Vinyl Sulfonate Esters and Vinyl Sulfonamides: Potent, Irreversible Inhibitors of Cysteine Proteases

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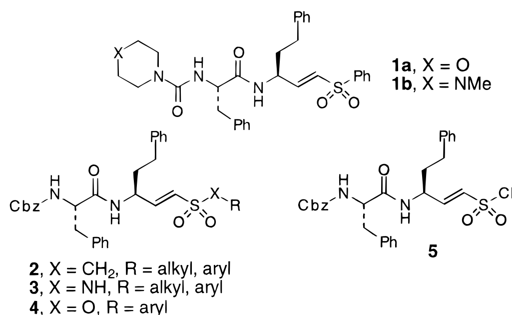
Cysteine proteases are an important class of enzymes involved in the degradative processing of peptides and proteins.[1,2] They are ubiquitous in nature and play vital roles in numerous physiological processes including arthritis, osteoporosis, Alzheimer's disease, cancer cell invasion, and apoptosis.[1−3] Cysteine proteases are also essential to the life cycles of many pathogenic protozoa.[4,5] One such parasite is *Trypanosoma cruzi*, the etiologic agent of Chagas' disease. Cruzain,[6,7] the major cysteine protease of *T. cruzi*, has been identified as a potential therapeutic target for treatment of Chagas' disease.[7−9]

Several strategies have been pursued in the design of cysteine protease inhibitors.[2,10,11] Peptidyl aldehydes,[12] diamino ketones,[13] and nitriles[14] are reversible inhibitors that form hemithioacetals, peptide ketals, and thioimidates, respectively, with the thiol of the active site cysteine residue, mimicking the initial covalent enzyme adduct in normal proteolytic turnover. Epoxysuccinyl derivatives,[15] peptidyl Michael acceptors,[16−18] (acyloxy)methyl ketones,[19] and halomethyl ketones are examples of inhibitors which irreversibly inactivate cysteine proteases via alkylation of the active site cysteine residue. Several classes of nonpeptidic reversible inhibitors of cysteine proteases also have been described.[20−22]

In connection with efforts to develop potent and selective inhibitors of cruzain, we became interested in the vinyl sulfone inhibitor series first introduced by Hanzlik[17] and further developed by Palmer et al.[18,23] Compound **1a** is a potent and selective inhibitor of cruzain, with a second-order rate constant (k_{inact}/K_i) of 203 000 s^{-1} M^{-1}.[18] Inhibitors **1a** and especially **1b** have also proven highly effective against *T. cruzi*, both in tissue culture and in vivo experiments (mouse model).[24] Although considerable effort has been devoted to the optimization of interactions of inhibitors with the cruzain S_1 and S_2 binding sites,[18,23,25] virtually nothing is known about the interactions of substrates or inhibitors with the S_1' and S_2' sites. The prime site region in cruzain contains a large open surface defined by Trp 177, and available X-ray structures suggest that there is considerable room for prime site inhibitor binding.[7,13,22,25] A recent X-ray structure of cathepsin K, the active site of which is homologous to that of cruzain, with covalently bound APC3328, a dipeptidyl phenyl vinyl sulfone inhibitor related to **1b**, reveals that the phenyl residue of the phenyl sulfonyl unit does not make optimal interactions with prime site residues.[26] Accordingly, we decided to probe the possibility that additional selectivity and potency in the vinyl sulfonyl series could be achieved by extending the inhibitor structure into the prime site region, via modification of the sulfonyl substituent as suggested by structure **2**. However, we anticipated that it might be easier to synthesize a family of vinyl sulfonamides **3** or vinyl sulfonate esters **4**, using the vinyl sulfonyl chloride **5** as a common precursor. Vinyl sulfonamides are well established as peptidomimetics,[27,28] but we are unaware of any reports of their use as inhibitors of cysteine proteases. Vinyl sulfonate esters[29] and vinyl sulfonamides,[30,31] like vinyl sulfones,[32,33] are known to be excellent Michael acceptors.



Vinyl sulfonyl chloride **5** was synthesized by using the general sequence reported by Gennari.[27] Thus, Horner−Wadsworth−Emmons reaction of *N*-Boc-L-homophenylalanal (**6**) with triethyl α-phosphorylmethanesulfonate (**7**)[34,35] provided ethyl vinyl sul-

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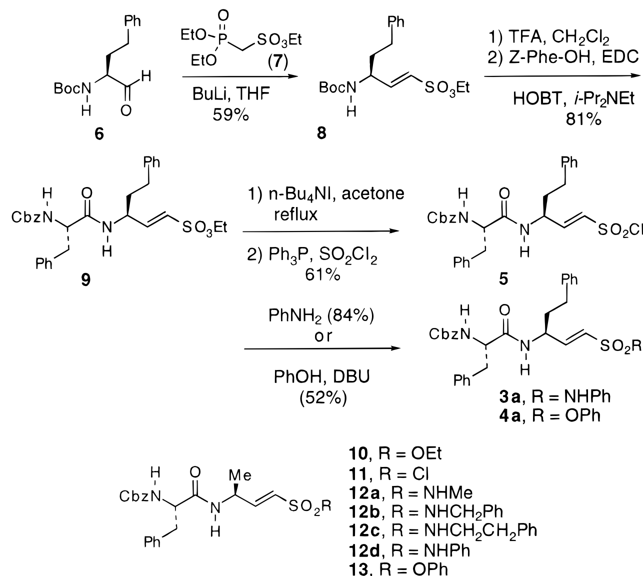
fonate **8**. Treatment of **8** with TFA in CH_2Cl_2 provided the corresponding amine, which was coupled with Z-Phe-OH, thereby giving **9** in 81% overall yield. Treatment of **9** with *n*-Bu_4NI in refluxing acetone gave the corresponding tetrabutylammonium sulfonate, which was converted to the targeted sulfonyl chloride **5** via Widlanski's procedure.[36] A small series of vinyl sulfonamides and sulfonate esters were then prepared by treatment of **5** with the appropriate amine or phenol, as illustrated here by the synthesis of **3a** and **4a**. A series of vinyl sulfonamides and sulfonates **12** and **13** were similarly prepared by way of **10**, which was synthesized by the HWE olefination of Z-Phe-Ala-H with **7**.



