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Advances in the Understanding of Mechanisms and Therapeutic Use of Bortezomib

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Abstract

The ubiquitin-proteasome pathway regulates many basic cellular processes and has been proven to be a promising target for cancer therapy. Bortezomib is the first U.S. Food and Drug Administration (FDA) approved proteasome inhibitor used in the treatment of newly diagnosed multiple myeloma, relapsed/refractory multiple myeloma, and mantle cell lymphoma. The anti-cancer mechanisms of bortezomib elucidated by preclinical studies include: upregulation of proapoptotic proteins (e.g., Noxa, IκB), inhibition of NFκB and its anti-apoptotic target genes, suppression of several anti-apoptotic proteins (e.g., Bcl-XL, Bcl-2, and STAT-3), down-regulation of expression of several proteins involved in DNA repair pathways, and induction of endoplasmic reticulum (ER) stress and pro-apoptotic Unfolded Protein Response (UPR). Bortezomib has potent chemo-/radio-sensitizing effects and can overcome traditional drug resistance in tumors when used in combination with potential chemotherapies. Although bortezomib has been successful in improving clinical outcomes when used in hematological malignancies, relapse may occur in those patients who responded initially. Furthermore, some cytotoxicities (such as peripheral neuropathy) were found to be associated with bortezomib treatment. These observations have encouraged researchers to search for the next generation proteasome inhibitors (including carfilzomib and marizomib) that could overcome bortezomib resistance and have improved properties, reduced toxicities, and broader anticancer activities, based on the lessons learned from the mechanisms and use of bortezomib. This review summarizes the current status of bortezomib as well as several other proteasome inhibitors that are currently under clinical and preclinical investigation.

Introduction

Cellular homeostasis and regulation of cellular functions depend on finely orchestrated intracellular processes, such as systematic degradation of regulatory proteins. In eukaryotic cells, the ubiquitin-proteasome pathway (UPP) is primarily responsible for degrading the majority of cellular proteins and plays an essential role in many basic cellular processes (Adams, 2003). The UPP is comprised of a ubiquitin conjugating system and the proteasome

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(Figure 1). Cellular proteins that govern important functions including signal transduction, cell cycle control, transcriptional regulation, and apoptosis are substrates of the UPP. Furthermore, the UPP selectively eliminates mutant, misfolded, and damaged proteins and is indispensable for cellular housekeeping (Ciechanover, 1994).

Given the significance of the UPP, aberrations in this pathway have been implicated in the pathogenesis of various human diseases including cancer (Ciechanover, 1998). Cancer cells frequently harbor defects in some regulatory proteins whose degradation is either overabundant or insufficient. For instance, excessive degradation of the tumor suppressor p53 or IκB (inhibitor of NF-κB) in tumor cells could promote cancer cell growth. Thus, targeting key features of the UPP responsible for the mechanisms underlying tumorigenesis and progression in cancer cells has been the subject of intense investigation. This review summarizes the development and applications of bortezomib, the first U.S. FDA approved selective inhibitor of the proteasome, for the treatment of multiple myeloma and mantle cell lymphoma. Furthermore, the second generation proteasome inhibitors as anticancer drugs currently being tested in clinical trials and several other proteasome inhibitors currently being developed preclinically are also discussed here.

The Ubiquitin-Proteasome Pathway

A basic understanding of the proteasome structure and function in the UPP is necessary to understand the mechanism of action of bortezomib. Degradation of proteins by the UPP involves two discrete and important steps: (i) the activation of ubiquitin and subsequent covalent attachment of multiple moieties to a protein substrate by the E1, E2, and E3 enzymes, followed by (ii) degradation of the tagged protein by the 26S proteasome (Figure 1) (Adams, 2003). Structurally, the 26S proteasome is a proteolytic complex comprised of a hollow cylindrical multi-catalytic 20S core and two 19S regulatory caps (Groll *et al.*, 1997). The 19S cap recognizes, binds, and cleaves the polyubiquitin chain of the target protein thereby directing it into the 20S catalytic core (Peters *et al.*, 1993). The 20S catalytic core consists of two identical alpha subunit (α1-α7) rings and two identical beta subunit (β1-β7) rings. The catalytic cleavage of peptides is facilitated by three proteolytically active beta subunits, β1 (caspase-like activity), β2 (trypsin-like activity), and β5 (chymotrypsin-like activity). The catalytic site of β5 subunit is defined by several hydrophobic residues, and the hydroxyl group on the side chain of threonine 1 (Thr1) is responsible for catalyzing cleavage of peptides through nucleophilic attack (Groll *et al.*, 1999). Inhibition of the proteasomal chymotrypsin-like activity significantly affects protein processing by the proteasome (Groll *et al.*, 1999).

