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SUBSTITUTED 3-OXO-1,2,5-THIADIAZOLIDINE 1,1-DIOXIDES: A NEW CLASS OF POTENTIAL MECHANISM-BASED INHIBITORS OF HUMAN LEUKOCYTE ELASTASE AND CATHEPSIN G

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Abstract. A series of substituted 3-oxo-1,2,5-thiadiazolidine 1,1-dioxides has been synthesized and their in vitro inhibitory activity toward human leukocyte elastase and cathepsin G was investigated. These compounds were found to inactivate the two enzymes efficiently and in a time-dependent fashion.

Neutrophil recruitment and degranulation in inflammatory states results in the extracellular release of several enzymes, including the serine proteinases elastase (EC 3.4.21.37), cathepsin G (EC 3.4.21.20) and proteinase 3 (1-3). An imbalance between the levels of these proteinases and their physiological inhibitors results in poor regulation of these enzymes, ultimately leading to the degradation of various components of the extracellular matrix via the unrestrained proteolytic action of these enzymes (4). Consequently, there has been an intense effort in recent years to devise ways of arresting and/or

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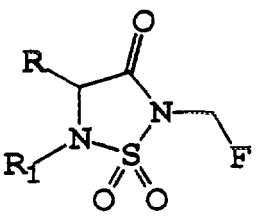
moderating the deleterious action of these enzymes using protein inhibitors (5-7) and/or low molecular weight synthetic inhibitors (8-9).

As part of an ongoing effort related to the development of novel mechanism-based inhibitors of the serine proteinases (10-14), we wish to describe the design, synthesis, and in vitro inhibitory activity of some substituted 3-oxo-1,2,5-thiadiazolidine 1,1-dioxides (represented by structure I).



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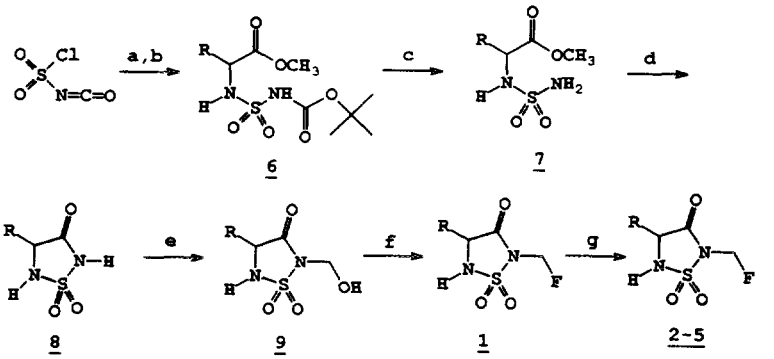
MATERIALS AND METHODS

Chemicals. Human leukocyte elastase was obtained from Elastin Products Co., Owensville, MO. Human leukocyte cathepsin G was 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 Chemical So., St. Louis, MO. Baker-analyzed 60-200 mesh silica gel was used for flash chromatography.

Synthesis. Compounds 1-5 were synthesized as shown in Scheme I (15-16). The NMR and IR spectra of the synthesized compounds were recorded on a Varian XL-300 NMR spectrometer and a Perkin-Elmer infrared spectrophotometer, respectively, and were consistent with the assigned structures. Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ.

(S)-Methyl-2-(tert-butoxycarbonylsulfamido) 3-phenyl propanoate 6. A solution of 10.5 g (74 mmol) of chlorosulfonyl isocyanate in 150 mL of dry methylene chloride was cooled in an ice bath under a nitrogen atmosphere and a solution of dry t-butyl alcohol (5.48 g; 74 mmol) in 75 mL methylene chloride was added dropwise. The resulting solution was added dropwise to a mixture of L-phenylalanine methyl ester hydrochloride salt (16.1 g; 74 mmol) and triethylamine (15.0 g; 148 mmol) in 150 mL methylene chloride kept at 0°C. The reaction mixture was allowed to warm to room temperature and stirred



Scheme I Synthesis of Inhibitors 1-5

[a]t-BuOH/CH_2Cl_2; [b](L)-Phe-OCH_3.HCl; [c]CF_3COOH; [d]NaH; [e]H-CO-H/methanol/heat; [f]DAST/CH_2Cl_2; [g]NaH followed by alkylating agent.

for two additional hours. The salt was filtered off and the filtrate was washed sequentially with water (2 x 30 mL) and brine (2 x 30 mL), and dried over anhydrous sodium sulfate. Removal of the solvent gave compound 6 (25.6 g; 96% yield) as a white solid, mp 120-1°C. ^{1}H NMR ($CDCl_3$): δ 7.48 (s,1H), 7.4-7.1 (m,5H), 5.72 (d,1H), 4.52 (m,1H), 3.71 (s,3H), 3.13 (d,2H), 1.47 (s,9H); $[\alpha]_D$ + 46.9 (c 1, CH_2Cl_2). Anal. Calcd. for $C_{15}H_{22}N_2O_6S$. C, 50.26; H, 6.19; N, 7.82. Found: C, 50.16; H, 7.45; N, 8.76.

