Journal of Peptide Science
J. Peptide Sci. **6**: 478–488 (2000)

Proteasome Inhibitors: From *In Vitro* Uses to Clinical Trials

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Received 5 June 2000
Accepted 24 June 2000

Abstract: Proteasomes are multicatalytic proteinase complexes which play a central role in intracellular protein degradation. They catalyse key events in cell cycle regulation and in the activation of the transcription factor NFκB. Proteasome inhibitors have been useful for the characterization of proteasome catalytic components and in the elucidation of proteasome functions in animal cells. Potent small peptide inhibitors of proteasomes also represent a novel approach to the treatment of inflammatory diseases (which involve activation of NFκB) and cancer. Such compounds have recently been shown to be effective in a variety of animal models, and at least one is currently in use in clinical trials. Copyright © 2000 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: intracellular proteolysis; ubiquitin; antigen presentation; cancer; cell cycle; lactacystin; epoxomicin; peptidyl boronic acid

The proteasome plays a central role in protein degradation in eukaryotic cells. It is involved in cell cycle control, apoptosis, transcriptional regulation, and is the most likely route by which the majority of cytoplasmic and nuclear proteins are turned over [1–3]. It is also responsible for the ER-associated degradation of integral membrane proteins and of misfolded proteins from the secretory pathway [4,5]. In higher eukaryotes, it plays an important role in antigen processing for presentation by the MHC class I pathway [6,7]. In by far the majority of cases, proteins are degraded by proteasomes to small peptides [2,8,9], although there are exceptions, including the processing of the NFκB p105 protein to form the 50 KDa subunit of the active transcription factor [10]. Degradation occurs by ubiquitin-dependent [3,11] and ubiquitin-independent pathways [12], and proteasome inhibitors have played a key role in the elucidation of proteasome functions in such pathways, especially in higher eukaryotes. In the ubiquitin-proteasome pathway, substrate proteins become marked for degradation by the attachment of multiubiquitin chains, which are recognized by regulatory complexes of the proteasome [3,11].

Proteasomes are widely distributed, being found in all types of eukaryotic cells, in archaebacteria, and at least in some eubacteria [2]. All proteasomes (20S proteasomes) are composed of 28 subunits arranged in a cylindrical structure composed of four heptameric rings. The subunits range from 22–30 kDa, giving a total molecular weight of around 700 kDa. The proteasome from the archaebacterium, *Thermoplasma acidophilum*, has been particularly useful for structural studies, because it is composed of just two different types of subunits, α and β. The α subunits form the outer rings, while the β subunits make up the inner rings. The overall architecture of the complex is similar to that of the molecular chaperones, such as GroEL [2]. X-ray crystallographic studies with the *Thermoplasma* proteasome revealed that the catalytic nucleophile was the N-terminal threonine residue of the beta subunit, which is located inside the hollow cylindrical structure [13]. Thus, the proteasome is a member of a family of N-terminal hydrolases [14], and was the first known example of a threonine protease. The related *E. coli* protease HslU/V has a proteolytic subunit, HslV, which has the N-terminal

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catalytic threonine residue and sequence similarities to proteasome subunits [15]. This forms a complex in association with HslU ATPase subunits.

The *Thermoplasma* proteasome β subunits are processed during assembly to remove an 8 amino acid propeptide and expose the catalytic threonine. Recombinant *Thermoplasma* proteasome α and β subunits produced in *E. coli* form active proteasomes, and site directed mutagenesis demonstrated that replacement of the threonine by a serine residue produced an active protease, only when expressed without the propeptide [16]. The catalytic mechanism is believed to be similar to that of a serine protease, but with a lysine (Lys33) and glutamate (Glu17) contributing to the proposed charge relay system [17]. Interestingly, the lysine is conserved in many of the eukaryotic proteasome beta subunits, but the Glu is replaced by a conserved Asp [18].

Yeast proteasomes contain seven different types of α subunits and seven different types of β subunits, arranged with one of each of the seven different subunits around the rings and two-fold symmetry [17].

