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Utilization of the 1,2,5-Thiadiazolidin-3-one 1,1 Dioxide Scaffold in the Design of Potent Inhibitors of Serine Proteases: SAR Studies Using Carboxylates

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Abstract—A series of carboxylate derivatives based on the 1,2,5-thiadiazolidin-3-one 1,1 dioxide and isothiazolidin-3-one 1,1 dioxide scaffolds has been synthesized and the inhibitory profile of these compounds toward human leukocyte elastase (HLE), cathepsin G (Cat G) and proteinase 3 (PR 3) was then determined. Most of the compounds were found to be potent, time-dependent inhibitors of elastase, with some of the compounds exhibiting k_{inact}/K_I values as high as 4,928,300 M^{-1} s^{-1}. The inhibitory potency of carboxylate derivatives based on the 1,2,5-thiadiazolidin-3-one 1,1 dioxide platform was found to be influenced by both the pK_a and the inherent structure of the leaving group. Proper selection of the primary specificity group (R_1) was found to lead to selective inhibition of HLE over Cat G, however, those compounds that inhibited HLE also inhibited PR 3, albeit less efficiently. The predictable mode of binding of these compounds suggests that, among closely-related serine proteases, highly selective inhibitors of a particular serine protease can be fashioned by exploiting subtle differences in their S′ subsites. This study has also demonstrated that the degradative action of elastase on elastin can be abrogated in the presence of inhibitor **17**.

Introduction

The influx of activated neutrophils and other phagocytic cells to inflammatory sites is associated with the extracellular release of a range of proteolytic enzymes, including the serine endopeptidases human leukocyte elastase (HLE), cathepsin G (Cat G) and proteinase 3 (PR 3).[1–3] Poor regulation of the activity of these enzymes by endogenous serpins (serine proteinase inhibitors) leads to the degradation of the major components of the extracellular matrix.[4–6] Thus, considerable efforts have been devoted to the design of novel agents capable of redressing the protease/antiprotease imbalance[7-9] via the selective inhibition of these enzymes.[10–12]

We have recently described the structure-based design of a highly versatile heterocyclic scaffold (1,2,5-thiadiazolidin-3-one 1,1 dioxide, **I**) and have shown that potent mechanism-based inhibitors of the aforementioned enzymes can be realized by embellishing the scaffold with appropriate recognition and chemical reactivity elements.[13–16] We have furthermore reported the solution-phase construction of the first libraries of mechanism-based inhibitors of serine proteases based on this template and, more importantly, have obtained preliminary evidence in support of the notion that the structural motif embodied in structure (**I**) renders the platform capable of binding to the active site of many serine proteases with a (chymo)trypsin-like fold.[17,18] We wish to describe herein the results of SAR studies with carboxylate derivatives of the 1,2,5-thiadiazolidin-3-one 1,1 dioxide (**I**) and a closely related scaffold isothiazolidin-3-one 1,1 dioxide (**II**) as inhibitors of HLE, Cat G, and PR 3. The rationale leading to the design of these two inhibitor scaffolds is summarized in Figure 1. In vitro biochemical studies demonstrating the ability of a representative member of this class of inhibitors to block the degradation of elastin by human leukocyte elastase are also described.

Results

Chemistry

Carboxylate derivatives **1**–**39** were synthesized according to Scheme 1.

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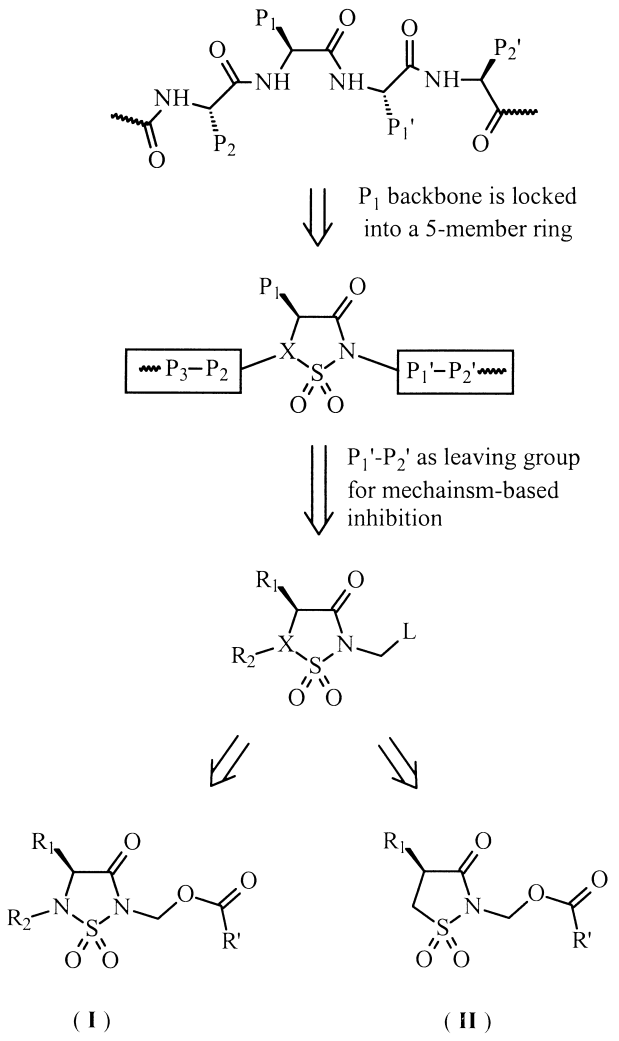


Figure 1. Design of templates (**I**) and (**II**).

Intermediate A (Scheme 1) was readily obtained via treatment of the appropriate amino acid ester with sulfamoyl chloride followed by cyclization with sodium hydride, while the synthesis of intermediate B involved an initial reductive alkylation of an amino acid ester precursor prior to the sulfamoylation and cyclization steps. Sequential alkylation of A yielded a series of inhibitors represented by structures C and D. An alternative procedure for the synthesis of C and D involved alkylation of A followed by treatment with sulfuryl chloride to yield chloromethyl compounds E and F. Treatment of E or F with sodium iodide in dry acetone gave the corresponding iodide derivatives which were then converted to C and D using an appropriate carboxylic acid in the presence of DBU. Compound D was also obtained from B by employing a novel chloromethylation protocol,[19] followed by sequential treatment with NaI/acetone and RCOOH/DBU. The synthesized compounds and their physical and spectral data are listed in Table 1.

Carboxylates **40**–**53** were obtained as shown in Scheme 2. The appropriate substituted malonic ester was used in the synthesis of a 2-alkyl substituted ethyl acrylate[20,21] which was elaborated further to yield the desired compounds (Table 2).

Biochemical studies

Progress curve method.[22] The inhibitory activity of compounds **1**–**44** toward HLE, Cat G and PR 3 was determined using the progress curve method. The apparent second-order inactivation rate constants (k_{inact}/K_I M^{-1} s^{-1}) were determined in duplicate and are listed in Table 3. Typical progress curves for the hydrolysis of MeOSuc-AAPV-pNA by HLE in the presence of inhibitor **14** are shown in Figure 2. The release of *p*-nitroaniline was continuously monitored at 410 nm. The pseudo first-order rate constants (k_{obs}) for the inhibition of HLE, Cat G and PR 3 by derivatives of (**I**–**II**) 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 $t=0$. The k_{obs} values were obtained by fitting the $A\sim t$ data into eq (1) using nonlinear regression analysis (SigmaPlot, Jandel Scientific). The second order rate constants (k_{inact}/K_I M^{-1} s^{-1}) were then determined by calculating $k_{obs}/[I]$, and then correcting for the substrate concentration using eq 2. Control curves in the absence of inhibitor were linear.

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

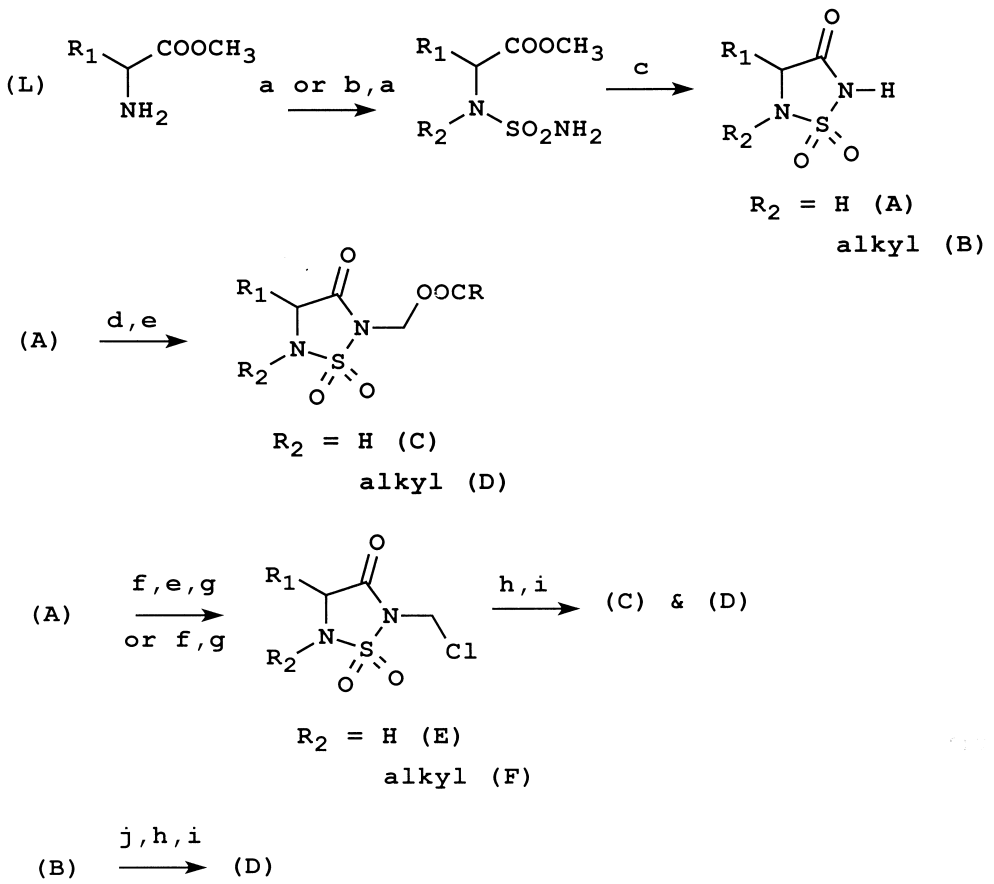
$$k_{inact}/K_I = (k_{obs}/[I])(1 + [S]/K_m) \quad (2)$$

