Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 2993–2996

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

General Solid-Phase Method to Prepare Novel Cyclic Ketone Inhibitors of the Cysteine Protease Cruzain

Lily Huang and Jonathan A. Ellman*

Center for New Directions in Organic Synthesis, Department of Chemistry, University of California, Berkeley, CA 94720, USA

Received 11 April 2002; accepted 25 June 2002

Abstract—A series of constrained ketone-based inhibitors has been developed that show low nanomolar K_i values. These ketone inhibitors showed promising activity towards cruzain, the cysteine protease implicated in Chagas' disease. This series of constrained inhibitors, which can be accessed quickly and efficiently using a solid-phase combinatorial strategy, should be applicable to other members of the cysteine protease class.

Cysteine proteases, which are characterized by an active-site cysteine thiol that attacks the carbonyl of an amide bond,[1] are essential to many biological processes. This class of proteases includes the calpains, which have been implicated in neurodegenerative disorders, cathepsin K, which has been linked to osteoporosis,[2] and the caspase family of proteases, which is involved in programmed cell death.[3] In addition, cysteine proteases are also crucial to the life cycles of many pathogenic protozoa.[4,5] The clear therapeutic relevance of cysteine proteases has resulted in substantial effort to develop novel and selective inhibitors of these enzymes.[6]

A common feature of virtually all cysteine protease inhibitors is an electrophilic functionality, such as a carbonyl or Michael acceptor, which is attacked by the cysteine thiol (Fig. 1).[7] The first class of reversible inhibitors to be reported was peptidyl aldehydes. However, the inherent reactivity of the aldehyde pharmacophore to nucleophilic attack and oxidation are considerable liabilities for achieving good pharmacokinetics and might result in toxicity. In addition, aldehyde-based inhibitors only allow display of functionality on one side of the carbonyl. In contrast, ketone-based pharmacophores are chemically more stable, and enable the display of functionality on both sides of the carbonyl to potentially achieve enhanced specificity through multiple interactions with the active site.

Highly potent and selective ketone-based reversible inhibitors have been identified for a variety of cysteine proteases.[1,8] In particular, Veber and co-workers have identified potent and highly selective reversible ketone-based inhibitors of cathepsin K,[9] such as **1** and **2** (Chart 1), which are efficacious in the treatment of osteoporosis in animal models of this disease. We have recently described the identification of potent and selective mercaptomethyl ketones, such as **3**, towards cruzain,[10] the cysteine protease implicated in Chagas' disease. Efforts in our laboratory have focused on cruzain since McKerrow and co-workers have demonstrated the therapeutic promise of inhibitors of cruzain for the treatment of Chagas' disease.[11]

We have previously reported the design and synthesis of a series of ketone-based inhibitors using solid-phase synthesis. Herein we expand that methodology to provide a series of constrained ketone-based cysteine protease inhibitors. Constrained ketone-based inhibitors, such as **1**, have previously been shown to be potent and selective inhibitors of cathepsin K by Veber and co-workers.[12]

The synthesis of constrained inhibitors **4** is detailed in Scheme 1. The scaffold is derived from *N*-allyloxycarbonyl (Alloc)-aspartic acid (β-benzyl ester) chloromethyl ketone, which is synthesized in a one-pot procedure[13] from the corresponding *N*-Alloc amino acid. The chloromethyl ketone scaffold **6** is attached to support using a hydrazine linker **5** to provide support-bound hydrazone **7**. The hydrazone not only provides the site of attachment to the solid support but also protects the carbonyl from nucleophilic attack. Displacement of

*Corresponding author. Fax: +1-510-642-8369; e-mail: jellman@uclink.berkeley.edu

0960-894X/02/$ - see front matter © 2002 Elsevier Science Ltd. All rights reserved.
PII: S0960-894X(02)00619-4

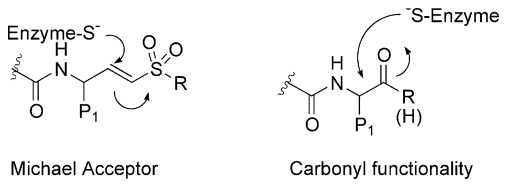


Figure 1. Cysteine protease pharmacophores.

the chloride with primary amines introduces the R^1 side chain. The resulting secondary amine **8** cyclizes upon heating at 60 °C for 24 h. Further functionalization can be achieved by removal of the Alloc protecting group from **9** followed by acylation with a carboxylic acid. The compounds were cleaved off support under mild acidic conditions to give the constrained inhibitors **4**. After column chromatography, the inhibitors were isolated in 20–55% overall yield for the six-step sequence.

