;

illuminating the mixture; and

measuring the amount of fluorescence in the mixture.

85. The method of claim 84, wherein R_{12} is selected from the group consisting of:



86. The method of claim 85, wherein R_{12} is  .

87. The method of any one of claims 61-83, wherein said mammal is a primate, equine, canine, feline, or bovine.

88. The method of any one of claims 61-83, wherein said mammal is a human.

89. The method of any one of claims 61-83, wherein the compound is administered to the mammal by inhalation, orally, intravenously, sublingually, ocularly, transdermally, rectally, vaginally, topically, intramuscularly, intra-arterially, intrathecally, subcutaneously, buccally, or intranasally.

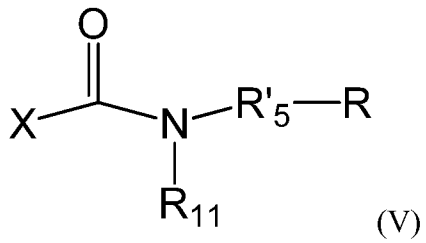
90. The method of any one of claims 61-83, wherein the compound is administered to the mammal intravenously.

91. A method for reducing local immunosuppression and/or tumor supporting-activity mediated by FAP+ stromal cells, comprising administering to a patient in need thereof a therapeutically effective amount of a prodrug of an active agent, wherein the active agent is

cytotoxic or inhibits protein expression or secretion to said FAP+ stromal cells, and is at least 2 fold more cytotoxic to the FAP+ stromal cells than the prodrug; and the prodrug (i) includes an FAP substrate sequence; (ii) is converted to the active agent by cleavage of the FAP substrate sequence by FAP, which substrate sequence has a k_{cat}/K_m for cleavage by FAP at least 10 fold more than for cleavage by prolyl endopeptidase EC 3.4.14.5 (PREP); and (iii) is selectively converted *in vivo* to the active agent by FAP+ stromal cells.

92. The method of claim 91, wherein the FAP substrate sequence has a k_{cat}/K_m for cleavage by FAP at least 10 fold more than for cleavage by other S9 prolyl endopeptidases.

93. The method of claim 91, wherein the prodrug is represented by formula V:



(V)

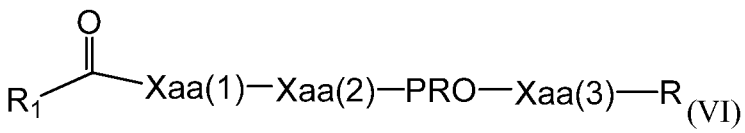
or a pharmaceutically acceptable salt thereof,

wherein

X-C(=O)NR_{11}-R'$_5$- represents the FAP substrate sequence, X is an N-acyl peptidyl group, -NR_{11}-R'$_5$ is an amino acid residue or analogue thereof that binds the P'$_1$ specificity subsite of FAP, and the FAP substrate sequence is cleaved by FAP to release NHR_{11}-R'$_5$-R; R_{11} represents H or lower alkyl; and

R'$_5$ and R taken together form the cytotoxic agent, or a moiety further metabolized at the site of the FAP+ stomal cells to form the cytotoxic agent.

94. The method of claim 93, wherein the prodrug is represented by formula VI:



(VI)

or a pharmaceutically acceptable salt thereof,

wherein

R_1-C(=O)- represents an acyl N-terminal blocking group;

Xaa(1) is an amino acid residue;

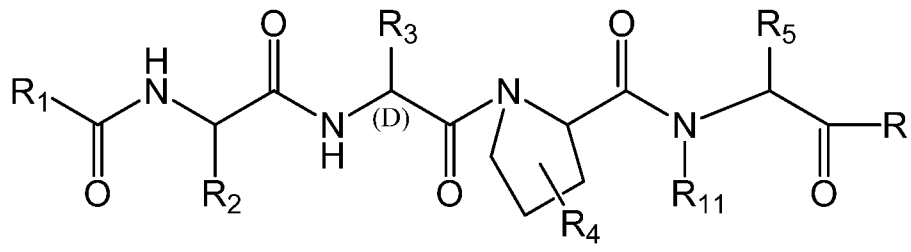
Xaa(2) is glycine, or a (D)-amino acid residue;

PRO represents a proline residue or an analogue thereof;

Xaa(3) is a large hydrophobic amino acid residue; and

the prodrug is cleaved by FAP to release Xaa(3)-R, and Xaa(3)-R is the cytotoxic agent.

95. The method of claim 93, wherein the prodrug is represented by formula VII:



(VII)

or a pharmaceutically acceptable salt thereof,

wherein

R_1-(C=O)- represents an acyl N-terminal blocking group;

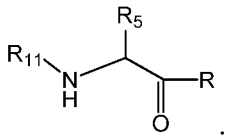
R_2 represents H, lower alkyl, or a mono- or di-hydroxy-substituted lower alkyl;

R_3 represents H, halogen, or lower alkyl;

R_4 is absent or represents lower alkyl, -OH, $-NH_2$ or halogen;

R_5 represents a large hydrophobic amino acid sidechain;

R_{11} represents H or lower alkyl; and

the prodrug is cleaved by FAP to release the cytotoxic agent,  .

96. The method of claim 94 or 95, wherein the acyl N-terminal blocking group is a moiety which, at physiological pH, reduces the cell permeability of said prodrug relative to said cytotoxic agent.

97. The method of claim 94 or 95, wherein the acyl N-terminal blocking group is selected from the group consisting of formyl, acetyl, benzoyl, trifluoroacetyl, succinyl and methoxysuccinyl.

98. The method of claim 94 or 95, wherein the acyl N-terminal blocking group includes one or more functional groups that are ionized at physiological pH.

99. The method of claim 94 or 95, wherein the acyl N-terminal blocking group includes one or more carboxyl groups.

100. The method of claim 94 or 95, wherein the acyl N-terminal blocking group is (lower alkyl)-C(=O)- substituted with one or more functional groups that are ionized at physiological pH.

101. The method of claim 94 or 95, wherein the acyl N-terminal blocking group is selected from the group consisting of aryl(C_1-C_6)acyl, and heteroaryl(C_1-C_6)acyl.

102. The method of claim 94 or 95, wherein the acyl N-terminal blocking group is an aryl(C_1-C_6)acyl, wherein aryl(C_1-C_6)acyl is a (C_1-C_6)acyl substituted with an aryl selected from the group consisting of benzene, naphthalene, phenanthrene, phenol and aniline.

103. The method of claim 94 or 95, wherein the acyl N-terminal blocking group is an heteroaryl(C_1-C_6)acyl, wherein heteroaryl(C_1-C_6)acyl is a (C_1-C_6)acyl substituted with a heteroaryl selected from the group consisting of pyrrole, furan, thiophene, imidazole, oxazole, thiazole, triazole, pyrazole, pyridine, pyrazine, pyridazine and pyrimidine.

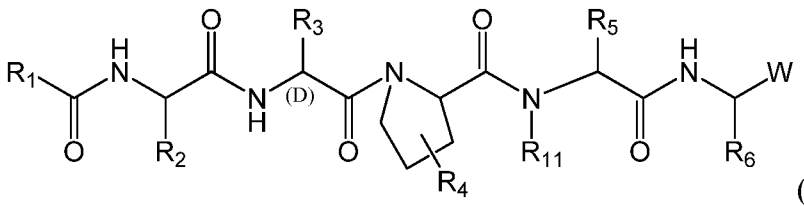
104. The method of claim 94 or 95, wherein the acyl N-terminal blocking group is represented by the formula –C(=O)-$(CH_2)_{1\text{-}10}$-C(=O)-OH.

105. The method of claim 104, wherein the acyl N-terminal blocking group is succinyl.

106. The method of any one of claims 93-95, wherein at least one of Xaa(1), Xaa(2) and Xaa(3) is a non-naturally occurring amino acid analogue.

107. The method of any one of claims 91-106, wherein the cytotoxic agent is a proteasome inhibitor.

108. The method of claim 95, wherein the proteasome inhibitor is represented by formula VIII:



(VIII)

or a pharmaceutically acceptable salt thereof,

wherein

R_1-(C=O)- represents an acyl N-terminal blocking group;

R_2 represents H, lower alkyl, or a mono- or di-hydroxy-substituted lower alkyl;

R_3 represents H, halogen, or lower alkyl;

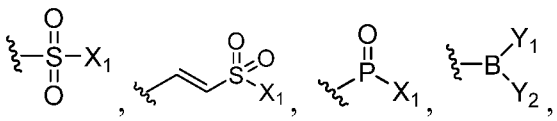
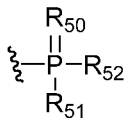
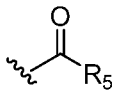
R_4 is absent or represents lower alkyl, -OH, $-NH_2$ or halogen;

R_5 represents a large hydrophobic amino acid sidechain;

R_6 is alkyl, cycloalkyl, aryl, heterocycle or $-(CH_2)_n-R_7$;

R_7 is aryl, aralkyl, cycloalkyl, alkoxy, alkylthio, -OH or -SH;

R_{11} represents H or lower alkyl;

W represents -CN, an epoxyketone, -CH=NR_5,  ,  , or  ;

R_8 represents H, an alkyl, an alkenyl, an alkynyl, $-C(X_1)(X_2)X_3$, $-(CH_2)_m-R_9$, $-(CH_2)_n$-OH, $-(CH_2)_n$-O-alkyl, $-(CH_2)_n$-O-alkenyl, $-(CH_2)_n$-O-alkynyl, $-(CH_2)_n$-O-$(CH_2)_m$-R_9, $-(CH_2)_n$-SH, $-(CH_2)_n$-S-alkyl, $-(CH_2)_n$-S-alkenyl, $-(CH_2)_n$-S-alkynyl, $-(CH_2)_n$-S-$(CH_2)_m$-R_9, $-C(=O)C(=O)NH_2$, $-C(=O)C(=O)OR_{10}$;

R_9 represents, independently for each occurrence, a substituted or unsubstituted aryl, aralkyl, cycloalkyl, cycloalkenyl, or heterocycle;

R_{10} represents, independently for each occurrence, hydrogen, or a substituted or unsubstituted alkyl, alkenyl, aryl, aralkyl, cycloalkyl, cycloalkenyl, or heterocycle;

Y_1 and Y_2 can independently or together be OH, or a group capable of being hydrolyzed to a hydroxyl group, including cyclic derivatives where Y_1 and Y_2 are connected via a ring having from 5 to 8 atoms in the ring structure;

R_{50} represents O or S;

R_{51} represents N_3, SH_2, NH_2, NO_2 or $-OR_{10}$;

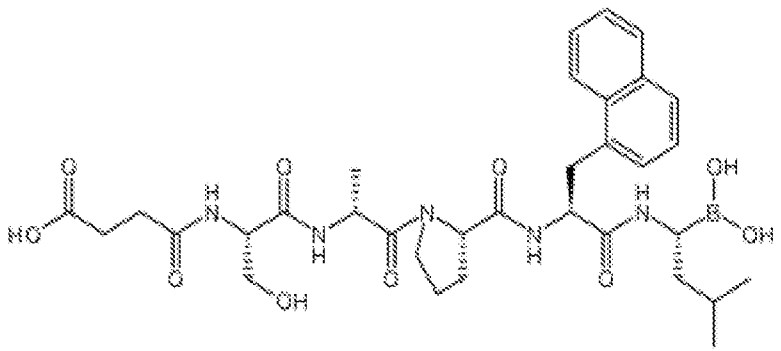
R_{52} represents hydrogen, lower alkyl, amine, $-OR_{10}$, or a pharmaceutically acceptable salt, or R_{51} and R_{52} taken together with the phosphorous atom to which they are attached complete a heterocyclic ring having from 5 to 8 atoms in the ring structure;

X_1 is halogen;

X_2 and X_3 each represent H or halogen; and

m is zero or an integer in the range of 1 to 8; and n is an integer in the range of 1 to 8.

109. The method of claim 108, wherein the proteasome inhibitor is



.

110. The method of claim 107, wherein the prodrug has a therapeutic index at least two times greater than the therapeutic index for the proteasome inhibitor when administered alone.

111. The method of any one of claims 91-110, wherein the prodrug is administered as a single agent therapy.

112. The method of any one of claims 91-110, wherein the prodrug is administered in a combination therapy with one or more anti-cancer agents.

