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USE OF THE 1,2,5-THIADIAZOLIDIN-3-ONE 1,1 DIOXIDE AND ISOTHIAZOLIDIN-3-ONE 1,1 DIOXIDE SCAFFOLDS IN THE DESIGN OF POTENT INHIBITORS OF SERINE PROTEINASES

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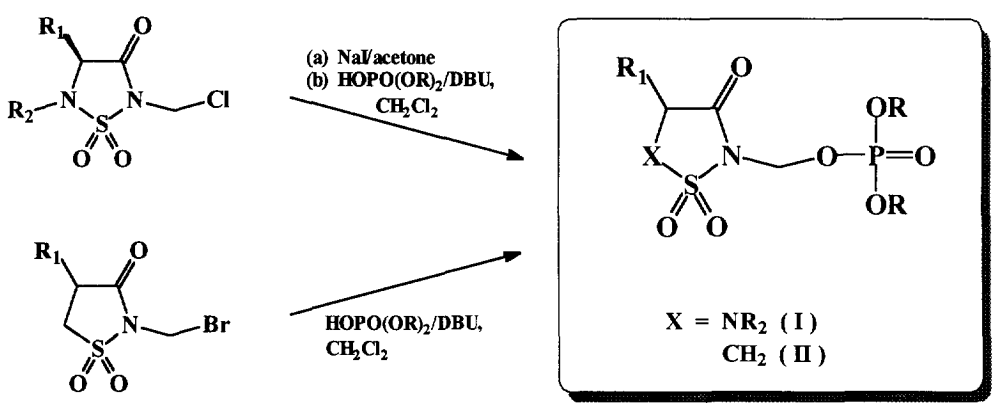
Abstract: The attachment of a phosphate leaving group to the 1,2,5-thiadiazolidin-3-one 1,1 dioxide and isothiazolidin-3-one 1,1 dioxide scaffolds was found to yield highly potent, time-dependent inhibitors of human leukocyte elastase (HLE).

The pathogenesis of a range of inflammatory diseases, such as rheumatoid arthritis and osteoarthritis,[1,2] pulmonary emphysema and bronchitis,[3,4] psoriasis[5] and others,[6,7] involves the interplay of multiple mediators, including proteolytic enzymes and pro-inflammatory mediators (IL-8, TNF-α, and LTB_4). Although the relative importance of each individual mediator remains to be determined, the preponderance of evidence leaves no doubt as to the damaging effects of proteolytic enzymes on extracellular matrix components.[8–10] The serine proteinases elastase (HLE), proteinase 3 (PR 3), and cathepsin G (Cat G) appear to play a pivotal role in inflammatory disease and, consequently, have been the focus of extensive studies aimed at modulating their activity and reestablishing a proteinase/antiproteinase balance.[11] We describe herein the use of a series of phosphate derivatives that utilize the 1,2,5-thiadiazolidin-3-one 1,1 dioxide (**I**) and isothiazolidin-3-one 1,1 dioxide (**II**) scaffolds in inhibiting HLE, Cat G and PR 3.[12,13]



Scheme 1. Inhibitor structures and synthesis

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Materials. A series of phosphate derivatives was synthesized by stirring (S)-4-isobutyl-5-benzyl-2-chloromethyl-1,2,5-thiadiazolidin-3-one 1,1 dioxide[12] or (S)-4-isobutyl-5-methyl-2-chloromethyl-1,2,5-thiadiazolidin-3-one 1,1 dioxide with sodium iodide in dry acetone at room temperature overnight. The solvent was removed and the residue was then dissolved in dry methylene chloride and treated with the appropriate phosphate $HOPO(OR)_2$ in the presence of 1,8-diazabicyclco[5.4.0]undec-7-ene (DBU). The reaction mixture was stirred at room temperature overnight. The crude products were isolated and purified using flash chromatography.[14] Compounds **6** and **7** were prepared by reacting (DL)4-isobutyl-2-bromomethyl-isothiazolidin-3-one 1,1 dioxide with di-n-butyl phosphate in the presence of DBU. The synthesized compounds are listed in Table 1.