Most of the eukaryotic proteasome β subunits are processed [2,18], but only three of them have a catalytic threonine, giving six catalytic sites in the mature proteasome (Figure 1). It is not clear why eukaryotic proteasomes have only the three different types of catalytic subunit, which are arranged in two clusters around the inner beta rings of the complex (Figure 1). Animal cells have three additional beta subunits which are induced by γ-interferon. These three inducible subunits, LMP2, LMP7 and MECL1, are closely related to the catalytic subunits, and can be incorporated in place of them to form immunoproteasomes which play a role in antigen processing [6,7]. The subunit arrangement in human proteasomes matches precisely that in the yeast [19], and it is assumed that in immunoproteasomes, γ-interferon inducible subunits replace their nearest homologue. Processing of the eukaryotic beta subunits accompanies proteasome assembly [20,21], although precise details of the assembly process differ in yeast [21,22] and higher eukaryotes [23,24]. Assembly occurs via half-molecules [23], involves a small chaperone protein [25], and results in autocatalytic removal of the beta subunit propeptides.

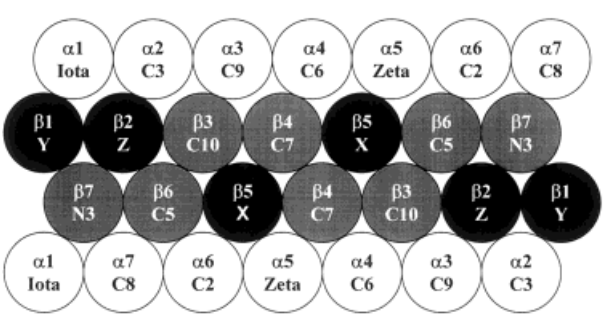

Figure 1 Arrangement of subunits in eukaryotic proteasomes. Eukaryotic proteasomes contain 14 different subunits, all of which are related. They are arranged in a stack of four heptameric rings. The nomenclature from the yeast crystal structure is α1–7 and β1–7 [17] and the homologous mammalian subunits (also shown) have the same arrangement [19]. Catalytic subunits are indicated in black (see Table 1).

MULTIPLE PEPTIDASE ACTIVITIES

The mammalian proteasome was first described as a multicatalytic endopeptidase complex, having several distinct peptidase activities, because protease inhibitors were found to have different effects depending upon the peptide substrate being used [[26], reviewed in [8]]. Three main activities are associated with all eukaryotic proteasomes. These have been described as the chymotrypsin-like, trypsin-like and peptidylglutamyl peptide hydrolase (PGPH) activities, based on their cleavage of bonds on the carboxyl side of basic, hydrophobic and acidic amino acids, respectively, although their precise specificity has still not been defined. Other activities have been ascribed to mammalian proteasomes, based on their synthetic peptide substrates and different sensitivities to a variety of inhibitors. These include a branched chain amino acid preferring (BrAAP) activity [27–29] and a small neutral amino acid preferring (SNAAP) activity [27], both of which seem to be distinct from the chymotrypsin-like activity assayed with substrates such as the commonly used Suc-LLVY-amidomethylcoumarin. Two peptidylglutamyl peptide hydrolase activities have been described [30], and conformational changes observed at high concentrations of the Cbz-LLE-β-naphthylamide substrate [31]. The existence of an acidic chymotrypsin-like activity has been demonstrated with bovine pituitary proteasomes [32]. The cleavage of most chymotrypsin-like substrates occurs optimally at neutral or slightly alkaline pH, while the hydrolysis of Boc-VEAL-β-naphthylamide was maximal at pH 5.5 [32].

Table 1 Catalytic Components in Proteasomes

Catalytic activity	Yeast catalytic subunits	Mammalian homologues
Chymotrypsin-like	β5 (PRE2)	*X*/MB1
		LMP7*
Trypsin-like	β2 (PUP1)	*Z*
		MECL1*
PGPH	β1 (PRE3)	*Y*/delta
		LMP2*

* The three γ-interferon-inducible subunits (LMP7, LMP2 and MECL1) replace subunits *X*, *Y* and *Z* in mammalian immunoproteasomes [6,7].
For the arrangement of catalytic subunits in the cylindrical structure, see Figure 1.

