Proteasome Inhibitors

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Contributor: Carlota Leonardo-Sousa, Rita Guedes, Andreia Carvalho, Pedro Fernandes, Natália Aniceto, Jorge Salvador, Maria Gama

Proteasome inhibitors have shown relevant clinical activity in several hematological malignancies, namely in multiple myeloma and mantle cell lymphoma, improving patient outcomes such as survival and quality of life, when compared with other therapies. However, initial response to the therapy is a challenge as most patients show an innate resistance to proteasome inhibitors, and those that respond to the therapy usually develop late relapses suggesting the development of acquired resistance. The mechanisms of resistance to proteasome inhibition are still controversial and scarce in the literature.

Keywords: ubiquitin–proteasome pathway ; proteasome inhibitors ; mechanisms of resistance ; innate resistance ; acquired resistance ; multiple myeloma ; cancer

1. Introduction

The quality and quantity of proteins within a cell must be tightly regulated according to cellular needs or physiological demand. The ubiquitin–proteasome pathway (UPP) is critical for the maintenance of intracellular protein homeostasis in physiological conditions, as well as during adaptive stress responses, and is responsible for the regulation of a wide variety of signaling pathways [1]. Accordingly, impairment of the UPP has been associated with several pathological conditions that include neoplastic disorders [2]. Cancer cells are characterized by the loss of cell cycle checkpoint control and are often subjected to elevated levels of stress because of hyperactivation of oncogenic signaling and/or adverse microenvironmental conditions. Therefore, transformed cells rely to a great extent on the correct function of UPP for survival and proliferation [2].

Just after the discovery of the UPP and its relevance to protein and cellular homeostasis, preclinical studies on the putative role of proteasome inhibitors as critical agents for modulating cancer cell death have begun [3]. The proteasome was identified and validated as a pivotal target in protein quality control and turnover, cell-cycle regulation, cell differentiation, and apoptosis. Since then, three proteasome inhibitors have been approved by the US Food and Drug Administration Agency (FDA) and the European Medicine Agency (EMA), Velcade® (bortezomib), Kyprolis® (carfilzomib), and Ninlaro® (ixazomib), as new drugs to treat multiple myeloma (MM) and mantle-cell lymphoma (MCL) [4][5][6][7][8][9].

MM is the second most frequent hematological malignancy with an age-adjusted incidence of approximately 7.1 per 100,000 persons per year in the USA (~1.8% of all cancers), based on 2014–2018 cases [10] and approximately 2.9 per 100,000 persons in Europe, based on 2020 cases [11] (**Figure 1**). This malignancy is described as an expansion of dysfunctional terminal differentiated plasma cells in the bone marrow. MM cells show strong bone marrow dependence, extensive somatic hypermutation of immunoglobulin genes and absence of IgM expression [12]. Therefore, MM is characterized by aberrant proliferation of bone marrow plasma cells that commonly produce a high amount of monoclonal immunoglobulin, leading to functional impairments in different organs, namely anemia, bone disease, renal dysfunction and hypercalcemia [13]. MM is a very heterogeneous disease with median survival ranging from 2 to 10 years and is characterized by remission periods alternating with relapse/progression phases, finally leading to refractory disease [13]. The improved understanding of the mechanisms involved in MM has led to more effective therapeutic strategies such as proteasome inhibitors (PIs), namely bortezomib, carfilzomib, and ixazomib, and immunomodulatory drugs (IMIDs), including thalidomide, lenalidomide, and pomalidomide, that allowed extension of the median overall patient survival to over 8 years [13][14] (**Figure 2**). These two classes of drugs revolutionized the treatment of MM due to their strong synergistic action. Despite this improvement in first-line therapy, almost all patients eventually relapse, the outcome progressively worsens, and the disease is still generally considered incurable.

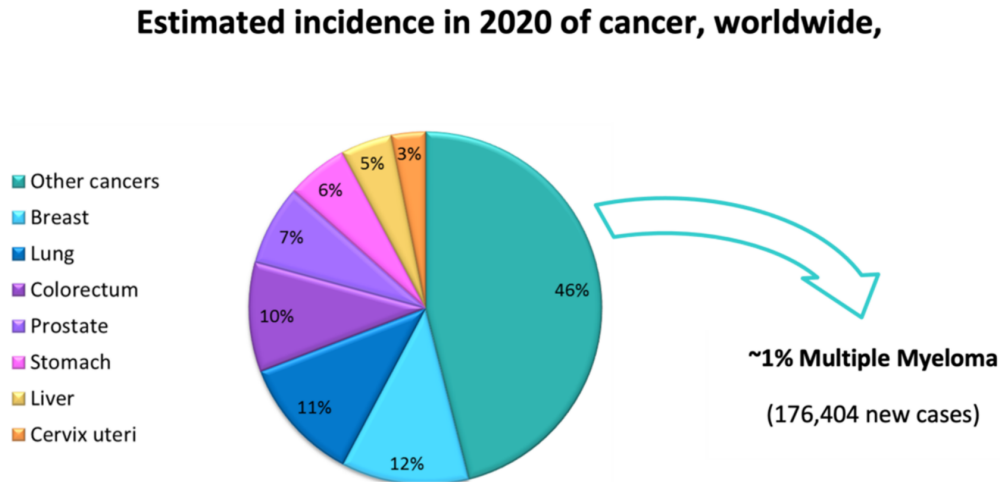

Figure 1. Estimated new cases of cancer in 2020, worldwide, both sexes and all ages. MM represents 1% of all types of cancer. Adapted from: Ferlay, J.; Ervik, M.; Lam, F.; Colombet, M.; Mery, L.; Piñeros, M.; Znaor, A.; Soerjomataram, I.; Bray, F. Global Cancer Observatory: Cancer Today; Lyon, France, 2020.

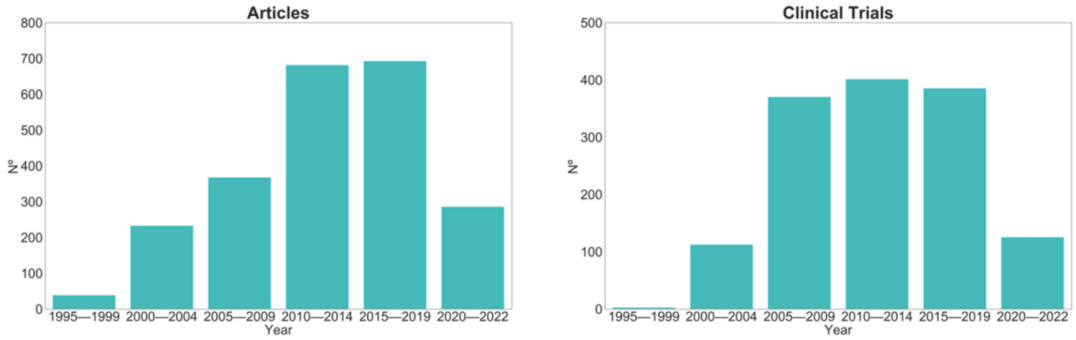

Figure 2. Articles published from 1995 reported on PubMed, entering the search terms “proteasome inhibitors” and “resistance” in all fields, and clinical trials whose intervention/treatment includes PIs (bortezomib, carfilzomib, ixazomib, marizomib, delanzomib or oprozomib) starting from 1995. Adapted from: ClinicalTrials.gov (https://clinicaltrials.gov/ct2/home accessed on 22 February 2022) and PubMed (https://pubmed.ncbi.nlm.nih.gov/ accessed on 22 February 2022).

MCL is a rare but aggressive disease, with a poor prognosis and limited survival, resulting from a malignant transformation of a B lymphocyte in the outer edge of a lymph node follicle (the mantle zone). MCL represents 3% to 10% of all newly diagnosed non-Hodgkin lymphoma (NHL) cases with an incidence of approximately 1 per 100,000 persons in the USA. This NHL is molecularly characterized by the chromosomal translocation t(11;14)(q13;q32) that results in a constitutional overexpression of the cell cycle regulator protein cyclin D1 with consequent cell cycle dysregulation [15]. This translocation is the initial event of the lymphomagenesis, but tumor cells can accumulate additional alterations that will ultimately produce the aggressive phenotype in disease progression. An increasing number of biologically targeted therapies are improving MCL treatment options in both first-line and relapsed conditions, namely the proteasome inhibitor bortezomib (Velcade®), the mechanistic target of rapamycin (mTOR) inhibitor temsirolimus (Torisel®), lenalidomide and ibrutinib, the four drugs currently licensed for MCL [15]. However, despite the recent advances in therapy, relapses are still frequent and associated with a poor prognosis. Generally, the disease is characterized by rapid relapses and poor long-term outcomes due to the development of resistance [16].

Both MM and MCL are aggressive diseases largely regarded as incurable, mostly due to the development of resistance. The results obtained in clinical trials with PIs grant them the status of promising therapeutics to be further investigated in a permanent search for new chemical entities able to offset the upregulation of the proteasome encountered in these diseases. MM patients showed considerably improved outcomes with the use of both first- and second-generation PIs that elicited deep initial responses in these patients. Additionally, primary resistance is also a drawback to the use of PIs (as demonstrated also for solid tumors) where, regardless of the promising pre-clinical data obtained, clinical data have been shown to be disappointing. Thus, this reinforces the importance of understanding drug resistance mechanisms associated with PIs, to acquire novel insights critical to further maximize the effectiveness of this class of drugs and improve therapies. Herein, researchers present a brief overview of the classes of PIs developed so far and critically discuss the advances and challenges related to the use of PIs in the clinic.

2. Ubiquitin–Proteasome Pathway (UPP)

The dynamic state of intracellular proteins is maintained by a perfect equilibrium between protein synthesis and protein degradation. The UPP is the primary proteolytic pathway responsible for the degradation of short-lived proteins, providing the specificity and temporal control needed for fine-tuning the steady-state levels of many regulatory proteins [17][18]. Therefore, in addition to mediating the degradation of damaged and misfolded intracellular proteins, through the regulation of protein turnover, the UPP also regulates the function of several proteins, including transcription factors, many of which are critical in the determination of cell fate [19][20]. The UPP plays a crucial role in numerous cellular functions including regulation of cell cycle and division, DNA damage repair, membrane trafficking, cellular stress response, intracellular signaling and apoptosis [17][18][20][21].

The degradation of proteins by the UPP is a sequential process involving an initial step of ubiquitin (Ub) conjugation to the protein substrate followed by the degradation of the polyubiquitinated protein through the 26S proteasome complex, with the release of free Ub, mediated by deubiquitinating enzymes (DUBs) (**Figure 3**) [17][18][20][21]. Ub conjugation, or ubiquitination, is a post-translational modification that consists of the covalent attachment of one (monoubiquitination) or several (polyubiquitination) Ub molecules to a protein and depends on the concerted, successive action of three types of enzymes: the ubiquitin-activating enzyme (E1), the ubiquitin-conjugating enzymes (E2) and the ubiquitin-protein ligases (E3) [17][21]. Polyubiquitination generally serves as a recognition signal for proteolytic degradation by the 26S proteasome. The 26S proteasome is a large (~2.5 MDa) multimeric protease complex, generally conserved in eukaryotes, both structurally and functionally. It is formed by a key 20S core particle (CP), which contains the protease subunits, capped on one or both ends by the 19S regulatory particles (RP), that regulate the proteolytic function of the protease core [17][22]. PIs typically target the 20S CP of the proteasome, so this will be expanded in further detail next.

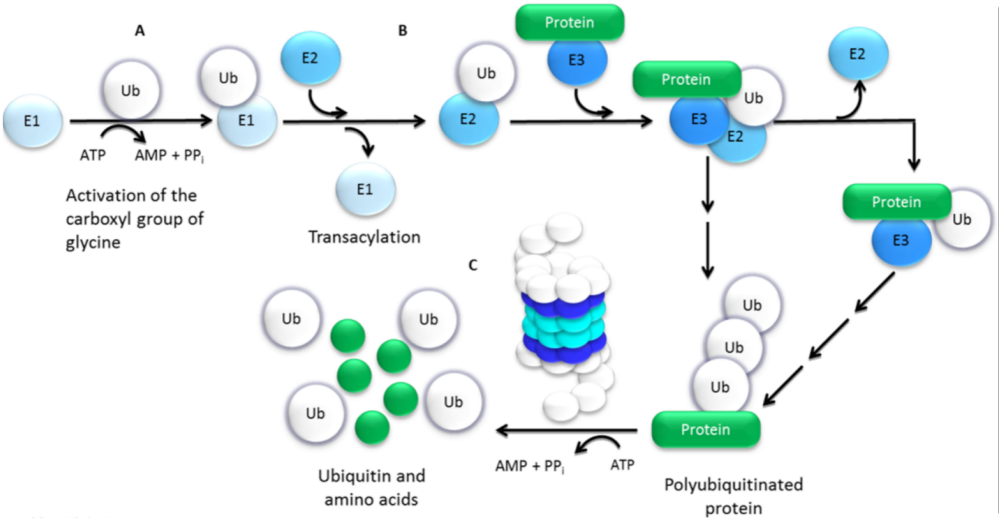

Figure 3. (**A**) Ubiquitin–proteasome pathway of protein degradation. In the ubiquitination process, there is activation of the carboxyl group of glycine found at the C-terminal residues of ubiquitin (Ub), catalyzed by E1 (formation of a thiol-ester bond between E1 and ubiquitin), with the hydrolysis of ATP to AMP and with the release of one PPi molecule. (**B**) After activated ubiquitin is transferred, by transacylation, to the thiol group of the enzyme E2. Then E3 recognizes the protein to be degraded and facilitates E2 to transfer the ubiquitin to the protein, with the formation of an isopeptide covalent bond between the C-terminal glycine residues of ubiquitin and a lysine residue of the protein. (**C**) From multiple cycles of ubiquitination, a polyubiquitinated protein is obtained. In the degradation process, the polyubiquitinated proteins are unfolded and recognized by the 26S proteasome, with ATP hydrolysis to AMP.