113. The method of claim 112, wherein the anti-cancer agent is a covalent proteasome inhibitor.

114. The method of claim 112, wherein the anti-cancer agent is a chemotherapeutic.

115. The method of claim 114, wherein the chemotherapeutic is docetaxel, paclitaxel, imatinib mesylate, gemcitabine, cis-platin, carboplatin, 5-fluorouracil, pemetrexed, methotrexate, doxorubicin, lenalidomide, dexamethasone, or monomethyl auristatin.

116. The method of claim 114, wherein the chemotherapeutic is docetaxel, gemcitabine, carboplatin, or doxorubicin.

117. The method of claim 114, wherein the chemotherapeutic is MG-132, PSI, fellutamide B, bortezomib, CEP-18770, MLN-2238, MLN-9708, epoxomicin, carfilzomib (PR-171), NC-005, YU-101, LU-005, YU-102, NC-001, LU-001, NC-022, PR-957 (LMP7), CPSI (β5), LMP2-sp-ek, BODIPY-NC-001, azido-NC-002, ONX-0912, omuralide, PS-519, marizomib, belactosin A, ^{125}I-NIP-L_3VS, NC-005-VS, or MV151.

118. The method of claim 112, wherein the anti-cancer agent is an immunotherapeutic agent.

119. The method of claim 118, wherein the immunotherapeutic agent is an anti-tumor antibody.

120. The method of claim 118, wherein the immunotherapeutic agent is a tumor antigen vaccine or anti-tumor dendritic cell vaccine.

121. Use of a prodrug of any one of claims 1-52 in the manufacture of a medicament for the treatment of a disorder for which inhibition of proteasome activity provides therapeutic benefit.

122. A packaged pharmaceutical, comprising a prodrug of any one of claims 1-52 formulated in a pharmaceutically acceptable excipient, in association with instructions (written and/or pictorial) describing the recommended dosage and/or administration of the formulation to a patient.

Figure 1

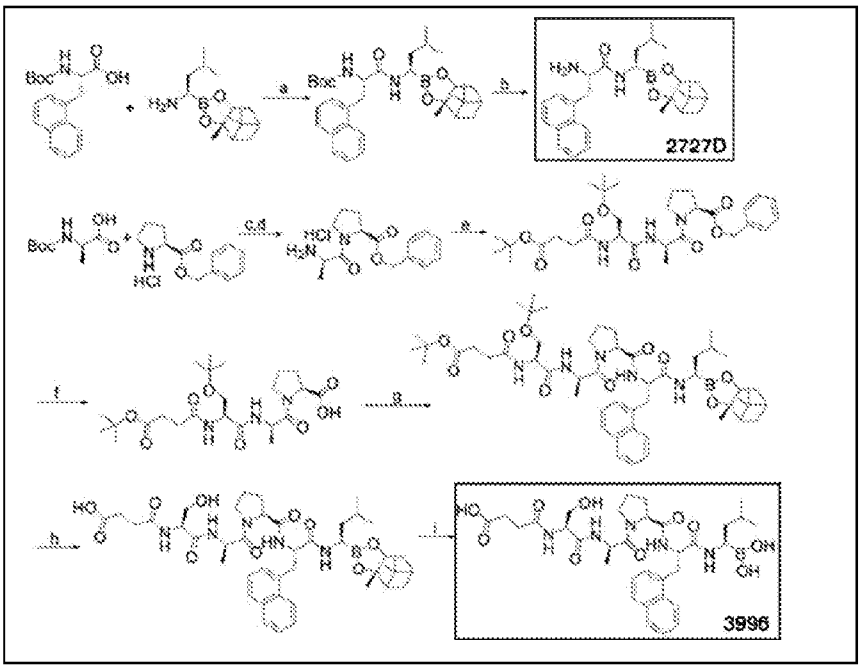


Figure 2

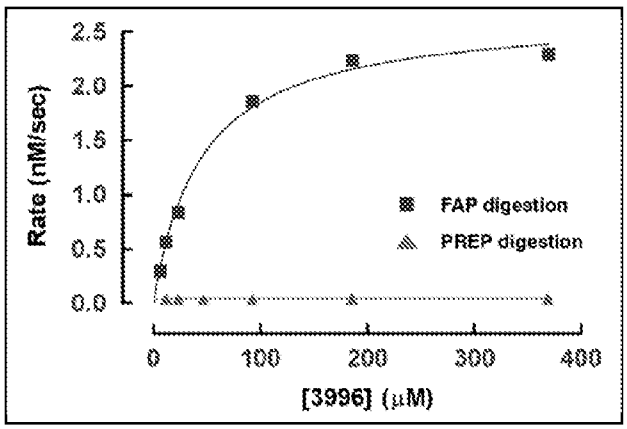

Rate (nM/sec)
2.5
2.0
1.5
1.0
0.5
0.0
0
100
200
300
400
[3996] (μM)
FAP digestion
PREP digestion

Figure 3

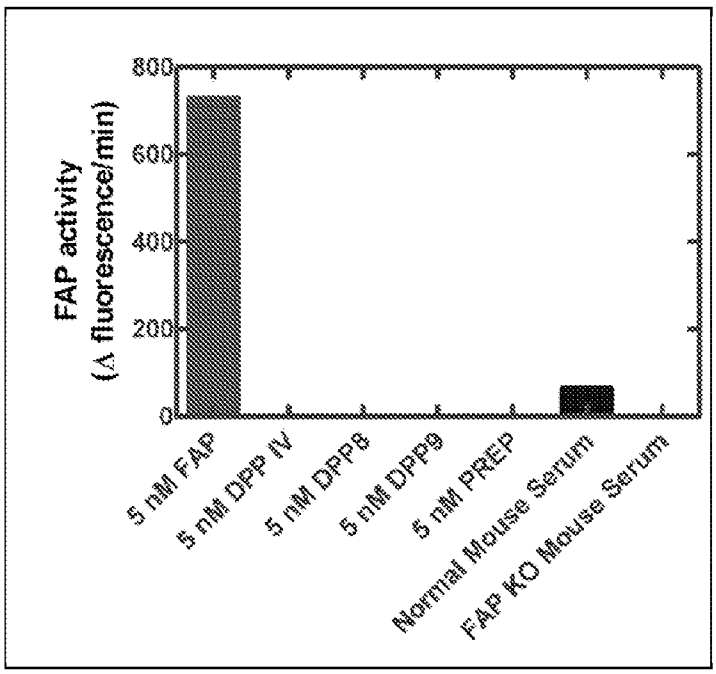

FAP activity
(Δ fluorescence/min)
800
600
400
200
0
5 nM FAP
5 nM DPP IV
5 nM DPP8
5 nM DPP9
5 nM PREP
Normal Mouse Serum
FAP KO Mouse Serum

Figure 4

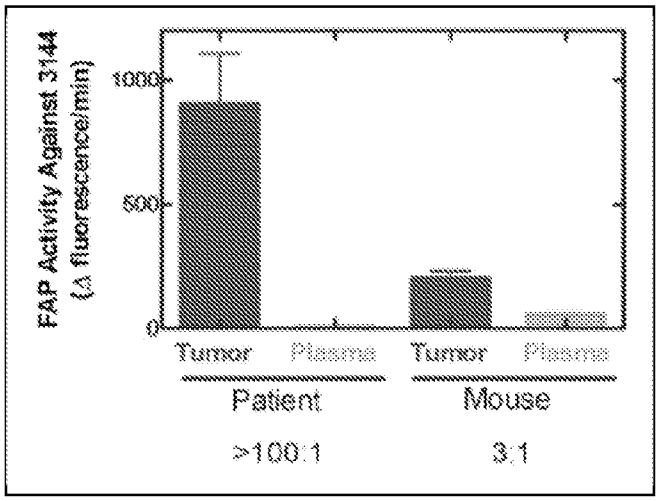

FAP Activity Against 3144
(Δ fluorescence/min)
1000
500
0
Tumor
Plasma
Tumor
Plasma
Patient
Mouse
>100:1
3:1

Figure 5

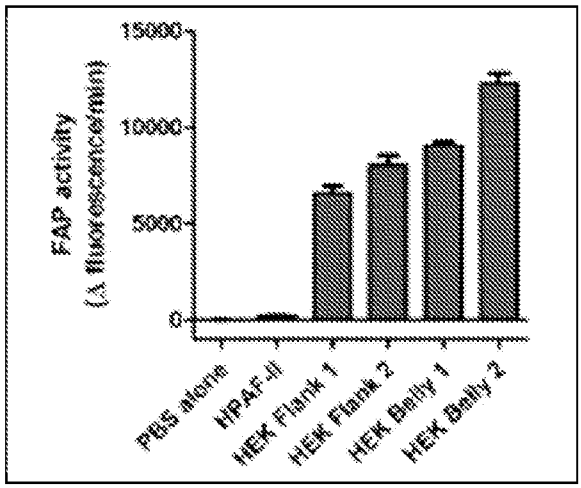

FAP activity
(Δ fluorescence/min)
15000
10000
5000
0
PBS alone
HPAF-II
HEK Flank 1
HEK Flank 2
HEK Belly 1
HEK Belly 2

Figure 6

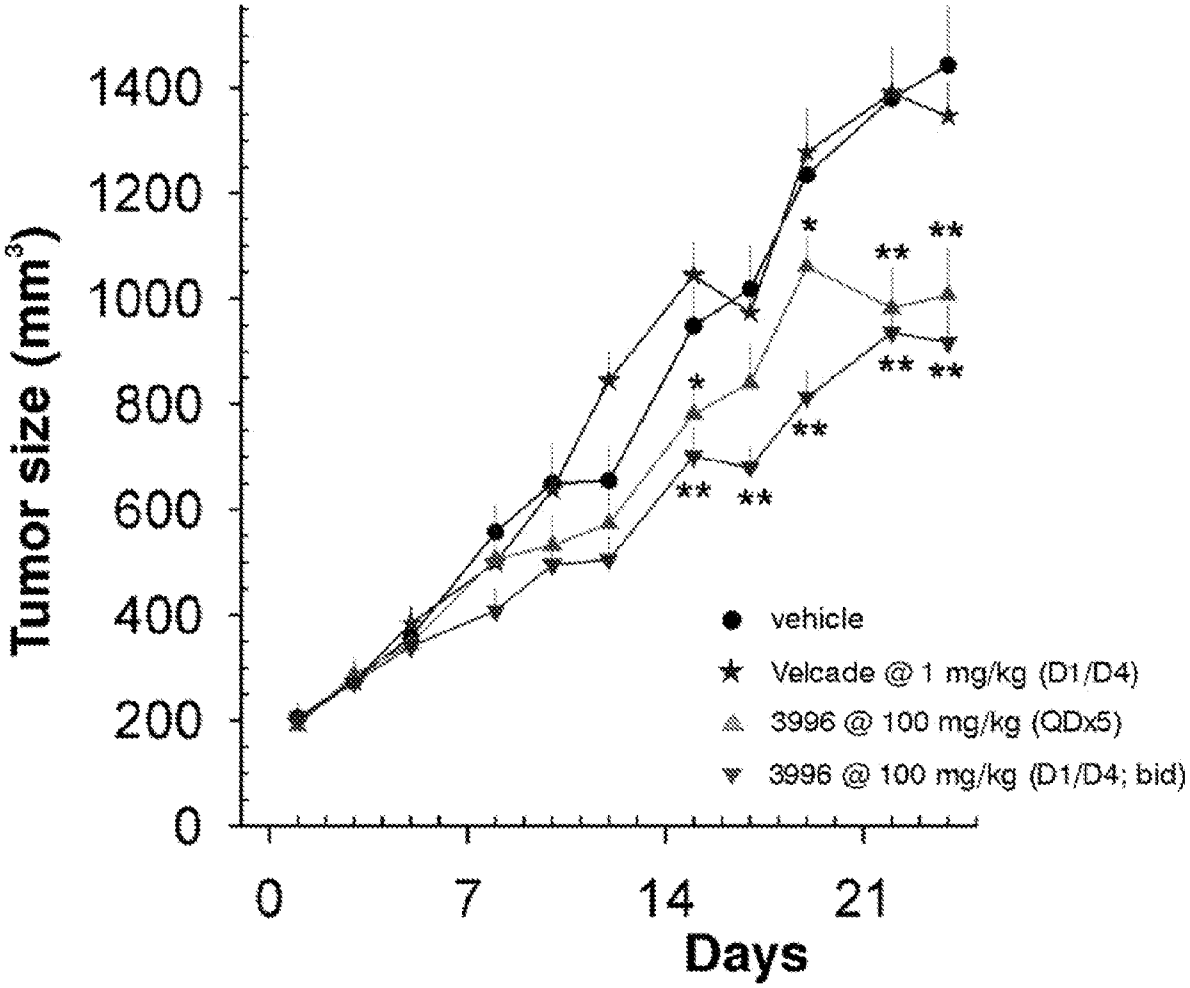

Tumor size (mm3)
1400
1200
1000
800
600
400
200
0
0
7
14
21
Days
vehicle
Velcade @ 1 mg/kg (D1/D4)
3996 @ 100 mg/kg (QDx5)
3996 @ 100 mg/kg (D1/D4; bid)
*
**

