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Significance of Hydrogen Bonding at the S_1' Subsite of Calpain I

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Abstract—α-Ketohydroxamates were synthesized as bioisosteres of α-ketoamides. The α-ketohydroxamates were generally more potent than the corresponding α-ketoamides. The potency of the compounds suggests that hydrogen bonding and steric bulk of substituents on the nitrogen atom of the ketoamide moiety influence calpain inhibition.

The implication of calpain I in neurological disorders (e.g., stroke) and other pathological conditions (e.g., platelet aggregation and cardiac ischemia) has attracted interest in the search for calpain inhibitors as potential therapeutic agents.[1–4] Most of the structure–activity relationship studies of calpain inhibitors have focused on modifications of the unprimed region of the inhibitors to probe for structural requirements for binding to the S subsites of calpain.[5,6] A comparatively limited number of studies have addressed the structural requirements for binding to the S′ subsites of the enzyme.[7,8] In one such study, Li et al.[7] demonstrated that hydrogen bonding between an inhibitor and the S_1' subsite of calpain is important for potent inhibition of the enzyme. The workers showed that compounds **1** and **3**, which are secondary α-ketoamides, are 380- and 1310-fold, respectively, more potent than compound **2**, which is a tertiary α-ketoamide. The difference in the calpain inhibitory potency of the compounds was attributed in part to the ability of **1** and **3** to undergo hydrogen bonding interactions with the S′ subsite of calpain and the inability of **2** to perform similar interactions with the enzyme (Fig. 1). To further explore the significance of hydrogen bonding interaction between an inhibitor and the S′ subsite of calpain, we synthesized and evaluated the calpain I inhibitory activity of compounds **3**–**9**. We reasoned that placing an oxygen atom alpha to the amide bond nitrogen of compounds **3** and **4** as in α-ketohydroxamates **5** and **6**, respectively, should increase the strength of the hydrogen bond between the inhibitor and the enzyme, and should result in potentiation of calpain inhibition. We also envisioned that *N*-alkyl α-ketohydroxamates **7**–**9** should be better inhibitors of calpain I compared to the tertiary α-ketoamide **2** if the oxygen atom served as a hydrogen bond acceptor at the S_1' subsite of calpain. While this work was in progress Mallamo et al.[9] disclosed compounds **5** and **6** in the patent literature.

Synthesis of the *N,O*-dialkyl hydroxylamines **14**–**16** required for the synthesis of compounds **7**–**9** is shown in Scheme 1. The compounds were prepared from commercially available di-*t*-butyl dicarbonate **10** and *O*-alkyl hydroxylamine **11** in aqueous base. The resulting carbamate **12** was treated with sodium hydride to effect proton abstraction followed by the addition of iodomethane or bromoethane to give *t*-butyl-*N*-alkyl carbamate **13**. Acidolysis of **13** gave the corresponding *N,O*-dialkyl hydroxylamines **14**–**16**.

Compounds **3**–**9** were synthesized as shown in Scheme 2. Boc-phenylalanine **17** was coupled with *N,O*-dimethyl hydroxylamine to give Weinreb amide **18**, which was reduced to aldehyde **19** with $LiAlH_4$.[10] The aldehyde was reacted with KCN to give cyanohydrin **20**. Deprotection of **20** and hydrolysis of the cyano group gave α-hydroxy-β-amino acid **21**. The amino group of **21** was Boc protected to give **22**, which was coupled with either *O*-alkyl hydroxylamine or *N,O*-dialkyl hydroxylamine using EDC and HOBT as the coupling agent to give the corresponding α-hydroxylamide or α-hydroxylhydroxamate **23**. Cleavage of the Boc protecting group of **23** followed by coupling with Cbz-leucine and oxidation of the hydroxyl group with Dess–Martin reagent gave target compounds **3**–**9**.

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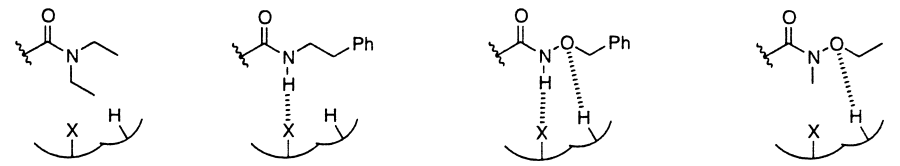
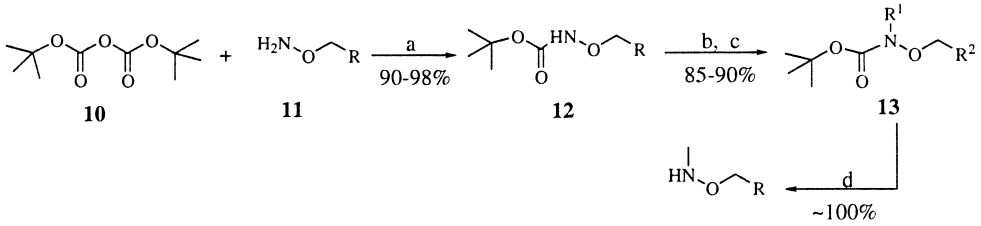