The 20S Proteasome Core Particle

The 20S CP is a barrel-shaped structure that corresponds to the catalytic component of the proteolytic machinery of the 26S proteasome. It is formed by four stacked heptameric rings, each ring consisting of seven α- or β-type subunits (**Figure 4**). The two inner β-rings contain the proteolytic active sites (β1–7), facing inward into the proteolytic chamber (the inside of the “barrel”). Three of which—β1, β2 and β5—are endowed with caspase-, trypsin- and chymotrypsin-like activities, respectively [22]. The β5 subunit with chymotrypsin-like activity is responsible for the cleavage of peptide bonds after a hydrophobic residue; the β2 subunit displays trypsin-like activity and cleaves the peptide bonds after a basic residue; and the β1 subunit has caspase-like or post-acidic-like activity cleaving the peptide bonds after an acidic residue [3][22]. The three catalytic subunits contain an N-terminal residue, Thr1, whose hydroxyl group acts as a nucleophile and interacts with the peptides of the proteins to be degraded (**Figure 4** and **Figure 5**) [22][23]. The α-subunits (α1–7) in the outer rings of the 20S CP can recognize and direct polyubiquitinated substrates into the proteolytic chamber. One or two

19S RP can be attached to the surface of the outer α-rings of the 20S CP to form the 26S proteasome holoenzyme (**Figure 4**).

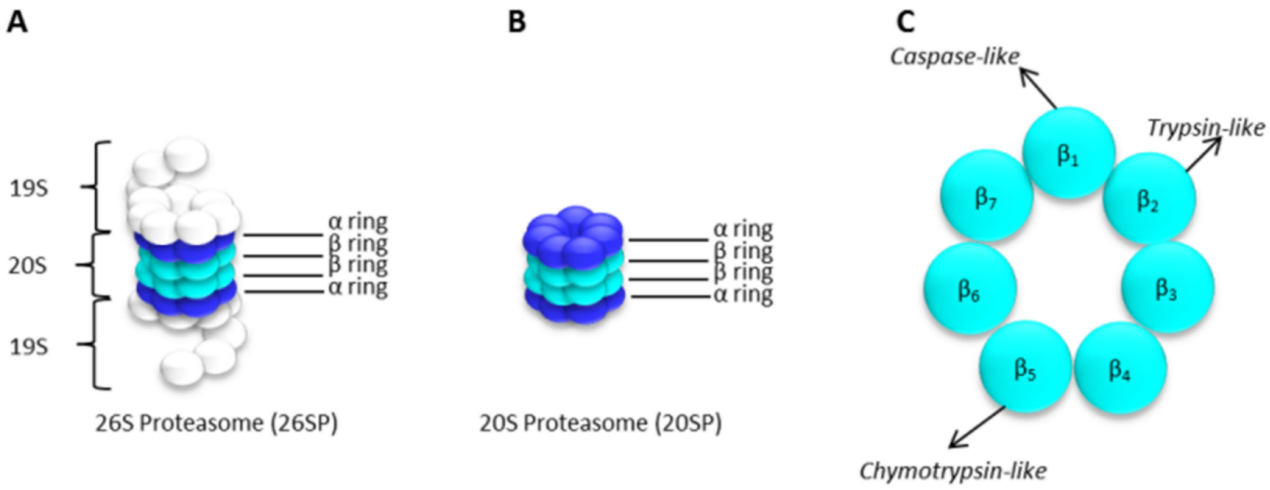

Figure 4. (**A**) 26S proteasome. The 26S proteasome consists of a multimeric protease with 2 19S RP and the 20S CP. (**B**) The 20S CP is constituted by 2 α heptameric rings and 2 β heptameric rings. (**C**) The catalytic subunits are located in 3 distinct β subunits, in both β heptameric rings.

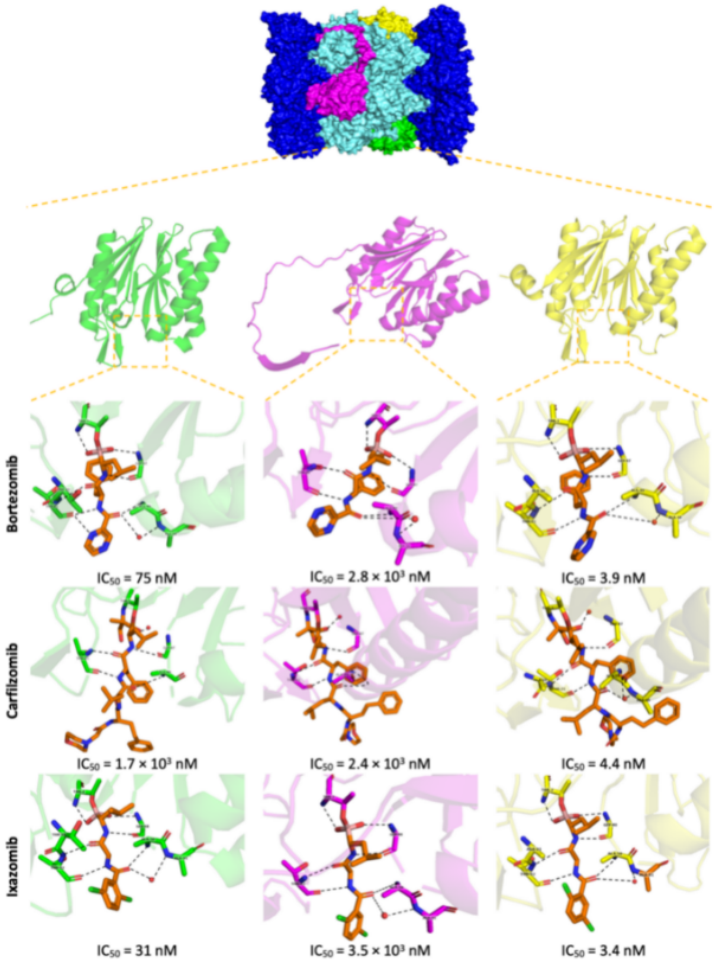

Figure 5. Three β catalytic subunits of 20S CP complexed or not with the three approved inhibitors. The β1, β2, and β5 subunits are colored in green, magenta, and yellow, respectively. The inhibitors are colored in orange; hydrogen bonds are shown as black dashed lines and water molecules are represented as red spheres. These images were generated with the crystallographic structures whose PDB IDs are 4R67, 5LE5, 5LF3, and 5LF7. Source: Report RPT-01200 Amendment 2 and Study TR-0004-171.

The 19S RP is a 700 kDa ring-shaped complex also called proteasome activator 700 (PA700) and is formed by two substructures, a lid and a base, with multiple subunits, as shown in **Figure 4**A. The 19S RP recognizes polyubiquitinated proteins and promotes either their ATP-dependent unfolding or the dismantling of ubiquitin chains, catalyzed by proteasome-associated DUBs [2]. The 20S CP generally mediates the cleavage/degradation of polyubiquitinated protein substrates that have been unfolded by the 19S RP into small peptides and amino acids [2][22]. However, in some cases,

the 20S CP can act alone through ubiquitin-independent degradation pathways, still being functional towards certain proteins [3][22][24][25].

3. Proteasome Inhibitors (PIs) in Cancer Therapy

3.1. Aldehydes

3,4-dichloroisocoumarin is a potent irreversible inhibitor of serine proteases and was one of the first compounds demonstrated to inhibit the 20S CP activity by covalently binding to the N-terminal Thr1 of the catalytic subunits. The exact mechanism of interaction between this inhibitor and the proteasome is not fully known, but because it contains a cyclic ester, such as β-lactone, it is suggested that the proteasome inhibition occurs through the formation of a non-hydrolysable acyl (**Figure 6**A). However, this compound demonstrated high toxicity and low selectivity for 20S CP in in vivo studies and human clinical trials [26][27][28]. Since then, analogs of 3,4-dichloroisocoumarin were synthesized, however, with poor inhibition directed at 20S CP [26].

Later, calpain inhibitors I and II, the first synthetic inhibitors of serine and cysteine proteases, also demonstrated efficiency in reversible inhibition of proteasome's proteolytic activity. However, these compounds also present the disadvantage of not being selective for the 20S CP. For instance, the calpain I inhibitor ALLN is 25-fold more potent against cathepsin B and calpain than against proteasome [27][28].

This drawback prompted the development of peptide aldehyde inhibitors, known for having a fast cellular uptake and slow binding to the β5 proteasome subunit (**Figure 6**B). However, these compounds dissociate rapidly from 20S CP and are easily inactivated by oxidation [27][28].

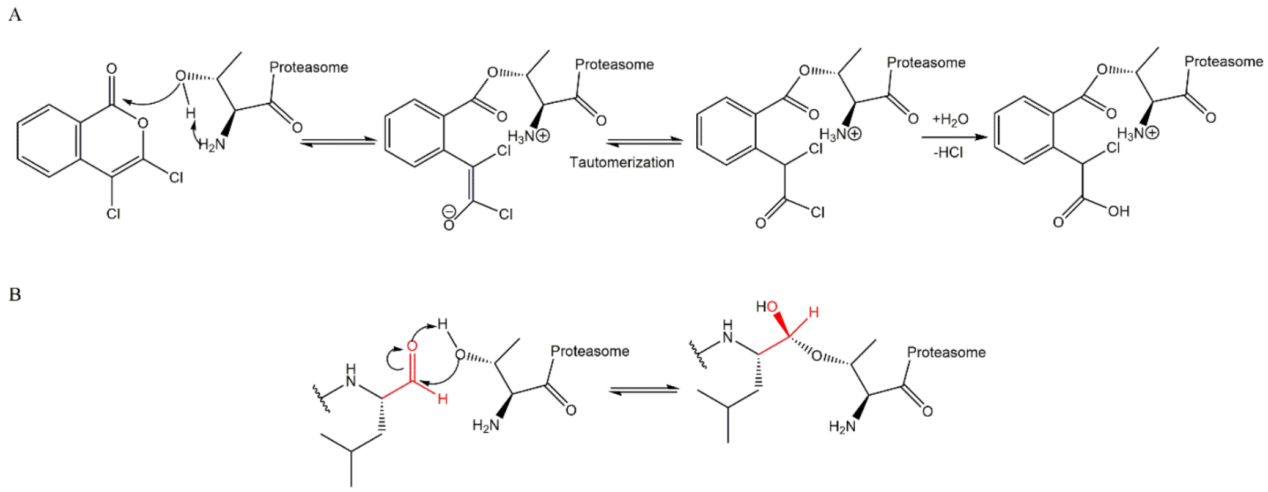

Figure 6. Mechanism of 20S CP inhibition by 3,4-dichloroisocoumarin (**A**) and by a peptide aldehyde (**B**). The hydroxyl group from Thr1 reacts with the carbonyl group of the inhibitor with the formation of a hemiketal, which is similar to a transition state of enzymatic reaction [27][29][30].

The peptide aldehyde inhibitors CEP1612, MG115, MG132 and PSI (**Figure 7**) are inhibitors of serine and cysteine proteases; however, they also inhibit the 20S CP and have increased selectivity to it when compared to the previously described inhibitors [27]. This has led to their frequent use in pre-clinical studies to evaluate the effects of PIs in several experimental models.

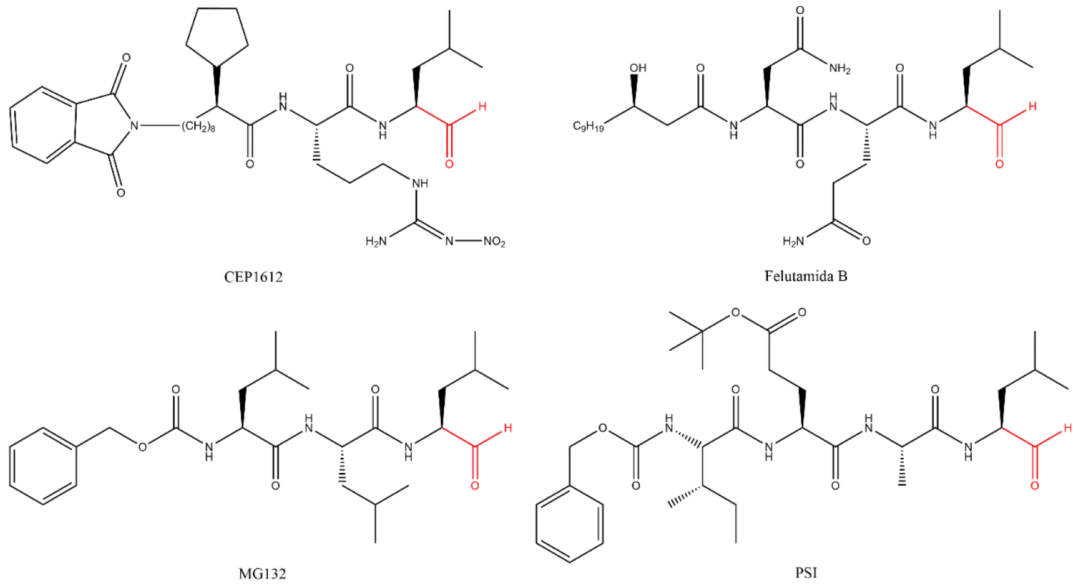

Figure 7. Examples of aldehydes inhibitors of 20S CP [27][31].

