Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 27–30

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

6-Acylamino-2-[(alkylsulfonyl)oxy]-1*H*-isoindole-1,3-dione Mechanism-Based Inhibitors of Human Leukocyte Elastase

John E. Kerrigan,[a,b,*] Matthew C. Walters,[a] Kerri J. Forrester,[a] Jason B. Crowder[a] and Lisa J. Christopher[a,†]

[a]*Department of Chemistry, University of South Alabama, Mobile, AL 36688, USA*
[b]*Department of Pharmaceutical Chemistry, College of Pharmacy, Rutgers University, 160 Frelinghuysen Road, Piscataway, NJ 08854, USA*

Received 25 August 1999; accepted 8 October 1999

Abstract—A study of various 2-[(alkylsulfonyl)oxy]-6-substituted-1*H*-isoindole-1,3-diones' inhibition of chymotrypsin compared to inhibition of HLE reveals that acylamino substitution in the 6-position increases selectivity and potency of these inhibitors for HLE. The best HLE inhibitor in this series was 6-(methylglutaryl)amino-2-[(ethylsulfonyl)oxy]-1*H*-isoindole-1,3-dione with a $k_{obs}/[I] =$ 220,000 M^{-1} s^{-1}.

Chronic obstructive pulmonary disease (COPD) is a growing concern as the nation's population steadily ages. In the United States alone, more than 15 million Americans suffered from COPD in 1994.[1] The 1994 figure is a substantial increase from the 1986 figure of 10 million.[2] Pulmonary emphysema and chronic bronchitis are two major components of COPD. Human leukocyte elastase (HLE; EC 3.4.21.37), a digestive serine protease found in polymorphonuclear leukocytes, has been implicated as a causative agent of emphysema.[3,4] A delicate balance between HLE and α-1-antitrypsin, a natural inhibitor of HLE, can be upset by a long history of cigarette smoking.[5,6] Cigarette smoking reduces the association rate constant of α-1-antitrypsin for HLE in the lower respiratory tract.[7]

A wide variety of different types of inhibitors of HLE are known.[8] Mechanism-based inhibitors have the advantage of being, chemically, fairly inactive until they reach their target enzyme.[9] These inhibitors take advantage of binding specificity to accomplish their goal. The enzyme recognizes the inhibitor as a substrate. The enzyme cleaves the inhibitor, releasing a reactive functionality. The reactive functional group covalently bonds to the enzyme, and effectively blocks the enzyme's active site. A wide range of mechanism-based inhibitors have been developed to inhibit serine proteases: 3,4-dichloroisocoumarin,[10] 3-alkoxy-7-amino-4-chloroisocoumarins,[11,12] haloenol lactones,[13] *N*-((alkylsulfonyl)oxy)succinimides,[14,15] saccharin derivatives,[16] 1,2,5-thiadiazolidin-3-one-1,1-dioxides,[17,18] isoxazolines,[19] sulfonyloxyketones[20] and *N*-((alkylsulfonyl)oxy)phthalimides.[21]

The *N*-((alkylsulfonyl)oxy)phthalimides are an attractive structural target for further development. Neumann and Gütschow found that simple *N*-((alkylsulfonyl)oxy)phthalimides are very effective inhibitors of chymotrypsin (ChT) and HLE in vitro.[21] While Neumann's compounds are indeed very good inhibitors of HLE, they are not selective for HLE.

The *N*-((alkylsulfonyl)oxy)succinimide inhibitors, upon acylation of the catalytic serine residue, release a sulphone amide anion group (Fig. 1) that undergoes a Lossen rearrangement to release a latent isocyanate **A**.[22] The released isocyanate reacts with the catalytic histidine residue forming an imidazole-*N*-carboxamide **B**, irreversibly inactivating the enzyme. Experimental evidence in support of the formation of the imidazole-*N*-carboxamide **B** in the inhibition of α-chymotrypsin by *N*-((methylsulfonyl)oxy)succinimides has been demonstrated by ^{13}C NMR studies.[23] The reactivation studies of chymotrypsin inactivated by *N*-((alkylsulfonyl)oxy)-phthalimides provides additional support for this

*Corresponding author. Tel.: +1-732-445-5763; fax: +1-732-445-6312.
†Present address: Bristol-Myers Squibb Co., PO Box 191, New Brunswick, NJ 08903-0191, USA.

