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REVIEW

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.[1,2] 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.[3–5] 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).[2] 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.[6]

Proteasome, the prominent part of UPS, is a large protein complex containing multicatalytic protease subunits.[7–9] Studies have validated close connection between proteasome dysfunction and various diseases including cancer,[10,11] infectious diseases,[12] immune diseases[13] and neurodegenerative diseases,[14] 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)[15,16] and mantel cell lymphoma.[17] Besides, various candidates are evaluated in clinical trials for the

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treatment of malignancies[18] and autoimmune diseases.[19] 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.[20,21] The 28 protein subunits are arranged as a cylindrical stack of four rings with seven subunits each (α7-β7-β7-α7) to form a barrel-shaped structure.[22] Three of the seven β subunits (β1, β2 and β5) encode three distinct proteolytic activities: caspase-like activity (β1), trypsin-like activity (β2) and chymotrypsin-like activity (β5).[23] In vertebrates or in response to interferon (IFN)-γ or tumor necrosis factor (TNF)-α, the catalytic active β-subunits (β1, β2, and β5) are replaced by their inducible counterparts low molecular mass polypeptide 2 (LMP2, β1i), multicatalytic endopeptidase complex-like 1 (MECL-1, β2i) and low molecular mass polypeptide 7 (LMP7, β5i), respectively, thereby forming the immunoproteasome[24,25] (Figure 1).

To avoid uncontrolled protein degradation, access to the chamber of the core particle with proteolytic activities is well regulated.[21,26] 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[27–29] (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 endoplasmatic reticulum (ER) stress,[30] NF-κB inhibition,[31] cell cycle arrest and proapoptotic factors increase,[32] thus making this protein complex an important drug target for various diseases.[33–35]

Targeting Proteasome for Various Diseases

The application of proteasome inhibitors for the therapy of hematological malignancies has been validated.[36–38] Besides, recent studies also suggest that proteasome targeting is a potential strategy for parasite infectious and bacterial/fungal infections diseases,[39] 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.[40,41]

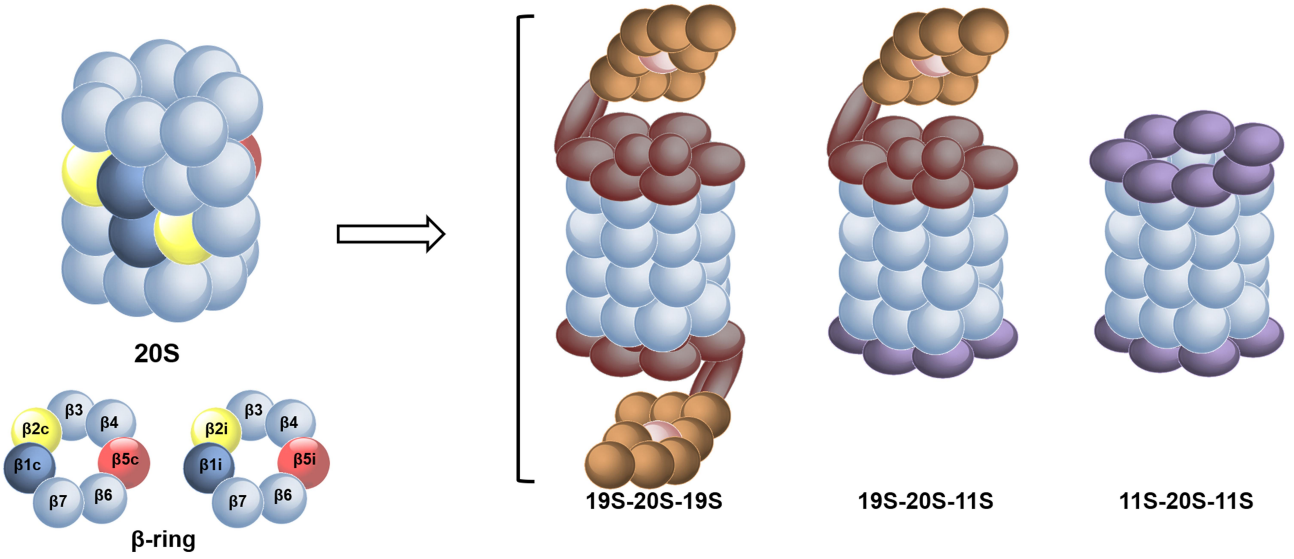


Figure 1 The 20S proteasome is comprised of four assembled rings, and the internal β-ring involves constitutive or immune-catalytic subunits. The 20S proteasome binds with 19S or 11S particle to form different proteasome assemblies.

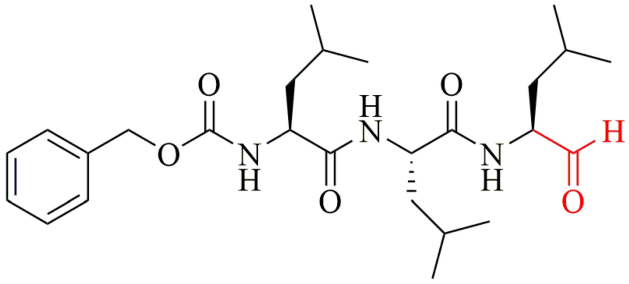
Parasite Infectious Diseases

The structure and function of pathogen genomes encode proteasomes are similar to the mammalian complex.[42] 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.[43–45] 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.[46] 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.[47] 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.[48] 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.[49,50]

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.[51] 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.[51] MG-132 could inhibit hemoglobin degradation, and it is most likely due to inhibition



