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Potent Second Generation Vinyl Sulfonamide Inhibitors of the Trypanosomal Cysteine Protease Cruzain

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Abstract—A new family of potent *N*-alkoxyvinylsulfonamide inhibitors of cruzain have been developed. Inhibitor **13** has a second order inactivation rate constant of 6,480,000 $s^{-1} M^{-1}$ versus cruzain, and is also highly effective against *Trypanosoma cruzi* trypomastigotes in a tissue culture assay.

Cysteine proteases are an important class of enzymes involved in the degradative processing of peptides and proteins.[1,2] They are ubiquitous in nature and play vital roles in numerous physiological processes including arthritis, osteoporosis, Alzheimer's disease, cancer cell invasion, and apoptosis.[1–5] Cysteine proteases are also essential to the life cycles of many pathogenic protozoa.[6–8] Consequently, considerable effort has been devoted to the development of novel and selective inhibitors of cysteine proteases.[2,9–11]

Cruzain,[12,13] the major cysteine protease of *Trypanosoma cruzi*, has been identified as a potential therapeutic target for treatment of Chagas' disease.[13–15] The McKerrow group has reported an experimental cure of Chagas' disease in a mouse model using the vinyl sulfone inhibitors **1** and **2** (Fig. 1) which target cruzain.[16] Similarly falcipain,[17] a cysteine protease isolated from *Plasmodium falciparum*, one of the major malaria parasites, and the cathepsin B-like cysteine protease from *Leishmania major*,[18] one of the parasites that causes leishmaniasis, have also been identified as potential chemotherapeutic targets.[18,19]

We recently described the synthesis of vinyl sulfonate ester **3**, which has a second order inactivation constant of $>14{,}000{,}000\ s^{-1} M^{-1}$ against cruzain.[20] In comparison, the phenyl vinyl sulfones **1** and **2** have second order inactivation constants of 181,000–420,000 $s^{-1} M^{-1}$.[21] Although the *N*-phenyl sulfonamide **4** also is a relatively weak cruzain inhibitor (242,000 $s^{-1} M^{-1}$), the benzyl vinyl sulfone **5** proved to be quite potent versus cruzain (1,400,000 $s^{-1} M^{-1}$).[20,22] Unfortunately, all of the inhibitors from this earlier study, including **3**–**5**, were inactive against *T. cruzi* in tissue culture assays.

In view of these results, we sought to develop second generation inhibitors with improved activity in in vitro studies. Taking a lead from the success realized with **1** and **2**,[16] we decided to incorporate the P_3 morpholinyl and *N*-methylpiperazinyl urea residues in second generation inhibitors. These groups contributed greatly to the increased in vivo activity of **1** and **2** as well as to the oral bioavailability of **2**, compared to the analogous inhibitor containing a Cbz group.[16] Accordingly, we

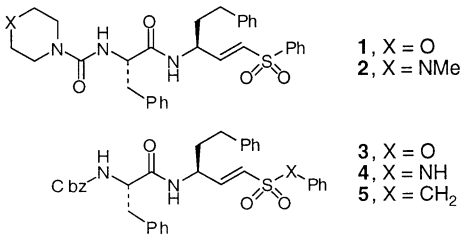

Figure 1.

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initially targeted **6**–**11** (Fig. 2) which were prepared by appropriate modifications of our syntheses of **3**–**5**.

Second order rate constants[23–25] for inactivation of cruzain by inhibitors **1**–**11** are summarized in Table 1. These data establish that the P_3 substituent has a significant influence on the activity of these compounds. For example, while the phenyl vinylsulfonates **3** and **6** are comparably active against cruzain, inhibitor **7** with a terminal *N*-methylpiperazinyl unit is more than an order of magnitude less potent. Similarly, the *N*-phenyl vinylsulfonamide **4** with a terminal P_3 Cbz group is a relatively weak inhibitor of cruzain, but compound **8** with a P_3 morpholinyl urea residue is approximately 20-fold more potent. Only for the benzyl sulfone inhibitors **5**, **10**, and **11** does the P_3 residue play an insignificant role in the activity against the enzyme. Additional studies designed to probe the P_1' and P_3 structural requirements of the vinyl sulfone, vinylsulfonamide and vinylsulfonate ester cysteine protease inhibitors will be pursued via a combinatorial strategy.

