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insights

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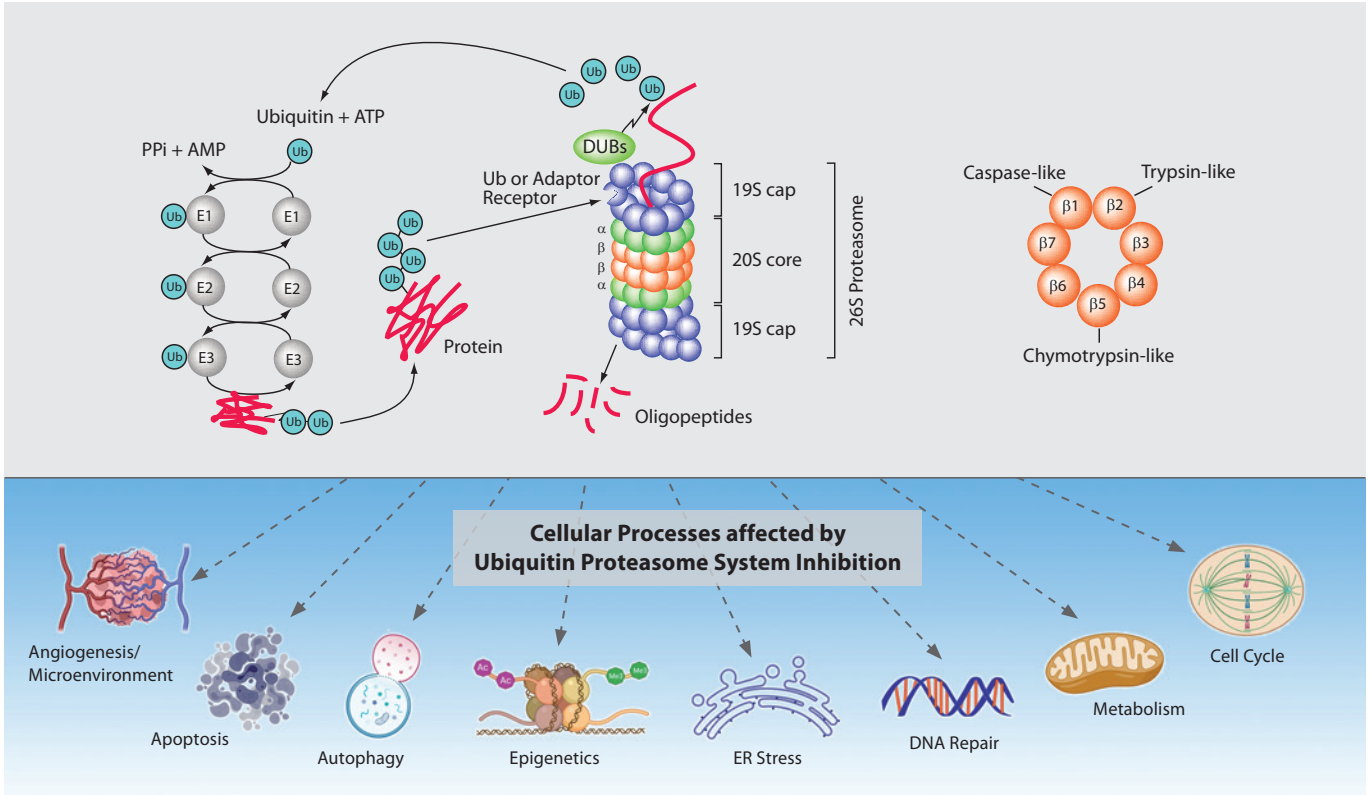
Ubiquitin-Proteasome System (UPS)

The **ubiquitin-proteasome system (UPS)** and the autophagic-lysosomal pathway are the two major **degradation systems** for both native and misfolded proteins in eukaryotic cells. They do not act independently from each other. Defective autophagy results in accumulation of ubiquitinated proteins, impacting the flux of the UPS, while dysfunction of the UPS can promote a compensatory induction of autophagy. Through protein degradation and the maintenance of protein homeostasis, the UPS regulates many normal cellular processes including signal transduction, cell cycle control, transcription and apoptosis (see Figure). The regulated proteolysis of bulk and misfolded proteins is strictly controlled by the 26S proteasome complex.