Incubation method.[23] The inhibitory activity of compounds **43**–**50** was determined by the incubation method and is expressed in terms of the bimolecular rate constant $k_{obs}/[I]$ M^{-1} s^{-1}. Briefly, in this method the enzyme is incubated with excess inhibitor, and the loss of enzymatic activity is followed by withdrawing aliquots at different time intervals and assaying for enzymatic activity. The observed rate constant (k_{obs}) is then calculated according to eq 3, where $[I]$ is the concentration of the inhibitor in the incubation mixture and $[E]_t/[E]_o$ is the amount of active enzyme remaining at t.

$$\ln\left([E]_t/[E]_o\right) = k_{obs}t \quad (3)$$

Molecular modeling

Enzyme-inhibitor modeling studies were carried out using the Tripos force field of SYBYL, version 6.5 (Tripos Associates, St. Louis, MO) and a Silicon graphics O2 workstation. Inhibitors were built using the SYBYL software package (SKETCH mode) and energy-minimized using the SYBYL force field. The crystal structure of HLE bound to the turkey ovomucoid inhibitor third domain (Brookhaven, 1PPF) was used in the modeling studies. The energy-minimized inhibitor was superimposed on the -Thr-Leu-Glu-Tyr-(-P_2-P_1-P_1'-P_2'-) segment of TOMI (alpha-carbon and



Scheme 1. Synthesis of derivatives of (**I**). [a]NH_2SO_2Cl/TEA; [b]R_2CHO/$NaBH(OAc)_3$/DCE; [c]NaH/THF; [d]$ClCH_2OOCR$/TEA; [e]NaH/CH_3CN then R_2Br; [f]$ClCH_2SPh$/DBU; [g]SO_2Cl_2; [h]NaI/acetone; [i]RCOOH/DBA/CH_2Cl_2; [j]$SOCl_2$/$HOCH_2SO_3Na$.

carbonyl group of inhibitor overlapping the corresponding alpha-carbon and carbonyl group of Leu-18 (P_1 residue) of TOMI. TOMI and water molecules were deleted and a shaded surface for HLE was generated.

Inhibition of elastinolysis

Inhibition of elastin degradation by HLE was determined using a published procedure.[24] In this protocol elastin and HLE in buffer solution were incubated at 37 °C, 1-mm aliquots were withdrawn at 10-min intervals, filtered and the absorbance determined at 276 nm. The change in absorbance was linear with time over 60 min. The rate of solubilization of elastin was determined by a linear least-squares fit to the data. The effect of an inhibitor on this reaction was then determined by varying the inhibitor concentration in the incubation mix. The percent inhibition is a reflection of the decrease in the slope in the presence of inhibitor divided by the control slope.

Discussion

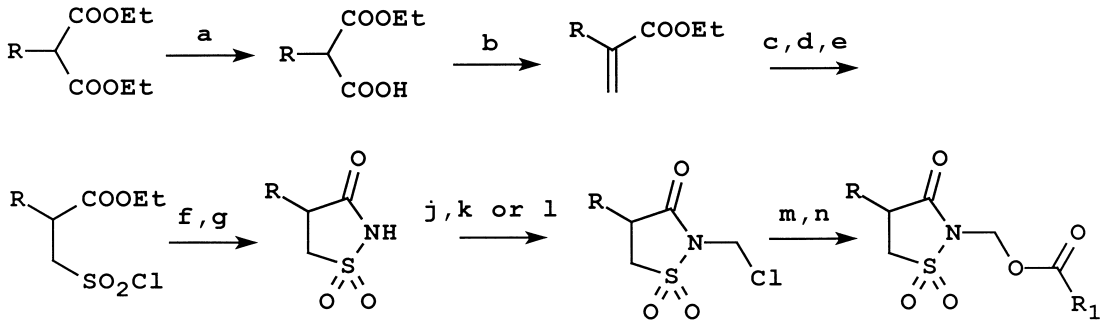
Recent studies in our laboratory have demonstrated that the motif embodied in the 1,2,5-thiadiazolidin-3-one 1,1 dioxide scaffold is ideally suited for the design of inhibitors of (chymo)trypsin-like serine proteinases.[16–18] These earlier results also suggested that inhibitors derived from this scaffold might bind a target serine protease in a highly predictable fashion, namely, with the R_1 residue accommodated at the primary specificity site (S_1)[25] and the R_2 and L groups oriented toward the S_2 and S_n' subsites, respectively. Thus, the carboxylate component in scaffolds (**I**) and (**II**) was expected to bind to the S_n' subsites of the target enzymes. Based on previous observations,[26] the use of carboxylate leaving groups was anticipated to yield highly potent inhibitors. With the widely diverse structures of available carboxylates, this series of inhibitors provides an ideal system to study S_n'–P_n' interactions, which are often less understood and less emphasized in inhibitor design, than the corresponding S_n–P_n interactions for most serine proteases.

This report details our early studies with carboxylate inhibitors derived from (**I**) and (**II**) which aimed at (a) gaining further insight into the interaction of (**I**) and (**II**) with serine proteases and, (b) optimizing inhibitory potency and enzyme selectivity through variations in R_1, R_2, and especially the leaving group L. While all three neutrophil-derived serine proteases (HLE, PR 3, and Cat G) were studied with the synthesized inhibitors, efforts were focused on the development of inhibitors of HLE suitable for further pharmacological evaluation.

Inhibitors synthesized in this study were those with hydrophobic R_1 groups which parallel the known primary substrate specificity (P_1) of the target enzyme, namely, HLE (Val, Leu),[27] PR 3 (Abu, Norval)[28] and

