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Potent and Specific Inhibition of Human Leukocyte Elastase, Cathepsin G and Proteinase 3 by Sulfone Derivatives Employing the 1,2,5-Thiadiazolidin-3-one 1,1 Dioxide Scaffold

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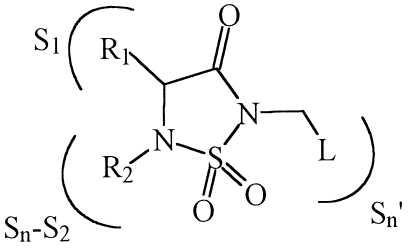
Abstract—This paper describes the results of structure–activity relationship studies in a series of heterocyclic mechanism-based inhibitors based on the 1,2,5-thiadiazolidin-3-one 1,1 dioxide scaffold **I** and capable of interacting with the S_n and S_n' subsites of a serine proteinase. Sulfone derivatives of **I** were found to be highly effective, time-dependent inhibitors of human leukocyte elastase (HLE), cathepsin G (Cat G) and proteinase 3 (PR 3). The judicious selection of an R_1 group (accommodated at the primary specificity site S_1) that is based on the known substrate specificity of a target serine proteinase, was found to yield highly selective inhibitors. The presence of a benzyl group (R_2 = benzyl) at the S_2 subsite was found to lead to a pronounced enhancement in inhibitory potency. Furthermore, the effective use of computer graphics and modeling has led to the design of potent, water-soluble inhibitors. The results of these studies demonstrate that the 1,2,5-thiadiazolidin-3-one 1,1, dioxide platform provides an effective means for appending recognition elements in a well-defined vector relationship, and in fashioning highly-selective and potent inhibitors of serine proteinases.

Introduction

The neutrophil-derived serine endopeptidases human leukocyte elastase (HLE), cathepsin G (Cat G), and proteinase 3 (PR 3) have been implicated in the etiology and/or pathophysiology of a range of inflammatory diseases, including pulmonary emphysema,[1] bronchitis,[2] psoriasis,[3] and ischemia-reperfusion injury.[4] Selective inhibitors of these enzymes may be of potential therapeutic value for these and related ailments.

We have recently described the structure-based design of a novel and general class of heterocyclic mechanism-based inhibitors of serine proteinases based on the 1,2,5-thiadiazolidin-3-one 1,1 dioxide scaffold **I**.[5,6] Exploratory biochemical studies have shown that the heterocyclic platform in **I** functions as a highly effective and versatile scaffold for appending peptidyl or nonpeptidyl recognition elements in a well-defined spatial arrangement, thereby optimizing favorable binding interactions with both the S_n and S_n' subsites. It was also demonstrated that high or near absolute selectivity for a target serine proteinase could be achieved through the selection of an R_1 group that is based on the known substrate specificity of the enzyme.[6] We wish to describe herein the results of structure–activity relationship studies using sulfone-based derivatives of **I** that delineate further the scope and utility of the heterocyclic template in **I** in the general design of serine proteinase inhibitors.



I

Key words: 1,2,5-Thiadiazolidin-3-one 1,1 dioxide; protease inhibitors; elastase; cathepsin G; proteinase 3.

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Results

Chemistry

The reaction sequence shown in Scheme 1 was used in the synthesis of compounds **1**–**33**. (L)-Leu-OCH_3 and (L) or (D)-Phe-OCH_3 were used as the starting materials for the synthesis of compounds **1**–**24** and **25**–**33**, respectively. Alkylation of compound **34** with an appropriate chloromethylalkyl or aryl sulfide, followed by N-5 alkylation and subsequent oxidation with *m*-chloroperbenzoic acid, yielded compounds **1**–**17** and **24**–**33**. Treatment of compound **35** with sulfuryl chloride yielded the corresponding chloromethyl derivative **36**, which was then reacted with the appropriate thiol in the presence of base. Subsequent oxidation yielded compounds **18**–**23**. The synthesized compounds and their physical and spectral data are listed in Table 1. For comparative purposes, a series of hydantoin derivatives **II** were also synthesized according to Scheme 2.

Biochemical studies

The inhibitory activity of compounds **1**–**33** toward HLE, Cat G, and PR 3 was determined as described in detail elsewhere.[6] Briefly, the apparent second-order inactivation rate constants (k_{inact}/K_i M^{-1} s^{-1}) were determined in duplicate or triplicate by the progress curve method[6,7] and are listed in Table 2. Typical progress curves for the hydrolysis of MeOSuc-AAPV-pNA by HLE in the presence of inhibitor **13** are shown in Figure 1. 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 inhibitor **I** as a function of time were determined according to eq (1), where A is the absorbance at 410 nm, v_0 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_0 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.

$$A = v_s t + (v_0 - v_s)(1 - e^{-k_{obs} t})/k_{obs} + A_0 \quad (1)$$

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

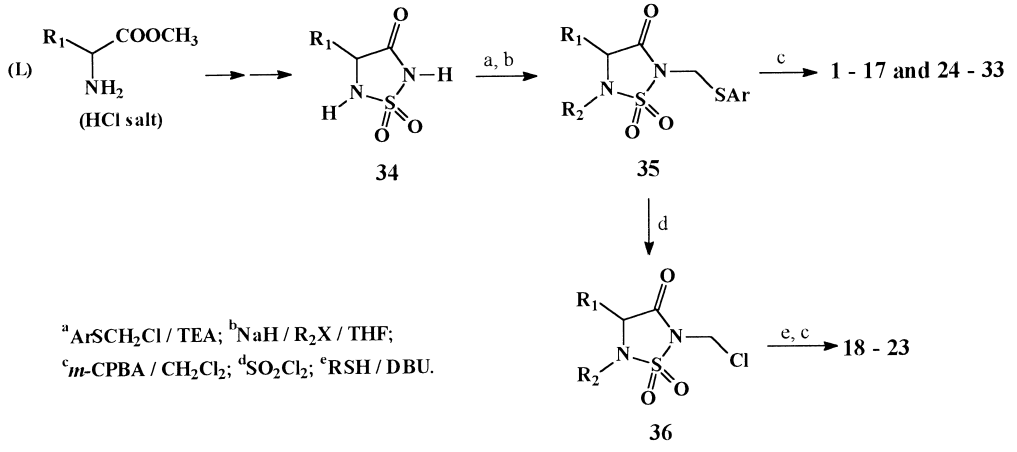
Molecular modeling

Enzyme–inhibitor modeling studies were carried out using the Tripos force field of SYBYL, version 6.1a (Tripos Associates, St Louis, MO) and a Silicon Graphics INDY workstation.

Discussion

Structure–activity relationship studies

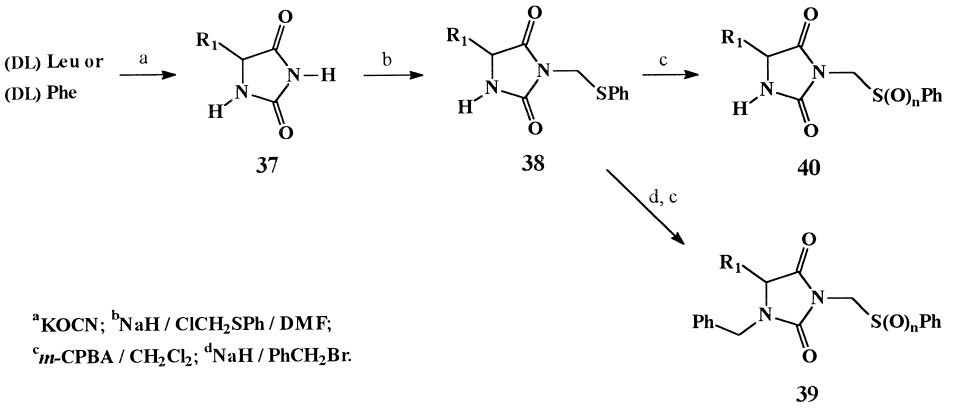
We have recently described the structure-based design of a novel and general class of mechanism-based inhibitors of serine proteinases based on the 1,2,5-thiadiazolidin-3-one 1,1 dioxide scaffold.[5,6] The results of specificity and modeling studies using the X-ray crystal structure of the HLE–TOMI (turkey ovomucoid inhibitor) complex suggested that the binding of **I** to the active site of HLE is similar to that of a peptidyl substrate, namely, the R_1 group is accommodated at the primary specificity site (S_1), while R_2 and L (SO_2R) extend into the S_2–S_n and



Scheme 1. Synthesis of derivatives **I**.