MG-132

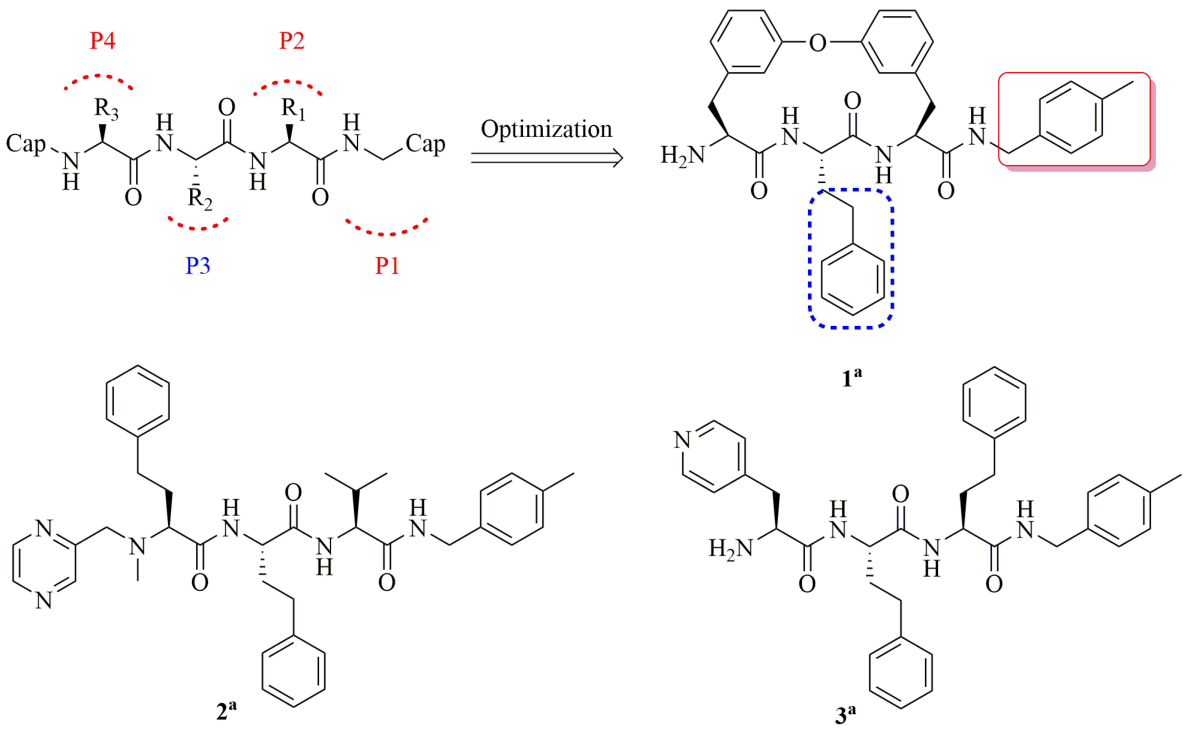
Figure 2 Structure of anti-malaria peptidyl aldehyde analogue MG-132.

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_{50}: 0.0476 μM) against PBMCs (IC_{50}: 10.8 μM) was achieved.[52]

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.[53] 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 *β5* 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 *β5* active subunit of *Plasmodium*.

Compounds **1, 2** and **3** (Figure 3) displayed potent activities against *P. falciparum* proteasome with EC_{50} values ranging from 0.0345 μM to 0.357 μM 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.[43] Although the structure of PR3 was highly



[a]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 μM and 28.8 nM, respectively. However, PR3 was not toxic for host HFF cells at the concentration of up to 50 μM.

Proteasome inhibitors have shown potent inhibitory activities against *P. falciparum* at all stages of its life cycle,[54–56] 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.[57]

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 *β5 P. falciparum* proteasome inhibitory activity but comparable *β2* inhibitory activity to LLL-vs Alternating the P3 position to Trp (WLL-vs) resulted in potent inhibitory activities against both *β2* and *β5* 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 *β2*-subunit of *P. falciparum* proteasome inhibitory activity and considerable selectivity.[57] 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 *β2 P. falciparum* proteasome, which was able to accommodate bulky side chains like Trp at the P1 and P3 positions, while the human *β2* 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} (μM)		EC_{50} (μM)		Selectivity[c]
	h20S[a]	Pf20S[a]	HFF[b]	Pf	
1	9.22	1.25	>50[c]	0.0345	>1450
2	1.97	0.0789	61	0.104	587
3	0.171	0.00924	68	0.357	189

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

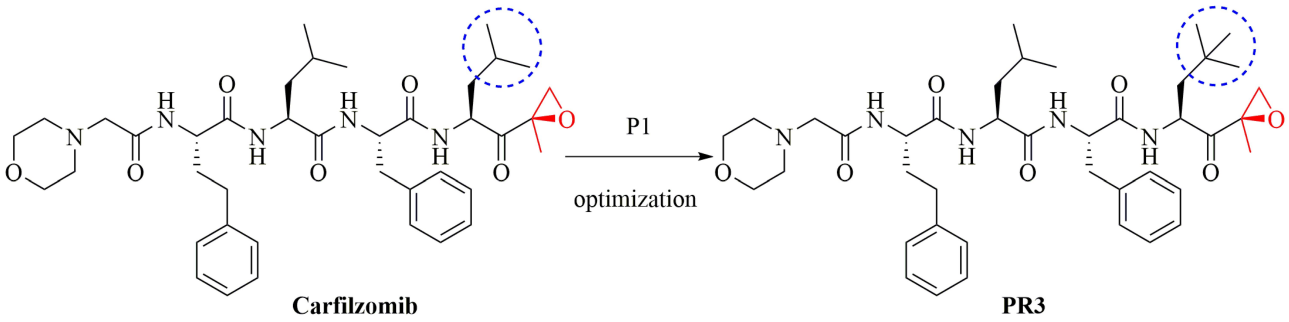


Figure 4 Structures of Carfilzomib and its derivative PR3.