(S)-Methyl 2-sulfamido-3-phenylpropanoate 7. Compound 6 (10.5 g; 29 mmol) was treated with trifluoroacetic acid (32 mL) in 8 mL of methylene chloride and stirred for 2 h at room temperature. The solvent and excess trifluoroacetic acid were removed in vacuo, leaving an oily residue that was taken up in methylene chloride (50 mL) and washed with 5% aqueous sodium bicarbonate (2 x 30 mL), brine (2 x 30 mL) and dried. Removal of the solvent left 7 as an oily product (7.2 g; 96% yield). ^{1}H NMR ($CDCl_3$): δ 7.37-7.15 (m,5H), 4.74 (s,2H), 4.38 (m,1H), 3.37 (s,3H), 3.2-3.0 (m,2H); $[\alpha]_D$ + 10.85. Calcd. for $C_{10}H_{14}N_2O_4S$: C, 46.50; H, 5.46; N, 10.85. Found: C, 46.41; H, 5.55; N, 10.70.

(S)-4-Benzyl-3-oxo-1,2,5-thiadiazolidine 1,1-dioxide 8. Compound 7 (6.2 g; 25 mmol) in 50 mL dry THF was treated with 60% NaH (1.1 g; 27 mmol) at 0°C and the reaction mixture was stirred at room temperature overnight. The solvent was removed in vacuo and the solid residue was triturated with ether, yielding the sodium salt of 7. This was dissolved in methanol (50 mL) and stirred with DOWEX 50X8-200 resin (20 g) for 2 h. The resin was filtered off and the solvent was removed, leaving behind crude 8 which was recrystallized from methanol (4.5 g; 80% yield), mp 196-7°C. ^{1}H NMR ($CDCl_3$): δ 8.28 (s,1H), 7.4-7.2 (m,6H), 3.45 (dd,1H), 3.14 (dd,1H), 2.88 (dd,1H); $[\alpha]_D$ -86.3 (c 1, CH_3OH).

Anal. Calcd. for $C_9H_{10}N_2O_3S$: C, 47.73; H, 4.61; N, 12.38. Found: C, 47.47; H, 4.61; N, 12.17.

(S)-4-Benzyl-2-hydroxymethyl-3-oxo-1,2,5-thiadiazolidine 1,1-dioxide 9. To a solution of compound 8 (0.45 g; 2 mmol) in 2 mL of methanol was added 37% formaldehyde (0.38 g; 4.7 mmol). The solution was refluxed for two minutes and the solvent was allowed to evaporate off at room temperature. A crystalline material formed which was collected and washed with ether, yielding pure 9 (0.15 g; 30% yield). ^{1}H NMR (acetone-d_6): δ 7.46-7.22 (m,5H), 7.14 (d,1H), 5.77 (d,1H), 5.12 (d,2H), 4.55 (m,1H), 3.30 (dd,1H), 3.00 (dd,1H). Anal. Calcd. for $C_{10}H_{12}N_2O_4S$: C, 46.86; H, 4.72; N, 10.93. Found: C, 47.00; H, 4.81; N, 10.83.

(S)-4-Benzyl-2-fluoromethyl-3-oxo-1,2,5-thiadiazolidine 1,1-dioxide 1. To a suspension of compound 9 (0.70 g; 2.73 mmol) in 20 mL of dry methylene chloride kept in an ice bath was added dropwise a solution of diethylaminosulfur trifluoride (DAST) (0.484 g; 3.00 mmol) in 20 mL methylene chloride. The reaction mixture was allowed to warm to room temperature and stirred for 0.5 h. Methylene chloride (15 mL) and water (15 mL) were added and the layers separated. The organic layer was dried and evaporated to give a crude product which was purified by flash chromatography: 1 (0.55 g; 78% yield), mp 92-93°C; ^{1}H NMR ($CDCl_3$): δ 7.45-7.18 (m,5H), 5.70 (d,1H), 5.53 (d,1H), 4.93 (s,1H), 4.48 (m,1H), 3.33 (dd,1H), 3.18 (dd,1H); $[\alpha]_D$ -130.8 (c 2; methanol). Anal. Calcd. for $C_{10}H_{11}N_2O_3FS$: C, 46.50; H, 4.29; N, 10.85. Found: C, 46.50; H, 4.29; N, 10.88.