Biochemical Studies. Compounds **1–7** were evaluated for their inhibitory activity toward HLE using the progress curve method.[12,15] The apparent second-order inactivation rate constants (k_{inact}/K_I M^{-1} s^{-1}) were determined in duplicate and are listed in Table 1. A typical progress curve for the hydrolysis of MeOSuc-Ala-Ala-Pro-Val-pNA by HLE in the presence of inhibitor **1** is shown in Figure 1. The release of *p*-nitroaniline was

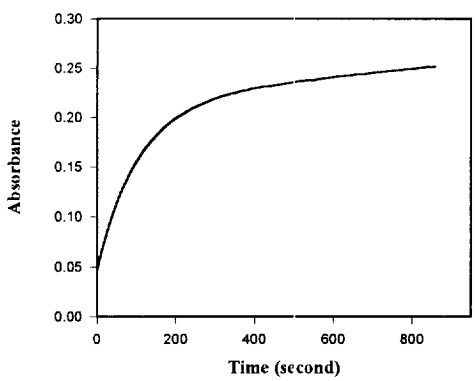


Figure 1. A typical progress curve for the inhibition of HLE by inhibitor **1**. Absorbance was recorded at 410nm for reaction solution containing 20 nM HLE, 1 mM MeOSuc-Ala-Ala-Pro-Val-pNA, and 200 nM of inhibitor in 0.1 M HEPES buffer, pH 7.25, and 3.6% DMSO. The temperature was maintained at 25 °C, and the reaction were initiated by the addition of enzyme.

continuously monitored at 410 nm. The pseudo first-order rate constants, k_{obs}, for the inhibition of HLE by compounds **1–7** as a function of time were determined according to eq 1, where A is the absorbance at 410 nm, v_o is the reaction velocity at t = 0, v_s is the final steady-state velocity, k_{obs} is the observed first-order rate constant, and A_o is the absorbance at t = 0. Fitting the A ~ t data into eq 1 using nonlinear regression analysis (SigmaPlot, Jander Scientific) yielded k_{obs}. The second-order rate constants (k_{inact}/K_I M^{-1} s^{-1}) were determined by calculating $k_{obs}/[I]$, and then correcting for the substrate concentration and Michaelis constant using eq 2. Control curves in the absence of inhibitor were linear.

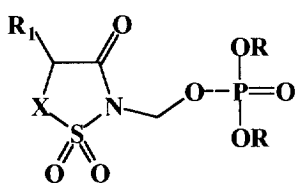
$$A = v_s t + (v_o - v_s)(1-e^{-k_{obs} t})/k_{obs} + A_o \qquad (1)$$

$$k_{obs}/[I] = k_{inact}/\{K_I (1 + [S]/[K_m])\} \qquad (2)$$

Molecular Modeling. Modeling studies of the enzyme-inhibitor binding interaction were performed using the Tripos force field of SYBYL, version 6.1a (Tripos Associates, St. Louis, MO), and a Silicon Graphics INDY workstation. The HLE active site model was generated using the coordinates of HLE-Turkey Ovomucoid Inhibitor (TOMI) complex.[16]

Results and Discussion. We have recently described the design and use of two novel and general classes of mechanism-based inhibitors of serine proteinases derived from the nonpeptidyl pharmacophores 1,2,5-thiadiazolidin-3-one 1,1 dioxide (**I**) and isothiazolidin-3-one 1,1 dioxide (**II**).[12,13] It was furthermore demonstrated that the heterocyclic scaffolds allow the attachment and optimal spatial orientation of peptidyl and nonpeptidyl recognition elements, leading to the exploitation of favorable binding interactions with both the S_n and S_n' subsites of a target serine proteinase. Earlier studies also suggested that while potency is related to the pK_a of the leaving group, binding interactions involving the leaving group and the S_n' subsites have a profound effect on inhibitory potency. Consequently, it was hypothesized that the use of the leaving group as a highly flexible design element could lead to enhanced binding affinity. Thus, in order to optimize inhibitory potency and selectivity, a series of inhibitors with phosphates as the leaving group[17] were synthesized and their interaction with HLE was investigated.

Table 1. Inhibitory activity of **I** and **II** toward HLE

Compd[a]	Structure	X	R	k_{inact}/K_I[b] $M^{-1} s^{-1}$
1		$NCH_2C_5H_6$	methyl	430,000
2		NCH_3	*n*-butyl	2,700,000
3		$NCH_2C_5H_6$	*n*-butyl	2,500,000
4		$NCH_2C_5H_6$	benzyl	6,000,000
5		$NCH_2C_5H_6$	phenyl	4,200,000
6[c]		CH_2	*n*-butyl	82,000
7[c]		CH_2	benzyl	95,000

[a] R_1 = isobutyl; [b] k_{inact}/K_I values are reproducible to within ±10%; [c] racemic.