The **26S proteasome complex** recognizes polyubiquitinated proteins, which were marked for elimination by the E1, E2 and E3 ubiquitinating enzymes (see Figure). Upon recognition, unfolding and transfer of the de-ubiquitinated target protein by the **19S regulatory cap** into the interior of the cylindrical **20S proteasome core** particle, protein degradation is facilitated by catalytic β-subunits having nucleophilic N-terminal threonine (Thr1) residues. Although eukaryotic 20S proteasomes harbor seven different β-subunits in their two-fold symmetrical α7β7β7α7 stacked complexes, only three β-subunits per β-ring **[subunits β1 (caspase-like), β2 (trypsin-like) and β5 (chymotrypsin-like)]** are proteolytically active. These three β-subunits are major targets for small molecule proteasome inhibitors. **Proteasome inhibition** has implications in a number of human diseases such as cancer (e.g. multiple myeloma (MM)), inflammation and ischemic stroke and is an important therapeutic target.

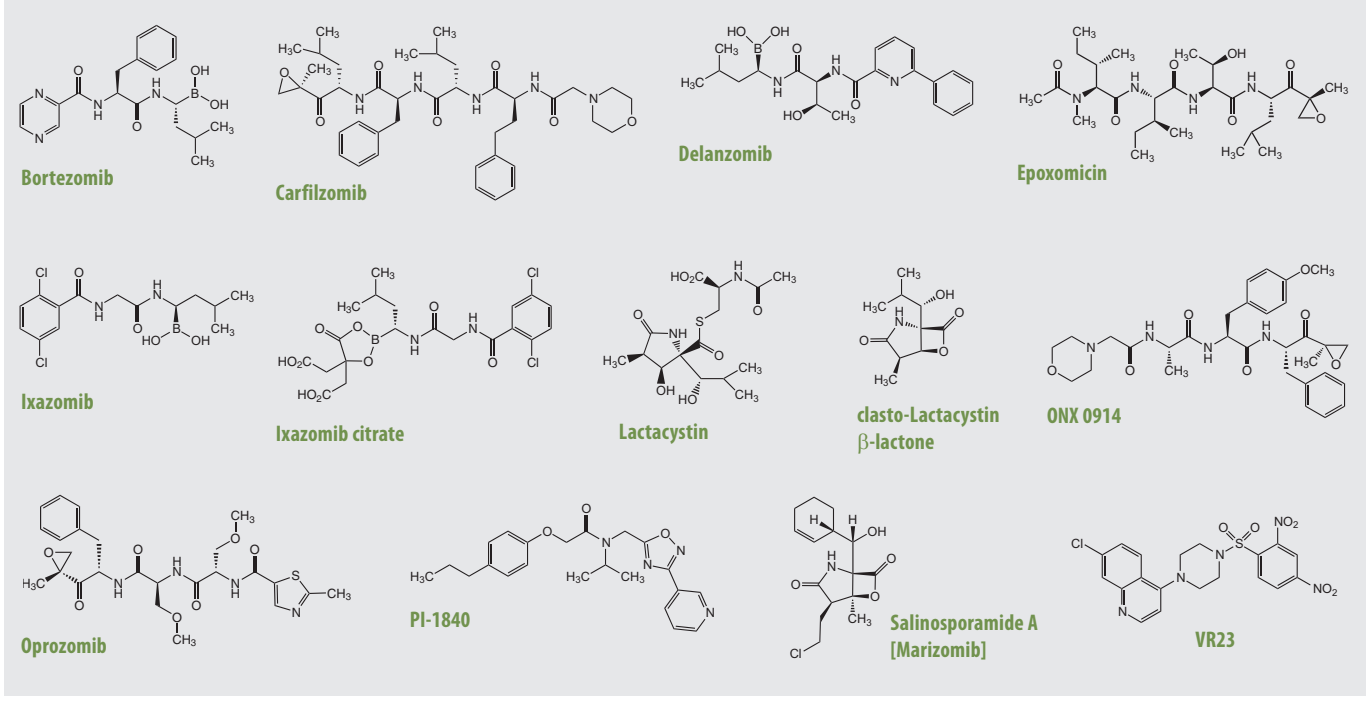
Several components of the UPS have been validated as potential anticancer targets, including 20S proteasomes, 19S proteasome-associated deubiquitinases (DUBs) and ubiquitin ligases (E3s). One of the strategies to improve the current status of cancer treatment is to repurpose old drugs with UPS-inhibitory properties as new anticancer agents.