Results of assays of inhibitors **3**–**11** against *T. cruzi* in the J744 macrophage culture system[16] are summarized in Table 2. Although very potent cruzain inhibitors from three different structural classes (vinyl sulfonate esters, vinyl sulfonamides, and vinyl sulfones) were examined, only one—benzyl vinyl sulfone **10**—exhibited significant activity in the cell culture assay. However, **10** still was not as effective as the original lead inhibitor **1**.

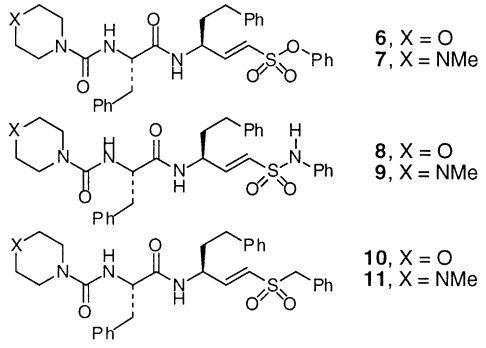

Figure 2.

Table 1. Second order inactivation constants for compounds **1**–**13** versus cruzain[a,b]

Compd	Second order inactivation constants (s^{-1} M^{-1})
1	181,000 ± 12,500
2	419,500 ± 87,500
3	16,800,000 ± 340,000
4	242,000 ± 84,000
5	1,400,000 ± 225,000
6	10,200,000 ± 4,320,000
7	720,000 ± 120,000
8	4,920,000 ± 660,000
9	1,260,000 ± 190,000
10	1,670,000 ± 360,000
11	3,850,000 ± 930,000
12	2,270,000 ± 230,000
13	6,480,000 ± 1,680,000

[a]It was not possible to demonstrate saturation kinetics for these inhibitors, owing to the range of inhibitor concentrations that could be studied. Consequently, inactivation rate constants are reported as k_{ass} values (see refs 23–25).
[b]Results reported here for **3**–**5** differ slightly compared to previously published data; see ref 22.

During the course of these studies, X-ray structures of inhibitors **1**, **5**, and the *p*-nitrophenyl sulfonate ester analogue of **3** (and ultimately also of **13**) covalently bound in the active site of cruzain were solved.[26] These structures established the mechanism of action of these inhibitors as Michael acceptors for the active site cysteine, and also show that one of the sulfonyl oxygen atoms participates in a hydrogen bond with the indole N–H of tryptophan-177, which defines the floor of the S_1'–S_2' region of the active site. Reasoning that the electron density at sulfonyl oxygen contributes to the strength of this hydrogen bond, and also that the partial positive character at the sulfonyl sulfur atom contributes to the reactivity of these inhibitors as Michael acceptors for the active site thiol (Cys-25), we suspected that the aryl sulfonate esters represented by **3**, **6**, and **7** would be better Michael acceptors than the *N*-phenyl sulfonamides (**4**, **8**, and **9**), whereas the *N*-aryl sulfonamides should be better hydrogen bond acceptors than the sulfonate esters. Because both properties—binding constant and reactivity as Michael acceptors—presumably contribute to the potency of these compounds as enzyme inhibitors, we targeted several additional structures whose electronic properties at the sulfonyl group would be intermediate between those of the sulfonate esters and sulfonamides. Specifically, we identified the *N*-sulfonyl hydroxylamine derivatives **12** and **13** as potential inhibitor structures. The possibility that the *N*-sulfonyl hydroxylamine unit might be partially ionized at physiological pH,[27] leading to improved solubility characteristics, was viewed as another potential advantage to this structural motif. *N*-Alkoxysulfonamides have found application in herbicides,

Table 2. Results of in vitro assays of inhibitors versus *T. cruzi*

Compd	Survival of *T. cruzi* treated J744 macrophage[a,b] (days)
None	6 (control)
1	>24
2	>24
3	Toxic
4	6
5	6
6	Toxic
7	9
8	9
9	9
10	20
11	13
12	>24
13	>24

[a]Effect of inhibitors on survival of J744 macrophages infected with *T. cruzi* trypomastigotes, treated daily with a solution of inhibitor (10 μM). Survival time is defined as the time before the cell monolayer is destroyed by the infection.[16] Untreated cells are completely destroyed after 6 days in this assay.
[b]Inhibitors **3** (30 μM) and **6** had no effect on the survival of the parasite-treated J744 cells. Control experiments established that **3** and **6** are not soluble under the assay conditions and that the insoluble inhibitor suspension is toxic to J744 cells.

