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P'-EXTENDED α-KETOAMIDE INHIBITORS OF PROTEASOME

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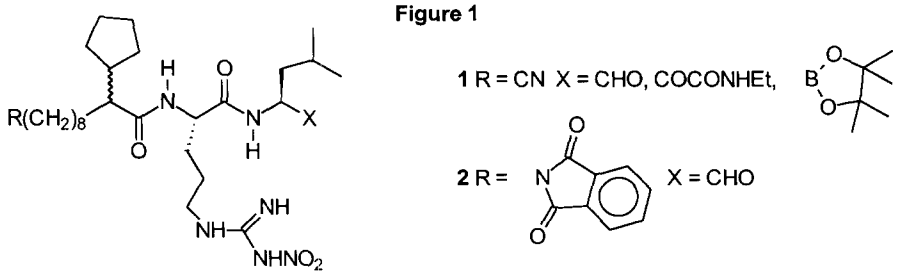
Abstract. A series of potent P'-extended α-ketoamide inhibitors of chymotrypsin-like activity of proteasome is described.

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



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 oxidation[5] 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

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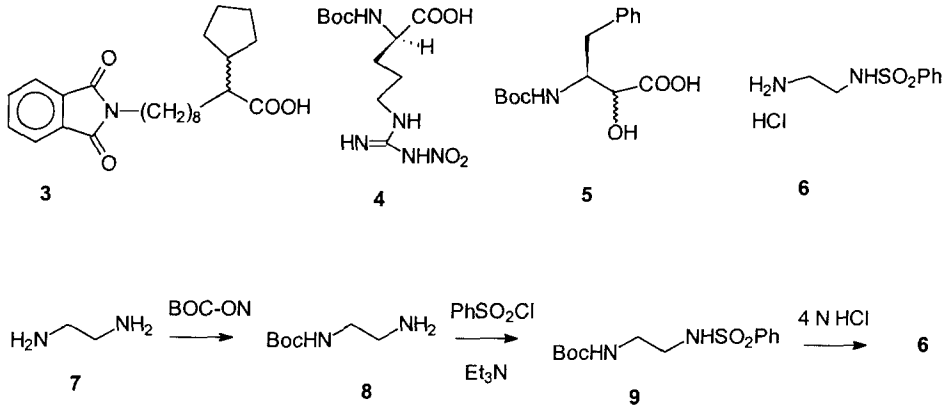
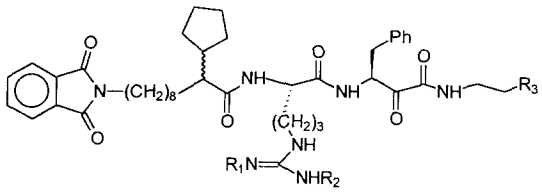
compound **15** by a phenylsulfonamido group generated compound **16**, with a tenfold enhancement of potency, revealing the importance of a H-bond donor/acceptor group in the region.

Scheme 1



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-(NO_2) at the P_2 site by Arg-(t-Boc)$_2$ (compound **19**) or Arg (HCl salt) (**20**) also diminished the activity.

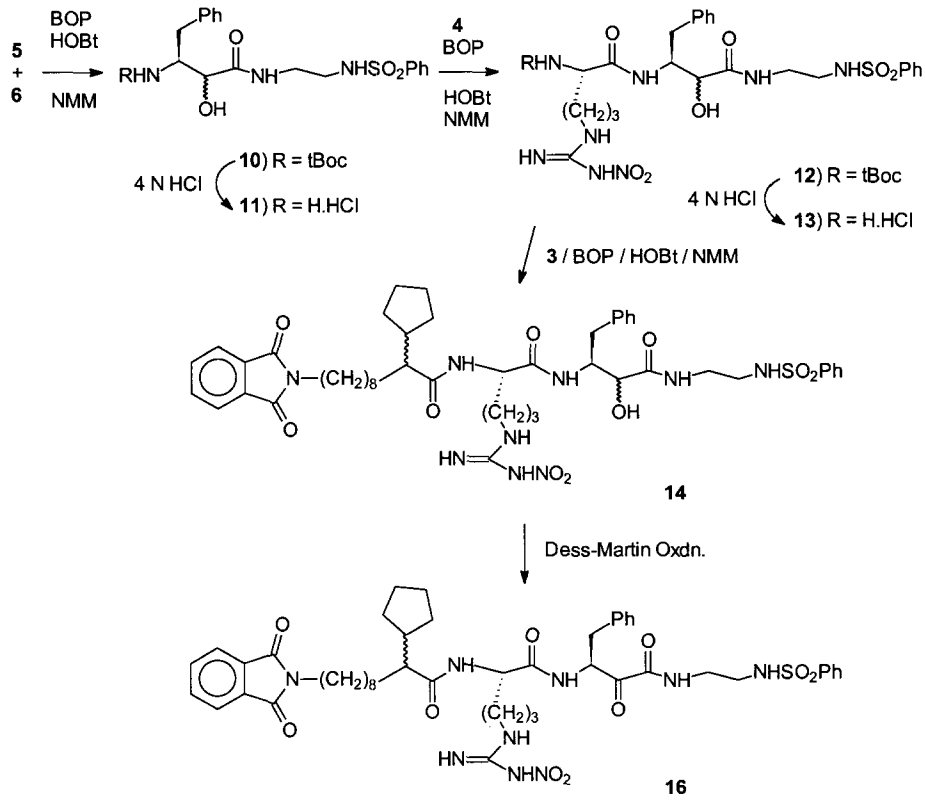
Table 1. Inhibitory Activity of the P_2-Arginine Derivatives[a]