As discussed earlier, degradation of misfolded and unfolded proteins is a major function of the UPP. Misfolded or unfolded proteins are recognized by the endoplasmic reticulum (ER) stress or unfolded protein response (UPR) pathway and are guided to degradation by the 26S proteasome (Tsai *et al.*, 2002). Disruption of proteasome activity causes accumulation of otherwise degradable proteins within the cell and constitutive ER stress blocks cellular growth and division, eventually leading to cell death (Obeng *et al.*, 2006). Cells in general may not respond similarly to proteasome inhibition and several types of cancer cells have actually been found to be far more sensitive to proapoptotic effects of proteasome inhibition

than normal cells (Dou and Li, 1999), and this provides the essential basis for proteasome inhibitors as anticancer drugs. Additionally, inhibition of the tumor cellular proteasome could potentially disrupt the mechanisms of *de novo* and acquired resistance, sensitizing them to chemo- or radiotherapy (Voorhees *et al.*, 2003). Therefore, development of small molecule proteasome inhibitors has received considerable attention as an important therapeutic strategy for a range of cancers, including multiple myeloma, lymphoma, and some solid tumors (Meiners *et al.*, 2008; Orlowski and Zeger, 2006).

Bortezomib: Biological Effects and Possible Mechanisms of Action

Bortezomib (also known as Velcade® and PS-341) was originally developed by Myogenics, and after successful completion of Phase I clinical trials in multiple myeloma patients it was later bought by Millennium Pharmaceuticals. After further clinical development, it had become a first-in-class U.S. FDA approved proteasome inhibitor for the treatment of multiple myeloma and mantle cell lymphoma (Kane *et al.*, 2003; 2007; 2006). The molecular formula of bortezomib is $C_{19}H_{25}BN_4O_4$ (Figure 2) and its chemical IUPAC name is [3-methyl-1-(3-phenyl-2-pyrazin-2-ylcar-bonylamino-propanoyl) amino-butyl] boronic acid. It is a dipeptidyl boronic acid-based specific, reversible inhibitor of the chymotrypsin-like activity of the 20S proteasome (Jackson *et al.*, 2005; Papandreou *et al.*, 2004). The boronic acid moiety of bortezomib forms a (pseudo)covalent bond with the nucleophilic hydroxyl side chain of Thr1 in the S1 pocket of the β5 subunit (Groll *et al.*, 2006a).

Bortezomib has been reported to possess potent antitumor activity in a wide variety of cancer cell lines including multiple myeloma, prostate cancer, pancreatic cancer, renal cell carcinoma, and squamous cell carcinoma both *in vitro* and in various animal xenograft models (Adams, 2002; Jagannath *et al.*, 2005; Kondagunta *et al.*, 2004; Richardson *et al.*, 2005; Shah *et al.*, 2001; Sunwoo *et al.*, 2001). In both solid tumor-and hematological malignancy-derived cell lines bortezomib has demonstrated equal potency in terms of anticancer effect (Frankel *et al.*, 2000).