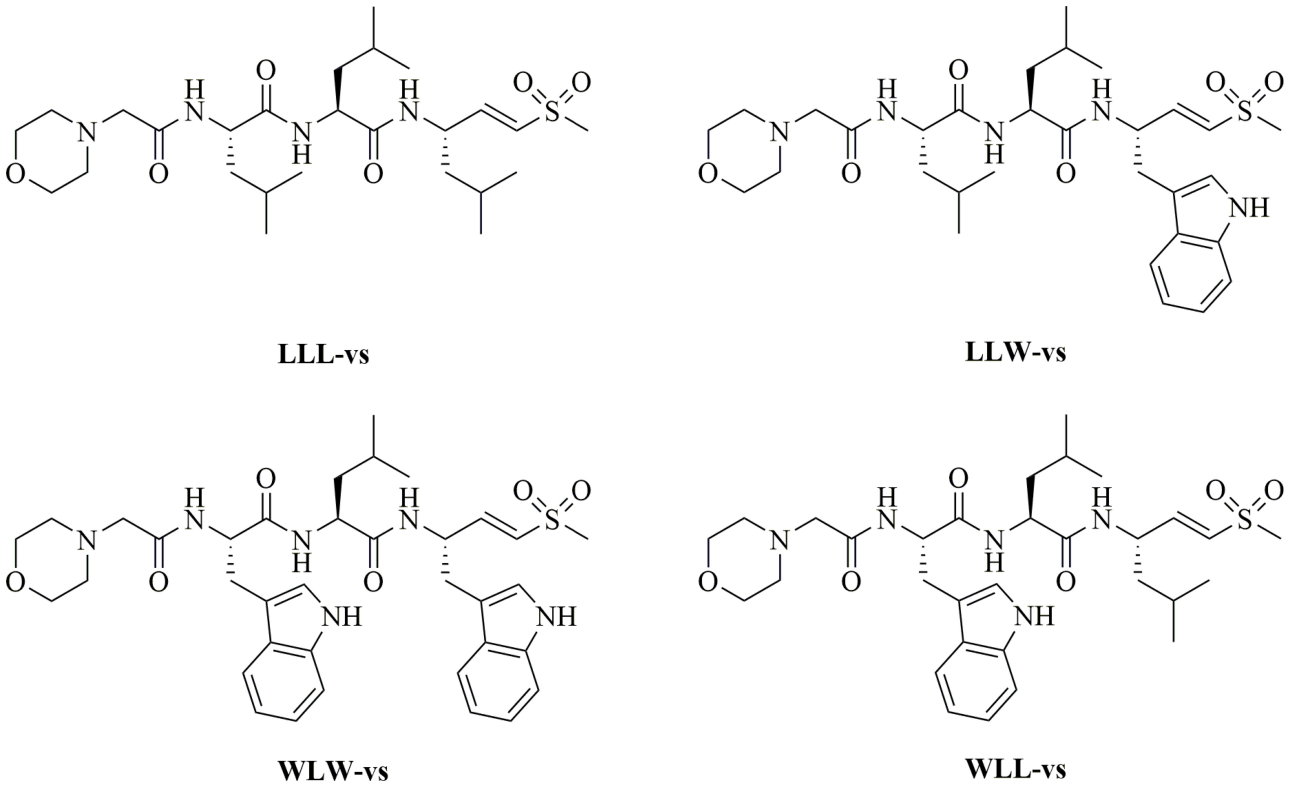


Figure 5 Vinyl sulfone derivatives LLL-vs, LLW-vs, WLW-vs and WLL-

A versatile class of peptidomimetic proteasome inhibitors with an asparagine ethylenediamines (AsnEDAs) scaffold was reported recently.[58] 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_{50} and EC_{50} Values of LLL-Vs, LLW-Vs, WLW-Vs and WLL-Vs in *P. falciparum*

Compound	Pf20S (IC_{50}, μM)			EC_{50}, μM		Selectivity[a]
	β1	*β2*	*β5*	HFF	*P. falciparum*	
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: [a]Selectivity: HFF/*P. falciparum*.

