

P'-EXTENDED α-KETOAMIDE INHIBITORS OF PROTEASOME

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Abstract. A series of potent P'-extended α-ketoamide inhibitors of chymotrypsin-like activity of proteasome is described. © 1999 Elsevier Science Ltd. All rights reserved.

Introduction. The proteasome is an eukaryotic cytoplasmic proteinase complex that plays a major role in cellular pathways for the breakdown and processing of proteins to peptides and amino acids.¹ Proteasome has been shown to have multiple catalytic activities that, collectively, are capable of cleaving most peptide bonds. Increased levels of this enzyme and subsequent protein breakdown have been implicated in many disease states including inflammation and cancer. Recently, we reported potent, selective, and novel dipeptide aldehyde, boronic ester, and α -ketoamide inhibitors (1-2) of the chymotrypsin-like activity of the proteasome complex.^{2,3}

Figure 1

$$R(CH_2)_8$$

$$0$$

$$H$$

$$0$$

$$1 R = CN X = CHO, COCONHEt, B$$

$$0$$

$$2 R = N$$

$$NHNO_2$$

$$X = CHO$$

As a part of our continuing effort to develop novel and potent inhibitors of proteasome, we initiated a program to probe the importance of the P'-sites on the chymotrypsin-like inhibitory activity of the α -ketoamide class of inhibitors. This *Letter* discloses our preliminary observation.

Chemistry. The repesentative target compound 16 (Table 1) was assembled from four building blocks, namely 3, 4, 5, and 6 (Scheme 1). The synthesis of 3, as a racemate, had previously been disclosed by us. ^{2a} Compound 4 was commercially available (NovaBiochem, San Diego, CA). Compound 5 was synthesized following a general procedure of Harbeson et al. ⁴ The synthesis of compound 6 is depicted in Scheme 1. Thus, 1,2-ethylenediamine (7) was selectively monoprotected (with a *t*-Boc group) to give 8 that was sulfonylated to generate 9. Deprotection of the *t*-Boc group in 9 generated 6. Scheme 2 depicts the synthesis of the target compound 16. Thus, compounds 5 and 6 were coupled to generate 10 that was deprotected to give 11. The reaction between 4 and 11 yielded 12 that was subsequently deprotected to give 13. Coupling of 3 and 13 generated 14 that underwent Dess-Martin oxidation⁵ to produce the target compound 16.

Biology and Discussion. Methods for partial purification of the enzyme and determination of the biological activities of inhibitors were described.² The inhibitory activities of compounds 15–20 are shown in Table 1. The parent ethyl- α -ketoamide (15) was a good inhibitor. Replacement of one of the terminal hydrogen atoms in

compound 15 by a phenylsulfonamido group generated compound 16, with a tenfold enhancement of potency, revealing the importance of a H-bond donor/acceptor group in the region.

$$H_2N$$
 NH_2
 H_2N
 H_2N
 H_2
 H_2N
 H_2
 H_2
 H_2
 H_2
 H_2
 H_2
 H_2
 H_2
 H_3
 H_3

Replacement of the phenylsulfonyl group in compound 16 by a *t*-butoxycarbonyl (*t*-Boc) group resulted in twofold potency reduction (cf. 17 vs 16). However, deprotection of the above-mentioned *t*-Boc group in compound 17 to generate a terminal amine (as HCl salt) resulted in ca. 30-fold loss in potency (cf. 18 vs 17). Thus, it appears that the enzyme does not tolerate a charge in the region. Replacement of Arg-(NO₂) at the P₂ site by Arg-(t-Boc), (compound 19) or Arg (HCl salt) (20) also diminished the activity.

Table 1. Inhibitory Activity of the P₂-Arginine Derivatives^a

$N-(CH_2)_8$ $N+$ $N+$ $N+$ $N+$ $N+$ $N+$ $N+$ $N+$					
Compound	$\mathbf{R}_{_{1}}$	\mathbb{R}_2	\mathbb{R}_3	K_i nM	
15	Н	NO_2	H	12	
16	Н	NO_2	NHSO ₂ Ph	1.1	
17	Н	NO_2	NHCOOC(CH ₃) ₃	2.2	
18	Н	NO_2	NH ₂ (HCl salt)	67	
19	COOC(CH ₃) ₃	COOC(CH ₃) ₃	NHSO ₂ Ph	34% @ 100 nM	
20	Н	H (HCl salt)	NHSO ₂ Ph	31% @ 100 nM	

^a Values for IC₅₀ were determined and converted to K_i values using the expression $K_i = \text{IC}_{50}/(1+\text{S}/K_m)$, assuming a competitive mechanism of inhibition. $n \ge 3$ in all cases. Replicate determinations of K_i agree within 25%.

To explore the importance of the P_2 and P_3 (pseudo)-sites in this class of inhibitors, we generated a series of truncated analogs of compound 16 (Table 2). As shown, the complete lack of P_2 - P_3 moiety abolished the activity (cf. 21 and 22 vs 16). Incorporation of Cbz-Arg(NO₂) as the P_2 moiety brought back partial activity (cf. 23 vs 21 or 22). However, note that compound 23 was >500-times less potent than compound 16, revealing the energetically beneficial binding offered by the lipophilic pseudo- P_3 residue of 16.

To investigate the effect of the related amino acid Lys at the P_2 position, we also replaced $Arg(NO_2)$ in compound 16 to generate a set of compounds (Table 3). While compound 24, containing *t*-Boc protected lysine residue at P_2 , displayed reduced activity, pyrazinecarbonyl and methylsulfonyl protected lysine derivatives (compounds 25 and 26) were moderately active. Compound 27, the most potent compound of the series, contained an unprotected lysine (HCl salt). However, note that the compound 16 is 20 times more potent than compound 27; thus, $Arg(NO_2)$ remains the choice of residue at P_2 . It should be noted that compound 16 was uniformly unable to inhibit the trypsin-like activity of the enzyme complex at concentrations up to 1 μ M. It was also found to be >150-fold selective for the chymotrypsin-like activity of proteasome in comparison to calpain I, a cysteine protease sensitive to inhibition by α -ketoamides.

Conclusion. We have described in this *Letter*, P'-extended potent α -ketoamide inhibitors of the chymotrypsin-like activity of the proteasome complex. Compound 16 (K_i 1.1 nM) emerged as the most potent inhibitor of the series and provided the launching pad for further exploration of this class of inhibitors.

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Table 2. Inhibitory Activity of the Truncated Analogs of Compound 16

Compound	R	Activity
21	COOC(CH ₃) ₃	14% @ 10 μΜ
22	H (HCl salt)	12% @ 10 μΜ
23	Cbz-Arg(NO ₂)	34% @ 1 μM

Table 3. Inhibitory Activity of the P2-Lysine Analogs of Compound 16

$$\begin{array}{c|c} O & Ph \\ N-(CH_2)_8 & NH & NH \\ O & (CH_2)_4 \\ NHR & NHSO_2Ph \\ \end{array}$$

Compound	R	K_{i} nM
24	COOC(CH ₃) ₃	48% @ 1000 nM
25	CO-pyrazine	80
26	SO ₂ CH ₃	41
27	H (HCl salt)	22

References and Notes

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