While catalytic activities can be ascribed to individual components of yeast proteasomes [33] (Table 1), the situation in mammalian proteasomes is more complex, with more catalytic activities described. The precise relationship between these measured activities and the known catalytic components of mammalian proteasomes is still not entirely clear (see below), possibly because of some overlap in substrate and inhibitor profile, but also because of allosteric effects around the beta rings [26,30,34]. The analysis is also complicated in animal cell proteasomes by the presence of the γ-interferon-inducible subunits, which become incorporated into immunoproteasomes [6,35]. These three inducible subunits all have the catalytic threonine residue which results in altered peptidase activities of immunoproteasomes [36–39], and the proportion of immunoproteasomes varies in different tissues and cell types.

As mentioned above, proteins are generally degraded by proteasomes to small peptides [8,9]. This requires access of proteins into the central proteolytic core of the proteasome [2]. From studies with yeast mutants defective in their proteolytic activity, there appears to be a heirachy among the different catalytic sites in contributing to protein degradation [40]. A 'bite and chew' mechanism has also been proposed, based on similar observations [34]. The chymotrypsin-like activity is the most important for effective protein degradation and, as discussed below, is also the main target for proteasome inhibition in animal cells. The 20S proteasome is probably not the physiologically relevant form of the proteasome in eukaryotic cells, where they associate with regulatory complexes [41–44]. A 19S regulatory complex binds to each end of the 20S proteasome to form 26S proteasomes which are required for ubiquitin-dependent degradation [3,11]. The 19S complexes are composed of about 20 different proteins, including six ATPase subunits [41], which probably play a role in protein unfolding and translocation.

INHIBITORS OF THE PROTEASOME

Understanding the structure, the novel catalytic mechanism and the complexity of mammalian proteasomes explains early difficulties in the classification of the protease, and in finding effective inhibitors of proteasome activities [reviewed in [8]]. Recent efforts in this area have produced a number of useful compounds (Table 2 and Figure 2). Of the mechanism based serine protease inhibitors, 3,4-dichloroisocoumarin [26] was found to be the most effective on proteasomes, but it does not inhibit all proteasome peptidase activities, and is not a very potent inhibitor compared with recently characterized compounds [30,46,47]. Peptidyl aldehydes, which form a reversible covalent adduct with the hydroxyl of the catalytic N-terminal threonine [13], were recognized as inhibitors from early work by Wilk and Orlowski [26]. Leupeptin (acetyl-Leu-Leu-argininal, Ac-LLR-H) is specific for trypsin-like activity [26,48], while compounds such as Cbz-LLF-H [49] and Cbz-LLL-H [50] are effective against the chymotrypsin-like activity. The Ki values of the best inhibitors are around 1–10 μM. Recently bivalent inhibitors have been designed, consisting of peptide aldehydes directed at chymotryptic and tryptic active sites on adjacent beta rings, with a polyoxyethylene spacer [53]. The potency of LLnL-H and RVR-H as bivalent inhibitors were each enhanced by two orders of magnitude compared with the pegylated monovalent inhibitor [53].

Peptidyl boronic acids [54–56] are much more potent inhibitors of the proteasome chymotrypsin-like activity than their corresponding peptide aldehydes. The trypsin-like and peptidylglutamyl peptide hydrolase activities are also affected, but to a lesser extent [56]. Modification of the peptidyl portion of the boronic acid inhibitors has allowed development of very potent proteasome inhibitors with some degree of specificity. Some of the best inhibitors, including compounds such as X-PheBoroLeu and X-Leu-Leu-BoroLeu, which have Ki values in the low nanomolar range [54–56], are relatively non-toxic and have potential therapeutic value (see below).

Irreversible inhibitors with appropriate peptidyl portions designed to target individual activities can be used to identify the catalytic components of proteasomes (see below), especially for mammalian proteasomes, where mutational analysis is not a straightforward approach. Peptidyl chloromethanes and peptidyl diazomethanes, although potentially suitable as covalent modifiers of catalytic residues, were only effective on proteasomes at rather high concentrations (around 100 μM) [58]. Peptidyl vinyl sulphones, which act as suicide substrates and irreversibly covalently modify the catalytic threonines, have proved to be more effective inhibitors [59].