The mechanism of action of bortezomib was found to be unique when it was compared with 60,000 other compounds by the National Cancer Institute (NCI) (Adams *et al.*, 1999). Though its detailed mechanism of action is still not completely understood some preclinical studies have proposed possible anticancer mechanisms of bortezomib. Proteasome inhibition by bortezomib induces ER stress and constitutive ER stress causes calcium release, which is subsequently taken up by the mitochondria, leading to cytochrome c release and subsequent activation of effector caspases, followed by cleavage of Bid to tBid, ultimately resulting in apoptosis (Landowski *et al.*, 2005). Recently, Gu *et al.* (2008) reported that bortezomib-triggered apoptosis in multiple myeloma cells was dependent on caspase-2 activation. This study showed that caspase-2, which is associated with ER stress, acts as a proximal caspase and functions upstream of mitochondrial signaling and is required for breakdown of mitochondrial transmembrane potential, release of cytochrome c, and down-stream activation of caspase-9. As myeloma cells produce and secrete large amounts of immunoglobulin, their threshold for induction of ER stress and proapoptotic UPR following proteasome inhibition may be lower. Not surprisingly myeloma cells have been shown to be very sensitive to proteasome inhibition (Meister *et al.*, 2007).

Mitsiades *et al*. (2002) described the molecular sequelae of proteasome inhibition by bortezomib in human multiple myeloma cells. In these cells bortezomib was reported to stimulate and up-regulate genes involved in proapoptotic cascades and down-regulate pro-survival genes. Inhibition of the proteasomal catalytic activity by bortezomib suppresses an important survival mechanism: the activation of nuclear factor-κB (NF-κB) pathway (Adams, 2004b; Palombella *et al.*, 1994). NF-κB is a heterodimeric transcription factor found in the cytoplasm bound to its inhibitory counterpart protein IκB in its inactive state. Ubiquitination and degradation of IκB by the UPP leads to release and translocation of NF-κB to the nucleus (Karin *et al.*, 2004). Once in the nucleus NF-κB can up-regulate the expression of genes that promote cell growth and survival [e.g., insulin-like growth factor 1 (IGF1) and its receptor IGF1R, NF-κB, Bcl-2 family members, and inhibitor-of-apoptosis proteins (IAPs)]. Therefore, it is thought that bortezomib prevents degradation of IκB and activation of NF-κB, in turn suppressing the production of survival factors. Recently it has been reported that NF-κB activity levels are different in drug-sensitive and drug-resistant multiple myeloma cells (Ma *et al.*, 2003). Furthermore, the presence of elevated NF-κB activity levels has been observed in patients with relapsed multiple myeloma (Feinman *et al.*, 1999). These studies suggest that NF-κB is a key target of bortezomib in multiple myeloma cells.

More recent studies have also identified other possible mechanisms involved in bortezomib's anticancer activity. NOXA, a pro-apoptotic protein belonging to the Bcl-2 family, appears to be another key modulator of bortezomib's anticancer effects (Qin *et al.*, 2005). In p53-mediated apoptosis, up-regulation of p53 expression with subsequent *Noxa* gene expression has been observed. Tumor suppressor p53 has been shown to interact directly with and activate the promoter for *Noxa* gene expression (Oda *et al.*, 2000). Induction of apoptosis by NOXA involves its direct interaction with anti-apoptotic proteins like Bcl-2 and Bcl-X_L or by stimulating other apoptosis-promoting factors (Adams and Cory, 1998; Gross *et al.*, 1999; Oda *et al.*, 2000). In a variety of tumor cell lines with defective p53 signaling, bortezomib can still induce NOXA expression and block tumor growth (Adams, 2004a; Caravita *et al.*, 2006; Nikiforov *et al.*, 2007). Bortezomib causes 20- to 60-fold induction of NOXA expression selectively in cancer cells but not in normal cells (Fernandez *et al.*, 2005; Qin *et al.*, 2005). This selectivity of proteasome inhibitors is directly dependent on c-Myc binding sites in the *Noxa* promoter and depletion of c-Myc blocks the tumor cell-selective induction of NOXA by bortezomib (Nikiforov *et al.*, 2007).

