

# Proteasome, a Promising Therapeutic Target for Multiple Diseases Beyond Cancer

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**Abstract :** Proteasome is vital for intracellular protein homeostasis as it eliminates misfolded and damaged protein. Inhibition of proteasome has been validated as a powerful strategy for anti-cancer therapy, and several drugs have been approved for treatment of multiple myeloma. Recent studies indicate that proteasome has potent therapeutic effects on a variety of diseases besides cancer, including parasite infectious diseases, bacterial/fungal infections diseases, neurodegenerative diseases and autoimmune diseases. In this review, recent developments of proteasome inhibitors for various diseases and related structure activity relationships are going to be summarized.

**Keywords:** proteasome inhibitor, infectious diseases, autoimmune diseases, neurodegenerative diseases, drugs

Protein turnover is mainly achieved by different degradation systems in cells, of which the ubiquitin-proteasome system (UPS) and the autophagosomal-lysosomal system are involved in the degradation of most cellular proteins.<sup>1,2</sup> The UPS is essential for the regulation of various cellular functions by breakdown of more than 80% of cellular proteins, ensuring that misfolded, oxidized or damaged proteins as well as proteins whose functions are no longer needed, to be degraded.<sup>3-5</sup> This system contributes to maintain normal cell functions and cellular homeostasis in eukaryotic cells. In this system, proteins are tagged for degradation by covalent linkage to polyubiquitin chain, which involves the orchestrated action of three classes of enzymes-E1 (ubiquitin-activating enzyme), E2 (ubiquitin-conjugating enzyme) and E3 (ubiquitin ligase).<sup>2</sup> Most ubiquitylated proteins are degraded through a multistep process including recognition of the polyubiquitin chain, unfolding proteins and translocation of the substance into the chamber of the proteasome. For mislabeled proteins, ubiquitylation could be reversed with deubiquitinating enzymes (DUBs), through which were regenerated for reuse by the cell.<sup>6</sup>

Proteasome, the prominent part of UPS, is a large protein complex containing multicatalytic protease subunits.<sup>7-9</sup> Studies have validated close connection between proteasome dysfunction and various diseases including cancer,<sup>10,11</sup> infectious diseases,<sup>12</sup> immune diseases<sup>13</sup> and neurodegenerative diseases,<sup>14</sup> thus guaranteeing its development prospect as a desirable drug target. Till now, three human constitutive proteasome inhibitors Bortezomib, Carfilzomib and Ixazomib have been approved for the treatment of multiple myeloma (MM)<sup>15,16</sup> and mantle cell lymphoma.<sup>17</sup> Besides, various candidates are evaluated in clinical trials for the

treatment of malignancies<sup>18</sup> and autoimmune diseases.<sup>19</sup> Actually, proteasome inhibition may be solutions for a variety of diseases, and with the development of inhibitors against different forms of proteasome, novel therapeutic options for these diseases will be exploited.

## Structure and Functions of Proteasome

The 20S proteasome (core particle, CP) is the most common form of this highly complex proteolysis machine, which consists of 28 subunits and has a mass of ~700kDa.<sup>20,21</sup> The 28 protein subunits are arranged as a cylindrical stack of four rings with seven subunits each ( $\alpha$ 7- $\beta$ 7- $\beta$ 7- $\alpha$ 7) to form a barrel-shaped structure.<sup>22</sup> Three of the seven  $\beta$  subunits ( $\beta$ 1,  $\beta$ 2 and  $\beta$ 5) encode three distinct proteolytic activities: caspase-like activity ( $\beta$ 1), trypsin-like activity ( $\beta$ 2) and chymotrypsin-like activity ( $\beta$ 5).<sup>23</sup> In vertebrates or in response to interferon (IFN)- $\gamma$  or tumor necrosis factor (TNF)- $\alpha$ , the catalytic active  $\beta$ -subunits ( $\beta$ 1,  $\beta$ 2, and  $\beta$ 5) are replaced by their inducible counterparts low molecular mass polypeptide 2 (LMP2,  $\beta$ 1i), multicatalytic endopeptidase complex-like 1 (MECL-1,  $\beta$ 2i) and low molecular mass polypeptide 7 (LMP7,  $\beta$ 5i), respectively, thereby forming the immunoproteasome<sup>24,25</sup> (Figure 1).

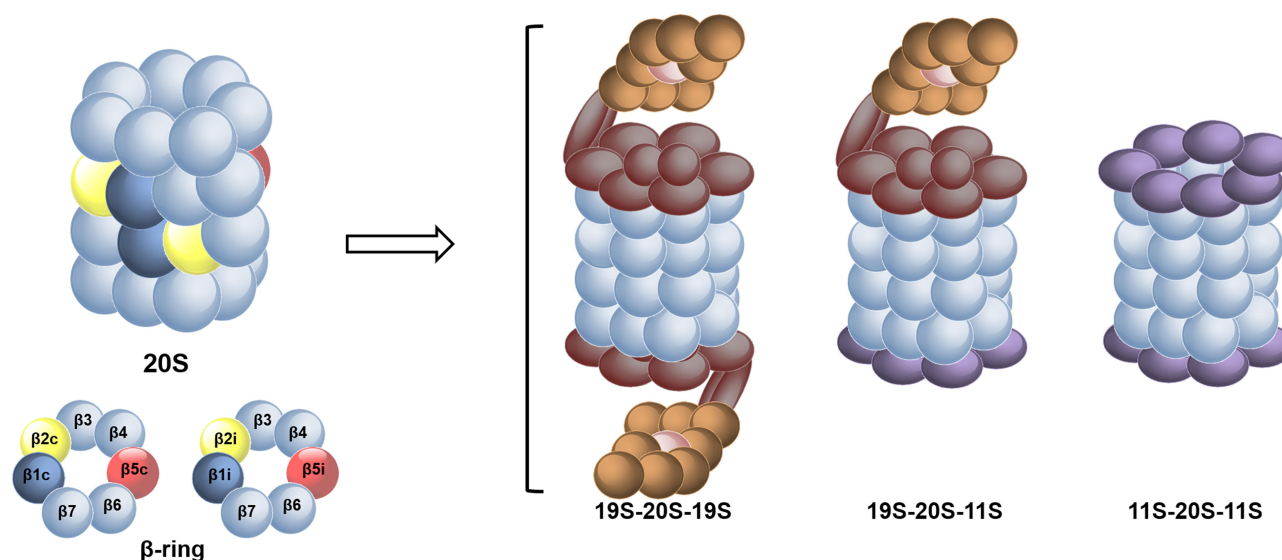
To avoid uncontrolled protein degradation, access to the chamber of the core particle with proteolytic activities is well regulated.<sup>21,26</sup> Two proteasome activators, the regulatory particle (19S) and the PA28 heptamer (11S), are identified till now, which help damaged or misfolded proteins to

remove the ubiquitin and unfold the protein or degrade unstructured proteins. The 20S proteasome binds with two 19S particle at both ends to form the prominent constitutive 26S proteasome (19S-20S-19S), while with an 11S-20S-11S assembly or 19S-20S-11S hybrid structure primarily in immunoproteasome<sup>27-29</sup> (Figure 1).

With the ability in controlling the levels of critical proteins in various physiological processes, the proteasome is significant in maintaining proteostasis. Proteasome inhibition induces a variety of cellular responses including endoplasmic reticulum (ER) stress,<sup>30</sup> NF- $\kappa$ B inhibition,<sup>31</sup> cell cycle arrest and proapoptotic factors increase,<sup>32</sup> thus making this protein complex an important drug target for various diseases.<sup>33-35</sup>

## Targeting Proteasome for Various Diseases

The application of proteasome inhibitors for the therapy of hematological malignancies has been validated.<sup>36-38</sup> Besides, recent studies also suggest that proteasome targeting is a potential strategy for parasite infectious and bacterial/fungal infections diseases,<sup>39</sup> for the rapid protein turnover of these pathogens through UPS system during development in its human host is quite crucial. Additionally, with the development of various proteasome inhibitors, treatment of immunologic and autoimmune diseases, neurodegenerative diseases may have more options in the future.<sup>40,41</sup>



**Figure 1** The 20S proteasome is comprised of four assembled rings, and the internal  $\beta$ -ring involves constitutive or immune-catalytic subunits. The 20S proteasome binds with 19S or 11S particle to form different proteasome assemblies.

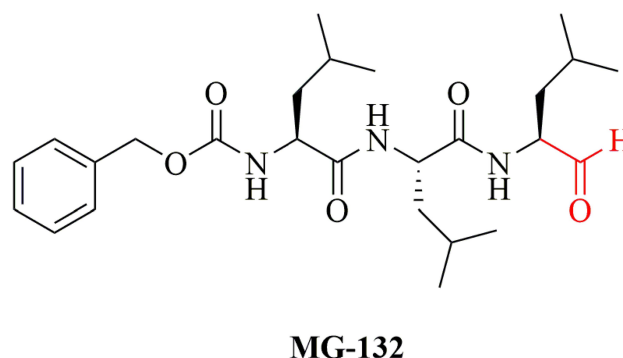
## Parasite Infectious Diseases

The structure and function of pathogen genomes encode proteasomes are similar to the mammalian complex.<sup>42</sup> Recently, Bortezomib and Carfilzomib have also been evaluated as anti-parasite drugs for targeting parasite proteasome, however, the results of these studies revealed that they were toxic to host cells.<sup>43–45</sup> Along with the deepening of research on parasite proteasome, the application of proteasome inhibitors for parasite infectious diseases has been reported.

### Malaria

Malaria has been ranked as one of the greatest global health problems by the World Health organization. Despite many effective molecules have been developed and approved for treating malaria, the morbidity and mortality from malaria remain increased in many countries in Africa, which creates enormous social and economic burdens.<sup>46</sup> Malaria in humans can be infected by 6 different species of *Plasmodium*, of which *P. falciparum* causes the deadliest form of infection and *P. vivax* is the most widespread.<sup>47</sup> Currently, the treatment of malarial highly depends on artemisinin and its derivatives combination therapies (ACTs). However, the emerging resistance to ACTs and other previous standard antimalarial drugs emphasize the need for developing novel targets and drugs.<sup>48</sup> Recent researches indicate that the *Plasmodium* proteasome has been validated as a novel target for exploring antimalarial drugs. It is well known that the proteasome plays a significant role in controlling protein quality in cells. Because of the high replication rate of the erythrocytic stage parasites, protein quality control is of great significance for *P. falciparum*. To avoid accumulation of misfolded or nonfunctional proteins, proteasome mediated protein turnover is tightly controlled, thus making *P. falciparum* proteasome potential for anti-malaria drug discovery.<sup>49,50</sup>

MG-132 (Figure 2), a widely used peptidyl aldehyde proteasome inhibitor, was the first choice for studying the UPS in some organisms including malaria parasites.<sup>51</sup> Falcipain, belonging to a family of hemoglobin-degrading cysteine proteases, is also an important antimalaria drug target against *Plasmodium falciparum*. Actually, this analogue was a dual-targeted inhibitor against proteasome and falcipain, which displayed higher efficacy and less risk of drug resistance compared with individual inhibitors of the two targets.<sup>51</sup> MG-132 could inhibit hemoglobin degradation, and it is most likely due to inhibition