Table 1. Physical constants and spectral data of inhibitors **1–33**

Compd	Mp (°C)	^{1}H NMR δ	MF (Anal.)
1	125.5–126.0	0.94 (m, 9H), 1.39 (m, 2H), 1.56–1.82 (m, 5H), 3.06 (ddd, 1H), 3.33 (ddd, H), 3.89 (t, 1H), 4.88 (s, 2H), 7.56–7.76 (m, 3H), 8.00 (dd, 2H)	$C_{17}H_{26}N_2O_5S_2$ (C, H, N)
2	145.0–147.0	0.70 (dd, 6H), 1.45–1.70 (m, 3H), 3.84 (t, 1H), 3.93 (s, 3H), 4.28 (d, 1H), 4.57 (d, 1H), 4.89 (s, 2H), 7.42–7.77 (m, 5H), 7.97–8.11 (m, 4H)	$C_{22}H_{26}N_2O_7S_2$ (C, H, N)
3	153.0–154.0	0.72 (dd, 6H), 1.50–1.70 (m, 3H), 3.88 (t, 1H), 4.31 (d, 1H) and 4.61 (d, 1H), 4.92 (s, 2H), 7.48–7.74 (m, 5H), 7.97–8.17 (m, 4H)	$C_{21}H_{24}N_2O_7S_2$ (C, H, N)
4	133.0–134.0	0.63 (d, 3H), 0.74 (d, 3H), 1.45–1.70 (m, 3H), 3.82 (t, 1H), 3.94 (s, 3H), 4.27 (d, 1H), 4.57 (d, 1H), 4.89 (s, 2H), 7.47–7.73 (m, 5H), 8.03 (m, 4H)	$C_{22}H_{26}N_2O_7S_2$ (C, H, N)
5	167.0–169.0	0.70 (dd, 6H), 1.37–1.70 (m, 3H), 4.18 (t, 1H), 4.42 (d, 1H), 4.71 (d, 1H), 5.06 (d, 2H), 7.52–7.82 (m, 5H), 8.02 (m, 3H), 8.14 (s, 1H)	$C_{21}H_{24}N_2O_7S_2$ (C, H, N)
6	165.0–167.0	0.48 (d, 3H), 0.70 (d, 3H), 1.35–1.62 (m, 3H), 3.93 (s, 3H), 3.98 (t, 1H), 4.39 (d, 1H), 4.90 (s, 2H), 5.17 (d, 1H), 7.40–7.73 (m, 6H), 8.00 (m, 3H)	$C_{22}H_{26}N_2O_7S_2$ (C, H, N)
7	181.0–182.0	0.51 (d, 3H), 0.71 (d, 3H), 1.33–1.62 (m, 3H), 4.16 (t, 1H), 4.49 (d, 1H), 5.01 (dd, 2H), 5.22 (d, 1H), 7.48 (m, 1H), 7.58–7.79 (m, 5H), 8.02 (m, 3H)	$C_{21}H_{24}N_2O_7S_2$ (C, H, N)
8	136.0–137.0	0.69 (dd, 6H), 1.40–1.70 (m, 3H), 3.65 (s, 2H), 3.94 (t, 1H), 4.41 (dd, 2H), 4.96 (d, 2H), 7.30–8.05 (m, 9H)	$C_{22}H_{26}N_2O_7S_2$ (C, H, N)
9	107.0–108.0	0.92 (dd, 6H), 1.47 (s, 9H), 1.50–1.78 (m, 3H), 3.68 (d, 1H), 4.12 (t, 1H), 4.16 (d, 1H), 4.86 (d, 2H), 7.55–7.75 (m, 3H), 8.03 (d, 2H)	$C_{19}H_{28}N_2O_7S_2$ (C, H, N)
10	111.0–112.0	0.90 (dd, 6H), 1.50–1.78 (m, 3H), 3.82 (d, 1H), 4.11 (dd, 1H), 4.32 (d, 1H), 4.77 (d, 2H), 5.19 (d, 2H), 7.32–7.45 (m, 5H), 7.52–7.73 (m, 3H), 8.01 (d, 2H)	$C_{22}H_{26}N_2O_7S_2$ (C, H, N)
11	140 (dec.)	(acetone-d_6) 0.93 (dd, 6H), 1.55 (m, 1H), 1.67–1.85 (m, 2H), 4.20 (dd, 2H), 4.40 (dd, 1H), 4.98 (dd, 2H), 7.62–8.05 (m, 5H)	$C_{15}H_{20}N_2O_7S_2$ (C, H, N)
12	oil	0.99 (dd, 6H), 1.70–2.00 (m, 3H), 2.97 (s, 3H), 3.08 (s, 3H), 4.01 (t, 1H), 4.78 (d, 2H)	$C_9H_{19}N_2O_5S_2$ (C, H, N)
13	102.0–103.0	0.93 (dd, 6H), 1.60–1.85 (m, 3H), 2.88 (s, 3H), 3.82 (t, 1H), 4.89 (s, 2H), 7.55 (d, 2H), 7.91 (d, 2H)	$C_{14}H_{19}N_2O_5S_2Cl$ (C, H, N)
14	102.0–103.0	0.69 (dd, 6H), 1.41–1.68 (m, 3H), 3.84 (t, 1H), 4.38 (dd, 2H), 4.88 (s, 2H), 7.38 (m, 5H), 7.53 (d, 2H), 7.90 (d, 2H)	$C_{20}H_{23}N_2O_5S_2Cl$ (C, H, N)
15	133.0–134.0	0.85 (dd, 6H), 1.55–1.80 (m, 3H), 2.81 (s, 3H), 4.75 (t, 1H), 4.82 (s, 2H), 7.50–7.68 (m, 3H), 7.93 (d, 2H)	$C_{14}H_{20}N_2O_5S_2$ (C, H, N)
16	103.0–104.0	0.63 (d, 3H), 0.77 (d, 3H), 1.44–1.70 (m, 3H), 3.85 (t, 1H), 4.28 (d, 1H), 4.50 (d, 1H), 4.90 (s, 2H), 7.30–8.05 (m, 10H)	$C_{22}H_{24}N_2O_5S_2$ (C, H, N)
17	118.0–120.0	0.82 (t, 6H), 1.50–1.80 (m, 3H), 2.97 (t, 2H), 3.31 (m, 1), 3.60 (m, 1H), 3.84 (t, 1H), 4.87 (d, 2H), 7.20–7.37 (m, 5H), 7.57 (t, 2H), 7.71 (t, 1H), 8.01 (d, 2H)	$C_{21}H_{26}N_2O_5S_2$ (C, H, N)
18	87.0–88.0	0.99 (dd, 6H), 1.84 (m, 2H), 1.95 (m, 1H), 2.97 (s, 3H), 4.00 (t, 1H), 4.64 (d, 2H), 4.65 (s, 2H), 7.41 (m, 3H), 7.52 (m, 2H)	$C_{15}H_{22}N_2O_5S_2$ (C, H, N)
19	108.0–109.0	0.99 (dd, 6H), 1.83 (m, 2H), 1.94 (m, 1H), 2.98 (s, 3H), 4.01 (t, 1H), 4.43 (s, 2H), 4.63 (d, 2H), 7.43 (dd, 4H)	$C_{15}H_{21}N_2O_5S_2Cl$ (C, H, N)
20	135.0–136.0	0.69 (d, 3H), 0.80 (d, 3H), 1.62–1.80 (m, 3H), 4.04 (t, 1H), 4.36 (d, 1H), 4.59 (d, 1H), 4.44 (s, 2H), 4.65 (d, 2H), 7.40 (d, 2H), 7.41 (s, 5H), 7.48 (d, 2H)	$C_{21}H_{25}N_2O_5S_2Cl$ (C, H, N)
21	65.0–67.0	0.97 (dd, 6H), 1.80 (m, 2H), 1.91 (m, 1H), 2.21 (m, 2H), 2.78 (t, 2H), 2.93 (s, 3H), 3.15 (m, 2H), 3.95 (t, 1H), 4.71 (d, 2H), 7.15–7.35 (m, 5H)	$C_{17}H_{26}N_2O_5S_2$ (C, H, N)
22	77.0–78.0	0.67 (d, 3H) and 0.79 (d, 3H), 1.58–1.78 (m, 3H), 2.22 (m, 2H), 2.78 (t, 2H), 3.13 (m, 2H), 4.00 (t, 1H), 4.31 (d, 1H), 4.54 (d, 1H), 4.71 (dd, 2H), 7.18–7.34 (m, 5H), 7.39 (s, 5H)	$C_{23}H_{30}N_2O_5S_2$ (C, H, N)
23	78.0–80.0	0.93 (dd, 6H), 1.58–1.59 (m, 3H), 2.88 (s, 3H), 3.81 (dd, 1H), 4.91 (s, 2H), 7.70 (d, 1H), 7.77 (t, 1H), 8.19 (d, 1H), 8.24 (s, 1H)	$C_{15}H_{19}N_2O_5S_2F_3$ (C, H, N)
24	102.0–103.0	0.59 (d, 3H), 0.73 (d, 3H), 1.43 (dd, 1H), 1.52–1.70 (m, 2H), 3.86 (dd, 1H), 4.20 (d, 1H), 4.50 (d, 1H), 4.92 (s, 2H), 7.38 (m, 5H), 7.74 (t, 1H), 7.95 (d, 1H), 8.18 (d, 1H), 8.26 (s, 1H)	$C_{21}H_{23}N_2O_5S_2F_3$ (C, H, N)
25	190.0–191.5	2.90 (m, 1H), 3.12 (dd, 1H), 4.28 (m, 1H), 4.88 (s, 2H), 7.20–8.10 (m, 10H), 8.12 (d, 1H)	$C_{16}H_{16}N_2O_5S_2$ (C, H, N)
26	116.0–118.0	2.66 (s, 3H), 3.04 (dd, 1H), 3.19 (dd, 1H), 4.03 (dd, 1H), 4.83 (s, 2H), 7.18–7.98 (m, 10H)	$C_{17}H_{18}N_2O_5S_2$ (C, H, N)
27	133.5–134.5	3.00 (m, 2H), 3.91 (d, 1H), 4.04 (dd, 1H), 4.35 (d, 1H), 4.85 (d, 2H), 7.00–8.00 (m, 15H)	$C_{23}H_{22}N_2O_5S_2$ (C, H, N)
28	114.0–115.0	3.00 (m, 2H), 3.92 (d, 1H), 4.03 (dd, 1H), 4.35 (d, 1H), 4.85 (d, 2H), 7.00–8.00 (m, 15H)	$C_{23}H_{22}N_2O_5S_2$ (C, H, N)
29	113.0–114.0	0.78 (t, 3H), 1.05–1.40 (m, 4H), 2.78 (m, 1H), 3.03 (dd, 1H), 3.16 (m, 2H), 4.07 (dd, 1H), 4.88 (d, 2H), 7.20–7.98 (m, 10H)	$C_{20}H_{24}N_2O_5S_2$ (C, H, N)
30	124.0–125.0	2.99 (dd, 1H), 3.18 (dd, 1H), 3.58 (ddd, 1H), 3.85 (ddd, 1H), 4.21 (dd, 1H), 4.88 (d, 2H), 5.77 (ddd, 1H), 6.30 (d, 1H), 7.20–8.00 (m, 15H)	$C_{25}H_{24}N_2O_5S_2$ (C, H, N)
31	143.0–144.0	1.43 (s, 9H), 2.89 (dd, 2H), 2.97 (d, 1H), 3.11 (dd, 1H), 3.90 (d, 1H),4.30 (dd, 1H), 4.88 (s, 2H), 7.20–7.40 (m, 5H), 7.55–7.80 (m, 3H), 8.00–8.10 (m, 2H)	$C_{22}H_{26}N_2O_7S_2$ (C, H, N)
32	144.0–145.0	1.42 (s, 9H), 2.89 (dd, 2H), 2.96 (d, 1H), 3.11 (dd, 1H), 3.90 (d, 1H), 4.30 (dd, 1H), 4.87 (s, 2H), 7.20–7.40 (m, 5H), 7.55–7.80 (m, 3H), 8.00–8.10 (m, 2H)	$C_{22}H_{26}N_2O_7S_2$ (C, H, N)
33	118.0–120.0	3.00 (ddd, 2H), 3.63 (s, 2H), 3.92 (d, 1H) and 4.29 (d, 1H), 4.02 (dd, 1H), 4.85 (dd, 2H), 6.98 (d, 2H) and 7.15 (d, 2H), 7.08 (m, 2H), 7.36 (m, 3H), 7.54 (m, 2H), 7.68 (m, 1H), 7.93 (d, 2H)	$C_{25}H_{24}N_2O_7S_2$ (C, H, N)



