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2-[(ALKYLSULFONYL)OXY]-6-SUBSTITUTED-1*H*-ISOINDOLE-1,3(2*H*)-DIONE MECHANISM-BASED INHIBITORS OF HUMAN LEUKOCYTE ELASTASE.

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Abstract: Derivatives of 2-[(alkylsulfonyl)oxy]-1*H*-isoindole-1,3(2*H*)-diones (*N*-(sulfonyloxy)phthalimides) with ring substituents in the 6-position were found to exhibit enhanced inhibitory potency and selectivity for HLE. The most potent inhibitor of HLE in this series was 6-amino-2-[(methylsulfonyl)oxy]-1*H*-isoindole-1,3(2*H*)-dione **10** with a k_{obs}/[I] of 110,000 M^{-1} s^{-1}.

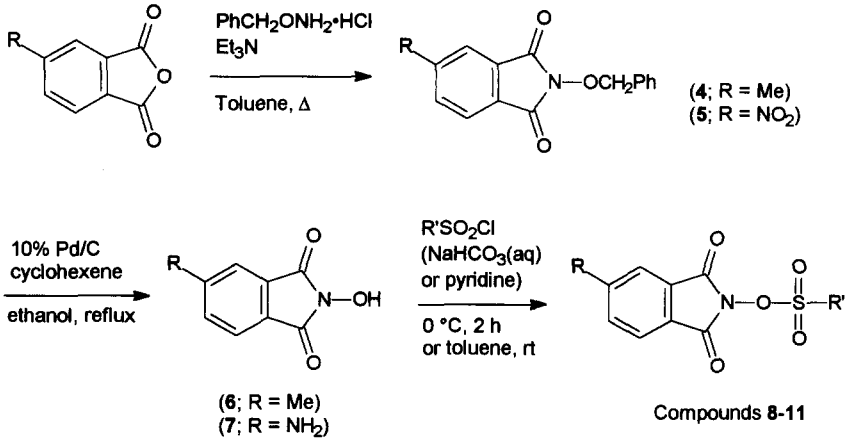
Human Leukocyte Elastase (HLE; EC 3.4.21.37) is a serine protease produced by polymorphonuclear leukocytes. HLE plays a major role in phagocytosis; however, it is potentially hazardous due to its high specificity for lung tissue (elastin) and other connective tissue proteins. HLE has been implicated in a variety of disease states including: emphysema,[1,2] cystic fibrosis,[3-5] acute respiratory distress syndrome,[6,7] (ARDS) bronchial secretory cell metaplasia,[8,9] and rheumatoid arthritis.[10] A natural inhibitor of HLE, α-1-antitrypsin (α-1-AT), is present to help maintain levels of HLE. Deficiency of α-1-AT has been linked to emphysema in cigarette smokers[11,12] and individuals with a genetic deficiency of α-1-AT.[13]

The development of highly specific mechanism-based inhibitors of HLE has remained an active area of research.[14] Powers et al. found that incorporation of hydrophobic substituents in the 7-position of the isocoumarin ring greatly enhances potency and selectivity of these inhibitors for HLE.[15,16] These compounds take advantage of favorable interactions with the hydrophobic S_n' sites of HLE. In comparison, incorporation of a polar amino group in 7-amino-4-chloro-3-ethoxyisocoumarin resulted in a considerable decrease in inhibitory potency for HLE.[17]

Neumann and Gütschow reported that simple *N*-(sulfonyloxy)phthalimides are potent mechanism-based inhibitors of serine proteases.[18] Based on the similarities between the isocoumarin and phthalimide ring systems, we reasoned that substituents placed on the phthalimide ring might enhance the potency and selectivity of these inhibitors for elastase.