Lactacystin is a naturally occurring non-peptide compound which is produced by actinomycetes. It was isolated originally by its ability to promote neurite outgrowth from cultured neurons [60] and, until recently, has been the most specific proteasome inhibitor known. Treatment of cells with [^{3}H]-lactacystin was first shown to target proteasomes, and to result in the covalent modification of the N-terminal threonine of the proteasome *X* subunit [61]. At higher concentrations, [^{3}H]lactacystin was later found to also modify the other catalytic subunits [62]. The active form of the compound is the β-lactone derivative, *clasto*-lactacystin β-lactone

Table 2 Proteasome Inhibitors

Inhibitor	Structure	Type of inhibition/k_{obs}/I or K_i value*	References
3,4-dichloro-isocoumarin	[45]	Irreversible k_{obs}/I 120 $M^{-1}s^{-1}$	[46–48]
Peptidyl aldehyde	Peptide-CHR-CHO	Reversible covalent modification of catalytic threonine [13]. K_i values of best inhibitors in the range 0.1-10 μM [49,51]. Include Ac-Leu-Leu-Nle-H (ALLN) Cbz-Ile-Glu(O-*t*Bu)Ala-Leu-H (PSI) Ac-Leu-Leu-Leu-H (MG132)	[49–52]
Bivalent peptidyl aldehydes	CO–Leu–Leu–Nle–H | (PEG)n | CO–Leu–Leu–Nle–H	IC50 17 nM compared to 1.8 μM for monovalent inhibitor	[53]
Peptidyl boronic acid	Peptide-CHR-$B(OH)_2$	Best inhibitors have K_i values in the low nM range	[54–56]
Peptidyl glyoxals	Peptide-CHR-CO-COOH	E.g. BzLeu-Leu-Tyr- or BzLeu-Leu-Leu- derivatives. Reversible covalent modifiers. K_i, approximately 3 nM	[57]
Peptidyl chloromethanes and diazomethanes	Peptide-CHR-CO-CH_2Cl	Irreversible, low k_{obs}/I, e.g. 1–10 $M^{-1}s^{-1}$	[58]
Peptidyl vinyl sulphones	Peptide-CHR-CH = CH-SO_2-CH_3	Irreversible inhibitors nitrophenylacetyl-Leu-Leu-Leu-vinyl sulphone (NLVS) best for chymotrypsin-like (k_{obs}/I 13 500 $M^{-1}s^{-1}$), Leu-leu-Leu-Leu-vinylsulphone best for trypsin-like activity (k_{obs}/I 1500 $M^{-1}s^{-1}$)	[59]
Lactacystin, *clasto*-lactacystin β-lactone	See Figure 2	Irreversible inhibitor k_{obs}/I, approximately 3000 $M^{-1}s^{-1}$ for 20S proteasomes, 8000 $M^{-1}s^{-1}$ for 26S proteasomes, the active form is *clasto*-lactacystin β-lactone	[60–64]
Epoxomicin	See Figure 2	Irreversible inhibitor k_{obs}/I 35 400 $M^{-1}s^{-1}$	[65]
Aclacinomycin A		Non-competitive inhibitor	[66]
Gliotoxin		Non-competitive inhibitor	[67]

* Chymotrypsin-like activity assayed with suc-LLVY-amidomethylcoumarin as substrate. R = amino acid side chain.

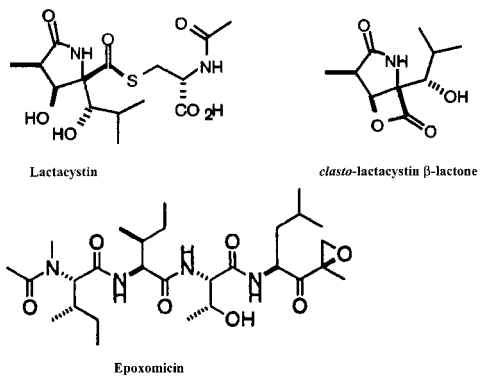

Figure 2 Structures of proteasome inhibitors lactacystin and epoxomicin. Lactacystin, which hydrolyses in aqueous solution to *clasto*-lactacystin β-lactone, and epoxomicin are naturally occurring compounds isolated for their ability to inhibit the cell cycle and for anti-tumour activity, respectively. Both are potent irreversible inhibitors of proteasomes (see Table 2).