Scheme 2. Synthesis of hydantion derivatives **II**.

Table 2. Inhibitory activity of derivatives of **I** toward human leukocyte elastase, proteinase 3, and cathepsin G

Compd[a]	R_1	R_2	L	k_{inact}/K_I M^{-1} s^{-1}[b]		
				HLE	PR3	Cat G
1	isobutyl	*n*-butyl	SO_2Ph	8230	430	inactive
2	isobutyl	(*p*-$COOCH_3$)Bzl	SO_2Ph	67,800	1280	790
3	isobutyl	(*p*-COOH)Bzl	SO_2Ph	7800	840	70
4	isobutyl	(*m*-$COOCH_3$)Bzl	SO_2Ph	63,600	7080	80
5	isobutyl	(*m*-COOH)Bzl	SO_2Ph	38,700	10,300	350
6	isobutyl	(*o*-$COOH_3$)Bzl	SO_2Ph	25,800	2440	60
7	isobutyl	(*o*-COOH)Bzl	SO_2Ph	7690	1050	150
8	isobutyl	(*p*-CH_2COOH)Bzl	SO_2Ph	26,700	1770	290
9	isobutyl	CH_2COO-*t*-Bu	SO_2PH	2020	1830	30
10	isobutyl	CH_2COO-Bzl	SO_2Ph	1710	—[c]	inactive
11	isobutyl	CH_2COOH	SO_2Ph	1050	310	inactive
12	isobutyl	methyl	SO_2CH_3	4590	370	inactive
13	isobutyl	methyl	SO_2(*p*-Cl phenyl)	32,200	6280	inactive
14	isobutyl	benzyl	SO_2(*p*-Cl phenyl	219,000	16,200	180
15[d]	isobutyl	methyl	SO_2Ph	9490	2250	inactive
16[d]	isobutyl	benzyl	SO_2Ph	95,200	5240	110
17	isobutyl	CH_2CH_2Ph	SO_2Ph	6590	—	inactive
18	isobutyl	methyl	SO_2CH_2Ph	22,300	9580	100
19	isobutyl	methyl	SO_2CH_2(*p*-Cl phenyl)	47,500	16,900	160
20	isobutyl	benzyl	SO_2CH_2(*p*-Cl phenyl)	165,000	20,300	70
21	isobutyl	methyl	$SO_2(CH_2)_3Ph$	15,400	8020	20
22	isobutyl	benzyl	$SO_2(CH_2)_3Ph$	9990	3250	60
23	isobutyl	methyl	SO_2(*m*-CF_3 phenyl)	38,200	4780	inactive
24	isobutyl	benzyl	SO_2(*m*-CF_3 phenyl)	240,000	2250	inactive
25	benzyl	H	SO_2Ph	inactive	—	30
26	benzyl	methyl	SO_2Ph	inactive	—	120
27	benzyl	benzyl	SO_2Ph	inactive	—	11,200
28	(D) benzyl	benzyl	SO_2Ph	inactive	—	1580
29	benzyl	*n*-butyl	SO_2Ph	inactive	—	320
30	benzyl	*t*-cinnamyl	SO_2Ph	inactive	—	760
31	benzyl	CH_2COO-*t*-Bu	SO_2Ph	inactive	—	1130
32	(D) benzyl	CH_2COO-*t*-Bu	SO_2Ph	inactive	—	280
33	benzyl	(*p*-CH_2COOH)Bzl	SO_2Ph	inactive	inactive	3760