Since aldehyde inhibitors demonstrated a moderate reactivity and were not sufficiently selective to the 20S CP (also inhibiting serine and cysteine proteases), other inhibitor classes were explored [32].

3.2. Boronates

Widely used in the synthesis of serine protease inhibitors, the boronic esters and acids were also demonstrated to reversibly inhibit the 20S CP. Consequently, potent and selective di- and tripeptidyl boronic acid inhibitors were developed and shown to be more potent than aldehydes. The boronate inhibitors are also not easily inactivated by oxidation and are more selective to the 20S CP in comparison to common proteases [27][33].

Bortezomib (PS341/MG341) (**Figure 8**), an analog of the dipeptide boronic acid, was synthesized in 1995 by Myogenics and later acquired by Millennium Pharmaceuticals, Inc. (now acquired by Takeda Pharmaceutical Company Limited) [34][35]. Bortezomib inhibits the β5 subunit of 20S CP reversibly through the presumable formation of a complex between the boronic acid and the Thr1 hydroxyl group which results in the formation of a tetrahedral adduct similar to peptide aldehydes (**Figure 9**) [27][28]. To a lesser extent, bortezomib also targets the β1 subunit, while the β2 site is left relatively untouched [28]. Bortezomib was the first 20S CP inhibitor approved in 2003 by the FDA for the treatment of MM and, in 2006, for the treatment of MCL in patients who have received at least one prior therapy [6][36]. It received the first authorization by EMA in 2004 and is currently authorized as a monotherapy or in combinatory therapies (with melphalan, prednisone, dexamethasone, thalidomide and pegylated liposomal doxorubicin) for the treatment of MM; combinations with rituximab, cyclophosphamide, doxorubicin, and prednisone can be used for the treatment of MCL in untreated patients who cannot have blood stem-cell transplantation [9].

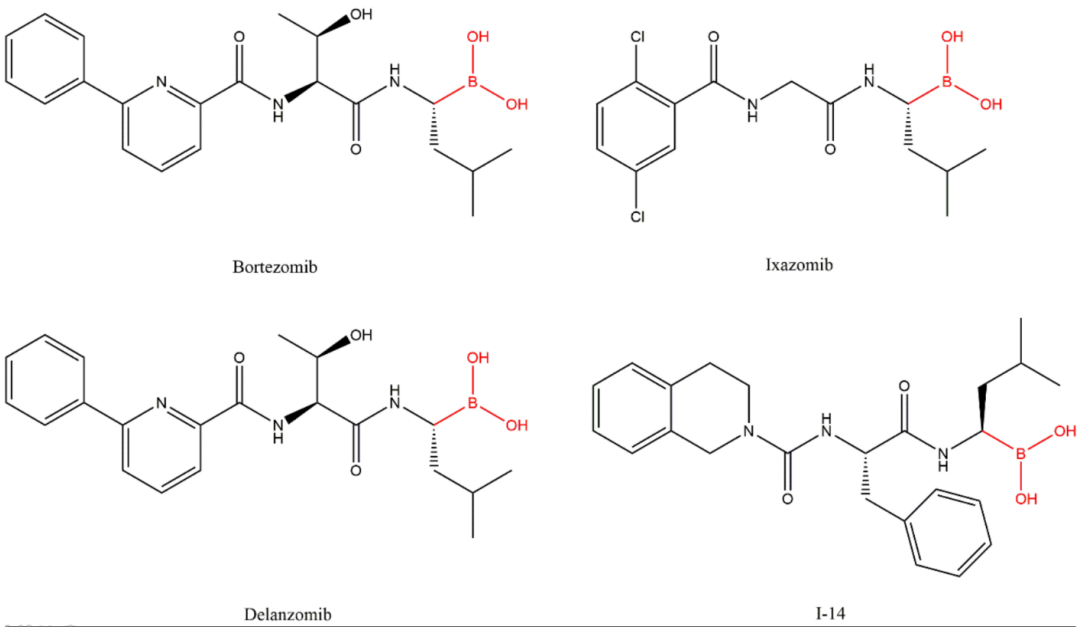

Figure 8. Examples of boronate inhibitors of 20S CP [31][37][38].

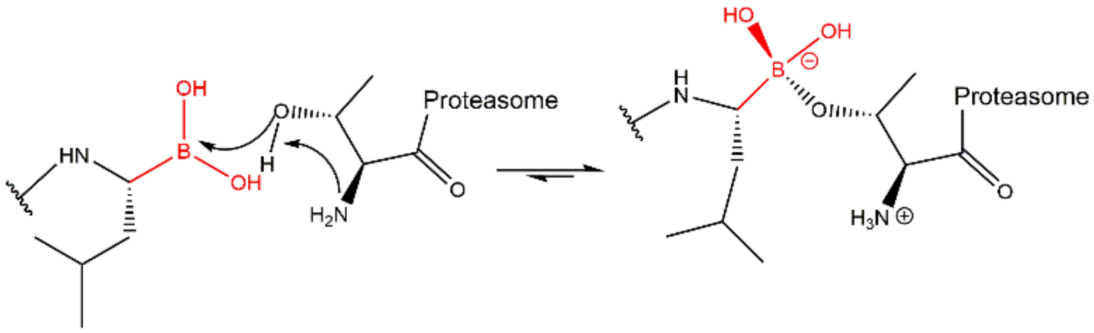

Figure 9. Mechanism of 20S CP inhibition by boronate inhibitors. The hydroxyl group from Thr1 reacts with the boronate from the inhibitor, with the formation of a borate, a tetrahedral boron anion [27][29].

However, bortezomib shows toxicity related to proteasomal inhibition in non-target tissues (e.g., 28% of the patients have grade 3 thrombocytopenia and induce peripheral neuropathy, of grade 3 in 12%, and any grade in 31% of the patients [39]), limited activity in solid tumors, innate and acquired resistance (being necessary to combine other chemotherapeutic agents to increase the cytotoxicity) and the necessity for subcutaneous or intravenous administration, since it is not orally bioavailable.

In order to overcome these limitations, second-generation inhibitors with improved ADME properties were developed, namely ixazomib and delanzomib [34].

Ixazomib (MLN-9708/2238) (**Figure 8**) is a reversible inhibitor that binds to proteasome's β5 subunit. It was approved by the FDA in 2015, received the first authorization in 2016 by the EMA and, in 2017, it was given "conditional approval" (more information about its benefits is still required) for the treatment of MM in combination with lenalidomide and dexamethasone in patients who have received at least one prior therapy [4][7]. It is the first 20S CP inhibitor approved for oral delivery. However, adverse effects such as peripheral neuropathy were also reported. It is administered in the form of a prodrug (ixazomib citrate), and it is rapidly hydrolyzed in the plasma. Compared with bortezomib, this inhibitor displays similar selectivity and potency for the β5 subunit; however, it has a substantially shorter half-life which may improve biodistribution [34][36].

Delanzomib (CEP-18770) (**Figure 8**) is a 20S CP inhibitor selective to the β5 subunit, with reversible inhibition comparable to bortezomib, which can be administered orally or intravenously. Phase I clinical trials for the treatment of MM, solid tumors and lymphomas, in patients with advanced solid tumors and MM, and results published in 2013 show that this inhibitor has a favorable safety profile with less neurotoxicity compared with bortezomib [40][41]. In 2016, a phase I/II study was conducted to determine the maximum tolerated dose of delanzomib and the efficacy and safety in patients with relapsed and refractory MM. The authors observed that the disappointing efficacy does not warrant the introduction of delanzomib for the treatment of MM [42][43]. In 2016, a phase I/II study with the objective of determining the maximum tolerated dose of delanzomib in combination with lenalidomide and dexamethasone in patients with relapsed or refractory MM was carried out. However, the study was terminated [44].

Because some inhibitors such as syringoline, α',β'-epoxyketone and vinyl sulfone (discussed below) exhibit urea in their structure, a class of peptide boronic acid inhibitors containing urea were synthesized. It was found that the inhibitor I-14 (**Figure 8**) showed excellent in vitro and in vivo antitumor activities, with relatively low toxicity and with appropriate pharmacologic properties. Compared with bortezomib, this compound demonstrated higher potency in inhibition of the β5 subunit of the 20S CP and better pharmacokinetic profile in in vivo assays with mice (it is metabolically more stable than bortezomib).

3.3. α',β'-Epoxyketones

In the search for antitumor agents with specific activity against B16 murine melanoma, the natural α',β'-epoxyketone eponomycin from Streptomyces hygroscopicus and epoxomicin (**Figure 10**) from the actinomycete strain Q996-17 were identified. These compounds demonstrated antitumor activity by inhibiting 20S CP [28].

Carfilzomib (PR-171) (**Figure 10**) is an α',β'-epoxyketone inhibitor which was approved by the FDA in 2012 and received the first authorization by the EMA in 2015, currently being authorized for the treatment of MM together with the lenalidomide plus dexamethasone or with dexamethasone alone or daratumumab plus dexamethasone in patients who have received at least one previous treatment [5][8]. According to the FDA, it can also be used in monotherapy for the treatment of MM in patients who have received one or more lines of therapy.

When compared to bortezomib, carfilzomib exhibits equal potency and greater selectivity to the β5 subunit. Although carfilzomib also requires intravenous administration, it presents lower neurotoxicity, most likely due to the higher selectivity to the β5 subunit [34][36].

Oprozomib (ONX-0912, PR-047) (**Figure 10**), another α',β'-epoxyketone inhibitor, is orally bioavailable and exhibits similar potency to carfilzomib in cytotoxicity assays. It resembles the in vitro anti-tumor activity to carfilzomib (cancer cell lines and primary cells), and it enhances the anti-myeloma activity of bortezomib [34][36]. Two clinical trials have been completed for this compound: a phase I study to evaluate the safety and tolerability of oprozomib in patients with advanced refractory or recurrent solid tumors; a phase Ib/II study to evaluate the combination therapy of oprozomib with melphalan and prednisone in transplant-ineligible patients with newly diagnosed MM [45][46]. Five clinical trials were terminated due to the identification that the safety profile and pharmacokinetic characteristics of the formulation used in all oprozomib studies required further optimization: two studies of phase Ib/II to determine the maximum tolerated dose, activity and safety of oprozomib in patients with hematologic malignancies and only in relapsed and/or refractory MM; a phase I study to evaluate the effect of food on the pharmacokinetics of oprozomib, the drug–drug interaction of oprozomib with midazolam, and the safety and tolerability of oprozomib in patients with advanced malignancies; and two phase Ib/II and Ib/III studies to evaluate combinatorial therapies of oprozomib with other chemotherapeutic drugs (dexamethasone; lenalidomide; cyclophosphamide; pomalidomide) in patients with MM [47][48][49][50][51]. Currently, a phase I clinical trial is ongoing (but not yet recruiting participants) to evaluate the safety, tolerability, pharmacokinetics, and efficacy of two formulations of oprozomib (immediate release and gastro-retentive formulations) plus pomalidomide and dexamethasone in patients with relapsed/refractory MM [52].

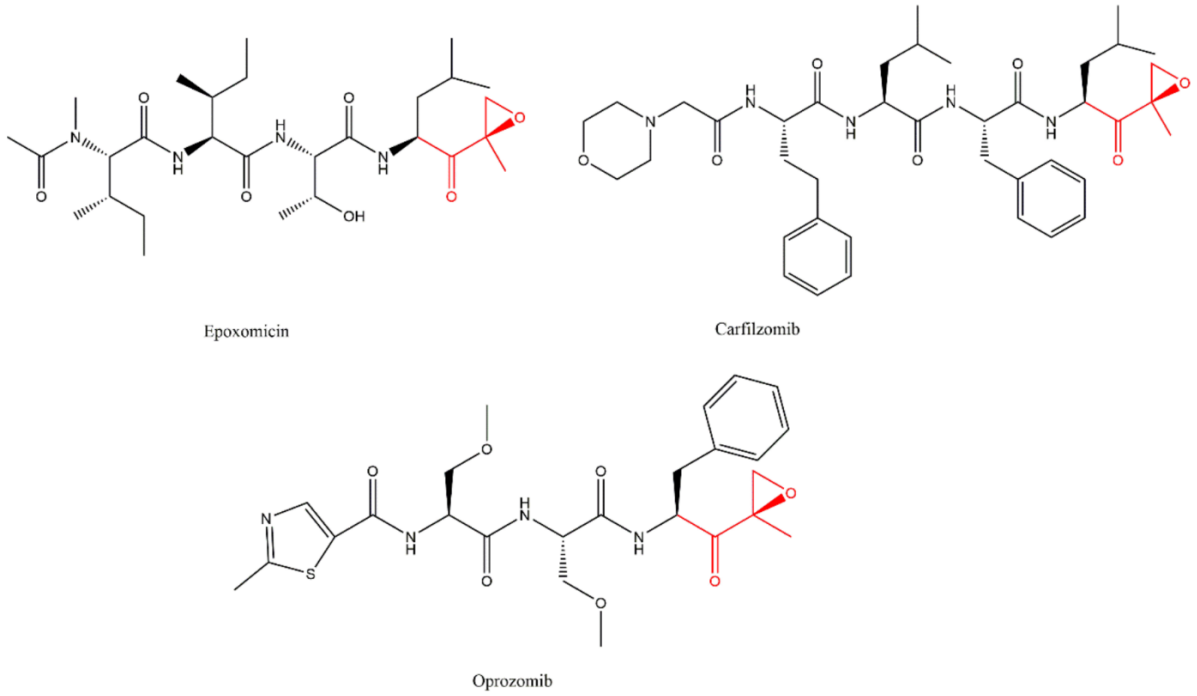

Figure 10. Examples of α',β'-epoxyketone inhibitors of 20S CP [27][31][53].