[63]. Lactacystin spontaneously hydrolyses to the lactone in aqueous solutions. Another naturally occurring compound, epoxomicin, which has known anti-tumour activity, is an even more potent inhibitor of proteasomes than lactacystin, and may be more specific [65]. Like lactacystin, epoxomicin also preferentially targets the chymotrypsin-like activity [65]. The measured k_{obs}/I was 35400 $M^{-1}s^{-1}$ compared with 8530 $M^{-1}s^{-1}$ and 6790 $M^{-1}s^{-1}$, respectively, for lactacystin and nitrophenylacetyl-Leu-Leu-Leu vinyl sulphone (NLVS) [65]. Trypsin-like and PGPH activities are also inactivated by epoxomicin, but much more slowly. Other non-peptide inhibitors of proteasomes include another anti-tumour agent, aclacinomycin A, and the fungal metabolite, gliotoxin (Table 2).

IDENTIFICATION OF CATALYTIC COMPONENTS ASSOCIATED WITH DISTINCT PEPTIDASE ACTIVITIES

The use of radiolabelled irreversible inhibitors of proteasome peptidase activities has allowed analysis of the relationship between measured peptidase activities and catalytic components. Analysis of radiolabelled subunits (Table 3) has shown that [^{3}H]AAF-CH2Cl, [^{3}H]lactacystin and [^{125}I]NLVS (all of which inhibit the chymotrypsin-like activity) all label the *X* and LMP7 subunits. These are the two mammalian homologues of the yeast PRE2 subunit (Table 1), which is known from mutational analysis to be responsible for chymotrypsin-like activity [33]. However, not all of the assignments based on results of labelling studies are so clear and, in some cases, additional subunits are labelled (Table 3), suggesting some possible overlap in reactivity of the different catalytic components. There is, therefore, not a simple model to precisely relate the distinct kinetic activities of mammalian proteasomes with the known catalytic components. As for the yeast proteasome, it is also not clear what the function of the non-catalytic beta subunits is. The C7 component, which lies adjacent to the *X* subunit which catalyses chymotrypsin-like activity in both β subunit rings (Figure 1), was labelled with peptidyl chloromethane inhibitors, as were the catalytic subunits themselves [68].

INHIBITION OF PROTEASOME FUNCTIONS IN CELLS

One of the major uses of proteasome inhibitors has been to establish the many functions of proteasomes in animal cells. Where measured the 26S proteasomes, which play a major role in intracellular protein degradation, have been found to have similar χ values to 20S proteasomes [10,56], and many of the cell permeable proteasome inhibitors are effective in cells (Table 4). Proteasome inhibitors have been used extensively to demonstrate the involvement of the proteasome or ubiquitin-proteasome pathway in the degradation of many diverse proteins in many different cell types. Substrates include short-lived proteins and some long-lived proteins [51], and ER-associated proteins, such as the cystic fibrosis transmembrane regulator (CFTR) [71,72]. Proteasome inhibitors also inhibit antigen processing [51,73]. Proteasomes (immunoproteasomes) are responsible for the generation of most peptides presented on MHC class I molecules. However, several studies have shown that proteasome inhibition does not completely block the presentation of class I restricted epitopes [77–79], and it seems that another large protease can substitute for immunoproteasomes in antigen processing [77,78]. It is not clear yet whether the different functional forms of proteasomes which have different subcellular distributions [80] can be selectively inhibited.

The specificity of the inhibitors is clearly a concern when inhibitors are being used as evidence for proteasome involvement in a cellular function. Until recently, lactacystin was believed to be specific for proteasomes, and has been used very widely in

studies of proteasome function in animal cells. However, other proteases, such as cathepsin A and the tripeptidylpeptidase II, have also been found to be inhibited by lactacystin [76,77]. Care must also be taken because inhibition of proteasomes can often cause apoptosis [81–83], depending on the cell type.