[a]All compounds have the L-configuration, unless indicated otherwise.
[b]Reproducibility: ±10%.
[c]Not determined.
[d]Reported in 6 and listed here for comparative purposes.

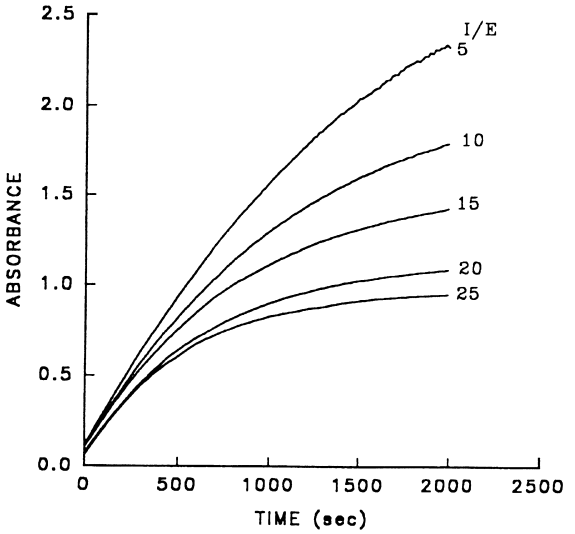


Figure 1. Progress curves for the inhibition of human leukocyte elastase (HLE) by compound **13**. Absorbance was recorded at 410 nm for reaction solutions containing 21.2 nM HLE, 1 mM MeOSuc-AAPV-pNA, and the indicated concentrations of inhibitor in 0.1 M HEPES buffer, pH 7.25, and 3.6% dimethyl sulfoxide. The temperature was maintained at 25 °C, and the reactions were initiated by the addition of enzyme.

S_n' subsites.[8] Thus, it was anticipated that appropriate derivatives of **I** capable of exploiting favorable binding interactions with both the S_n and S_n' subsites would function as highly selective and effective inhibitors of a target serine proteinase. In order to gain further insight into the interaction of **I** with serine proteinases, as well as optimize inhibitory activity and selectivity, a series of sulfone derivatives was obtained (Scheme 1) by varying the nature of R_1, R_2, and L (Table 1), and their inhibitory activity toward HLE, Cat G, and PR 3 determined.

The inactivation of HLE by a representative member of this class (compound **13**, Table 2) was found to be rapid and time-dependent. Furthermore, the progress curves also indicate that the interaction of **13** with HLE involves the active site (Figure 1). These inhibitors inactivated HLE with high efficiency, that is, their partition ratios were close to zero (unpublished observations). The apparent K_i^* (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 range.

Based on the results summarized in Table 2, the following inferences can be made:

Nature of R_1 and R_2. (1) Derivatives of **I** are potent inhibitors of HLE, PR 3, and Cat G. Compounds derived from (L)-Leu were found to be highly effective in inhibiting HLE (Table 2), reflecting the known preference of HLE for P_1 residues with small hydrophobic alkyl chains (Val, Leu), but showed little or no activity toward Cat G, a chymotrypsin-like proteinase. Conversely, compounds derived from (L)-Phe were found to inhibit Cat G, but not HLE or PR 3 (Table 2, compounds **25**–**33**). These results indicate that the selection of an R_1 group (corresponding to the side chain of the P_1 residue) that is based on the known substrate specificity of a serine proteinase results in both high potency and enzyme selectivity.[6] (2) Compounds that inhibited HLE also inhibited PR 3, albeit with lower efficiency. The primary structure of HLE shows considerable homology with PR 3 (54%), however, the active site of PR 3 is more constricted due to the substitution of Val-190 with Ile, which reduces the size of the S_1 pocket, and is smaller than that of HLE.[9–11] Nevertheless, because the active sites of the two enzymes are similar, leucine-derived compounds were found to inhibit both HLE and PR 3. (3) Previous in vivo studies with peptidyl and nonpeptidyl trifluoromethylketones,[12] β-lactam,[13] and saccharin[14] derivatives have shown that, despite their high inhibitory potency, highly lipophilic inhibitors lack oral bioavailability, an observation ascribed to inappropriate physicochemical properties, namely, poor absorption and protein binding. However, oral bioavailability could be achieved by introducing acidic or basic polar functionalities. As a prelude toward the design of orally bioavailable inhibitors of HLE based on **I**, inhibitors with a polar functionality (Table 2, compounds **3**, **5**, **7**, **8**, and **11**) were synthesized and shown to be efficient inhibitors of the enzyme. Although the potency of these compounds was lower than that of the more lipophilic precursor esters (Table 2, compounds **2**, **4**, **6**, **10**), nevertheless these compounds had high potency. In the case of compound **8**, where the P_2 residue consists of a (*p*-carboxymethyl)benzyl group at N-5, molecular modeling was used to predict the preferred location of the polar functionality. The modeling studies suggested that the carboxyl group in **8** is oriented away from the hydrophobic surface of the enzyme and toward the aqueous milieu, while the P_2 residue is nestled into the hydrophobic S_2 subsite of the enzyme, accounting for its high potency. (4) With respect to the nature of the P_2 residue, a benzyl group is preferred over an alkyl group (Table 2, compounds **15** versus **16**, **19** versus **20**, and **23** and **24**). Interestingly, and for reasons that are not intuitively obvious at the moment, the stability of the acyl enzyme(s) formed by the interaction of **I** with a serine proteinase is primarily controlled by the nature of the R_2 group, with small alkyl P_2 residues such as methyl, for example, giving rise to acyl enzymes that deacylate extremely slowly.[6] These observations suggest that R_2 can serve as an exquisite means of controlling the *stability*, and hence the rate of reactivation, of the acyl enzyme(s) formed from **I**. This distinct feature of **I**

could, in principle, be used as a sensitive 'switch' in regulating the recovery of enzymatic activity and, hence, the duration of action, of a potential medicinal agent.