General Procedure for Preparing Compounds 2-5. To a solution of compound 1 (0.2 g; 0.774 mmol) in 3 mL dry acetonitrile kept in an ice bath, was added 60% sodium hydride (0.027 g; 0.70 mmol), followed by the appropriate alkylating agent (7.74 mmol). The reaction mixture was stirred overnight and the solvent was removed in vacuo. The residue was dissolved in 20 mL methylene chloride, washed with water (2 x 20 mL) and dried over anhydrous sodium sulfate. The isolated products were purified by flash chromatography.

(S)-4-Benzyl-2-fluoromethyl-5-methyl-3-oxo-1,2,5-thiadiazolidine 1,1-dioxide 2: mp 88.5-90°C. ^{1}H NMR: δ 7.42-7.20 (m,5H), 5.76-5.50 (m,2H), 4.10 (dd,1H), 3.32 (dd,1H), 3.13 (dd,1H), 2.71 (s,3H). $[\alpha]_D$ -89.4 (c 1, methanol:methylene chloride 5:1). Anal. Calcd. for $C_{11}H_{13}N_2O_3FS$: C, 48.52; H, 4.81; N, 10.29. Found: C, 48.62; H, 4.90; N, 10.24.

(S)-4,5-Dibenzyl-2-fluoromethyl-3-oxo-1,2,5-thiadiazolidine 1,1-dioxide 3: oil; ^{1}H NMR: δ 7.40-7.05 (m,10H), 5.69 (s,1H), 5.52 (s,1H), 4.35 (d,1H), 4.13 (dd,1H), 4.07 (d,1H), 3.11 (m,2H). $[\alpha]_D$ -92.2 (c 1, $CHCl_3$). Anal. Calcd. C, 58.60; H, 4.92; N, 8.05. Found: C, 58.42; H, 4.89; N, 7.96.

(S)-4-Benzyl-5(tert-butoxycarbonylmethyl)-2-fluoromethyl-3-oxo-1,2,5-thiadiazolidine 1,1-dioxide 4: mp 124-124.5°C. ^{1}H NMR: δ 7.45-7.25 (m,5H),

5.76 (q, 1H), 5.59 (q, 1H), 4.41 (dd,1H), 3.93 (dd,1H), 3.30 (dd,1H), 3.07 (dd,1H), 3.03 (d,1H), 1.42 (s,9H). $[\alpha]_D$ -62.6 (c 1, $CHCl_3$). Anal. Calcd. C, 51.60; H, 5.68; N, 7.52. Found: C, 51.51; H, 5.71; N, 7.52.

(S)-Benzyl-5(benzyloxycarbonylmethyl)-2-fluoromethyl-3-oxo-1,2,5-thiadiazolidine 1,1-dioxide 5: mp 79.79.5°C. ^{1}H NMR: δ 7.45-7.20 (m,10H), 5.68 (q, 1H), 5.50 (q,1H), 5.09 (d,2H), 4.36 (dd,1H), 4.09 (d,1H), 3.31 (dd,1H), 3.16 (d,1H), 3.04 (dd,1H). $[\alpha]_D$ -55.5 (c 1, $CHCl_3$). Anal. Calcd. C, 56.15; H, 4.71; N, 6.89. Found: C, 55.98; H, 4.73; N, 6.82.

Enzyme Assays and Inhibition Studies. Human leukocyte elastase (HLE) and cathepsin G (Cath G) were assayed as described in detail elsewhere (17-18).

RESULTS AND DISCUSSION

We have recently shown that a variety of heterocyclic structures embodying appropriate recognition and reactivity elements function as highly effective time-dependent inhibitors of the proteolytic enzymes human leukocyte elastase (HLE) and cathepsin G (Cath G) (10-14). Based on these observations, we reasoned that substituted thiadiazolidine 1,1-dioxides (structure I) may function as inhibitors of serine proteinases via a cascade of steps involving enzyme-induced nucleophilic ring-opening by a target proteinase, followed by tandem loss of fluoride ion to form a highly reactive electrophilic center. Further reaction with an active site nucleophilic residue (His-57) was anticipated to lead to irreversibly inactivated enzyme (Figure 1).

