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Relative Rates of Michael Reactions of 2′-(Phenethyl)thiol with Vinyl Sulfones, Vinyl Sulfonate Esters, and Vinyl Sulfonamides Relevant to Vinyl Sulfonyl Cysteine Protease Inhibitors

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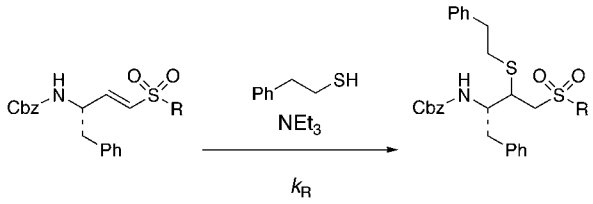
ABSTRACT



The relative rates of Michael additions of 2′-(phenethyl)thiol to representative vinyl sulfonyl Michael acceptors were measured. The dependence of the reactivity of the Michael acceptor on the nature of the sulfonyl R substituent was determined in order to evaluate the effect of these substituents on the inactivation kinetics of comparably substituted vinyl sulfonyl cysteine protease inhibitors. The rates of these Michael additions vary over 3 orders of magnitude, with phenyl vinyl sulfonate esters (R = OPh) being ca. 3000-fold more reactive than *N*-benzyl vinyl sulfonamides (R = NHBn).

Cysteine proteases are essential to the life cycles of the parasites that cause malaria, Chagas' disease, and leishmaniasis, making them attractive targets for the treatment of these diseases.[1−3] Numerous groups have made important contributions toward the development of cysteine protease inhibitors, and several classes of potent, irreversible inhibitors are now available (including those based on Michael acceptors that target the active site cysteine residue).[4,5] Following up on leads provided by the work of Hanzlik and Palmer,[6,7] our laboratory has developed potent irreversible vinyl sulfonyl inhibitors of the cysteine proteases associated with several parasites.[8−10] The active site cysteine reacts with the vinyl sulfonyl unit of the inhibitors by conjugate addition,[11] leading to irreversible binding of the inhibitor in the active

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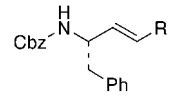


1, R = SO_2OPh
2, R = COMe
3, R = SO_2Ph
4, R = SO_2OEt
5, R = $SO_2N(Me)OBn$
6, R = SO_2NHOBn
7, R = CO_2Me
8, R = $SO_2N(Me)Bn$
9, R = SO_2NHBn

Figure 1. Michael acceptors employed in this study.

site.[12] This mechanism was unambiguously demonstrated for cruzain, the major cysteine protease associated with *Trypanosoma cruzi* (Chagas' disease), by three-dimensional structures of several enzyme−inhibitor complexes solved by X-ray crystallography, which show the active site Cys-25 covalently bound in 1,4-fashion with respect to the vinyl sulfonyl group of the inhibitor.[12]

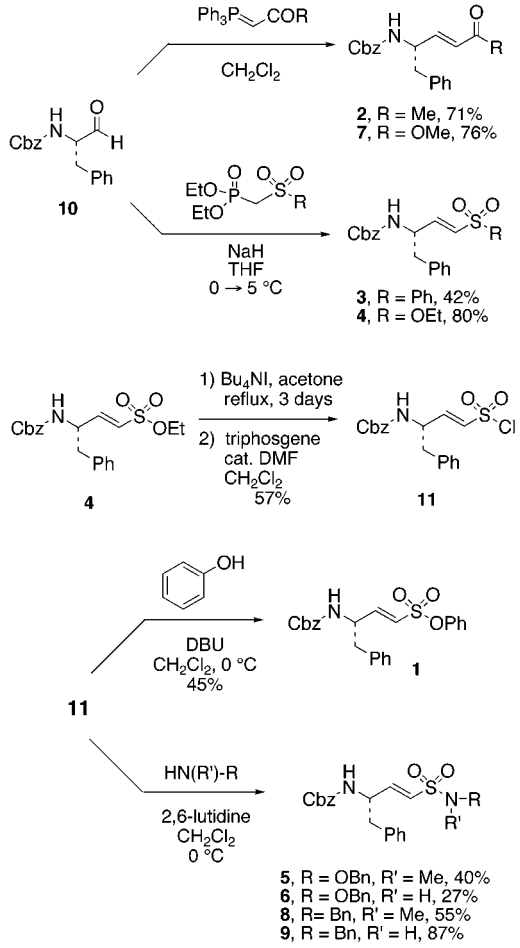
Several vinyl sulfonyl inhibitors exhibit strikingly potent inhibition kinetics against cruzain, while also demonstrating potent *in vivo* activities against *T. cruzi*.[8,9] In fact, one vinyl sulfone inhibitor targeting cruzain is capable of curing mice infected by *T. cruzi*.[13] A continuing goal of our collaboration with the McKerrow and Rosenthal groups at UCSF is to develop viable therapeutics for Chagas' disease and malaria by designing improved inhibitors that have better bioavailability, lower host toxicity, and increased selectivity for the parasites.

