Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 2647–2652

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

P_2-PROLINE-DERIVED INHIBITORS OF CALPAIN I

Rabindranath Tripathy,[a] * Zi-Qiang Gu,[a] * Derek Dunn, [a] Shobha E. Senadhi,[b] Mark A. Ator,[b] and Sankar Chatterjee[a] *

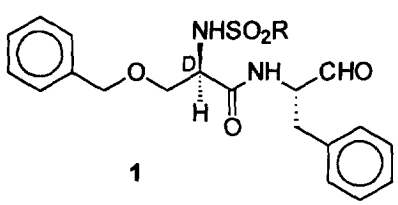
Departments of [a]Chemistry and [b]Biochemistry
Cephalon, Inc., 145 Brandywine Parkway, West Chester, PA 19380-4245, U.S.A.

Received 28 May 1998; accepted 3 August 1998

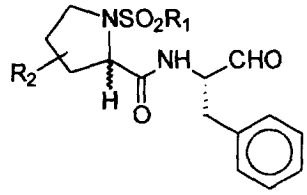
Abstract. The syntheses and biological activities of a series of calpain I inhibitors, derived from D- and L-Pro, are described.

Introduction. The possible role of calpain I, a calcium-activated intracellular neutral protease,[1] in the pathology of stroke (focal cerebral ischemia) has been suggested.[2] Stroke is one of the leading causes of mortality in the western hemisphere; in the US alone, more than half a million strokes occur each year killing more than one-third of the victims. Potent peptide-based reversible[3-6] aldehyde and α-ketocarbonyl, and irreversible[7-12] halomethyl ketone, diazomethyl ketone, epoxysuccinate, and acyloxymethyl ketone inhibitors of calpains have been reported. In all of these inhibitors, calpain 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; compound **1** (Figure 1; R = CH_3) is a member of this series.[13]

Figure 1



1



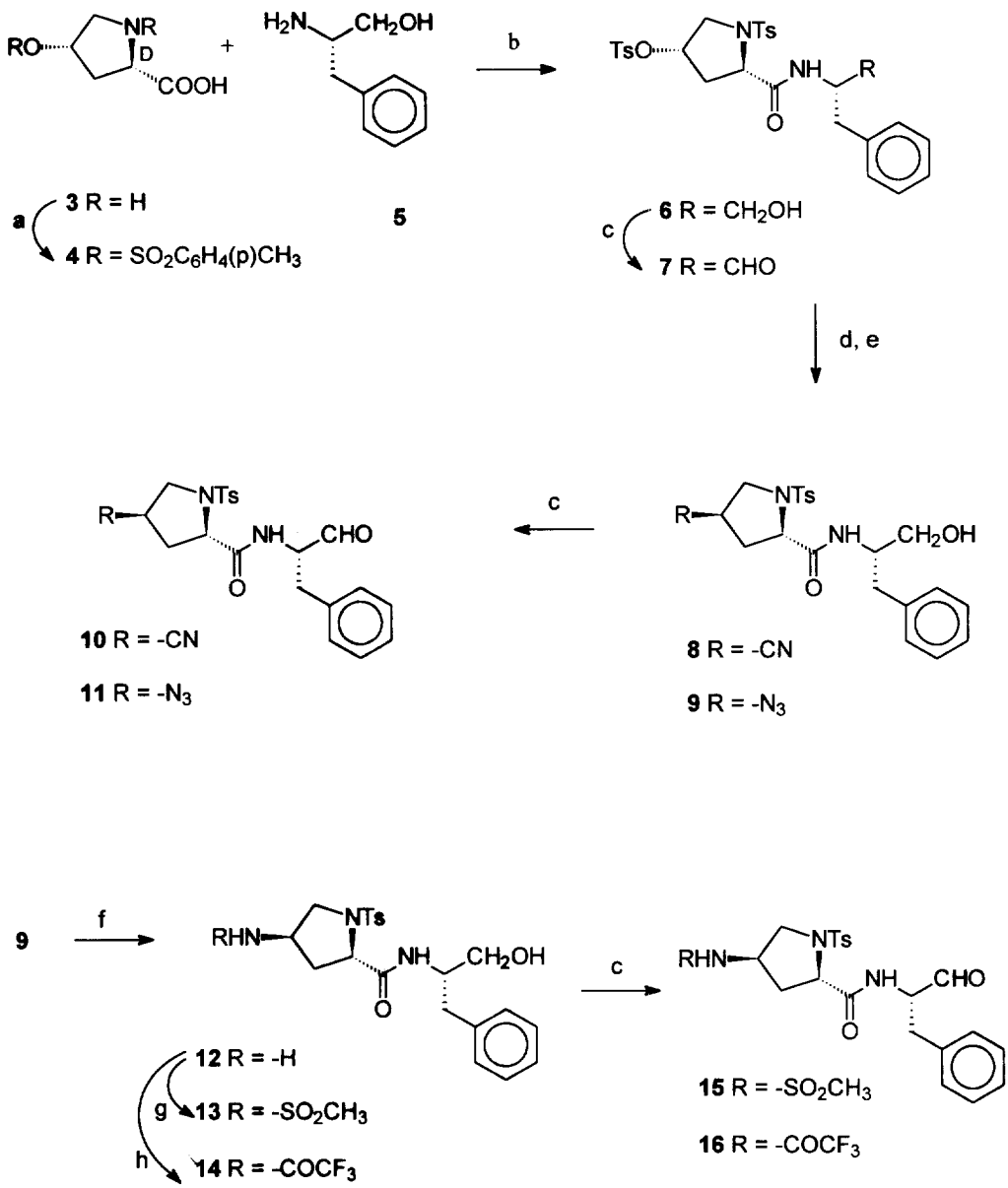
2 A = ····· H (D-Pro series)