This inhibitor class binds covalently and irreversibly to 20S CP through the interaction of the hydroxyl and the amide groups from Thr1 (**Figure 11**) where the N-terminal amine attacks the epoxide α-carbon, yielding a 6-membered ring (**Figure 11**A) [27][29][54][55][56]. However, high-resolution crystallography of human 20S CP in complex with oprozomib, dihydroeponemycin (epoxomicin analog), and epoxomicin performed by Schrader et al. [57] suggests that the inhibition reaction yields a 7-membered ring product through a nucleophilic attack by the N-terminal amine of the epoxide β carbon (**Figure 11**B). This mechanism is different from other classes and characterizes the α',β'-epoxyketone inhibitors as more selective against the proteasome (because other proteases, which are common targets for many 20S CP inhibitors, do not contain a nucleophilic amino terminal residue) [27][54].

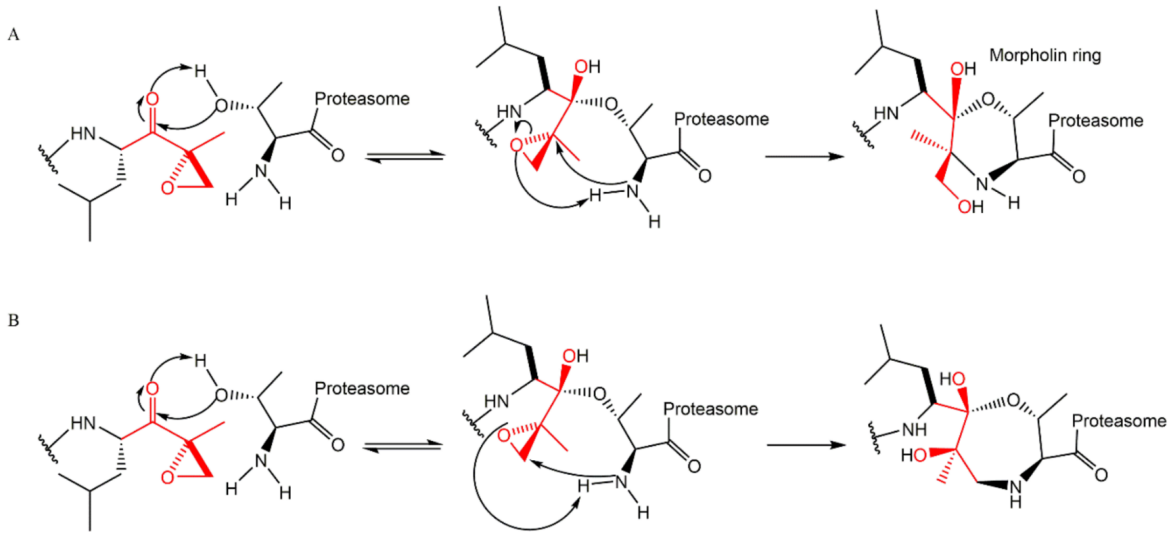

Figure 11. Mechanism of proteasome inhibition by α',β'-epoxyketone inhibitors. The hydroxyl and amine groups from Thr1 react, respectively, with the carbonyl group and α-carbon or β-carbon of epoxide group from inhibitor, promoting the formation of a 6-membered ring (**A**) and 7-membered ring (**B**), respectively [27][29][57].

3.4. Non-Covalent Macrocyclics

In 2000, several 20S CP inhibitors from Apiospora montagnei Sacc. TC 1093 (TMC-95 A to D) were isolated. In spite of the fact that these inhibitors exhibit uncommon macrocyclic characteristics, they demonstrated a capacity to inhibit the β5 subunit of 20S CP and did not inhibit other proteases (such as calpain II, cathepsin L, and trypsin) [58][59]. Groll et al. [60] described crystal structures with the TMC-95A inhibitor bound to yeast 20S CP, where the inhibitor is bound non-covalently to all proteolytic active β-subunits, without modifying their N-terminal threonines, binding through a tight network of hydrogen bonds which connects the ligand with the β subunits [27][28][60]. **Figure 12** illustrates the chemical structure of the inhibitors TMC-95 A to D.

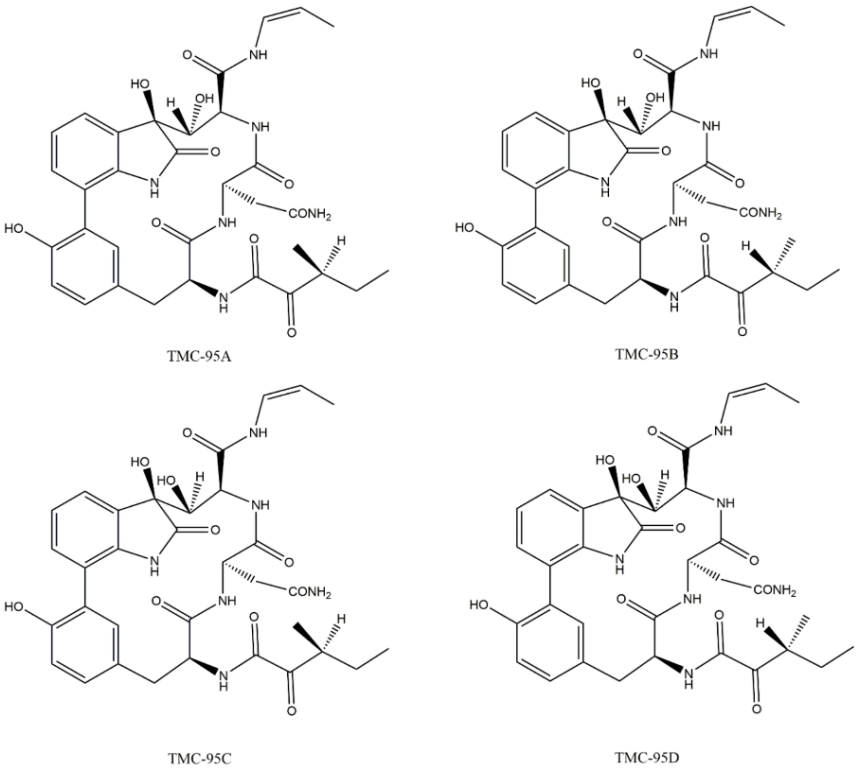

Figure 12. Examples of macrocyclic inhibitors of 20S CP [59].

3.5. α-Ketoaldehydes and α-Ketoamides

The α-ketoaldehyde and α-ketoamide peptides are covalent reversible inhibitors of the 20S CP which have been widely ignored for a long time because their benefits were not as evident as other classes of inhibitors. However, a study by Stein et al. [56] concluded that α-ketoamides are the most potent reversible PIs. These compounds may be able to penetrate deeper into solid tissue, thereby making them promising candidates for a range of tumor subtypes broader than those

targeted by bortezomib and carfilzomib. These compounds may also have applications as autoimmune disorder therapies. The mechanism of proteasome inhibition by α-ketoamides involves the formation of a reversible hemiketal (**Figure 13**A). The mechanism of proteasome inhibition by α-ketoaldehydes resembles the mechanism of inhibition by α',β'-epoxyketones due to the interaction of the inhibitor with the hydroxyl and the amide groups from Thr1 of the 20S CP, leading to the formation of a 6-membered ring (**Figure 13**B). Like α',β'-epoxyketones, peptide α-ketoaldehydes also have major selectivity to the 20S CP. They show Ki values more than 1000-fold higher in the inhibition of serine proteases, which is the case for chymotrypsin and subtilisin (for example), compared to aldehyde peptides because those serine proteases lack the amino terminal nucleophilic residue as part of their active sites. However, α-ketoaldehydes are reversible inhibitors and are thus less potent than α',β'-epoxyketone, β-lactone and boronate inhibitor classes [27][29]. **Figure 14** illustrates examples of α-ketoaldehyde and α-ketoamide inhibitors.

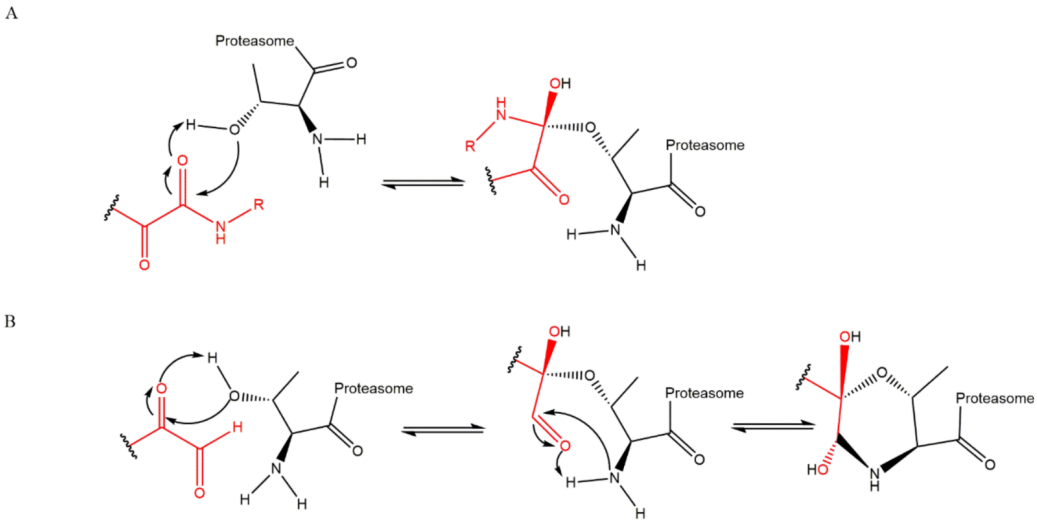

Figure 13. (**A**) Mechanism of proteasome inhibition by α-ketoamide inhibitor, where the hydroxyl from Thr1 reacts with ketoamide group from inhibitor, with the formation of a hemiketal. (**B**) Mechanism of proteasome inhibition by α-ketoaldehyde inhibitor, where the hydroxyl and amine groups from Thr1 react, respectively, with ketone and aldehyde groups from inhibitor, promoting the formation of a 6-membered ring [29][30][55].

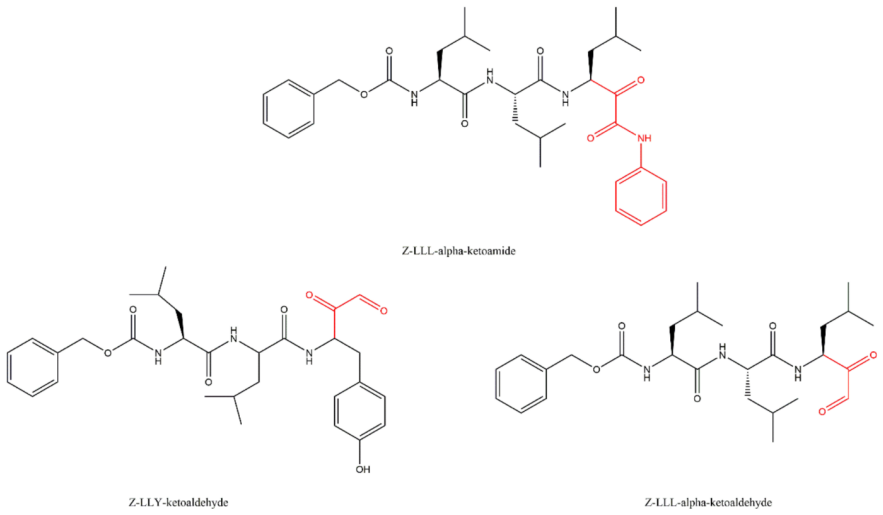

Figure 14. Examples of α-ketoaldehyde (Z-LLL-α-ketoamide) and α-ketoamide (Z-LLY-ketoaldehyde and Z-LLL-α-ketoaldehyde) inhibitors. Adapted from: [56][57].

3.6. Peptide Vinyl Derivatives

The vinyl peptide derivatives have electron withdrawing groups (sulfone or ester) in the C-terminal which behave as Michael acceptors of the catalytic Thr1 hydroxyl group, promoting the formation of a covalent irreversible bond (**Figure 15**) [61].

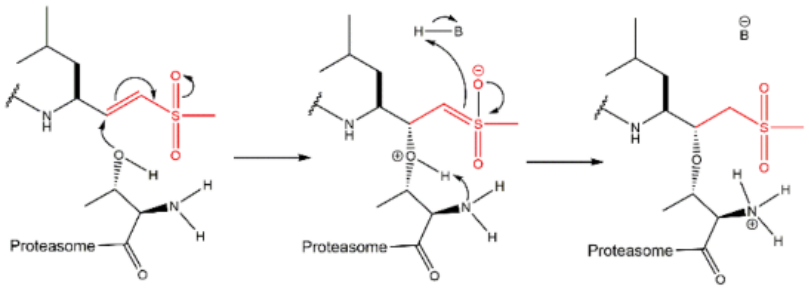

Figure 15. Mechanism of proteasome inhibition by a peptide vinyl derivate inhibitor, in the example, with sulfone as an electron withdrawing group and which function as Michael acceptor of the hydroxyl group from Thr1 [27][29].

The peptide vinyl sulfones were first described by Nazif and Bogyo [62] and are characterized by their lower reactivity when compared to aldehydes. The compounds AdaAhx3-Leu-Leu-Leu-VS, NIP-Leu-Leu-Asn-VS, and NLVS (NIP-Leu-Leu-Leu-vinyl-sulfone) are three examples of peptide vinyl sulfones (**Figure 16**).