To understand the structural effects that give rise to very potent inhibition of cruzain and related cysteine protases, it is necessary to separate the binding constant (K_i) of the inhibitor scaffold and rate constant of the irreversible chemical step of inhibition (k_{inact}) of various vinyl sulfonyl derivatives. However, very fast inhibition rates for many of our vinyl sulfonyl cysteine protease inhibitors have precluded determination of k_{inact}/K_i values; the majority of inhibition constants from our previous work have been reported as k_{assoc} values,[8,9] from which it is impossible to extract K_i and k_{inact}.[14]

To assess the intrinsic reactivities of the Michael acceptors, which presumably contribute to k_{inact} for the enzyme inhibition reaction, we have carried out pseudo-first-order kinetic analyses of Michael additions of 2′-(phenethyl)thiol to inhibitor analogues **1**−**9** (Figure 1).

Mechanistic studies of base-catalyzed conjugate additions of alcohols[15] and the uncatalyzed addition of amines[16] to vinyl sulfones and vinyl sulfonamides have previously been reported. The kinetics of the base-catalyzed Michael addition

Scheme 1. Synthesis of Michael Addition Substrates



of 2-hydroxyethanethiol to *N*-methyl-*N*-phenylvinylsulfonamide have also been reported.[15] However, we are not aware of any kinetic evaluation of the full array of vinyl sulfonyl units reported in the cysteine protease inhibition literature. An analysis of the Michael reactivity of the vinyl sulfonyl derivatives should allow us to anticipate the magnitude of k_{inact} in the design of future generations of cysteine protease inhibitors. We also expect that the data reported here may be useful in situations where the Michael acceptor reactivities of different vinyl sulfonyl functional groups can be exploited in organic synthesis.[11,17]

The vinyl sulfonyl derivatives used in this study were synthesized as summarized in Scheme 1, using chemistry patterned after our syntheses of vinyl sulfonyl cysteine protease inhibitors.[8−10] Enone, enoate, and vinyl sulfonyl groups were installed by stabilized Wittig and Horner−Wadsworth−Emmons reactions to furnish compounds **2**, **3**, **4**, and **7**. The phenyl sulfonate ester **1** and the sulfonamides **5**, **6**, **8**, and **9** were installed via base-promoted coupling of phenol or the appropriate amine with the sulfonyl chloride **11**.

Relative rates of base-promoted Michael additions of 2′-phenethylthiol to acceptors **1**−**9** were measured by ^{1}H NMR

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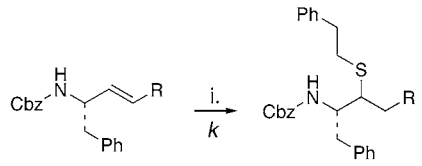
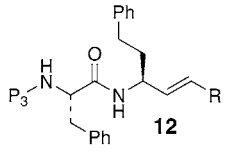
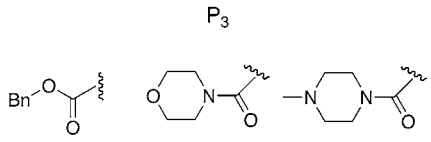
Table 1. (A) Relative Pseudo-First-Order Rate Constants of Michael Addition of 2′-(Phenethyl) Thiol to Various Michael Acceptors. (B) Relative Apparent Second-Order Rate Constants for Inhibition of Cruzain by Inhibitors **12**

A



B





compound	R	relative k^a	relative $k_{ass}{}^b$		
1	SO_2OPh	3000	1500	1100	70
2	COMe	2700			
3	SO_2Ph	120	65	19	40
4	SO_2OEt	90	9		
5	$SO_2N(Me)OBn$	150			
6	SO_2NHOBn	50		230	670
7	CO_2Me	17			
8	$SO_2N(Me)Bn$	3			
9	SO_2NHBn	1	1	500[c]	130[c]

[a] Relative pseudo-first-order rate constant. [b] Apparent second-order rate constants of inhibition of cruzain for known inhibitors.[8,9] [c] Data for the appropriate inhibitor **12** with R = SO_2NHBn (Bn = CH_2Ph) are not available; indicated data are for the *N*-phenyl vinyl sulfonamide (R = SO_2NHPh).[9]

spectroscopy (Table 1A). 2′-(Phenethyl)thiol and triethylamine served as the most convenient model for the practical time scale. The reaction solvent was methanol-d_4, and CH_2Cl_2 was used as an internal integration standard. Reaction rates were measured by integration of a vinylic proton signal (δ = 6.7 ppm) of the starting material against the CH_2Cl_2 internal standard. A large excess (10 equiv.) of 2′-(phenethyl)-thiol was employed to ensure pseuo-first-order kinetics for consumption of the Michael acceptor.