antibiotics,[28] enzyme inhibitors,[27,29] and receptor agonists,[30] and the patent literature reveals a growing number of applications of this functional group in peptidomimetic enzyme inhibitors.[31] However, to the best of our knowledge, this is the first application of the *N*-alkoxy sulfonamide unit in an active site targeted, mechanism-based inhibitor design strategy.[32]

Inhibitors **12**–**13** were synthesized as summarized in Figure 3. Horner–Wadsworth–Emmons olefination of *N*-Boc-L-homophenylalanal (**14**) with phosphonate **15**[33,34] using NaH in THF provided vinyl sulfonate **16** in 65–87% yield, an improvement over the 59% yield previously obtained using BuLi in THF for this HWE reaction.[20] Exposure of **16** to *n*-Bu_4NI in acetone[34] followed by treatment of the tetrabutylammonium sulfonate salt with triphosgene and catalytic DMF in CH_2Cl_2 provided the vinylsulfonyl chloride **17** in 70–78% yield.[35] Sulfonyl chloride **17** was then converted to the vinyl *N*-sulfonyl hydroxylamine derivative **18** in 67% yield by treatment with *O*-benzylhydroxylamine and 2,6-lutidine in CH_2Cl_2. The efficiency of this step was compromised by the propensity of **17** to undergo competitive Michael addition of the hydroxylamine prior to acylation. Finally, deprotection of the Boc group and coupling of the resulting amine with phenylalanine derivatives **19**–**20** provided inhibitors **12** and **13**.

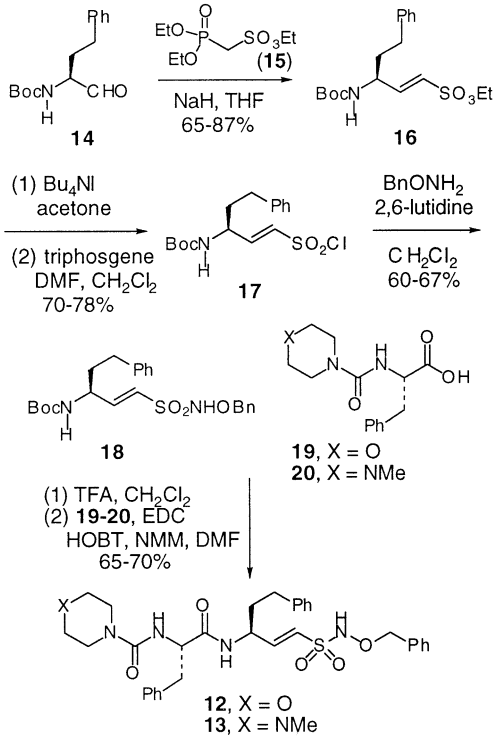

Figure 3.

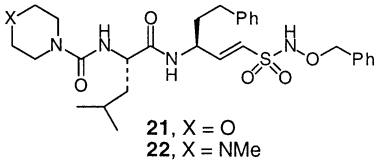

Figure 4.

Enzyme inhibition studies established that the dipeptidyl vinyl *N*-sulfonyl hydroxylamines **12**–**13** are potent, irreversible inhibitors of cruzain (see Table 1). Compound **12** is also potent inhibitor of the cathepsin B-like cysteine protease from *L. major*,[36] and **13** is an exceptionally potent inhibitor of rhodesain, the major cysteine protease isolated from *Trypanosoma brucei rhodesiense*, the causative agent of African sleeping sickness.[37] Two additional analogues in this series (**21** and **22**) are potent inhibitors of falcipain, a cysteine protease isolated from *P. falciparum* (Fig. 4).[38]

Significantly, *N*-alkoxy vinylsulfonamides **12** and **13** are excellent inhibitors of *T. cruzi* in the J744 macrophage cell culture assay system (Table 2). The results of this assay showed that at 10 μM concentration, inhibitors **12** and **13** were as effective as the reference compound **1** in inhibiting the growth of *T. cruzi*, as evidenced by the survival of the macrophages through the end of the experiment. One difference, however, is that parasites reappeared in the J744 cultures 4 days after the treatment with **12** and **13** was terminated, whereas no parasites reappeared in cultures treated with **1**.

In summary, we have established that the *N*-alkoxy vinylsulfonamides **12** and **13** are novel, potent, and highly effective inhibitors of cruzain, with significant activity in tissue culture experiments. Studies of **12** and **13** in mouse models of Chagas' disease are in progress, and additional congeners in the *N*-alkoxy vinylsulfonamides series are being examined as potential chemotherapeutic agents targeting *T. cruzi*, *T. brucei*, and *P. falciparum*. The results of these efforts will be reported in due course.