0960-894X/00/$ - see front matter
PII: S0960-894X(99)00588-0

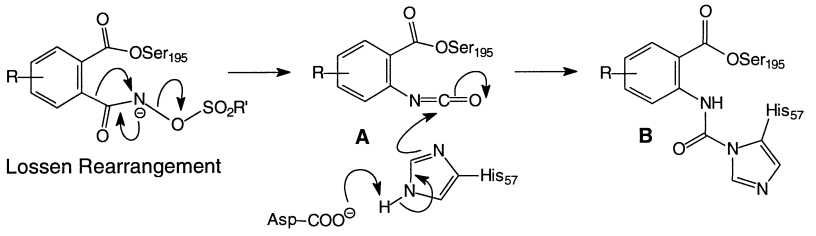


Figure 1. Mechanism of inhibition.

mechanism.[21] It was found that only a maximum of 25% activity was restored for the mesyloxy (R′ = Me) and 18% activity restored for the dansyloxy (R′ = Dansyl).[21] Neumann and Gütschow have suggested a similar mechanism for their *N*-((alkylsulfonyl)oxy)-phthalimides (R = H).[21]

Powers et al. observed that hydrophobic side-chains enhanced potency and selectivity of their mechanism-based isocoumarin inhibitors for HLE.[11] Based on structural similarity between the isocoumarin and phthalimide ring systems (Fig. 2), we surmised that 6-acylamino-substituted-1*H*-isoindole-1,3-diones might bind to HLE in a similar fashion and benefit from hydrophobic substituents in the 6-position.

Synthesis

Compound **4** was synthesized via our previously published procedure.[24] Compounds **5**–**11** were synthesized from **4** via reaction with the appropriate acid chloride.[25] For the preparation of compound **5**, tosyl-L-phenylalanine acid chloride was prepared from tosyl-L-phenylalanine in refluxing $SOCl_2$. Compound **12** (R = ethyl) was prepared from **3** via the same procedure used to prepare compound **4** (R = methyl). Compounds **13**–**15** were prepared from **12** as shown in Scheme 1 below.

Discussion

Most of the compounds in this study exhibited good selectivity for HLE over chymotrypsin (see Table 1). The notable exception was the phenylalanine mimic **6**, which was only a 2-fold better inhibitor of HLE over chymotrypsin and was the least potent. The result with compound **6** indicates that the (tosyl)amino group in the Tos-L-Phe derivative **5** may play an important role in binding to the enzyme. Inclusion of a *trans* double bond (R′ = *trans*-PhCH=CHCONH) in derivative **7** moderately increased inhibitor selectivity and potency for HLE relative to compound **6**. The (R′ = methylglutaryl/ R = CH_3CH_2) derivative **15** was the most potent and

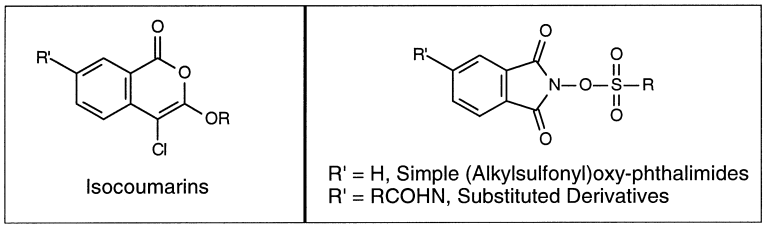
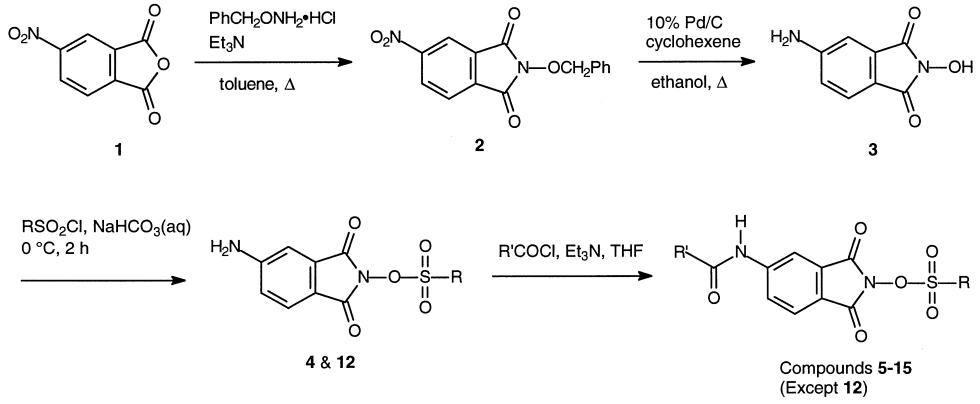
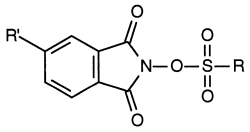