Table 1. Physical constants and spectral data of inhibitors **1**–**39**

Compound	mp (°C)	^{1}H NMR (δ)	MF (anal.)
1	Oil	1.09 (t, 3H), 1.20 (s, 9H), 1.88 (m, 1H), 2.02 (m, 1H) 4.22 (m, iH), 5.35 (d, 1H), 5.62 (d, 2H)	$C_{10}H_{18}N_2O_5S$ (C, H, N)
2	Oil	0.98 (t, 3H), 1.21 (s, 9H), 1.90 (m, 1H), 2.06 (m, 1H) 2.88 (s, 3H), 3.89 (t, 1H), 5.62 (d, 2H)	$C_{11}H_{20}N_2O_5S$ (C, H, N)
3	Oil	0.86 (t, 3H), 1.22 (s, 9H), 1.67 (m, 1H), 1.91 (m, 1H) 3.97 (t, 1H), 4.42 (dd, 2H), 5.67 (d, 2H), 7.38 (s, 5H)	$C_{17}H_{24}N_2O_5S$ (C, H ,N)
4	Oil	1.06 (t, 3H), 1.87 (m, 1H), 2.02 (m, 1H), 4.22 (m, 1H) 5.59 (d, 1H), 5.87 (d, 2H), 7.21 (s, 3H)	$C_{12}H_{12}Cl_2N_2O_5S$ (C, H, N)
5	Oil	0.99 (t, 3H), 1.21 (s, 9H), 1.45–1.62 (m, 2H), 1.72–1.86 (m, 1H), 1.93–2.06 (m, 1H), 4.24 (m, 1H), 5.20 (d, 1H) 5.62 (d, 2H)	$C_{11}H_{20}N_2O_5S$ (C, H, N)
6	91.0–94.0	0.79 (t, 3H), 1.22 (s, 9H), 1.30 (m, 2H), 1.59 (m, 1H), 1.84 (m, 1H), 3.66 (s, 2H), 3.95 (t, 1H), 4.39 (dd, 2H) 5.66 (d, 2H), 7.33 (dd, 4H), 11.5 (br s, 1H)	$C_{20}H_{28}N_2O_7S$ (C, H, N)
7	95.0–96.0	1.00 (d, 3H), 1.09 (d, 3H), 1.20 (s, 9H), 2.38 (m, 1H), 4.20 (d, 1H), 5.64 (s, 2H)	$C_{11}H_{20}N_2O_5S$ (C, H ,N)
8	85.0–88.0	0.99 (dd, 6H), 1.21 (s, 9H), 1.71 (m, 1H), 1.88 (m, 2H), 4.25 (dd, 1H), 5.62 (d, 2H)	$C_{12}H_{22}N_2O_5S$ (C, H, N)
9	Oil	0.90 (dd, 6H), 1.14 (s, 9H), 1.74 (m, 2H), 1.85 (m, 1H), 2.82 (s, 3H), 3.79 (t, 1H), 5.57 (d, 2H)	$C_{13}H_{24}N_2O_5S$ (C, H, N)
10	Oil	0.74 (dd, 6H), 1.23 (s, 9H), 1.52–1.78 (m, 3H), 3.99 (t, 1H), 4.40 (dd, 2H), 5.65 (d, 2H), 7.37 (s, 5H)	$C_{19}H_{28}N_2O_5S$ (C, H, N)
11	Oil	0.75 (dd, 6H), 1.21 (s, 9H), 1.57–1.82 (m, 3H), 3.90 (t, 1H), 3.92 (s, 3H), 4.44 (dd, 2H), 5.65 (d, 2H), 7.47 (d, 2H), 8.04 (d, 2H)	$C_{21}H_{30}N_2O_7S$ (C, H, N)
12	Oil	2.08 (s, 3H), 3.05 (m, 2H), 4.04 (m, 1H), 4.40–4.30 (dd, 2H), 5.58 (dd, 2H), 7.20 (m, 10H)	$C_{19}H_{20}N_2O_5S$ (C, H, N)
13	82–86	0.98 (dd, 1H), 1.75 (m, 1H), 1.88 (m, 2H), 4.28 (dd, 1H), 5.5 (br s, 1H), 5.85 (s, 2H), 7.46 (t, 2H), 7.61 (t, 1H), 8.03 (dd, 2H)	$C_{14}H_{18}N_2O_5S$ (C, H, N)
14	Oil	0.98 (dd, 6H), 1.87 (m, 2H), 1.97 (m, 1H), 2.92 (s, 3H), 3.91 (t, 1H), 5.90 (dd, 2H), 7.37 (m, 2H), 7.60 (m, 1H), 8.08 (d, 2H)	$C_{15}H_{20}N_2O_5S$ (C, H, N)
15	Oil	0.73 (dd, 6H), 1.62 (m, 1H), 1.75 (m, 2H), 3.95 (t, 1H), 4.42 (dd, 2H), 5.88 (dd, 2H), 7.37 (m, 5H), 7.46 (m, 2H), 7.6 (m, 1H), 8.08 (dd, 2H)	$C_{21}H_{24}N_2O_5S$ (C, H, N)
16	75–77	0.98 (dd, 6H), 1.72 (m, 1H), 1.88 (m, 2H), 4.29 (ddd, 1H), 5.24 (br d, 1H), 5.88 (dd, 2H), 7.32 (s, 3H)	$C_{14}H_{16}N_2Cl_2O_5S$ (C, H, N)
17	Oil	0.97 (dd, 6H), 1.82 (m, 2H), 1.92 (m, 1H), 2.91 (s, 3H), 3.90 (t, 1H), 5.89 (dd, 2H), 7.32 (s, 3H)	$C_{15}H_{18}Cl_2N_2O$ (C, H, N)
18	Oil	0.73 (dd, 6H), 1.62 (m, 1H), 1.73 (m, 2H), 3.93 (t, 1H), 4.42 (dd, 2H), 5.89 (dd, 2H), 7.32 (s, 3H), 7.38 (s, 5H)	$C_{21}H_{22}Cl_2N_2O_5S$ (C, H, N)
19	Oil	0.71 (dd, 6H), 1.6–1.8 (m, 3H), 3.65 (s, 2H), 3.92 (t, 1H), 4.40 (dd, 2H), 5.89 (dd, 2H), 7.32 (m, 5H)	$C_{23}H_{24}Cl_2N_2O_5S$ (C, H, N)
20	92–93	1.0 (dd, 6H), 1.74 (m, 1H), 1.93 (m, 2H), 4.29 (ddd, 1H), 5.17 (br d, 1H), 5.78 (s, 2H), 6.44 (d, 1H), 7.42 (m, 3H), 7.54 (m,2H), 7.78 (d, 1H)	$C_{16}H_{20}N_2O_5S$ (C, H, N)
21	Oil	0.98 (dd, 6H), 1.82 (m, 2H), 1.93 (m, 1H), 2.92 (s, 3H), 3.88 (t, 1H), 5.28 (d, 1H), 5.76 (dd, 2H), 5.93 (d, 1H), 7.39 (m, 3H), 7.49 (m, 2H)	$C_{17}H_{22}N_2O_5S$ (C, H, N)
22	74–75	0.98 (dd, 6H), 1.78 (m, 1H), 1.9 (m, 2H), 4.31 (dd, 1H), 5.35 (br d, 1H), 5.8 (s, 2H), 6.98 (d, 1H),7.39 (m, 3H), 7.63 (m, 2H)	$C_{16}H_{19}FN_2O_5S$ (C, H, N)
23	66–70	1.0 (dd, 6H), 1.84 (m, 2H), 1.96 (m, 1H), 2.93 (s, 3H), 3.92 (t, 1H), 5.86 (dd, 2H), 7.0 (d,1H), 7.42 (m, 3H), 7.68 (m, 2H)	$C_{17}H_{21}FN_2O_5S$ (C, H, N)
24	Oil	0.98 (dd, 6H), 1.82 (m, 2H), 1.93 (m, 1H), 2.68 (t, 2H), 2.88 (s, 3H), 2.96 (t, 2H), 3.84 (t, 1H), 5.64 (dd, 2H), 7.2 (m, 3H), 7.28 (m, 2H)	$C_{17}H_{24}N_2O_5S$ (C, H, N)
25	Oil	0.73 (dd, 6H), 1.54–1.78 (m, 3H), 2.69 (t, 2H), 2.98 (t, 2H), 3.91 (t, 1H), 4.38 (dd, 2H), 5.64 (dd, 2H), 7.17–7.30 (s,5H)	$C_{23}H_{28}N_2O_5S$ (C, H, N)
26	Oil	0.98 (dd, 6H), 1.8 (m, 2H), 1.92 (m, 1H), 2.98 (s, 3H), 3.68 (s, 2H), 3.85 (t, 1H), 5.67 (dd, 2H), 7.29 (m, 5H)	$C_{16}H_{22}N_2O_5S$ (C, H, N)
27	59–61	0.72 dd, 6H), 1.57 (m, 1H), 1.7 (m, 2H), 3.68 (s, 2H), 3.89 (t, 1H), 4.35 (dd, 2H), 5.66 (dd, 2H), 7.3 (m, 5H), 7.37 (s, 5H)	$C_{22}H_{26}N_2O_5S$ (C, H, N)
28	Oil	1.0 (dd, 6H), 1.87 (m 2H), 1.96 (m, 1H), 2.92 (s, 3H), 3.93 (t, 1H), 5.94 (dd,2H), 7.42 (dd, 1H), 8.32 (dt, 1H), 7.61 (dt, 1H), 8.05 (dd, 1H)	$C_{14}H_{19}N_3O_5S$ (C, H, N)
29	Oil	0.97 (dd, 6H), 1.8 (m, 2H), 1.9 (m, 1H), 2.97 (s, 3H), 4.15 (t, 1H), 4.29 (br s, 2H), 4.48 (s, 3H), 5.75 (dd, 2H), 7.9 (d, 2H), 8.87 (d, 2H)	$C_{16}H_{24}IN_3O_5S_2$ (C, H, N)
30	Oil	0.93 (dd, 6H), 1.8 (m, 2H), 1.91 (m, 1H), 2.88 (s, 3H), 3.68 (s, 2H), 3.87 (t, 1H), 5.65 (dd, 2H), 7.2–7.33 (m, 3H), 7.41 (m, 2H)	$C_{16}H_{22}N_2O_5S_2$ (C, H, N)
31	Oil	0.95 (dd, 6H), 1.79 (m, 2H), 1.9 (m, 1H), 2.88 (s, 3H), 3.86 (t, 1H), 4.18 (s, 2H), 5.62 (dd, 2H), 7.61 (m, 2H), 7.7 (m, 1H), 7.98 (d, 2H)	$C_{16}H_{22}N_2O_7S_2$ (C, H, N)
32	175d	0.98 (dd, 6H), 1.78 (m, 2H), 1.9 (m, 1H), 2.9 (s, 3H), 3.8 (s, 2H), 3.86 (t, 1H), 5.71 (s, 2H), 7.18 (d, 2H), 8.42 (d, 2H)	$C_{15}H_{21}N_3O_5S_2$ (C, H, N)
33	Oil	0.97 (dd, 6H), 1.8 (m, 2H), 1.9 (m, 1H), 2.97 (s, 3H), 4.15 (t, 1H), 4.29 (br s, 2H), 4.48 (s, 3H), 5.75 (dd, 2H), 7.9 (d, 2H), 8.87 (d, 2H)	$C_{16}H_{24}N_3O_5S_2$ (C, H, N)
34	Oil	0.94 (dd, 6H), 1.72 (m, 2H), 1.87 (m, 1H), 2.86 (s, 3H), 3.5 (br s, 1H), 3.81 (t, 1H), 5.23 (s, 1H), 5.67 (dd, 2H), 7.30–7.48 (m, 5H)	$C_{16}H_{22}N_2O_6S$ (C, H, N)
35	Oil	0.98 (dd, 6H), 1.72 (m, 2H), 1.88 (m, 1H), 2.86 (s, 3H), 3.43 (br s, 1H), 3.81 (t, 1H), 5.25 (br s, 1H), 5.68 (dd, 2H), 7.30–7.48 (m, 5H)	$C_{16}H_{22}N_2O_6S$ (C, H, N)
36	Oil	0.97 (dd, 6H), 1.81 (m, 2H), 1.92 (m, 1H), 2.90 (s, 3H), 3.38 (s, 3H), 3.68 (3.73 (t, 2H), 3.87 (t, 1H), 4.21 (s, 2H), 5.72 (d, 2H)	$C_{13}H_{24}N_2O_7S$ (C, H, N)
37	Oil	0.97 (dd, 6H), 1.78–1.97 (m, 3H), 2.90 (s, 3H, 3.38 (s, 3H), 3.53–3.77 (m, 8H), 3.67 (t, 2H), 4.21 (s, 2H), 5.70 (d, 2H)	$C_{15}H_{28}N_2O_8S$ (C, H, N)
38	Oil	3.10 (t, 2H), 4.05–4.35 (dd, 2H), 4.12 (m, 1H), 5.88 (s, 2H), 7.05–7.35 (m, 10H)	$C_{24}H_{20}Cl_2N_2SO_5$ (C, H, N)
39	Oil	2.08 (s, 3H), 3.05 (m, 2H), 4.04 (m, 1H), 4.04–4.30 (dd, 2H), 5.58 (dd, 2H), 7.20 (m, 10H)	$C_{19}H_{20}N_2O_5S$ (C, H, N)