Figure 7

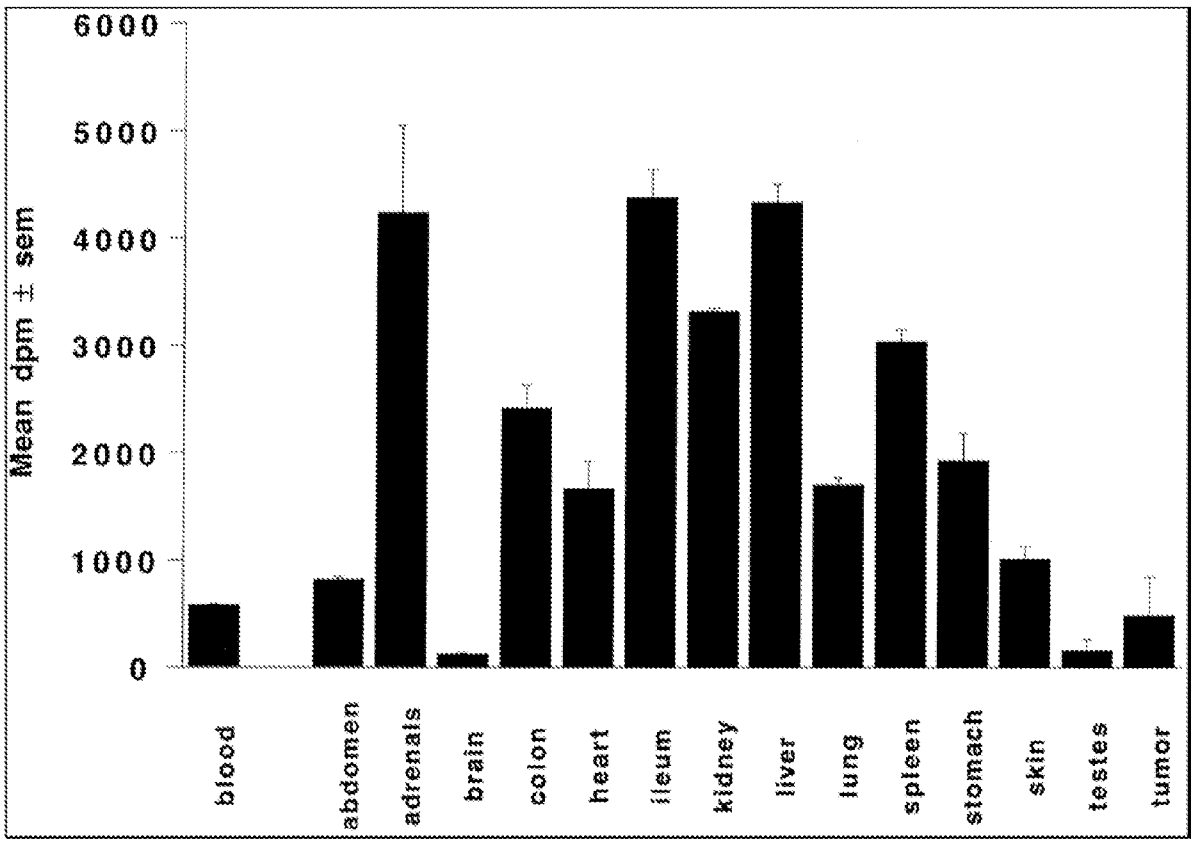

Mean dpm ± sem
6000
5000
4000
3000
2000
1000
0
blood
abdomen
adrenals
brain
colon
heart
ileum
kidney
liver
lung
spleen
stomach
skin
testes
tumor

Figure 8

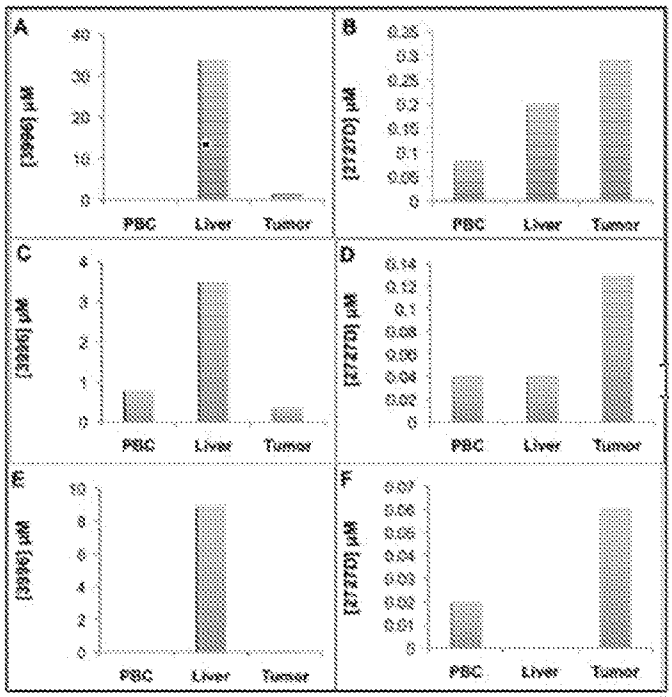


Figure 9

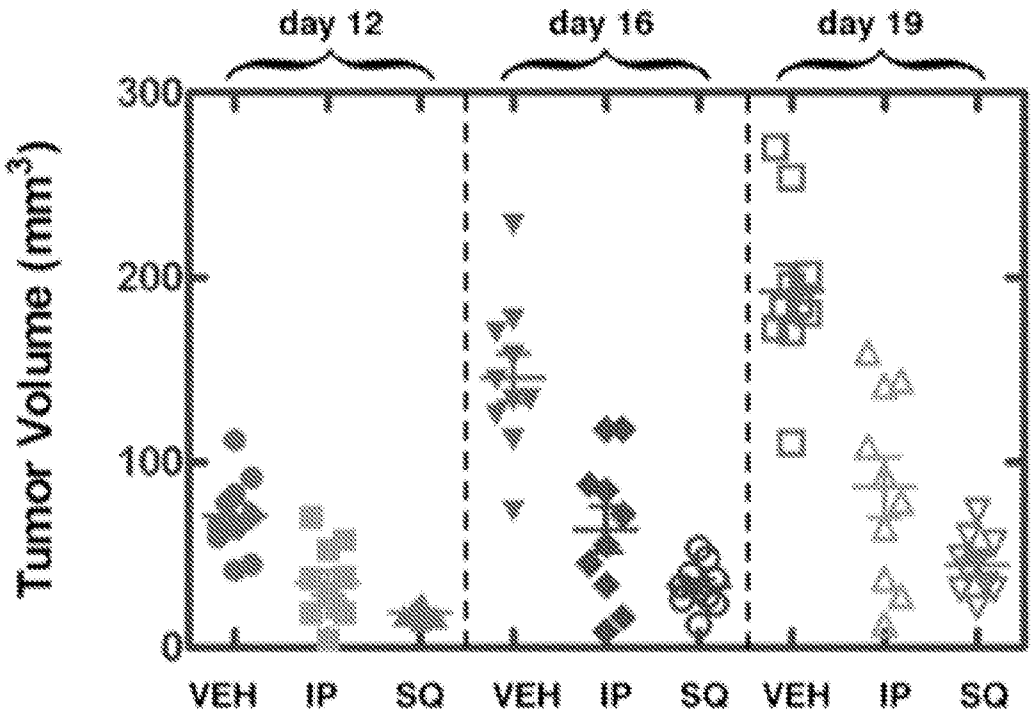
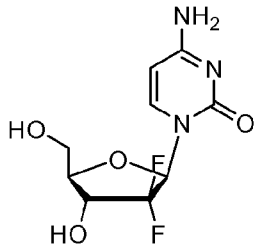

day 12
day 16
day 19
Tumor Volume (mm3)
300
200
100
0
VEH
IP
SQ
VEH
IP
SQ
VEH
IP
SQ

Figure 10



Gemcitabine

* Gemcitabine is the current standard of care for pancreatic cancer

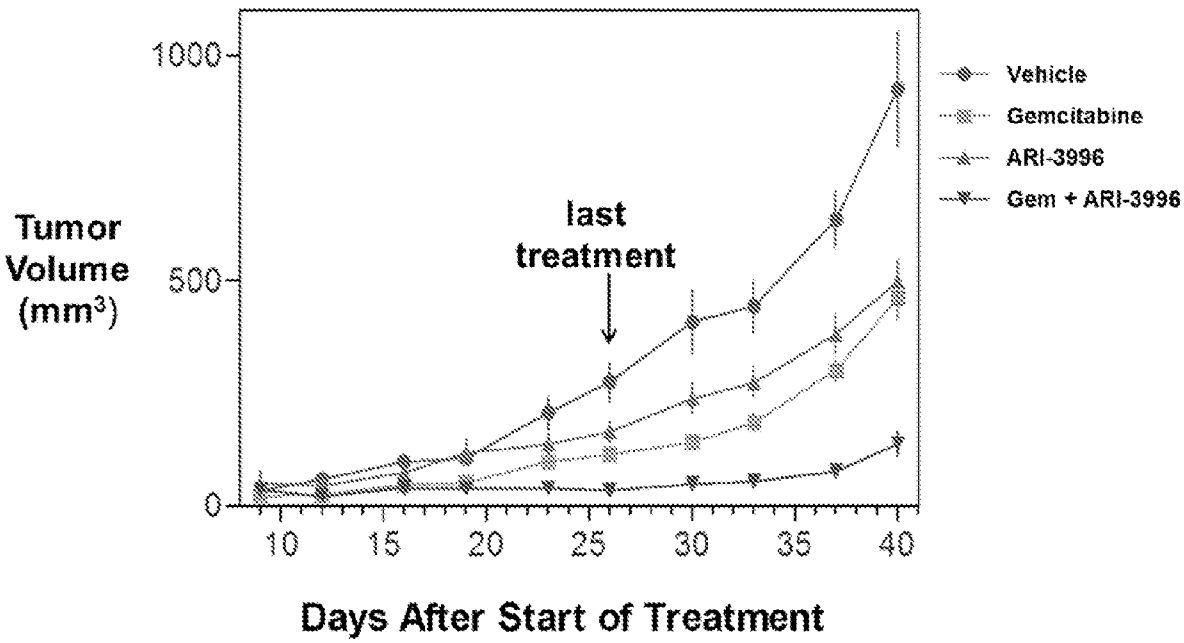


Figure 11

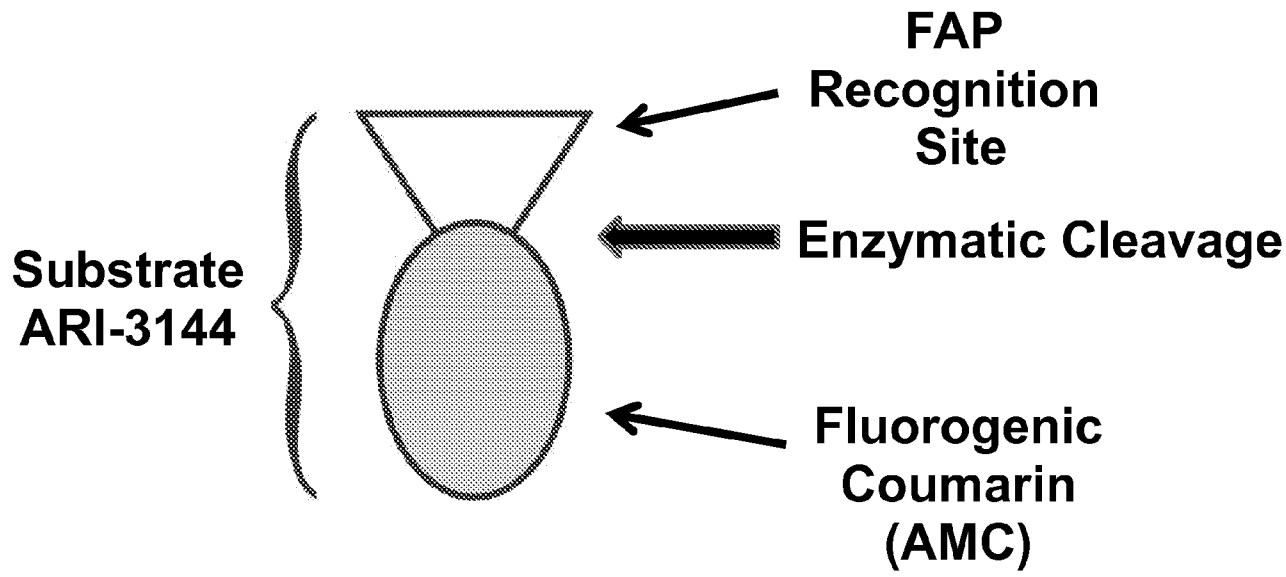

FAP
Recognition
Site
Enzymatic Cleavage
Substrate
ARI-3144
Fluorogenic
Coumarin
(AMC)