In contrast to the isolation of acyclic amidomethyl ketones and mercaptomethyl ketones where no epimerization is observed, the constrained cyclic ketones are obtained with $\geq$20% epimerization. This is consistent with the observations of Marquis that cyclic ketone inhibitors of cathepsin K (e.g., **1** in Chart 1) are not configurationally stable and readily epimerize.[12]

A series of fully functionalized ketones was synthesized using the sequence outlined in Scheme 1. Cbz was incorporated in the P_3 and Phe in the P_2 position based upon SAR from prior inhibitor efforts.[10,14–17] A collection of side chains at R^1 were chosen based on the previous SAR obtained from both mercaptomethyl ketone libraries[10] and amidomethyl ketone libraries[18] synthesized in this laboratory. These compounds were screened against cruzain and the results are summarized in Table 1. Interestingly, the most active cyclic inhibitors,

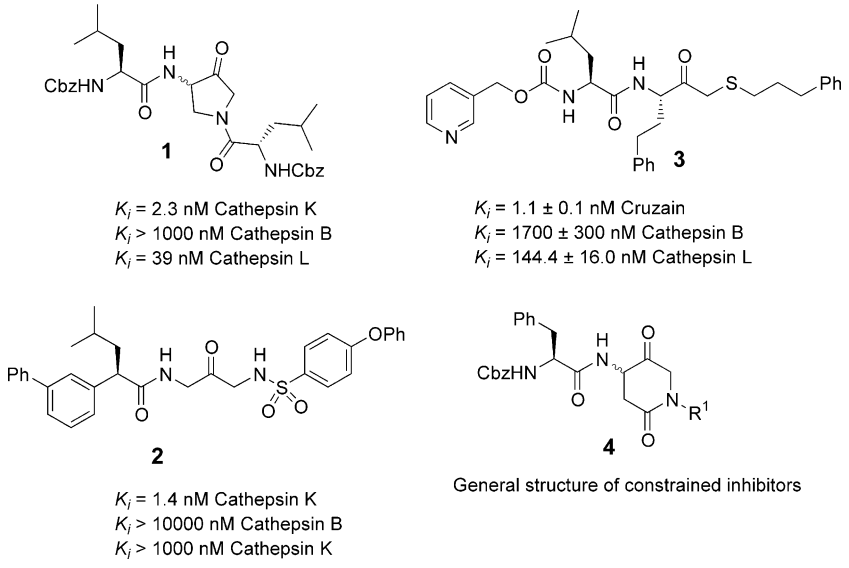
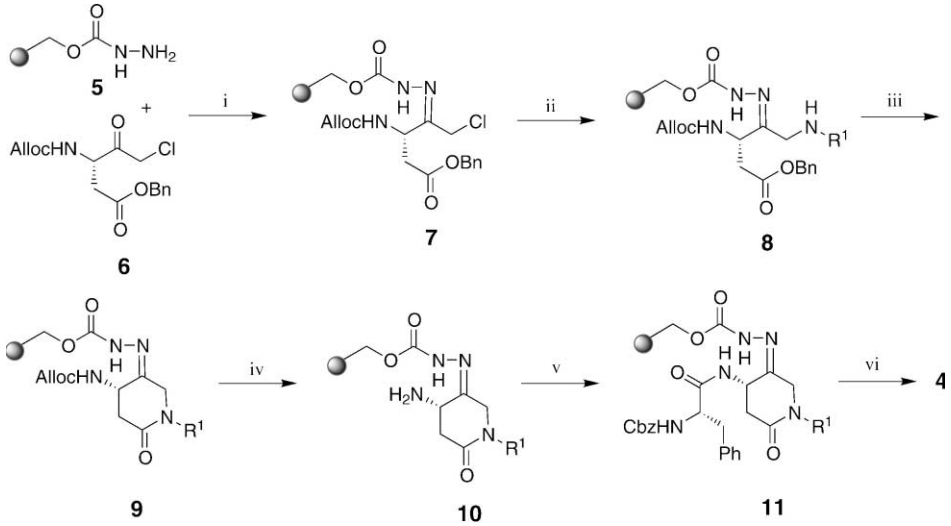


Chart 1.