Acknowledgements

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References and Notes

1. McKerrow, J. H.; James, M. N. G. *Perspec. Drug Discov. Des.* **1996**, *6*, 1.
2. Otto, H.-H.; Schirmeister, T. *Chem. Rev.* **1997**, *97*, 133.
3. Miller, D. K. *Annu. Rep. Med. Chem.* **1996**, *31*, 249.
4. Thornberry, N. A.; Miller, D. K.; Nicholson, D. W. *Perspec. Drug Discov. Des.* **1995**, *2*, 389.
5. Nicholson, D. W.; Ali, A.; Thornberry, N. A.; Vaillancourt, J. P.; Ding, C. K.; Gallant, M.; Gareau, Y.; Griffin, P. R.; Labelle, M.; Lazebnik, Y. A.; Munday, N. A.; Raju, S. M.; Smulson, M. E.; Yamin, T.-T.; Yu, V. L.; Miller, D. K. *Nature* **1995**, *376*, 37.
6. McKerrow, J. H.; Sun, E.; Rosenthal, P. J.; Bouvier, J. *Annu. Rev. Microbiol.* **1993**, *47*, 821.
7. McKerrow, J. H. *Perspec. Drug Discov. Des.* **1994**, *2*, 437.
8. Robertson, C. D.; Coombs, G. H.; North, M. J.; Mottram, J. C. *Perspec. Drug Discov. Des.* **1996**, *6*, 99.
9. Rasnick, D. *Perspec. Drug Discov. Des.* **1996**, *6*, 47.