Hypoxia-inducible factor-1α (HIF-1α), which is known to support tumor growth, is one of the most studied and promising molecular targets for anti-cancer therapy. In both androgen-dependent and androgen-independent prostate cancer cell lines, bortezomib was reported to reduce HIF-1α protein synthesis through suppression of PI3K/Akt/mTOR and MAPK pathways (Befani *et al.*, 2011). Very recently, studies in breast cancer cell lines by Ishii *et al.* (2006) reported Cyclin D1 levels to be an important marker or target for predicting the response to bortezomib treatment. Cyclin D1 is a short-lived protein that is regulated by the UPP (Diehl *et al.*, 1998; Germain *et al.*, 2000). Cyclin D1 was found to be over-expressed in approximately 25% and 90% of all multiple myeloma and mantle cell lymphoma patients, respectively (Lesage *et al.*, 2005; O'Connor *et al.*, 2005). Cyclin D1 expression levels

correlated with the overall response rate to bortezomib treatment in each disease. This study showed that in a variety of human tumors including breast cancer cells, Cyclin D1 levels inversely correlated with the levels of anti-apoptotic transcription factor Signal Transducer and Activator of Transcription 3 (STAT3). STAT3 is known to regulate the expression of anti-apoptotic Bcl-X_L and has been found to be overexpressed in many human cancers. Bortezomib treatment was more effective in Cyclin D1-overexpressing cells and therefore the authors proposed an additional mechanism of action for bortezomib's anti-cancer activity which is the stabilization of Cyclin D1 protein levels in order to inhibit the STAT3/Bcl-X_L survival axis (Ishii *et al.*, 2006).

Bortezomib has also been tested in combination with other chemotherapeutic drugs. In doxorubicin-, mitoxantrone-, and melphalan-sensitive and -resistant RPMI-8226 human multiple myeloma cells, bortezomib was found to possess potent growth inhibitory effects *in vitro* (Hideshima *et al.*, 2001). In these combinations bortezomib had a profound sensitization effect on cancer cells resistant to chemotherapeutic drugs such as melphalan and doxorubicin. The mechanism of sensitization was found to be down-regulation of expression of several proteins involved in DNA repair pathways (Mitsiades *et al.*, 2003).

Bortezomib: Reports from Clinical Trials

The FDA approval of bortezomib as a front-line treatment for patients with newly diagnosed and relapsed/refractory multiple myeloma reinforces the UPP as a valid target in the treatment of malignant diseases. Bortezomib, either alone or in combination, has an overall positive response in the clinic. For example, in a Phase II clinical trial conducted by Oakervee and colleagues, relapsed multiple myeloma patients were treated with a combination therapy of bortezomib, doxorubicin, and dexamethasone. Nearly partial response (PR) was achieved in 20 of 21 patients (95%), including complete response (CR) in 43%, near CR in 14%, very good PR in 24%, and PR in 14% (Oakervee *et al.*, 2005). In another Phase II trial conducted by Jagannath *et al.* (2005), 32 consecutive patients with untreated symptomatic multiple myeloma patients were given bortezomib in combination with dexamethasone, yielding a response rate (CR+PR) of 88%. Additionally, in the Phase II Study of Uncontrolled Multiple Myeloma Managed with Proteasome Inhibition Therapy (SUMMIT), 202 relapsed and refractory multiple myeloma patients were treated with 1.3 mg/m2 bortezomib for a 3-week cycle for up to eight cycles. The combined overall response rate was 35% with bortezomib treatment alone (Richardson *et al.*, 2003). The most recent phase III VISTA trial, which included patients with previously treated multiple myeloma, reported that the combination of bortezomib with melphalan and prednisone improves overall patient survival (Mateos *et al.*, 2010). Analysis of the data from this trial showed that bortezomib-based drugs as first-line treatments had a greater survival advantage over conventional drugs followed by bortezomib treatments for salvage (Mateos *et al.*, 2010). It also showed that in patients treated with bortezomib plus melphalan-prednisone the rate of improvement of peripheral neuropathy was 79%, indicating this adverse side effect to be generally manageable and reversible in most patients with relapsed myeloma (Mateos *et al.*, 2010).