THERAPEUTIC APPLICATIONS OF PROTEASOME INHIBITORS

Proteasome inhibitors have potential therapeutic uses in two main areas. The first is in the treatment of inflammatory diseases. Because of the critical function of proteasomes in the activation of NFκB in

Table 3 Assignment of Peptidase Activities to Mammalian Catalytic β-Subunits

Peptidase activity	Assigned β-subunits	Basis for assignment
Chymotrypsin-like	LMP7, *X*/MB1 (LMP2)	(a) Lactacystin is a much more effective inhibitor of the chymotrypsin-like activity than the trypsin-like or PGPH activities. [^{3}H] Lactacystin preferentially modified the *X*/MB1 subunit [61] (b) [^{3}H]acetyl-Ala-Ala-Phe-CH_2Cl primarily inhibits the chymotrypsin-like activity of 20S and 26S proteasomes. This inhibitor predominately labelled subunits *X*/MB1 and LMP7 [68]. C7 (β4) was also labelled (c) Studies using [^{14}C]dichloroisocoumarin (DCI) and peptidyl aldehydes suggested that the chymotrypsin-like activity was catalysed by *X*/MB1 and LMP2 subunits [69] (d) [^{125}I]NLVS modified LMP7 and *X*/MB1 subunits to a much greater extent than [^{125}*I*]-Tyr-Leu-Leu-Leu-VS. NLVS displayed a nine times higher k_{obs}/I value for the chymotrypsin-like activity compared to Tyr-Leu-Leu-Leu-VS [70]
Trypsin-like	MECL1, *Z*	(a) [^{125}*I*]Y-Leu-Leu-Leu-VS modified MECL1 and *Z* subunits to a much greater extent than [^{125}I]NLVS. Y-Leu-Leu-Leu-VS displayed a 56 times higher k_{obs}/I value for the trypsin-like activity compared to NLVS [70] (b) The more effective trypsin-like inhibitors Leu-Leu-Leu-Leu-VS and Bz-Phe-Leu-Leu-Leu-VS, competed with [^{125}I]Tyr-Leu-Leu-Leu-VS for binding to MECL1 and *Z* subunits, to a greater extent than Tyr-Leu-Leu-Leu-VS or NLVS [70] (c) [^{125}I]Tyr-Gly-Arg-CH_2Cl, which inhibited trypsin-like, as well as chymotrypsin-like activity, labelled C7 and to a much lesser extent, MB1(*X*), and LMP7 [68]
PGPH (LLE1, high affinity)	LMP2, Y/δ	(a) [^{125}I]Gly-Leu-Leu-Leu-VS-PhOH exclusively labelled the LMP2 and Y/δ subunits. This inhibitor displayed relatively low k_{obs}/I values for the chymotrypsin-like and trypsin-like activities [70] (b) NLVS displayed a similar k_{obs}/I value to Gly-Leu-Leu-Leu-VS-PhOH for the PGPH activity. [^{125}I]NLVS also labelled the LMP2 subunit to a similar level as [^{125}I]Gly-Leu-Leu-Leu-VS-PhOH [70]
PGPH (LLE2, low affinity)	Y/δ	(a) Studies using [^{14}C]DCI and peptidyl aldehydes suggested that the PGPH activity measured with 0.64 mM Cbz-Leu-Leu-Glu-NAP was catalysed by Y/δ [69]
BrAAP (branched chain amino acid preferring)	(a) LMP7	(a) Studies using [^{14}C]DCI and peptidyl aldehydes suggested that the BrAAP activity was catalysed by the LMP7 subunit [69]
	(b) LMP7, *X*/MB1, LMP2, Y/δ	(b) Studies using lactacystin and peptidyl aldehydes suggested that the BrAAP activity was catalysed by the active sites responsible for the chymotrypsin-like and PGPH activities [28]
SNAAP (small neutral amino acid preferring)	–	–
Acidic chymotrypsin-like	–	–

Table 4 Proteasome Inhibitors Commonly Used to Inhibit Proteasomes in Cultured Cells