The data in Table 1A demonstrate that the pseudo-first-order rates of Michael addition to this class of compounds vary by up to 3 orders of magnitude as a function of the nature of the sulfonyl unit. Vinyl sulfonamide **9** was the least reactive substrate, while the phenyl vinyl sulfonate ester **1** underwent conjugate addition at a rate ca. 3000-fold higher. We also tested the *N*-methylsulfonamides **5** and **8** since we were concerned that triethylamine would deprotonate the sulfonamide units of **6** and **9**, artificially depressing the apparent conjugate addition rates. The data for compound pairs **5**/**6** and **8**/**9** show that this effect depresses the rates by a factor of 3. We have also included data for enone **2** and enoate **7** to establish the reactivities of vinyl sulfonyl compounds compared to conventional enoyl Michael acceptors.

In polar protic solvents, the triethylamine-catalyzed addition of thiols to enoyl compounds involves formation of an enolate ion intermediate, followed by protonation by the triethylammonium conjugate acid.[18] The relative rates of Michael addition to α,β-unsaturated esters, amides, and ketones are readily understood by consideration of the electrophilicity of the enoyl β-carbon, which tracks the energy of the transition state for formation of the initial enolate intermediate upon addition of the nucleophile to the Michael acceptor. The relative energies of the enolate species is highly dependent on the inductive and/or resonance effects of the substituents bonded to the carbonyl carbon.[19,20]

However, the situation differs for conjugate addition reactions of vinyl sulfonyl acceptors. As these compounds undergo conjugate addition, the reactive intermediate is an α-carbanion, which is stabilized by the sulfur atom of the sulfonyl unit. The origin of this α-stabilization has been attributed to the polarizability of the sulfur atom and by σ effects of antibonding orbitals on the sulfur atom.[21−24] In contrast to enoyl Michael acceptors, electronic effects arising from the linking oxygen or nitrogen atoms of the sulfonate ester and sulfonamide groups are exclusively inductive, since poor sp^3/d orbital overlap between oxygen or nitrogen with sulfur precludes an electron-donating resonance effect. Therefore, strong σ-electron-withdrawing inductive effects would be expected to stabilize α-carbanion formation. Indeed, sulfonate ester **1** is a highly reactive Michael acceptor, comparable to that of conventional enone electro-

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philes (see **2**). Our results also show that the vinyl sulfonamides **5**, **6**, **8**, and **9** are substantially less reactive Michael acceptors than the sulfonate ester **1** and have absolute reactivity comparable to that of α,β-unsaturated ester **7**. Since the NH of sulfonamide **9** is reasonably acidic (pK_a of phenyl sulfonamide = 10.1 and pK_a of triethylammonium ion = 11.0),[25] **9** is likely in equilibrium with the anionic N-deprotonated form, thereby suppressing the rate of Michael addition by destabilizing the corresponding α-carbanion product of the initial Michael addition step. This effect should be absent in the *N*-methyl vinyl sulfonamide **8**, which still shows substantially lower reactivity as a Michael acceptor than the vinyl sulfonate esters. This may be attributed to the lower electronegativity of nitrogen relative to oxygen.[26] An identical effect is seen in the *N*-alkoxy sulfonamides **5** and **6**, which have higher Michael reactivities than **8** and **9**, possibly due to an additive electron-withdrawing effect of the *N*-alkoxy group. However, it is unclear at present why the vinyl sulfonamides **8** and **9** are so much less reactive than the phenyl vinyl sulfone **3**. On the basis of the inductive effect arguments (O > N > C), we would have expected that **3** would be the least reactive substrate in this series.[26]

At the outset of these investigations, we had hoped that it might be possible to correlate the relative Michael reactivity of **1**−**9** with the k_{inact} values for analogously substituted inhibitors **12** against the cysteine protease cruzain. However, we have not been able to obtain k_{inact} data for most of our inhibitors;[8−10] instead, we have second-order k_{assoc} values.[14] Normalized second-order rate constants of inactivation of cruzain for a known set of inhibitors containing the vinyl sulfonyl substituents examined in this study are given in Table 1B. The data summarized in Table 1B clearly indicate that changes in the inhibitor P_3 substituent, distal to the site of the enzymatic Michael addition, often result in a dramatic change in the inhibition kinetics. This points toward the fundamentally important role of enzyme binding (K_i) in the inhibition event. In addition, changes in the P_1' substituent of the inhibitor ("R" in structure **12**) may also affect enzyme binding and ultimately also the absolute potency as an enzyme inhibitor (best approximated as k_{inact}/K_i values). We hope that k_{inact} values for our highly potent inhibitors can be obtained by using stopped-flow or rapid-quench kinetic techniques.

Whether the relative reactivity profile of the various vinyl sulfonyl derivatives examined in this study ultimately correlates with k_{inact} values for enzyme inhibition remains to be determined. Nevertheless, we expect that the reactivity effects demonstrated here will be useful as a general preliminary gauge of Michael reactivity in the design of new cysteine protease inhibitors containing the vinyl sulfonyl functional groups.

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Supporting Information Available: Details of the kinetics measurements and procedures for synthesis of **1**−**9** are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

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