Compound	R_1	R_2	R_3	K_i nM
15	H	NO_2	H	12
16	H	NO_2	$NHSO_2Ph$	1.1
17	H	NO_2	$NHCOOC(CH_3)_3$	2.2
18	H	NO_2	NH_2 (HCl salt)	67
19	$COOC(CH_3)_3$	$COOC(CH_3)_3$	$NHSO_2Ph$	34% @ 100 nM
20	H	H (HCl salt)	$NHSO_2Ph$	31% @ 100 nM

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

Scheme 2



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(NO_2) 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 μM. 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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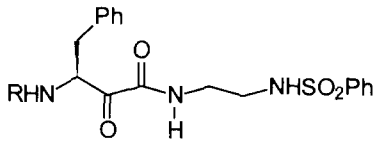
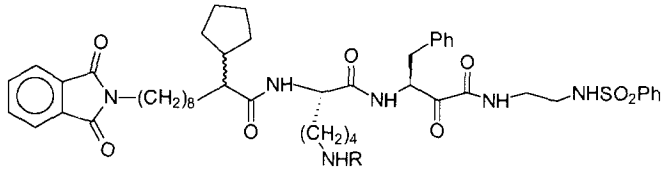
Table 2. Inhibitory Activity of the Truncated Analogs of Compound **16**



Compound	R	Activity
21	$COOC(CH_3)_3$	14% @ 10 μM
22	H (HCl salt)	12% @ 10 μM
23	Cbz-Arg(NO_2)	34% @ 1 μM

Table 3. Inhibitory Activity of the P_2-Lysine Analogs of Compound **16**



Compound	R	K_i nM
24	$COOC(CH_3)_3$	48% @ 1000 nM
25	CO-pyrazine	80
26	SO_2CH_3	41
27	H (HCl salt)	22

References and Notes

1. Adams, J.; Stein, R. *Annu. Rep. Med. Chem.* **1996**, *31*, 279.
2. (a) Iqbal, M.; Chatterjee, S.; Kauer, J. C.; Das, M.; Messina, P.; Freed, B.; Biazzo, W.; Siman, R. *J. Med. Chem.* **1995**, *38*, 2276; (b) Iqbal, M.; Chatterjee, S.; Kauer, J. C.; Mallamo, J. P.; Messina, P. A.; Reiboldt, A.; Siman, R. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 287.
3. For some recent examples from other laboratories, see (a) Lynas, J. F.; Harriott, P.; Healy, A.; McKervey, M. A.; Walker, B. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 373; (b) Adams, J.; Behnke, M.; Chen, S.; Cruickshank, A. A.; Dick, L. R.; Grenier, L. Klunder, J. M.; Ma, Y-T.; Plamondon, L.; Stein, R. L. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 333; (c) Lum, R. T.; Nelson, M. G.; Joly, A.; Horsma, A. G.; Lee, G.; Meyer, S. M.; Wick, M. M.; Schow, S. R. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 209.
4. Harbeson, S. L.; Abelleira, S. M.; Akiyama, A.; Barrett, R., III,; Carroll, R. M.; Straub, J. A.; Tkacz, J. N.; Wu, C.; Musso, G. F. *J. Med. Chem.* **1994**, *37*, 2918.
5. Burkhart, J. P.; Peet, N. P.; Bey. P. *Tetrahedron Lett.* **1988**, *29*, 3433.