Figure 2. Isocoumarins and sulfonyloxy phthalimides.



Scheme 1. Synthesis of acylaminosulfonyloxy phthalimides.[25]

Table 1. Inhibition of chymotrypsin and human leukocyte elastase by 6-acylamino-2-[(alkylsulfonyl)oxy]-1*H*-isoindole-1,3-diones[a]



Compound	k_{obs}/[I] (M^{-1} s^{-1})				
	R′	R	Chymotrypsin	HLE	k_{obs}/[I] Ratio HLE/ChT
4	NH_2	CH_3	12,000	110,000	9.2
5	Tos-L-PheCONH	CH_3	11,000	**160,000**	15
6	$PhCH_2CH_2CONH$	CH_3	4,800	9,700	2.0
7	*trans*-PhCH=CHCONH	CH_3	1,600	20,000	13
8	PhCONH	CH_3	3,600	25,000	6.9
9	(1-Naphthyl)CONH	CH_3	3,300	25,000	7.6
10	$CH_3OCOCONH$	CH_3	5,400	30,000	5.6
11	$CH_3OCO(CH_2)_3CONH$	CH_3	6,600	57,000	8.6
12	NH_2	CH_2CH_3	13,000	**60,000**	4.6
13	$CH_3OCOCONH$	CH_2CH_3	5,300	56,000	11
14	$CH_3OCO(CH_2)_2CONH$	CH_2CH_3	9,600	25,000	2.6
15	$CH_3OCO(CH_2)_3CONH$	CH_2CH_3	14,000	**220,000**	16

[a]Conditions: 0.1 M HEPES, 0.5 M NaCl, pH 7.5 at 25 °C and <6% DMSO. Substrate used for chymotrypsin was Suc-Ala-Ala-Pro-Phe-pNA ([S] = 80 μM; [E] = 4 nM). Substrate used for HLE was MeO-Suc-Ala-Ala-Pro-Val-pNA ([S] = 100 μM; [E] = 30 nM). All values were calculated from the initial part of the progress curve via the method of Tian and Tsou (Tian, W.; Tsou, C. *Biochemistry* **1982**, *21*, 1028). All data was collected in triplicate with an average relative standard deviation of ±20%. All log plots had $R^2 \geq 0.99$.

most selective inhibitor of HLE in this series of compounds. The tosyl-L-Phe derivative **5** is the next most selective and potent HLE inhibitor. Most of the other analogues in this series of compounds were at least sevenfold more potent inhibitors of HLE relative to inhibition of chymotrypsin. Changing the R group from methyl (**10** and **11**) to ethyl (**13** and **15**) resulted in a 2- to 4-fold increase in HLE inhibition with the parent amino compound being the exception. The ethyl analogues **13** and **15** show promise for further development.

In conclusion, we have demonstrated that hydrophobic substituents in the 6-position enhance potency and selectivity of mechanism-based sulfonyloxy phthalimide inhibitors of HLE over chymotrypsin. The aromatic ring moiety in phenylalanine is well tolerated, as is the flexible methyl ester chain of the methylglutaryl group.

