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POTENT α-KETOCARBONYL AND BORONIC ESTER DERIVED INHIBITORS OF PROTEASOME+

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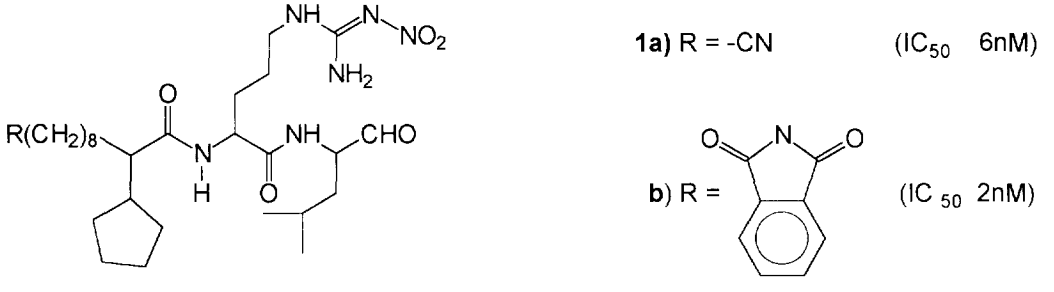
Abstract. Potent and selective α-ketocarbonyl (**8a-b**) and boronic ester (**11**) derived inhibitors of the chymotrypsin-like activity of *proteasome*, first member of a newly identified class of threonine proteases, are described.

Introduction. The proteasome is an eukaryotic cytoplasmic proteinase complex which 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 which, collectively, are capable of cleaving most peptide bonds.[2] Increased levels of this enzyme and subsequent protein breakdown have been implicated in many disease states including muscular dystrophy, cachexia accompanying cancer and malnutrition, emphysema, acute leukemia, and Alzheimer's disease. Recently, we reported potent, selective and novel dipeptide aldehyde inhibitors **1a-b** (**Figure 1**) of the chymotrypsin-like activity of the proteasome complex.[3]

Figure 1



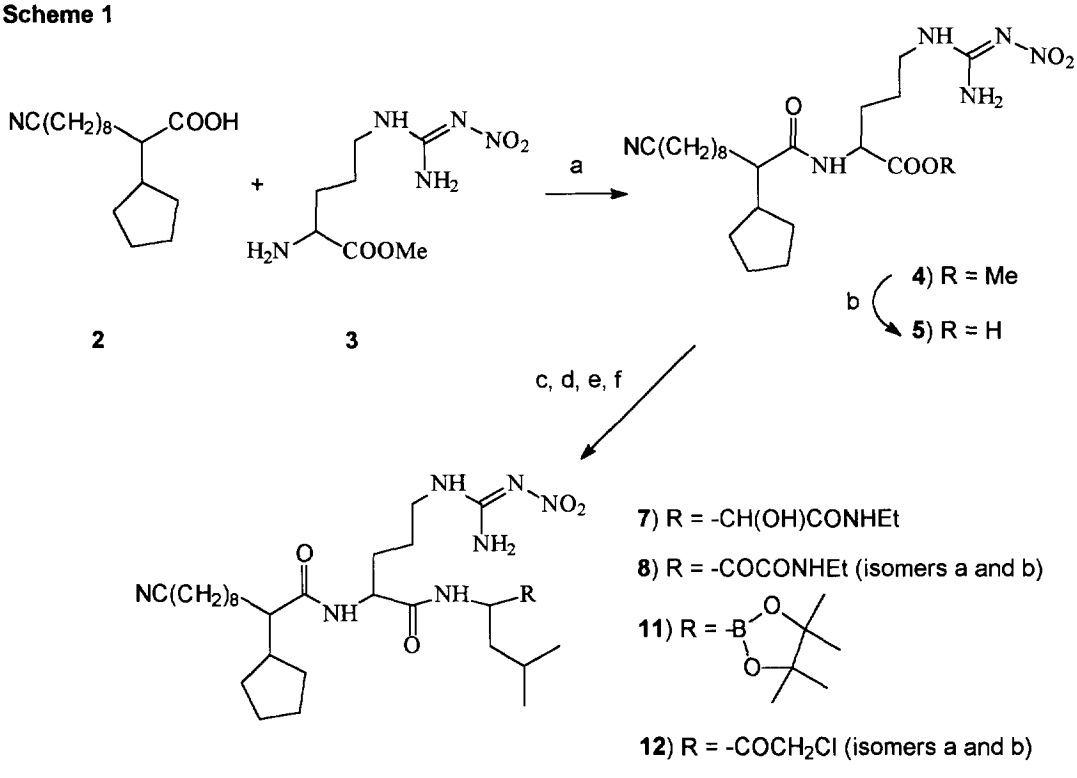
The X-ray crystal structure of archaebacterial proteasome, has recently been reported.[4] In another recent publication, Schreiber et al. described the inhibition of proteasome by the natural product lactacystin.[5] Based on these reports, the proteasome appears to be the first member of a newly identified class of proteolytic enzymes, namely, the threonine proteases. As a part of our continuing interest in developing potent, selective inhibitors of