Synthesis. Compounds **1-3** (Table I) were synthesized by known methods.[18-20] Compounds **8-11** were synthesized via the procedure shown in Scheme 1. The appropriate phthalic anhydride was reacted with *O*-benzylhydroxylamine hydrochloride in toluene at reflux in the presence of triethylamine to produce

the desired *N*-(benzyloxy)phthalimide product (**4** & **5**). The benzyl protecting group was removed reductively via a transfer hydrogenation procedure.[21] For compound **5** where R = NO_2, *bis*-reduction (removal of the benzyl group coupled with reduction of the NO_2 group) proceeded smoothly to give compound **7**. Coupling of the *N*-hydroxyphthalimides (**6** & **7**) to the appropriate alkylsulfonyl chloride was accomplished using aqueous $NaHCO_3$ at 0 °C or pyridine in toluene for *p*-toluenesulfonyl chloride. See Notes for experimental procedures and data on compounds.[22-25]

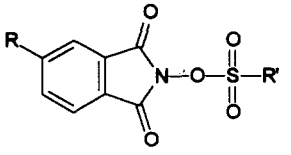


Scheme 1. Synthesis of Compounds **8-11**.

Discussion. Seven compounds were prepared and tested as inactivators of chymotrypsin (ChT), porcine pancreatic elastase (PPE; EC 3.4.21.36) and HLE (Table I). Compounds **1-3** (lacking the 6-substituent) were synthesized for comparison to derivatives **8-11** (possessing either a CH_3 or NH_2 6-substituent). Compound **10** (R = NH_2) was the most potent inhibitor of HLE in this series. It was an approximately twofold more effective inhibitor of HLE compared to its unsubstituted analogue **1** (R = H). Incorporation of a methyl substituent in the 6-position (compounds **8** & **9**) led to decreased inhibition of HLE; however, increased inhibition of PPE was observed for these compounds. Compound **8** was a fivefold more potent inhibitor of PPE than compound **3** (R = H). A marked increase in inhibition of PPE was observed for the NH_2 substituted compounds **10** & **11**. Inhibition of chymotrypsin decreased with the addition of a substituent to the 6-position.

The mechanism of inhibition of serine proteases by sulfonyloxy phthalimides (Figure 1) and succinimides has been established by fluorescence spectroscopy[18] and ^{13}C NMR[26] studies of inhibitor-chymotrypsin complexes. These compounds are true mechanism-based inhibitors of serine proteases in that upon acylation of the serine hydroxyl group, a reactive intermediate is released that undergoes a Lossen rearrangement to give isocyanate **A**. The isocyanate intermediate **A** reacts with the catalytic histidine residue to give the imidazole-*N*-carboxamide **B**.

Table I. Inhibition of Chymotrypsin, Porcine Pancreatic Elastase and Human Leukocyte Elastase by 2-[(Alkylsulfonyl)oxy]-6-substituted-1*H*-isoindole-1,3(2*H*)-diones.



			k_{obs}/[I] (M^{-1} s^{-1})		
Compd.	R	R'	chymotrypsin	PPE	HLE
1	H	CH_3	2,700	6,500	61,000
2	H	$CH(CH_3)_2$	160,000	370	63,000
3	H	4-$(CH_3)C_6H_4$	90,000	1,400	78,000†
8	CH_3	4-$(CH_3)C_6H_4$	27,000	7,400	58,000
9	CH_3	CH_3	16,000	21,000†	30,000
10	NH_2	CH_3	12,000	24,000†	110,000†
11	NH_2	$CH(CH_3)_2$	11,000	22,000†	82,000†

†Second Order Constants.

Conditions: 0.1 M HEPES, 0.5 M NaCl, pH 7.5 at 25 °C and < 6% DMSO. Substrate used for ChT was Suc-Ala-Ala-Pro-Phe-pNA ([S] = 80 μM; [E] = 4 nM). Substrate used for PPE was Suc-Ala-Ala-Ala-pNA ([S] = 430 μM; [E] = 106 nM). For ChT, [I] = 54 nM to 1.2 μM. For PPE, [I] = 500 nM - 14 μM. The substrate used for HLE was MeO-Suc-Ala-Ala-Pro-Val-pNA ([S] = 100 μM; [E] = 30 nM). For HLE, [I] = 100 nM to 360 nM. All values were calculated from the initial part of the progress curve via the method of Tian and Tsou.[27] All log plots had $r^2 > 0.99$.