Scheme 2. Synthesis of derivatives of (**II**). KOH/ethanol/reflux; [b]Et_2NH/37% HCHO; [c]$HSCOCH_3$; [d]H_2O_2/HCOOH; [e]PCl_3; [f]NH_4OH/toluene; [g]NaH/THF; [h]37% HCHO/aq ethanol; [i]DAST; [j]$ClCH_2SPh$/TEA; [k]SO_2Cl_2; [l]$SOCl_2$/$HOCH_2SO_3Na$; [m]NaI/Acetone; [n]RCOOH/DBU/CH_2Cl_2.

Table 2. Physical data and spectral data of inhibitors **40**–**53**

Compound	mp (°C)	^{1}H NMR (δ)	MF (anal.)
40	Oil	0.97 (dd, 6H), 1.58 (m, 1H), 1.7 (m, 1H), 2.01 (m, 1H), 3.28 (m, 2H), 3.77 (q, 1H), 5.83 (dd, 2H), 7.42 (t, 2H), 7.59 (t, 1H), 8.04 (d, 2H)	$C_{15}H_{19}NO_5S$ (C, H, N)
41	Oil	0.98 (dd, 6H), 1.58 (m, 1H), 1.7 (m, 1H), 2.03 (m, 1H), 3.31 (m, 2H), 3.8 (m, 1H), 5.87 (s, 2H), 7.32 (s, 3H)	$C_{15}H_{17}Cl_2NO_5S$ (C, H, N)
42	Oil	0.96 (dd, 6H), 1.53 (m, 1H), 1.68 (m, 1H), 1.99 (m, 1H), 2.35 (s, 3H), 3.22–3.32 (m, 2H), 3.75 (m, 1H), 5.78 (dd, 2H), 7.12 (dd, 1H), 7.33 (dt, 1H), 7.60 (dt, 1H), 8.03 (dd, 1H)	$C_{17}H_{21}NO_7S$ (C, H, N)
43	Oil	0.98 (dd, 6H), 1.57 (m, 1H), 1.7 (m, 1H), 2.03 (m, 1H), 3.24–3.40 (m, 2H), 3.78 (m, 5.72 (dd, 2H), 6.42 (d, 1H), 7.39 (m, 3H), 7.52 (m, 2H), 7.75 (d, 1H)	$C_{17}H_{21}NO_5S$ (C, H, N)
44	Oil	1.0 (dd, 6H), 1.57 (m, 1H), 1.73 (m, 1H), 2.01 (m, 1H), 2.71 (t, 2H), 2.98 (t, 2H), 3.23–3.33 (m, 2H), 3.77 (m, 1H), 5.62 (dd, 2H), 7.21 (m, 3H), 29 (m, 2H)	$C_{17}H_{23}NO_5S$ (C, H, N)
45	129–131	2.93 (dd, 1H), 3.28 (dd, 1H), 3.66 (dd, 1H), 3.78–3.93 (m, 2H), 5.82 (dd, 2H), 7.28 (m, 5H), 7.6 (m, 3H)	$C_{18}H_{15}Cl_2NO_5S$ (C, H, N)
46	79–80	2.95 (m, 1H), 3.30 (m, 1H), 3.45–3.60 (m, 3H), 5.74 (d, 2H), 6.43 (d, 1H), 7.2–7.6 (m, 10H), 8.80 (d, 1H)	$C_{20}H_{19}NO_5S$ (C, H, N)
47	Oil	2.66 (t, 2H), 2.95 (m, 3H), 3.25 (m, 1H), 3.40–3.55 (m, 3H), 5.59 (d, 2H), 7.18–7.40 (m, 10H)	$C_{20}H_{21}NO_5S$ (C, H, N)
48	Oil	2.92 (t, 1H), 3.25 (m, 1H), 3.40–3.55 (m, 3H), 3.65 (s, 2H), 5.60 (d, 2H), 7.15–7.40 (m, 10 H)	$C_{19}H_{19}NO_5S_2$ (C, H, N)
49	Oil	2.95 (m, 1H), 3.32–3.35 (m, 4H), 3.70 (d, 1H), 3.9 (d, 1H), 5.57 (m, 2H), 7.2 (m, 2H), 7.33 (m, 3H), 7.53 (m, 3H), 7.69 (m, 2H)	$C_{19}H_{19}NO_6S_2$ (C, H, N)
50	Oil	2.92 (m, 1H), 3.25–3.58 (m, 4H), 4.16 (s, 2H), 5.57 (d, 2H), 7.18 (m, 2H), 7.32 (m, 7.58 (m, 2H), 7.70 (m, 1H), 7.95 (m, 2H)	$C_{19}H_{19}NO_7S_2$ (C, H, N)
51	Oil	2.95 (m, 1H), 3.30 (m, 1H), 3.50 (m, 1H), 5.58 (d, 1H), 5.61 (d, 2H), 7.15–7.50 (m, 10H), 7.89 (d, 1H)	$C_{20}H_{19}NO_5S_2$ (C, H, N)
52	83–84	2.95 (m, 1H), 3.30 (m, 1H), 3.4–3.6 (m, 3H), 5.72 (m, 2H), 5.91 (d, 1H), 7.2 (m, 2H), 7.25–7.40 (m, 7H), 7.48 (m, 2H)	$C_{20}H_{19}NO_5S_2$ (C, H, N)
53	55–56	2.95 (m, 1H), 3.35 (m, 1H), 3.45–3.60 (m, 3H), 5.80 (s, 2H), 6.54 (d, 1H), 7.15–7.32 (m, 6H), 7.60–7.75 (m, 3H), 8.0 (m, 2H)	$C_{20}H_{19}NO_7S_2$ (C, H, N)

Cat G (Phe).[29] R_2 and L groups were chosen mainly from hydrophobic groups in order to explore the favorable interactions with secondary subsites S_2 and S_1'–S_2' of HLE, which are also known to be hydrophobic in nature.

The inactivation of HLE by derivatives of (**I**) and (**II**) was found to be time-dependent, rapid and highly efficient. For example, total inactivation of HLE by compound **17** (Table 3) was achieved by using only ~2 eq of inhibitor and led to the formation of a highly stable E-I complex (Fig. 3). Typical progress curves for the hydrolysis of methoxysuccinyl-Ala-Ala-Pro-Val *p*-nitroanilide by HLE in the presence of varying concentrations of inhibitor **14** are shown in Figure 3. The results summarized in Tables 3 and 4 indicate that derivatives of 1,2,5-thiadiazolidin-3-one 1,1 dioxide (**I**) and isothiazolidin-3-one 1,1 dioxide (**II**) with carboxylate leaving groups are very potent inhibitors of HLE, PR 3 and cathepsin G. In general, inhibitors derived from scaffold (**I**) are more potent than those from scaffold (**II**). A discussion of the results in Tables 3 and 4 in terms of R_1, R_2, and L follows.