Figure 12

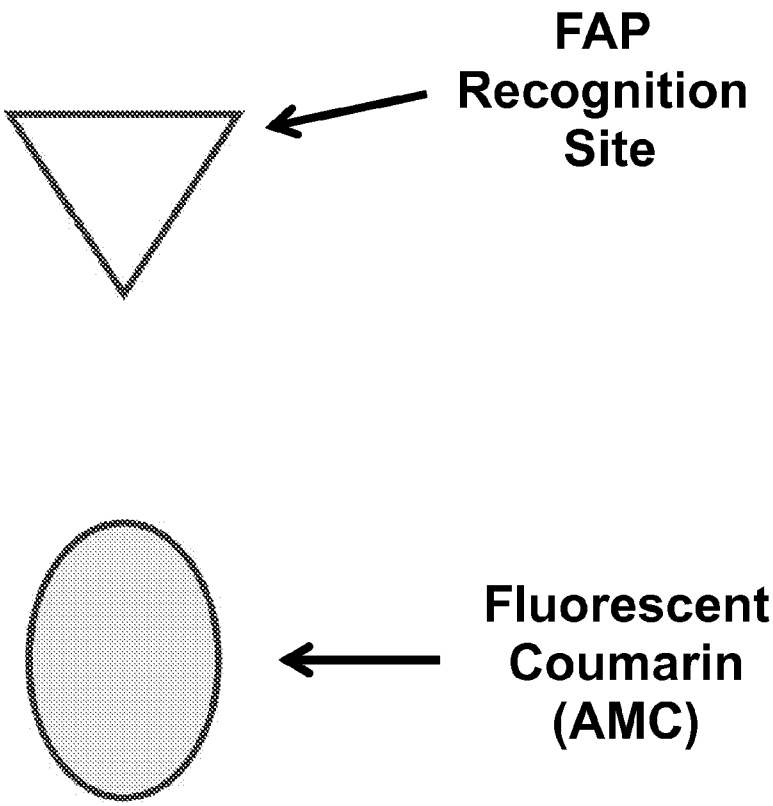


Figure 13

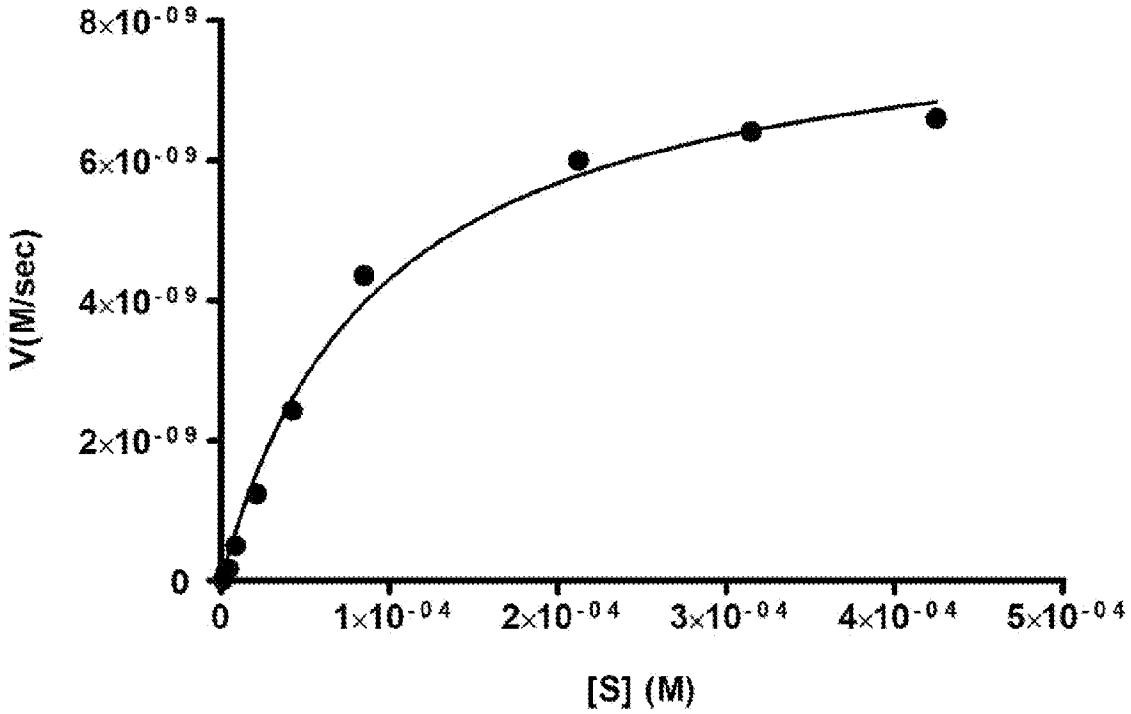

8×10-09
6×10-09
4×10-09
2×10-09
0
V(M/sec)
0
1×10-04
2×10-04
3×10-04
4×10-04
5×10-04
[S] (M)

Figure 14

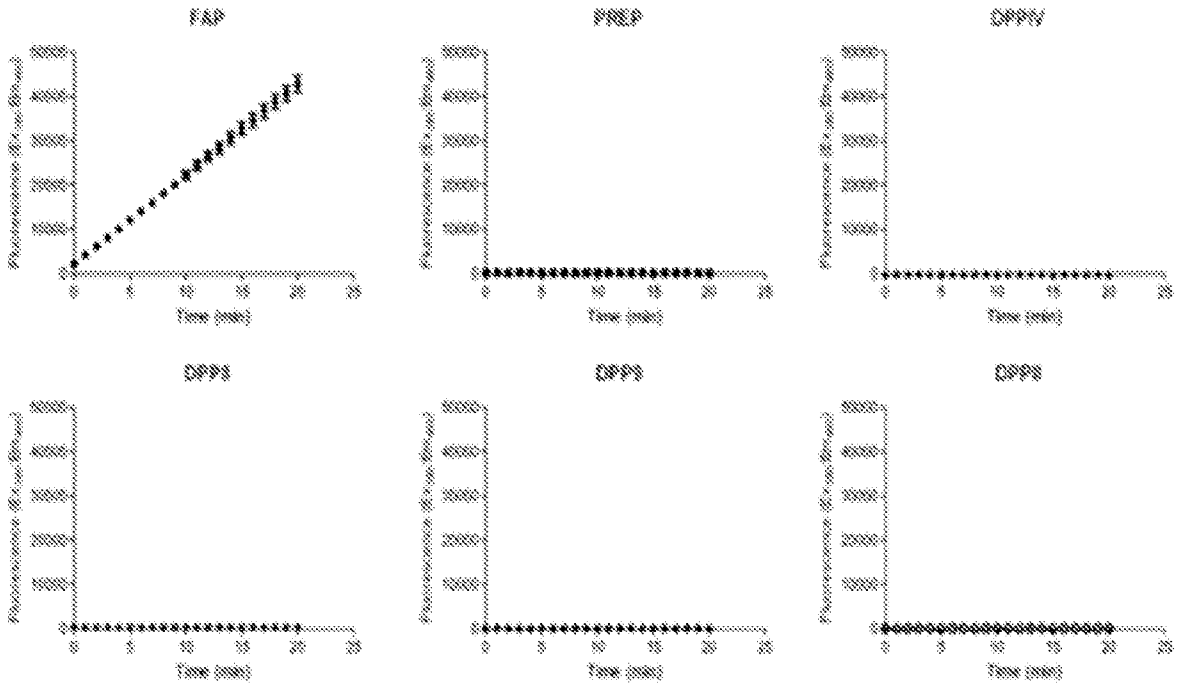


Figure 15

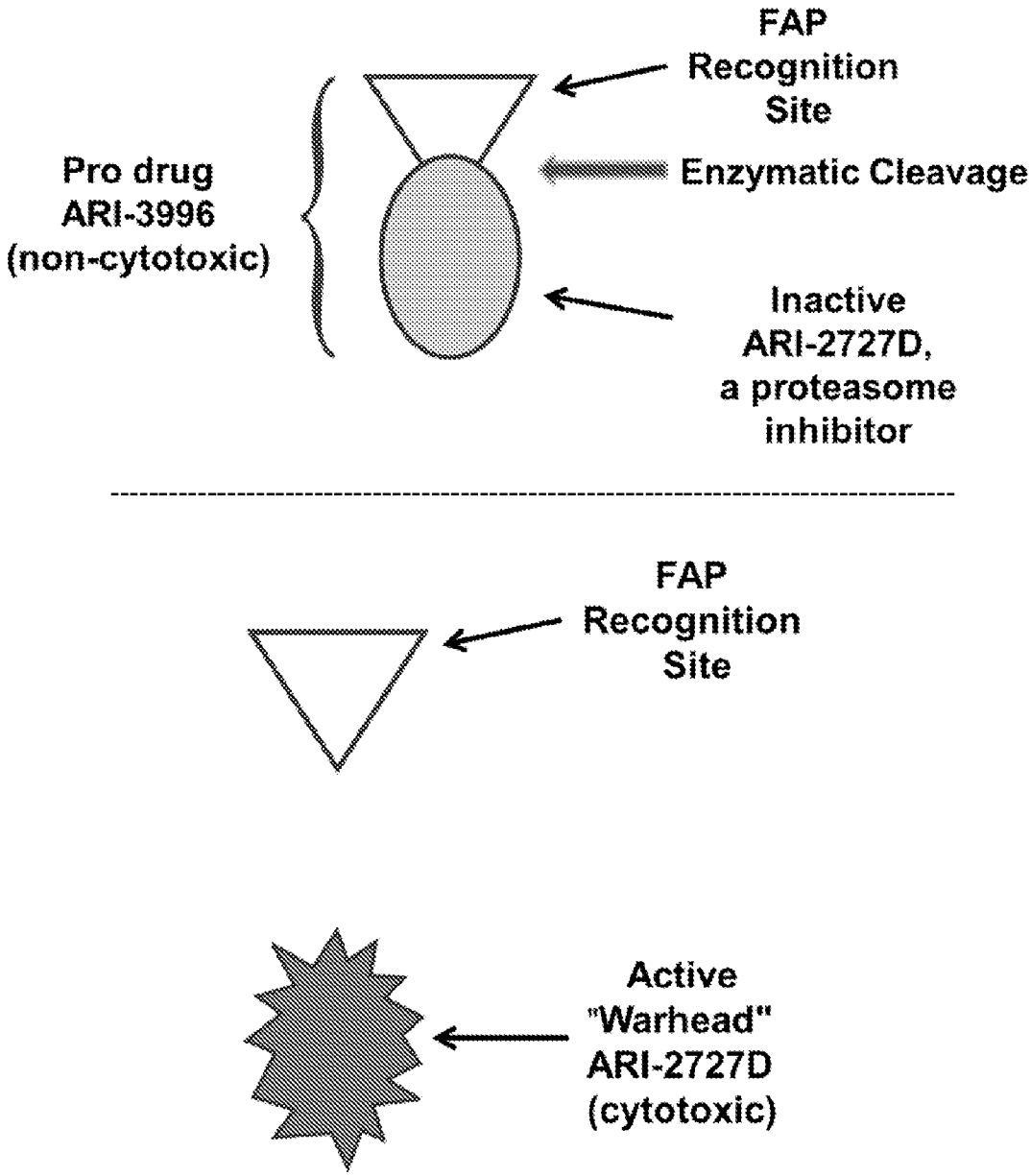

FAP
Recognition
Site
Enzymatic Cleavage
Pro drug
ARI-3996
(non-cytotoxic)
Inactive
ARI-2727D,
a proteasome
inhibitor
FAP
Recognition
Site
Active
"Warhead"
ARI-2727D
(cytotoxic)