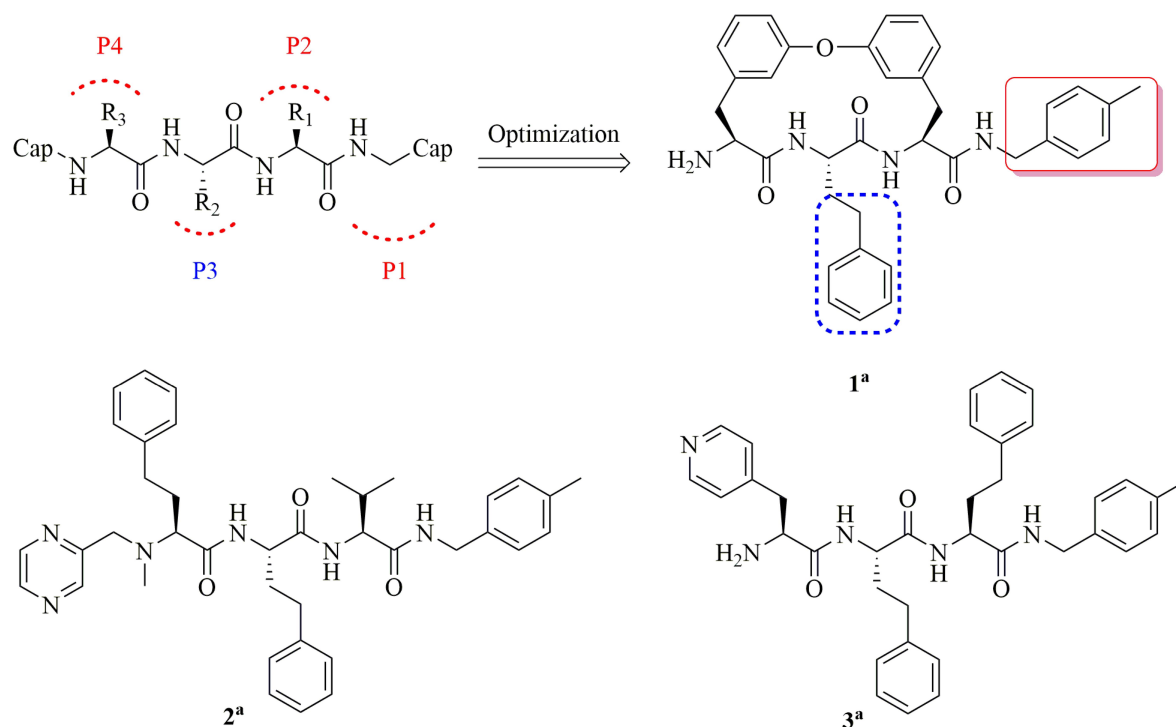
**Figure 2** Structure of anti-malaria peptidyl aldehyde analogue MG-132.

of hemoglobin-degrading falcipain cysteine proteases. The *N*-terminal aldehyde group of MG-132 could react with the catalytic cysteine residue of falcipain or threonine residue of proteasome to form covalent interactions. Besides, the P2 leucine was a falcipain preferred residue, through which a more than 227-fold selectivity for *P. falciparum* (IC<sub>50</sub>: 0.0476  $\mu$ M) against PBMCs (IC<sub>50</sub>: 10.8  $\mu$ M) was achieved.<sup>52</sup>

In another study, nine short *N*, *C*-capped peptides were screened from a library of 1600 non-covalent proteasome inhibitors, and these compounds showed potent activities in culture with no toxicity in host cells.<sup>53</sup> All of the nine compounds possessed a common 4-methylbenzyl group at the P1 position, indicating that the hydrophobic side chains in the S1 pocket of the  $\beta$ 5 subunit were important to the activities against *plasmodium*. Furthermore, eight of the nine inhibitors had a bulky homo-Phe in the P3 position, and the molecular docking and homology model revealed that homo-Phe was quite suitable for the S3 pocket in the  $\beta$ 5 active subunit of *Plasmodium*.

Compounds **1**, **2** and **3** (Figure 3) displayed potent activities against *P. falciparum* proteasome with EC<sub>50</sub> values ranging from 0.0345  $\mu$ M to 0.357  $\mu$ M and the selectivity was greater than 100-fold for the parasite over the host cell (Table 1). In particular, compound **1** was a non-natural cyclic peptide, which displayed significant antiparasitic activity. This analogue showed a more than 1450-fold selectivity for *P. falciparum* relative to human foreskin fibroblasts (HFF) cells but weak proteasome inhibition towards mammalian cells.

PR3 was identified through screening of a library containing 670 carfilzomib analogues for inhibition of ring-stage 72-hr *P. falciparum* replication assay, which showed selectively for *P. falciparum* proteasome against human proteasome.<sup>43</sup> Although the structure of PR3 was highly



<sup>a</sup>The compound numbers in the paper (1, 2, 3 etc.) were reordered and uncorrelated to the analogue numbers in the original articles.

**Figure 3** Anti-malaria N, C-capped non-covalent peptidyl derivatives.

similar to carfilzomib with only a tert-butyl group instead of isopropyl at P1 position (Figure 4), the anti-parasite activity of PR3 was 100-fold less potent than Carfilzomib, with  $EC_{50}$  values of 2.90  $\mu$ M and 28.8 nM, respectively. However, PR3 was not toxic for host HFF cells at the concentration of up to 50  $\mu$ M.

Proteasome inhibitors have shown potent inhibitory activities against *P. falciparum* at all stages of its life cycle,<sup>54–56</sup> but most inhibitors lacked selectivity against mammalian proteasome. Hence, a substrate profiling method was applied to identify the substrate specificity and structural properties of the *P. falciparum* as well as

to uncover differences in the specificities of the human and *P. falciparum* proteasome. The results revealed a clear preference for tryptophan (Trp) in P3 and P1 positions for inhibitors against *P. falciparum* proteasome compared to the human constitutive proteasome.<sup>57</sup>

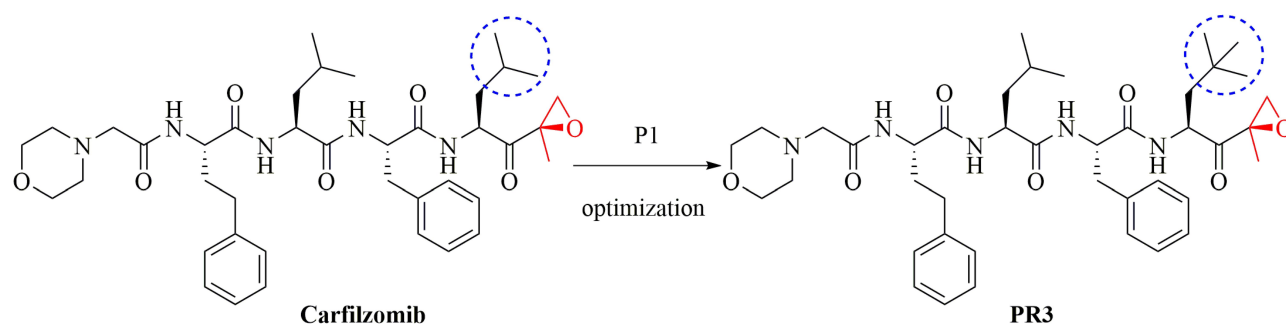
Compounds WLL-vs, WLW-vs and LLW-vs were designed based on the tri-leucine scaffold and the Leu residues were replaced with Trp at the positions of P1 and P3 (Figure 5). Analogue LLW-vs showed reduced  $\beta 5$  *P. falciparum* proteasome inhibitory activity but comparable  $\beta 2$  inhibitory activity to LLL-vs Alternating the P3 position to Trp (WLL-vs) resulted in potent inhibitory activities against both  $\beta 2$  and  $\beta 5$  subunits of *P. falciparum* proteasome (Table 2). Furthermore, compound WLW-vs was produced by substitution of leucine with tryptophan at both P1 and P3 positions, which exhibited potent  $\beta 2$ -subunit of *P. falciparum* proteasome inhibitory activity and considerable selectivity.<sup>57</sup> The high-resolution cryo-EM analysis of WLW-vs binding with pf 20S revealed that the main reason for the selectivity is the bigger binding pocket of  $\beta 2$  *P. falciparum* proteasome, which was able to accommodate bulky side chains like Trp at the P1 and P3 positions, while the human  $\beta 2$  pocket cannot.

**Table 1** The  $IC_{50}$  and  $EC_{50}$  Values of Compounds 1, 2 and 3 Against *P. falciparum* 20S Proteasome

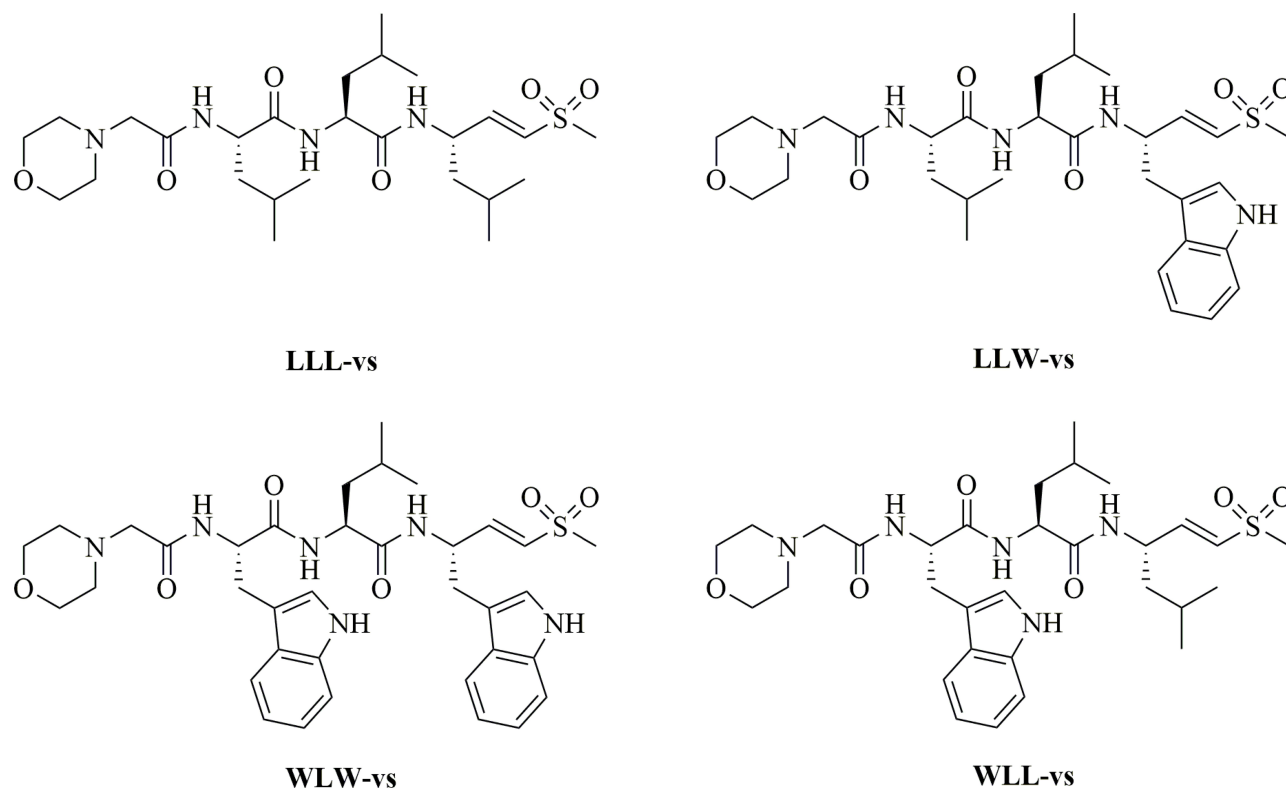
Compound	$IC_{50}$ ( $\mu$ M)		$EC_{50}$ ( $\mu$ M)		Selectivity <sup>c</sup>
	h20S <sup>a</sup>	Pf20S <sup>a</sup>	HFF <sup>b</sup>	Pf	
1	9.22	1.25	>50 <sup>c</sup>	0.0345	>1450
2	1.97	0.0789	61	0.104	587
3	0.171	0.00924	68	0.357	189

**Notes:** <sup>a</sup>h20S: The human 20S proteasome, pf20S: *P. falciparum* 20S proteasome. <sup>b</sup>HFF (Human foreskin fibroblast) were non-confluent. <sup>c</sup>Determined as the ratio of HFF  $EC_{50}$  to Pf  $EC_{50}$  for a 72h treatment period.