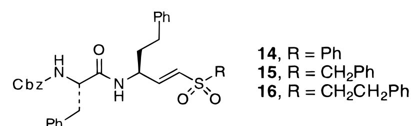
The vinyl sulfonate ester and vinyl sulfonamide inhibitors were screened vs cruzain (see Supporting Information for details). Kinetic analyses[37,38] were performed on the most interesting compounds in the series, which confirmed that the vinyl sulfonamides and sulfonate esters are time-dependent inhibitors of cruzain and several other cysteine proteases (see Table 1).[39] Compound **12b** is representative of the *N*-alkyl vinyl sulfonamides examined, which proved to be relatively weak inhibitors of cruzain. On the other hand, the simple sulfonate ethyl esters **9** and **10** proved to be much more potent, with **9** having a second-order inhibition rate constant of 112 000 s^{-1} M^{-1}. Because alkyl sulfonates are

Table 1. Second-Order Rate Constants for Inhibition of Cysteine Proteases[a]

inhibitor	inhibitor class	enzyme[b]	second-order inactivation rates (s^{-1} M^{-1})
12b	vinyl sulfonamide	cruzain	9700 ± 2000[c]
3a	vinyl sulfonamide	cruzain	289000 ± 6000[c]
9	vinyl sulfonate ester	cruzain	90600 ± 6000[c]
9	vinyl sulfonate ester	papain	4800 ± 1000[c]
9	vinyl sulfonate ester	cathepsin B	<1000
13	vinyl sulfonate ester	cruzain	5100000 ± 170000[d]
13	vinyl sulfonate ester	papain	343000 ± 33000[c]
13	vinyl sulfonate ester	cathepsin B	43700 ± 4400[c]
4a	vinyl sulfonate ester	cruzain	14280000 ± 1250000[c]
4a	vinyl sulfonate ester	papain	325000 ± 187000[d]
4a	vinyl sulfonate ester	cathepsin B	112200 ± 6400[d]
14	vinyl sulfone	cruzain	634000 ± 133000[d]
14	vinyl sulfone	papain	7000 ± 1100[c]
14	vinyl sulfone	cathepsin B	<2000
15	vinyl sulfone	cruzain	1956000 ± 116000[c]
16	vinyl sulfone	cruzain	149000 ± 15000[c]

[a] See Supporting Information for details of the kinetic analyses. [b] Cruzain: purified recombinant protein lacking the C terminal domain (ref 6). Papain: EC 3.4.22.2, Sigma. Cathepsin B: bovine spleen, EC 3.4.22.1, Sigma. [c] k_{ass} (see ref 37). [d] k_{inact}/K_i.

highly reactive alkylating agents, we decided to examine several aryl sulfonate esters—which should not be alkylating agents. We were delighted to discover that the phenyl vinyl sulfonates **13** (k_{inact}/K_i = 5 200 000 s^{-1} M^{-1}) and especially **4a** (k_{ass} = 14 000 000 s^{-1} M^{-1})[38] are extremely potent inhibitors of cruzain. To the best of our knowledge, **4a** ranks as the most potent cruzain inhibitor reported to date.[18] This observation prompted us to synthesize several *N*-aryl vinyl sulfonamides, of which **3a** proved to be an excellent inhibitor of cruzain (k_{ass} = 289 000 s^{-1} M^{-1}). It is interesting to note that throughout this series, the selectivity for inhibition of cruzain vs cathepsin B is at least 100-fold.



Comparative enzyme inhibition data are provided in Table 1 for phenyl vinyl sulfone **14**, benzyl vinyl sulfone **15**, and phenethyl vinyl sulfone **16**. These data show convincingly that the benzyl vinyl sulfone is the most potent of the three (k_{ass} = 1 956 000 s^{-1} M^{-1} for **15** vs cruzain).[38] This result, together with the data reported for vinyl sulfonate esters **4a** vs **9** and sulfonamides **3a** vs **12b**, indicates that a one-atom spacer between the sulfonyl unit and an aromatic ring is preferred, and that maximal activity is obtained when the spacer is an oxygen atom (e.g., phenyl vinyl sulfonate **4a**). Efforts to probe more fully the structural requirements of the sulfonate or sulfonamide units for maximal activity as cysteine protease inhibitors are in progress and will be reported in due course.

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Supporting Information Available: Experimental procedures for synthesis of **3a**, **4a**, **5**, and **8**−**16**; details of enzymatic kinetic assays of inhibitors **3a**, **4a**, **9**, **12b**, and **13**−**16**; and tables of percent inhibition of cruzain at different inhibitor concentrations (22 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

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(39) Cathepsin B and papain were screened for comparative purposes. Both enzymes are highly homologous to cruzain.