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P_2-ACHIRAL, P'-EXTENDED α-KETOAMIDE INHIBITORS OF CALPAIN I

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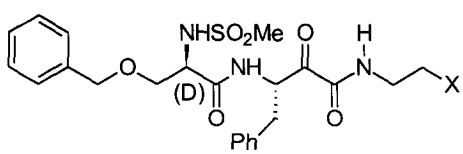
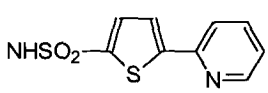
Abstract. A series of potent P_2-achiral, P'-extended α-ketoamide inhibitors of calpain I is described.

Introduction. Calpain I, a calcium–activated intracellular neutral protease,[1] has been implicated in the pathology of stroke.[2] Stroke is the third leading cause of mortality in the US. Potent peptide-based reversible[3] and irreversible[4] inhibitors of calpains have been reported. In all of these inhibitors, calpain I tolerated a range of amino acids at P_1. However, the P_2-amino acid was uniformly either L-Leu or -Val indicating this could be a strict structural requirement of calpain I at the P_2-site. Recently, we described a series of potent calpain I inhibitors incorporating *N*-alkyl- or *N*-arylsulfonyl-D-amino acids at P_2.[5] Therein we reported compound **1** (K_i = 130 nM), an *N*-ethyl α-ketoamide incorporating Ms-D-Ser(OBn) at P_2. Subsequent SAR studies based on **1** generated α-ketoamide **2** (K_i = 10 nM), a P'-extended biarylsulfonamide.[6] In continuing our work, we explored whether we could retain the potency of this class of inhibitors by replacing the P_2-amino acid residue with an achiral moiety *en route* to nonpeptidic inhibitors. We now report the results of our effort by disclosing a series of potent P_2-achiral, P'-extended α-ketoamide inhibitors (Tables 1-2) of calpain I.



1) X = H

2) X = 

Chemistry. The synthesis of one of the representative target compounds **9** is shown in Scheme 1. Commercially available 1,2-ethylenediamine (**23**) was selectively monoprotected (BOC-ON/THF) with a *t*-Boc group to generate **24**. Sulfonylation of **24** with 5-(pyrid-2-yl)thiophene-2-sulfonyl chloride generated **25**. Deprotection of **25** with 4 N HCl in dioxane gave **26**. Coupling (NMM/HOBt/BOP) of **26** and **27** produced **28** that was deprotected (4 N HCl in dioxane) to give **29**. Compound **29** was capped with 2,6-dichlorobenzoyl chloride to generate **30**. Dess-Martin oxidation of **30** produced **9**.

Biology and discussion. Methods for enzyme (recombinant human calpain I) preparation and assay conditions were described previously.[7] Initially, we replaced the P_2-D-amino acid residue of compound **2** with a benzoyl group as the P_2-moiety. Benzoyl in place of the *N*-protected P_2 subunit of **2** significantly reduced the activity (cf. **3** vs **2**). 3,4- or 3,5-Disubstitution on the benzene ring also did not have any beneficial effect (cf. **4** and **5** vs **2**). However, 2,6-dimethyl substitution on the benzene ring offered improved potency (**6** vs **3**). Replacement of the methyl groups on benzene in **6** by chlorine atoms was beneficial (cf. **9** vs **6**). Note that the 2,6-difluorobenzoyl moiety of compound **10** was also tolerated as the P_2-moiety. On the other hand, changing the substitution pattern from 2,6– to 2,5- resulted in loss of activity (cf. **7** and **8** vs **9**). Replacement of the benzene nucleus in **9** by a pyridine nucleus (compound **11**) provided a less active analog.

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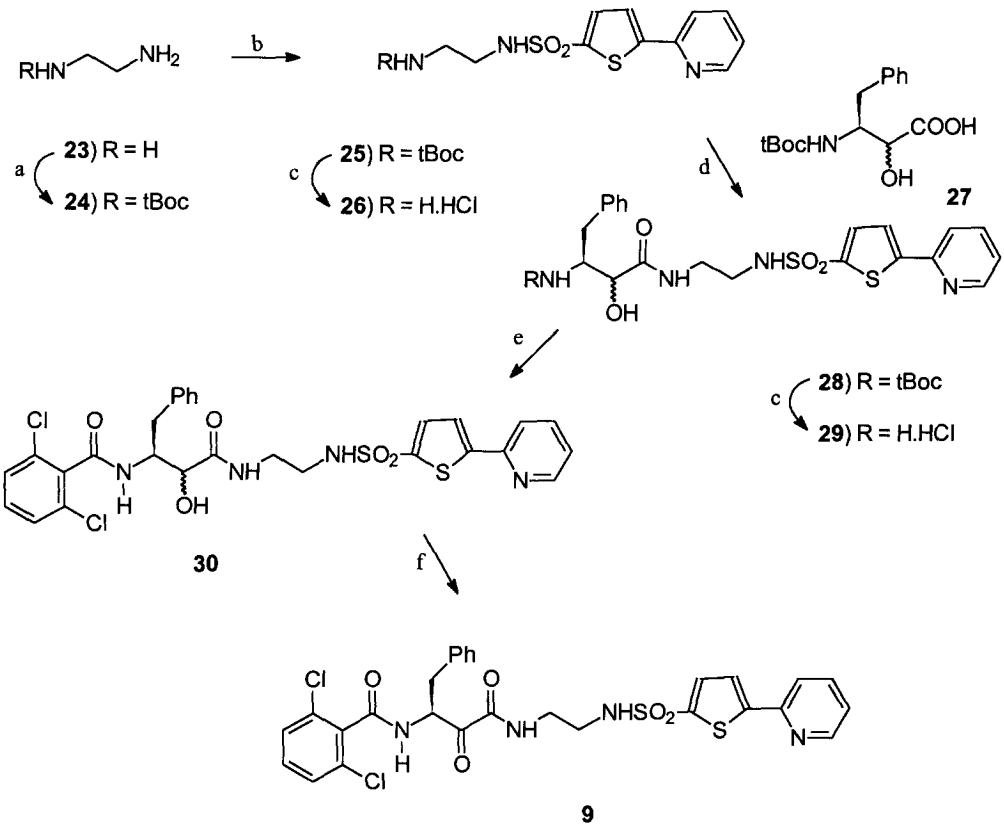
Scheme 1