Inhibitor	Comments/references
MG132*	[10]
(Ac-Leu-Leu-Leu-H)	Also inhibits other proteases?
ALLN* (calpain inhibitor 1)	[51,71–74]
(Ac-Leu-Leu-Nle-H)	Also inhibits calpain 1
PSI	[52]
(Cbz-Ile-Glu(O-*t*Bu)Ala-Leu-H)	Effect on other proteases?
Peptidyl boronic acids	[56,75]
	Some can also inhibit other protease(s) [56]
Lactacystin* *clasto*-lactacystin β-lactone (see Figure 2)	Irreversible. Predominantly inhibits chymotrypsin-like activity at lower concentrations. Also inhibits cathepsin A [76] and tripeptidyl peptidase II [77].
NLVS (Nitrophenyacetyl-Leu-Leu-Leu-vinyl sulphone)	Irreversible. Probably also inhibits cysteine proteases [59,70]
Epoxomicin (see Figure 2)	Irreversible. So far seems specific [65]

* There are numerous papers in which several of these inhibitors have been used to investigate proteasome-mediated intracellular protein degradation (concentrations used often 10–100 μM).

Table 5 Effective Proteasome Inhibitors in Animal Models of Cancer, Autoimmune and Inflammatory Diseases

Proteasome inhibitor	Animal model	References
Cbz-Leu-Leu-Phe-CHO	Murine model of human Burkitt's lymphoma	[84]
Epoxomicin	Murine B16 melanoma tumours Inflammation in murine ear edema	[85,65]
Peptidyl boronic acid	• Asthma models-inflammation in	[86]
PS519	(a) allergen-induced pulmonary eosinophilia in rats	
	(b) mouse delayed-type hypersensitivity model	
	• Relapsing experimental autoimmune encephalomyelitis	[87]
Peptidyl boronic acid PS341 (2-pyrazinylcarbonylPheBoroLeu)	Streptococcal cell wall induced arthritis	[88]
	PC3 murine tumour model	[89]
PSI (Cbz-Ile-Glu(O-tBu)Ala-Leu-H)	Sepsis induced muscle proteolysis	[90]
Lactacystin	Cachexia (mouse model)	[91]

animal cells, proteasome inhibitors may be useful for the treatment of inflammatory diseases, such as asthma and arthritis. NFκB is required for the regulation of many genes involved in the inflammatory response. Proteasome inhibitors can have toxic effects. The fungal metabolite gliotoxin, which targets the proteasome [67], has toxic effects, such as suppression of antigen processing, induction of macrophage apoptosis and inhibition of NFκB activation. There are potential toxic side effects of proteasome inhibitors, either a result of unwanted effects on other functions of proteasomes themselves, or of inhibition of other proteases or non-specific effects. The non-proteasome effects can hopefully be minimized by using high potency proteasome inhibitors, which are effective at low doses, and by optimizing treatment by careful administration of appropriate doses. Trials of several compounds using a variety of animal models (Table 5) have yielded some encouraging results, especially those with high potency peptidyl boronic acid inhibitors [86].

The second area of potential therapeutic value is cancer. The discovery that the proteasome is a cellular target for inhibitors of cell division (lactacystin) and for anti-tumour compounds, such as epoxomicin, suggested that proteasome inhibitors may provide a novel approach for the treatment of cancer. The observed pro-apoptotic effect of proteasome inhibitors in certain cells may be

advantageous for treatment and, remarkably, several groups have found that proteasome inhibitors induce apoptosis of transformed cells, but not of normal cells [83,84]. A number of compounds have been tested in animal model systems. Suitable peptidyl aldehyde and peptidyl boronic acid inhibitors cause early tumour regression, and delay progression in more advanced tumours [65,84,89].

CONCLUSIONS AND FUTURE PROSPECTS

Proteasomes are involved in a wide range of cellular functions, and it is not straightforward to predict the effects of proteasome inhibitors. Already, some highly potent and selective inhibitors have been found for the proteasome, and significant advances made in establishing their potential therapeutic uses. They provide an exciting new approach for the treatment of tumours and various inflammatory diseases, although not enough is known yet to predict their likely effectiveness in any new model system. As proteasome inhibitors can be toxic to cells, compounds may only be effective therapeutic agents within a narrow range. Nevertheless, proteasome inhibitors can reach their target when administered to animals, they have recently been shown to be effective in a variety of disease models, and clinical trials are currently underway [91].

Acknowledgements

AJR was the recipient of a Wellcome Trust University Award and RCG was the recipient of a Biotechnology and Biological Sciences Research Council studentship.

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