**Figure 4** Structures of Carfilzomib and its derivative PR3.



**Figure 5** Vinyl sulfone derivatives LLL-vs, LLW-vs, WLW-vs and WLL-vs.

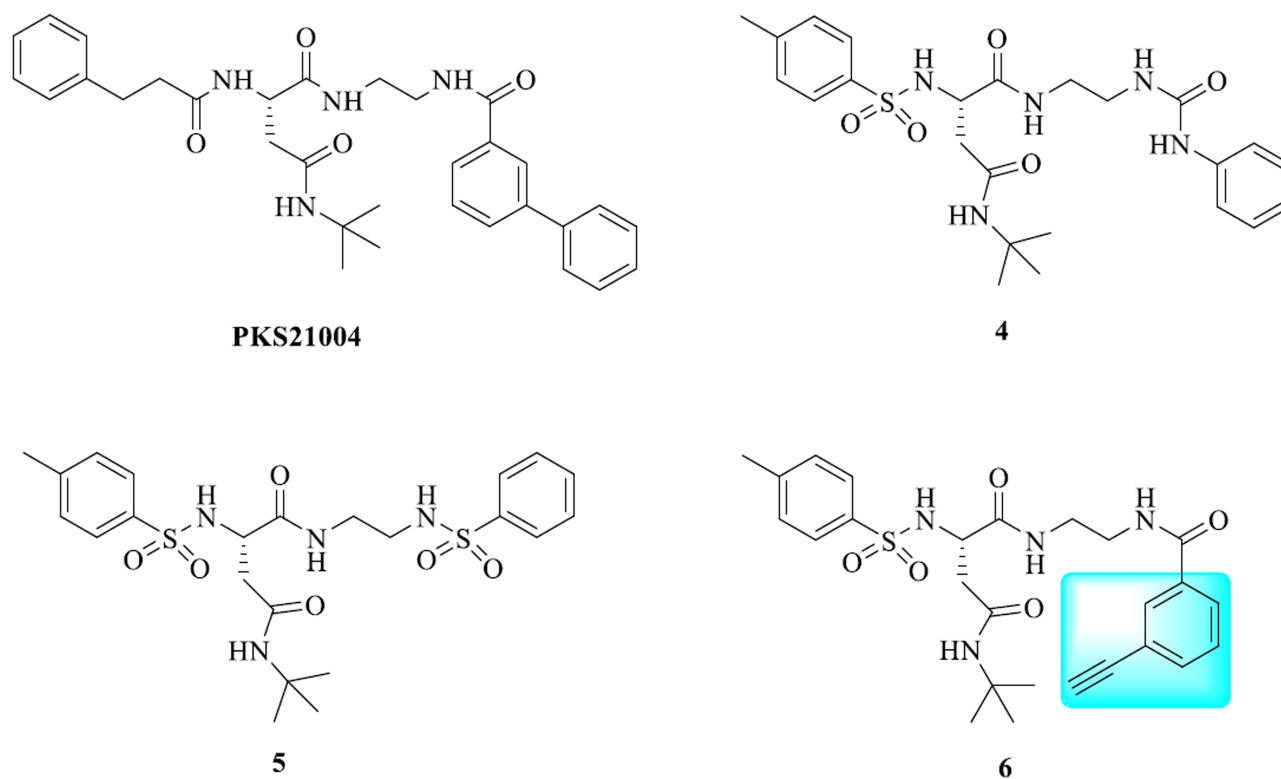
A versatile class of peptidomimetic proteasome inhibitors with an asparagine ethylenediamines (AsnEDAs) scaffold was reported recently.<sup>58</sup> It revealed that the

hydrophilic moieties introduced in this scaffold enhanced the selectivity for *P. falciparum* proteasome over human proteasome, as well as the anti-parasite activity against

**Table 2** IC<sub>50</sub> and EC<sub>50</sub> Values of LLL-Vs, LLW-Vs, WLW-Vs and WLL-Vs in *P. falciparum*

Compound	Pf20S (IC <sub>50</sub> , μM)			EC <sub>50</sub> , μM		Selectivity <sup>a</sup>
	β1	β2	β5	HFF	<i>P. falciparum</i>	
LLL-vs	>50	3.3	2.2	94	3.9	24
LLW-vs	>50	5.0	>50	>250	81	>3
WLL-vs	>50	0.9	0.8	129	0.191	675
WLW-vs	>50	0.8	>50	>250	15.4	>16

**Note:** <sup>a</sup>Selectivity: HFF/*P. falciparum*.



**Figure 6** AsnEDA constructed peptidomimetic analogue PKS21004 and its derivatives.

erythrocytic stages of *P. falciparum*. Compound **4** and its derivatives **5**, **6** (Figure 6) were optimized starting from PKS21004. At P1 position, amide was replaced by phenylurea and sulfonamide group to obtain compound **4** and **5** with  $IC_{50}$  values of 2.576  $\mu$ M and 15.135  $\mu$ M, respectively (Table 3). Furthermore, replacing phenylurea (**4**) and sulfonamide (**5**) with 3-ethynylbenzene (compound **6**) enhanced potency by 548- and 3220-fold, respectively. Compound **6** was the most potent AsnEDA-based *P. falciparum* proteasome inhibitor ( $IC_{50}$ : 4.7 nM), which exhibited good selectivities over  $\beta 5c$  and  $\beta 5i$  with  $IC_{50}$  values of 430 nM and 112 nM, respectively (Table 3).

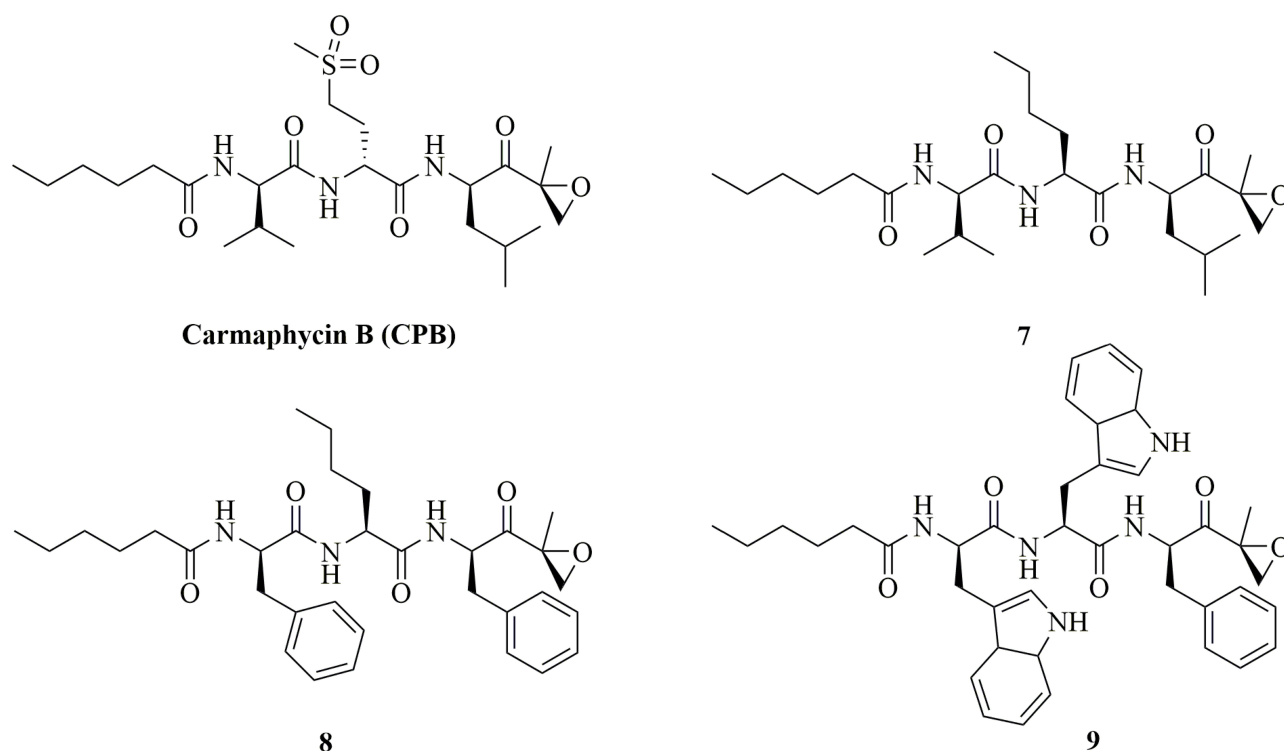
### Schistosomiasis

As a potential drug target for the treatment of malaria, proteasome has also been found with potent inhibitory activities on other parasitic infections, such as schistosomiasis. Proteolytic enzymes in schistosome are vital in invasion of mammalian host, digestion of host proteins and regulation of host's immune response and physiology. Hence proteasome in this protease system is a potential target for developing anti-schistosomiasis drugs. Carmaphycin B (Figure 7) was isolated from a Curaçao collection of *Symploca* sp. marine cyanobacteria, which featured a leucine-derived  $\alpha$ ,  $\beta$ -epoxyketone warhead, an amino acid residue with methionine sulfone, and an *N*-hexanoyl amino terminus capping

**Table 3**  $IC_{50}$  Values of AsnEDAs Against *P. falciparum* Proteasome, Human  $\beta 5i$  and  $\beta 5c$

Compound	$IC_{50}$ (nM)			$EC_{50}$ (nM)	
	$\beta 5i$	$\beta 5c$	Pf20S $\beta 5$	Pf 3D7	HepG2
PKS21004	58	326	3.6	4.6	3,670
4	2090 $\pm$ 29	>100,000	2576 $\pm$ 326	>2770	>11,000
5	2892 $\pm$ 1004	>100,000	15,135 $\pm$ 4565	>2770	>11,000
6	112 $\pm$ 11	430 $\pm$ 130	4.7 $\pm$ 1.4	3.1 $\pm$ 0.1	>11,000

**Abbreviation:** Pf 3D7, *P. falciparum* heparin binding protein 3D7.



**Figure 7** Anti-schistosomiasis peptidyl epoxyketone derivatives.

group. This analogue showed *S. mansoni* proteasome (Sm20S)  $\beta 2$  and  $\beta 5$  inhibitory activities with  $IC_{50}$  values of 0.6 nM and 9.8 nM, respectively, as well as weak  $\beta 1$  inhibitory activity with  $IC_{50}$  value of up to 500 nM.<sup>59</sup> However, Carmaphycin B was cytotoxic against HepG2 cell with a 24 h  $EC_{50}$  value of 12.6 nM.<sup>60</sup> To decrease the cytotoxicity and obtain more active inhibitors, analogues of Carmaphycin B **7**, **8**, and **9** (Figure 7) were identified. Compared to Carmaphycin B, the P2 position of compound **7** was replaced with norleucine (Nle), and the cytotoxicity against HepG2 cell was 11-fold decreased ( $IC_{50}$  values for **7** of 134 nM and Carmaphycin B of 12.6 nM). However, no difference was observed in potency between human constitutive proteasome (c-h20S) and Sm20S for the  $\beta 5$ ,  $\beta 2$  and  $\beta 1$  subunits (Table 4). Compound **8** differed from **7** with substitutions of Phe for

Leu and Val in P1 and P3 position, and this analogue showed a similar inhibitory activity for the  $\beta 5$  subunits of c-h20S and Sm20S. However, for the  $\beta 2$  subunit of Sm20S, **8** displayed a 3.3-fold and at least 19-fold potency for c20S and i20S, respectively. Similar to **7** and **8**, compound **9** was comprised of Phe-Trp-Trp at the P1, P2, and P3 position, and it showed at least 12.5-fold more potent activity for  $\beta 2$  subunit of Sm20S compared with human proteasome (Table 4). Meanwhile, compound **9** was 27.4-fold less cytotoxic for HepG2 cell than Carmaphycin B ( $IC_{50}$  values for **9** and Carmaphycin B of 346 nM and 12.6 nM, respectively)