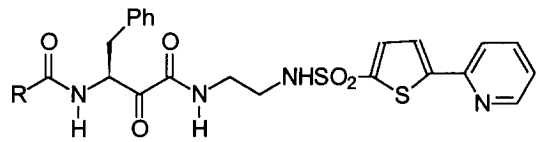


Reagents: (a) BOC-ON, THF, 23 °C, overnight; (b) 5-(Pyrid-2-yl)thiophene-2-sulfonyl chloride, Et_3N, CH_2Cl_2, 0 °C to 23 °C, 1 h; (c) 4 N HCl in dioxane, 23 °C, 1 h; (d) BOP, HOBt, NMM, DMF, 0 °C to 23 °C, 2 h; (e) 2,6-Dichlorobenzoyl chloride, Et_3N, CH_2Cl_2, 0 °C to 23 °C, 1 h; (f) Dess-Martin periodinane, CH_2Cl_2, 23 °C, 1 h.

We also employed alkanoyl groups as P_2-achiral moieties. In a series of homologous alkyl group-containing compounds, increase of the chain-length resulted in a significant increase in potency (cf. **12**, **13** and **14**). It should be noted that P_2-moieties in compounds **13** and **14** could be considered as des-amino Val and Leu, respectively. It is conceivable that the terminal isopropyl and isobutyl groups from compounds **13** and **14**, respectively, occupy the same three-dimensional space, as occupied by corresponding alkyl side chains from P_2-Val and –Leu containing dipeptide inhibitors. However, the manner in which this class of compounds binds to calpain I awaits X-ray crystal structure determination of a corresponding enzyme-inhibitor complex. The chain-length also had a marked effect in a pair of phenylalkylene containing P_2-groups (cf. **15** and **16**). Thus, it appears that the calpain I accommodates bulky lipophilic groups from the P_2-region of this class of inhibitors.

Table 2 displays variation in the P'-sulfonamide moiety in a series of P_2-2,6-dichlorobenzoyl-derived inhibitors. As shown, the enzyme tolerated different biaryl motifs (cf **17-20**). However, separating the aromatic moieties by a spacer (e.g., in compound **21**) resulted in reduced activity. In a similar way, replacement of the terminal biaryl motif with a single aromatic (substituted) moiety (compound **22**) was also detrimental to the inhibitory activity. Compound **20** (K_i = 8 nM) emerged as the most potent member of the series. Note that the inhibitory activity of **20** is comparable to that of the parent α-ketoamide **2**.

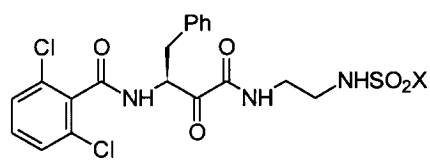
Table 1. Inhibitory Activity of α-Ketoamides Containing Achiral P_2 Mimetics[a]

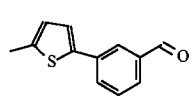
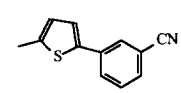
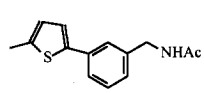
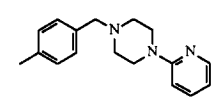
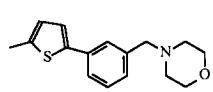
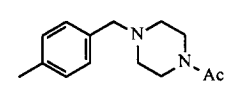


Compound	R	K_i nM	Compound	R	K_i nM
3	Phenyl	570	**10**	2,6-Difluorophenyl	14
4	3,4-Methylenedioxyphenyl	>1000	**11**	2,6-Dichloronicotinyl	57
5	3,5-Bis(trifluoromethyl)phenyl	430	**12**	$(CH_3)_2CH$-	1100
6	2,6-Dimethylphenyl	130	**13**	$(CH_3)_2CHCH_2$-	420
7	2-Chloro-5-methoxyphenyl	46	**14**	$(CH_3)_2CHCH_2CH_2$-	21
8	2,5-Dichlorophenyl	49	**15**	$Ph(CH_2)_3$-	1100
9	2,6-Dichlorophenyl	26	**16**	$PhCH(CH_3)(CH_2)_2$-	62

[a]Values for IC_{50} were determined and converted to K_i values using the expression $K_i = IC_{50}/(1+S/K_m)$, assuming a competitive mechanism of inhibition[5]. n ≥ 3 in all cases. Replicate determinations of K_i agree within 25%.