Indeed, incubation of an equimolar mixture of Cath G with compound 5 led to rapid acylation of the enzyme, followed by slow deacylation (with an approximate half-life of 3 h), ultimately leading to full recovery of enzymatic activity after 24 h (Figure 2). We were unable to determine the $k_{obs}/[I]$ M^{-1} s^{-1} values for compounds 3-5 under pseudo first-order conditions due to the

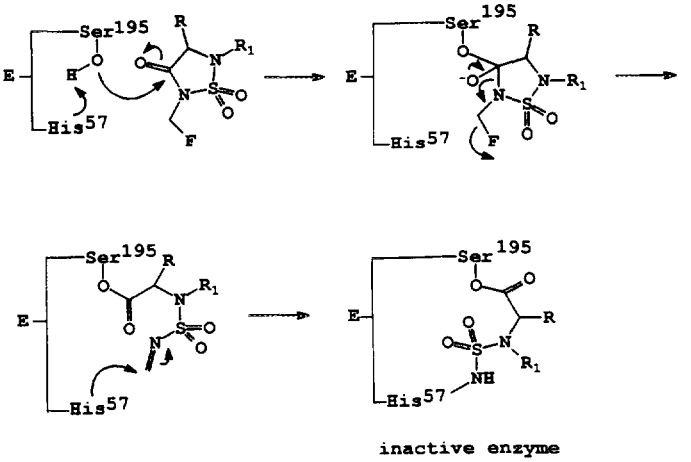


Figure 1. Postulated Mechanism of Action of Compound I.

rapid nature of the interaction between Cath G and the inhibitors. With the exception of compound **2**, these compounds were found to inactivate Cath G much more efficiently than HLE (Table I). In order to probe the effect of structure on inhibitory activity the nature of R_1 was varied. It is clear from

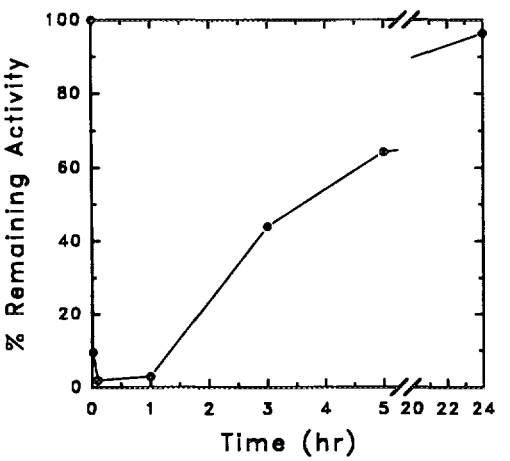


Figure 2. Time-dependent loss of enzymatic activity. Human leukocyte cathepsin G (1.48 μM) was incubated with inhibitor **5** (1.48 μM) in 0.1 M HEPES buffer, pH 7.5, and 1% DMSO. Aliquots were withdrawn at different time intervals and assayed for enzymatic activity using methoxysuccinyl Ala-Ala-Pro-Phe p-nitroanilide.

TABLE 1. INHIBITION OF HUMAN LEUKOCYTE ELASTASE AND CATHEPSIN G BY SUBSTITUTED 3-OXO-1,2,5-THIADIAZOLIDINE 1,1-DIOXIDES

Compound	R	R_1	$k_{obs}/[I]$ M^{-1} s^{-1}	
			HLE	Cath G
1	benzyl	H	NA	600
2	benzyl	methyl	1315	750
3	benzyl	benzyl	930	*
4	benzyl	CH_2COOt-Bu	490	*
5	benzyl	$CH_2COOBzl$	810	*

NA = no activity;
*inactivation was too fast to measure by sampling techniques at an [I]/[E] = 5.

Table I that while variations in R_1 effected modest changes in inhibitory activity toward HLE, there was a dramatic increase in the inhibitory activity of the compounds toward Cath G.

In a separate experiment, Cath G was incubated with an equivalent amount of compound 5. Following near total inactivation of the enzyme (5% remaining activity), excess hydroxylamine was added (0.045 M final concentration) and the regain in enzymatic activity was monitored by withdrawing aliquots at different time intervals. Total regain in enzymatic activity was observed after 1 h, suggesting the presence of labile acyl linkages.

The interaction of **5** with Cath G involves the active site, as evidenced by the observed decrease in the $k_{obs}/[I]$ M^{-1} s^{-1} value (550 M^{-1} s^{-1}) when the experiment was repeated in the presence of substrate. The mechanism by which these compounds inactivate HLE and Cath G remains unknown at the moment. Further studies aimed at unravelling the mechanism of action of these compounds and probing the effect of structure on inhibitory activity are in progress and will be reported in due course.

In conclusion, this study has demonstrated that substituted 3-oxo-1,2,5-thiadiazolidine 1,1-dioxides constitute a new class of heterocyclic inhibitors of HLE and cathepsin G.

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