Toxicity, Resistance, and Limitations of Bortezomib

In spite of its improved efficacy compared to traditional chemotherapies, bortezomib still possesses some toxicity in the clinic and about 60% of patients will eventually not respond to bortezomib due to the emergence of resistance. The average time between the start of bortezomib treatment and the occurrence of resistance to it is about one year (Richardson *et al*., 2003). The most frequent toxicities associated with bortezomib are gastrointestinal symptoms, anemia, thrombocytopenia, asthenia (fatigue, malaise, and weakness), elevated calcium levels, and peripheral neuropathy (Orlowski *et al*., 2005).

Some of the molecular mechanisms for the emergence of resistance have been found to be mutations in the bortezomib binding pocket of the β5 subunit, overexpression of the proteasomal β5 subunit (Oerlemans *et al*., 2008), over-expression of the anti-apoptotic protein Bcl-2 (Smith *et al*., 2011), high secretion of GRP-78, a chaperone protein of the unfolded protein response (Kern *et al*., 2009), and over-expression of heat shock proteins (HSPs) 27, 70, and 90 as well as T-cell factor 4 (Shringarpure *et al*., 2006). For example, de Wilt *et al*. (2011) showed that in non-small cell lung cancer (NSCLC) intrinsic bortezomib resistance correlated with high basal levels of proteasome activity, whereas acquired resistance was associated with proteasome subunit over-expression and emergence of mutant β5-subunits. Additionally, prevalence of proteasome inhibition- resistant, constitutive NF-κB activity in RPMI 8226 multiple myeloma cells and stem-like cells of mantle cell lymphoma has been reported (Jung *et al*., 2011; Yang *et al*., 2008). Furthermore, down-regulation of XBP1, a major regulator of UPR, has been observed in myeloma cells resistant to bortezomib (Ling *et al*., 2011).

Beyond bortezomib's approved indications for the treatment of multiple myeloma and mantle cell lymphoma, it has been investigated for the treatment of solid tumors in clinical studies. It was found that bortezomib had decreased efficacy in solid tumors, possibly due to induction of stress granule formation by bortezomib. This involves phosphorylation of translation initiation factor eIF2α by heme-regulated inhibitor kinase (HRI) (Fournier *et al*., 2010).

Recent preclinical studies have shown that green tea polyphenol epigallocatechin-3-gallate (EGCG) (Golden *et al*., 2009; Kim *et al*., 2009) and vitamin C (Perrone *et al*., 2009; Zou *et al*., 2006) have the potential to inhibit the cytotoxicity of bortezomib. These two natural compounds contain vicinal diols that can bind and inactivate boronic acid present in bortezomib, thereby inhibiting the proteasome-inhibitory and anticancer activities of bortezomib (Figure 3). Further clinical studies are needed for evidence-based recommendations on consumption of green tea and vitamin C for patients receiving bortezomib.

The Second Generation Proteasome Inhibitors: Carfilzomib and Marizomib

The acquired resistance to bortezomib therapy seen in patients encouraged researchers to search for other new proteasome inhibitors as well as novel natural compounds with proteasome-inhibitory activity. Carfilzomib (Figure 2), a peptide epoxyketone derived from epoxomicin, was first developed by Proteolix, Inc. and currently by Onyx Pharmaceuticals.

Carfilzomib binds irreversibly to the proteasome and preferentially inhibits the chymotrypsin-like activity over the caspase-like or trypsin-like activities (Kuhn *et al.*, 2007). Results from preclinical studies demonstrate that carfilzomib induced higher levels of apoptosis than bortezomib in primary plasma cell models, and was able to overcome resistance to bortezomib. In animal models, carfilzomib had anti-cancer activity in both dose- and time-dependent manners, and again the anti-cancer efficacy of carfilzomib was stronger than that of bortezomib when tested on its clinical dosing schedule (Demo *et al.*, 2007). Carfilzomib is currently being investigated in both Phase II and III clinical trials for the treatment of recurrent multiple myeloma and solid tumors (Kuhn *et al.*, 2011). Phase II clinical trials in patients with previously treated multiple myeloma showed response rates in the range of 25–54% (Yang *et al.*, 2009). Another clinical trial has been initiated to evaluate the efficacy and safety of carfilzomib with lenalidomide and dexamethasone in patients with relapsed multiple myeloma (NCT00603447, Proteolix).