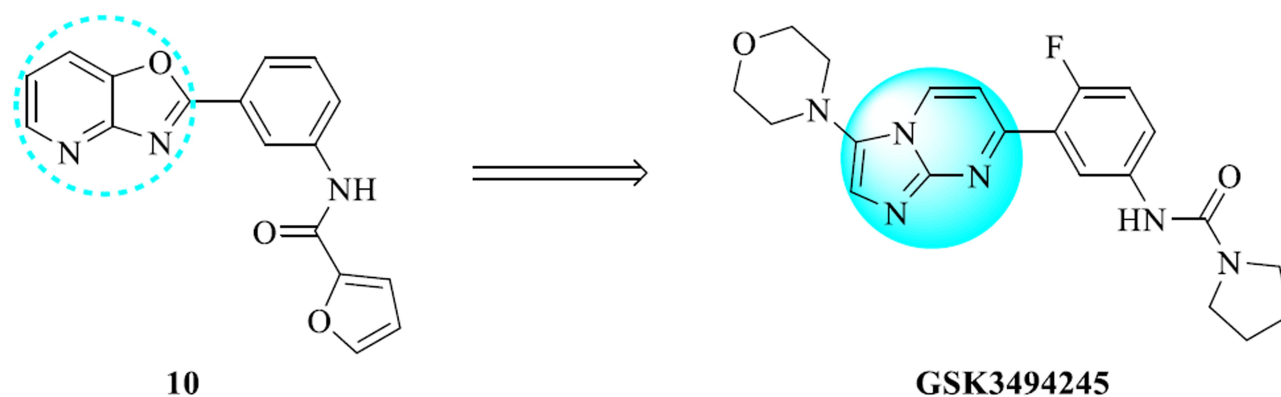
### Visceral Leishmaniasis

The proteasome was also suggested as a target for exploring anti-Visceral Leishmania (VL) drugs. The hit compound **10**

**Table 4** Inhibitory Activities and Cytotoxicities of Carmaphycin B and Its Analogues

Compound	$IC_{50}$ (nM)						HepG2 Cytotoxicity, $IC_{50}$ (nM)
	$\beta 5i$	$\beta 5c$	Sm20S $\beta 5$	$\beta 2i$	$\beta 2c$	Sm20S <sup>a</sup> $\beta 2$	
CPB	4.5	2.0	0.6	62.0	12.0	9.8	12.6
<b>7</b>	14.5	2.7	1.9	201	26.0	25.2	134
<b>8</b>	12.8	2.3	1.3	>500	85.2	25.5	30.8
<b>9</b>	5.3	3.8	5.4	>500	488	38.8	346

**Abbreviation:** Sm20S, *S. mansoni* proteasome.



**Figure 8** Optimization of GSK3494245 with anti-visceral leishmaniasis activity.

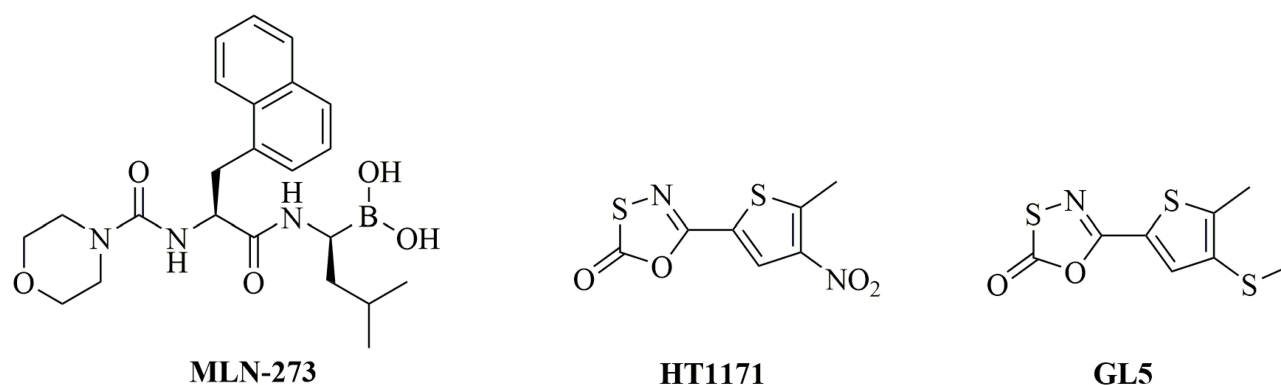
was identified through phenotypic screening from a diversity library (15,659 compounds) against the related kinetoplastid parasite *Trypanosoma cruzi* (*T. cruzi*) with an  $EC_{50}$  value of 0.22  $\mu$ M, which also displayed a favorable selectivity over mammalian cell growth inhibition (THP-1 cells,  $EC_{50} > 50$   $\mu$ M) but with poor in vitro metabolic stability owing to the rapid degradation ( $CL_{int} = 24$  mL/min per gram).<sup>61</sup> To tackle toxicity and also improve bioavailability, GSK3494245 (Figure 8) was obtained with imidazo[1,2-a]pyrimidine scaffold, which showed better in vitro metabolic stability ( $CL_{int} = 0.8$  mL/min per gram) and selectivity over mammalian cells (THP-1 cells,  $EC_{50} > 50$   $\mu$ M). However, this analogue showed lower potency against *T. cruzi* with  $EC_{50}$  of 1.6  $\mu$ M. Furthermore, GSK3494245 could inhibit the  $\beta 5$  of the *L. donovani* proteasome in a dose-dependent manner with  $IC_{50}$  value of 0.16  $\mu$ M but had no effect on  $\beta 1$  or  $\beta 2$  subunit. The structures of Apo and GSK3494245 bounding to *L. tarentolae* 20S proteasome were determined by single particle Cryo-EM at 3.3Å resolution, which revealed a previously undiscovered binding site for inhibitors of the  $\beta 5$ , and the site lies between the  $\beta 4$  and  $\beta 5$  subunits. The

discovery exploited an induced cavity that is lined on one side by  $\beta 4$  residues that are different between human and kinetoplastid protozoan. What's more, GSK3494245 is currently undergoing preclinical development, and it is now being progressed toward human clinical trials.

## Bacterial/Fungal Infectious Diseases

With the deepening study on proteasome, intimate correlations between this target and bacterial or fungal infections have been clarified. Tuberculosis (TB) is responsible for 1.3 million deaths worldwide in 2017<sup>62,63</sup> *Mycobacterium tuberculosis* (Mtb) is the causative agent of tuberculosis, which is rare among bacterial pathogens in expressing a functional 20S proteasome.<sup>64–69</sup> Furthermore, the Mtb epidemic has been aggravated by the drug-resistant strains in recent years.<sup>70</sup> Hence, Mtb 20S proteasome has gained much attention for exploring effective treatments for TB. Several classes of Mtb proteasome inhibitors with varied degrees activity and selectivity have been reported (Figure 9).

MLN-273 (Figure 9), a dipeptidyl boronate proteasome inhibitor, was found as a tool for studying the mechanism



**Figure 9** Structures of MLN-273, HT1171 and GL5 against Mtb proteasome.

**Table 5** Kinetic Parameters of GL5 and HT1171

Compound	$k_{\text{obs}}/[I], \text{M}^{-1}\text{s}^{-1}$			
	Mtb 20SOG	h20S $\beta 5$	h20S $\beta 2$	h20S $\beta 1$
GL5	376.4	0.4	NI	0.03
HT1171	2,134	10.1	6.9	2.8

**Abbreviations:** Mtb 20SOG, *Mycobacterium tuberculosis* proteasome open gate form; NI, no inhibition.

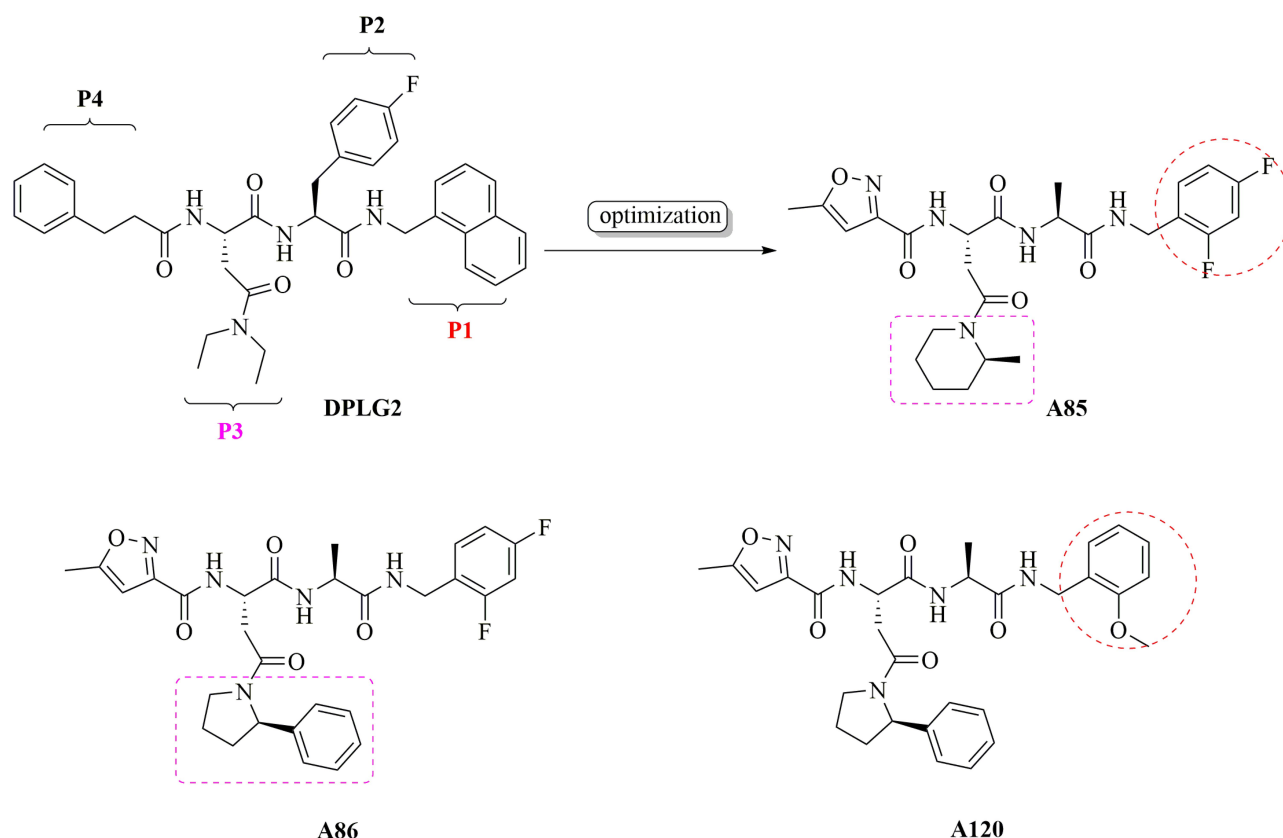
of *Rhodococcus* 20S proteasome. This analogue showed potent inhibitory activity against Mtb proteasome with  $\text{IC}_{50}$  value of 1.6 nM.<sup>64,71</sup> The crystal structure of MLN-273 binding to Mtb20S indicated that the P1 leucine side-chain seemed to be important due to its location in a hydrophobic S1 pocket formed by Val31, Ile45, Ala49, Ala52 and Val53. In addition, the naphthyl moiety at P2 position has little interaction with the protein, while the P3 side chain of morpholino group and the dipeptide backbone shows no specific interaction with the protein.<sup>65</sup>

GL5 and HT1171 (Figure 9) belong to a class of oxathiazole-2-one derivatives, which were identified through screening of a library containing 20,000 compounds.<sup>72–74</sup> GL5 and HT1171 were >1000-fold more effective against

Mtb proteasome than human proteasome by cyclocarbonylating the threonine residue of Mtb proteasome active site. The two compounds showed abilities in inhibiting mycobacterial proteasomes and killed non-replicating Mtb at the concentrations ranging from 12.5 to 50  $\mu\text{M}$  with no apparent toxicity to mammalian cells. The results of the kinetic analysis of inactivation of Mtb 20SOG (“open-gate” mutant) and human proteasome (h20S) by oxathiazol-2-ones are illustrated in Table 5.