Since they are easier to synthesize than other irreversible PIs, and can be coupled with radioisotopes and fluorescent probes, there is interest in their use as probes to evaluate proteasome activity for in vitro studies in different cells and tissues. For this purpose, various vinyl sulfone inhibitors have been synthesized containing tyrosine or a nitrophenyl group in order to facilitate the radioiodation process, obtaining, e.g., [^{125}I]NIP-Leu-Leu-Asn-VS, [^{125}I]Tyr-Leu-Leu-Leu-VS e Ada-[^{125}I]Tyr-Ahx3-Leu-Leu-Leu-VS, which react with all three catalytic β subunits [27][28][63].

Based on the synthesis of an arecoline derivative (1,2,5,6-Tetrahydropyridine-3-carbonyl-Val-Ser-Leu-benzylamide) which inhibits the β2 and β5 subunits, the Tomatis group [64] identified tripeptide vinyl esters as a class of selective inhibitors of proteasome trypsin-like activity. In this class, HMB−Val-Ser-Leu-VE (**Figure 16**), HMB-Leu-Leu-Leu-VE and Z-Val-Ser-Leu-VE were the most potent inhibitors, from which other vinyl ester pseudotripeptide analogs were developed [64][65][66][67]. Because several of vinyl ester tripeptides synthesized by this group demonstrated conformational similarities with the cyclic inhibitor TMC-95A and because the cyclization restricts the conformation which could provide an increase in the potency and/or selectivity, they cyclized some of their inhibitors. They observed that the cyclization did indeed increase the affinity of the inhibitors to the β1 or β5 subunit (**Figure 16**) [68][69]. They also synthesized analogs with different functional groups: ketone which demonstrated lower potency than the esters [70], and α,β-unsaturated N-acylpyrrole which demonstrated to be more selective to β1 subunit [71].

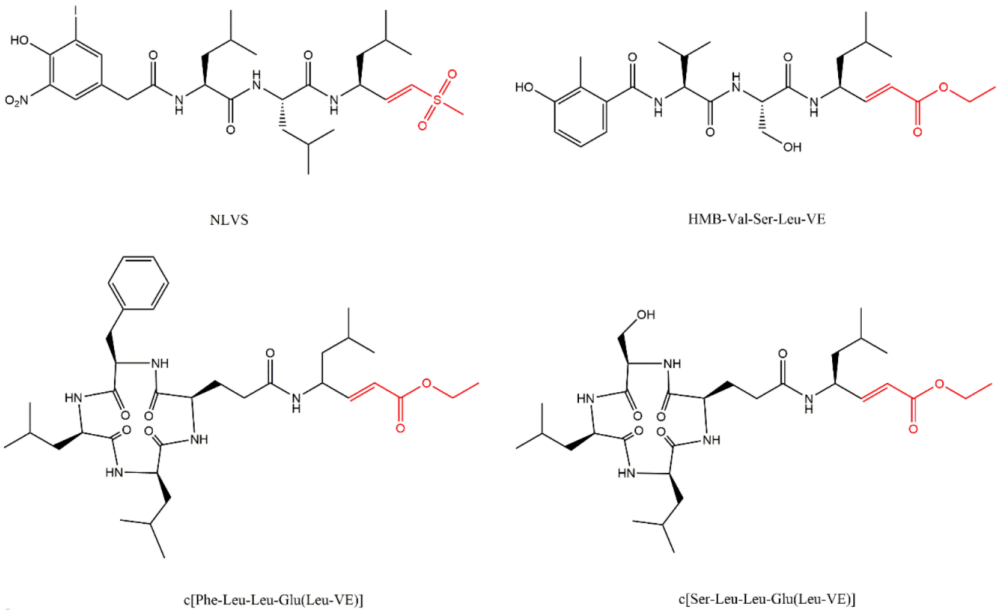

Figure 16. Example of vinyl sulfone inhibitor (NLVS) and vinyl esters inhibitors, 2 cyclics (c[Phe-Leu-Leu-Glu(Leu-VE)] and c[Ser-Leu-Leu-Glu(Leu-VE)], which present more selectivity for β5 and β1 subunits, respectively) [33][64][68][69].

3.7. β-Lactones

Lactacystin (a metabolite from Streptomyces gram-positive) was the first natural non-peptide-like proteasome inhibitor to be found in nature, and it bears a β-lactone moiety. In vivo, it acts as a prodrug which is hydrolyzed at neutral pH into clasto-lactacystin-β-lactone (also called omuralide) (**Figure 17**), which can cross the plasma membranes of mammalian cells (whereas the lactacystin form cannot) and it is covalently and irreversibly bound to the β5 subunit's Thr1, resulting in

the opening of the β-lactone ring and acylation of the hydroxyl group in Thr1 (**Figure 18**). Omuralide does not inhibit various serine and cysteine proteases, except for cathepsin A and cytosolic tripeptidyl peptidase II [27][28][61].

In 2000, belactosin A and C were isolated from Streptomyces sp. by Asai et al. [72] (**Figure 17**). These compounds exhibited antitumor activity attributed to the inhibition of proteasome activity. Additionally, with the goal of increasing the potency of belactosin A, a benzyl group was introduced (KF33955, **Figure 17**) [73]. Other derivatives were synthesized in 2013 by Kawamura et al. [74], who identified the 3e derivative (**Figure 17**) as an inhibitor comparable to bortezomib (IC50 value of 5.7 nM for the β5 subunit). Belactosin C analogs of the boronate inhibitors class were synthesized with the purpose of developing reversible inhibitors [75]. However, the most potent boronate inhibitor developed exhibited a value of IC50 for the β5 subunit of 20S CP, 10-fold than bortezomib's value (IC50 = 280 nM).

Marizomib (also named salinosporamide A or NPI-0052) (**Figure 17**) is a secondary metabolite of the marine actinomycete Salinispora tropica. This is the only non-peptidic proteasome inhibitor for which the Committee for Orphan Medicinal Products has issued a positive opinion regarding the orphan drug designation for the treatment of MM (2014) and for the treatment of glioma (2018), because marizomib crosses the blood-brain barrier [76][77]. According to the Triphase Accelerator Corporation, the orphan drug designation was also granted by the FDA, for the treatment of glioblastoma and MM [78][79][80]. It is an irreversible proteasome inhibitor whose carbonyl group interacts with Thr1′s hydroxyl group (it inhibits the three catalytic subunits of the 20S CP quickly and for a long period of time). Although it is orally bioavailable [36], all related (completed and ongoing) clinical studies have reported the drug administration as intravenous [81][82][83][84][85][86][87][88][89][90][91]. Marizomib was first tested in a phase I clinical trial conducted in patients with advanced solid tumor malignancies or refractory lymphoma whose disease had progressed after standard treatment [91]. Afterwards, two studies of phases I and II were conducted to evaluate the safety, pharmacokinetics and pharmacodynamics of escalating maximum tolerated and recommended doses of marizomib (and low dose dexamethasone) in patients with advanced malignancies including solid tumors, lymphomas, leukemias and MM (one of the studies only analyzed MM). These studies demonstrated that marizomib does not induce severe peripheral neuropathy or hematologic toxicity associated with bortezomib and carfilzomib, and it was verified that it is well-tolerated in heavily pretreated relapsed and/or refractory MM patients [82][84][92]. A phase I clinical trial has been completed to assess marizomib in combination with the histone deacetylase inhibitor vorinostat, in patients with melanoma, non-small cell lung cancer, pancreatic cancer or lymphoma [85]. This study demonstrated that this combination therapy is feasible and well-tolerated. Albeit confirmed responses were not reported, 61% of the evaluable patients reported a stable disease and 39% had decreases in tumor measurements (up to 25%) [93]. In a phase I clinical trial in patients with relapsed/refractory MM, a combination of marizomib, pomalidomide and low-dose dexamethasone demonstrated that this combination is well tolerated and promising in heavily pre-treated patients, including those who were refractory to prior treatment with carfilzomib, bortezomib and/or lenalidomide, and patients with high-risk cytogenetics (17p deletion and/or 4:14 chromosome translocation) [86][94][95]. The safety and preliminary efficacy of marizomib, alone or in combination with bevacizumab, were evaluated in patients with recurrent glioblastoma in a phase I/II clinical trial. This study demonstrated that marizomib is safe, as monotherapy or in combination with bevacizumab, for patients with recurrent glioblastoma. However, it did not show a benefit to patients from the addition of marizomib to bevacizumab. Marizomib was also shown to inhibit the proteolytic activity of all three subunits, with repeated dosing, at all doses assessed [87][96]. The detailed results from a completed phase I clinical trial to evaluate the combination of marizomib with Optune™, temozolomide and radiotherapy in patients with newly diagnosed WHO Grade IV malignant glioma are expected [88][97]. Three studies are ongoing, but not yet recruiting participants: (1) a phase III trial to evaluate marizomib in combination with standard temozolomide-based radiochemotherapy versus standard temozolomide-based radiochemotherapy alone in patients with newly diagnosed glioblastoma; (2) a phase II clinical trial to evaluate nanoparticle albumin-bound rapamycin as a single agent or combined with standard therapies (including marizomib) in bevacizumab-naïve subjects with progressive high grade glioma following prior therapy and subjects with newly diagnosed glioblastoma; and (3) a phase I study to evaluate the safety, tolerability, pharmacokinetic parameters and preliminary efficacy of the drugs marizomib and panobinostat in pediatric patients with diffuse intrinsic pontine glioma [81][83][89]. A phase II study to evaluate the efficacy of treatment with marizomib for recurrent low-grade and anaplastic supratentorial, infratentorial was terminated because the pharmaceutical company leading the study closed their program evaluating marizomib [90]. A phase II study will be carried out to evaluate the combination of marizomib, more pomalidomide and dexamethasone in patients with relapsed/refractory MM patients and patients with central nervous system involvement [98].

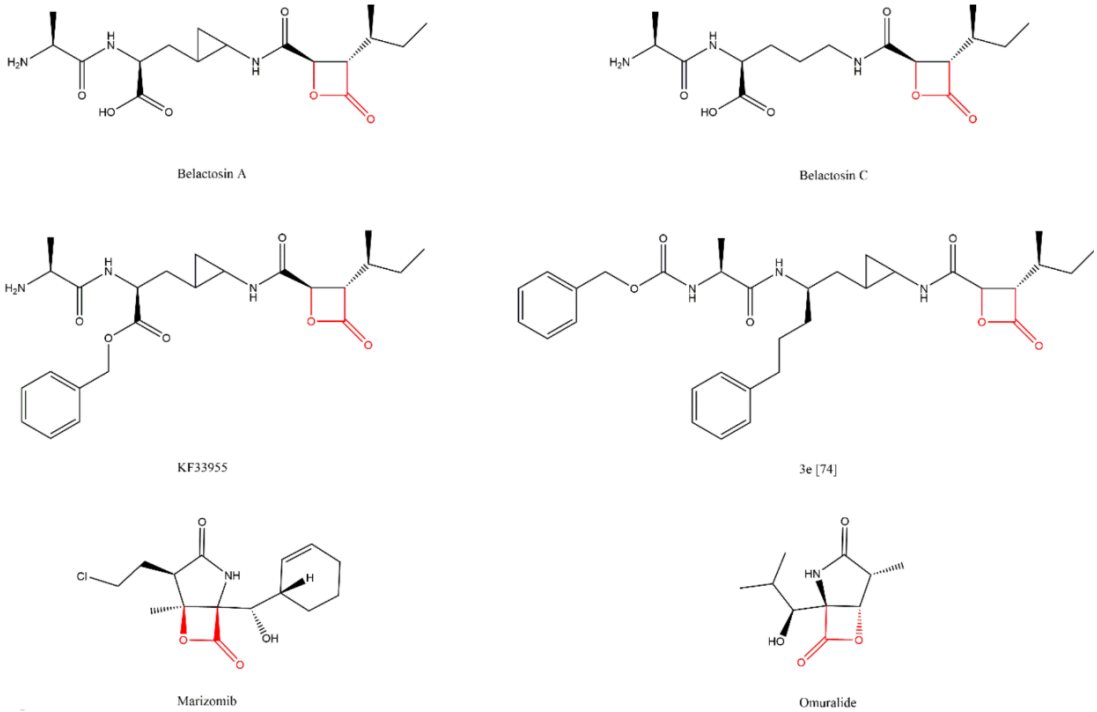

Figure 17. Examples of β-lactone inhibitors of 20S CP [27][36][73][74].

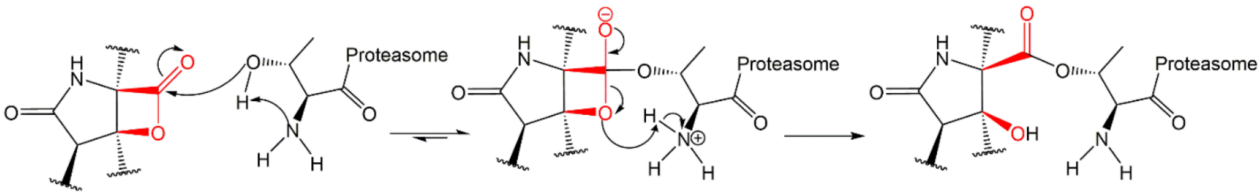

Figure 18. Mechanism of proteasome inhibition by β-lactone. The hydroxyl group from Thr1 reacts with the carbonyl group from the inhibitor, inducing the opening of the ring and acylation of hydroxyl from Thr1 [27][29].