B = —— H (L-Pro series)

To investigate how the introduction of an element of constraint in compound **1** affects the inhibitory activity, we incorporated an *N*-alkyl/arylsulfonyl-D-proline moiety at P_2 in **1** to generate compounds of general structure **2A** (Fig. 1). In order to compare how the configuration of proline influences the inhibitory activity, we also generated a limited series of *N*-alkyl/arylsulfonyl-L-proline-derived compounds of general structure **2B** (Fig. 1).[14] Herein we report the results of our observation.

0960-894X/98/$ - see front matter
PII: S0960-894X(98)00470-3

Scheme 1



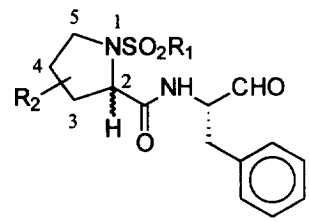
Reagents: (a) p-toluenesulfonyl chloride, THF, $NaOH-H_2O$; (b) NMM/HOBt/BOP/DMF; (c) Dess-Martin periodinane, CH_2Cl_2; (d) **6**, KCN, DMF; (e) **6**, NaN_3, DMF; (f) 10% Pd-C, H_2, EtOH; (g) CH_3SO_2Cl, Et_3N, CH_2Cl_2; (h) $(CF_3CO)_2O$; Et_3N, CH_2Cl_2.

Chemistry. The syntheses[15] of the representative target compounds **7**, **10-11**, and **15-16** are shown in Scheme 1. Commercially available *cis*-4-hydroxy-D-proline (**3**) was simultaneously N- and O-sulfonylated to generate **4**. Compound **4** was coupled with (*s*)-phenylalaninol (**5**) to yield the dipeptide alcohol, **6**. Dess-Martin oxidation of **6** generated the target compound **7**. In separate experiments, the OTs group in **6** was displaced in S_N2 fashion by cyano and azido groups to produce compounds **8** and **9**, respectively. Oxidation of **8-9** generated the target compounds **10-11**. The azido group in **9** was hydrogenated to the amino group of compound **12**, which was subsequently sulfonylated and carbonylated to generate **13** and **14**, respectively. Oxidation of **13-14** produced **15-16**.

Biology and discussion. The biological activities of the compounds were determined using recombinant human calpain I, prepared as described by Meyer et al.[16] with Suc-Leu-Tyr-MNA (Enzyme Systems Products, Dublin, CA) as substrate. Table 1 displays the activity of the target compounds. As shown, in the D-Pro series, the arylsulfonyl moiety on proline-*N* is ca. four times preferred over an alkylsulfonyl moiety (cf. **20** vs **17**). Sterically demanding groups (e.g., tosyl, mesyl) at the 4-position diminish the inhibitory activity (cf. **20** vs **18**, **19**, **7**, **21**, and **15**). However, the enzyme tolerates relatively smaller-sized groups (e.g., cyano, azido) at the 4-position (cf. **20** vs **10**, and **11**). Also, the enzyme prefers a trifluoroacetamido group over a methanesulfonamido group at the 4-position (cf. **16** vs **15**). Surprisingly, the enzyme accommodates both D- and L-*N*-methanesulfonylproline (cf. **17** vs **22**). Note, in the L-proline series, the enzyme does not discriminate between alkylsulfonyl and arylsulfonyl moieties on proline-*N* (**22** and **23**). Interestingly, while the *trans*-tosyl group at the 4-position in the L-proline series improves the inhibitory activity (cf. **23** vs **24**), the *trans*-benzoyloxy group at the same position diminishes the activity (cf. **23** vs **25**) indicating the beneficial effect of a sulfonyl group in the region. It should be noted that D-Pro-derived **20** and L-Pro-derived **24** are equipotent to the reference compound **1**.