STANDARD Proteasome Inhibitors – *From the Source!*

PRODUCT NAME	DESCRIPTION	PID
Bortezomib [PS-341]	Inhibits chymotrypsin-like and caspase-like activity (IC_{50}=3-5nM).	AG-CR1-3602
Carfilzomib [PR-171]	Inhibits the chymotrypsin-like β5 subunit of the constitutive 20S proteasome (IC_{50}=5.2nM) and the β5i subunit [LMP7] of the 20S immunoproteasome (IC_{50}=14nM).	AG-CR1-3669
Delanzomib [CEP-18770]	Inhibits the chymotrypsin-like β5 subunit of the constitutive 20S proteasome (IC_{50}=3.8nM) and the caspase-like β1 subunit (IC_{50}=~70nM).	AG-CR1-3673
(-)-Epigallocatechin gallate	Inhibits chymotrypsin-like activity (IC_{50}~200nm).	AG-CN2-0063
Epoxomicin	Inhibits predominant chymotrypsin-like activity (IC_{50}=4nM).	AG-CN2-0422
Ixazomib [MLN2238]	Inhibits all the three catalytic activities of the constitutive 20S proteasome: chymotrypsin-like β5 subunit (IC_{50}=3.4nM), trypsin-like β2 subunit (IC_{50}=3.5μM) and the caspase-like β1 subunit (IC_{50}=0.03μM).	AG-CR1-3670
Ixazomib citrate [MLN9708]	Inhibits all the three catalytic activities of the constitutive 20S proteasome: chymotrypsin-like β5 subunit (IC_{50}=3.4nM), trypsin-like β2 subunit (IC_{50}=3.5μM) and the caspase-like β1 subunit (IC_{50}=0.03μM).	AG-CR1-3671
clasto-Lactacystin β-lactone	Chymotrypsin-like, trypsin-like and caspase-like activity inhibitor (IC_{50}~1μM).	AG-CN2-0442
Lactacystin	Chymotrypsin-like, trypsin-like and caspase-like activity inhibitor (IC_{50}=4.8μM).	AG-CN2-0104
ONX 0914	Inhibits the β5i subunit [LMP7] of the 20S immunoproteasome (IC_{50}=73nM) with minimal cross-reactivity to the chymotrypsin-like β5 subunit of the constitutive 20S proteasome (IC_{50}=1.04μM).	AG-CR1-3674
Oprozomib [ONX 0912]	Inhibits the chymotrypsin-like β5 subunit of the constitutive 20S proteasome (IC_{50}=36nM) and the β5i subunit [LMP7] of the 20S immunoproteasome (IC_{50}=82nM).	AG-CR1-3672
PI-1840 [Proteasome Inhibitor]	Inhibits the chymotrypsin-like β5-subunit of the constitutive 20S proteasome (IC_{50}=27nM), with minimal trypsin-like (β2) and caspase-like (β1) activity (IC_{50}= >100μM, for both).	AG-CR1-3675
Piperlongumine	Inhibits the β5i subunit (LMP7) (IC_{50}=15μM) with minimal inhibition of the human constitutive 20S proteasome.	AG-CN2-0024
Salinosporamide A [Marizomib]	Inhibits all the three catalytic activities of the constitutive 20S proteasome: chymotrypsin-like (IC_{50}=3.5nm); trypsin-like (IC_{50}=28nm); caspase-like (IC_{50}=430nm).	AG-CN2-0444
VR23 [Proteasome Inhibitor]	Inhibits all the three catalytic activities of the constitutive 20S proteasome: chymotrypsin-like (IC_{50}=50-100nm); trypsin-like (IC_{50}=1nm); caspase-like (IC_{50}=3μm).	AG-CR1-3676
Z-Leu-Leu-Phe-CHO [MG-110]	Chymotrypsin-like activity inhibitor.	AG-CP3-0021
Z-Leu-Leu-Nva-CHO [MG-115]	Chymotrypsin-like activity inhibitor.	AG-CP3-0015
Z-Leu-Leu-Leu-CHO [MG-132]	Chymotrypsin-like and caspase-like activity inhibitor (IC_{50}~1μM).	AG-CP3-0011
Z-Leu-Leu-Leu-B(OH)2 [MG-262]	Chymotrypsin-like and caspase-like activity inhibitor (IC_{50}~150nM).	AG-CP3-0024