R_1 as a primary specificity determinant

According to the design rationale, R_1 residues bind to the primary specificity pockets (S_1) of serine proteases. This is indeed the case as suggested by the results listed in Tables 4 and 5. Highly potent inhibitors of HLE were obtained with templates (**I**) and (**II**), when an isobutyl group was chosen as R_1, consistent with the primary selectivity of HLE (leucine). On the other hand, a phenyl group is clearly the group of choice as R_1 for inhibition of Cat G (phenylalanine). Selectivity for PR 3 over HLE was only observed with smaller R_1 groups (compounds **1** and **5**). This is also consistent with the observation from X-ray crystallographic studies, in which the S_1 pocket of PR 3 was found to be slightly smaller than that of HLE. Interestingly, no inhibition of HLE was observed when an isopropyl group was used as R_1, which is in contrast with the strong preference of

Table 3. Inhibitory activity of derivatives of (I) toward human leukocyte elastase, proteinase 3, and cathepsin G

Compound	R_1	R_2	L	HLE	PR 3	CAT G
				(k_{inact}/K_I M^{-1} s^{-1})		
1	Ethyl[a]	H	$OOCCMe_3$	300	900	Inactive
2	Ethyl[a]	Methyl	$OOCCMe_3$	400	300	Inactive
3	Ethyl[a]	Benzyl	$OOCCMe_3$	1500	400	Inactive
4	Ethyl[a]	H	2,6-Dichlorobenzoate	3700	3400	Inactive
5	*n*-Propyl	H	$OOCCMe_3$	1300	5100	Inactive
6	*n*-Propyl	(*p*-$HOOCCH_2$) benzyl	$OOCCMe_3$	4500	2000	200
7	Isopropyl	H	$OOCCMe_3$	Inactive	Inactive	Inactive
8	Isobutyl	H	$OOCCMe_3$	4100	2400	70
9	Isobutyl	Methyl	$OOCCMe_3$	42,700	5900	Inactive
10	Isobutyl	Benzyl	$OOCCMe_3$	60,500	1800	200
11	Isobutyl	(*p*-CH_3OOC) Benzyl	$OOCCMe_3$	13,700	—[b]	300
12	Isobutyl	Benzyl	$OOCCH_3$	163,300	—	—
13	Isobutyl	H	Benzoate	91,600	2600	1100
14	Isobutyl	Methyl	Benzoate	267,500	12,800	300
15	Isobutyl	Benzyl	Benzoate	335,900	26,000	90
16	Isobutyl	H	2,6-Dichlorobenzoate	711,800	88,200	500
17	Isobutyl	Methyl	2,6-Dichlorobenzoate	4,928,300	33,400	60
18	Isobutyl	Benzyl	2,6-Dichlorobenzoate	2,381,000	14,400	30
19	Isobutyl	($HOOCCH_2$) benzyl	2,6-Dichlorobenzoate	1,220,000	196,400	2300
20	Isobutyl	H	*trans*-Cinnamate	31,300	9500	1200
21	Isobutyl	Methyl	*trans*-Cinnamate	318,200	9700	100
22	Iisobutyl	H	α-Fluorocinnamate	56,400	—	—
23	Isobuty	Methyl	α-Fluorocinnamate	628,200	—	100
24	Isobutyl	Methyl	Dihydrocinnamate	296,300	—	—
25	Isobutyl	Benzyl	Dihydrocinnamate	3200	—	—
26	Isobutyl	Methyl	Phenylacetate	357,100	—	200
27	Isobutyl	Benzyl	Phenylacetate	63,800	—	—
28	Isobutyl	Methyl	3-Nicotinate	105,100	—	90
29	Isobutyl	Methyl	3-Nicotinate (methyl iodide)	12,500	—	Inactive
30	Isobutyl	Methyl	Phenylthioacetate	395,400	—	70
31	Isobutyl	Methyl	Phenylsulfonyl acetate	753,200	—	—
32	Isobutyl	Methyl	(4-Pyridylthio) acetate	161,300	—	—
33	Isobutyl	Methyl	4-(*N*-Methyl-4-pyridyl) acetate	108,300	—	Inactive
34	Isobutyl	Methyl	(R) Mandelate	440,500	—	20
35	Isobutyl	Methyl	(S) Mandelate	308,100	—	100
36	Isobutyl	Methyl	Oxaacid-1[c]	71,100	—	—
37	Isobutyl	Methyl	Oxaacid-2[d]	79,700	—	—
38	Benzyl	Benzyl	2,6-Dichlorobenzoate	3600	3500	2200
39	Benzyl	Benzyl	$OOCCH_3$	—	—	10,600

[a]racemic.
[b]Not determined.
[c]$CH_3OCH_2CH_2OCH_2COOH$.
[d]$CH_3OCH_2CH_2OCH_2CH_2OCH_2COOH$.

HLE for valine at its primary specificity site. Molecular modeling studies suggest that the isopropyl group lacks the flexibility needed for the inhibitor molecule to fit properly into the active site pocket of HLE when it is attached to a five-member ring. With respect to the effect of R_1 on inhibition, the results from this carboxylate series are remarkably consistent with previous observations with the sulfone series of inhibitors, which again confirm the highly predictable manner in which these inhibitor templates bind to their target proteases with R_1 as the primary specificity determinant.[15–18]

Enzyme selectivity design with R_2 and L

Although selective inhibition between HLE and Cat G can be achieved with proper choice of R_1, it is a challenge to selectively inhibit highly similar proteases such as HLE and PR 3. While recent X-ray crystallographic studies have confirmed the similar topographical features of their active sites, they have also revealed that the S_2 and S_2' pockets of HLE are more hydrophobic than those of PR 3. This suggests a strategy for selective inhibition of HLE over PR 3 by using hydrophobic R_2 and L groups to provide favorable interactions with the secondary binding pockets S_2 and S_2' of HLE. Indeed, the compounds in Table 3 with the highest selectivity toward HLE are **17** and **18** (selectivity ratios for HLE over PR 3 are 150 and 165, respectively), both compounds having hydrophobic R_2 and L groups. The difference in the nature of S_2 between HLE and PR 3 was further evidenced with compound **19**, which has an extra hydrophilic (CH_2CO_2H) group in R_2 when compared to compound **18**. In this instance k_{inact}/K_I decreased by half for HLE but there was a corresponding 14-fold increase for PR 3.

Modifications in the leaving group L using hydrophilic functionality in order to increase aqueous solubility were found to diminish potency (compounds **28** and **29**, **36** and **37**). The hydrophilic nature of the S_1' and S_2'

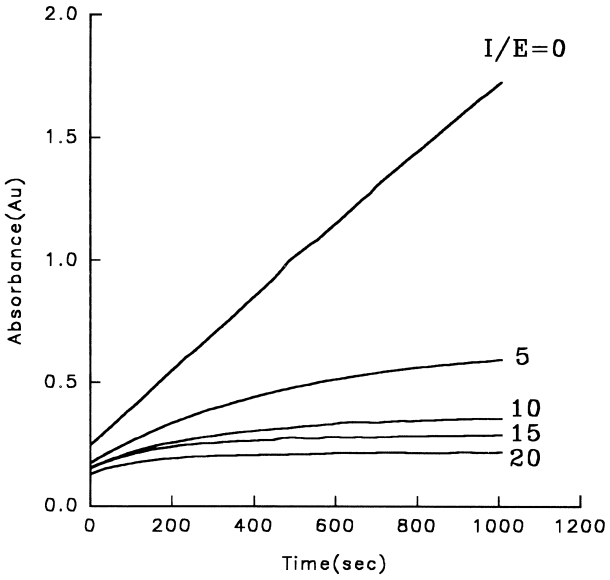


Figure 2. Progress curves for the inhibition of human leukocyte elastase (HLE) by compound **14**. Absorbance was recorded at 410 nm for reaction solutions containing HLE (20 nM), MeOSuc-AAPV-pNA (1 mM) and the indicated concentrations of inhibitor **14** in 0.1 M HEPES buffer, pH 7.25, and 3.6% DMSO. The temperature was maintained at 25 °C, and the reactions were initiated by the addition of enzyme.

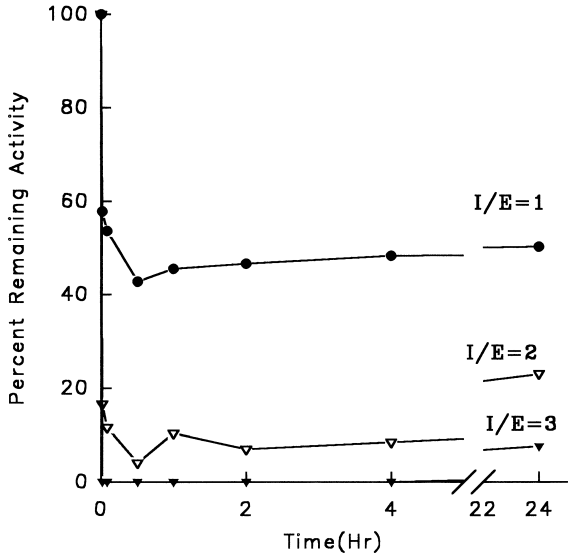


Figure 3. Percent remaining activity versus time plot obtained by incubating inhibitor **17** with human leukocyte elastase (138 nM) at different inhibitor to enzyme ratios in 0.1 M HEPES buffer containing 0.5 M NaCl, pH 7.25, and 1% DMSO. Aliquots were withdrawn at different time intervals and assayed for enzymatic activity using MeOSuc-AAPV pNA by monitoring the absorbance at 410 nm for 1 min.

subsites of Cat G, as revealed by recent crystallographic studies,[28] may be the reason for the poor inhibitory activity of compound **38** against Cat G, although the same hydrophobic leaving group (2,6-dichlorobenzoate) gives some of the most potent inhibitors of HLE reported today (compounds **17** and **18**). In the case of HLE, inhibitors with dihydrocinnamate and phenylacetate as leaving groups (compounds **24** and **25**, **26** and **27**), displayed an unexpected drop in potency in going from R_2 = methyl to R_2 = benzyl. The available evidence suggests that a non-productive hydrophobic collapse[30] occurs with inhibitors **25** and **27**. This serendipitous observation and its effect on molecular recognition is the subject of a future report.

