Potent Inhibitors of Proteasome

Mohamed Iqbal,* Sankar Chatterjee,*
James C. Kauer, Manoj Das, Patricia Messina,
Bethany Freed, William Biazzo, and Robert Siman

Departments of Chemistry and Biochemistry, Cephalon, Inc., 145 Brandywine Parkway, West Chester, Pennsylvania 19380

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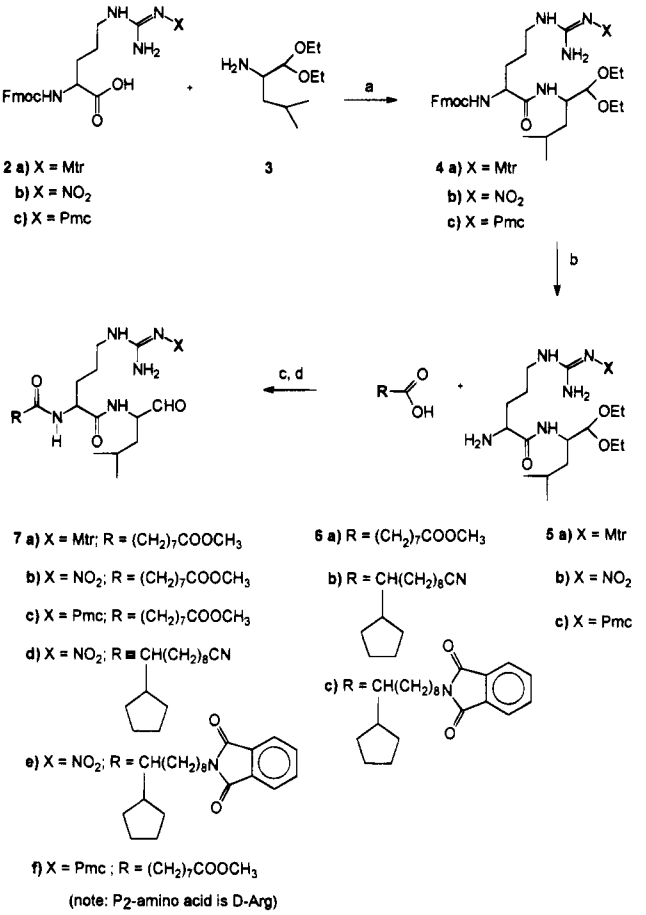
Introduction. The proteasome (also known as multicatalytic proteinase complex, neutral endopeptidase, 20S proteasome and ingensin) is a large molecular weight (700 kD) eukaryotic cytoplasmic proteinase complex which plays a major role in cellular pathways for the rapid breakdown and processing of proteins to peptides and amino acids.[1] The proteins to be degraded are conjugated to the polypeptide ubiquitin and then degraded by an ATP-dependent proteinase complex that contains proteasome as its proteolytic core.[2] Proteasome has been shown to have multiple catalytic activities which, collectively, are capable of cleaving most peptide bonds.[3] Increased levels of this enzyme and subsequent protein breakdown have been implicated in many disease states including muscular dystrophy, the cachexia accompanying cancer and malnutrition, emphysema, leprosy, and acute leukemia. The role of proteasome in muscle wasting in denervation atrophy[4] and in cell-cycle progression[5] has been reported. The abnormally high expression of proteasome in leukemic cell lines[6] and the presence of autoantibodies against proteasome in patients with systemic lupus erythematosus[7] have also been documented. Recently, the formation of the β-amyloid peptide-containing senile plaque in Alzheimer's disease patients following the cleavage of amyloid precursor protein (APP) has been suggested to result, in part, from the action of the chymotrypsin-like activity of this enzyme.[8] Determination of biological functions of the different catalytic activities of proteasome has been hindered by the lack of potent and selective inhibitors.

Synthetic inhibitors of proteasome are thus important targets since they may have potential as therapeutics for a variety of disease states. In our effort to develop agents which are capable of inhibiting the proteasome complex, we systematically designed a set of potent and specific substrates and inhibitors of the chymotrypsin-like activity of this enzyme complex. Here we report the design and synthesis of a series of potent dipeptide inhibitors, **7a–e** (Scheme 1), produced by modifying an early tetrapeptide aldehyde lead molecule, **1**, N-methoxysuccinyl-Glu-Val-Lys-Phe-H (Table 1). Compound **7e** displays the highest inhibitory potency for the proteasome ever reported. This compound has been shown to block the presentation of peptide antigens via the major histocompatibility complex class I (MHC-I) pathway[9,10] and, in addition, reduces the rate of turnover of mutant forms of Cu/Zn superoxide dismutase-1.[11]

Chemistry. Compound **1** was synthesized by coupling the side-chain-protected dipeptides, *N*-methoxysuccinyl-Glu(Boc)-Val-OH and Lys(Boc)-Phe-H diethyl acetal, followed by acid treatment. The syntheses of the inhibitors **7a–e** are depicted in Scheme 1. Fmoc-Arg-(X)-OH (**2a–c**) was coupled with Leu-H diethyl acetal (**3**) to produce the compounds **4a–c**. Removal of the Fmoc group in **4a–c** generated the free amines **5a–c**. Coupling of **5a–c** with the acids **6a–c** produced the intermediate acetals which on acid hydrolysis yielded the corresponding aldehydes **7a–e**. The acid **6a** is commercially available from the Aldrich Chemical Co.; the syntheses of the racemic acids **6b** and **6c** are shown in Scheme 2. Benzylation of cyclopentylacetic acid (**8a**) produced the ester **8b** which on alkylation with 1,8-diiodooctane generated the corresponding iodo ester **9**. Displacement of the iodo group in **9** by sodium cyanide and potassium phthalimide respectively produced **10a,b**. Hydrogenation of **10a,b** yielded the acids **6b,c**. The diastereomers of **7d** and **7e** were assayed as a mixture. Compound **7f** was synthesized in the same manner as in **7c** except that D-Arg was used.