Figure 16

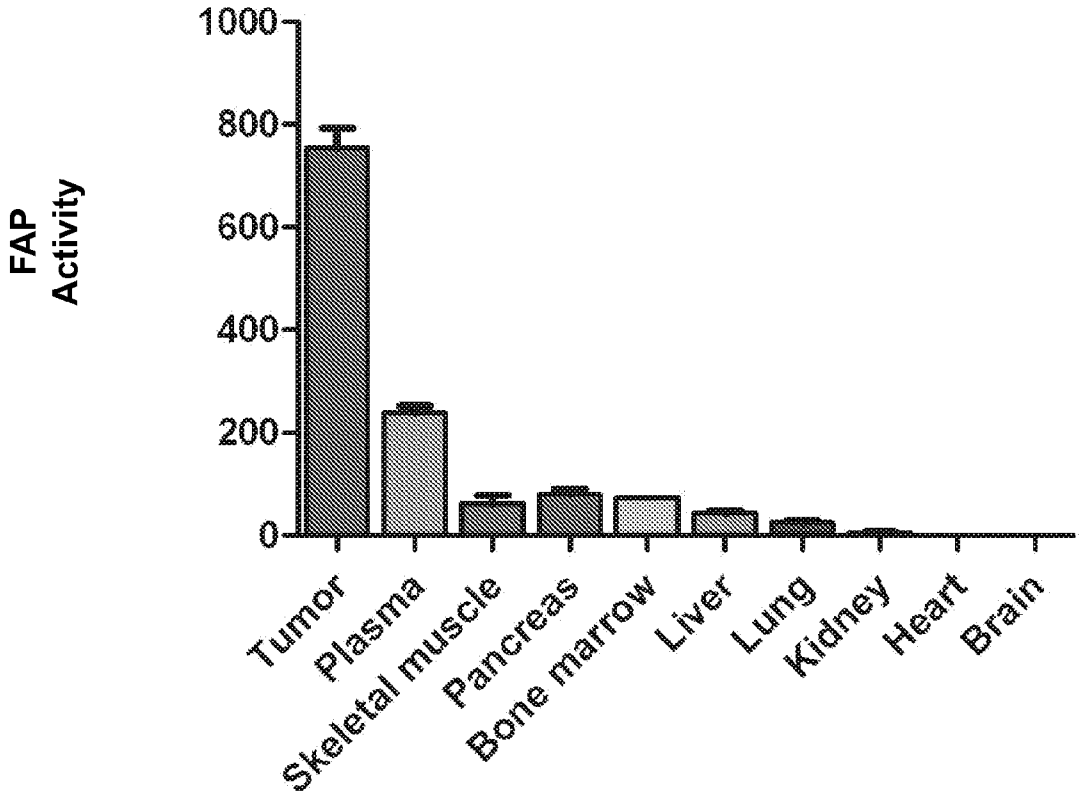

FAP Activity
1000
800
600
400
200
0
Tumor
Plasma
Skeletal muscle
Pancreas
Bone marrow
Liver
Lung
Kidney
Heart
Brain

Figure 17

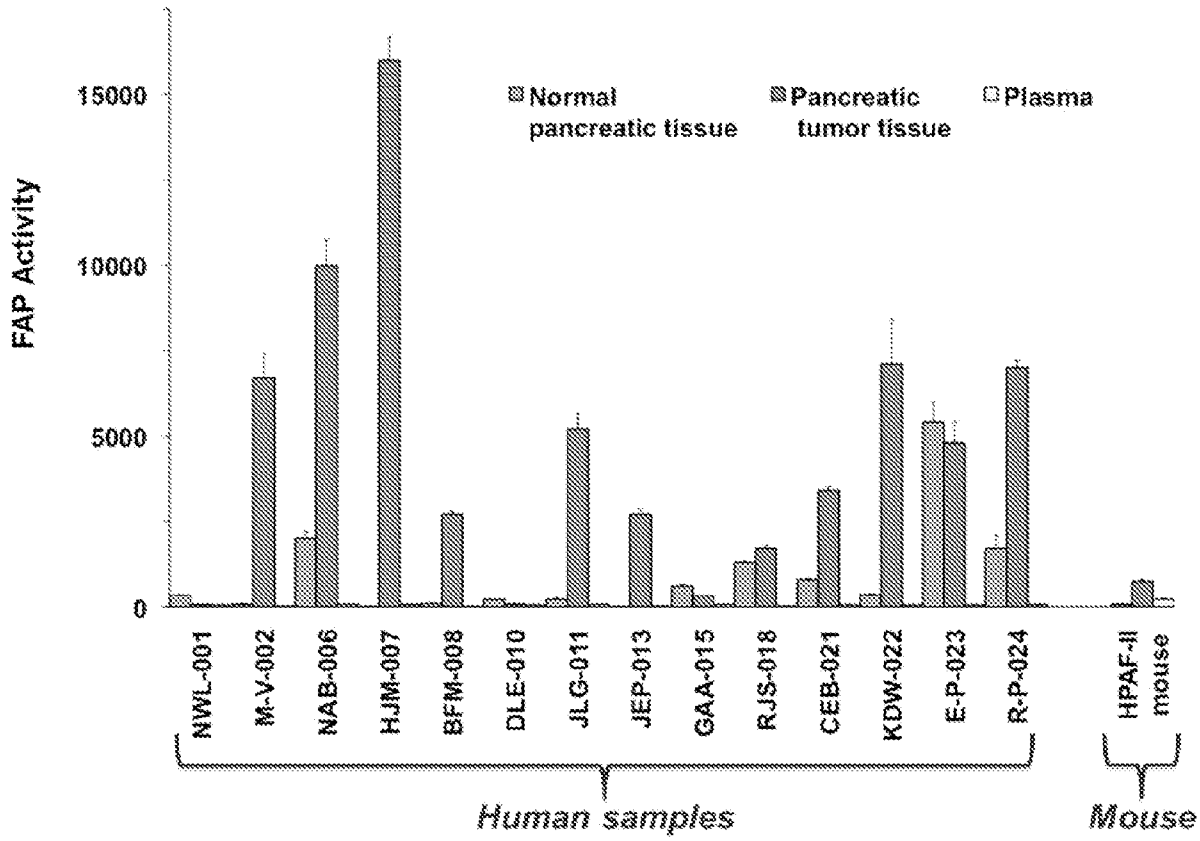

FAP Activity
15000
10000
5000
0
Normal pancreatic tissue
Pancreatic tumor tissue
Plasma
NWL-001
M-V-002
NAB-006
HJM-007
BFM-008
DLE-010
JLG-011
JEP-013
GAA-015
RJS-018
CEB-021
KDW-022
E-P-023
R-P-024
HPAF-II mouse
Human samples
Mouse

Figure 18

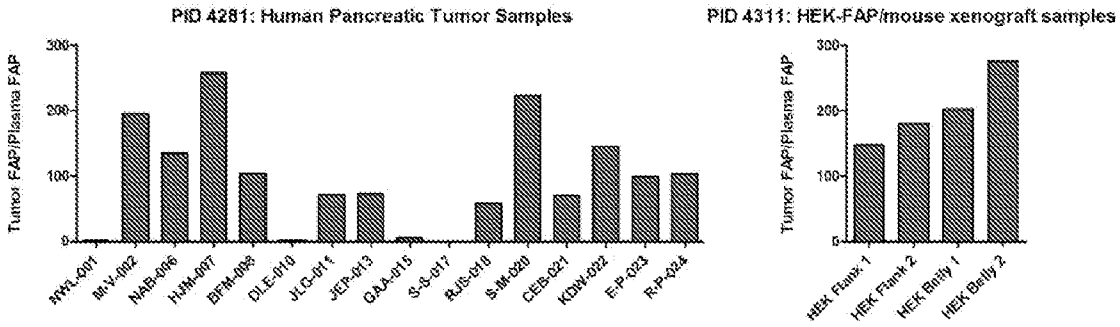

PID 4281: Human Pancreatic Tumor Samples
Tumor FAP/Plasma FAP
300
200
100
0
MWL-001
MV-002
NAB-006
HAM-007
BFM-008
DLE-010
JLG-011
JEP-013
GAA-015
S-S-017
RJB-018
S-M-020
CEB-021
KDM-022
F-P-023
R-P-024
PID 4311: HEK-FAP/mouse xenograft samples
Tumor FAP/Plasma FAP
300
200
100
0
HEK Flank 1
HEK Flank 2
HEK Belly 1
HEK Belly 2

Figure 19

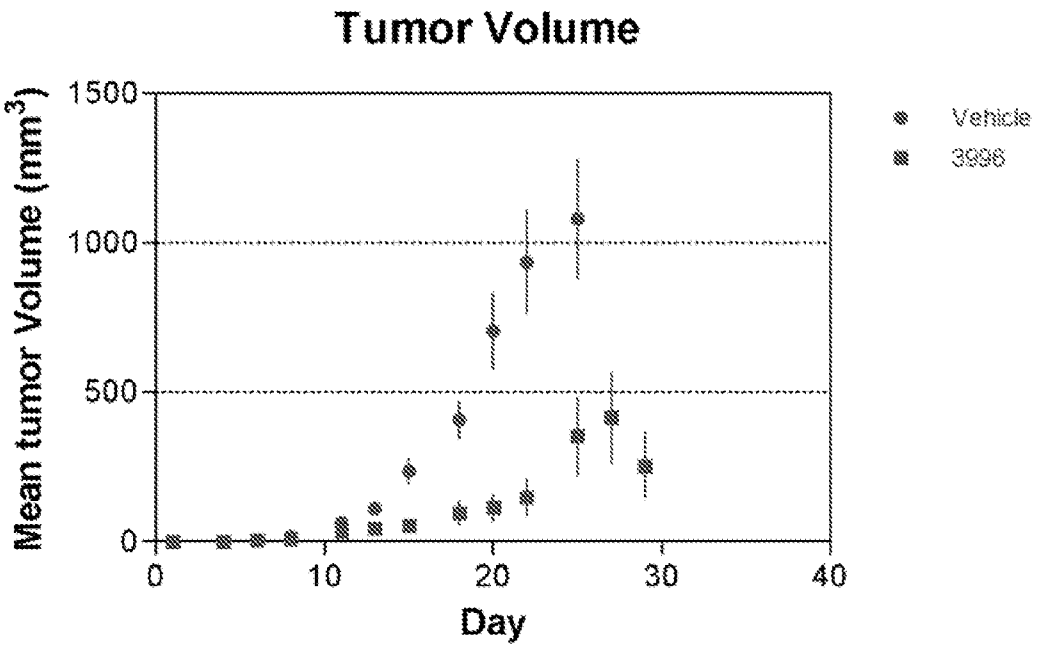

Tumor Volume
Mean tumor Volume (mm3)
1500
1000
500
0
0
10
20
30
40
Day
Vehicle
3996