Table 2. Variation in the Terminal Sulfonamide Moiety



Compound	X	K_i nM	Compound	X	K_i nM
17		15	**20**		8
18		14	**21**		59
19		21	**22**		71

Finally, replacement of the entire α-ketoamide moiety of compound **9** (K_i = 26 nM) by an aldehyde group generated the less potent compound, 2,6-dichlorobenzoyl-Phe-H (**31**, K_i ~ 360 nM). Thus, it appears that the greater potency of **9** over **31** arises primarily due to the energetically beneficial binding offered by the P'-extended moiety of **9**.

Conclusion. In this *Letter*, we disclosed a series of potent, P_2-achiral, P'-spanning α-ketoamide inhibitors of calpain I. This study expands the scope of our previous observation that the presence of a L-Leu or L-Val residue at P_2 is not a preferred structural requirement for a potent calpain I inhibitor. The study also reveals that energetically beneficial binding offered by the P'-spanning moiety of this class of α-ketoamide inhibitors is sufficient enough to offset the absence of a P_2-amino acid residue.

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References and Notes

1. Croall, D. L.; DeMartino, G. N. *Physiol. Rev.* **1991**, *71*, 813.

2. Wang, K. K. W.; Yuen, P. *Trends Pharm. Sci.* **1994**, *15*, 412.

3. (a) Iqbal, M.; Messina, P. A.; Freed, B.; Das, M.; Chatterjee, S.; Tripathy, R.; Tao, M.; Josef, K. A.; Dembofsky, B.; Dunn, D.; Griffith, E.; Siman, R.; Senadhi, S. E.; Biazzo, W.; Bozyczko–Coyne, D.; Meyer, S. L.; Ator, M. A.; Bihovsky, R. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 539; (b) Mehdi, S. *Trends Biol. Sci.* **1991**, *16*, 150; (c) Harbeson, S. L.; Abelleira, S. M.; Akiyama, A.; Barrett, R., III,; Carroll, R. M.; Straub, J. A.; Tkacz, J. N.; Wu, C.; Musso, G. F. *J. Med. Chem.* **1994**, *37*, 2918. (d) 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. (e) Li, Z.; Ortega-Vilain, A-C.; Patil, G. S.; Chu, D-L.; Foreman, J. E.; Eveleth, D. D.; Powers, J. C. *J. Med. Chem.* **1996**, *39*, 4089.

4. (a) Chatterjee, S.; Josef, K.; Wells, G.; Iqbal, M.; Bihovsky, R.; Mallamo, J. P.; Ator, M. A.; Bozyczko–Coyne, D.; Mallya, S.; Senadhi, S.; Siman, S. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1237. (b) Chatterjee, S.; Ator, M. A.; Bozyczko–Coyne, D.; Josef, K.; Wells, G.; Tripathy, R.; Iqbal, M.; Bihovsky, R.; Senadhi, S. E.; Mallya, S.; O'Kane, T. M.; McKenna, B. A.; Siman, R.; Mallamo, J. P. *J. Med. Chem.* **1997**, *40*, 3820; (c) Angliker, H.; Anagli, J.; Shaw, E. *J. Med. Chem.* **1992**, *35*, 216; (d) Crawford, C.; Mason, R. W.; Wickstrom, P.; Shaw, E. *Biochem. J.* **1988**, *253*, 751; (e) McGowan, E. B.; Becker, E.; Detwiler, T. C. *Biochem. Biophys. Res. Commun.* **1989**, *158*, 432; (f) Huang, Z.; McGowan, E. B.; Detwiler, T. C. *J. Med. Chem.* **1992**, *35*, 2048; (g) Harris, A. L.; Gregory, J. S.; Maycock, A. L.; Graybill, T. L.; Osifo, I. K.; Schmidt, S. L.; Dolle, R. E. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 393.

5. Chatterjee, S.; Gu, Z-Q.; Dunn, D.; Tao, M.; Josef, K.; Tripathy, R.; Bihovsky, R.; Senadhi, S. E.; O'Kane, T. M.; McKenna, B. A.; Mallya, S.; Ator, M. A.; Bozyczko–Coyne, D.; Siman, R.; Mallamo, J. P. *J. Med. Chem.* **1998**, *41*, 2663.

6. Manuscript *in preparation*.

7. Meyer, S. L.; Bozyczko-Coyne, D.; Mallya, S. K.; Spais, C. M.; Bihovsky, R.; Kawooya, J. K.; Lang, D. M.; Scott, R. W; Siman, R. *Biochem. J.* **1996**, *314*, 511.