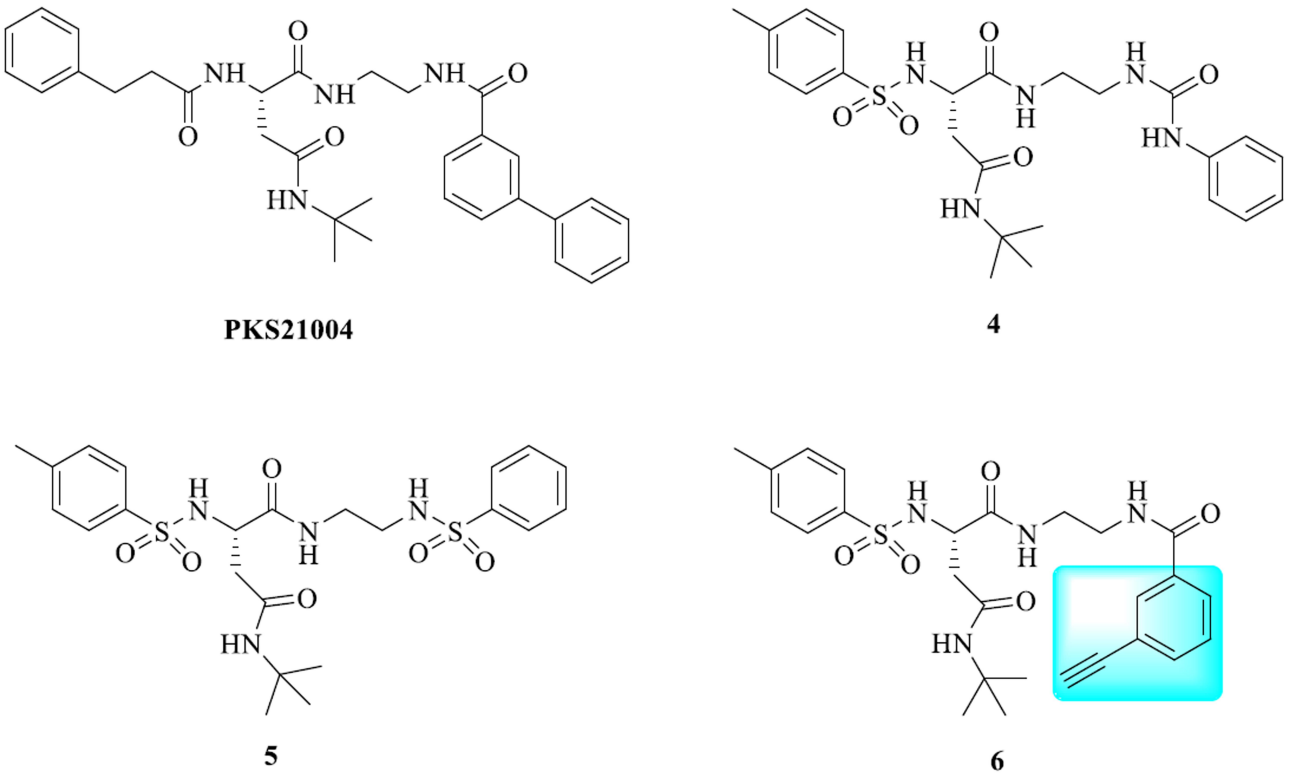


Figure 6 AsnEDA constructed peptidomimetic analogue PKS21004 and its derivatives.

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 μM and 15.135 μM, 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 *β*5c and *β*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 *α, β*-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* Proteasoe, Human *β*5i and *β*5c

Compound	IC_{50} (nM)			EC_{50} (nM)	
	β5i	β5c	Pf20S β5	Pf 3D7	HepG2
PKS21004	58	326	3.6	4.6	3,670
4	2090±29	>100,000	2576±326	>2770	>11,000
5	2892±1004	>100,000	15,135±4565	>2770	>11,000
6	112±11	430±130	4.7±1.4	3.1±0.1	>11,000

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

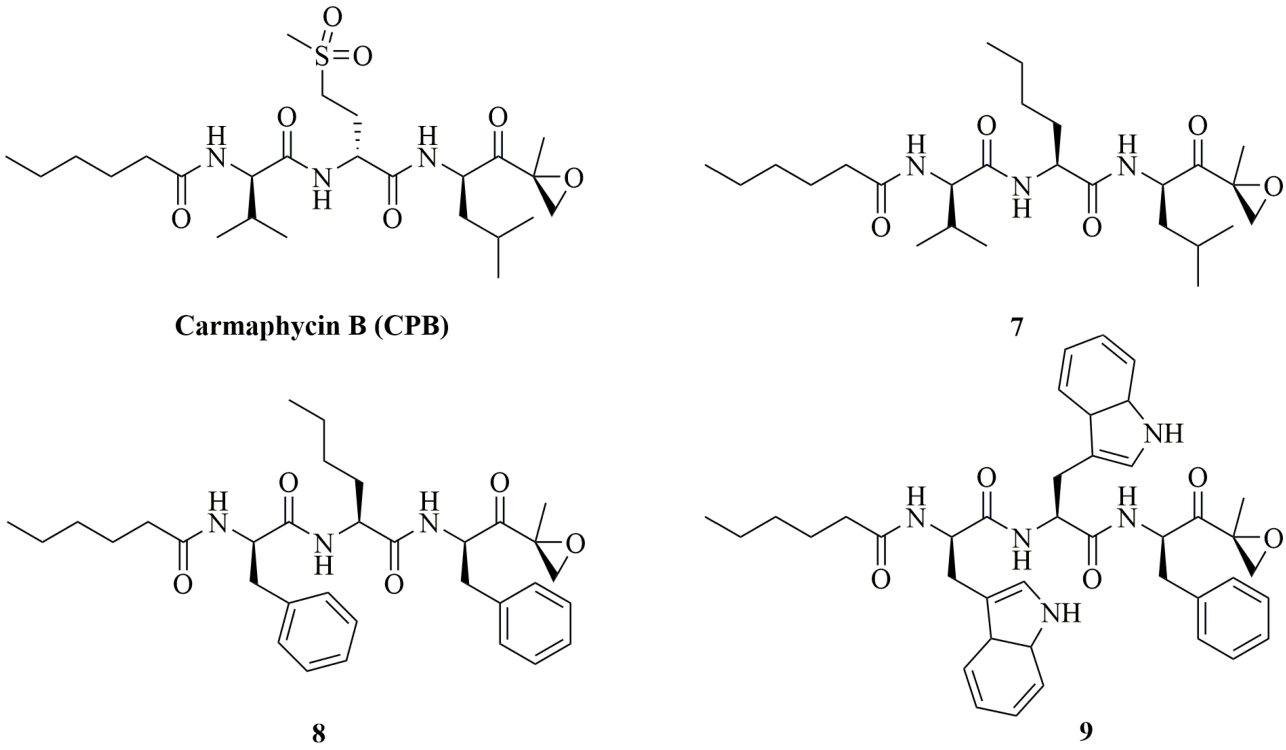


Figure 7 Anti-schistosomiasis peptidyl epoxyketone derivatives.