10. Rich, D. H. In *Comprehensive Medicinal Chemistry*; Sammes, P. G. Ed.; Pergamon: Oxford, 1990; Vol. 2; p 391.
11. Brown, W. M.; Dziegielewska, K. M. *Protein Sci.* **1997**, *6*, 5.
12. Eakin, A. E.; McGrath, M. E.; McKerrow, J. H.; Fletterick, R. J.; Craik, C. S. *J. Biol. Chem.* **1993**, *268*, 6115.
13. McGrath, M. E.; Eakin, A. E.; Engel, J. C.; McKerrow, J. H.; Craik, C. S.; Fletterick, R. J. *J. Mol. Biol.* **1995**, *247*, 251.
14. Harth, G.; Andrews, N.; Mills, A. A.; Engel, J. C.; Smith, R.; McKerrow, J. H. *Mol. Biochem. Parasitol.* **1993**, *58*, 17.
15. Serveau, C.; Lalmanach, G.; Juliano, M. A.; Scharfstein, J.; Juliano, L.; Gauthier, F. *Biochem. J.* **1996**, *313*, 951.
16. Engel, J. C.; Doyle, P. S.; Hsieh, I.; McKerrow, J. H. *J. Exp. Med.* **1998**, *188*, 725.
17. Rosenthal, P. J.; McKerrow, J. H.; Aikawa, M.; Nagasawa, H.; Leech, J. H. *J. Clin. Invest.* **1988**, *82*, 1560.
18. Selzer, P. M.; Chen, X. W.; Chan, V. J.; Cheng, M. S.; Kenyon, G. L.; Kunz, I. D.; Sakanari, J. A.; Cohen, F. E.; McKerrow, J. H. *Exp. Parasitol.* **1997**, *87*, 212.
19. Rosenthal, P. J. *Emerg. Infect. Dis.* **1998**, *4*, 49.
20. Roush, W. R.; Gwaltney, S. L.; Cheng, J., III; Scheidt, K. A.; McKerrow, J. H.; Hansell, E. *J. Am. Chem. Soc.* **1998**, *120*, 10994.
21. Palmer, J. T.; Rasnick, D.; Klaus, J. L.; Brömme, D. *J. Med. Chem.* **1995**, *38*, 3193.
22. Second order inactivation rate constants reported here for **3**–**5** differ slightly from the k_{ass} data reported previously,[20] owing to modifications of the kinetic assay procedure that was used (see ref 25).
23. Beith, J. G. *Methods Enzymol.* **1995**, *248*, 59.
24. Tian, W.-X.; Tsou, C.-L. *Biochemistry* **1982**, *21*, 1028.
25. Kinetic analyses were performed as follows. Cruzain (2 nM) in 100 μL of assay buffer was added to inhibitor dilutions in 100 μL of 5 μM Z-Phe-Arg-AMC ($K_m = 1$ μM) in buffer A. Progress curves were obtained for 5 min at room temperature (less than 5% of substrate consumed) with tenfold dilutions of inhibitor, starting at 10 μM. Inhibitor dilutions which gave simple exponential progress curves over a wide range of k_{obs} were used to determine kinetic parameters. The value of k_{obs}, the rate constant for loss of enzyme activity, was determined from an equation for pseudo first order dynamics using UltraFit (Biosoft) or Prism 3.16 (GraphPad). When k_{obs} varied linearly with inhibitor concentration, k_{ass} was determined by linear regression analysis. If the variation was hyperbolic, indicating saturation inhibition kinetics, k_{inact} and K_i were determined from an equation describing a two-step irreversible inhibitor mechanism [$k_{obs} = k_{inact}$ [I]o/([I]o + K_i* (1 + [S]o/K_m))] and non-linear regression analysis using UltraFit or Prism (see ref 23).
26. Brinen, L. S.; Hansell, E.; Cheng, J.; Roush, W. R.; McKerrow, J. H.; Fletterick, R. J. *Structure* **2000**, *8*, 831.
27. Briganti, F.; Pierattelli, R.; Scozzafava, A.; Supuran, C. T. *Eur. J. Med. Chem.* **1996**, *31*, 1001.
28. Mewshaw, R. E.; Commons, T. J. *J. Antibiot.* **1987**, *40*, 1563.
29. Nagasawa, H. T.; Kawle, S. P.; Eberling, J. A.; DeMaster, E. G.; Fukuto, J. M. *J. Med. Chem.* **1995**, *38*, 1865.
30. Shue, H.-J.; Chen, X.; Blythin, D. J.; Carruthers, N. I.; Spitler, J. M.; Wong, S.-C.; Chapman, R. W.; Rizzo, C.; West, R.; She, H. S. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1709.
31. (a) See, for example: Serine and cysteine protease inhibitors: Chatterjee, S.; Mallamo, J. P.; Bihovsky, R.; Wells, G. J. *Chem. Abs.* **1999**, *130*, 312099. (b) HIV aspartyl protease: Sherrill, R. G.; Hale, M. R.; Spaltenstein, A.; Furfine, E. S.; Andrews, C. W.; Lowen, G. T. *Chem. Abs.* **2000**, *132*, 49801. (c) Aspartyl protease inhibitors: Hale, M. R.; Tung, R.; Price, S.; Wilkes, R. D.; Schairer, W. C.; Jarvis, A. N.; Spaltenstein, A.; Furfine, E. S.; Samano, B.; Kaldor, I.; Miller, J. F.; Beriger, M. S. *Chem. Abs.* **2001**, *134*, 56676. (d) MEK inhibitors: Bridges, A. J.; Booth, R. J.; Tecle, H.; Scaggs, Y.; Kaufman, M.; Barrett, S. D.; Dixon, A.; Lee, K.; Pinnock, R. D. *Chem. Abs.* **2001**, *134*, 131318.
32. (a) *O*-Acyl hydroxamates are established cysteine protease inhibitors: Robinson, V. J.; Coles, P. J.; Smith, R. A.; Krantz, A. *J. Am. Chem. Soc.* **1991**, *113*, 7760. (b) Brömme, D.; Schierhorn, A.; Kirschke, H.; Wiederanders, B.; Barth, A.; Fittkau, S.; Demuth, H.-U. *Biochem. J.* **1989**, *263*, 861.
33. Carretero, J. C.; Demillequand, M.; Ghosez, L. *Tetrahedron* **1987**, *43*, 5125.
34. Musicki, B.; Widlanski, T. S. *Tetrahedron Lett.* **1991**, *32*, 1267.
35. Reynolds, R. C.; Crooks, P. A.; Maddry, J. A.; Akhtar, M. S.; Montgomery, J. A.; Secrist, J. A. *J. Org. Chem.* **1992**, *57*, 2983.
36. Hansell, E.; McKerrow, J. H.; Roush, W. R. Unpublished research results.
37. Caffrey, C.; Hansell, E.; McKerrow, J. H.; Roush, W. R. Unpublished research results.
38. Rosenthal, P. J.; Roush, W. R.; Neitz, R. J. Unpublished research results.