Neumann detected the fluorescence (415 nm) of the covalent chymotrypsin-inhibitor complex **B** (R = H) at an excitation wavelength of 285 nm along with almost complete quench of the tryptophan residue fluorescence of the enzyme.[18] Our compounds pose an interesting dilemma as two covalent complexes **B** are possible resulting from two possible isocyanates **A**. The placement of an R group on the ring destroys the symmetry of the ring system resulting in the possibility of either a *para*-complex (R group *para* to imidazole-*N*-carboxamide) or a *meta*-complex. Additional study (most likely in the form of an X-ray crystal structure of an enzyme-inhibitor complex) will be needed to resolve the question of which complex is formed.

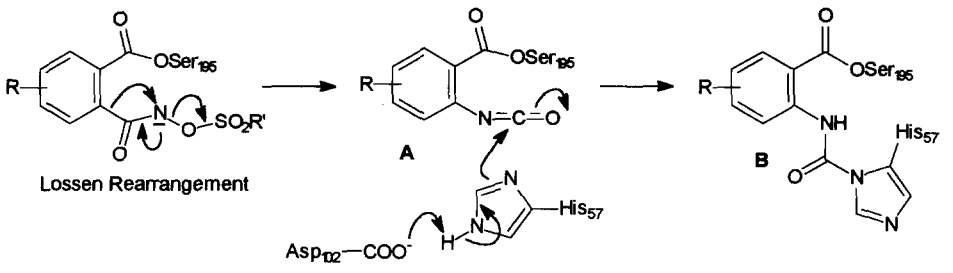


Figure 1. Mechanism of inhibition of serine proteases by 2-[(sulfonyl)oxy]-6-substituted-1*H*-isoindole-1,3-diones.

In summary, 6-amino-2-[(sulfonyl)oxy]-1*H*-isoindole-1,3-diones (**10** & **11**) were found to be fairly potent inhibitors of HLE. Unlike the 7-amino-4-chloro-3-ethoxyisocoumarin of Harper and Powers,[17] the polar amino group was found to enhance inhibitor potency in our substituted *N*-(sulfonyloxy)phthalimides. This result clearly indicates that the amino-substituted sulfonyloxy phthalimides bind in a very different manner than the aminoisocoumarins. This might be an indication of the additional binding mode accessible to the sulfonyloxy phthalimides (Figure 1). Incorporation of a 6-methyl substituent was found to enhance inactivation of PPE, but depress the inactivation of HLE. Future efforts will focus on the study of acyl/urea derivatives of compounds **10** and **11**.

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22. **General Procedure for Preparation of Compounds 4 & 5**. Triethylamine (2.4 mL, 17.1 mmol) was added to a heating mixture of the appropriate 4-substituted phthalic anhydride (3.0 g, 15.5 mmol) and *O*-benzylhydroxylamine hydrochloride (2.72 g, 17.1 mmol) in dry toluene (20 mL). The mixture was allowed to reflux for 1 h. The solvent was removed in vacuo followed by recrystallization of the crude product from ethanol.

4 (502 mg; 64%): colorless powder; mp 117 °C; IR (KBr) 1784, 1722 (C=O) cm^{-1}; Anal ($C_{16}H_{13}NO_3$) Calcd C, 71.90; H, 4.90; N, 5.24. Found C, 71.64; H, 4.98; N, 5.19.

5 (2.8 g; 56%): colorless powder; mp 188-190 °C; ^1H NMR (DMSO) δ 5.21 (s, 2 H), 7.40 (m, 5 H), 8.13 (d, J = 8.2 Hz, 1 H), 8.49 (d, J = 1.8 Hz, 1 H), 8.63 (dd, J = 8.1, 2.1 Hz, 1 H) ppm; IR (KBr) 1792, 1745 (C=O) cm^{-1}; EIMS *m/z* 299 (M^+ + H); HREIMS Calcd for $C_{15}H_{11}N_2O_5$ 299.0668; Found 299.0688.

23. **General Procedure for Preparation of 2-Hydroxy-6-methyl-1*H*-isoindole-1,3(2*H*)-dione (6) and 6-Amino-2-hydroxy-1*H*-isoindole-1,3(2*H*)-dione (7)**. To a suspension of **4** (1.0 g, 3.9 mmol) and Pd (2.1 g of a 10% mixture on carbon) in ethanol (20 mL) was added cyclohexene (2.3 mL, 23 mmol). The mixture was allowed to reflux for 15 min. The reaction mixture was filtered over celite to remove the catalyst. The filtrate was concentrated in vacuo followed by recrystallization from cold ethanol. The product was used without any further purification.