+ This paper is dedicated to the memory of Mrs. Annette K. Altschuler.

proteasome, we now report that related α-ketocarbonyl (**8a-b**) and boronic ester derived (**11**) compounds are also potent inhibitors of the chymotrypsin-like activity of proteasome. The corresponding chloromethyl ketones (**12a-b**) have significantly lower inhibitory activity.

Chemistry. Syntheses of the target compounds are depicted in **Scheme 1**. Previously disclosed racemic acid **2**[3] was coupled with N^g-nitro-L-arginine methyl ester dihydrochloride (**3**) to generate the compound **4**, which on hydrolysis yielded the acid **5**. Compound **5** was coupled with 3-(S)-amino-2-hydroxy-5-methyl-hexanoic acid-N-ethylamide hydrochloride, **6**, (prepared by the method of Harbeson et al.[6]) to generate α-hydroxyamide **7**. Dess-Martin oxidation of 7 gave α-ketoamide **8**; diastereomers **8a** and **8b** were separated by preparative reverse-phase HPLC. Similarly, compound **5** was combined with boroleucine pinacol ester hydrochloride, **9**[7] and leucine chloromethylketone hydrochloride **10** (Bachem Biosciences, Inc., King of Prussia, Pa) to generate compounds **11** and **12** respectively. Compound **11** was assayed as a mixture; diastereomers (**a** and **b**) of compound **12** were separated by preparative reverse-phase HPLC.

Scheme 1



Reagents (a) BOP / HOBt / NMM / DMF; (b) 1(N) NaOH / MeOH; (c) **6** / BOP / HOBt / NMM / DMF; (d) Dess-Martin periodinane / CH_2Cl_2; (e) **9** / BOP / HOBt / NMM / DMF; (f) **10** / BOP / HOBt / NMM / DMF

Biology. Isolation and partial purification of the proteasome was achieved from postmortem human liver and brain.[8, 9] Biological activities of the inhibitors were determined according to the procedure previously described using the chromogenic substrate, Succinyl-Leu-Leu-Val-Tyr-amidomethylcoumarin (Bachem Biosciences, Inc., King of Prussia, Pa).[3] The inhibitory activities of the compounds (**8a-b**, **11**, **12a-b**) are shown in Table 1.

Table 1. Proteasome Inhibitory Activities of the Compounds **8a-b**, **11**, **12a-b**

Compound	IC_{50} nM
8a	22(n = 3)
8b	13(n = 3)
11	8(n = 3)
12a	>1000(n = 3)
12b	>1000(n = 3)

Discussion. As shown in Table 1, both α-ketocarbonyl (**8a-b**) and boronic ester derived (**11**) inhibitors show activity comparable with the corresponding aldehyde (cf. **1a**); the chloromethylketone derived inhibitors **12a-b** are less potent. Interestingly, inhibitory activity is less dependent on the stereochemistry of the pseudo-P_3 site (cf. **8a** and **8b**: the former is approximately two times less potent than the latter). This is in sharp contrast with the strict stereochemical requirement at the P_2 site. We have shown previously[3] that incorporation of a (D)-Arginine at P_2 in the aldehyde series diminishes inhibitory activity. It should be noted that compounds **8a-b** and **11** were uniformly unable to inhibit the trypsin-like activity of the enzyme complex at concentrations up to 1 μM and found to be >100-fold selective for the chymotrypsin-like activity of the proteasome in comparison to α-chymotrypsin, a related protease sensitive to inhibition by peptidyl α-ketoamides and boronic esters. Thus, the chymotrypsin-like activity of the multicatalytic proteasome is inhibited by dipeptide aldehydes, α-ketoamides and boronic esters. Other protease inhibitory moieties are currently being investigated.

Conclusion. We have described in this letter, potent α-ketocarbonyl and boronic ester-derived inhibitors of the chymotrypsin-like activity of the proteasome complex. This activity is catalyzed by an active-site threonine, and the results described here, and previously, demonstrate that several classic protease inhibitory moieties are capable of inhibiting a threonine protease. These inhibitors also provide useful probes for defining the emerging role of the proteasome complex in different biological functions.[10]

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10. Abbreviations used: BOP: benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate; HOBt: 1-hydroxybenzotriazole; NMM: N-methylmorpholine; DMF: N,N-dimethylformamide.

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