* Note: All IC_{50} values (where indicated) are from literature and might vary based on the experimental setup.



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Other Proteasome Inhibitors / Modulators

PRODUCT NAME	DESCRIPTION	PID
Apigenin	Inhibits chymotrypsin-like and trypsin-like proteasome catalytic activity.	CDX-A0438
Betulinic acid (>99%)	Chymotrypsin-like activity activator at low micromolar concentration.	AG-CN2-0415
Betulinic acid (>97%)	Chymotrypsin-like activity activator at low micromolar concentration.	AG-CN2-0417
Celastrol	Inhibits 20S proteasome chymotrypsin-like activity.	AG-CN2-0460
Curcumin (high purity)	Inhibits all three catalytic activities (IC_{50}~10μM). Inhibits DUB activity.	AG-CN2-0059
Kendomycin	Inhibits 20S proteasome chymotrypsin-like activity.	BVT-0001
Luteolin	Inhibits chymotrypsin-like and trypsin-like proteasome catalytic activity.	AG-CN2-0098
Nelfinavir . mesylate	Pan-proteasome inhibition in AMO-1 and U266 myeloma cells; 60 % inhibition of the chymotrypsin-like activity of 26S proteasome at 5μM.	AG-CR1-3726
Quercetin . dihydrate	Inhibits all three catalytic activities (IC_{50}~15μM).	AG-CN2-0409
Ritonavir	Inhibits 20S proteasome chymotrypsin-like activity.	AG-CR1-3683
Saquinavir . mesylate	Inhibits chymotrypsin-like and caspase-like activity of the 26S proteasome and purified 20S proteasome.	AG-CR1-3727
Shikonin	Proteasome inhibitor.	AG-CN2-0487
Terrein	Inhibits chymotrypsin- and trypsin-like activity (IC_{50}~0.3mM).	BVT-0193
Withaferin A	Inhibits 20S proteasome β5 subunit chymotrypsin-like activity.	AG-CN2-0490