3.8. Syrbactins

Syrbactins are a highly potent class of 20S CP inhibitors that consist of a 12-membered lactam, with an α,β-unsaturated amide system which reacts irreversibly with the hydroxyl from Thr1, through a Michael-type 1,4-addition (**Figure 19**).

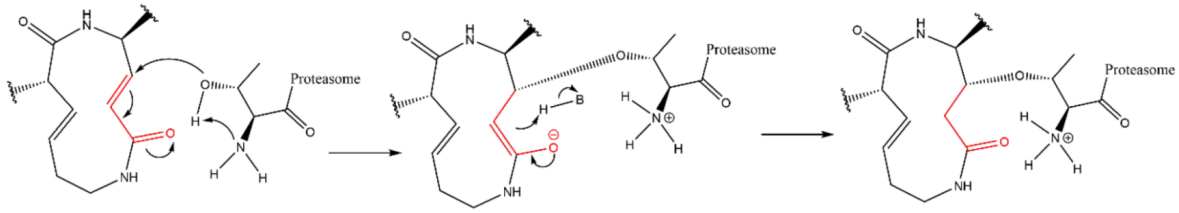

Figure 19. Mechanism of proteasome inhibition by syrbactin inhibitor, via a Michael-type 1,4-addition [29].

This class of inhibitors is comprised of structurally related families of natural products, distinct in their own lactam macrocyclic systems and exocyclic chains (namely in the presence of urea): syringolins and glidobactins.

Syringolins A and B (**Figure 20**), produced by strains of the vegetal pathogen Pseudomonas syringae pv. Syringae, and the glidobactin A, isolated from various bacteria, are natural inhibitors that belong to this class.

Syringolin A proved to be a more potent inhibitor than syringolin B, being able to inhibit irreversibly the three catalytic subunits of the eukaryotic 20S CP, and showed anticancer activity, pointing to the existence of apoptosis in human neuroblastoma and ovarian cancer cells. Various analogs were also synthesized with the help of their total synthesis.

Glidobactin A, isolated from the bacterial strain Polyangium brachsporum, was described as an antitumoral drug, and its cellular target (20S CP) was only identified 20 years later, because of its similar structure to that of syringolin A. It showed an inhibitory activity 15-fold higher than syringolin A for the β2 and β5 subunits, but there was no evidence that it could inhibit the β1 subunit [99][100][101][102]. The hybrid inhibitor syringolin A-glidobactin A (**Figure 20**) showcased inhibitory activity to the β1 subunit [103].

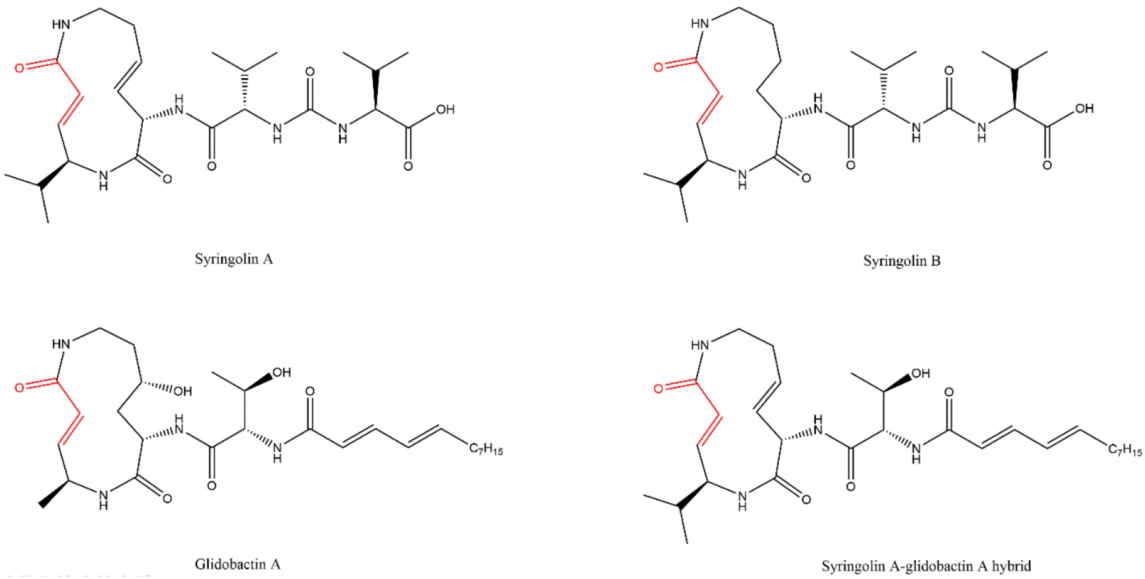

Figure 20. Examples of syrbactin inhibitors [99][103].

4. Resistance Mechanisms to Proteasome Inhibitors (PIs)

Acquired or innate PI resistance is a major obstacle in the treatment of MM and MCL. Resistance to bortezomib is particularly relevant since its therapeutic effect heavily depends on interpatient variability: only around 35% of patients with MM respond to bortezomib therapy [104]; the newly diagnosed patients did not achieve a partial or better response [39] and the ability of patients, who previously demonstrated sensitivity to bortezomib, to return to positive responses to bortezomib ranges between 31% and 60% [105], emerging as a limitation to continued clinical use.

As a strategy to partially overcome bortezomib resistance, irreversible inhibitors, such as carfilzomib (or more potent ones) were used because the prolonged 20S CP inhibition would induce a less resistant antitumor response and several studies have shown less pronounced cross-resistance compared to bortezomib [39][106][107][108][109]. Two phase II clinical trials demonstrated that carfilzomib had inhibitory activity in patients with relapsed and/or refractory MM who had already received bortezomib treatment [110][111]. A phase I/II clinical trial demonstrated that replacing bortezomib with carfilzomib, in patients with MM, who failed to bortezomib-containing combination regimens is safe and can be effective [112]. In 2018 a similar phase I/II clinical trial was initiated to evaluate the efficacy and safety of ixazomib as a replacement for bortezomib or carfilzomib among MM patients who are non-responsive to proteasome inhibitor-containing combination regimens [113]. Preliminary results show that the replacement of bortezomib or carfilzomib with ixazomib rarely leads to responses among the participants [114]. Marizomib is an irreversible inhibitor and inhibits all three catalytic subunits of 20S CP, and therefore it can help overcome bortezomib and carfilzomib resistance, associated with the upregulation or mutation of the β5 subunit [115].

Different mechanisms have been suggested to lead to drug resistance e.g., mutations and overexpression of proteasome subunit β5 [116], alterations in genes associated with stress response such as heat shock proteins [117], and up-regulation of cell survival pathways such as the insulin-like growth factor 1/insulin-like growth factor type 1 receptor axis (IGF-1/IGF-1R) [118] and peptidylprolyl isomerase A (PPIA) [119].

To know more about some of the main mechanisms associated with innate and acquired resistance to PIs and some of the approaches developed to overcome this therapeutic drawback, namely by using combination therapies, please access the article.

References

1. Hershko, A.; Ciechanover, A. The ubiquitin system. Annu. Rev. Biochem. 1998, 67, 425–479.
2. Obrist, F.; Manic, G.; Kroemer, G.; Vitale, I.; Galluzzi, L. Trial Watch: Proteasomal inhibitors for anticancer therapy. Mol. Cell. Oncol. 2015, 2, e974463.
3. Manasanch, E.E.; Orlowski, R.Z. Proteasome inhibitors in cancer therapy. Nat. Rev. Clin. Oncol. 2017, 14, 417–433.
4. EMA Ninlaro®: EPAR—Product Information. Available online: https://www.ema.europa.eu/en/documents/product-information/ninlaro-epar-product-information_en.pdf (accessed on 5 April 2021).

5. EMA Kyprolis®: EPAR—Product Information. Available online: https://www.ema.europa.eu/en/documents/product-information/kyprolis-epar-product-information_en.pdf (accessed on 5 April 2021).
6. FDA Highlights of Prescribing Information Velcade® (Bortezomib). Available online: https://www.accessdata.fda.gov/drugsatfda_docs/label/2019/206927s000lbl.pdf (accessed on 5 April 2021).
7. FDA Highlights of Prescribing Information Ninlaro® (Ixazomib). Available online: https://www.accessdata.fda.gov/drugsatfda_docs/label/2021/208462s010lbl.pdf (accessed on 5 April 2021).
8. FDA Highlights of Prescribing Information Kyprolis® (Carfilzomib). Available online: https://www.accessdata.fda.gov/drugsatfda_docs/label/2020/202714s030lbl.pdf (accessed on 5 April 2021).
9. EMA Velcade: EPAR—Product Information. Available online: https://www.ema.europa.eu/en/documents/product-information/velcade-epar-product-information_en.pdf (accessed on 5 April 2021).
10. Cancer Stat Facts: Myeloma. Available online: https://seer.cancer.gov/statfacts/html/mulmy.html (accessed on 24 February 2022).
11. Ferlay, J.; Ervik, M.; Lam, F.; Colombet, M.; Mery, L.; Piñeros, M.; Znaor, A.; Soerjomataram, I.; Bray, F. Global Cancer Observatory: Cancer Today; International Agency for Research on Cancer: Lyon, France, 2020.
12. Kuehl, W.M.; Bergsagel, P.L. Molecular pathogenesis of multiple myeloma and its premalignant precursor. J. Clin. Investig. 2012, 122, 3456–3463.
13. Offidani, M.; Corvatta, L.; Morè, S.; Olivieri, A. Novel experimental drugs for treatment of multiple myeloma. J. Exp. Pharmacol. 2021, 13, 245–264.
14. Goldman-Mazur, S.; Kumar, S.K. Current approaches to management of high-risk multiple myeloma. Am. J. Hematol. 2021.
15. Roué, G.; Sola, B. Management of drug resistance in mantle cell lymphoma. Cancers 2020, 12, 1565.
16. Pérez-Galán, P.; Dreyling, M.; Wiestner, A. Mantle cell lymphoma: Biology, pathogenesis, and the molecular basis of treatment in the genomic era. Blood 2011, 117, 26–38.
17. Glickman, M.H.; Ciechanover, A. The ubiquitin-proteasome proteolytic pathway: Destruction for the sake of construction. Physiol. Rev. 2002, 82, 373–428.
18. Ciechanover, A.; Brundin, P. The ubiquitin proteasome system in neurodegenerative diseases. Neuron 2003, 40, 427–446.
19. Thompson, S.; Loftus, L.; Ashley, M.; Meller, R. Ubiquitin–proteasome system as a modulator of cell fate. Curr. Opin. Pharmacol. 2008, 8, 90–95.
20. Bhat, K.P.; Greer, S.F. Proteolytic and non-proteolytic roles of ubiquitin and the ubiquitin proteasome system in transcriptional regulation. Biochim. Biophys. Acta Gene Regul. Mech. 2011, 1809, 150–155.
21. Schwartz, A.L.; Ciechanover, A. Targeting proteins for destruction by the ubiquitin system: Implications for human pathobiology. Annu. Rev. Pharmacol. Toxicol. 2009, 49, 73–96.
22. Kim, H.M.; Yu, Y.; Cheng, Y. Structure characterization of the 26S proteasome. Biochim. Biophys. Acta Gene Regul. Mech. 2011, 1809, 67–79.
23. Orlowski, N.; Wilk, S. A multicatalytical protease complex from pituitary that forms enkephalin and enkephalin containing peptides. Biochem. Biophys. Res. Commun. 1981, 101, 814–822.
24. Young, P.; Deveraux, Q.; Beal, R.E.; Pickart, C.M.; Rechsteiner, M. Characterization of two polyubiquitin binding sites in the 26 S protease subunit 5a. J. Biol. Chem. 1998, 273, 5461–5467.
25. Yao, T.; Cohen, R.E. A cryptic protease couples deubiquitination and degradation by the proteasome. Nature 2002, 419, 403–407.
26. Harer, S.L.; Bhatia, M.S.; Bhatia, N.M. Proteasome inhibitors mechanism; source for design of newer therapeutic agents. J. Antibiot. 2012, 65, 279–288.
27. Kisselev, A.F.; Goldberg, A.L. Proteasome inhibitors: From research tools to drug candidates. Chem. Biol. 2001, 8, 739–758.
28. Borissenko, L.; Groll, M. 20S proteasome and its inhibitors: Crystallographic knowledge for drug development. Chem. Rev. 2007, 107, 687–717.
29. Huber, E.M.; Groll, M. Inhibitors for the immuno- and constitutive proteasome: Current and future trends in drug development. Angew. Chemie Int. Ed. 2012, 51, 8708–8720.

