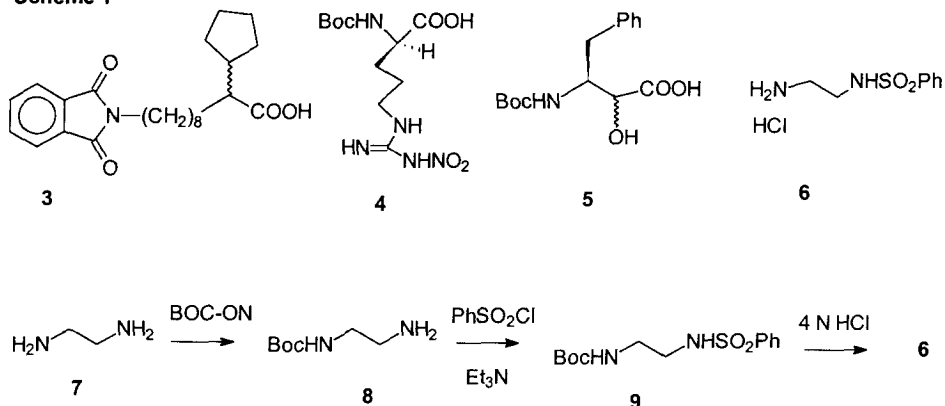


PII: S0960-894X(99)00443-6

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



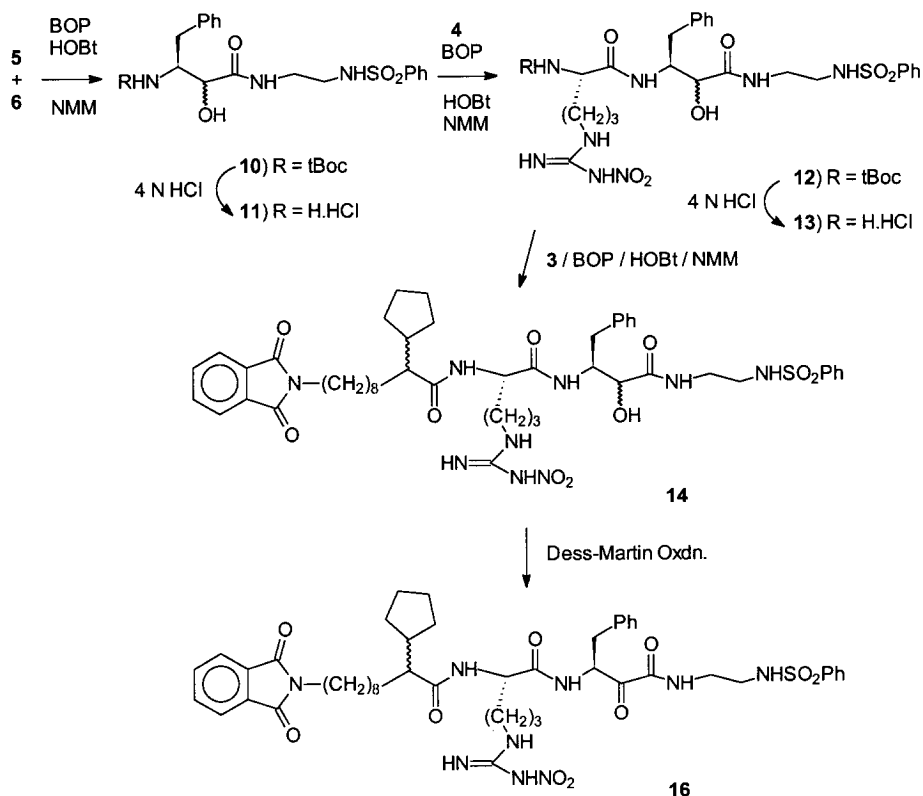
Replacement of the phenylsulfonyl group in compound **16** by a *t*-butoxycarbonyl (*t*-Boc) group resulted in twofold potency reduction (cf. **17** vs **16**). However, deprotection of the above-mentioned *t*-Boc group in compound **17** to generate a terminal amine (as HCl salt) resulted in ca. 30-fold loss in potency (cf. **18** vs **17**). Thus, it appears that the enzyme does not tolerate a charge in the region. Replacement of Arg-(NO₂) at the P₂ site by Arg-(*t*-Boc)₂ (compound **19**) or Arg (HCl salt) (**20**) also diminished the activity.

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

Compound	R ₁	R ₂	R ₃	K _i nM
15	H	NO ₂	H	12
16	H	NO ₂	NHSO ₂ Ph	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 ₂ Ph	34% @ 100 nM
20	H	H (HCl salt)	NHSO ₂ Ph	31% @ 100 nM

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

Scheme 2

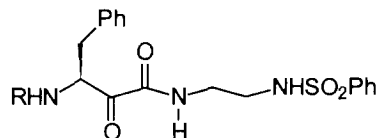


To explore the importance of the P₂ and P₃ (pseudo)-sites in this class of inhibitors, we generated a series of truncated analogs of compound 16 (Table 2). As shown, the complete lack of P₂-P₃ moiety abolished the activity (cf. 21 and 22 vs 16). Incorporation of Cbz-Arg(NO₂) as the P₂ moiety brought 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-P₃ residue of 16.

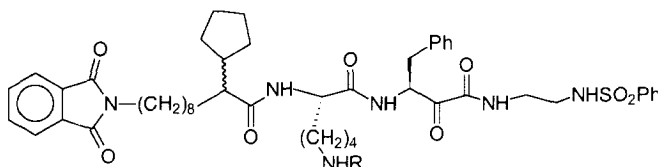
To investigate the effect of the related amino acid Lys at the P₂ position, we also replaced Arg(NO₂) in compound 16 to generate a set of compounds (Table 3). While compound 24, containing *t*-Boc protected lysine residue at P₂, displayed reduced activity, pyrazinecarbonyl and methylsulfonyl protected 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(NO₂) remains the choice of residue at P₂. 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 P₂-Lysine Analogs of Compound 16

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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