Nature of the leaving group. (1) As mentioned earlier, the binding of **I** to the active site orients the leaving group toward the hydrophobic S_n' subsites. The results listed in Table 2 suggest that both the pK_a and inherent structure of L exert a profound effect on inhibitory potency. As a design element, the inherent structure of L can be used to optimize binding affinity. For example, molecular modeling was used to gain insight into the binding of compound **20** to the active site of HLE and to optimize the hydrophobic binding interaction of the phenyl ring (in L) in the inhibitor and a phenylalanine residue (Phe-41) located in the vicinity of the S_2' subsite (Figure 2(a)). The overlay of energy-minimized inhibitor **20** and the P_2-P_2' segment (-Thr-Leu-Glu-Tyr-) of TOMI shows the isobutyl (P_1) and benzyl (P_2) groups in the inhibitor nestled into the corresponding S_1 and S_2 enzyme subsites, with the phenyl ring of the leaving group occupying the S_2' subsite, reflecting fairly good structural and electronic complementarity (Figure 2(b)).

Increasing the hydrophobic character of the sulfone leaving group was found to augment hydrophobic interactions with the S_n' subsites, yielding compounds with higher inhibitory potency (Table 2, compounds **13**/**15**, **18**/**19**, **15**/**23**). Potency was also found to be sensitive to the pK_a of the leaving group (vide infra). (2) In general, sulfone-based inhibitors of Cat G were less potent than the corresponding HLE inhibitors (Table 2, compounds **25**–**33**). The best inhibitors of Cat G were those with $R_1 = R_2 =$ benzyl (Table 2, compound **27**). (3) The corresponding sulfoxides also inhibited HLE. For example, the diastereomeric *sulfoxides* corresponding to compound **15** inactivated HLE with k_{inact}/K_i values of 1,110 and 1390 M^{-1} s^{-1}. In contrast, the inactivation of HLE by the corresponding *sulfides* was found to be slow. For example, incubation of a 200-fold excess of the sulfide corresponding to compound **16** with HLE led to time-dependent inactivation of the enzyme with

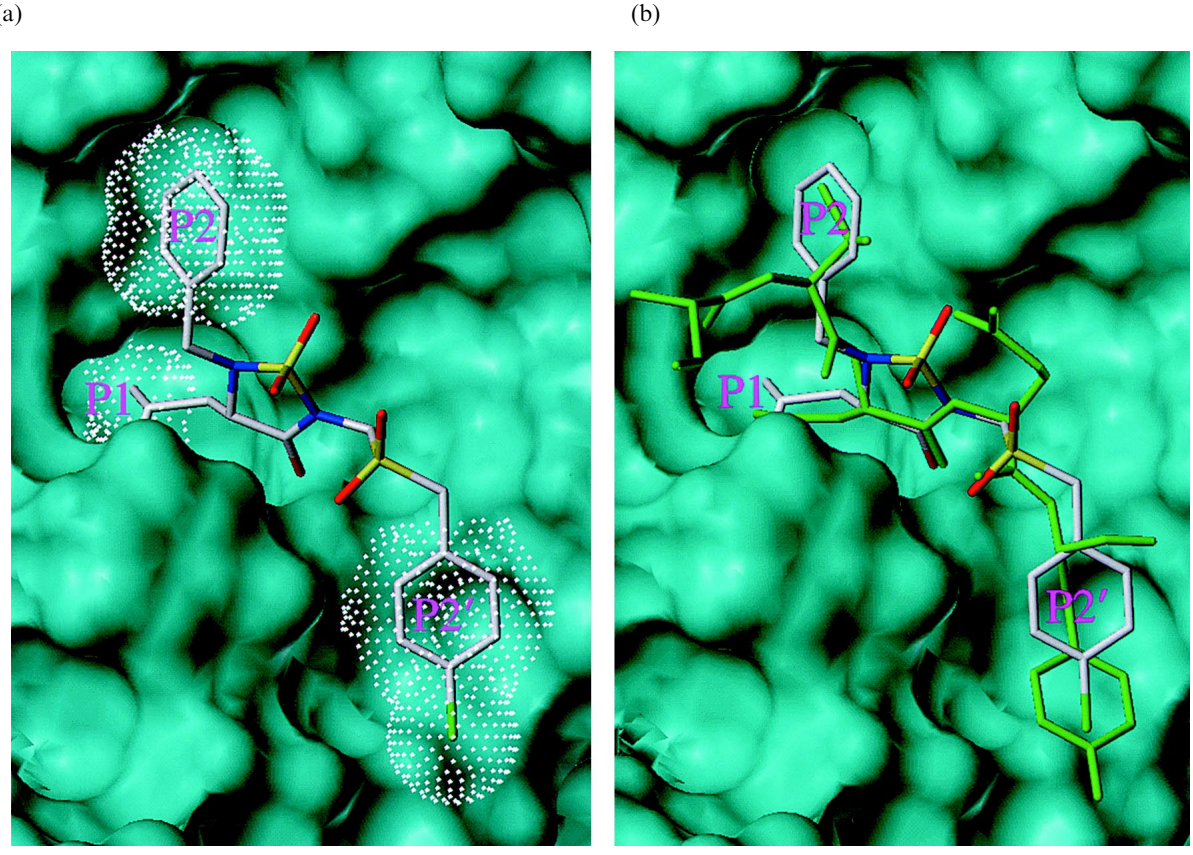


Figure 2. (a) Energy-minimized inhibitor **20** (Table 1) docked to active site of human leukocyte elastase (HLE) with the isobutyl group occupying the S_1 pocket. The benzyl group is nestled into the hydrophobic S_2 pocket, and the *N*-(4-chlorobenzyl)sulfonyl leaving group extends into the hydrophobic S_2' pocket, providing specific lipophilic interactions. (b) Overlay of inhibitor **20** and the P_2-P_2'

minimal recovery of enzymatic activity after 24 h (Figure 3).

It is tentatively proposed that, unlike sulfone derivatives of **I** that inactivate a serine proteinase via a mechanism involving the formation of a highly reactive sulfonyl imine that partitions via two possible pathways[6] (Figure 4(a)), sulfide derivatives lead to acylation of the enzyme without loss of the leaving group (Figure 4(b)). Definitive resolution of the mechanistic issues will have to await the results of ongoing studies.

Nature of the template. Exploratory attempts to gain insight into the nature of the interaction of **I** with serine proteinases, included the synthesis and in vitro evaluation of a series of hydantoin derivatives **II**. None of the hydantoin derivatives displayed any inhibitory activity toward HLE, Cat G, or PR 3, suggesting that the manifestation of time-dependent inhibitory activity involves a delicate interplay between binding interactions, chemical reactivity, and leaving group ability.

In summary, the results presented herein describe (a) the efficient inhibition of HLE, Cat G, and PR 3 by sulfone derivatives of **I** and (b) demonstrate that the heterocyclic scaffold in **I** has built-in flexibility that permits the optimization of inhibitory potency, specificity and the stability of the enzyme–inhibitor complex(es) via the use of an array of recognition elements appended in a well-defined spatial arrangement.