Another second generation proteasome inhibitor is marizomib (Figure 2) which is currently being developed by Nereus Pharmaceuticals, Inc. (Potts *et al.*, 2011). It is a natural product derivative resembling lactacystin, the first identified natural proteasome inhibitor. In contrast to bortezomib and carfilzomib, marizomib irreversibly targets all three active sites of the proteasome (Joazeiro *et al.*, 2006). Marizomib showed significantly stronger and more durable effects on the proteasomal chymotrypsin-like and trypsin-like activities than bortezomib in preclinical studies (Chauhan *et al.*, 2005; Groll *et al.*, 2006b). The increased potency of marizomib may be related to its specificity toward caspase 8-mediated apoptosis compared to bortezomib (Chauhan *et al.*, 2005). Consistent with these properties, marizomib was able to overcome bortezomib resistance and work synergistically with conventional therapy in multiple myeloma and chronic lymphocytic leukemia (CLL) cell models (Chauhan *et al.*, 2008; 2010; Sterz *et al.*, 2008). Phase I studies aimed at establishing optimal dosing of marizomib against advanced solid tumors or refractory lymphomas and multiple myeloma have been conducted (Yang *et al.*, 2009). Furthermore, clinical studies investigating marizomib in combination with vorinostat are ongoing in a Phase Ib open-label study in patients with advanced non-small lung cancer (NCT00667082, Nereus Pharmaceuticals).

In addition, CEP-18770 (developed by Cephalon) and MLN-9708 (developed by Millennium Pharmaceutics) are two other reversible, peptide boronic acid-based proteasome inhibitors (Dick and Fleming, 2010; Kupperman *et al.*, 2010). CEP-18770 is being investigated in Phase I and II clinical trials for the treatment of recurrent, advanced stage solid tumors, lymphoblastic leukemia and non-Hodgkin's lymphoma, while MLN-9708 is in Phase I and II clinical trials for lymphoma and solid tumors (www.cancer.gov/clinicaltrials/search; www.clinicaltrials.gov). Furthermore, ONX-0912 (by Onyx Pharmaceuticals), another peptide epoxyketone proteasome inhibitor, is currently in Phase I and II clinical trials for patients with solid tumors and hematological cancers (www.cancer.gov/clinicaltrials/search; www.clinicaltrials.gov).

Further clinical studies are needed to define the profiles of toxicity and anticancer efficacy of these second generation proteasome inhibitor drugs.

Other Proteasome Inhibitors Currently Being Developed

The immunoproteasome ($20S_i$ and $26S_i$) is a cytokine-inducible form of the constitutive proteasome, with the replacement of β1, β2, and β5 subunits with the immunoproteasome-specific $\beta1_i$, $\beta2_i$, and $\beta5_i$ subunits, respectively. It has been found that myeloma cells express increased levels of the immunoproteasome complex. Also, lower levels of the immunoproteasome and increased levels of the constitutive proteasome are associated with relapsed myeloma and bortezomib resistance (Kuhn *et al.*, 2011). IPSI-001, an immunoproteasome-specific inhibitor, has been investigated and found to preferably inhibit immunoproteasome $20S_i$ activity over constitutive 20S proteasome activity. The main mechanism involves binding of IPSI-001 to the $\beta1_i$ subunit. This inhibition leads to enhanced apoptotic cell death in human cancer cells generated from a hematologic origin. Other IPSIs, PR-924, PR-957, and different $\beta1_i$ specific inhibitors, are also currently being developed. It has been shown that IPSIs were able to overcome bortezomib-resistance in the preclinical setting, suggesting that they may provide an alternative therapeutic option for cancer patients resistant to bortezomib (Dou, 2011; Kuhn *et al.*, 2011).