DPLG2 (Figure 10) is an *N*, *C*-Capped dipeptide non-covalent proteasome inhibitor, which was discovered by screening against Mtb20S with 1,600 *N*, *C*-capped dipeptides.<sup>76,77</sup> P1 naphthyl and P3 *N*, *N*-diethyl Asn amide were incorporated in the peptide skeleton of DPLG-2, and the co-crystal structure of DPLG2 with Mtb20S revealed that the P1 and P3 dictated the species selectivity. Furthermore, the peptide backbone of DPLG2 was able to form 6 hydrogen bonds in binding with Mtb20S. Hence, DPLG-2 potently inhibited Mtb20S with a  $K_i$  value of 15 nM and displayed over 3,600-fold selectivity against human  $\beta 5$  and  $\beta 5i$ .

Recently, a series of proteasome-specific dipeptidyl inhibitors with methylisoxazole capped at *N*-terminus

**Figure 10** Asn amide containing peptidyl analogue DPLG2 and its derivatives.



**Table 6** IC<sub>50</sub> Values of A85 and Its Derivatives Against Mtb20S, Human  $\beta$ 5i and  $\beta$ 5c

Compound	IC <sub>50</sub> ( $\mu$ M)			Selectivity Index <sup>a</sup>
	Mtb20S	i-h20S $\beta$ 5i	c-h20S $\beta$ 5c	
A85	0.007	0.026	1.412	3.714/201.429
A86	0.003	7.89	>100	2,630/> 33,333
A120	0.065	>100	>100	> 1,500/>1,500

Note: <sup>a</sup> Selectivity Index: (i-h20S + Mtb)/(c-h20S + Mtb).

have been reported.<sup>78</sup> A85 (Figure 10) was discovered starting from DPLG2 by an iterative, automated microfluidic system termed CyclOpsTM.<sup>79–83</sup> Compared to DPLG2, A85 showed potent activity over Mtb20S with an IC<sub>50</sub> value of 7 nM, while the IC<sub>50</sub> values against human  $\beta$ 5c (3.7-fold) and human  $\beta$ 5i (202-fold) were 26 nM and 1.412  $\mu$ M, respectively (Table 6).

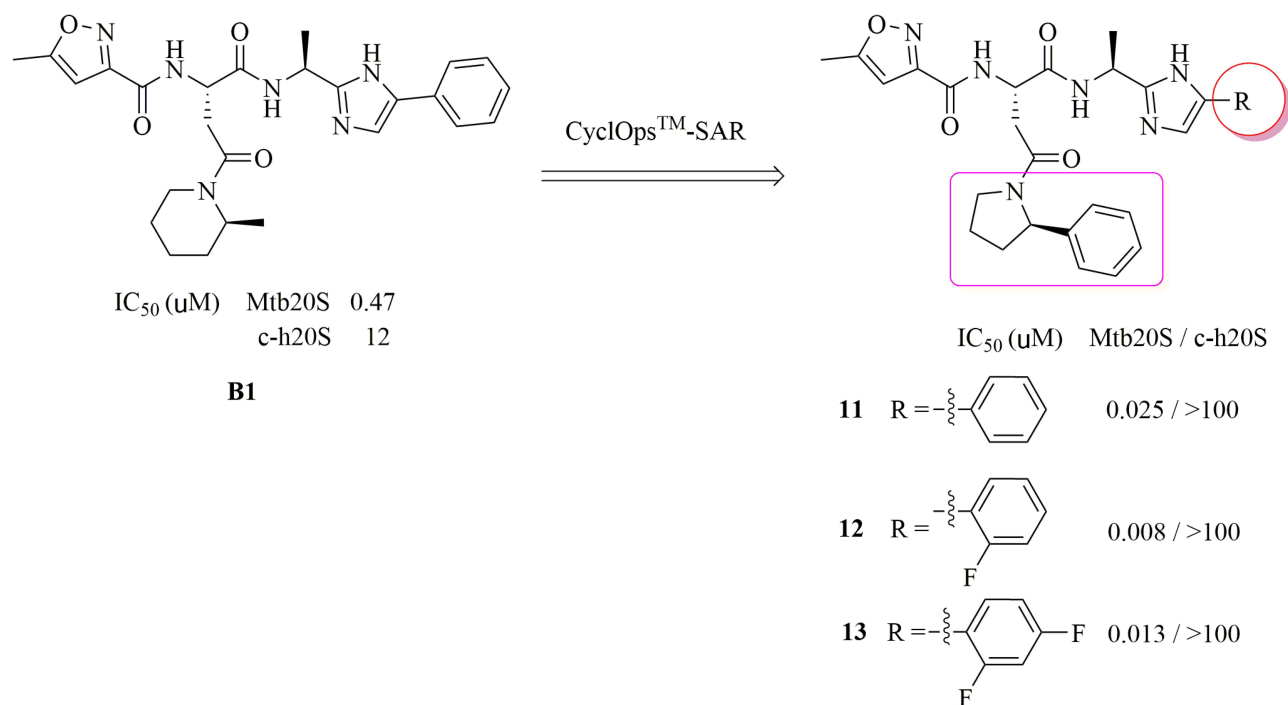
A86, with improved inhibitory activity and selectivity, was afforded by replacing the 2-methylpiperidin-1-yl of A85 with 2-phenylpyrrolidinyl (Table 6). Subsequently, it's discovered that the inhibitory potency for Mtb20S and human proteasomes were all reduced while 2, 4-difluorinebenzyl of A86 was replaced by 2-methoxybenzyl in A120 (Table 6). The results of X-ray structures of Mtb20S in complex with A85 and A86 revealed that the two compounds can bind to

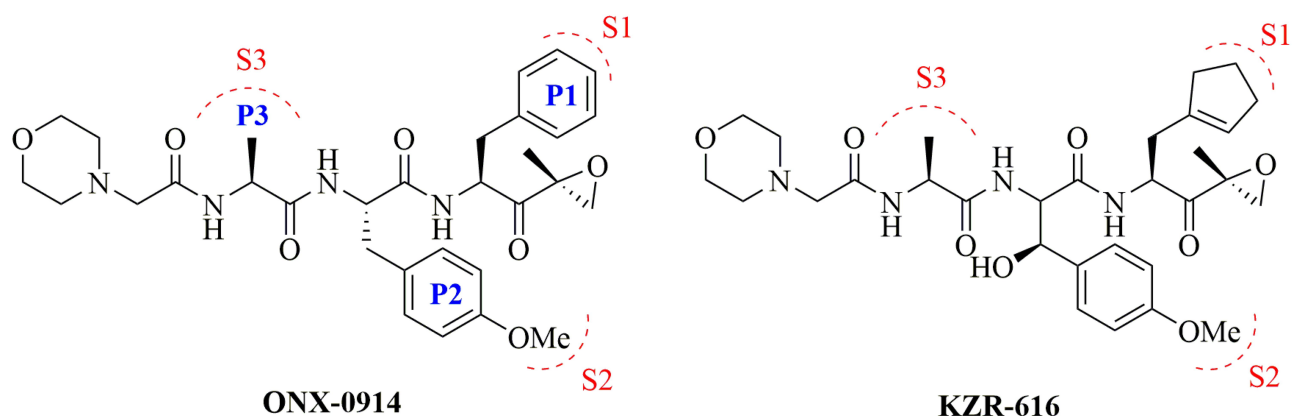
Mtb20S non-covalently, in which a short antiparallel  $\beta$ -strand between the compounds and the backbone atoms of Thr-21, Gly-47, and Ala-49 was formed.<sup>78</sup> These results indicated that 2-phenylpyrrolidinyl at P3 position was necessary to maintain the potency and selectivity for Mtb20S over human proteasomes.

With the optimization of C-terminal amide with heterocyclic rings, compound B1 (Figure 11) was identified with a phenylimidazole scaffold maintaining modest inhibitory activity against Mtb20S. Besides, B1 also showed weak inhibitory activity for  $\beta$ 5c with IC<sub>50</sub> value of about 10  $\mu$ M. Compounds 11, 12 and 13 (Figure 11) were derived from B1, and all the three compounds were much potent for Mtb20S with IC<sub>50</sub> values of 25 nM, 8 nM and 13 nM, respectively. Moreover, the IC<sub>50</sub> values of both  $\beta$ 5c and  $\beta$ 5i were more than 100  $\mu$ M.<sup>78</sup>

## Immunologic and Autoimmune Diseases

Immunoproteasome, a variant proteasome, is expressed in immune cells abundantly. It plays an important role in antigen presentation and participates in a majority of immune processes such as the regulation of cytokine production, the expansion and survival of T cells and the differentiation of T helper cells.<sup>84</sup> It's observed that the dysfunction of immunoproteasome leads to various immunological diseases and

**Figure 11** Peptidomimetic phenylimidazoles with Mtb20S inhibitory activities.



**Figure 12** Immunoproteasome selective inhibitors ONX-0914 and KZR-616.

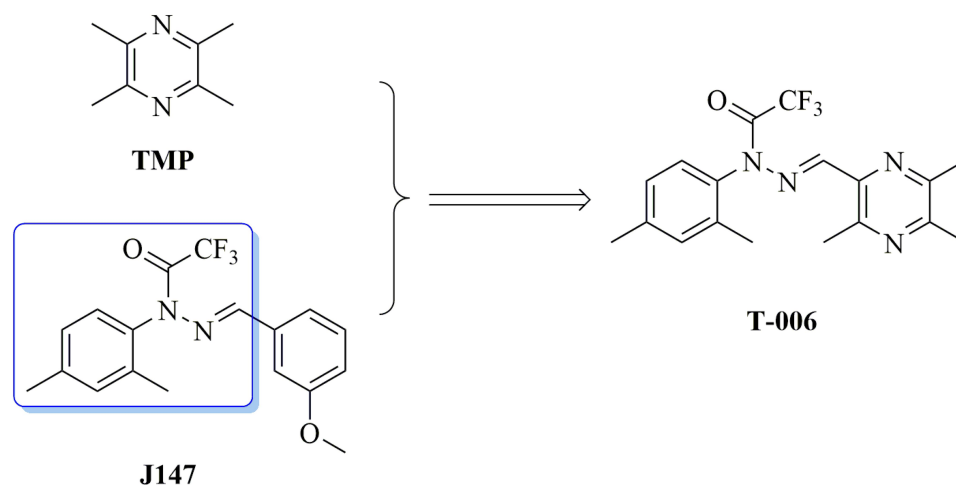
the upregulation may increase cytokine secretion which is relevant to autoimmune diseases.<sup>85</sup> The strategy of immunoproteasome inhibitors utilized for the treatment of immunologic and autoimmune diseases has been verified by multiple clinical trials. Till now, three drugs Bortezomib, Carfilzomib and Ixazomib target constitutive proteasome and immunoproteasome simultaneously have been approved by FDA. However, inhibition of the wide distributed constitutive proteasome results in toxicities that require dose reductions or even cessation of the treatment. Therefore, considerable efforts have been made for developing immunoproteasome-specific inhibitors that could be used as therapeutic agents for the treatment of autoimmune disorders.<sup>86</sup>

ONX-0914 (Figure 12) was the first selective epoxyketone-based peptidyl immunoproteasome inhibitor, which showed potent inhibitory activity for  $\beta 5i$  and moderated activity against  $\beta 5c$  with  $IC_{50}$  values of 5.7 nM and 54 nM, respectively. In addition, it also displayed moderate inhibitory activities against  $\beta 1i$  ( $IC_{50}$ : 460 nM) and  $\beta 2i$  ( $IC_{50}$ : 590 nM). Early treatment of lupus-prone mice with ONX-0914 can prevent disease progression, and therapy of mice with established disease dramatically abrogated nephritis.<sup>87</sup> Therefore, ONX-0914 has shown bright therapeutic prospect in the models of systemic lupus erythematosus, rheumatoid arthritis, multiple sclerosis, myasthenia gravis and etc.<sup>88</sup> KZR-616 (Figure 12), a selective immunoproteasome inhibitor with a tripeptide epoxyketone scaffold, was identified based on the optimization of ONX-0914 by Kezar Life Sciences. Compared with other epoxyketone compounds, an R-hydroxyl group substitution at the  $\beta$  position of the P2 methyltyrosine side chain would be well tolerated and resulted in hydrogen-bonding with backbone carbonyl of Ser21. It's reported that KZR-616 could inhibit  $\beta 1i$  and  $\beta 5i$  simultaneously

with  $IC_{50}$  values of 0.039  $\mu$ M and 0.623  $\mu$ M, respectively, which was necessary for producing anti-inflammatory effect in vitro and in vivo. Furthermore, KZR-616 has been approved for various clinical trials for the treatment of systemic lupus erythematosus at the stage of Phase Ib/II (NCT03393013, August, 2016),<sup>89</sup> and two other clinical trials are posted for the treatment of active polymyositis or dermatomyositis (NCT04033926, July, 2019) and active autoimmune hemolytic anemia or immune thrombocytopenia (NCT04039477, July, 2019) at the stage of phase II for the evaluation of the safety, tolerability, efficacy, pharmacokinetics and pharmacodynamics.