6 (298 mg, 43%): colorless crystals; mp 205-208 °C.

7 (390 mg, 27%): yellow powder; mp 268 °C dec (Lit.[28] 272-273 °C).

24. **Preparation of 2-[[(4-Methylphenyl)sulfonyl]oxy]-6-methyl-1*H*-isoindole-1,3(2*H*)-dione (8) and 2-[[(Methyl)sulfonyl]oxy]-6-methyl-1*H*-isoindole-1,3(2*H*)-dione (9).** Pyridine (0.1 mL, 1.2 mmol) was added to a mixture of **6** (160 mg, 0.9 mmol) and the appropriate alkyl or aryl sulfonyl chloride (172 mg, 0.9 mmol) in toluene (3 mL). The mixture was heated to 50 °C for 1 h and then allowed to stir at rt overnight. The precipitated product was removed by filtration and recrystallized from cold ethanol.

8 (202 mg, 68%): colorless crystals; mp 170-171 °C; ^{1}H NMR ($CDCl_3$) δ 2.50 (s, 3 H), 2.52 (s, 3 H), 7.40 (d, J = 8.0 Hz, 2 H), 7.59 (d, J = 7.8 Hz, 1 H), 7.66 (s, 1 H), 7.73 (d, J = 7.7 Hz, 1 H), 7.95 (d, J = 8.4 Hz, 2 H) ppm; IR (KBr) 1792, 1755 (C=O) cm^{-1}; EIMS *m/z* 331 (M^+); HREIMS Calcd for $C_{16}H_{13}NO_5S$ 331.0514; Found 331.0480; Anal. ($C_{16}H_{13}NO_5S$) Calcd C, 58.00; H, 3.95; N, 4.23. Found C, 57.74; H, 3.99; N, 4.19.

9 (120 mg, 61%): colorless crystals; mp 194-196 °C; ^{1}H NMR (DMSO) δ 3.34 (s, 3 H), 3.64 (s, 3 H), 7.75 (d, J = 7.7 Hz, 1 H), 7.82 (s, 1 H), 7.87 (d, J = 7.7 Hz, 1 H) ppm; IR (KBr) 1797, 1736 (C=O) cm^{-1}; EIMS *m/z* 255 (M^+); HREIMS Calcd for $C_{10}H_9NO_5S$ 255.0201; Found 255.0232; Anal. ($C_{10}H_9NO_5S$) Calcd C, 47.06; H, 3.55; N, 5.49. Found C, 46.94; H, 3.55; N, 5.42.

25. **Preparation of 6-Amino-2-[[(methyl)sulfonyl]oxy]-1*H*-isoindole-1,3(2*H*)-dione (10) and 6-Amino-2-[[(isopropyl)sulfonyl]oxy]-1*H*-isoindole-1,3(2*H*)-dione (11).** To a suspension of **7** (256 mg, 1.4 mmol) in water (10 mL) was added $NaHCO_3$ (120 mg, 1.4 mmol). The mixture was cooled to 0 °C followed by addition of the appropriate alkylsulfonyl chloride (0.1 mL, 1.4 mmol) dropwise. The mixture was allowed to stir for 2 h at 0 °C. The precipitated product was collected, and washed with water. In both cases, the crude product was recrystallized from ethyl acetate/hexanes.

10 (265 mg, 72%): yellow powder; mp 197-199 °C (Lit.[28] 198-199 °C); IR (KBr) 3508, 3404 (NH_2), 1792, 1723 (C=O) cm^{-1}; EIMS *m/z* 256 (M^+).

11 (200 mg, 94%): yellow powder; mp 200 °C dec (Lit.[28] 156-157 °C); IR (KBr) 3475, 3367 (NH_2), 1771, 1692 (C=O) cm^{-1}; EIMS *m/z* 284 (M^+); HREIMS Calcd for $C_{11}H_{12}N_2O_5S$ 284.0467; Found 284.0425.

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