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.[59] However, Carmaphycin B was cytotoxic against HepG2 cell with a 24 h EC_{50} value of 12.6 nM.[60] 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)
	β5i	β5c	Sm20S β5	β2i	β2c	Sm20S[a] β2	
CPB	4.5	2.0	0.6	62.0	12.0	9.8	12.6
7	14.5	2.7	1.9	201	26.0	25.2	134
8	12.8	2.3	1.3	>500	85.2	25.5	30.8
9	5.3	3.8	5.4	>500	488	38.8	346

Abbreviation: Sm20S, *S. mansoni* proteasome.

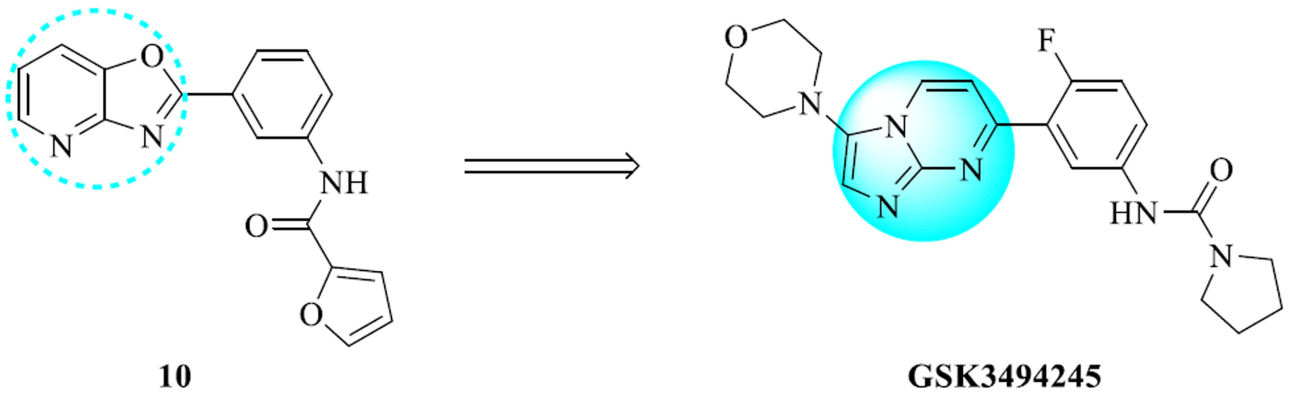


Figure 8 Optimization of GSK3494245 with anti-visceral leishmaniasis activity.

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 μM, which also displayed a favorable selectivity over mammalian cell growth inhibition (THP-1 cells, EC_{50} > 50 μM) but with poor in vitro metabolic stability owing to the rapid degradation (CL_{int} = 24 mL/min per gram).[61] 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 μM). However, this analogue showed lower potency against T. cruzi with EC_{50} of 1.6 μM. Furthermore, GSK3494245 could inhibit the *β*5 of the *L. donovani* proteasome in a dose-dependent manner with IC_{50} value of 0.16 μM but had no effect on *β*1 or *β*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 *β*5, and the site lies between the *β*4 and *β*5 subunits. The discovery exploited an induced cavity that is lined on one side by *β*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[62,63] *Mycobacterium tuberculosis* (Mtb) is the causative agent of tuberculosis, which is rare among bacterial pathogens in expressing a functional 20S proteasome.[64–69] Furthermore, the Mtb epidemic has been aggravated by the drug-resistant strains in recent years.[70] 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

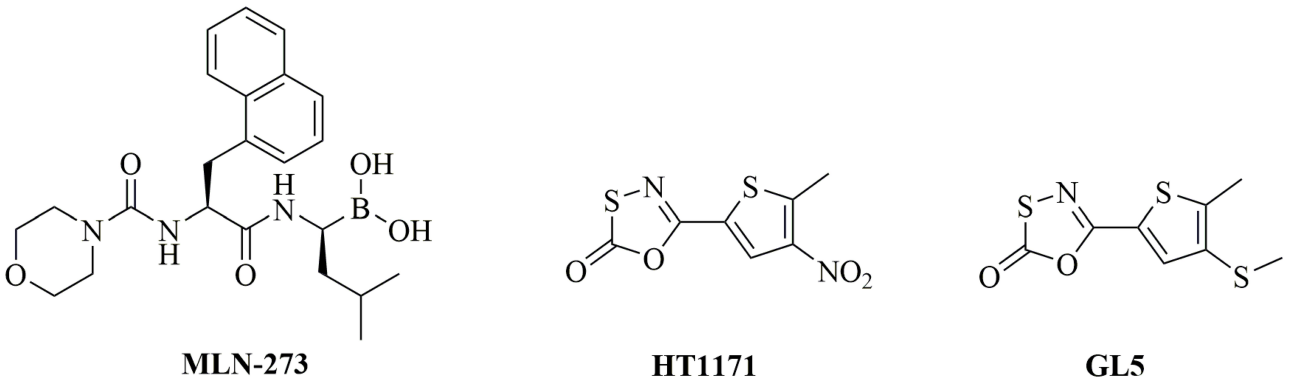


Figure 9 Structures of MLN-273, HT1171 and GL5 against Mtb proteasome.

Table 5 Kinetic Parameters of GL5 and HT1171

Compound	k_{obs}/[I], $M^{-1}s^{-1}$			
	Mtb 20SOG	h20S β5	h20S β2	h20S β1
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 IC_{50} value of 1.6 nM.[64,71] 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.[65]

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.[72–74] 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 μM 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.[76,77] 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 β5 and β5i.