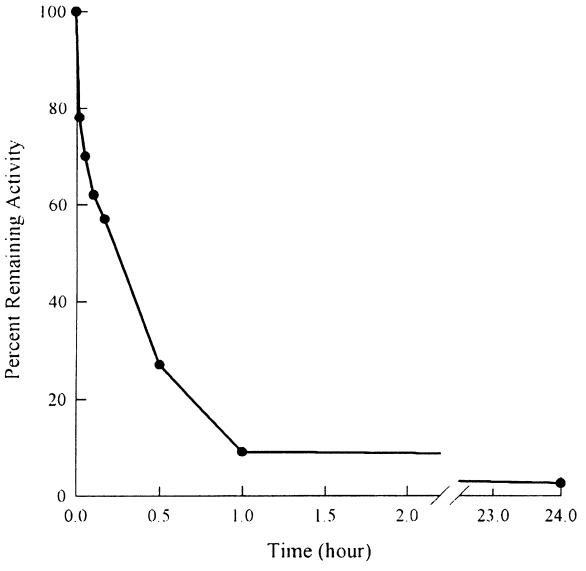
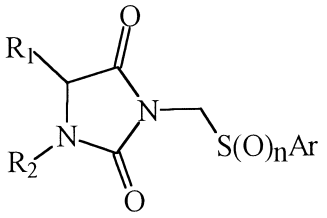


Figure 3. Percent remaining activity versus time plot constructed by incubating a 200-fold excess of (L)-4-isobutyl-2-(phenylthiomethyl)-5-benzyl 1,2,5-thiadiazoli-din-3-one 1,1 dioxide (2.76 nM) with HLE (13.8 μM) in 0.1 M HEPES buffer, pH 7.25 at 25 °C. Aliquots were withdrawn at different time intervals and assayed for enzymatic activity using MeOSuc-AAPV-pNA (4.25 mM) by monitoring the absorbance at



II

R_1 = isobutyl, benzyl
R_2 = H, benzyl
n = 0, 2

Experimental

Melting points were recorded on a Mel-Temp apparatus and are uncorrected. The NMR spectra of the synthesized compounds were recorded on a Varian XL-300 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, USA. Human leukocyte cathepsin G and proteinase 3 were purchased from Athens Research and Technology Co., Athens, GA, USA. Methoxysuccinyl Ala-Ala-Pro-Val *p*-nitroanilide and methoxysuccinyl Ala-Ala-Pro-Phe *p*-nitroanilide were purchased from Sigma Chemicals Co., St Louis, MO, USA.

Representative syntheses

(*S*)-4-Isobutyl-2-[(phenylthio)methyl]-1,2,5-thiadiazolidin-3-one 1,1 dioxide (35, R_1 = isobutyl, R_2 = H, Ar = Ph). A solution of (*S*)-4-isobutyl-1,2,5-thiadiazolidin-3-one 1,1 dioxide[6] (1.92 g, 10 mmol), chloromethyl phenyl sulfide (1.80 g, 12 mmol), and triethylamine (1.01 g, 10 mmol) in 15 mL of dry acetonitrile was refluxed for 20 h. The solvent was removed by in vacuo, and the residue was taken up in 30 mL of ethyl acetate, washed with water, 5% aq HCl (10 mL), 5% aq sodium bicarbonate, and dried over anhydrous sodium sulfate. Evaporation of the solvent left a crude product which was purified by flash chromatography on silica gel, using a hexane/methylene chloride gradient, mp 48–49 °C (1.64 g, 52% yield).[6]

(*S*)-4-Isobutyl-5-benzyl-2-[(phenylthio)methyl]-1,2,5-thiadiazolidin-3-one 1,1 dioxide (35, R_1 = isobutyl, R_2 = benzyl, Ar = Ph). Benzyl bromide (14.0 g, 82.0 mmol) was added to a solution of compound (*S*)-4-isobutyl-

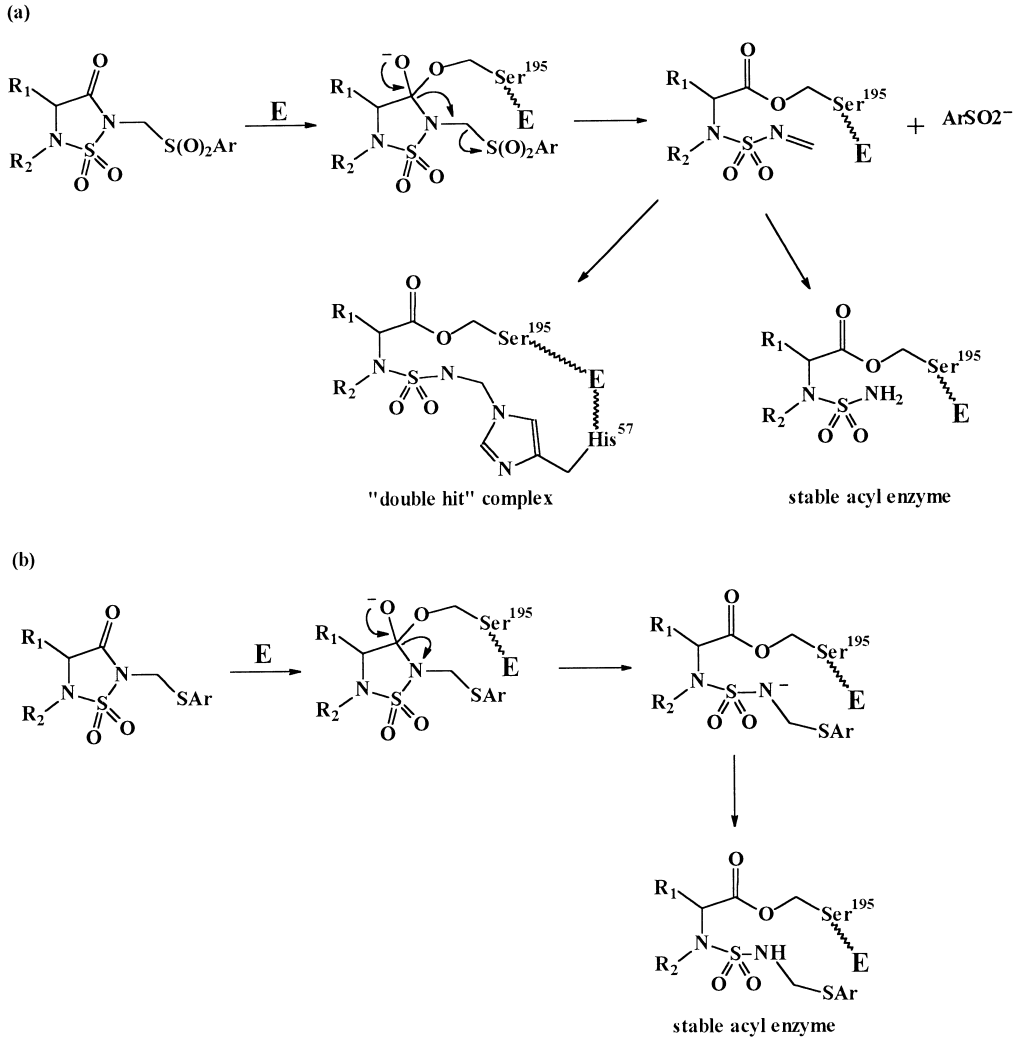


Figure 4. (a) Postulated mechanism of action of sulfone derivatives of **I** involving enzyme-induced formation of an *N*-sulfonyl imine and concomitant loss of the leaving group. (b) Interaction of a sulfide derivative of **I** whereby nucleophilic ring opening leads to the formation of an acyl enzyme without loss of the leaving group.

2-[(phenylthio)methyl]-1,2,5-thiadiazolidin-3-one 1,1 dioxide (23.4 g, 74.4 mmol) in dry acetonitrile (150 mL). The solution was cooled in an ice bath, and 60% NaH (3.0 g, 74.9 mmol) was added in small portions over a period of 5 min. The reaction mixture was stirred at room temperature overnight, and the solvent and excess benzyl bromide were removed in vacuo. The residue was dissolved in methylene chloride (100 mL) and washed with water. The organic layer was dried over anhydrous sodium sulfate. Removal of the solvent left a crude product, which was purified using flash chromatography (90% yield). ^{1}H NMR ($CDCl_3$) δ 0.69 (dd,6H), 1.48 (m,1H), 1.62 (m,2H), 3.77 (t,1H), 4.23 (d,1H), 4.45 (d,1H), 4.97 (d,2H), 7.30–7.65 (m,10H); ^{13}C NMR δ 22.05, 22.28, 24.28, 39.75, 45.43, 52.10, 62.63, 128.40, 128.80, 128.96, 129.08, 129.18, 132.39, 133.09, 133.44, 167.08.