30. Zwickl, P.; Baumeister, W. (Eds.) The Proteasome—Ubiquitin Protein Degradation Pathway; Current Topics in Microbiology and Immunology; Springer: Berlin/Heidelberg, Germany, 2002; Volume 268, ISBN 978-3-642-63971-5.
31. Guedes, R.A.; Serra, P.; Salvador, J.; Guedes, R. Computational approaches for the discovery of human proteasome inhibitors: An overview. Molecules 2016, 21, 927.
32. Moore, B.S.; Eustáquio, A.S.; McGlinchey, R.P. Advances in and applications of proteasome inhibitors. Curr. Opin. Chem. Biol. 2008, 12, 434–440.
33. Adams, J. (Ed.) Proteasome Inhibitors in Cancer Therapy; Humana Press: Totowa, NJ, USA, 2004; ISBN 978-1-61737-452-4.
34. Dou, Q.; Zonder, J. Overview of proteasome inhibitor-based anti-cancer therapies: Perspective on bortezomib and second generation proteasome inhibitors versus future generation inhibitors of ubiquitin-proteasome system. Curr. Cancer Drug Targets 2014, 14, 517–536.
35. Chen, D.; Frezza, M.; Schmitt, S.; Kanwar, J.; Dou, Q.P. Bortezomib as the first proteasome inhibitor anticancer drug: Current status and future perspectives. Curr. Cancer Drug Targets 2011, 11, 239–253.
36. Crawford, L.J.; Walker, B.; Irvine, A.E. Proteasome inhibitors in cancer therapy. J. Cell Commun. Signal. 2011, 5, 101–110.
37. Achanta, G. A boronic-chalcone derivative exhibits potent anti-cancer activity through inhibition of the proteasome. Mol. Pharmacol. 2006, 579, 3932–3940.
38. Han, L.-Q.; Yuan, X.; Wu, X.-Y.; Li, R.-D.; Xu, B.; Cheng, Q.; Liu, Z.-M.; Zhou, T.-Y.; An, H.-Y.; Wang, X.; et al. Urea-containing peptide boronic acids as potent proteasome inhibitors. Eur. J. Med. Chem. 2017, 125, 925–939.
39. Ruschak, A.M.; Slassi, M.; Kay, L.E.; Schimmer, A.D. Novel proteasome inhibitors to overcome bortezomib resistance. JNCI J. Natl. Cancer Inst. 2011, 103, 1007–1017.
40. ClinicalTrials.gov. Phase I Study of the Proteosome Inhibitor CEP 18770 in Patients with Solid Tumours or Non-Hodgkin's Lymphomas. Available online: https://www.clinicaltrials.gov/ct2/show/study/NCT00572637?term=delanzomib&rank=3 (accessed on 28 February 2017).
41. Gallerani, E.; Zucchetti, M.; Brunelli, D.; Marangon, E.; Noberasco, C.; Hess, D.; Delmonte, A.; Martinelli, G.; Böhm, S.; Driessen, C.; et al. A first in human phase I study of the proteasome inhibitor CEP-18770 in patients with advanced solid tumours and multiple myeloma. Eur. J. Cancer 2013, 49, 290–296.
42. ClinicalTrials.gov. Study to Determine the Maximum Tolerated Dose and Evaluate the Efficacy and Safety of CEP-18770 (Delanzomib) in Patients with Relapsed Multiple Myeloma Refractory to the Most Recent Therapy. Available online: https://www.clinicaltrials.gov/ct2/show/NCT01023880?term=delanzomib&rank=1 (accessed on 28 February 2017).
43. Vogl, D.T.; Martin, T.G.; Vij, R.; Hari, P.; Mikhael, J.R.; Siegel, D.; Wu, K.L.; Delforge, M.; Gasparetto, C. Phase I/II study of the novel proteasome inhibitor delanzomib (CEP-18770) for relapsed and refractory multiple myeloma. Leuk. Lymphoma 2017, 58, 1872–1879.
44. ClinicalTrials.gov. Delanzomib (CEP-18770) in Combination with Lenalidomide and Dexamethasone in Relapsed or Refractory Multiple Myeloma. Available online: https://www.clinicaltrials.gov/ct2/show/NCT01348919?term=delanzomib&rank=2 (accessed on 28 February 2017).
45. ClinicalTrials.gov. A Study of Oprozomib, Melphalan, and Prednisone in Transplant Ineligible Patients with Newly Diagnosed Multiple Myeloma. Available online: https://clinicaltrials.gov/ct2/show/study/NCT02072863?cond=oprozomib&rank=6 (accessed on 5 October 2017).
46. ClinicalTrials.gov. Phase 1 Study of Oprozomib Administered Orally in Patients with Advanced Refractory or Recurrent Solid Tumors. Available online: https://clinicaltrials.gov/ct2/show/study/NCT01129349?cond=oprozomib&rank=5 (accessed on 5 October 2017).
47. ClinicalTrials.gov. Study of Oprozomib and Dexamethasone, in Combination with Lenalidomide or Oral Cyclophosphamide in Patients With Newly Diagnosed Multiple Myeloma. Available online: https://clinicaltrials.gov/ct2/show/NCT01881789?recrs=d&cond=oprozomib&rank=1 (accessed on 5 October 2017).
48. ClinicalTrials.gov. Phase 1b/2, Multicenter, Open-label Study of Oprozomib and Dexamethasone in Patients with Relapsed and/or Refractory Multiple Myeloma. Available online: https://clinicaltrials.gov/ct2/show/NCT01832727?recrs=d&cond=oprozomib&rank=2 (accessed on 5 October 2017).
49. ClinicalTrials.gov. Open-label Study of the Safety and Activity of Oprozomib in Patients with Hematologic Malignancies. Available online: https://clinicaltrials.gov/ct2/show/NCT01416428?recrs=d&cond=oprozomib&rank=3 (accessed on 5 October 2017).

50. ClinicalTrials.gov. A Study of Oprozomib, Pomalidomide, and Dexamethasone in Subjects with Primary Refractory or Relapsed and Refractory Multiple Myeloma. Available online: https://clinicaltrials.gov/ct2/show/NCT01999335?recrs=d&cond=oprozomib&rank=4 (accessed on 5 October 2017).
51. ClinicalTrials.gov. A Phase 1 Study of Oprozomib to Assess Food Effect, Drug-Drug Interaction with Midazolam, and Safety and Tolerability in Patients with Advanced Malignancies. Available online: https://clinicaltrials.gov/ct2/show/NCT02244112?recrs=d&cond=oprozomib&rank=5 (accessed on 5 October 2017).
52. ClinicalTrials.gov. Phase 1b Study Evaluating OPomD in Relapsed or Refractory Multiple Myeloma. Available online: https://www.clinicaltrials.gov/ct2/show/NCT02939183?term=Oprozomib&rank=9 (accessed on 28 February 2017).
53. Basmadjian, C.; Zhao, Q.; Bentouhami, E.; Djehal, A.; Nebigil, C.G.; Johnson, R.A.; Serova, M.; de Gramont, A.; Faivre, S.; Raymond, E.; et al. Cancer wars: Natural products strike back. Front. Chem. 2014, 2, 20.
54. Groll, M.; Nazif, T.; Huber, R.; Bogyo, M. Probing structural determinants distal to the site of hydrolysis that control substrate specificity of the 20S proteasome. Chem. Biol. 2002, 9, 655–662.
55. Gräwert, M.A.; Gallastegui, N.; Stein, M.; Schmidt, B.; Kloetzel, P.-M.; Huber, R.; Groll, M. Elucidation of the α-keto-aldehyde binding mechanism: A lead structure motif for proteasome inhibition. Angew. Chemie Int. Ed. 2011, 50, 542–544.
56. Stein, M.L.; Cui, H.; Beck, P.; Dubiella, C.; Voss, C.; Krüger, A.; Schmidt, B.; Groll, M. Systematic comparison of peptidic proteasome inhibitors highlights the α-ketoamide electrophile as an auspicious reversible lead motif. Angew. Chemie Int. Ed. 2014, 53, 1679–1683.
57. Schrader, J.; Henneberg, F.; Mata, R.A.; Tittmann, K.; Schneider, T.R.; Stark, H.; Bourenkov, G.; Chari, A. The inhibition mechanism of human 20 S proteasomes enables next-generation inhibitor design. Science 2016, 353, 594–598.
58. Koguchi, Y.; Kohno, J.; Nishio, M.; Takahashi, K.; Okuda, T.; Ohnuki, T.; Komatsubara, S. TMC-95A, B, C, and D, novel proteasome inhibitors produced by Apiospora Montagnei Sacc. TC 1093. taxonomy, production, isolation, and biological activities. J. Antibiot. 2000, 53, 105–109.
59. Kohno, J.; Koguchi, Y.; Nishio, M.; Nakao, K.; Kuroda, M.; Shimizu, R.; Ohnuki, T.; Komatsubara, S. Structures of TMC-95A-D: Novel proteasome inhibitors from Apiospora montagnei Sacc. TC 1093. J. Org. Chem. 2000, 65, 990–995.
60. Groll, M.; Koguchi, Y.; Huber, R.; Kohno, J. Crystal structure of the 20 S proteasome:TMC-95A complex: A non-covalent proteasome inhibitor. J. Mol. Biol. 2001, 311, 543–548.
61. Génin, E.; Reboud-Ravaux, M.; Vidal, J. Proteasome inhibitors: Recent advances and new perspectives in medicinal chemistry. Curr. Top. Med. Chem. 2010, 10, 232–256.
62. Nazif, T.; Bogyo, M. Global analysis of proteasomal substrate specificity using positional-scanning libraries of covalent inhibitors. Proc. Natl. Acad. Sci. USA 2001, 98, 2967–2972.
63. Masters, E.I. Structural Studies of the Proteasome and Implications for Proteasome Activation. Ph.D. Thesis, Department of Biochemistry, University of Utah, Salt Lake City, UT, USA, 2008.
64. Marastoni, M.; Baldisserotto, A.; Cellini, S.; Gavioli, R.; Tomatis, R. Peptidyl vinyl ester derivatives: New class of selective inhibitors of proteasome trypsin-like activity. J. Med. Chem. 2005, 48, 5038–5042.
65. Marastoni, M.; Baldisserotto, A.; Trapella, C.; Gavioli, R.; Tomatis, R. Synthesis and biological evaluation of new vinyl ester pseudotripeptide proteasome inhibitors. Eur. J. Med. Chem. 2006, 41, 978–984.
66. Marastoni, M.; Baldisserotto, A.; Canella, A.; Gavioli, R.; De Risi, C.; Pollini, G.P.; Tomatis, R. Arecoline tripeptide inhibitors of proteasome. J. Med. Chem. 2004, 47, 1587–1590.
67. Baldisserotto, A.; Destro, F.; Vertuani, G.; Marastoni, M.; Gavioli, R.; Tomatis, R. N-Terminal-prolonged vinyl ester-based peptides as selective proteasome β1 subunit inhibitors. Bioorg. Med. Chem. 2009, 17, 5535–5540.
68. Baldisserotto, A.; Marastoni, M.; Fiorini, S.; Pretto, L.; Ferretti, V.; Gavioli, R.; Tomatis, R. Vinyl ester-based cyclic peptide proteasome inhibitors. Bioorg. Med. Chem. Lett. 2008, 18, 1849–1854.
69. Baldisserotto, A.; Marastoni, M.; Gavioli, R.; Tomatis, R. New cyclic peptide proteasome inhibitors. Bioorg. Med. Chem. Lett. 2009, 19, 1966–1969.
70. Franceschini, C.; Trapella, C.; Sforza, F.; Gavioli, R.; Marastoni, M. Synthesis and biological properties of C-terminal vinyl ketone pseudotripeptides. J. Enzyme Inhib. Med. Chem. 2013, 28, 560–564.
71. Baldisserotto, A.; Ferretti, V.; Destro, F.; Franceschini, C.; Marastoni, M.; Gavioli, R.; Tomatis, R. α,β-unsaturated N -acylpyrrole peptidyl derivatives: New proteasome inhibitors. J. Med. Chem. 2010, 53, 6511–6515.
72. Asai, A.; Hasegawa, A.; Ochiai, K.; Yamashita, Y.; Mizukami, T. Belactosin A, a Novel antitumor antibiotic acting on cyclin/CDK mediated cell cycle regulation, produced by Streptomyces sp. J. Antibiot. 2000, 53, 81–83.

73. Asai, A.; Tsujita, T.; Sharma, S.V.; Yamashita, Y.; Akinaga, S.; Funakoshi, M.; Kobayashi, H.; Mizukami, T. A new structural class of proteasome inhibitors identified by microbial screening using yeast-based assay. Biochem. Pharmacol. 2004, 67, 227–234.

74. Kawamura, S.; Unno, Y.; List, A.; Mizuno, A.; Tanaka, M.; Sasaki, T.; Arisawa, M.; Asai, A.; Groll, M.; Shuto, S. Potent proteasome inhibitors derived from the unnatural cis-cyclopropane isomer of Belactosin A: Synthesis, biological activity, and mode of action. J. Med. Chem. 2013, 56, 3689–3700.

75. Nakamura, H.; Watanabe, M.; Ban, H.S.; Nabeyama, W.; Asai, A. Synthesis and biological evaluation of boron peptide analogues of Belactosin C as proteasome inhibitors. Bioorg. Med. Chem. Lett. 2009, 19, 3220–3224.

76. COMP Marizomib for the Treatment of Plasma Cell Myeloma. Available online: http://www.ema.europa.eu/docs/en_GB/document_library/Orphan_designation/2014/09/WC500171952.pdf (accessed on 6 April 2017).

77. COMP Marizomib for the Treatment of Glioma. Available online: https://www.ema.europa.eu/en/documents/orphan-designation/eu/3/18/2119-public-summary-opinion-orphan-designation-marizomib-treatment-glioma_en.pdf (accessed on 21 February 2020).

78. Triphase Accelerator Corporation Marizomib. Available online: http://triphaseco.com/marizomib/ (accessed on 2 October 2017).

79. Triphase Accelerator Corporation Triphase Receives FDA Orphan Drug Designation for Marizomib in Multiple Myeloma. Available online: http://triphaseco.com/wp-content/uploads/2014/02/20140226_Triphase_orphan_drug_news-release_final.pdf (accessed on 4 October 2018).

80. Triphase Accelerator Corporation Triphase Accelerator Corporation Announces FDA Orphan Drug Designation for Marizomib in Malignant Glioma. Available online: http://triphaseco.com/wp-content/uploads/2015/11/MRZ-Orphan-Press-Release-11192015_FINAL.pdf (accessed on 4 October 2018).