Leaving group L and inhibitory potency

The leaving group L not only serves as a recognition element that interacts with the S_n' subsites, but its chemical reactivity is also correlated with inhibitory potency. As shown in Table 5, more potent inhibition of HLE was observed with inhibitors having lower pK_a values. The large number of commercially available carboxylic acids encompassing a wide range of pK_a values provide, in principle, a means of fine-tuning the potency and stability of this series of serine protease inhibitors.

Inhibition of elastinolysis

The ability of one of the inhibitors to block the degradation of elastin by elastase was investigated. It is evident from Figure 4 that compound **17** (Table 3) is effective in preventing the degradation of elastin by elastase, suggesting that this class of compounds are of potential therapeutic value. The results of this study also demonstrate that, in contrast to protein inhibitors of HLE that do not inhibit HLE pre-adsorbed on elastin, low molecular weight inhibitors are highly effective in inhibiting HLE pre-adsorbed on elastin.

In summary, the findings described herein demonstrate that a range of carboxylate derivatives based on the 1,2,5-thiadiazolidin-3-one 1,1 dioxide and isothiazolidin-3-one 1,1 dioxide scaffolds are highly effective inhibitors of HLE, PR 3 and Cat G. Furthermore, the compounds are effective in blocking the degradative action of elastase on elastin.

Experimental

Melting points were recorded on a Mel-Temp apparatus and are uncorrected. Purification of compounds by flash chromatography was carried out using Merck grade silica gel (grade 60, 230–400 mesh, 60 A) purchased from Aldrich Chemical Co. Thin layer chromatography was performed using Analtech silica gel plates and the TLC plates were visualized by iodine vapor and/or UV light. When necessary, solvents were dried and/or distilled before use. Tetrahydrofuran was distilled from sodium benzophenone. Methylene chloride was distilled from calcium hydride. Acetonitrile and triethylamine (Aldrich) were dried over freshly activated Linde 3 A molecular sieves. Anhydrous acetone was purchased from Aldrich Chemical Co. ^{1}H and ^{13}C NMR spectra of the synthesized compounds were recorded on a Varian XL-400 NMR spectrometer. A Hewlett-Packard diode array UV–VIS spectrophotometer was used in the enzyme assays and inhibition studies. Human leukocyte elastase was purchased from Elastin Products Co., Owensville, MO. Human leukocyte cathepsin G and

Table 4. Inhibitory activity of derivatives of (II) toward human leukocyte elastase and cathepsin G

Compd	R_1	L	HLE	CAT G
			k_{inact}/K_I M^{-1} s^{-1}	
40	Isobutyl	Benzoate	34,200	2900
41	Isobutyl	2,6-Dichlorobenzoate	469,900	2200
42	Isobutyl	Acetylsalicylate	35,700	—
43	Isobutyl	*trans*-Cinnamate	31,700	5500
44	Isobutyl	Dihydrocinnamate	23,200	—
45	Benzyl	2,6-Dichlorobenzoate	2100	3700
46	Benzyl	*trans*-Cinnamate	300	3200
47	Benzyl	Dihydrocinnamate	700	600
48	Benzyl	(Phenylthio)acetate	1000	1300
49	Benzyl	(Phenylsulfoxide)acetate	300	800
50	Benzyl	(Phenylsulfonyl)acetate	300	1100
51	Benzyl	*trans*-3-(Phenylthio)acrylate	200	5500
52	Benzyl	*cis*-3-(Phenylthio)acrylate	200	7300
53	Benzyl	*cis*-3-(Phenylsulfonyl)acrylate	400	5000

Table 5. Correlation of k_{inact}/K_I M^{-1} s^{-1} and leaving group pK_a

Compound[a]	R′	pK_a	k_{inact}/K_I M^{-1} s^{-1}
10	Me_3C-	5.03	60,500
12	Me-	2.76	163,000
15	Ph-	4.20	336,000
18	2,6-Cl_2-Ph-	1.59	2,380,000

[a]All compounds are derived from scaffold (**I**), with isobutyl as R_1, benzyl as R_2 and k_{inact}/K_I values for inhibition of HLE.

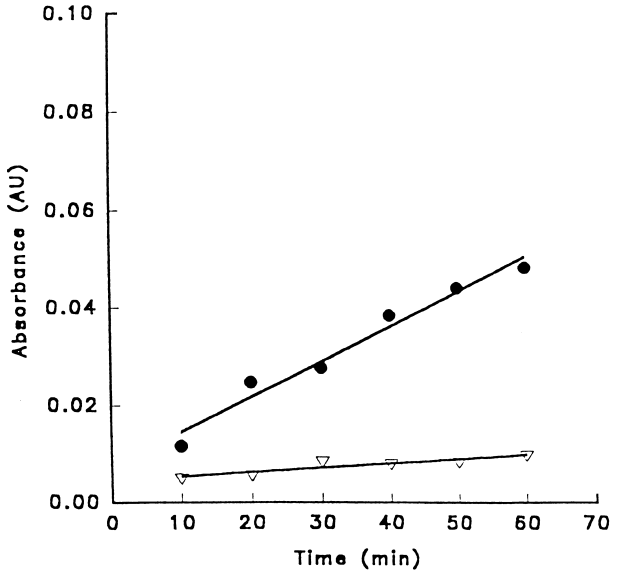


Figure 4. Inhibition of elastinolysis. Elastin (1.5 mg/mL) was incubated with human leukocyte elastase (199 nM) in 0.05 M TES buffer, pH 7.5, 37 °C, using the following conditions: (a) no inhibitor; (b) inhibitor **17**, at an inhibitor to enzyme ratio of 5. Aliquots were withdrawn at 10 min intervals and filtered through Millipore Millex GV filters. The optical density was monitored at 276 nm.

proteinase 3 were purchased from Athens Research and Technology Co., Athens, GA. Methoxysuccinyl Ala-Ala-Pro-Val *p*-nitroanilide and methoxysuccinyl Ala-Ala-Pro-Phe *p*-nitroanilide were purchased from Sigma Chemicals Co., St. Louis, MO.

Representative syntheses

Synthesis of (*S*)-4-isobutyl-2-[(trimethylacetyl)oxymethyl]-1,2,5-thiadiazolidin-3-one 1,1 dioxide 8. A solution of (*S*)-4-isobutyl-1,2,5-thiadiazolidin-3-one 1,1 dioxide[15] (6.2 g; 30 mmol), chloromethyl pivaloate (4.7 g; 31 mmol) and triethylamine (3.0 g; 30 mmol) in 40 mL dry acetonitrile was refluxed for 12 h. The solvent was removed in vacuo, and the residue was taken up in ethyl acetate (100 mL), washed with 5% aq HCl (50 mL), 5% aq $NaHCO_3$ (35 mL), and dried (Na_2SO_4). Evaporation of the solvent left a crude product, which was purified by flash chromatography, using a hexane:methylene chloride gradient, mp 85–88 °C (4.8 g; 52% yield).

Synthesis of (*S*)-4-isobutyl-5-methyl-2[(trimethylacetyl)-oxymethyl]-1,2,5-thiadiazolidin-3-one 1,1 dioxide 9. Sodium hydride (60%, 0.04 g; 1 mmol) was added to a mixture of compound **8** (0.31 g; 1 mmol) and iodomethane (0.43 g; 3 mmol) in dry acetonitrile (2 mL) at 0 °C under nitrogen. The resulting mixture was then stirred at room temperature for 5 h. The solvent was removed in vacuo and the residue was taken up in methylene chloride (20 mL), washed with water (10 mL) and dried (Na_2SO_4). Evaporation of the solvent left a crude product which was purified by flash chromatography[31] using a hexane:methylene chloride gradient (0.14 g; 43% yield).

(*S*) 4-Isobutyl-2-phenylthiomethyl-1,2,5-thiadiazolidin-3-one 1,1 dioxide. (*S*)-4-Isobutyl-1,2,5-thiadiazolidin-3-one 1,1 dioxide (17.8 g; 100 mmol) and chloromethylphenyl sulfide (17.43 g; 110 mmol) were dissolved in dry acetonitrile (150 mL). DBU (15.2 g; 100 mmol) was added and the resulting mixture was refluxed overnight. The solvent was removed, the residue was dissolved in ethyl acetate (300 mL) and washed successively with 5% aq HCl (100 mL), 5% aq $NaHCO_3$ (100 mL) and water (50 mL). The organic phase was dried (Na_2SO_4) and the solvent removed. The crude product was purified by flash chromatography using a hexane:methylene chloride gradient (17.37 g; 55% yield), mp 53–54 °C. ^1H NMR ($CDCl_3$): δ 0.92 (dd, 6H), 1.58 (m, 1H), 1.73 (m, 2H), 4.1 (ddd, 1H), 4.93 (dd, 2H), 5.32 (br d, 1H), 7.3 (m, 3H), 7.55 (m, 2H).

