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INHIBITION OF CALPAIN BY PEPTIDYL HETEROCYCLES

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Abstract: Dipeptidyl and tripeptidyl heterocycles designed to mimic peptide ketoamides and ketoacids were prepared and evaluated in vitro as inhibitors of human calpain I. Boc-Leu-Leu-imidazole (12) inhibited calpain I at low micromolar concentrations. Copyright © 1996 Elsevier Science Ltd

Calpains are calcium-dependent cysteine proteases found in most mammalian cells.¹ There are two distinct subclasses of calpains: Calpain I, a low Ca²⁺-requiring form, and calpain II, a high Ca²⁺-requiring form.² Calpains are involved in physiological processes such as the development of long-term memory, breakdown of neurofilaments at axon terminals, muscle protein turnover, metabolism of neuropeptides, and regulation of meiosis.³ Calpain has also been associated with several pathophysiological conditions such as neurodegeneration, stroke, Alzheimer's disease, and amyotrophy. Thus a number of therapeutic areas could benefit from the clinical development of calpain inhibitors.

A number of reversible^{4a-j} and irreversible inhibitors^{4a-d,4j-1} have been reported to inhibit calpains. Reversible calpain inhibitors include peptide derivatives containing an electron-deficient carbonyl group in place of the scissile amide unit. Aldehydes, α -ketoacids, and α -ketoamides have been successfully incorporated into calpain inhibitors.⁴ Powers et al. demonstrated that α -ketoamides are potent reversible calpain inhibitors.^{4f} α -Ketoheterocycle derivatives have been recently reported as potent inhibitors against the serine protease elastase.⁵ In search of novel calpain inhibitors, we introduced α -ketoheterocycles in place of the α -ketoacid and α -ketoamide functionalities in dipeptide and tripeptide derivatives. We now report the synthesis and evaluation of α -ketoheterocyclic derivatives **1** (peptidyl heterocycles) as calpain inhibitors.



The synthesis of the peptidyl imidazole, thiazole, and tetrazole derivatives was accomplished as outlined in Schemes I-III. Conversion of Boc-L-phenylalanine methyl ester **2a** to Boc-L-Phe-thiazole **4a** with 2-lithiothiazole,⁶ followed by deprotection of **4a** with hydrochloric acid, and coupling with Cbz-Leu-Leu-OH afforded α -ketothiazole derivative **5** (Scheme I). The Boc-L-leucine *N*-methoxy-*N*-methyl amide⁷ **2b** was converted to α -keto *N*-trimethylsilylethoxymethyl imidazole **4b**, which was treated with TFA,

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followed by coupling with Cbz-L-leucine to afford *N*-Cbz protected α -keto *N*-SEM-imidazole 6. Deprotection of 6 with tetrabutylammonium fluoride gave *N*-Cbz protected α -ketoimidazole 7 (Scheme I). As shown in Scheme II, the reaction of Boc-L-leucinal 8 with lithiated *N*-benzylimidazole gave 2-substituted imidazole 9. Deprotection of 9 with hydrochloric acid, followed by coupling with Cbz-L-leucine or Boc-L-leucine, afforded the peptidyl alcohols 10a and 10b. Oxidation of 10a with the Dess-Martin periodinane⁸ gave α -keto-*N*-benzyl imidazole 11. Hydrogenation of 10b with Pearlman's catalyst, followed by oxidation gave the *N*-Boc protected α -ketoimidazole 12 (Scheme II). The reaction yields were not optimized. No racemizations were observed within the detection limits of ¹H NMR and HPLC analyses.



Inhibition of calpain

Peptidyl α -ketotetrazole 15 was prepared as shown in Scheme III. Condensation of Cbz-Leu-Leu-H 13 with potassium cyanide in the presence of sodium bisulfite, followed by the addition of acetic anhydride gave the cyanohydrin acetate derivative 14. Treatment of 14 with sodium azide and aluminum chloride, gave the corresponding tetrazole alcohol, Jones oxidation of which gave α -ketotetrazole derivative 15.⁹

Compd.	Сар	Рер	Heterocycle	% Inhibition at 10 uM
5	Cbz	Leu-Leu-Phe	N S	54
6	Cbz	Leu-Leu		0
7	Cbz	Leu-Leu	N N N N N N N N N N N N N N N N N N N	0
11	Cbz	Leu-Leu	N N CH2C6H5	39
12	Boc	Leu-Leu	N N N N N N N N N N N N N N N N N N N	77
15	Cbz	Leu-Leu	× → × × × ×	11



pep

het

^a SEM = 2-(trimethylsilyl)ethoxymethyl

Peptidyl heterocycles were tested as inhibitors of human recombinant calpain I at 20 °C, using Suc-Leu-Tyr-NMA (90.2 mM, $K_m = 0.5$ mM) as substrate, as previously described.¹⁰ The inhibitory activities of the compounds are shown in Table I.

Tripeptidyl thiazole derivative 5 gave moderate inhibitory activity. In the α -ketoimidazoles 6, 7, 11, and 12, the activity is dependent on the peptide capping group and the protecting group on imidazole.

Compound 12, having Boc as the capping group, exhibits good inhibitory activity compared to compound 7 with Cbz as the capping group (no inhibitory activity was observed at 10 uM). Interestingly, *N*-benzyl protected compound 11 exhibited some activity at 10 uM. However, *N*-SEM protected imidazole 6, and free imidazole compound 7 show no inhibition at this concentration. The α -ketotetrazole 15, which may be considered an α -ketoacid isostere, demonstrated very weak inhibition of calpain.

In summary, α -ketoheterocycle-based inhibitors of calpain have been synthesized. Boc-Leu-Leuimidazole 12 is the most potent compound in this series. Other activated ketone moieties are being

investigated and the results will be reported soon.

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