## Neurodegenerative Disease

Parkinson's disease (PD) is one of the most common neurodegenerative diseases with distinct clinical symptoms. The pathogenesis of PD is characterized in part by the degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNc), the intraneuronal accumulation of mis-folded  $\alpha$ -synuclein ( $\alpha$ -syn) is the hallmark of PD.<sup>90</sup> Studies have confirmed that the degradation of ubiquitylated protein by UPS (with activators of 26S proteasome or inhibitors of deubiquitylating enzymes) or by autophagy would facilitate alleviating or prevention of neurodegenerative diseases.<sup>91</sup> It has been verified that the inhibition of USP14, a deubiquitylating enzyme, would accelerate the degradation of aberrant proteins in cell by enhancing the activity of proteasome.<sup>92</sup> IU1, a selective small-molecular inhibitor, prevents USP14 with  $IC_{50}$  value of 4–5  $\mu$ M but failed to inhibit other DUBs.<sup>93,94</sup> T-006 (Figure 13), a Chinese medicinal component with a new tetramethylpyrazine (TMP) scaffold, was designed from two multifunctional neuroprotective chemicals TMP and J147 with combination strategy.<sup>95,96</sup> It was reported that T-006 could



**Figure 13** Combination of TMP and J147 to form proteasome activator T-006.

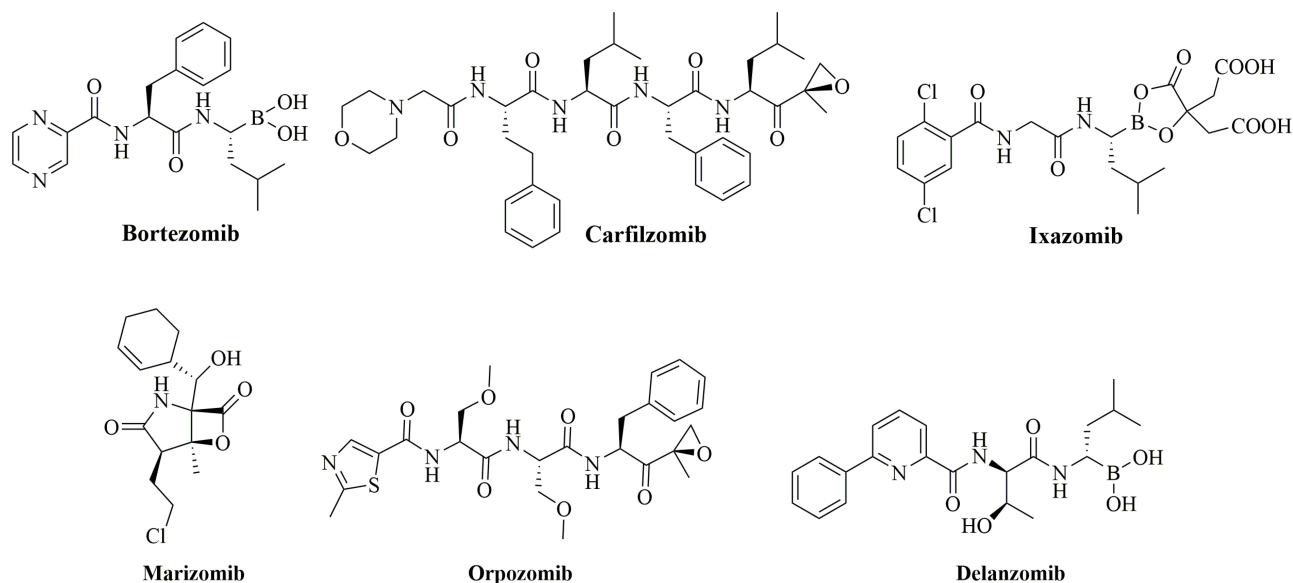
prevent glutamate-induced excitotoxicity in Cerebellar granule neurons (CGNs) by regulating the PI3K/AKT pathway. Besides, this analogue could selectively mutate  $\alpha$ -syn via increasing  $\beta$ 5i gene expression and correspondingly enhancing its chymotrypsin-like proteasome activity. These results indicated that T-006 could be a potent therapeutic agent as a proteasome activator for the treatment of PD and related diseases.<sup>95</sup>

## Malignancies

Proteasome is closely correlated to intracellular protein degradation and many important physiological functions, which influences the development of tumor. The inhibition

of proteasome presents a dysregulation of crucial regulatory proteins including NF- $\kappa$ B, P53, cyclins and CDK, which are relevant to various signaling pathways.<sup>33,97</sup> Accordingly, proteasome inhibitors have been proved to be effective anti-cancer drugs.

Bortezomib, a boronate dipeptide, was the first proteasome inhibitor approved by FDA for the therapy of MM in 2003 and mantle cell lymphoma in 2006. It is a reversible covalent inhibitor primarily acting with the CT-L activity of the constitutive proteasome (Figure 14). Bortezomib showed strong inhibitory activities for  $\beta$ 1i ( $IC_{50}$ : 5.5 nM),  $\beta$ 5c ( $IC_{50}$ : 7 nM) and  $\beta$ 5i ( $IC_{50}$ : 3.3 nM) subunits.<sup>98</sup> However, there are restrictions to its clinical



**Figure 14** Representative proteasome inhibitors approved or under clinical trials.

application due to its severe toxicities and adverse effects including peripheral neuropathy.<sup>99</sup> Due to peripheral neuropathy, the dosage of Bortezomib cannot be increased to overcome poor tissue penetration and rapid clearance from blood which may result in the limitation of therapy in the solid tumors.<sup>100</sup> In the clinical treatment of diseases, Bortezomib is often considered in combination with other therapies (like chemotherapy and radiotherapy).<sup>101</sup>

Carfilzomib (Figure 14) is an  $\alpha'$ ,  $\beta'$ -epoxyketone tetrapeptide with an N-acyl morpholine cap, which was obtained through optimization of a natural product epoxomicin. Unlike bortezomib, carfilzomib covalently and irreversibly binds to the  $\beta 5c$  subunit, and results with sustained proteasome inhibition. Carfilzomib existed better activity for both  $\beta 5c$  and  $\beta 5i$  subunits with  $IC_{50}$  values of 6 nM and 33 nM, respectively, while the  $IC_{50}$  values against other subunits were greater than 600 nM.<sup>102</sup> Compared with Bortezomib, it renders less neurotoxic side effects and also has a good therapeutic prospect in solid tumor models. However, drug resistance was found in Carfilzomib treatment.<sup>101</sup>

Ixazomib (Figure 14) was also identified from a panel of boronic acid analogues, and it was the first oral available proteasome inhibitor approved for the therapy of MM in 2015 in association with Lenalidomide and Dexamethasone. Ixazomib is a prodrug, which is able to hydrolyze quickly and transform to MLN2238, and reversibly inhibits the proteasome. Remarkably, it selectively targets the  $\beta 5c$  subunit with an  $IC_{50}$  value of 3.4 nM, while shows moderate inhibitory activities against  $\beta 1c$  and  $\beta 2c$  with  $IC_{50}$  values of 31 nM and 3.5  $\mu$ M, respectively.<sup>86,103</sup> Despite structural similarity, Ixazomib demonstrates lower incidence of peripheral neuropathy and better efficacy in solid tumor models.<sup>101</sup>

In addition to the above described three approved proteasome inhibitors, currently there are other proteasome inhibitors under evaluation for cancer therapy in various clinical trials.<sup>101</sup> The  $\beta$ -lactone marizomib (Figure 14) isolated from the marine actinomycete *Salinispora tropica*, is in a Phase III trial combined with standard temozolomide-based radiochemotherapy.<sup>104</sup> Oprozomib (Figure 14) is an orally bioavailable epoxyketone proteasome inhibitor and a new oral formulation is being investigated in a phase I/II study.<sup>105</sup> Delanzomib (Figure 14) is a boronic acid, and Phase I clinical trials have been completed in patients with MM and solid tumors.<sup>106</sup>

## Conclusions and Perspectives

With nearly 20 years' development of proteasome inhibitors in clinical, the old drug target seems still active and with potential for developing more therapeutic drugs. In addition to the great success in treating hematological malignancies, proteasome inhibitors also show prospect for the therapy of other diseases, including treatment of organ transplant patients with acute allograft rejection,<sup>107</sup> treatment of reperfusion injury after stroke,<sup>108</sup> therapeutics in cardiac diseases,<sup>109</sup> Japanese encephalitis,<sup>110</sup> bone disease,<sup>111</sup> oxidative stress<sup>112</sup> and so on. However, there were fewer inhibitors with clear actions because of lacking of the thorough study, and which need to be further studied.

Currently, the research of proteasome inhibitors is mainly focused on infectious diseases, although none has entered clinical trials, and the relevant research needs to be further deepened. The proteasome inhibitors on immune diseases progressed rapidly, and compound KZR-616 is now evaluated in multiple clinical trials at different stages. However, human constitutive proteasome inhibition also induces severe toxicities and adverse effects, meanwhile the drug resistance limited their clinical application. For developing therapeutics for other diseases beyond cancer, selectivity is the most concerned issue. With the clarification of cocrystal structures of various forms of proteasome and inhibitors, rational design of selective drug candidates would be more effective.

## Disclosure

The authors confirm that this article content has no conflicts of interest.

## References

1. Hanna J, Guerra-Moreno A, Ang J, Micoogullari Y. Protein degradation and the pathologic basis of disease. *Am J Pathol*. 2019;189(1):94–103. doi:10.1016/j.ajpath.2018.09.004
2. Bedford L, Lowe J, Dick LR, Mayer RJ, Brownell JE. Ubiquitin-like protein conjugation and the ubiquitin-proteasome system as drug targets. *Nat Rev Drug Discov*. 2011;10(1):29–46. doi:10.1038/nrd3321
3. Dohmen RJ, Huibregtse J, Scheffner M. Ubiquitin, Ubiquitin-Like Proteins, and Proteasome-Mediated Degradation. *Encyclopedia Cell Biol*. 2016;1:582–595.
4. Meyer-Schwesinger C. The ubiquitin-proteasome system in kidney physiology and disease. *Nat Rev Nephrol*. 2019;15(7):393–411. doi:10.1038/s41581-019-0148-1
5. Kar G, Keskin O, Fraternali F, Gursoy A. Emerging role of the ubiquitin-proteasome system as drug targets. *Curr Pharm Des*. 2013;19(18):3175–3189. doi:10.2174/1381612811319180002
6. Mata-Cantero L, Lobato-Gil S, Aillet F, et al. *The Ubiquitin-Proteasome System (UPS) as a Cancer Drug Target: Emerging Mechanisms and Therapeutics*. 2015.