(*S*)-4-Isobutyl-2-chloromethyl-1,2,5-thiadiazolidin-3-one 1,1 dioxide. (*S*)-4-Isobutyl-2-phenylthiomethyl-1,2,5-thiadiazolidin-3-one 1,1 dioxide (9.42 g; 30 mmol) was dissolved in dry methylene chloride (75 mL) and cooled to 0 °C. Sulfuryl chloride (8.09 g; 60 mmol) was added dropwise and the resulting solution was allowed to warm to room temperature. The solvent was removed and the crude product was purified by flash chromatography using a hexane:methylene chloride gradient to yield an oily product (0.27 g; 87% yield). ^{1}H NMR ($CDCl_3$): δ 0.98 (dd, 6H), 1.72 (m, 1H), 1.88 (m, 2H), 4.25 (ddd, 1H), 5.18 (br d, 1H), 5.36 (s, 2H) ppm. ^{13}C NMR: δ 169.49, 59.16, 47.57 ppm.

Synthesis of (*S*)-4-isobutyl-2-(benzoyloxy)methyl-1,2,5-thiadiazolidin-3-one 1,1 dioxide 13. (*S*)-4-Isobutyl-2-chloromethyl-1,2,5-thiadiazolidin-3-one 1,1 dioxide (0.48 g; 2 mmol) was dissolved in dry acetone (6 mL), sodium iodide (0.33 g; 2.2 mmol) was added, and the reaction mixture was stirred at room temperature overnight under nitrogen. A precipitate formed which was removed by filtration through a small amount of silica gel in a disposable glass pipette. The solvent was then removed and the iodomethyl intermediate was redissolved in dry methylene chloride (4 mL). To this solution was added a solution of benzoic acid (0.27 g; 2.2 mmol) and DBU (0.33 g; 2.2 mmol) in dry methylene chloride (4 mL), and the resulting mixture was stirred at room temperature overnight. The solvent was removed, and the residue dissolved in ethyl acetate (75 mL) and extracted successively with water (25 mL), 5% aq HCl (20 mL), 5% aq $NaHCO_3$ (25 mL) and brine (25 mL). The organic phase was dried (Na_2SO_4) and the solvent removed, leaving a crude product which was purified on a Chromatotron plate using methylene chloride as the eluent (0.15 g; 30.6% yield).

(*S*)-4-Isobutyl-2-phenylthiomethyl-5-benzyl-1,2,5-thiadiazolidin-3-one 1,1 dioxide. (*S*)-4-Isobutyl-2-phenylthiomethyl-1,2,5-thiadiazolidin-3-one 1,1 dioxide (3.36 g; 10.7 mmol) and benzyl bromide (2.01 g; 11.77 mmol) were dissolved in dry acetonitrile (25 mL), and 60% sodium hydride (0.28 g; 10.7 mmol) added. The reaction mixture was stirred at room temperature overnight. The solvent was then removed and the residue was dissolved in ethyl acetate (10 mL). The organic phase was extracted with water (2×30 mL) and dried over anhydrous sodium sulfate. The solvent was removed and the crude product was purified by flash chromatography[31] using a hexane:methylene chloride gradient, to yield an oily product (2.79 g; 71%). ^{1}H NMR ($CDCl_3$): δ 0.69 (dd, 6H), 1.48 (m, 1H), 1.63 (m, 2H), 3.78 (t, 1H), 4.33 (dd, 2H), 4.97 (dd, 2H), 7.34 (m, 8H), 7.58 (m, 2H) ppm.

(*S*)-4-Isobutyl-2-chloromethyl-5-benzyl-1,2,5-thiadiazolidin-3-one 1,1 dioxide. This was prepared using the same procedure as that used in the preparation of (*S*)-4-isobutyl-2-chloromethyl-1,2,5-thiadiazolidin-3-one 1,1 dioxide. An oily product was obtained in 54% yield. ^{1}H NMR ($CDCl_3$): δ 0.72 (dd, 6H), 0.60 (m, 1H), 1.73 (m, 2H), 3.92 (t, 1H), 4.41 (dd, 2H), 5.37 (dd, 2H), 7.37 (s, 5H) ppm.

Synthesis of (*S*)-4-isobutyl-5-benzyl-2-(benzoyloxy)methyl-1,2,5-thiadiazolidin-3-one 1,1 dioxide 15. Compound **15** was synthesized using the same procedure as that used in the preparation of compound **9**.

Synthesis of Compound 36. 4-Isobutyl-2-chloromethyl-5-methyl-1,2,5-thiadiazolidin-3-one 1,1 dioxide (0.51 g; 2 mmol) was mixed with sodium iodide (0.33 g; 2.2 mmol) in dry acetone (3 mL), and the resulting mixture was stirred at room temperature overnight. The by-product, sodium chloride, was removed by filtration through a small amount of silica gel in a disposable glass pipette. The solvent was removed in vacuo and the iodo intermediate was redissolved in dry methylene chloride (2 mL). A solution of 3,6-dioxaheptanoic acid (0.32 g; 2 mmol) and DBU (0.30 g; 2 mmol) in methylene chloride (2 mL) was added, and the resulting mixture was stirred at room temperature overnight. The reaction mixture was then diluted with methylene chloride (40 mL) and washed with 5% aq HCl (20 mL), 5% aq $NaHCO_3$ (20 mL) and brine (20 mL). The organic phase was dried (Na_2SO_4) and then concentrated in vacuo. The crude product was purified by flash chromatography using a hexane:methylene chloride gradient, yielding an oily product.

Diethylisobutyl malonate. Sodium metal (28.78 g; 1.25 mol) was added in small pieces to dry ethanol (300 mL) under a nitrogen atmosphere. After the reaction had subsided, an additional 600 mL of dry ethanol was added and the reaction mixture brought to reflux until all the sodium metal had reacted. The solution was then cooled to 80 °C and diethyl malonate (200.2 g; 1.25 mol) was added dropwise over a period of 1 h. To the resulting solution was added 2-methyl-1-bromopropane (171.25 g; 1.25 mol) and the solution was then refluxed for 24 h. The mixture was cooled to room temperature and ethanol was removed in vacuo. The product was isolated by vacuum distillation (82–90 °C, ~0.2 torr) (50% yield). ^{1}H NMR ($CDCl_3$): δ 0.91 (dd, 6H), 1.27 (t, 6H), 1.58 (m, 1H), 1.8 (t, 2H), 3.42 (t, 1H), 4.19 (q, 4H) ppm.

Synthesis of monoester. A solution of potassium hydroxide (24.91 g; 444 mmol) in absolute alcohol (400 mL) was treated with a solution of diethyl isobutyl malonate (96.41 g; 446 mmol) in absolute ethanol (400 mL). The reaction mixture was stirred at room temperature for 48 h. The solvent was removed in vacuo and the residue was dissolved in water (300 mL) and extracted with diethyl ether (2×150 mL). The aqueous phase was acidified to pH 1 with 12 N HCl and extracted with diethyl ether (3×100 mL). The organic extracts were combined, dried (Na_2SO_4) and evaporated, leaving 67.47 g (76% yield) of pure product. ^{1}H NMR ($CDCl_3$): δ 0.92 (d, 6H), 1.29 (t, 3H), 1.62 (m, 1H), 1.82 (m, 2H), 3.48 (t, 1H), 4.22 (q, 2H), 10.4 (br s, 1H) ppm.

Synthesis of ethyl 2-isobutylacrylate. Diethylamine (27.57 g; 377 mmol) was added to the above monoester (68.24 g; 363 mmol) at room temperature and the solution was cooled to 0 °C. Aqueous formaldehyde (38 mL of a 37% formaldehyde solution) was added dropwise

over a period of 0.5 h, after which time the ice bath was removed and the reaction mixture stirred overnight. The solution was extracted with diethyl ether (3×150 mL). The organic extracts were combined and washed successively with 5% aq HCl (3×100 mL), 5% aq $NaHCO_3$ (100 mL) and brine (100 mL). The organic phase was dried (Na_2SO_4) and the solvent removed, leaving 34.0 g (60% yield) of product. ^{1}H NMR ($CDCl_3$): δ 0.88 (d, 6H), 1.29 (t, 3H), 1.80 (m, 1H), 2.18 (d, 2H), 4.19 (q, 2H), 5.47 (d, 1H), 6.16 (d, 1H) ppm.

Synthesis of thiolacetic acid adduct. Thiolacetic acid (33.1 g; 435 mmol) was added to ethyl 2-isobutyl acrylate (31.33 g; 217.6 mmol) and stirred at room temperature for 1 week. The reaction mixture was diluted with diethyl ether (100 mL) and 5% aq $NaHCO_3$ (100 mL) and the resulting mixture was stirred for 1 h. After the reaction had subsided, sodium bicarbonate (15 g) was added in small portions with vigorous stirring. Ethyl ether (100 mL) was added, the mixture was transferred to a separatory funnel, agitated and the two phases separated. The organic phase was washed with water (100 mL), dried (Na_2SO_4) and evaporated, leaving a quantitative yield of pure product. ^{1}H NMR ($CDCl_3$): δ 0.92 (d, 6H), 1.26 (t, 3H), 1.38 (m, 1H), 1.60 (m, 2H), 2.33 (s, 3H), 2.68 (m, 1H), 2.98 (dd, 1H), 3.13 (dd, 1H), 4.16 (q, 2H) ppm.