In addition to the proteasome inhibitors mentioned above, some other specific proteasome inhibitors (e.g., TMC-95A and PR39) have also been developed, which, however, have not yet been tested clinically. Some natural flavonoids (e.g., EGCG, apigenin) and medicinal compounds (e.g., celastrol, withaferin A) have shown to possess tumor proteasome-inhibitory capabilities *in vitro* and *in vivo* and these compounds have already been used in various human studies. Also, some old drugs that can bind to copper or zinc (e.g., 5-amino-8-hydroxyquinoline, clioquinol, and disulfiram) and inhibit the tumor proteasome in preclinical settings have been used in various clinical studies. The above mentioned proteasome inhibitors could also chemo-/radio-sensitize human cancer cells, but whether they could overcome bortezomib-resistance needs to be studied (Dou, 2010; Ruschak *et al.*, 2011).

Conclusions and Future Perspectives

Most targeted therapies are designed with the intent of inhibiting a single protein target or signaling pathway to dampen tumor progression and have shown limited anti-cancer efficacy. Alternatively, targeting the UPP, which regulates multiple events and involves many protein targets, represents one of the most promising anticancer strategies. Bortezomib is the first U.S. FDA approved proteasome inhibitor in clinical use. It possesses potent antitumor activity and acts as a chemo-/radio-sensitizing agent when combined with conventional therapy or radiation. However, side effects and the eventual emergence of drug-resistance in a significant portion of cancer patients encouraged the development of second generation proteasome inhibitors like carfilzomib, marizomib, CEP-18770, MLN-9708, and ONX-0912 that are currently being investigated in clinical trials. Furthermore, IPSIs were shown to overcome bortezomib-resistance in the preclinical setting, and are ready to move to clinical studies. Natural products EGCG, apigenin, celastrol, and withaferin A also possess tumor proteasome-inhibitory effects *in vitro* and *in vivo* and these compounds have already been investigated in various human studies. Moreover, some copper- or zinc-binding drugs (e.g., 5-amino-8-hydroxyquinoline, clioquinol, and

disulfiram) inhibit the tumor proteasome in both preclinical and clinical studies. However, all the new proteasome inhibitors' efficacy, their ability to overcome bortezomib-resistance, and whether they themselves develop resistance remain to be investigated and elucidated. Finally, the importance of the UPP has incited researchers to target other aspects of the pathway such as E1, E2, and, especially E3 enzymes, as well as the ubiquitin receptors and deubiquinating enzymes (DUBs) toward the goal of improving cancer therapy. There are high hopes in the field that the discovery of novel anticancer agents targeting the ubiquitin-proteasome pathway will help illuminate the future of cancer treatment.

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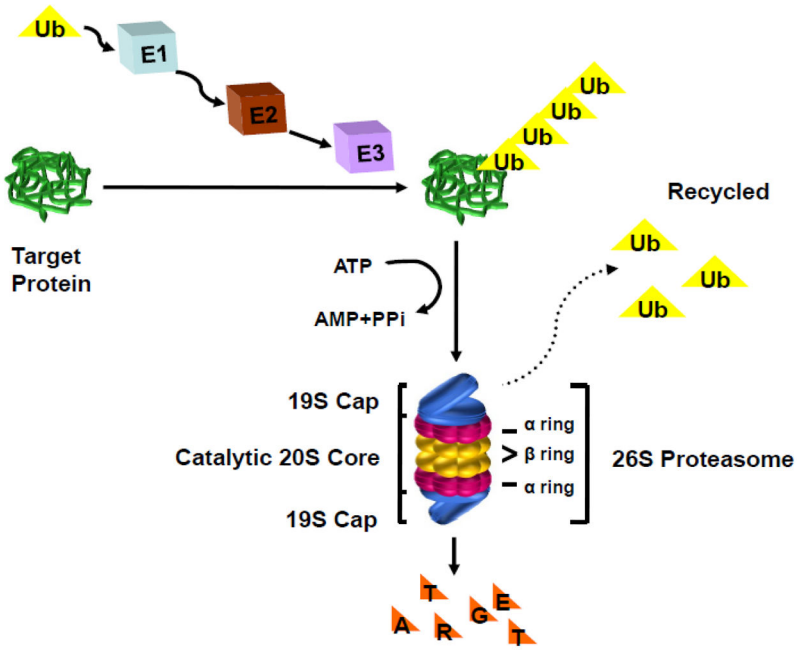

Figure 1.