Scheme 1. (i) THF, 45–50 °C; (ii) NH_2R^1, DMF; (iii) DMF, 60 °C, 24 h; (iv) 8:3 $TMSN_3$/TBAF, $Pd(PPh_3)_4$, CH_2Cl_2; (v) Cbz-L-Phe-OH, PyBOP, HOAt, *i*-Pr_2EtN, DMF; (vi) TFA, H_2O, CF_3CH_2OH.

Table 1. Cyclized ketone inhibitors

Inhibitor	R^1	% Yield[a]	Diastereomer ratio[b]	Cruzain K_i (nM)[c]	Cathepsin B K_i (nM)[c]	Cathepsin L K_i (nM)[c]
4a	$-CH_2CH_2CH_3$	38	2.1:1	366±30	10,510±1000	232±15
4b	$-CH(CH_3)_2$	20	4.3:1	384±20	12,920±1500	597±31
4c	$-(CH_2)_2OCH_3$	55	2.9:1	983±90	>10,000	865±53
4d	$-CH_2CH(CH_3)_2$	41	1.5:1	274±20	4550±400	299.4±25
4e	$-(CH_2)_3Ph$	37	1.1:1	16±0.4	693±37	19±1
4f	$-(CH_2)_2Ph$	39	1.1:1	53±2	6170±290	93±3
4g	$-CH_2Ph$	55	2.4:1	202±8	13,220±730	377±20

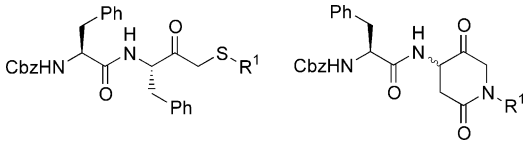
[a]Overall yield of purified product for six steps.
[b]Diastereomer ratio determined by ^1H NMR analysis.
[c]K_i values determined at four to eight concentrations in duplicate using the linear regression analysis of Williams and Morrison.[19]

4e–**4g**,[20] incorporated the side chains present in the most potent mercaptomethyl ketones. In addition, the SAR of the side chains was consistent with the SAR of the previously reported mercaptomethyl ketones (Table 2). For example, the most potent cyclized ketone [$R^1 = -(CH_2)_3Ph$, $K_i = 16.3$ nM] contained the same side chain as the most potent mercaptomethyl ketone [$R^1 = -(CH_2)_3Ph$, $K_i = 2.0$ nM].

To probe the selectivity of these inhibitors for cruzain over human cysteine proteases, the cyclized inhibitors were screened against human cathepsin B and human cathepsin L. These compounds were especially poor inhibitors of cathepsin B (Table 1), with most compounds having K_i values in the μM range. For cathepsin L, the cyclized inhibitors shared similar K_i values with cruzain, with the most potent inhibitor again containing the phenylpropyl side chain.

The potency and selectivity of these inhibitors is quite promising, since in contrast to the acyclic mercaptomethyl ketone inhibitors, the constrained inhibitors do not display functionality that would interact with the S_1 pocket of the enzyme. Indeed, substrate specificity studies indicate that small amino acids, such as glycine, alanine and threonine, are least preferred at the P_1 position, while the basic amino acids, arginine and lysine, are most preferred.[21] In addition, inhibitor design studies have demonstrated that quite large substituents, such as the hPhe and Phe side chains, are well accommodated by the enzyme in the S_1 pocket.[10] Further efforts to improve the potency and selectivity of the inhibitors, in particular by introducing functionality on the piperidone ring to interact with the S_1 pocket, is currently in progress.

Table 2. SAR of mercaptomethyl ketones and cyclized ketones



R^1	Cruzain K_i (nM)[a]	Cruzain K_i (nM)[a]
$-(CH_2)_3Ph$	2.0±0.2	16.3±0.4
$-(CH_2)_2Ph$	4.2±0.2	53.2±1.7
$-CH_2Ph$	44.0±2.6	202±8

[a]K_i values determined at 4–8 concentrations in duplicate using the linear regression analysis of Williams and Morrison.[19]

In this paper we have disclosed a new series of constrained inhibitors of the cysteine protease cruzain that have low nanomolar K_i values. This series of constrained inhibitors, which can be accessed quickly and efficiently using a solid-phase combinatorial strategy, should be applicable to other members of the cysteine protease class.