2 (K_i = 76.0 μM) **3** (K_i = 0,072 μM) **5** (K_i = 0.045 μM) **8** (K_i = 0.750 μM)

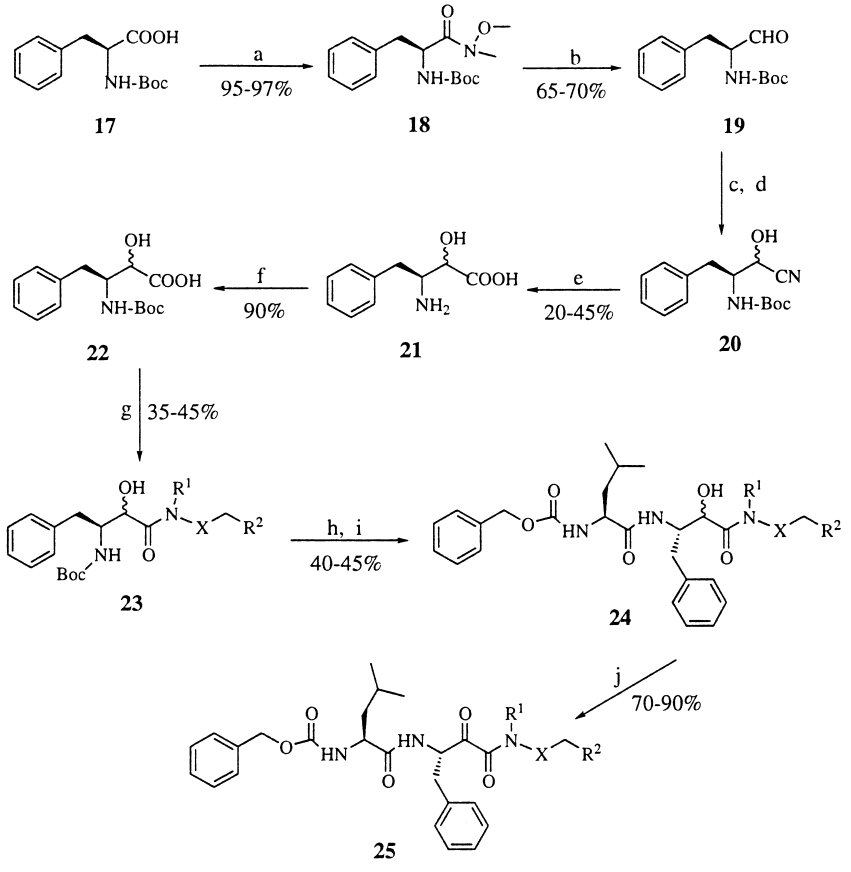
Figure 1. Proposed hydrogen bonding interactions between the compounds and the S′ subsite of calpain. The calpain inhibitory potency of the compounds mirror the hydrogen bonding pattern of the inhibitors at the S′ subsite of the enzyme.



10 **11** **12** **13**

14, R^1 = Me, R^2 = Ph; **15**, R^1 = Me, R^2 = Me
16, R^1 = Et, R^2 = Ph

Scheme 1. Reagents: (a) aqueous base; (b) NaH/DMF; (c) MeI or EtBr; (d) HCl.

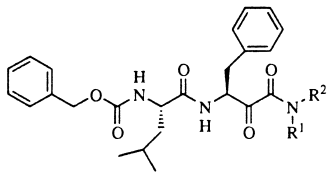


17 **18** **19** **20** **21** **22** **23** **24** **25**

R^1 = H, Me or Et R^2 = Me or Ph X = O or CH_2

Scheme 2. Reagents: (a) CDI, CH_3NHOCH_3; (b) $LiAlH_4$; (c) $NaHSO_3$; (d) KCN; (e) HCl/dioxane; (f) $(t\text{-ButOCO})_2O$, NaOH; (g) R^1-NH-X-CH_2-R^2, EDC, HOBT; (h) HCl/dioxane; (i) Cbz-leucine, EDC, HOBT; (j) Dess–Martin reagent.

