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INHIBITION OF CALPAIN BY PEPTIDYL HETEROCYCLES

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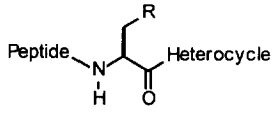
Abstract: Dipeptidyl and tripeptidyl heterocycles designed to mimic peptide ketoamides and ketoacids were prepared and evaluated in vitro as inhibitors of human calpain I. Boc-Leu-Leu-imidazole (**12**) inhibited calpain I at low micromolar concentrations. Copyright © 1996 Elsevier Science Ltd

Calpains are calcium-dependent cysteine proteases found in most mammalian cells.[1] There are two distinct subclasses of calpains: Calpain I, a low Ca^{2+}-requiring form, and calpain II, a high Ca^{2+}-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-l] 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.

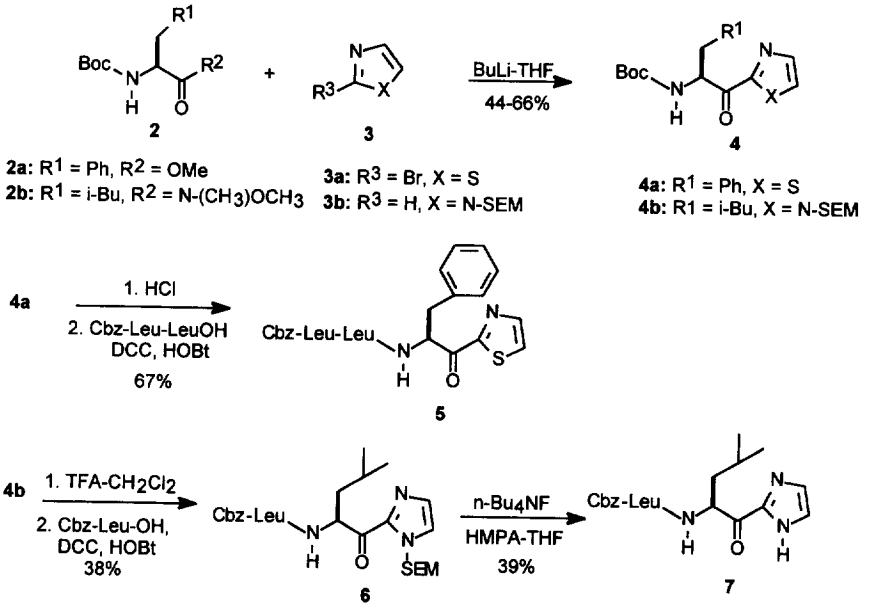


1

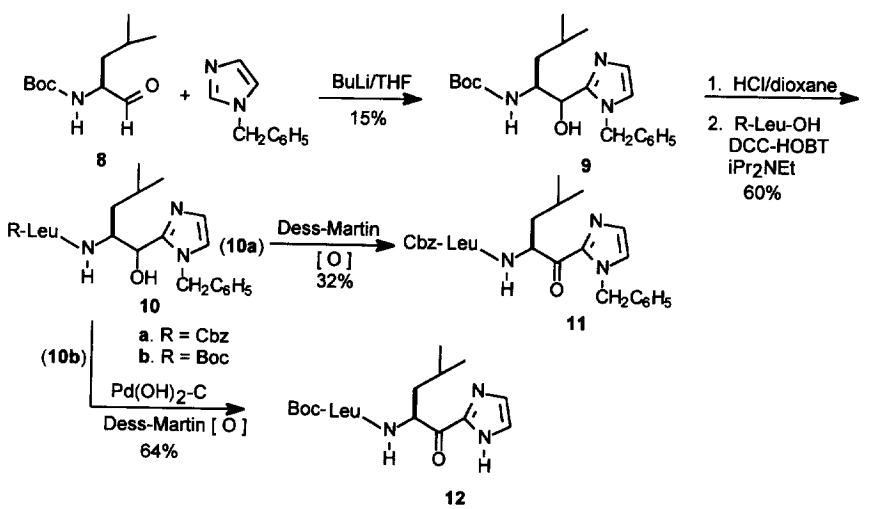
The synthesis of the peptidyl imidazole, thiazole, and tetrazole derivatives was accomplished as outlined in Schemes I-III. Conversion of Boc-L-phenylalanine methyl ester **2a** to Boc-L-Phe-thiazole **4a** with 2-lithiothiazole,[6] followed by deprotection of **4a** with hydrochloric acid, and coupling with Cbz-Leu-Leu-OH afforded α-ketothiazole derivative **5** (Scheme I). The Boc-L-leucine *N*-methoxy-*N*-methyl amide[7] **2b** was converted to α-keto *N*-trimethylsilylethoxymethyl imidazole **4b**, which was treated with TFA,

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 periodinane[8] 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 ^{1}H NMR and HPLC analyses.

Scheme I



Scheme II



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]

Scheme III

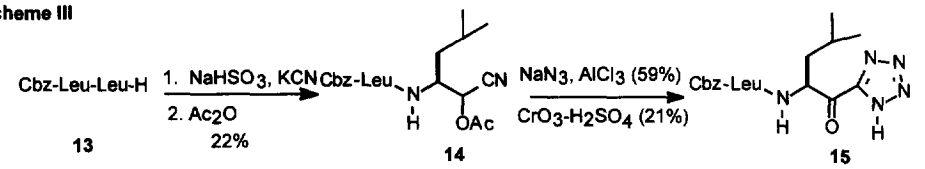
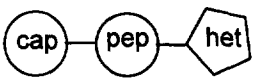
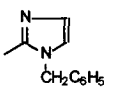


Table I. Inhibition of Calpain I by Peptidyl Heterocycles.[a]



Compd.	Cap	Pep	Heterocycle	% Inhibition at 10 uM
5	Cbz	Leu-Leu-Phe		54
6	Cbz	Leu-Leu		0
7	Cbz	Leu-Leu		0
11	Cbz	Leu-Leu		39
12	Boc	Leu-Leu		77
15	Cbz	Leu-Leu		11

[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-Leu-imidazole **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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