(*S*)-4-Isobutyl-5-benzyl-2-chloromethyl-1,2,5-thiadiazolidin-3-one 1,1 dioxide (36, R_1 = isobutyl, R_2 = benzyl). (*S*)-4-Isobutyl-2-[(phenylthio)methyl]-1,2,5-thiadiazolidin-3-one 1,1 dioxide (31.2 g, 74 mmol) was treated with sulfuryl chloride (20 g, 148 mmol) in 50 mL dry CH_2Cl_2 and the reaction mixture was stirred for 5 h at room temperature. The solvent and excess sulfuryl chloride were removed in vacuo. The crude product was purified by flash chromatography (silica gel/hexane/methylene chloride as eluents), yielding 16.7 g (68% yield) of pure product. ^{1}H NMR ($CDCl_3$) δ 0.67 (d, 3H), 0.78 (d, 3H), 1.55–1.80 (m, 3H), 3.92 (t, 1H), 4.28 (d, 1H), 4.53 (d,

1H), 5.36 (d, 2H), 7.38 (s, 5H). ^{13}C NMR ($CDCl_3$) δ 21.92, 22.27, 24.27, 39.61, 46.53, 52.51, 62.97, 128.98, 129.04, 29.23, 133.02, 166.67.

(*S*)-4-Isobutyl-2-[(*p*-chlorobenzylthio)methyl]-5-benzyl-1,2,5-thiadiazolidin-3-one 1,1 dioxide (I, R_1 = isobutyl, R_2 = benzyl, L = (*p*-chlrobenzylthio)methyl). A solution of (*S*)-4-isobutyl-5-benzyl-2-chloromethyl-1,2,5-thiadiazolidin-3-one 1,1 dioxide (0.99 g, 3 mmol) in 10 mL dry acetonitrile was reacted with 4-chlorobenzylthiol (0.48 g, 3 mmol) in the presence of 1,8-diazabicyclo[5.4.0]undecen-7-ene (0.46 g, 3 mmol) at room temperature overnight. The solvent was removed on a rotary evaporator and the residue was taken up in methylene chloride (30 mL) and washed with 5% HCl (20 mL), 5% $NaHCO_3$ and brine. The organic solution was dried over anhydrous sodium sulfate and evaporated, leaving a crude product, which was purified by flash chromatography using silica gel (hexane/methylene chloride). Pure compound **39** was obtained in 84% yield (1.14 g). ^{1}H NMR ($CDCl_3$) δ 0.70 (d, 3H), 0.80 (d, 3H), 1.53–1.81 (m, 3H), 3.87 (t, 1H), 3.91 (s, 2H), 4.28 (d, 1H), 4.52 (d, 1H), 4.56 (d, 2H), 7.33 (dd, 4H), 7.39 (s, 5H).

(*S*)-4-Isobutyl-*N*-[(4-chlorobenzylsulfonyl)methyl]-5-benzyl-1,2,5-thiadiazolidin-3-one 1,1 dioxide (19). A solution of (*S*)-4-isobutyl-2-[(*p*-chlorobenzylthio)methyl]-5-benzyl-1,2,5-thiadiazolidin-3-one 1,1 dioxide (0.47 g, 1 mmol) in 3 mL dry CH_2Cl_2 was reacted with 70% *m*-chloroperbenzoic acid (0.62 g, 3.60 mmol) at room temperature overnight. Methylene chloride (20 mL) was added and the organic phase was washed with saturated sodium bicarbonate (25 mL) and dried over anhydrous sodium sulfate. The solvent was removed in vacuo and the crude product purified by flash chromatography (silica gel/hexane/methylene chloride), yielding 0.46 g (98% yield) of pure product.

(*S*)-4-Isobutyl-5-[(*m*-carboxymethyl)benzyl]-2-[(phenylthio)methyl]-1,2,5-thiadiazolidin-3-one 1,1 dioxide (35, R_1 = isobutyl, R_2 = *m*-(carboxymethyl)benzyl, Ar = Ph). A solution of (*S*)-4-isobutyl-5-benzyl-2-[(phenylthio) methyl-1,2,5-thiadiazolidin-3-one 1,1 dioxide (0.94 g, 3 mmol) and *m*-(carboxymethyl)benzyl bromide (0.69 g, 3 mmol) in dry acetonitrile (10 mL) was cooled to 0 °C and treated with 60% sodium hydride (0.12 g, 3 mmol). The mixture was stirred at room temperature for 1 h. The solvent was removed in vacuo and the residue was taken up in ethyl acetate (35 mL), and washed with 5% hydrochloric acid and brine. The organic layer was dried over anhydrous sodium sulfate and evaporated. The crude product was purified using flash chromatography (silica gel/hexane/CH_2Cl_2), yielding 0.97 g (70% yield) of pure product. ^{1}H NMR ($CDCl_3$) δ 0.69 (dd, 6H), 1.45–1.70 (m, 3H), 3.78 (t, 1H), 3.93 (s, 3H), 4.22 (d, 1H), 4.52 (d, 1H), 4.98 (d, 2H), 7.32 (m, 3H), 7.46 (t, 1H), 7.58 (m, 3H), 8.02 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 22.02, 22.22, 24.25, 39.61, 45.46, 51.78, 52.29, 63.01, 128.47, 129.07, 129.17, 129.96, 130.07, 130.84, 132.20, 133.14, 133.43, 134.08, 166.31, 166.76.

(*S*)-4-Isobutyl-5-[(*m*-carboxymethyl)benzyl]-2-[(phenylsulfonyl)methyl]-1,2,5-thidiazolidin-3-one 1,1 dioxide (4). A solution of (*S*)-isobutyl-5-[(*m*-carboxymethyl)benzyl]-2-phenylthio)methyl]-1,2,5-thiadiazolidin-3-one 1,1 dioxide (0.87 g, 1.88 mmol) in 10 mL dry CH_2Cl_2 was reacted with 70% *m*-chloroperbenzoic acid (1.1 g, 6.38 mmol) at room temperature overnight. Methylene chloride (20 mL) was added and the organic phase was washed with saturated $NaHCO_3$ (25 mL) and dried over anhydrous Na_2SO_4. The solvent was removed in vacuo, and the crude product was purified using flash chromatography (silica gel/hexane/CH_2Cl_2), yielding 0.74 g (80% yield) of pure product. ^{1}H NMR ($CDCl_3$) δ 0.63 (d, 3H), 0.74 (d, 3H), 1.45–1.70 (m, 3H), 3.82 (t, 1H), 3.94 (s, 3H), 4.27 (d, 1H), 4.57 (d, 1H), 4.89 (s, 2H), 7.47–7.73 (m, 5H), 8.03 (m, 4H).

(*S*)-4-Isobutyl-5-[(*m*-carboxyl)benzyl]-2-[(phenylsulfonyl)methyl)]-1,2,5-thiadiazolidin-3-one 1,1 dioxide (5). A mixture of the above ester (0.62 g, 1.25 mmol), iodotrimethylsilane (0.31 g, 1.50 mmol), and iodine (32 mg) in dry $CDCl_3$ (5 mL) was kept at 55 °C for 24 h. NMR analysis showed the presence of starting material. An additional 0.1 g of iodotrimethylsilane was added and the mixture kept at 55 °C for an additional 24 h. The solvent was removed in vacuo and ethyl acetate (25 mL) was added to the residue. The organic layer was washed with 5% HCl, 10% aq sodium thiosulfite and water (20 mL). It was dried over anhydrous sodium sulfate and then evaporated leaving a crude product that was purified by flash chromatography (silica gel/hexane/ethyl acetate/HOAC), yielding 0.33 g (59% yield) of pure **5**. ^{1}H NMR (acetone-d_6) δ 0.70 (dd, 6H), 1.37–1.70 (m, 3H), 4.18 (t, 1H), 4.42 (d, 1H), 4.71 (d, 1H), 5.06 (d, 2H), 7.52–7.82 (m, 5H), 8.02 (m, 3H), 8.14 (s, 1H).