Figure 20

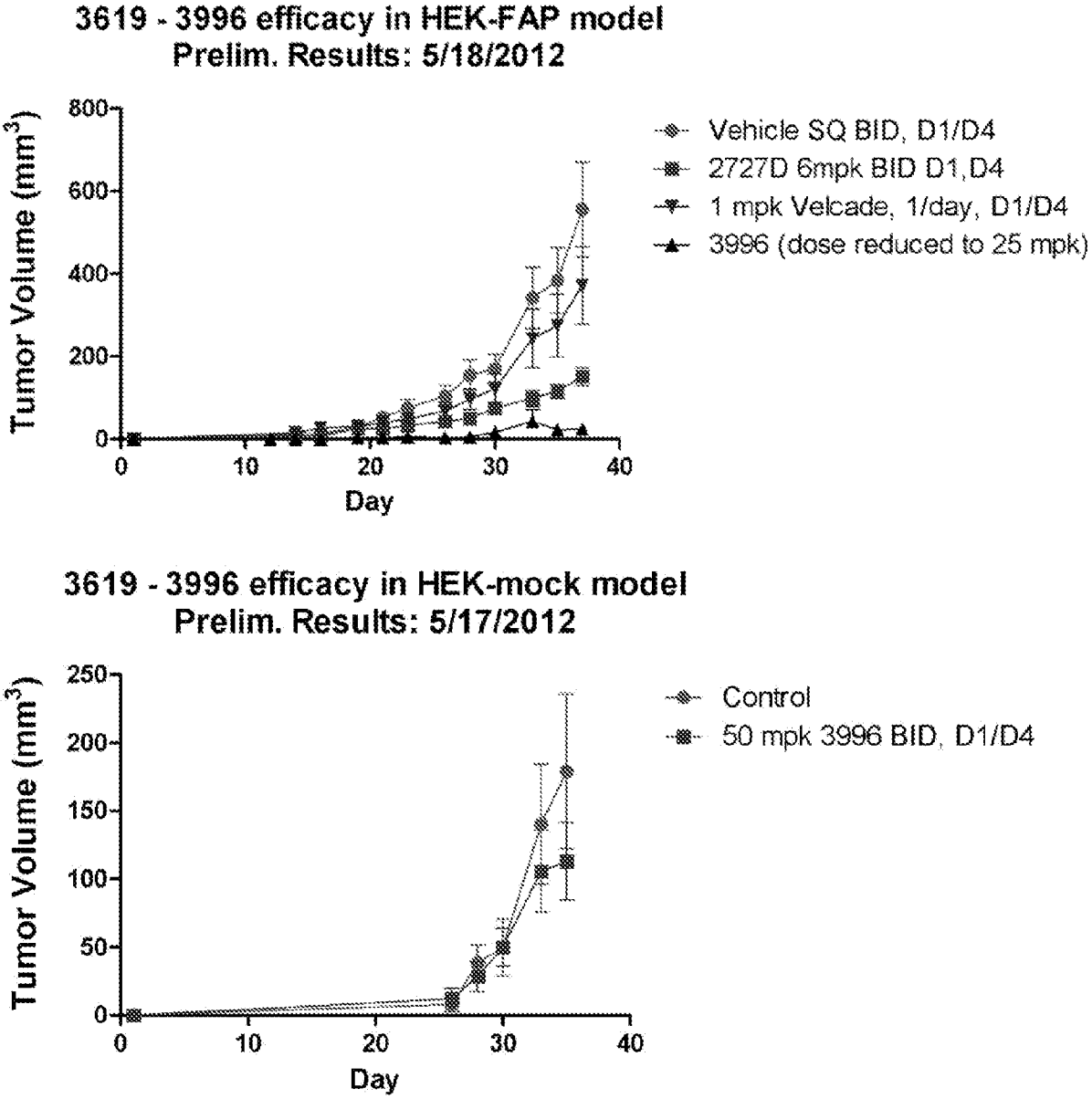

3619 - 3996 efficacy in HEK-FAP model
Prelim. Results: 5/18/2012
Tumor Volume (mm3)
800
600
400
200
0
0
10
20
30
40
Day
Vehicle SQ BID, D1/D4
2727D 6mpk BID D1,D4
1 mpk Velcade, 1/day, D1/D4
3996 (dose reduced to 25 mpk)
3619 - 3996 efficacy in HEK-mock model
Prelim. Results: 5/17/2012
Tumor Volume (mm3)
250
200
150
100
50
0
0
10
20
30
40
Day
Control
50 mpk 3996 BID, D1/D4

Figure 21

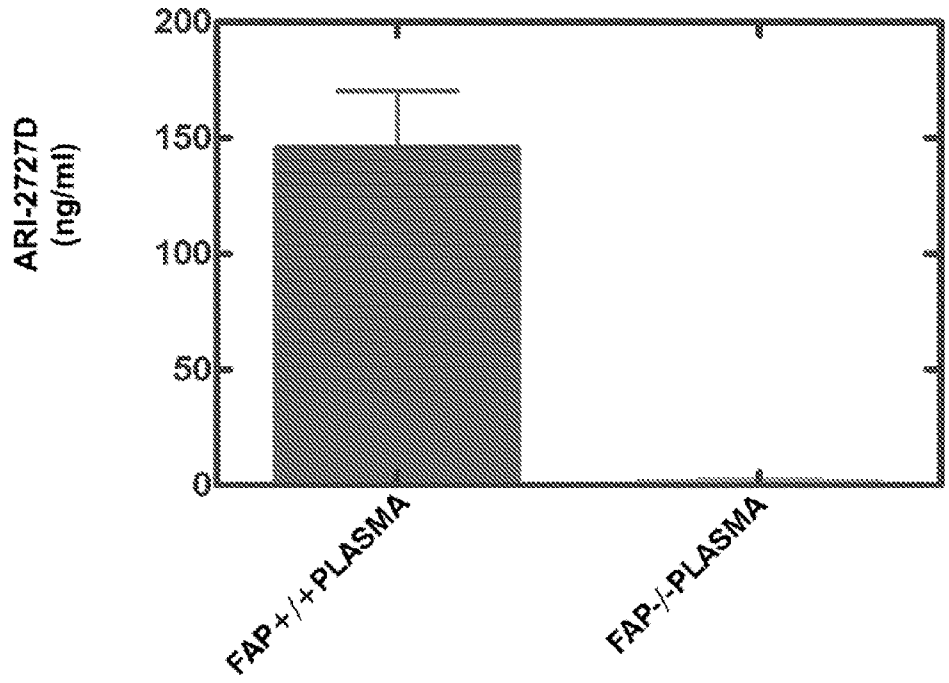


Figure 22

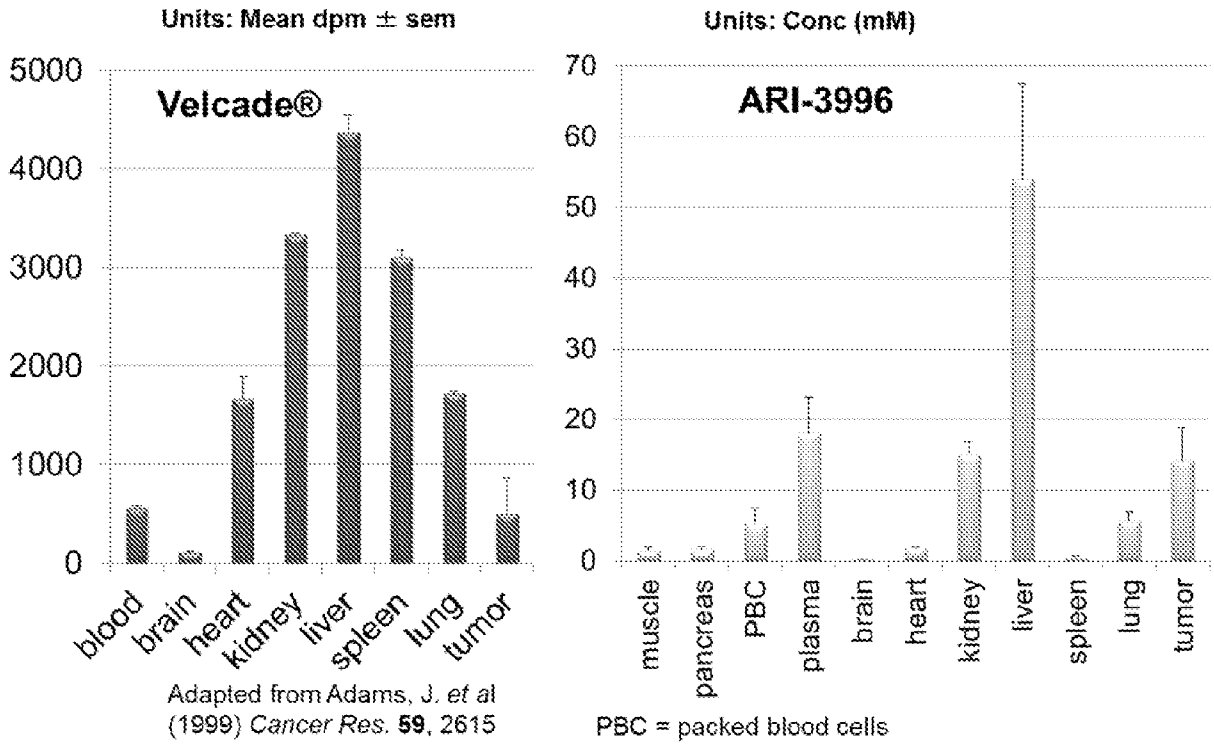

Units: Mean dpm ± sem
Velcade®
5000
4000
3000
2000
1000
0
blood
brain
heart
kidney
liver
spleen
lung
tumor
Adapted from Adams, J. et al (1999) Cancer Res. 59, 2615
Units: Conc (mM)
ARI-3996
70
60
50
40
30
20
10
0
muscle
pancreas
PBC
plasma
brain
heart
kidney
liver
spleen
lung
tumor
PBC = packed blood cells