Acknowledgements

The authors acknowledge support from the NIH (Grant GM 54051) and the Burroughs Wellcome Fund (New initiatives in Malaria Award). Dr. J. H. McKerrow and E. Hansell are also gratefully acknowledged for supplying cruzain. The Center for New Directions in Organic Synthesis is supported by Bristol-Myers Squibb as a Sponsoring Member and Novartis as a Supporting Member.

References and Notes

1. For a review, see: Otto, H.-H.; Schirmeister, T. *Chem. Rev.* **1997**, *97*, 133.
2. Bossard, M. J.; Tomaszek, T. A.; Thompson, S. K.; Amegadzie, B. Y.; Hanning, C. R.; Jones, C.; Kurdyla, J. T.; McNulty, D. E.; Drake, F. H.; Gowen, M.; Levy, M. A. *J. Biol. Chem.* **1996**, *271*, 12517.
3. Thornberry, N. A. *Chem. Biol.* **1998**, *5*, R97.
4. McKerrow, J. H.; Sun, E.; Rosenthal, P. J.; Bouvier, J. *Annu. Rev. Microbiol.* **1993**, *47*, 821.
5. McKerrow, J. H. *Perspec. Drug Discov. Des.* **1994**, *2*, 437.
6. McKerrow, J. H.; Engel, J. C.; Caffrey, C. R. *Bioorg. Med. Chem.* **1999**, *7*, 639.
7. See also the symposium in print on cysteine protease inhibitors featured in: *Bioorg. Med. Chem.* **1999**, *7*, 571.
8. Leung, D.; Abbenante, G.; Fairlie, D. P. *J. Med. Chem.* **2000**, *43*, 305.
9. For leading references, see: Marquis, R. W.; Ru, Y.; LoCastro, S. M.; Zeng, J.; Yamashita, D. S.; Oh, H.-J.; Erhard, K. F.; Davis, L. D.; Tomaszek, T. A.; Tew, D.; Salyers, K.; Proksch, J.; Ward, K.; Smith, B.; Levy, M.; Cummings, M. D.; Haltiwanger, R. C.; Trescher, G.; Wang, B.; Hemling, M. E.; Quinn, C. J.; Cheng, H.-Y.; Lin, F.; Smith, W. W.; Janson, C. A.; Zhao, B.; McQueney, M. S.; D'Alessio, K.; Lee, C.-P.; Marzulli, A.; Dodds, R. A.; Blake, S.; Hwang, S.-M.; James, I. E.; Gress, C. J.; Bradley, B. R.; Lark, M. W.; Gowen, M.; Veber, D. F. *J. Med. Chem.* **2001**, *44*, 1380.