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

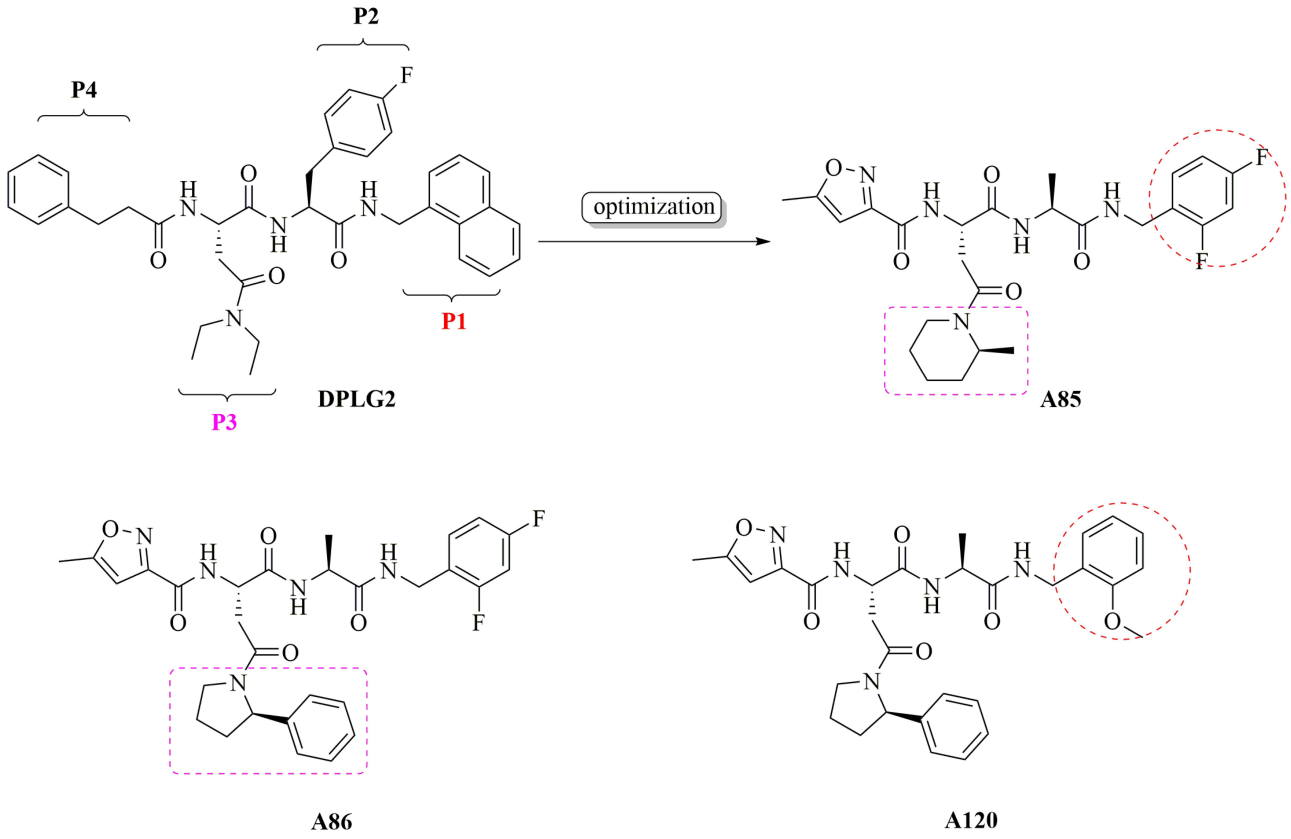


Figure 10 Asn amide containing peptidyl analogue DPLG2 and its derivatives.

Table 6 IC_{50} Values of A85 and Its Derivatives Against Mtb20S, Human β5i and β5c

Compound	IC_{50} (μM)			Selectivity Index[a]
	Mtb20S	i-h20S β5i	c-h20S β5c	
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: [a] Selectivity Index: (i-h20S ÷ Mtb)/(c-h20S ÷ Mtb).

have been reported.[78] A85 (Figure 10) was discovered starting from DPLG2 by an iterative, automated microfluidic system termed CyclOpsTM.[79–83] Compared to DPLG2, A85 showed potent activity over Mtb20S with an IC_{50} value of 7 nM, while the IC_{50} values against human β5c (3.7-fold) and human β5i (202-fold) were 26 nM and 1.412 μM, 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 β-strand between the compounds and the backbone atoms of Thr-21, Gly-47, and Ala-49 was formed.[78] 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 β5c with IC_{50} value of about 10 μM. Compounds **11, 12** and **13** (Figure 11) were derived from B1, and all the three compounds were much potent for Mtb20S with IC_{50} values of 25 nM, 8 nM and 13 nM, respectively. Moreover, the IC_{50} values of both β5c and β5i were more than 100 μM.[78]

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.[84] It's observed that the dysfunction of immunoproteasome leads to various immunological diseases and