Figure 23

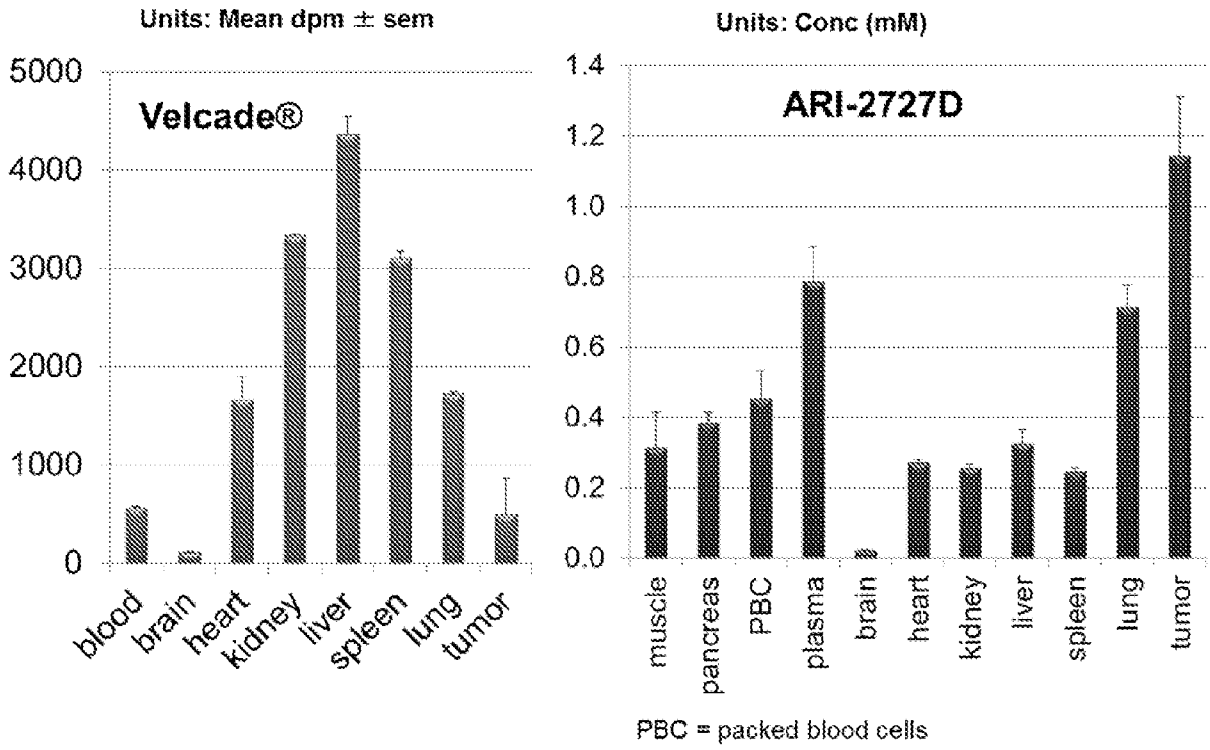
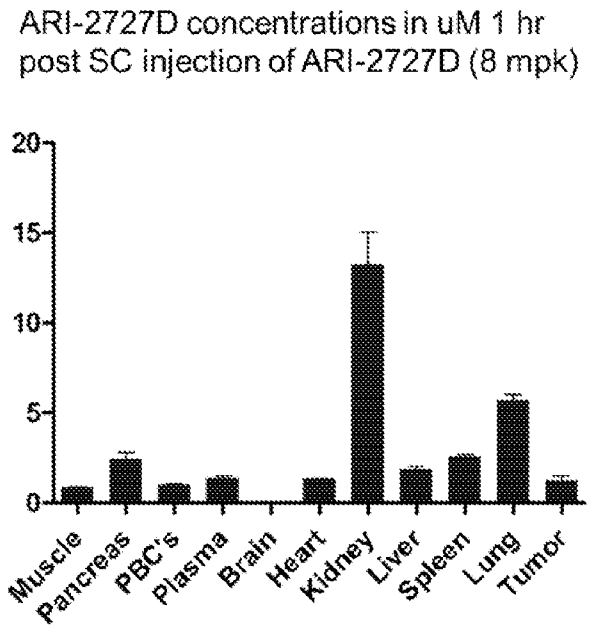
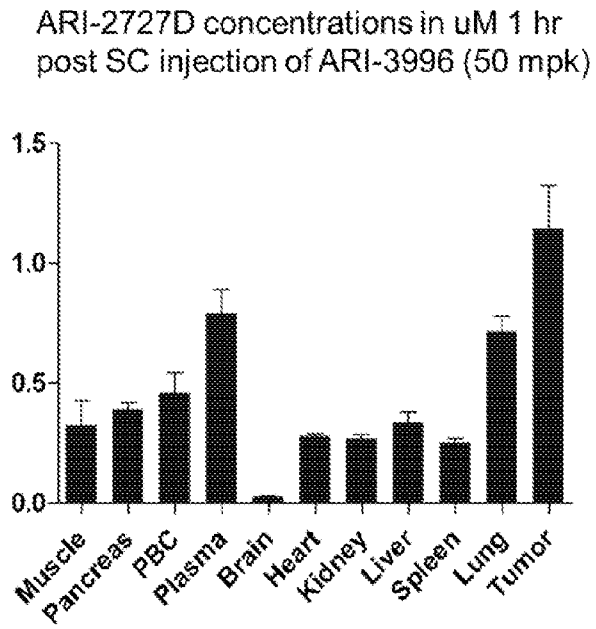

Units: Mean dpm ± sem
Velcade®
5000
4000
3000
2000
1000
0
blood
brain
heart
kidney
liver
spleen
lung
tumor
Units: Conc (mM)
ARI-2727D
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
muscle
pancreas
PBC
plasma
brain
heart
kidney
liver
spleen
lung
tumor
PBC = packed blood cells

Figure 24





Agent	Tumor conc. / Liver conc.
Velcade®	0.11
ARI-3996	0.26
ARI-2727D	3.5

Figure 25

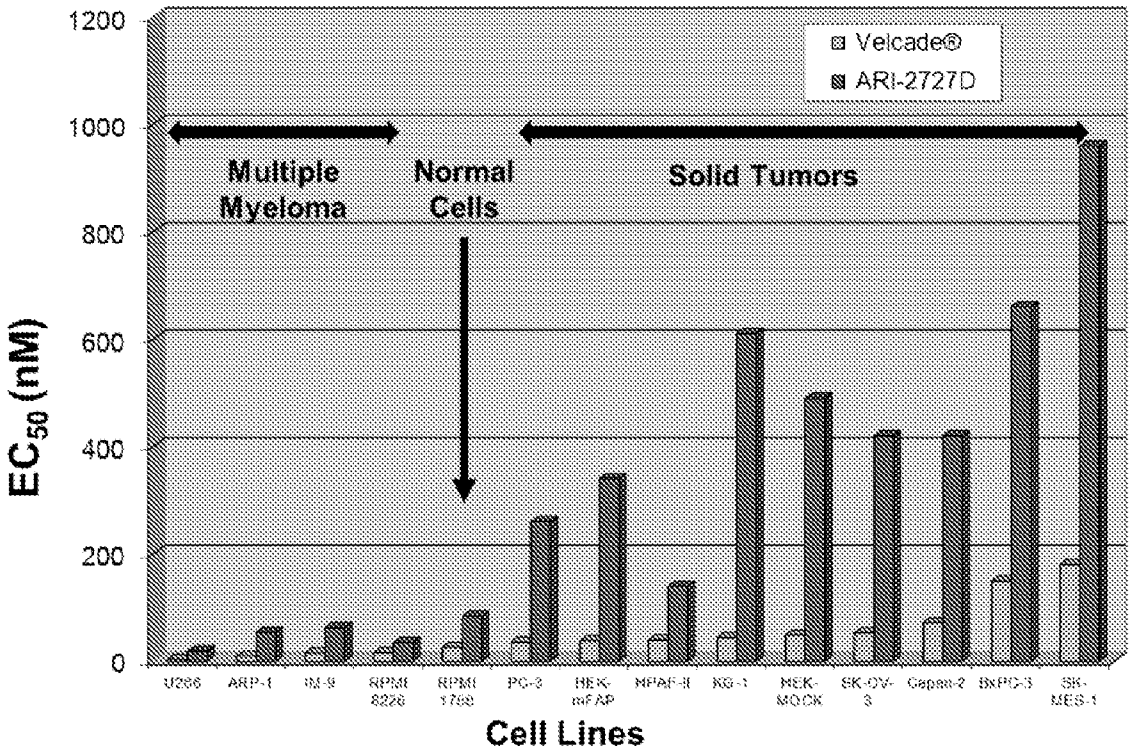

1200
1000
800
600
400
200
0
EC50 (nM)
Velcade®
ARI-2727D
Multiple Myeloma
Normal Cells
Solid Tumors
U266
ARP-1
IM-9
RPMI 8226
RPMI 1788
PC-3
HEK-mFAP
MPAF-II
KG-1
HEK-MOCK
SK-OV-3
Capan-2
BxPC-3
SK-MES-1
Cell Lines

Figure 26

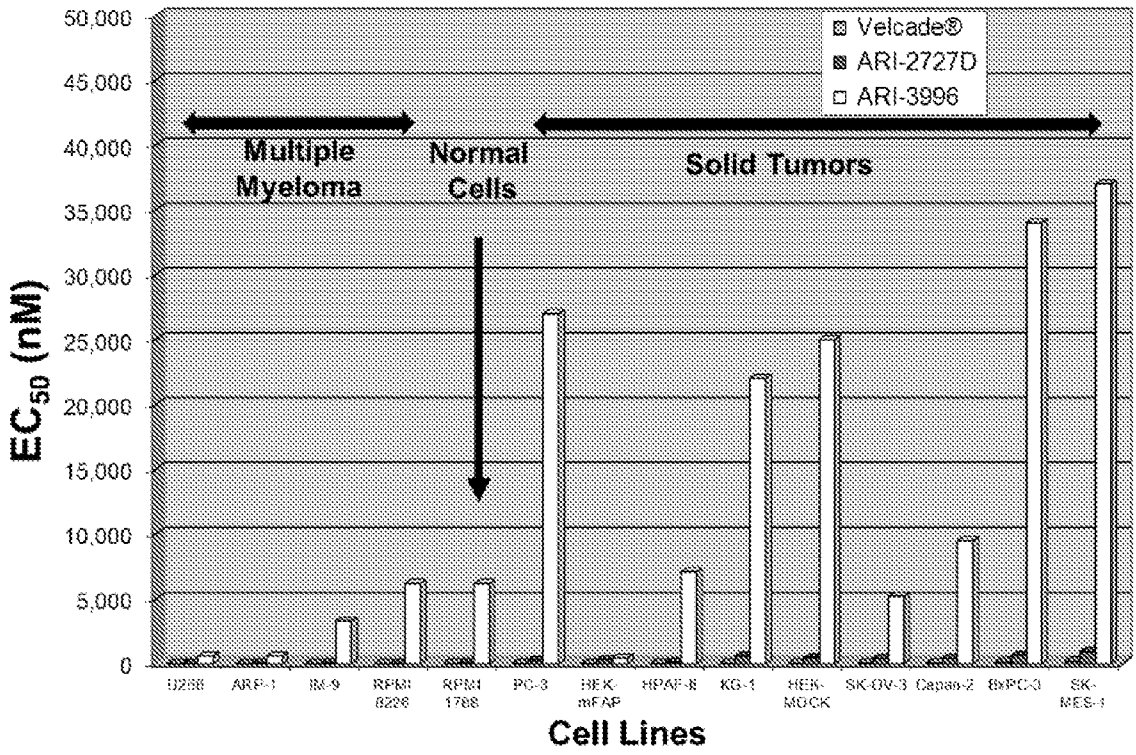

Velcade®
ARI-2727D
ARI-3996
Multiple Myeloma
Normal Cells
Solid Tumors
EC50 (nM)
50,000
45,000
40,000
35,000
30,000
25,000
20,000
15,000
10,000
5,000
0
U266
ARP-1
IM-9
RPMI 8226
RPMI 1788
PC-3
HEK-mFAP
HPAF-II
KG-1
HEK-MOCK
SK-OV-3
Capan-2
BxPC-3
SK-MES-1
Cell Lines

Figure 27

			FAP Activity (ΔFL/min/mg protein)	
Tissue Source	Tissue Spec No.	Serum Barcode	Tissue	Serum
Bladder	1007526	113388	1969	50
Bladder	1005013	106958	1182	33
Bladder	1005636	109017	474	42
Bladder	1006129	110335	4104	68
Bladder	1007027	110340	1847	87
Breast	1005395	108343	98	85
Breast	1005451	108499	1598	59
Breast	1008772	112035	1648	65
Breast	1009328	118686	1073	57
Breast	1009649	119507	360	64
Colon	1006685	111639	643	50
Colon	1006857	112000	1413	74
Colon	1006879	112112	1988	46
Colon	1007172	112887	569	45
Colon	1007407	113270	835	45
Lung	1009959	120228	118	56
Lung	1010842	122636	1374	55
Lung	1010756	122466	104	53
Lung	1009309	117908	313	58
Lung	1010231	120826	135	46

Figure 27 (Continued)

			FAP Activity (ΔFL/min/mg protein)	
Tissue Source	Tissue Spec No.	Serum Barcode	Tissue	Serum
Ovary	1005120	107660	5241	90
Ovary	1005431	108462	9	94
Ovary	1005817	109521	345	96
Ovary	1005896	109675	42	46
Ovary	1006594	111511	588	63
Renal	1005560	108834	29	56
Renal	1009391	118759	553	31
Renal	1009454	118987	471	52
Renal	1009526	119039	53	51
Renal	1009522	119183	101	35
Sarcoma	1005042	107420	309	59
Sarcoma	1005118	107612	21	117
Sarcoma	1005228	107876	1982	69
Sarcoma	1005746	109227	353	61
Sarcoma	1005744	109270	302	68
Uterine	1006279	110607	1840	46
Uterine	1010225	120790	1325	37
Uterine	1008368	116201	780	67
Uterine	1008591	116699	1606	75
Uterine	1009314	118623	230	61

Figure 27 (Continued)

	FAP Activity (ΔFL/min/mg protein)						
	Pancreatic Tissue		Plasma				
Sample ID	Normal	Tumor	Baseline	Pre-Op Labs	Pre-Surgery	Immediate Post-Follow Up	Follow Up
MVL-001	393	57		48	46	40	31
M-V-002	74	6700	34		38	35	32
E-G-003			35		38	35	44
HSB-004				40	41		
L-K-005				41	41		
NAB-006	2181	9878	75		65	71	129
HJM-007	21	16528	65		69	53	61
BFM-008	105	2726	29		32	21	23
JLG-009			49		43		
DLE-010	216	85	49		42	41	26
JLG-011	223	5400	71		69	71	54
JRP-012			49		50		
JEP-013	22	2713	36	30	27	18	
G-F-014			35		32	28	35
GAA-015	553	319	69		71	43	75
R-H-016			27			22	30
S-S-017	1818		56		55	41	49
RJS-018	1266	1678	32		31	21	
D-T-019			28		22		
S-M-020		7428	32		31	26	40
CEB-021	806	3430	51		50	35	52
KDW-022	340	7083	43		40	41	39
E-P-023	5289	4782	49		45	40	33
R-P-024	1773	6972	61		57	47	44