Incubation of HLE with any of the phosphate derivatives listed in Table 1 led to rapid and time-dependent loss of enzymatic activity. It is evident from Table 1 that phosphate derivatives of **I** are remarkably effective inhibitors of HLE. The values of the second-order rate constants are some of the highest reported for inhibitors of HLE, attesting to the inhibitory prowess of these compounds.[18] The apparent K_I*'s (defined herein as $K_I^* = k_{react}/k_{inact}^*$, where k_{react} is the first-order reactivation constant and k_{inact}^* is the apparent second-order inactivation constant, k_{inact}/K_I) for these inhibitors were in the nM to sub nM range[12]. The relative potencies of compounds **1**, **3**, and **4** reflect the importance of hydrophobic binding interactions involving the S_n' subsites of HLE and the two R groups in the inhibitors. This trend is congruent with the hydrophobic nature of the S_n' subsites of HLE, as revealed by its X-ray crystal structure.[16] As suggested by molecular modeling, the two R groups in the phosphate moiety can be used as substrate recognition elements P_1' and P_2', respectively. For instance, the two benzyl groups in inhibitor **4** provide the most favorable binding interaction with the S_1' and S_2' subsites, leading to optimal inhibitory activity (Figure 2).

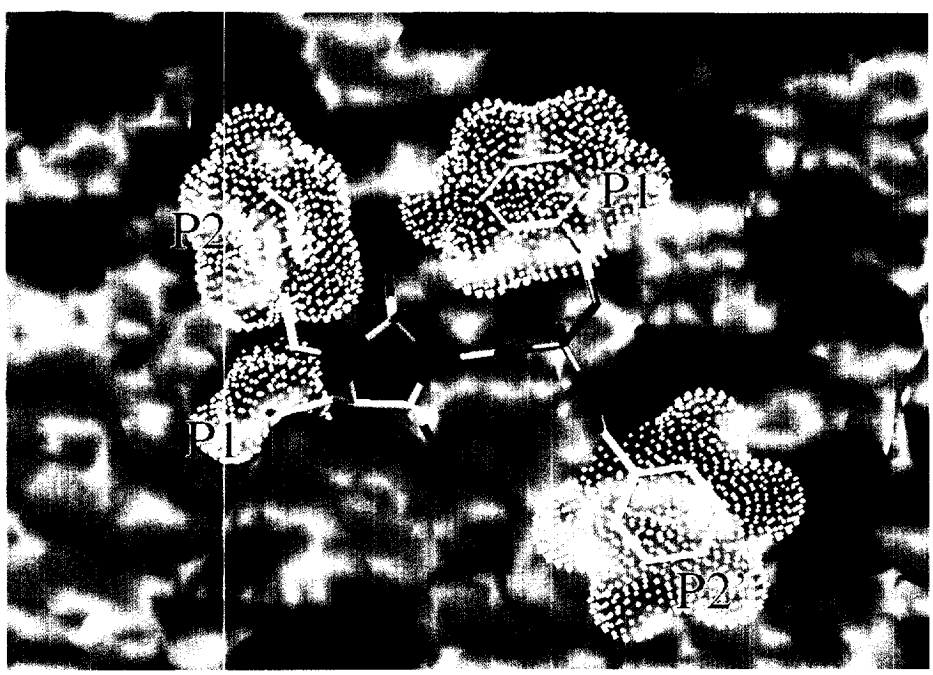


Figure 2. Energy minimized inhibitor **4** docked to the active site of HLE with the isobutyl group occupying the S_1 pocket. The N-benzyl group is nestled into the S_2 pocket, while the two O-benzyl groups extend into the hydrophobic S_1' and S_2' subsites, respectively.

As anticipated, inhibitors based on the isothiazolidin-3-one 1,1 dioxide scaffold were also fairly potent (Table 1, compounds **6** and **7**), however, their potency was considerably lower due to the lack of a P_2 residue. The P_2-S_2 lipophilic interaction is highly significant in HLE (as well as Cat G and PR 3).[12]

A representative member of this class (compound **4**) was used to probe the enzyme selectivity of the inhibitors. Compound **4** was a fairly efficient inhibitor of PR 3 (k_{inact}/K_I 89,120 M^{-1} s^{-1}) and a poor inhibitor of Cat G (k_{inact}/K_I 120 M^{-1} s^{-1}). These results are consistent with the known substrate specificities of the three enzymes[19] and, in the case of Cat G, its narrow S_1' subsite and the more hydrophilic nature of the S_1' and S_2' subsites.[20] As shown previously,[12] the primary specificity for a target serine proteinase is dictated by the nature of the R_1 group in **I** and **II**.

In summary, phosphate derivatives based on the 1,2,5-thiadiazolidin-3-one 1,1 dioxide and isothiazolidin-3-one 1,1 dioxide scaffolds have been shown to be highly potent inhibitors of HLE, providing cogent validation of the hypothesis that the nature of the leaving group in this series of compounds can serve as a flexible design element for optimizing inhibitory potency. The highly predictable binding interaction of these inhibitors with their target enzymes provides further assurance regarding the effectiveness of the structure-based approach used in the design of the two heterocyclic templates and the development of highly effective mechanism-based inhibitors of serine proteinases.

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