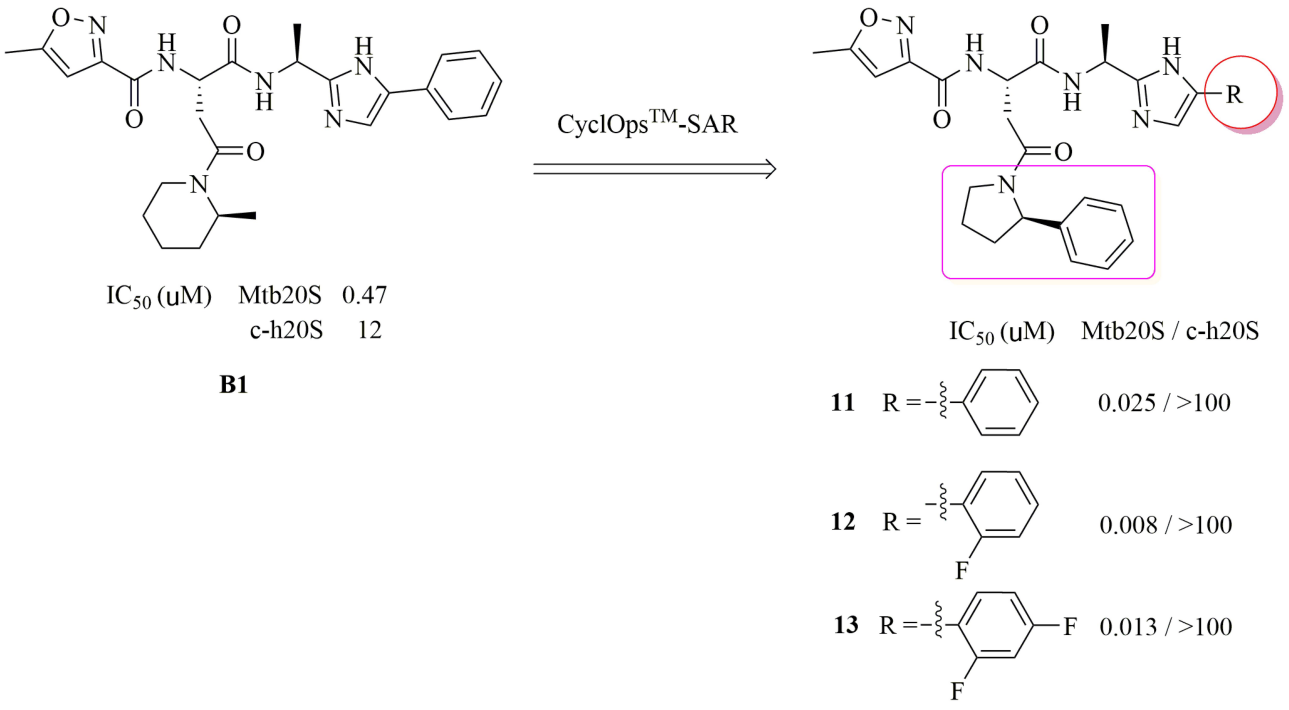


Figure 11 Peptidomimetic phenylimidazoles with Mtb20S inhibitory activities.

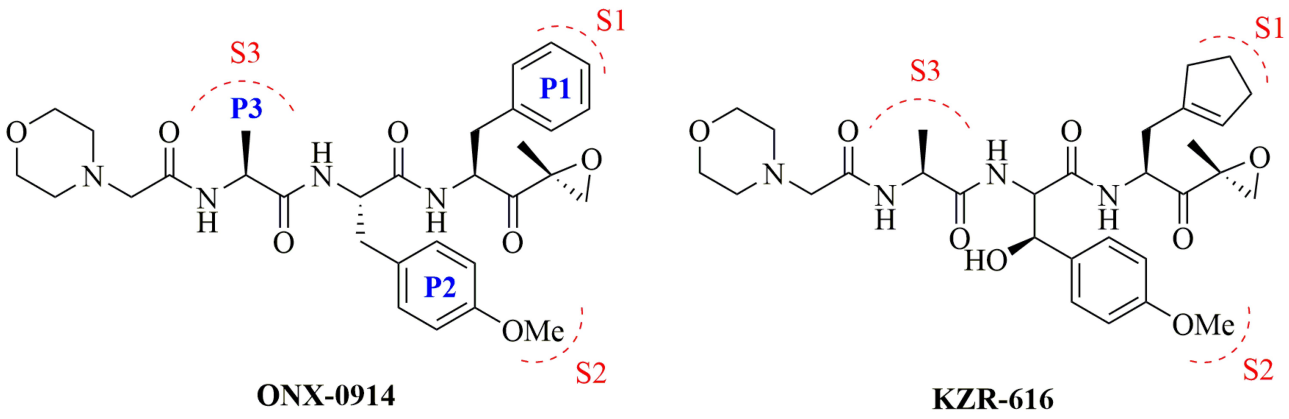


Figure 12 Immunoproteasome selective inhibitors ONX-0914 and KZR-616.

the upregulation may increase cytokine secretion which is relevant to autoimmune diseases.[85] 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.[86]

ONX-0914 (Figure 12) was the first selective epoxyketone-based peptidyl immunoproteasome inhibitor, which showed potent inhibitory activity for β5i and moderated activity against β5c with IC_{50} values of 5.7 nM and 54 nM, respectively. In addition, it also displayed moderate inhibitory activities against β1i (IC_{50}: 460 nM) and β2i (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.[87] Therefore, ONX-0914 has shown bright therapeutic prospect in the models of systemic lupus erythematosus, rheumatoid arthritis, multiple sclerosis, myasthenia gravis and etc.[88] 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 β 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 β1i and β5i simultaneously with IC_{50} values of 0.039 μM and 0.623 μM, 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),[89] 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 α-synuclein (α-syn) is the hallmark of PD.[90] 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.[91] 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.[92] IU1, a selective small-molecular inhibitor, prevents USP14 with IC_{50} value of 4–5 μM but failed to inhibit other DUBs.[93,94] 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.[95,96] It was reported that T-006 could