81. ClinicalTrials.gov. Phase 1 Trial of Marizomib Alone and in Combination with Panobinostat for Children with Diffuse Intrinsic Pontine Glioma. Available online: https://www.clinicaltrials.gov/ct2/show/NCT04341311?cond=marizomib&draw=3&rank=4 (accessed on 5 April 2021).

82. ClinicalTrials.gov. Phase 2 Clinical Trial of NPI-0052 in Patients with Relapsed or Relapsed/Refractory Multiple Myeloma. Available online: https://clinicaltrials.gov/ct2/show/NCT00461045?term=marizomib&draw=2&rank=7 (accessed on 21 February 2020).

83. ClinicalTrials.gov. ABI-009 (Nab-Rapamycin) in Recurrent High Grade Glioma and Newly Diagnosed Glioblastoma. Available online: https://clinicaltrials.gov/ct2/show/NCT03463265?term=marizomib&draw=2&rank=10 (accessed on 20 February 2020).

84. ClinicalTrials.gov. Phase 1 Clinical Trial of NPI-0052 in Patients with Advanced Malignancies. Available online: https://clinicaltrials.gov/ct2/show/NCT00629473?cond=marizomib&rank=5 (accessed on 6 October 2017).

85. ClinicalTrials.gov. NPI-0052 and Vorinostat in Patients with Non-small Cell Lung Cancer, Pancreatic Cancer, Melanoma or Lymphoma. Available online: https://clinicaltrials.gov/ct2/show/NCT00667082?term=marizomib&draw=2&rank=3 (accessed on 21 February 2020).

86. ClinicalTrials.gov. Combination Study of Pomalidomide, Marizomib, and Low-Dose Dexamethasone in Relapsed and Refractory Multiple Myeloma. Available online: https://www.clinicaltrials.gov/ct2/show/NCT02103335?term=Marizomib&rank=3 (accessed on 28 February 2017).

87. ClinicalTrials.gov. Stage 1: Marizomib + Bevacizumab in WHO Gr IV GBM; Stage 2: Marizomib Alone; Stage 3: Combination of Marizomib and Bevacizumab. Available online: https://clinicaltrials.gov/ct2/show/NCT02330562?cond=marizomib&rank=3 (accessed on 6 October 2017).

88. ClinicalTrials.gov. Study of Marizomib With Temozolomide and Radiotherapy in Patients with Newly Diagnosed Brain Cancer. Available online: https://clinicaltrials.gov/ct2/show/NCT02903069?cond=marizomib&rank=1 (accessed on 6 October 2017).

89. ClinicalTrials.gov. A Phase III Trial of With Marizomib in Patients with Newly Diagnosed Glioblastoma. Available online: https://www.clinicaltrials.gov/ct2/show/NCT03345095?term=NPI-0052&draw=2&rank=9 (accessed on 5 April 2021).

90. ClinicalTrials.gov. Marizomib for Recurrent Low-Grade and Anaplastic Supratentorial, Infratentorial, and Spinal Cord Ependymoma. Available online: https://clinicaltrials.gov/ct2/show/NCT03727841?term=marizomib&draw=2&rank=2 (accessed on 20 February 2020).

91. ClinicalTrials.gov. Phase 1 Clinical Trial of NPI-0052 in Patients with Advanced Solid Tumor Malignancies or Refractory Lymphoma. Available online: https://clinicaltrials.gov/ct2/show/NCT00396864?cond=marizomib&rank=7 (accessed on 6 October 2017).

92. Harrison, S.J.; Mainwaring, P.; Price, T.; Millward, M.J.; Padrik, P.; Underhill, C.R.; Cannell, P.K.; Reich, S.D.; Trikha, M.; Spencer, A. Phase I clinical trial of marizomib (NPI-0052) in patients with advanced malignancies including multiple myeloma: Study NPI-0052-102 final results. Clin. Cancer Res. 2016, 22, 4559–4566.
93. Millward, M.; Price, T.; Townsend, A.; Sweeney, C.; Spencer, A.; Sukumaran, S.; Longenecker, A.; Lee, L.; Lay, A.; Sharma, G.; et al. Phase 1 clinical trial of the novel proteasome inhibitor marizomib with the histone deacetylase inhibitor vorinostat in patients with melanoma, pancreatic and lung cancer based on in vitro assessments of the combination. Invest. New Drugs 2012, 30, 2303–2317.
94. Spencer, A.; Laubach, J.P.; Zonder, J.A.; Badros, A.Z.; Harrison, S.; Khot, A.; Chauhan, D.; Anderson, K.C.; Reich, S.D.; Trikha, M.; et al. Phase 1, multicenter, open-label, combination study (NPI-0052-107; NCT02103335) of pomalidomide (POM), marizomib (MRZ, NPI-0052), and low-dose dexamethasone (LD-DEX) in patients with relapsed and refractory multiple myeloma. Blood 2015, 126, 4220.
95. Spencer, A.; Harrison, S.; Laubach, J.P.; Zonder, J.; Badros, A.Z.; Bergin, K.; Khot, A.; Zimmerman, T.; Anderson, K.C.; MacLaren, A.; et al. Pmd-107: Marizomib, pomalidomide and low dose-dexamethasone combination study in relapsed/refractory multiple myeloma (NCT02103335): Full enrollment results from a phase-1 multicenter, open label study. Blood 2016, 128, 3326.
96. Bota, D.A.; Mason, W.; Kesari, S.; Magge, R.; Winograd, B.; Elias, I.; Reich, S.D.; Levin, N.; Trikha, M.; Desjardins, A. Marizomib alone or in combination with bevacizumab in patients with recurrent glioblastoma: Phase I/II clinical trial data. Neuro-Oncol. Adv. 2021, 3, 1–10.
97. Mason, W.P.; Kesari, S.; Stupp, R.; Aregawi, D.G.; Piccioni, D.E.; Roth, P.; Desjardins, A.; Reich, S.D.; Casadebaig, M.-L.; Elias, I.; et al. Full enrollment results from an extended phase I, multicenter, open label study of marizomib (MRZ) with temozolomide (TMZ) and radiotherapy (RT) in newly diagnosed glioblastoma (GBM). J. Clin. Oncol. 2019, 37, 2021.
98. ClinicalTrials.gov. Marizomib Central Nervous System (CNS). Available online: https://clinicaltrials.gov/ct2/show/NCT05050305?term=marizomib&draw=1&rank=4 (accessed on 22 February 2022).
99. Clerc, J.; Groll, M.; Illich, D.J.; Bachmann, A.S.; Huber, R.; Schellenberg, B.; Dudler, R.; Kaiser, M. Synthetic and structural studies on syringolin A and B reveal critical determinants of selectivity and potency of proteasome inhibition. Proc. Natl. Acad. Sci. USA 2009, 106, 6507–6512.
100. Clerc, J.; Schellenberg, B.; Groll, M.; Bachmann, A.S.; Huber, R.; Dudler, R.; Kaiser, M. Convergent synthesis and biological evaluation of syringolin A and derivatives as eukaryotic 20S proteasome inhibitors. Eur. J. Org. Chem. 2010, 2010, 3991–4003.
101. Kisselev, A.F.; van der Linden, W.A.; Overkleeft, H.S. Proteasome inhibitors: An expanding army attacking a unique target. Chem. Biol. 2012, 19, 99–115.
102. Krahn, D.; Ottmann, C.; Kaiser, M. The chemistry and biology of syringolins, glidobactins and cepafungins (syrbactins). Nat. Prod. Rep. 2011, 28, 1854.
103. Archer, C.R.; Groll, M.; Stein, M.L.; Schellenberg, B.; Clerc, J.; Kaiser, M.; Kondratyuk, T.P.; Pezzuto, J.M.; Dudler, R.; Bachmann, A.S. Activity enhancement of the synthetic syrbactin proteasome inhibitor hybrid and biological evaluation in tumor cells. Biochemistry 2012, 51, 6880–6888.
104. Richardson, P.G.; Mitsiades, C.; Hideshima, T.; Anderson, K.C. Bortezomib: Proteasome inhibition as an effective anticancer therapy. Annu. Rev. Med. 2006, 57, 33–47.
105. Orlowski, R.Z.; Kuhn, D.J. Proteasome inhibitors in cancer therapy: Lessons from the first decade. Clin. Cancer Res. 2008, 14, 1649–1657.
106. Dou, Q.P. (Ed.) Resistance to Proteasome Inhibitors in Cancer; Resistance to Targeted Anti-Cancer Therapeutics; Springer International Publishing: Los Angeles, CA, USA, 2014; ISBN 978-3-319-06751-3.
107. Suzuki, E.; Demo, S.; Deu, E.; Keats, J.; Arastu-Kapur, S.; Bergsagel, P.L.; Bennett, M.K.; Kirk, C.J. Molecular mechanisms of bortezomib resistant adenocarcinoma cells. PLoS ONE 2011, 6, e27996.
108. Verbrugge, S.E.; Al, M.; Assaraf, Y.G.; Niewerth, D.; Van Meerloo, J.; Cloos, J.; Van Der Veer, M.; Scheffer, G.L.; Peters, G.J.; Chan, E.T.; et al. Overcoming bortezomib resistance in human B cells by anti-CD20/rituximab-mediated complement-dependent cytotoxicity and epoxyketone-based irreversible proteasome inhibitors. Exp. Hematol. Oncol. 2013, 2, 1.
109. Rückrich, T.; Kraus, M.; Gogel, J.; Beck, A.; Ovaa, H.; Verdoes, M.; Overkleeft, H.S.; Kalbacher, H.; Driessen, C. Characterization of the ubiquitin–proteasome system in bortezomib-adapted cells. Leukemia 2009, 23, 1098–1105.
110. Vij, R.; Siegel, D.S.; Jagannath, S.; Jakubowiak, A.J.; Stewart, A.K.; McDonagh, K.; Bahlis, N.; Belch, A.; Kunkel, L.A.; Wear, S.; et al. An open-label, single-arm, phase 2 study of single-agent carfilzomib in patients with relapsed and/or

refractory multiple myeloma who have been previously treated with bortezomib. Br. J. Haematol. 2012, 158, 739–748.

111. Siegel, D.S.; Martin, T.; Wang, M.; Vij, R.; Jakubowiak, A.J.; Lonial, S.; Trudel, S.; Kukreti, V.; Bahlis, N.; Alsina, M.; et al. A phase 2 study of single-agent carfilzomib (PX-171-003-A1) in patients with relapsed and refractory multiple myeloma. Blood 2012, 120, 2817–2825.
112. Berenson, J.R.; Hilger, J.D.; Yellin, O.; Dichmann, R.; Patel-Donnelly, D.; Boccia, R.V.; Bessudo, A.; Stampleman, L.; Gravenor, D.; Eshaghian, S.; et al. Replacement of bortezomib with carfilzomib for multiple myeloma patients progressing from bortezomib combination therapy. Leukemia 2014, 28, 1529–1536.
113. ClinicalTrials.gov. Ixazomib as a Replacement for Carfilzomib and Bortezomib for Multiple Myeloma Patients. Available online: https://clinicaltrials.gov/ct2/show/NCT02206425?term=bortezomib&draw=4&rank=669 (accessed on 7 October 2018).
114. Berenson, J.R.; Cohen, A.; Spektor, T.M.; Bitran, J.D.; Chen, G.Q.; Moezi, M.M.; Bessudo, A.; Ye, J.Z.; Hager, S.J.; Moss, R.A.; et al. Replacement of ixazomib for relapsed/refractory multiple myeloma patients refractory to a bortezomib or carfilzomib-containing combination therapy. J. Clin. Oncol. 2017, 35, 8013.
115. Levin, N.; Spencer, A.; Harrison, S.J.; Chauhan, D.; Burrows, F.J.; Anderson, K.C.; Reich, S.D.; Richardson, P.G.; Trikha, M. Marizomib irreversibly inhibits proteasome to overcome compensatory hyperactivation in multiple myeloma and solid tumour patients. Br. J. Haematol. 2016, 174, 711–720.
116. Oerlemans, R.; Franke, N.E.; Assaraf, Y.G.; Cloos, J.; van Zantwijk, I.; Berkers, C.R.; Scheffer, G.L.; Debipersad, K.; Vojtekova, K.; Lemos, C.; et al. Molecular basis of bortezomib resistance: Proteasome subunit 5 (PSMB5) gene mutation and overexpression of PSMB5 protein. Blood 2008, 112, 2489–2499.
117. Chauhan, D.; Li, G.; Shringarpure, R.; Podar, K.; Ohtake, Y.; Hideshima, T.; Anderson, K.C. Blockade of Hsp27 overcomes Bortezomib/proteasome inhibitor PS-341 resistance in lymphoma cells. Cancer Res. 2003, 63, 6174–6177.
118. Kuhn, D.J.; Berkova, Z.; Jones, R.J.; Woessner, R.; Bjorklund, C.C.; Ma, W.; Davis, R.E.; Lin, P.; Wang, H.; Madden, T.L.; et al. Targeting the insulin-like growth factor-1 receptor to overcome bortezomib resistance in preclinical models of multiple myeloma. Blood 2012, 120, 3260–3270.
119. Cohen, Y.C.; Zada, M.; Wang, S.-Y.; Bornstein, C.; David, E.; Moshe, A.; Li, B.; Shlomi-Loubaton, S.; Gatt, M.E.; Gur, C.; et al. Identification of resistance pathways and therapeutic targets in relapsed multiple myeloma patients through single-cell sequencing. Nat. Med. 2021, 27, 491–503.