10. Huang, L.; Lee, A.; Ellman, J. A. *J. Med. Chem.* **2002**, *45*, 676.
11. Engel, J. C.; Doyle, P. S.; Hseih, I.; McKerrow, J. H. *J. Exp. Med.* **1998**, *188*, 725.
12. Marquis, R. W.; Ru, Y.; Zeng, J.; Trout, R. E. L.; LoCastro, S. L.; Gribble, A. D.; Witherington, J.; Fenwick, A. E.; Garnier, B.; Tomaszek, T.; Tew, D.; Hemling, M. E.; Quinn, C. J.; Smith, W. W.; Zhao, B.; McQueney, M. S.; Janson, C. A.; D'Alessio, K.; Veber, D. F. *J. Med. Chem.* **2001**, *44*, 725.
13. Krantz, A.; Copp, L. J.; Coles, P. J.; Smith, R. A.; Heard, S. B. *Biochemistry* **1991**, *30*, 4678.
14. Franke de Cazzulo, B. M.; Martinez, J.; North, M. J.; Coombs, G. H.; Cazzulo, J.-J. *FEMS Microbiol. Lett.* **1994**, *124*, 81.
15. Meirelles, M. N. L.; Juliano, L.; Carmona, E.; Silva, S. G.; Costa, E. M.; Murta, A. C. M.; Scharfstein, J. *Mol. Biochem. Parasitol.* **1992**, *52*, 175.
16. Palmer, J. T.; Rasnick, D.; Klaus, J. L.; Bromme, D. J. *J. Med. Chem.* **1995**, *38*, 3193.
17. Roush, W. R.; Gwaltney, S. L., II; Chen, J.; Scheidt, K. A.; McKerrow, J. H.; Hansell, E. *J. Am. Chem. Soc.* **1998**, *120*, 10994.
18. Lee, A.; Huang, L. University of California at Berkeley, unpublished results.
19. Williams, J. W.; Morrison, J. F. *Methods Enzymol.* **1979**, *63*, 437.
20. **Synthesis of 4e.** A solution of *N*-allyloxycarbonyl aspartic acid (β-benzyl ester) chloromethyl ketone (4 equiv, 0.109 g, 0.32 mmol) in 1.6 mL of THF was added to carbazate linker **5** (Lee, A.; Huang, L.; Ellman, J. A. *J. Am. Chem. Soc.* **1999**, *121*, 9907) (0.2 g, 0.4 mmol/g) presolvated in THF. After 6 h at 45–50 °C, the solution was removed, and the resin was rinsed with THF (5×5 mL). A solution of 3-phenyl-1-propylamine (0.57 mL, 4 mmol, 50 equiv) in 2 mL of DMF was added to the resin. The cartridge was gently rocked on the shaker table for 15 min. After filtration of the solution, the resin was rinsed with DMF (3×5 mL) and anhydrous DMF (2×5 mL). The resin was subsequently cyclized by heating the resin in 2 mL of DMF for 24 h at 60–70 °C. Following this reaction, the resin was rinsed with DMF (3×5 mL) and CH_2Cl_2 (3×5 mL). A solution of trimethylsilyl azide (0.09 mL, 0.7 mmol, 8 equiv), tetrabutylammonium fluoride (0.063 g, 0.24 mmol, 3 equiv), and $Pd(PPh_3)_4$ (0.018 g, 0.16 mmol, 0.2 equiv) in 5 mL of CH_2Cl_2 was added to the resin in a glovebag under N_2 atmosphere. The resin was gently rocked for 4 h and was protected from light. After removal of the solution, the resin was rinsed with CH_2Cl_2 (5×5 mL) and DMF (5×5 mL). An acylation stock solution of Cbz–Phe–OH (0.119 g, 0.4 mmol, 5 equiv), *i*-Pr_2EtN (0.14 mL, 0.8 mmol, 10 equiv), HOAt (0.054 g, 0.4 mmol, 5 equiv), and PyBOP (0.208 g, 0.4 mmol, 5 equiv) in 2 mL of DMF was prepared immediately before addition. The acylation solution was added to the resin, and the cartridge was gently rocked for 4 h. Upon removal of the solution, the resin was rinsed with DMF (5×5 mL) and the acylation reaction sequence was repeated. After excess reagents were filtered, the resin was rinsed with DMF (5×5 mL) and THF (5×5 mL) and dried overnight under vacuum. The material was cleaved using a 5 mL solution of 1:4:15 TFA/H_2O/trifluoroethanol. After the mixture sat at room temperature for 2–4 h, the solution was removed. The resin was washed with THF (3×5 mL), and the washings were combined and concentrated. Toluene was added to form an azeotrope with the residual water and TFA. The desired inhibitor was formed in a 37% yield, as determined by mass balance after purification by column chromatography. IR: 1659, 1710, 1727 cm^{-1}. ^1H NMR (500 MHz): δ 1.82–1.90 (m, 2H), 2.12–2.40 (m, 1H), 2.62–2.65 (m, 2H), 3.02–3.11 (m, 3H), 3.39–3.43 (m, 2H), 3.78–3.94 (m, 2H), 4.48–4.63 (m, 2H), 5.07 (s, 2H), 5.36–5.47 (m, 1H), 6.46–6.66 (m, 1H), 7.16–7.36 (m, 15H); ^{13}C NMR (125 MHz): δ 28.03, 28.06, 33.02, 33.03, 35.50, 35.63, 38.21, 38.65, 46.12, 46.16, 52.76, 52.94, 54.85, 54.89, 56.0, 67.12, 67.18, 126.13, 127.16, 127.17, 128.00, 128.16, 128.20, 128.22, 128.48, 128.51, 128.51, 128.75, 128.76, 129.23, 135.8, 136.0, 140.91, 140.93, 155.9, 166.92, 167.07, 170.8, 199.91, 200.08. FABHRMS: 528.2498 (MH^+, $C_{31}H_{33}N_3O_5$ requires 528.2501).
21. Harris, J. L.; Backes, B. J.; Leonetti, F.; Mahrus, S.; Ellman, J. A.; Craik, C. J. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 7754.