Accommodation of both D- and L-proline (compounds **17** and **22**) at P_2 implies similar binding modes for the two inhibitors to calpain I. Recently, Kempf et al. reported that the biological and pharmacokinetic properties of the HIV aspartic protease inhibitor ritanovir and its P_2-D-valinyl diastereomer were indistinguishable; the X–ray crystal structures of the enzyme-inhibitor complexes revealed similar binding modes for the two inhibitors.[17] In our case, the manner in which compounds **17** and **22** bind to the enzyme awaits the X-ray crystal structure determination of the respective enzyme-inhibitor complex. It is noteworthy to mention that the methylation of the reference compound **1** generates a 46 times less potent compound[13] whereas proline-derived *N*-methyl methanesulfonamides, **17** and **22**, are only 4-5 times less potent revealing the beneficial orientation of the C(2)-N(1)-S-O moiety. It should be mentioned that the replacement of the proline

Table 1. Inhibitory Activities of the Compounds[a]



Comp.	Config. of proline	R_1	R_2	Calpain I IC_{50} (nM)	Cathepsin B IC_{50} (nM)	α-chymotrypsin % inh @ 10 μM
7	D	*p*-CH_3-C_6H_4	*cis*-4-OTs	74% @ 1 μM	–	–
10	D	*p*-CH_3-C_6H_4	*trans*-4-CN	28	8700	6
11	D	*p*-CH_3-C_6H_4	*trans*-4-N_3	28	>10,000	9
15	D	*p*-CH_3-C_6H_4	*trans*-4-$NHSO_2CH_3$	40% @ 1 μM	–	–
16	D	*p*-CH_3-C_6H_4	*trans*-4-$NHCOCF_3$	100	–	–
17	D	Me	H	53	3000	2
18	D	Ph	*cis*-4-$PhSO_2O$	42% @ 1 μM	–	–
19	D	Ph	*trans*-4-OTs	48% @ 1 μM	–	–
20	D	*p*-CH_3-C_6H_4	H	14	3600	12
21	D	*p*-CH_3-C_6H_4	*cis*-4-OMs	79% @ 1 μM	–	–
22	L	Me	H	45	883	–
23	L	Ph	H	41	1400	–
24	L	Ph	*trans*-4-$PhSO_2O$	13	38	–
25	L	Ph	*trans*-4-$PhCH_2O$	125	–	–
1	–	–	–	11	42	23

[a] $n \geq 3$, in all cases.

moiety by a sarcosine moiety (N-methylglycine) diminishes the activity (data not shown).

Table 1 also contains inhibitory activity of a selected set of compounds (calpain I IC_{50} < 100 nM) against cathepsin B, a related cysteine protease. In the D-proline series, compounds **10**, **11**, and **20** displayed excellent selectivity for calpain I over cathepsin B. However, the selectivity of the L-proline-derived compounds for calpain I over cathepsin B was modest. Table 1 also contains inhibitory activity (at 10 μM) of the same set of compounds against α-chymotrypsin, a serine protease with P_1-Phe-specificity; compounds were less active in this assay.

Conclusion. We have described in this *Letter* a series of calpain I inhibitors incorporating D- and L-Pro at P_2. This work compliments our previous observation that contrary to earlier literature reports, L-Leu or -Val residue at P_2 might not be a strict requirement for a potent calpain I inhibitor. Work is currently underway to determine the cellular activities of these compounds and will be reported in due course.

Acknowledgment. We thank Drs. Jeffry L. Vaught, John P. Mallamo, and Robert Siman for their support and encouragement. We are grateful to Drs. Ron Bihovsky, Mohamed Iqbal, Gregory Wells and Ming Tao for many helpful discussions. We are pleased to acknowledge SmithKline Beecham Pharmaceuticals for partial support of this research.

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. 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.

4. Mehdi, S. *Trends Biol. Sci.* **1991**, *16*, 150.

5. 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.

6. (a) 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. (b) 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.

7. (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.

8. Angliker, H.; Anagli, J.; Shaw, E. *J. Med. Chem.* **1992**, *35*, 216.

9. Crawford, C.; Mason, R. W.; Wickstrom, P.; Shaw, E. *Biochem. J.* **1988**, *253*, 751.

10. McGowan, E. B.; Becker, E.; Detwiler, T. C. *Biochem. Biophys. Res. Commun.* **1989**, *158*, 432.

11. Huang, Z.; McGowan, E. B.; Detwiler, T. C. *J. Med. Chem.* **1992**, *35*, 2048.

12. 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.

13. 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.

14. Hosoda et al. mentioned Cbz–L–Pro–derived inhibitors in a patent application (Patent Application No. JP93–345753, 1993–12–27; Assignee: Fujirebio).

15. Synthetic details will be published in the full account of this work.

16. 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.

17. Kempf, D. J.; Molla, A.; Marsh, K. C.; Park, C.; Rodrigues, A. D.; Korneyeva, M.; Vasavanonda, S.; McDonald, E.; Flentge, C. A.; Muchmore, S.; Wideburg, N. E.; Saldivar, A.; Cooper, A.; Kati, W. M.; Stewart, K. D.; Norbeck, D. W. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 699.