7. Collins GA, Goldberg AL. The Logic of the 26S Proteasome. *Cell*. 2017;169(5):792–806. doi:10.1016/j.cell.2017.04.023
8. Micel LN, Tentler JJ, Smith PG, Eckhardt GS. Role of ubiquitin ligases and the proteasome in oncogenesis: novel targets for anticancer therapies. *J Clin Oncol*. 2013;31(9):1231–1238. doi:10.1200/JCO.2012.44.0958
9. Jung T, Grune T. The proteasome and the degradation of oxidized proteins: part I-structure of proteasomes. *Redox Biol*. 2013;1:178–182. doi:10.1016/j.redox.2013.01.004
10. Dou QP. Targeting tumor ubiquitin-proteasome pathway with new and old drugs. *Curr Cancer Drug Targets*. 2011;11(3):236–238. doi:10.2174/156800911794519789
11. Frezza M, Schmitt S, Dou QP. Targeting the ubiquitin-proteasome pathway: an emerging concept in cancer therapy. *Curr Top Med Chem*. 2011;11(23):2888–2905. doi:10.2174/156802611798281311
12. Berkers CR, Ovaa H. Drug discovery and assay development in the ubiquitin-proteasome system. *Biochem Soc Trans*. 2010;38(Pt1):14–20. doi:10.1042/BST0380014
13. Mattingly LH, Gault RA, Murphy WJ. Use of systemic proteasome inhibition as an immune-modulating agent in disease. *Endocr Metab Immune Disord Drug Targets*. 2007;7(1):29–34. doi:10.2174/187153007780059397
14. Hu H. Abnormal protein aggregation and neurodegenerative diseases. *Chin Sci Bull*. 2001;46(1):1–3.
15. Cao B, Mao X. The ubiquitin-proteasomal system is critical for multiple myeloma: implications in drug discovery. *Am J Blood Res*. 2011;1(1):46–56.
16. Hideshima T, Bradner JE, Wong J, et al. Small-molecule inhibition of proteasome and aggresome function induces synergistic antitumor activity in multiple myeloma. *Proc Natl Acad Sci U S A*. 2005;102(24):8567–8572. doi:10.1073/pnas.0503221102
17. Holkova B, Grant S. Proteasome inhibitors in mantle cell lymphoma. *Best Pract Res Clin Haematol*. 2012;25(2):133–141. doi:10.1016/j.beha.2012.04.007
18. Spano JP, Bay JO, Blay JY, Rixe O. Proteasome inhibition: a new approach for the treatment of malignancies. *Bull Cancer*. 2005;92(11):E61–66, 945–952.
19. Wang J, Maldonado MA. The ubiquitin-proteasome system and its role in inflammatory and autoimmune diseases. *Cell Mol Immunol*. 2006;3(4):255–261.
20. Kish-Trier E, Hill CP. Structural biology of the proteasome. *Annu Rev Biophys*. 2013;42:29–49. doi:10.1146/annurev-biophys-083012-130417
21. Jung T, Catalgol B, Grune T. The proteasomal system. *Mol Aspects Med*. 2009;30(4):191–296. doi:10.1016/j.mam.2009.04.001
22. Hirano Y, Kaneko T, Okamoto K, et al. Dissecting beta-ring assembly pathway of the mammalian 20S proteasome. *EMBO J*. 2008;27(16):2204–2213. doi:10.1038/emboj.2008.148
23. Saeki Y, Tanaka K. Assembly and function of the proteasome. *Methods Mol Biol*. 2012;832:315–337.
24. Huber EM, Basler M, Schwab R, et al. Immuno- and constitutive proteasome crystal structures reveal differences in substrate and inhibitor specificity. *Cell*. 2012;148(4):727–738. doi:10.1016/j.cell.2011.12.030
25. Basler M, Groettrup M. Immunoproteasome-specific inhibitors and their application. *Methods Mol Biol*. 2012;832:391–401.
26. Finley D. Recognition and processing of ubiquitin-protein conjugates by the proteasome. *Annu Rev Biochem*. 2009;78:477–513. doi:10.1146/annurev.biochem.78.081507.101607
27. Glickman MH, Rubin DM, Coux O, et al. A subcomplex of the proteasome regulatory particle required for ubiquitin-conjugate degradation and related to the COP9-signalosome and eIF3. *Cell*. 1998;94(5):615–623. doi:10.1016/S0092-8674(00)81603-7
28. Yao T, Cohen RE. A cryptic protease couples deubiquitination and degradation by the proteasome. *Nature*. 2002;419(6905):403–407. doi:10.1038/nature01071
29. Cromm PM, Crews CM. The proteasome in modern drug discovery: second life of a highly valuable drug target. *ACS Central Sci*. 2017;3(8):830–838.
30. Menéndez-Benito V, Verhoef LGGC, Masucci MG, Dantuma NP. Endoplasmic reticulum stress compromises the ubiquitin-proteasome system. *Hum Mol Genet*. 2005;14(19):2787–2799. doi:10.1093/hmg/ddi312
31. Hay RT, Vuillard L, Desterro JM, Rodriguez MS. Control of NF-kappa B transcriptional activation by signal induced proteolysis of I kappa B alpha. *Philos Trans R Soc Lond B Biol Sci*. 1999;354(1389):1601–1609. doi:10.1098/rstb.1999.0504
32. Adams J. The proteasome: structure, function, and role in the cell. *Cancer Treat Rev*. 2003;29(Suppl 1):3–9. doi:10.1016/S0305-7372(03)00081-1
33. Crawford LJ, Walker B, Irvine AE. Proteasome inhibitors in cancer therapy. *J Cell Commun Signal*. 2011;5(2):101–110. doi:10.1007/s12079-011-0121-7
34. Moreau P, Richardson PG, Cavo M, et al. Proteasome inhibitors in multiple myeloma: 10 years later. 2012;120(5):947–959.
35. Xie SC, Dick LR, Gould A, Brand S, Tilley L. The proteasome as a target for protozoan parasites. *Expert Opin Ther Targets*. 2019;23(11):903–914. doi:10.1080/14728222.2019.1685981
36. Mitsiades C, Mitsiades N, Hideshima T, Richardson P, Anderson K. Proteasome inhibition as a therapeutic strategy for hematologic malignancies. *Expert Rev Anticancer Ther*. 2005;5:465–476. doi:10.1586/14737140.5.3.465
37. Richardson P. Clinical update: proteasome inhibitors in hematologic malignancies. *Cancer Treat Rev*. 2003;29(Suppl 1):33–39. doi:10.1016/S0305-7372(03)00080-X
38. Mikhael J, Chang H. Bortezomib: proteasome inhibition as a novel mechanism of cancer therapy-implications for hematological malignancies. *Lett Drug Des Discov*. 2007;4:82–86. doi:10.2174/157018007779422541
39. Khare S, Nagle AS, Biggart A, et al. Proteasome inhibition for treatment of leishmaniasis, Chagas disease and sleeping sickness. *Nature*. 2016;537(7619):229–233. doi:10.1038/nature19339
40. Fierabracci A. Proteasome inhibitors: a new perspective for treating autoimmune diseases. *Curr Drug Targets*. 2012;13(13):1665–1675. doi:10.2174/138945012803530053
41. Ying Z, Wang H, Wang G. The ubiquitin proteasome system as a potential target for the treatment of neurodegenerative diseases. *Curr Pharm Des*. 2013;19(18):3305–3314. doi:10.2174/1381612811319180013
42. Bibo-Verdugo B, Jiang Z, Caffrey CR, O'Donoghue AJ. Targeting proteasomes in infectious organisms to combat disease. *FEBS J*. 2017;284(10):1503–1517.
43. Li H, Ponder EL, Verdoes M, et al. Validation of the proteasome as a therapeutic target in Plasmodium using an epoxyketone inhibitor with parasite-specific toxicity. *Chem Biol*. 2012;19(12):1535–1545. doi:10.1016/j.chembiol.2012.09.019
44. Reynolds JM, El Bissati K, Brandenburg J, Gunzl A, Mamoun CB. Antimalarial activity of the anticancer and proteasome inhibitor bortezomib and its analog ZL3B. *BMC Clin Pharmacol*. 2007;7:13. doi:10.1186/1472-6904-7-13
45. Dogovski C, Xie SC, Burgio G, et al. Targeting the cell stress response of Plasmodium falciparum to overcome artemisinin resistance. *PLoS Biol*. 2015;13(4):e1002132. doi:10.1371/journal.pbio.1002132
46. Gonzalez J, Bai GX, Frevert U, Corey EJ, Eichinger D. Proteasome-dependent cyst formation and stage-specific ubiquitin mRNA accumulation in Entamoeba invadens. *Eur J Biochem*. 1999;264(3):897–904. doi:10.1046/j.1432-1327.1999.00682.x
47. Luth MR, Gupta P, Otilie S, Winzeler EA. Using in vitro evolution and whole genome analysis to discover next generation targets for antimalarial drug discovery. *ACS Infect Dis*. 2018;4(3):301–314. doi:10.1021/acsinfecdis.7b00276