Scheme 1[a]



[a] Reagents: (a) BOP, HOBt, NMM, DMF, 25 °C; (b) 30% $(Et)_2NH$ in DMF, 25 °C; (c) BOP, HOBt, NMM, DMF, 25 °C; (d) 1 N HCl, THF, 25 °C.

Table 1. Proteasome Inhibitory Activities of the Compounds **1** and **7a–f**

compd	IC_{50} (nM)	compd	IC_{50} (nM)
1	1000 ($n = 6$)	**7d**	6 ($n = 6$)
7a	95 ($n = 3$)	**7e**	2 ($n = 10$)
7b	40 ($n = 3$)	**7f**	>3000 ($n = 3$)
7c	20 ($n = 3$)		

Biology. Isolation and partial purification of the proteasome was achieved from postmortem human liver and brain by ion-exchange chromatography, ammonium sulfate precipitation, and gel filtration.[12,13] Biological activities of the inhibitors were determined by preincubation of the enzyme at 37 °C with several concentra-

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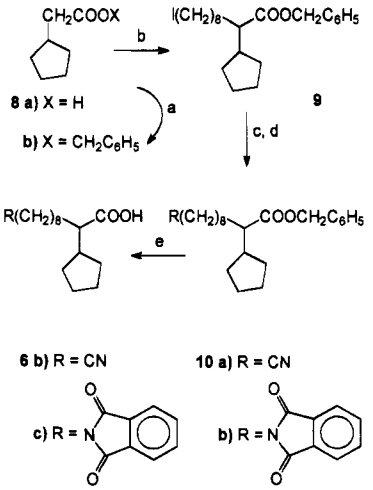
Scheme 2[a]



[a] Reagents: (a) $C_6H_5CH_2OH$, *p*-toluenesulfonic acid, C_6H_6, reflux; (b) LDA, THF–hexane, 1,8-diiodooctane, HMPA, −78 °C to 0 °C; (c) NaCN, DMSO, 70–75 °C; (d) potassium phthalimide, DMF, 70–75 °C; (e) 10% Pd–C (DeGussa), CH_3OH, H_2.

tions of the test compound for 15 min prior to addition of the chromogenic substrate (*N*-methoxysuccinyl-Glu-Val-Lys-Met-*p*-nitroanilide, Bachem). After incubating for various periods of time, the concentration of free *p*-nitroaniline was determined on a spectrophotometer. Protease activity was quantified under conditions in which substrate hydrolysis increased linearly with time, and the change in absorption was proportional to the concentration of free *p*-nitroaniline. The inhibitory activities of the compounds (**1**, **7a–f**) are listed in Table 1.

Discussion. As shown in Table 1, the lead tetrapeptide aldehyde (**1**) was modified to potent dipeptide aldehydes **7a–c** through incorporation of a hydrophobic alkyl chain at the amino terminal. Introduction of a branched alkyl group at the pseudo-P_3 site along with incorporation of polar groups at the end of the alkyl chain enhanced inhibitory potency (**7d** and **7e**). Also, it should be noted that the D-Arg modification at the P_2 site abolishes the activity of this class of inhibitors toward the enzyme complex (compare **7c** with **7f**). Compound **7e** (IC_{50} 2 nM) is the most potent inhibitor in this series. This compound was uniformly unable to inhibit the trypsin-like activity of the enzyme complex at concentrations up to 1 μM and found to be >45-fold selective for the chymotrypsin-like activity of the proteasome in comparison to cathepsin B or calpain I, two cysteine proteases sensitive to inhibition by peptidyl aldehydes.

Conclusion. We have described in this communication a series of potent and selective inhibitors of the chymotrypsin-like activity of the proteasome complex. These inhibitors should provide useful probes for the assessment of the role of the proteasome in different biological functions. The outcome of these studies will be the basis of future publications from our laboratories.[14]

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Supplementary Material Available: ^{1}H-NMR (300 MHz) and HPLC (reverse phase) spectra for the compound **7e** (2 pages). Ordering information is given on any current masthead page.

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(14) Abbreviations used: Fmoc, fluorenylmethoxycarbonyl; Mtr, 4-methoxy-2,3,6-trimethylbenzene-1-sulfonyl; Pmc, 2,2,5,7,8-pentamethylchroman-6-sulfonyl; BOP, (benzotriazol-1-yloxy)tris-(dimethylamino)phosphonium hexafluorophosphate; HOBt, 1-hydroxybenzotriazole; NMM, *N*-methylmorpholine; DMF, *N,N*-dimethylformamide; LDA, lithium diisopropylamide; HMPA, hexamethylphosphoramide; THF, tetrahydrofuran; DMSO, dimethyl sulfoxide.

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