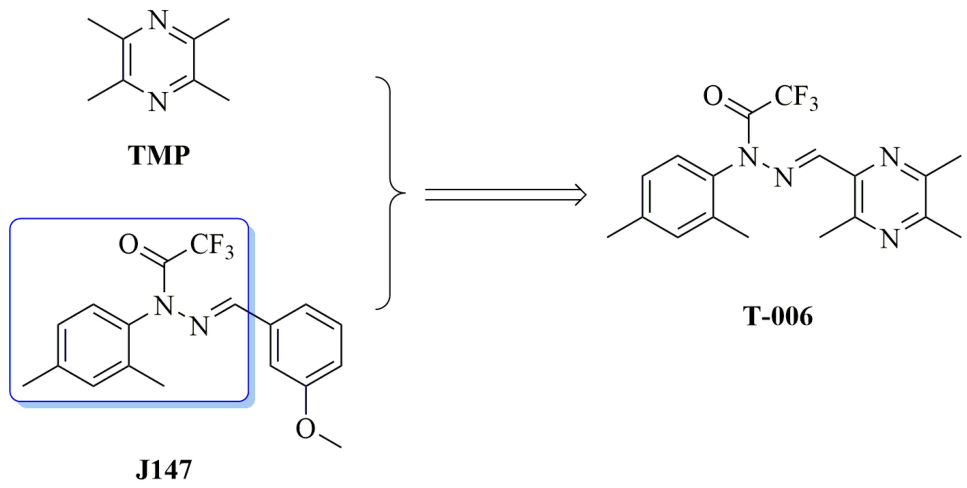


Figure 13 Combination of TMP and J147 to form proteasome activator T-006.

prevent glutamate-induced excitotoxicity in Cerebellar granule neurons (CGNs) by regulating the PI3K/AKT pathway. Besides, this analogue could selectively mutate α-syn via increasing β5i 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.[95]

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-κB, P53, cyclins and CDK, which are relevant to various signaling pathways.[33,97] 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 β1i (IC_{50}: 5.5 nM), β5c (IC_{50}: 7 nM) and β5i (IC_{50}: 3.3 nM) subunits.[98] However, there are restrictions to its clinical

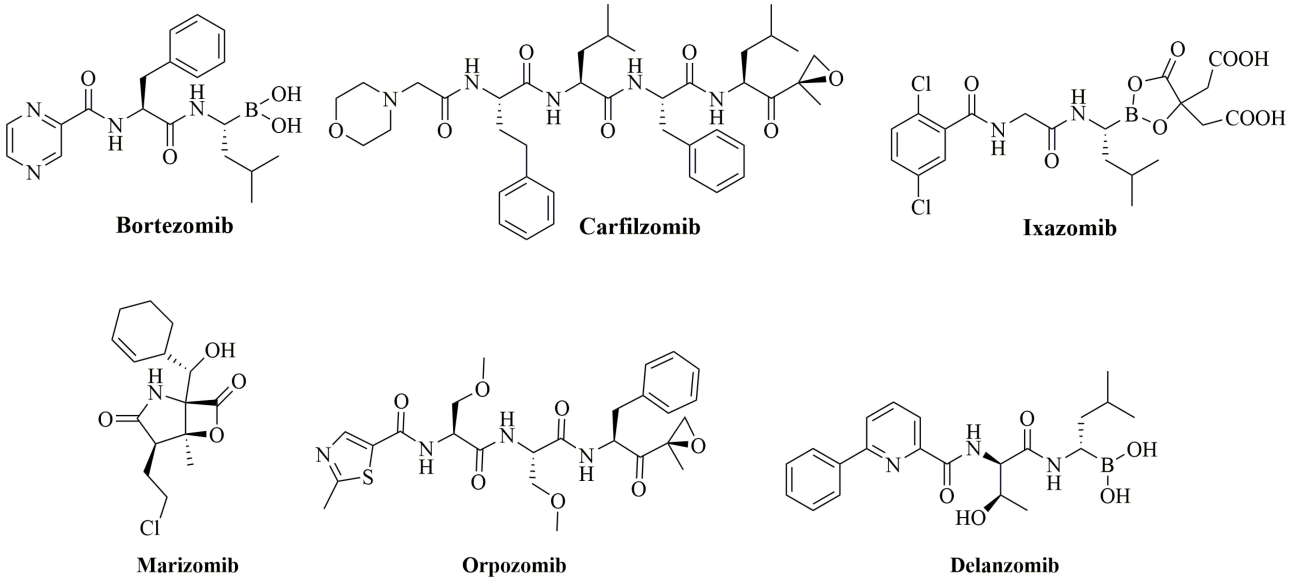


Figure 14 Representative proteasome inhibitors approved or under clinical trials.

application due to its severe toxicities and adverse effects including peripheral neuropathy.[99] 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.[100] In the clinical treatment of diseases, Bortezomib is often considered in combination with other therapies (like chemotherapy and radiotherapy).[101]

Carfilzomib (Figure 14) is an α', β'-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 β5c subunit, and results with sustained proteasome inhibition. Carfilzomib existed better activity for both β5c and β5i 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[102]. 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.[101]

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 β5c subunit with an IC_{50} value of 3.4 nM, while shows moderate inhibitory activities against β1c and β2c with IC_{50} values of 31 nM and 3.5 μM, respectively.[86,103] Despite structural similarity, Ixazomib demonstrates lower incidence of peripheral neuropathy and better efficacy in solid tumor models.[101]

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.[101] The β-lactone marizomib (Figure 14) isolated from the marine actinomycete *Salinispora tropica*, is in a Phase III trial combined with standard temozolomide-based radiochemotherapy.[104] Oprozomib (Figure 14) is an orally bioavailable epoxyketone proteasome inhibitor and a new oral formulation is being investigated in a phase I/II study.[105] Delanzomib (Figure 14) is a boronic acid, and Phase I clinical trials have been completed in patients with MM and solid tumors.[106]

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,[107] treatment of reperfusion injury after stroke,[108] therapeutics in cardiac diseases,[109] Japanese encephalitis,[110] bone disease,[111] oxidative stress[112] 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.

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