Table 1. Inhibition of porcine erythrocyte calpain I by compounds **3–9**[a]



Compound	R^1	R^2	K_i (μM)
1	H	Et	0.200[b]
2	Et	Et	76.00[b]
3	H	CH_2Bn	0.072 (0.052)[b]
4	H	CH_2Et	0.107
5	H	OBn	0.045
6	H	OEt	0.062
7	CH_3	OBn	0.673
8	CH_3	OEt	0.750
9	CH_2CH_3	OBn	28.00

[a]K_i values are the averages of triplicate determinations obtained by Dixon plots where 1/v were plotted against I to give intersecting lines with correlation coefficient >0.95. The assay mixture consisted of 50 mM Tris–HCl pH 7.4, 50 mM NaCl, 10 mM DTT, 1 mM EDTA, 1 mM EGTA, calpain I, and varying concentrations of inhibitors in DMSO. Total DMSO concentration = 2%.

The compounds were evaluated[11] as inhibitors of calpain I from porcine erythrocytes and the results are shown in Table 1. Secondary α-ketohydroxamates **5** and **6** were marginally better inhibitors than the corresponding secondary α-ketoamides **3** and **4**, respectively. Tertiary α-ketohydroxamates **7** and **8** were over 100-fold more potent than tertiary α-ketoamide **2**. However, tertiary α-ketohydroxamate **9** was only about 3-fold more potent than α-ketoamide **2**. Thus, as the size of the substituent on the ketoamide nitrogen was increased from hydrogen (as in **5**) to methyl (as in **7**) to ethyl (as in **9**) a significant decrease in calpain inhibitory potency was observed (i.e., the calpain inhibitory potency of the compounds decreased in the following order: **5** > **7** > **9**). The results suggest that, while hydrogen bonding may contribute to the enhanced potency of the α-ketohydroxamates compared to the α-ketoamides (Fig. 1), steric bulk intolerance at the S_1' subsite of the enzyme may also contribute to the observed rank order of calpain inhibitory potency of the compounds (i.e., **5** > **7** > **9**).

Acknowledgements

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References and Notes

1. Sorimachi, H.; Saido, T. C.; Suzuki, K. *FEBS Lett*. **1994**, *343*, 1.
2. Bartus, R. T. *Neuroscientist* **1997**, *3*, 314.
3. Wang, K. K. W.; Yuen, P.-W. *Trends Pharm. Sci*. **1994**, *15*, 412.
4. Wang, K. K. W.; Yuen, P.-W. *Adv. Pharm*. **1997**, *37*, 117.
5. Donkor, I. O. *Curr. Med. Chem*. **2000**, *7*, 1171.
6. Wells, G. J.; Bihovsky, R. *Expert Opin. Ther. Pat*. **1998**, *8*, 1707.
7. Li, Z.; Patil, G. S.; Golubski, Z. E.; Hori, H.; Tehrani, K.; Foreman, J. E.; Eveleth, D. D.; Bartus, R. T.; Powers, J. C. *J. Med. Chem*. **1993**, *36*, 3472.
8. Chatterjee, S.; Dunn, D.; Tao, M.; Wells, G.; Gu, Z.-Q.; Bihovsky, R.; Ator, M. A.; Siman, R.; Mallamo, J. P. *Bioorg. Med. Chem. Lett*. **1999**, *9*, 2371.
9. Mallamo, J. P.; Bihovsky, R.; Josef, K. A. International Application (PCT) WO 00/16767, 2000.
10. Zheng, X.; Donkor, I. O.; Miller, D. D.; Ross, C. R., II *Chirality* **2000**, *12*, 2.
11. Calpain activity was monitored in a reaction mixture containing 50 mM Tris–HCl (pH 7.4), 50 mM NaCl, 10 mM dithiothreitol, 1 mM EDTA, 1 mM EGTA, 0.2 or 1.0 mM Suc-Leu-Tyr-AMC (Calbiochem), 2 μg porcine erythrocyte calpain I (Calbiochem), varying concentrations of inhibitor dissolved in DMSO (2% total concentration) and 5 mM $CaCl_2$ in a final volume of 250 μL in a polystyrene microtiter plate. Assays were initiated by addition of $CaCl_2$ and the increase in fluorescence (λ_{ex} = 370 nm, λ_{em} = 440 nm) was monitored at ambient temperature using a SPECTRAmax Gemini fluorescence plate reader (Molecular Devices). K_i values were determined by Dixon plots.[12] The average of triplicate assays, plotted as 1/v versus *I*, gave intersecting lines with correlation coefficient ≥0.95. No other attempt was made to correct for slow binding or autolysis.
12. Dixon, M. *Biochem. J*. **1972**, *129*, 197.