4-Isobutyl-isothiazolidin-3-one 1,1 dioxide. Formic acid (460 mL) and 30% hydrogen peroxide (90 mL) were mixed at room temperature, stirred for 1 h, then cooled to 0 °C. A solution of the thioester (34.80 g; 150 mmol) in formic acid (50 mL) was added dropwise to the performic acid solution, and the mixture was stirred for 2 h at 0 °C. The ice bath was removed and the reaction mixture was stirred at room temperature for an additional 46 h. Excess formic acid was removed using a rotary evaporator and residual formic acid was removed by azeotropic distillation on the rotary evaporator using toluene (4×100 mL). Residual toluene was removed under high vacuum, leaving a near quantitative yield of sulfonic acid. This was used in the next step without further purification.

Phosphorus trichloride (41.00 g; 375 mmol) was added to the sulfonic acid (37.20 g; 150 mmol) under nitrogen and the solution was heated to gentle reflux for 2 h. The reaction mixture was cooled to room temperature and excess PCl_3 was removed on the rotary evaporator using an ice trap. The product was dissolved in methylene chloride (100 mL) and filtered through a short column of silica gel. The silica gel was washed with methylene chloride (150 mL), the eluents were combined and the solvent removed, leaving 34 g (88% yield) of product which was used in the next step. ^{1}H NMR ($CDCl_3$): δ 0.98 (dd, 6H), 1.29 (t, 3H), 1.46 (m, 1H), 1.68 (m, 2H), 3.21 (m, 1H), 3.68 (dd, 1H), 4.2–4.3 (m, 3H) ppm. ^{13}C NMR: δ 172.37, 66.59, 61.68 ppm.

The sulfonyl chloride (33.98 g; 132.5 mmol) was dissolved in toluene (200 mL) and cooled in an ice bath. Ammonium hydroxide (15 M) was added dropwise. The resulting suspension was stirred at room temperature for 1 h, the solvent was removed and the residue was taken up in ethyl acetate (400 mL). The organic phase was washed with water (75 mL), brine (75 mL) and dried (Na_2SO_4). The solvent was removed, leaving 21.84 g (69.5% yield) of product. This was used in the next step without further purification. ^{1}H NMR ($CDCl_3$): δ 0.94 (dd, 6H), 1.29 (t, 3H), 1.39 (m, 1H), 1.62 (m, 2H), 3.05 (m, 1H), 3.18 (dd, 1H), 3.63 (dd, 1H), 4.19 (q, 2H), 5.18 (s, 2H) ppm. ^{13}C NMR: δ 174.22, 61.20, 56.76 ppm.

A solution of the sulfonamide derivative (10.83 g; 45.7 mmol) in dry THF (75 mL) was added dropwise to a suspension of 60% NaH (1.5 g; 50 mmol) in dry THF. After stirring the mixture overnight at room temperature, the solvent was removed and the residue was washed with methylene chloride, yielding the sodium salt of the cyclic imide in quantitative yield. ^{1}H NMR (DMSO-d_6): δ 0.86 (dd, 6H), 1.22 (m, 1H), 1.53 (m, 1H), 1.67 (m, 1H), 2.71 (br m, 2H), 3.20 (br m,1H) ppm. ^{13}C NMR: 179.1, 54.05, 45.56 ppm.

The free imide was obtained using the following procedure: sodium salt (5 mmol) was dissolved in methanol (20 mL), Dowex-H^+ resin (2 g), and the resulting suspension was stirred for 1 h. The resin was filtered off and washed with additional methanol. Removal of the solvent left 0.89 g (93% yield) of pure product. ^{1}H NMR ($CDCl_3$): δ 0.97 (dd, 6H), 1.5–1.7 (m, 2H), 1.92 (m, 1H), 3.30 (m, 2H), 3.75 (m, 1H), 7.37 (br s, 1H) ppm. ^{13}C NMR: 172.97, 53.71, 41.89 ppm.

Synthesis of 4-isobutyl-2-(phenylthiomethyl)-isothiazolidin-3-one 1,1 dioxide. 4-Isobutyl-isothiazolidin-3-one 1,1 dioxide (0.96 g; 5 mmol) and tetrabutylammonium bromide (0.16 g; 0.5 mmol) were suspended in dry toluene (5 mL). Chloromethyl phenyl sulfide (2.03 g; 5 mmol) was added and the resulting mixture was refluxed for 24 h. The solvent was removed and the residue was dissolved in methylene chloride and filtered through a pad of silica gel. Evaporation of the solvent left a crude product which was purified using a Chromatotron plate (hexane:methylene chloride), yielding 1.03 g (66% yield) of pure oily product. ^{1}H NMR ($CDCl_3$): δ 0.92 (dd, 6H), 1.45 (m, 1H), 1.62 (m, 1H), 1.85 (m, 1H), 3.16 (m, 2H), 3.64 (m, 1H), 4.91 (dd, 2H), 7.32 (m, 3H), 7.57 (m, 2H) ppm. ^{13}C NMR: δ 168.51, 52.37, 44.11 ppm.

Synthesis of 4-isobutyl-2-chloromethyl-isothiazolidin-3-one 1,1 dioxide. 4-Isobutyl-2-phenylthiomethyl-isothiazolidin-3-one 1,1 dioxide (11.24 g; 35.9 mmol) was dissolved in dry methylene chloride (70 mL) under nitrogen and cooled to 0 °C. A solution of sulfuryl chloride (9.68 g; 71.72 mmol) in methylene chloride (40 mL) was added dropwise, and the resulting mixture was stirred and allowed to warm to room temperature over a period of 1 h. The solvent was removed and the residue was purified by flash chromatography using a hexane:methylene chloride gradient (4.75 g; 55% yield). ^{1}H NMR ($CDCl_3$): δ 0.97 (dd, 6H), 1.58 (m, 1H), 1.71 (m, 1H), 2.0 (m, 1H), 3.24–3.34 (m, 2H), 3.78 (m, 1H), 5.32 (dd, 2H) ppm. ^{13}C NMR: δ 168.17, 52.37, 45.44 ppm.

General procedure for the synthesis of compounds 39–44. 4-Isobutyl-2-chloromethyl-isothiazolidin-3-one 1,1 dioxide (0.48 g; 2 mmol) was dissolved in dry acetone (6 mL), sodium iodide (0.33 g; 2.2 mmol) was added, and the reaction mixture was stirred overnight at room temperature. The solvent was removed in vacuo and the iodomethyl intermediate was redissolved in dry methylene chloride (4 mL). A solution of the appropriate carboxylic acid (2.2 mmol) and DBU (0.33 g; 2.2 mmol) in methylene chloride (4 mL) was added, and the resulting mixture was stirred overnight at room temperature. The reaction mixture was diluted with methylene chloride (35 mL) and washed successively with water (15 mL), 5% aq HCl (15 mL), 5% aq $NaHCO_3$ (20 mL) and brine (25 mL). The solution was dried (Na_2SO_4) and the solvent evaporated, leaving a crude product which was purified by flash chromatography using a hexane:methylene chloride gradient (0.67 g; 58% yield).

4-Benzyl-2-hydroxymethyl-isothiazolidin-3-one 1,1 dioxide. 4-Benzylisothiazolidin-3-one 1,1 dioxide[26] (0.53 g; 2.35 mmol) and 37% formaldehyde (0.71 g; 7.05 mmol) in 20 mL of a mixture of water and ethanol (3:1) were refluxed for 1 h. The resulting solution was allowed to cool to room temperature. The precipitate was collected by suction filtration and washed with several portions of diethyl ether (5×10 mL) and dried to yield a pure product (0.53 g; 88% yield), mp 148–149 °C. ^{1}H NMR (acetone-d_6): δ 3.0 (dd, 1H), 3.40 (m, 2H), 3.70 (m, 2H), 5.10 (m, 2H), 5.62 (t, 1H), 7.25–7.40 (m, 5H).

4-Benzyl-2-chloromethyl-isothiazolidin-3-one 1,1 dioxide. A mixture of 4-benzyl-2-hydroxymethyl-isothiazolidin-3-one 1,1 dioxide (0.51 g; 2 mmol), thionyl chloride (2.38 g; 20 mmol) and dry diethyl ether (3 mL) was stirred at room temperature for 3 days. The excess reagent and solvent were removed in vacuo and the residue was taken up in methylene chloride (30 mL). The organic layer was washed with 5% aq $NaHCO_3$ (2×15 mL) and dried (Na_2SO_4). Removal of the solvent yielded the desired compound (0.5 g; 91% yield), mp 140–141 °C. ^{1}H NMR ($CDCl_3$): δ 2.93 (m, 1H), 3.30 (m, 1H), 3.40–3.60 (m, 3H), 5.32 (d, 2H), 7.18–7.40 (m, 5H).

General procedure for the synthesis of compounds 45–52. Compounds **43**–**50** were prepared by refluxing a solution of 4-benzyl-2-chloromethyl-isothiazolidin-3-one 1,1 dioxide (2 mmol), triethylamine (3 mmol) and the appropriate carboxylic acid (3 mmol) in dry acetonitrile (10 mL) for 12 h. The solvent was removed and the residue was taken up in diethyl ether (30 mL). The organic layer was washed with 5% aq $NaHCO_3$ (15 mL), 5% aq HCl (15 mL), water (20 mL) and dried (Na_2SO_4). The solvent was removed in vacuo, yielding a crude product which was purified by flash chromatography.

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