Synthesis of hydantoin derivatives

(DL)-5-Isobutylhydantoin (37, R_1 = isobutyl). (DL)-Leu hydrochloride (50 mmol) and potassium cyanate (60 mmol) were added to a solution of water (30 mL) and pyridine (25 mL). The solution was heated to 60 °C for 1 h, cooled to room temperature, and the pyridine extracted with ethyl acetate (4×100 mL). Glacial acetic (30 mL) and 6 N HCl (30 mL) were added to the glacial phase and the solution was refluxed for 0.5 h. The solution was kept at 5 °C overnight. The precipitated product was collected, washed with water, and air-dried (6.3 g, 80% yield), mp 215–216 °C. NMR(DMSO-d_6) δ 0.91 (dd,6H), 1.35–1.55 (m,2H), 1.78 (br m, 1H), 4.0 (m,1H), 8.02 (br s, 1H), 10.59 (br s,1H).

(DL)-5-Isobutyl-3-(phenylthiomethyl)hydantoin (38, R_1 = isobutyl). (DL)-5-Isobutylhydantoin (15 mmol) was dissolved in dry DMF (20 mL) under nitrogen and 60% sodium hydride (15 mmol) was added at room temperature. Chloromethylphenyl sulfide (16 mmol) was then added and the resulting solution heated to 80 °C for 2.5 h. The reaction mixture was then cooled to room temperature and diluted with water. The crystallized product was collected by suction filtration, dissolved in ethyl ether (100 mL), and extracted with saturated sodium chloride (30 mL). The organic phase was dried over anhydrous sodium sulfate and the solvent removed. The crude product was washed with hexane, leaving a pure product (1.76 g, 42% yield), mp 115–116 °C. NMR($CDCl_3$) δ 0.94 (dd,6H), 1.45 (m,1H), 1.7 (m,2H), 4.0 (dd,1H), 4.86 (dd,2H), 6.68 (br s, 1H), 7.3 (m,3H), 7.53 (m,2H).

(DL)-5-Isobutyl-3-(phenylsulfonylmethyl)hydantoin (40, R_1 = isobutyl). Oxidation of (DL)-5-isobutyl-3-(phenylthiomethyl)hydantoin with 70% *m*-chloroperbenzoic acid using a procedure similar to that used in the preparation of compound **19** yielded the desired product.

(DL)-5-Isobutyl-3-(phenylthiomethyl)-1-benzylhydantoin (39, R_1 = isobutyl, n = 0). A solution of (DL)-5-isobutyl-3-(phenylthiomethyl)hydantoin (2 mmol) in dry acetonitrile (6 mL) was treated with 60% sodium hydride (2 mmol) at room temperature under nitrogen with stirring. Benzyl bromide (2.2 mmol) was then added and the reaction mixture stirred overnight. Following removal of the solvent on the rotary evaporator, methylene chloride (35 mL) was added and the solution extracted with saturated sodium bicarbonate (10 mL). The organic phase was dried over anhydrous sodium sulfate and evaporated, leaving a crude product that was purified on a chromatotron plate using methylene chloride as the eluent (67% yield). ^{1}H NMR ($CDCl_3$) δ 0.83 (dd,6H), 1.62 (m,2H), 1.78 (m,1H), 3.69 (dd,1H), 4.01 (d,1H), 4.92 (dd,2H), 5.08 (d,1H), 7.19 (m,2H), 7.29–7.39 (m,6H), 7.55 (m,2H).

(DL)-5-Isobutyl-3-(phenylsulfonylmethyl)-1-benzylhydantoin (39, R_1 = isobutyl, n = 2). *m*-Chloroperbenzoic acid (2 mmol) was added to a solution of (DL)-5-isobutyl-3-(phenylthiomethyl)-1-benzylhydantoin (1 mmol) in 5 mL methylene chloride. The reaction mixture was stirred at room temperature overnight. An additional amount of methylene chloride (35 mL) was added and the solution was extracted with 5% aq sodium sulfite (10 mL) and 5% $NaHCO_3$ (10 mL). The organic phase was dried and evaporated, leaving a crude product that was purified on a chromatotron plate, yielding an oily product (22% yield). ^{1}H NMR (DMSO-d_6) δ 0.85 (dd, 6H), 1.63 (m, 2H), 1.82 (m, 1H), 3.77 (dd, 1H), 4.02 (d, 1H), 4.93 (d, 2H), 5.05 (d, 1H), 7.20 (m, 2H), 7.35 (m, 2H), 7.58 (m, 2H), 7.70 (m, 2H), 7.94 (m, 2H).

(DL)-5-Benzylhydantoin (40, R_1 = benzyl). This was synthesized from (DL)-Phe using a procedure similar to that used in the preparation of (DL)-5-isobutylhydantoin ^{1}H NMR(DMSO-d_6) δ 2.94 (dd,2H), 4.36 (m,1H), 7.23 (m,5H), 7.96 (br s,1H), 10.43 (br s, 1H).

(DL)-5-Benzyl-3-(phenylthiomethyl)hydantoin (38, R_1 = benzyl). This was prepared from (DL)-5-benzylhydantoin using a procedure similar to that used in the synthesis of (DL)-5-isobutyl-3-(phenylthiomethyl)-1-benzylhydantoin, mp 130–131 °C. ^{1}H NMR($CDCl_3$) δ 2.71 (dd,1H), 3.2 (dd,1H), 4.16 (dd,1H), 4.81 (s,2H), 5.69 (s,1H), 7.16 (m,2H), 7.30 (m,6H), 7.48 (m,2H).

(DL)-5-Benzyl-3-(phenylsulfonylmethyl)hydantoin (40, R_1 = benzyl). This was prepared from (DL)-5-benzyl-3-(phenylthiomethyl)hydantoin using a similar procedure as that used in the synthesis of (DL)-5-isobutyl-3-(phenylsulfonylmethyl)-1-benzylhydantoin, mp 136–138 °C. ^{1}H NMR ($CDCl_3$) δ 2.88 (dd, 1H), 3.2 (dd, 1H), 4.31 (m, 1H), 4.78 (d, 2H), 6.27 (s, 1H), 7.17–7.40 (m, 6H), 7.52–7.86 (m, 4H).

(DL)-5-Benzyl-3-(phenylthiomethyl)-1-benzylhydantoin (39, R_1 = benzyl, n = 0). Synthesized as described above. ^{1}H NMR ($CDCl_3$) δ 2.99 (dd, 1H), 3.11 (dd, 1H), 3.85 (d, 1H), 3.91 (dd, 1H), 4.77 (dd, 2H), 5.05 (d, 1H), 6.98 (m, 2H), 7.08 (m, 2H), 7.21–7.31 (m, 9H), 7.37 (m, 2H).

(DL)-5-Benzyl-3-(phenylsulfonylmethyl)-1-benzylhydantoin (39, R_1 = benzyl, n = 2). Obtained from (DL)-5-benzyl-3-(phenylthiomethyl)-1-benzylhydantoin by oxidation with 70% *m*-chloroperbenzoic acid. ^{1}H NMR ($CDCl_3$) δ 3.08 (dd, 1H), 3.17 (dd, 1H), 3.88 (d, 1H), 4.03 (t, 1H), 4.78 (dd, 2H), 4.99 (d, 1H), 7.0 (m, 2H), 7.12 (m, 2H), 7.33 (m, 7H), 7.49 (m, 2H), 7.67 (m, 2H).

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