48. Krishnan KM, Williamson KC. The proteasome as a target to combat malaria: hits and misses. *Transl Res*. 2018;198:40–47. doi:10.1016/j.trsl.2018.04.007
49. Blasco B, Leroy D, Fidock DA. Antimalarial drug resistance: linking *Plasmodium falciparum* parasite biology to the clinic. *Nat Med*. 2017;23(8):917–928.
50. Kreidenweiss A, Kremsner PG, Mordmüller B. Comprehensive study of proteasome inhibitors against *Plasmodium falciparum* laboratory strains and field isolates from Gabon. *Malar J*. 2008;7:187. doi:10.1186/1475-2875-7-187
51. Prasad R, Atul Kolla VK, et al. Blocking *Plasmodium falciparum* development via dual inhibition of hemoglobin degradation and the ubiquitin proteasome system by MG132. *PLoS One*. 2013;8(9):e73530–e73530.
52. Sinnis P, Prasad R, Atul, et al. Blocking *plasmodium falciparum* development via dual inhibition of hemoglobin degradation and the ubiquitin proteasome system by MG132. *PLoS One*. 2013;8(9):e73530.
53. Li H, Tsu C, Blackburn C, et al. Identification of potent and selective non-covalent inhibitors of the *Plasmodium falciparum* proteasome. *J Am Chem Soc*. 2014;136(39):13562–13565. doi:10.1021/ja507692y
54. Gantt SM, Myung JM, Briones MR, et al. Proteasome inhibitors block development of *Plasmodium* spp. *Antimicrob Agents Chemother*. 1998;42(10):2731–2738. doi:10.1128/AAC.42.10.2731
55. Czesny B, Goshu S, Cook JL, Williamson KC. The proteasome inhibitor epoxomicin has potent *Plasmodium falciparum* gametocytocidal activity. *Antimicrob Agents Chemother*. 2009;53(10):4080–4085. doi:10.1128/AAC.00088-09
56. Prudhomme J, McDaniel E, Pons N, et al. Marine actinomycetes: a new source of compounds against the human malaria parasite. *PLoS One*. 2008;3(6):e2335. doi:10.1371/journal.pone.0002335
57. Li H, O'Donoghue AJ, van der Linden WA, et al. Structure- and function-based design of *Plasmodium*-selective proteasome inhibitors. *Nature*. 2016;530(7589):233–236. doi:10.1038/nature16936
58. Zhan W, Visone J, Ouellette T, et al. Improvement of asparagine ethylenediamines as anti-malarial *plasmodium*-selective proteasome inhibitors. *J Med Chem*. 2019;62(13):6137–6145.
59. Bibo-Verdugo B, Wang SC, Almaliti J, et al. The proteasome as a drug target in the metazoan pathogen, *Schistosoma mansoni*. *ACS Infect Dis*. 2019;5(10):1802–1812. doi:10.1021/acsinfecdis.9b00237
60. LaMonte GM, Almaliti J, Bibo-Verdugo B, et al. Development of a potent inhibitor of the *plasmodium* proteasome with reduced mammalian toxicity. *J Med Chem*. 2017;60(15):6721–6732. doi:10.1021/acs.jmedchem.7b00671
61. Wyllie S, Brand S, Thomas M, et al. Preclinical candidate for the treatment of visceral leishmaniasis that acts through proteasome inhibition. *Proc Natl Acad Sci U S A*. 2019;116(19):9318–9323. doi:10.1073/pnas.1820175116
62. Saxena AK, Singh A. Mycobacterial tuberculosis enzyme targets and their inhibitors. *Curr Top Med Chem*. 2019;19(5):337–355. doi:10.2174/1568026619666190219105722
63. Organization WH. *Global Tuberculosis Report*. 2018.
64. Lin G, Hu G, Tsu C, et al. Mycobacterium tuberculosis prcBA genes encode a gated proteasome with broad oligopeptide specificity. *Mol Microbiol*. 2006;59(5):1405–1416. doi:10.1111/j.1365-2958.2005.05035.x
65. Hu G, Lin G, Wang M, et al. Structure of the Mycobacterium tuberculosis proteasome and mechanism of inhibition by a peptidyl boronate. *Mol Microbiol*. 2006;59(5):1417–1428. doi:10.1111/j.1365-2958.2005.05036.x
66. Darwin KH, Lin G, Chen Z, Li H, Nathan CF. Characterization of a Mycobacterium tuberculosis proteasomal ATPase homologue. *Mol Microbiol*. 2005;55(2):561–571. doi:10.1111/j.1365-2958.2004.04403.x
67. Darwin KH, Ehrt S, Gutierrez-Ramos JC, Weich N, Nathan CF. The proteasome of Mycobacterium tuberculosis is required for resistance to nitric oxide. *Science*. 2003;302(5652):1963–1966. doi:10.1126/science.1091176
68. Lin G, Tsu C, Dick L, Zhou XK, Nathan C. Distinct specificities of Mycobacterium tuberculosis and mammalian proteasomes for N-acetyl tripeptide substrates. *J Biol Chem*. 2008;283(49):34423–34431. doi:10.1074/jbc.M805324200
69. WH Organization. *Global Tuberculosis Report*. 2015.
70. Koul A, Arnoult E, Lounis N, Guillemont J, Andries K. The challenge of new drug discovery for tuberculosis. *Nature*. 2011;469(7331):483–490.
71. Mc Cormack T, Baumeister W, Grenier L, et al. Active site-directed inhibitors of Rhodococcus 20 S proteasome. Kinetics and mechanism. *J Biol Chem*. 1997;272(42):26103–26109. doi:10.1074/jbc.272.42.26103
72. Lin G, Li D, de Carvalho LP, et al. Inhibitors selective for mycobacterial versus human proteasomes. *Nature*. 2009;461(7264):621–626.
73. Totaro KA, Barthelme D, Simpson PT, et al. Rational design of selective and bioactive inhibitors of the mycobacterium tuberculosis proteasome. *ACS Infect Dis*. 2017;3(2):176–181. doi:10.1021/acsinfecdis.6b00172
74. Russo F, Gising J, Åkerbladh L, et al. Optimization and evaluation of 5-styryl-oxathiazol-2-one mycobacterium tuberculosis proteasome inhibitors as potential antitubercular agents. *ChemistryOpen*. 2015;4(3):342–362. doi:10.1002/open.201500001
75. Totaro K, Barthelme D, Simpson P, et al. Rational design of selective and bioactive inhibitors of the mycobacterium tuberculosis proteasome. *ACS Infect Dis*. 2016;3.
76. Lin G, Chidawanyika T, Tsu C, et al. N,C-Capped dipeptides with selectivity for mycobacterial proteasome over human proteasomes: role of S3 and S1 binding pockets. *J Am Chem Soc*. 2013;135(27):9968–9971. doi:10.1021/ja400021x
77. Hsu HC, Singh PK, Fan H, et al. Structural basis for the species-selective binding of N,C-capped dipeptides to the mycobacterium tuberculosis proteasome. *Biochemistry*. 2017;56(1):324–333. doi:10.1021/acs.biochem.6b01107
78. Zhan W, Hsu HC, Morgan T, et al. Selective phenylimidazole-based inhibitors of the mycobacterium tuberculosis proteasome. *J Med Chem*. 2019;62(20):9246–9253. doi:10.1021/acs.jmedchem.9b01187
79. Parry DM. Closing the loop: developing an integrated design, make, and test platform for discovery. *ACS Med Chem Lett*. 2019;10(6):848–856. doi:10.1021/acsmedchemlett.9b00095
80. Pant SM, Mukonoweshuro A, Desai B, et al. Design, synthesis, and testing of potent, selective hepsin inhibitors via application of an automated closed-loop optimization platform. *J Med Chem*. 2018;61(10):4335–4347. doi:10.1021/acs.jmedchem.7b01698
81. Ramjee MK, Patel S. Continuous-flow injection microfluidic thrombin assays: the effect of binding kinetics on observed enzyme inhibition. *Anal Biochem*. 2017;528:38–46. doi:10.1016/j.ab.2017.04.016
82. Desai B, Dixon K, Farrant E, et al. Rapid discovery of a novel series of Abl kinase inhibitors by application of an integrated microfluidic synthesis and screening platform. *J Med Chem*. 2013;56(7):3033–3047. doi:10.1021/jm400099d
83. Czechitzky W, Dedio J, Desai B, et al. Integrated synthesis and testing of substituted xanthine based dpp4 inhibitors: application to drug discovery. *ACS Med Chem Lett*. 2013;4(8):768–772. doi:10.1021/ml400171b
84. Ogorevc E, Schiffer ES, Sosic I, Gobec S. A patent review of immunoproteasome inhibitors. *Expert Opin Ther Pat*. 2018;28(7):517–540. doi:10.1080/13543776.2018.1484904
85. Eskandari SK, Seelen MAJ, Lin G, Azzi JR. The immunoproteasome: an old player with a novel and emerging role in alloimmunity. 2017;17(12):3033–3039.

86. Ettari R, Zappala M, Grasso S, Musolino C, Innao V, Allegra A. Immunoproteasome-selective and non-selective inhibitors: a promising approach for the treatment of multiple myeloma. *Pharmacol Ther.* **2018**;182:176–192. doi:10.1016/j.pharmthera.2017.09.001
87. Ichikawa HT, Conley T, Muchamuel T, et al. Beneficial effect of novel proteasome inhibitors in murine lupus via dual inhibition of type I interferon and autoantibody-secreting cells. *64(2):493–503.*
88. Liu RT, Zhang P, Yang CL, et al. ONX-0914, a selective inhibitor of immunoproteasome, ameliorates experimental autoimmune myasthenia gravis by modulating humoral response. *J Neuroimmunol.* **2017**;311:71–78. doi:10.1016/j.jneuroim.2017.08.005
89. Johnson HWB, Lowe E, Anderl JL, et al. Required Immunoproteasome Subunit Inhibition Profile for Anti-Inflammatory Efficacy and Clinical Candidate KZR-616 ((2S,3R)-N-((S)-3-(Cyclopent-1-en-1-yl)-1-((R)-2-methyloxiran-2-yl)-1-oxopropan-2-yl)-3-hydroxy-3-(4-methoxyphenyl)-2-((S)-2-(2-morpholinoacetamido)propanamido)propanamide). *J Med Chem.* **2018**;61(24):11127–11143.
90. Kevin SP, McNaught CWO, Barry H, Isacson O, Jenner P. Failure of the ubiquitin–proteasome system in Parkinson's disease. *NAT REV.* **2001**;2:589–593. doi:10.1038/35086067
91. Das S, Ramakrishna S, Kim KS. Critical Roles Of Deubiquitinating Enzymes In The Nervous System And Neurodegenerative Disorders. *Mol Cells.* **2020**;43(3):203–214.
92. Gadhave K, Kumar P, Kapuganti S, Uversky V, Giri R. Unstructured biology of proteins from ubiquitin-proteasome system: roles in cancer and neurodegenerative diseases. *Biomolecules.* **2020**;10:796. doi:10.3390/biom10050796
93. Lee B-H, Lee MJ, Park S, et al. Enhancement of proteasome activity by a small-molecule inhibitor of USP14. *Nature.* **2010**;467(7312):179–184. doi:10.1038/nature09299
94. Nag DK, Finley D. A small-molecule inhibitor of deubiquitinating enzyme USP14 inhibits Dengue virus replication. *Virus Res.* **2012**;165(1):103–106. doi:10.1016/j.virusres.2012.01.009
95. Zhou H, Shao M, Guo B, et al. Tetramethylpyrazine analogue T-006 promotes the clearance of alpha-synuclein by enhancing proteasome activity in parkinson's disease models. *Neurotherapeutics.* **2019**;16(4):1225–1236.
96. Chen HY, Xu DP, Tan GL, et al. A potent multi-functional neuroprotective derivative of tetramethylpyrazine. *J Mol Neurosci.* **2015**;56(4):977–987. doi:10.1007/s12031-015-0566-x
97. Veggiani G, Gerpe MCR, Sidhu SS, Zhang W. Emerging drug development technologies targeting ubiquitination for cancer therapeutics. *Pharmacol Ther.* **2019**;199:139–154.
98. Fonović M, Bogoy M. Activity based probes as a tool for functional proteomic analysis of proteases. *Expert Rev Proteomics.* **2008**;5:721–730. doi:10.1586/14789450.5.5.721
99. Beksac G. The safety of bortezomib for the treatment of multiple myeloma. *Expert Opin Drug Saf.* **2018**;17:953–962. doi:10.1080/14740338.2018.1513487
100. Wertz IE, Wang X. From discovery to bedside: targeting the ubiquitin system. *Cell Chem Biol.* **2019**;26(2):156–177. doi:10.1016/j.chembiol.2018.10.022
101. Thibautaud TA, Smith DM, Practical A. Review of proteasome pharmacology. *Pharmacol Rev.* **2019**;71(2):170–197. doi:10.1124/pr.117.015370
102. Demo SD, Kirk CJ, Aujay MA, et al. Antitumor activity of PR-171, a novel irreversible inhibitor of the proteasome. *Cancer Res.* **2007**;67(13):6383–6391. doi:10.1158/0008-5472.CAN-06-4086
103. Correction: evaluation of the Proteasome Inhibitor MLN9708 in Preclinical Models of Human Cancer. *Cancer Res.* **2010**;70(9):3852–3853.
104. Roth P JR, Gorlia T, Dhermain F, et al. A phase III trial of marizomib in combination with standard temozolomide-based radiochemotherapy versus standard temozolomide-based radiochemotherapy alone in patients with newly diagnosed glioblastoma. *Neuro-Oncology.* **2019**;21:iii98. doi:10.1093/neuonc/noz126.359
105. Zhu H, Wang T, Xin Z, et al. An oral second-generation proteasome inhibitor oprozomib significantly inhibits lung cancer in a p53 independent manner in vitro. *Acta Biochim Biophys Sin (Shanghai).* **2019**;51(10):1034–1040. doi:10.1093/abbs/gmz093
106. Isono M, Sato A, Asano T, Okubo K, Asano T. Delanzomib Interacts with Ritonavir Synergistically to Cause Endoplasmic Reticulum Stress in Renal Cancer Cells. *Anticancer Res.* **2018**;38(6):3493–3500. doi:10.21873/anticancer.12620
107. Trivedi HL, Terasaki PI, Feroz A, et al. Abrogation of anti-HLA antibodies via proteasome inhibition. *Transplantation.* **2009**;87(10):1555–1561. doi:10.1097/TP.0b013e3181a4b91b
108. Williams AJ, Dave JR, Tortella FC. Neuroprotection with the proteasome inhibitor MLN519 in focal ischemic brain injury: relation to nuclear factor kappaB (NF-kappaB), inflammatory gene expression, and leukocyte infiltration. *Neurochem Int.* **2006**;49(2):106–112. doi:10.1016/j.neuint.2006.03.018
109. Shukla SK, Rafiq K. Proteasome biology and therapeutics in cardiac diseases. *Transl Res.* **2019**;205:64–76. doi:10.1016/j.trsl.2018.09.003
110. Wang S, Liu H, Zu X, et al. The ubiquitin-proteasome system is essential for the productive entry of Japanese encephalitis virus. *Virology.* **2016**;498:116–127. doi:10.1016/j.virol.2016.08.013
111. Vriend J, Reiter RJ. Melatonin, bone regulation and the ubiquitin-proteasome connection: A review. *Life Sci.* **2016**;145:152–160. doi:10.1016/j.lfs.2015.12.031
112. Huseby NE, Ravuri C, Moens U. The proteasome inhibitor lactacystin enhances GSH synthesis capacity by increased expression of antioxidant components in an Nrf2-independent, but p38 MAPK-dependent manner in rat colorectal carcinoma cells. *Free Radic Res.* **2016**;50(1):1–13. doi:10.3109/10715762.2015.1100730

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