Fluorescent Substrates for Ubiquitin-Proteasome Activity Measurement

PRODUCT NAME	DESCRIPTION	PID
Ac-Ala-Asn-Trp-AMC	Fluorogenic substrate for specifically measuring chymotrypsin-like activity of the 20S immunoproteasome.	AG-CP3-0037
Ac-Arg-Leu-Arg-AMC	Fluorogenic substrate for measuring the trypsin-like peptidase activity of the 20S proteasome.	AG-CP3-0013
Ac-Pro-Ala-Leu-AMC	Fluorogenic substrate for specifically measuring caspase-like activity of the 20S immunoproteasome.	AG-CP3-0036
Ac-Trp-Leu-Ala-AMC	Fluorogenic substrate for measuring the chymotrypsin-like peptidase activity of the 20S proteasome, calpains and other chymotrypsin-like proteases.	AG-CP3-0035
Boc-Leu-Arg-Arg-AMC	Fluorogenic substrate for measuring the trypsin-like peptidase activity of the 20S proteasome.	AG-CP3-0014
Suc-Leu-Leu-Val-Tyr-AMC	Fluorogenic substrate for measuring the chymotrypsin-like peptidase activity of the 20S proteasome, calpains and other chymotrypsin-like proteases.	AG-CP3-0016
Suc-Leu-Tyr-AMC	Fluorogenic substrate for measuring the chymotrypsin-like peptidase activity of the 20S proteasome.	AG-CP3-0017
Z-Leu-Leu-Leu-AMC	Fluorogenic substrate for measuring the chymotrypsin-like peptidase activity of the 20S proteasome.	AG-CP3-0019
Z-Leu-Leu-Glu-AMC	Fluorogenic substrate for measuring the caspase-like activity of the 20S proteasome.	AG-CP3-0022
Z-Leu-Arg-Gly-Gly-AMC	Preferred substrate sequence of the human deSUMOylating enzymes SENP6 and SENP7.	AG-CP3-0023

Proteasome Complex Modulators

PRODUCT NAME	DESCRIPTION	PID
Apcin	APC/C E3 ubiquitin ligase inhibitor.	AG-CR1-3603
Auranofin	Proteasomal deubiquitinase (DUB) inhibitor.	AG-CR1-3611
BAY 11-7082	RBR E3 ligase inhibitor. Effects by inactivating the E2-conjugating enzymes Ubc13 and UbcH7 and the E3 ligase LUBAC, preventing the formation of Lys63-linked and linear polyubiquitin chains.	AG-CR1-0013
Lovastatin	SKP2 E3 ligase inhibitor.	AG-CN2-0051
NSC697923	Selective Ub-conjugating enzyme (E2) complex Ubc13-Uev1A inhibitor. Inhibits the formation of the Ubc13~Ub conjugate.	AG-CR1-3519
Oridonin	CRL/SCF RING E3 inhibitor. Inhibits Fbw7 an E3 ubiquitin ligase (CRL/SCF RING) of c-Myc and promotes proteasomal degradation.	CDX-O0131
Simvastatin	SKP2 E3 ligase inhibitor.	AG-CN2-0052
Suramin . hexasodium salt	Cullin-RING E3 ubiquitin ligase inhibitor.	AG-CR1-3575
Vitexin	Inhibits polyubiquitin synthesis by the ubiquitin-conjugating enzyme E2-25K.	AG-CN2-0425

SUMO-Related Inhibitors

PRODUCT NAME	DESCRIPTION	PID
Anacardic acid	SUMOylation inhibitor.	AG-CR1-0046
MLN4924 [NAE Inhibitor]	Inhibits ubiquitin-activating enzyme (UAE) and SUMO-activating enzyme (SAE) with IC_{50} values of 1.5 and 8.2μM, respectively.	AG-CR1-3703

Proteasome Assay Kits & 20S Proteasome/20S Immunoproteasome Complexes

The two proteasome kits are designed to test for specific activity of 20S immunoproteasome or 20S constitutive proteasome, and include purified proteasomes, AMC-conjugated substrates, specific inhibitors and necessary buffers and solutions. Fluorescence detection can be performed at Excitation/Emission (nm): 345/445, allowing for a real-time read out of specific activity.

All highly active and pure proteasomes offered by AdipoGen Life Sciences are able to proteolytically degrade substrates in an ATP-independent manner.