Figure 28

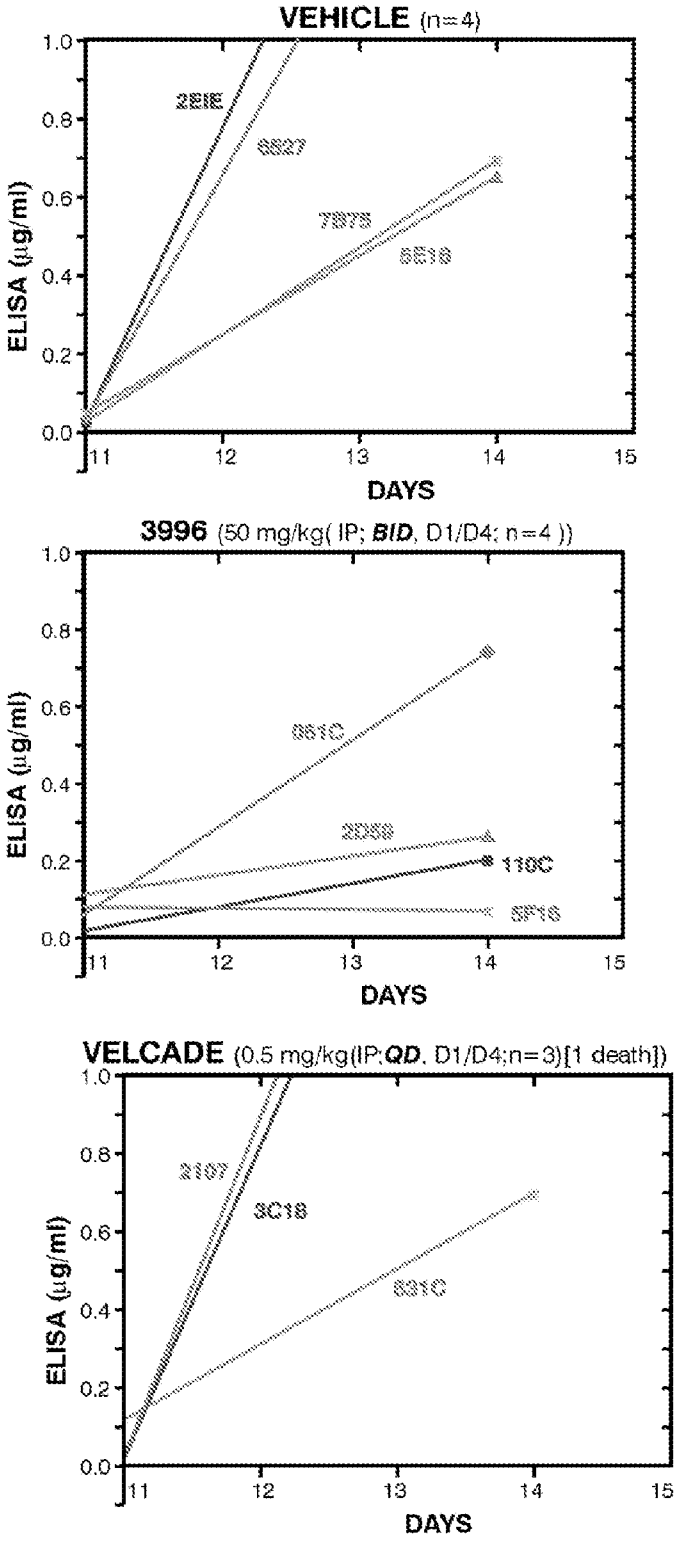

VEHICLE (n=4)
ELISA (µg/ml)
DAYS
2E8E
8827
7B7S
8E18
3996 (50 mg/kg(IP; BID, D1/D4; n=4))
ELISA (µg/ml)
DAYS
861C
2D88
110C
8F16
VELCADE (0.5 mg/kg(IP;QD, D1/D4;n=3)[1 death])
ELISA (µg/ml)
DAYS
3107
3C18
831C

Figure 28 (Continued)

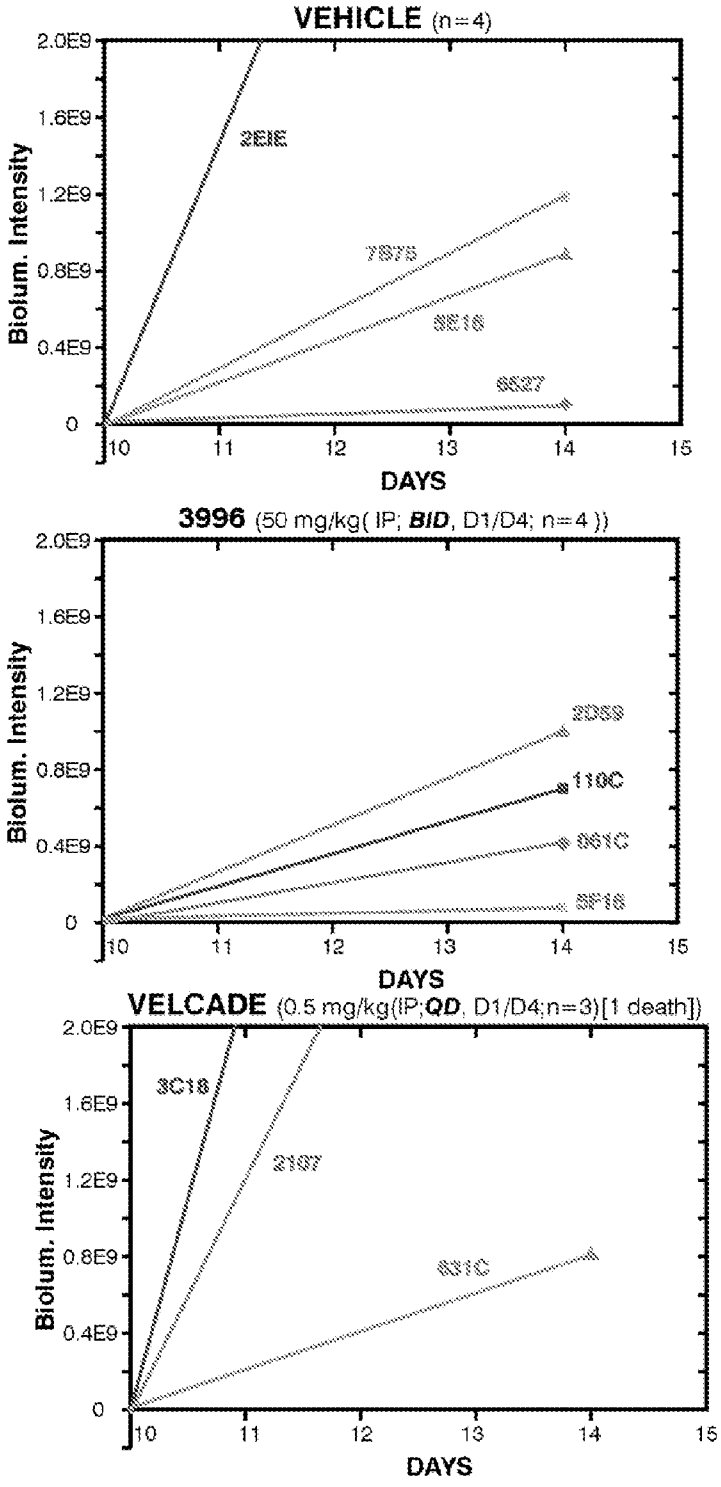


Figure 29

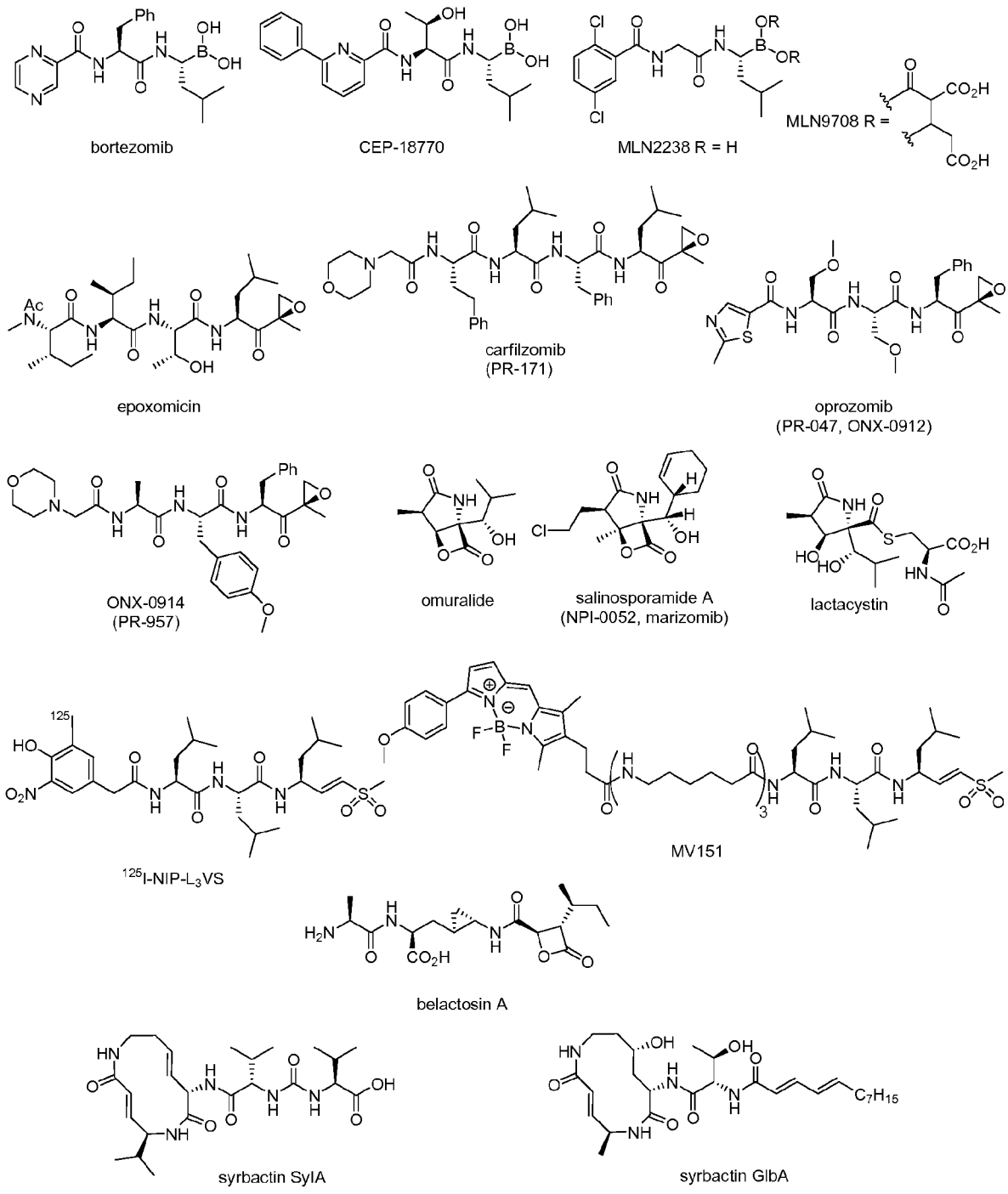

bortezomib
CEP-18770
MLN2238 R = H
MLN9708 R =
epoxomicin
carfilzomib
(PR-171)
oprozomib
(PR-047, ONX-0912)
ONX-0914
(PR-957)
omuralide
salinosporamide A
(NPI-0052, marizomib)
lactacystin
125I-NIP-L3VS
MV151
belactosin A
syrbactin SylA
syrbactin GlbA

Figure 29 (Continued)