The ubiquitin-proteasome pathway. Target proteins of the proteasome are tagged with polyubiquitin molecules in an ATP-dependent process through E1, E2, and E3 ligases. Polyubiquitinated proteins are then recognized by the 19S regulatory complex of the 26S proteasome and fed into the 20S catalytic core for degradation and the ubiquitin molecules recycled. The 20S proteasome contains four stacked rings (αββα): α subunits serve as a gate to regulate protein entry while the β subunits possess the enzymatic activities.

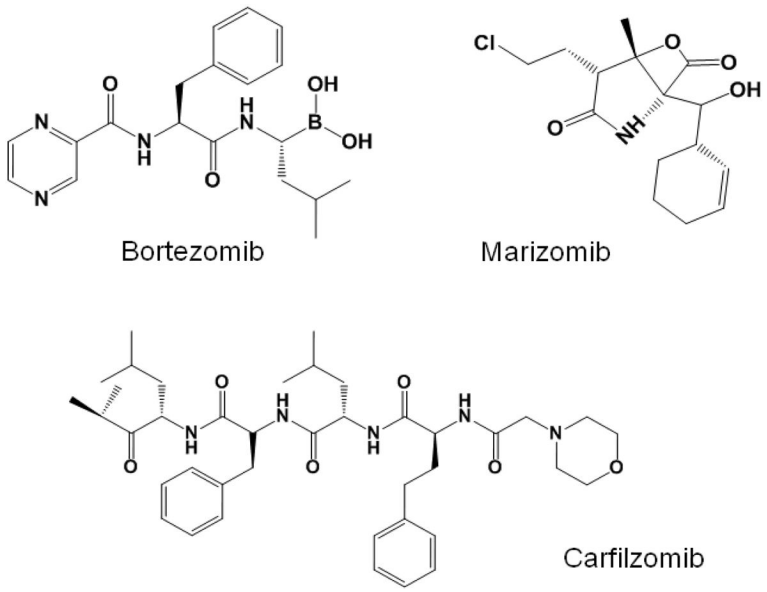

Figure 2.
Chemical structures of proteasome inhibitors bortezomib, carfilzomib, and marizomib.

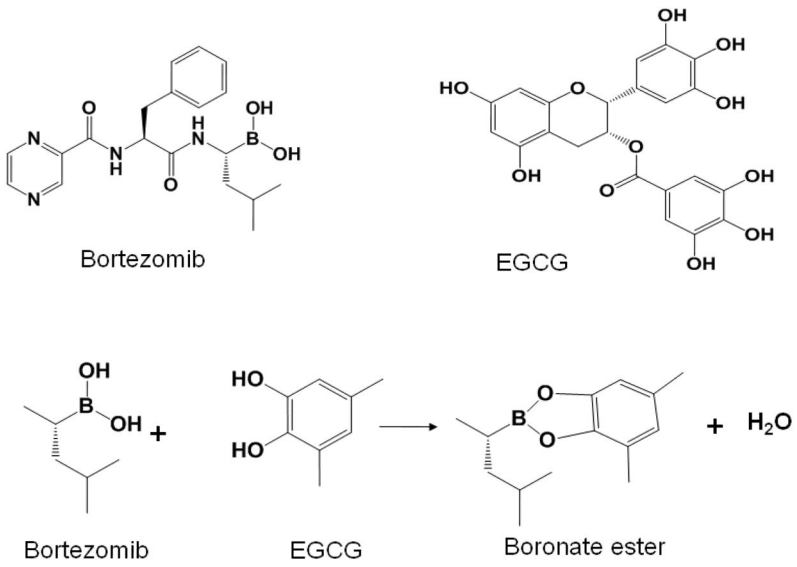

Figure 3.

Bortezomib interacts with certain natural compounds with vicinal diols. Postulated interaction between bortezomib and EGCG as an example is presented.