PRODUCT NAME	DESCRIPTION	PID
20S Immunoproteasome Assay Kit	Designed to test for specific activity of 20S immunoproteasome.	SBB-KP0037
20S Constitutive Proteasome Assay Kit	Designed to test for specific activity of 20S proteasome.	SBB-KP0038
20S Immunoproteasome (human) (untagged)	20S immunoproteasome is most active against Suc-LLVY-AMC (AG-CP3-0016), Ac-PAL-AMC (AG-CP3-0036), and Ac-ANW-AMC (AG-CP3-0037) substrates.	SBB-PP0004
20S Immunoproteasome (mouse) (untagged)	20S immunoproteasome is most active against Suc-LLVY-AMC (AG-CP3-0016), Ac-PAL-AMC (AG-CP3-0036), and Ac-ANW-AMC (AG-CP3-0037) substrates.	SBB-PP0083
20S Immunoproteasome (rat) (untagged)	20S immunoproteasome is most active against Suc-LLVY-AMC (AG-CP3-0016), Ac-PAL-AMC (AG-CP3-0036), and Ac-ANW-AMC (AG-CP3-0037) substrates.	SBB-PP0046
20S Proteasome (human) (untagged)	20S Proteasome is most active against Suc-LLVY-AMC (AG-CP3-0016), Z-LLE-AMC (AG-CP3-0022), and Ac-WLA-AMC (AG-CP3-0035) substrates.	SBB-PP0005
20S Proteasome (mouse) (untagged)	20S Proteasome is most active against Suc-LLVY-AMC (AG-CP3-0016), Z-LLE-AMC (AG-CP3-0022), and Ac-WLA-AMC (AG-CP3-0035) substrates.	SBB-PP0047
20S Proteasome (rat) (untagged)	20S Proteasome is most active against Suc-LLVY-AMC (AG-CP3-0016), Z-LLE-AMC (AG-CP3-0022), and Ac-WLA-AMC (AG-CP3-0035) substrates.	SBB-PP0086
Angiocidin (human) (rec.)	Angiocidin shows sequence similarity with proteasome components and is also being referred to as 26A proteasome regulatory subunit S5A.	AG-40B-0061
Ubiquitin (human) (rec.) (Europium-Cryptate)	Human ubiquitin (aa1-76) is site-specifically conjugated to a single Europium-Cryptate moiety.	SBB-TR0014
Ubiquitin (human) (rec.) (Cy5)	Human ubiquitin (aa1-76) is site-specifically conjugated to a single Cyanine 5 (Cy5) moiety.	SBB-TR0015
Ubiquitin (human) (rec.) (6-FAM)	Human ubiquitin (aa1-76) is site-specifically conjugated to a single fluorescein (6-FAM) moiety.	SBB-TR0016

Specialty Degradation Reagents

PRODUCT NAME	DESCRIPTION	PID
D-Biotin p-nitrophenyl ester (Biotin-ONP; BNP)	Exploits the intracellular ubiquitin-proteasome system to selectively degrade target proteins. D-Biotin p-nitrophenyl ester is commonly used as a biotin-tagged photoaffinity probe and an alkyl chain-based PROTAC linker that can be used in the synthesis of PROTACs.	CDX-B0307
Lipoyl-TRIM21 (human) (rec.) (His)	TRIM21 (tripartite motif-containing protein 21) is a cytosolic Fc receptor induced by interferon (IFN). TRIM21 functions as a E3 ligase. During infection, antibodies are delivered efficiently to the cytosol when bound to intracellular pathogens such as viruses and bacteria. The antibody-pathogen complex in the cytosol upon engagement of the protein TRIM21 is ubiquitinylated and degraded by the proteasome machinery.	AG-40B-0182

Buffers and Solutions

PRODUCT NAME	DESCRIPTION	PID
AMC Standard Solution	A fluorogenic standard useful for quantitating assays monitoring 7-amino-4-methylcoumarin (AMC) release.	SBB-RB0128
AFC Standard Solution	A fluorogenic standard useful for quantitating assays monitoring 7-amino-4-trifluoromethylcoumarin (AFC) release.	SBB-RB0129
Loading Buffer (5X)	Loading buffer for separation and visualization of proteins with SDS-PAGE and western blot analysis.	SBB-RB0126
MgATP (100X) Solution	Pre-coupled Mg-ATP is an ideal energy source for semi-purified conjugation/degradation reactions.	SBB-RB0127

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