Acknowledgements

The authors wish to thank the National Institutes of Health (HL58190-01) for generous support of this work. High field proton NMR spectra were recorded either at USA on a Jeol Eclipse 300 MHz NMR or on a Bruker 400 MHz NMR spectrometer at the UAB Comprehensive Cancer Center, NMR Core Facility, Birmingham, AL.

References and Notes

1. Peterson, M.; Rahr, R.; Blessing, D.; Ayachi, S. *Physician Assistant* **1995**, *19*, 39.
2. Higgins, M. W.; Thom, T. In *Incidence, Prevalence and Mortality: Intra and Intercounty Differences*; Hensley, M. J.; Saunders, N. A., Eds.; Marcel Dekker: New York, 1989, pp. 24–30.
3. Janoff, A. *Am. Rev. Respir. Dis.* **1985**, *132*, 417.
4. Snider, G. L.; Lucey, E. C.; Stone, P. J. *Am. Rev. Respir. Dis.* **1986**, *133*, 149.
5. Johnson, D.; Travis, J. *J. Biol. Chem.* **1978**, *253*, 7142.
6. Beatty, K.; Matteson, N.; Travis, J. *Hoppe-Seyler's Z. Physiol. Chem.* **1984**, *365*, 731.
7. Ogushi, F.; Hubbard, R. C.; Vogelmeier, C.; Fells, G. A.; Crystal, R. G. *J. Clin. Invest.* **1991**, *87*, 1060.
8. Bernstein, P. R.; Edwards, P. D.; Williams, J. C. *Inhibitors of Human Leukocyte Elastase*; Ellis, G. W., Luscombe, D. K., Eds.; Elsevier: Amsterdam, 1994; Vol. 31, pp. 59–120.
9. Powers, J. C.; Harper, J. W. In *Proteinase Inhibitors*; Barret, A. J.; Salvensen, G., Eds.; Elsevier: New York, 1986, pp. 131–152.
10. Harper, J. W.; Hemmi, K.; Powers, J. C. *Biochemistry* **1985**, *24*, 1831.
11. Kerrigan, J. E.; Oleksyszyn, J.; Kam, C.; Selzler, J.; Powers, J. C. *J. Med. Chem.* **1995**, *38*, 544.
12. Harper, J. W.; Powers, J. C. *Biochemistry* **1985**, *24*, 7200.
13. Daniels, S. B.; Cooney, E.; Sofia, M. J.; Chakravarty, P. K.; Katzenellenbogen, J. A. *J. Biol. Chem.* **1983**, *258*, 15046.
14. Groutas, W. C.; Brubaker, M. J.; Venkataraman, R.; Stanga, M. A. *Arch. Biochem. Biophys.* **1992**, *297*, 144.
15. Groutas, W. C.; Brubaker, M. J.; Chong, L. S.; Venkataraman, R.; Huang, H.; Epp, J. B.; Kuang, R.; Hoidal, J. R. *Bioorg. Med. Chem.* **1995**, *3*, 375.
16. Groutas, W. C.; Chong, L. S.; Venkataraman, R.; Epp, J. B.; Kuang, R.; Houser-Archield, N.; Hoidal, J. R. *Bioorg. Med. Chem.* **1995**, *3*, 187.
17. Groutas, W. C.; Kuang, R.; Ruan, S.; Epp, J. B.; Venkataraman, R.; Truong, T. M. *Bioorg. Med. Chem.* **1998**, *6*, 661.
18. Kuang, R.; Venkataraman, R.; Ruan, S.; Groutas, W. C. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 539.
19. Groutas, W. C.; Venkataraman, R.; Chong, L. S.; Yoder, J. E.; Epp, J. B.; Stanga, M. A.; Kim, E. *Bioorg. Med. Chem.* **1995**, *3*, 125.

20. Ohba, T.; Wakayama, J.; Ikeda, E.; Takei, H. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1127.
21. Neumann, U.; Gütschow, M. *J. Biol. Chem.* **1994**, *269*, 21561.
22. Groutas, W. C.; Giri, P. K.; Crowley, J. P.; Castrisos, J. C.; Brubaker, M. J. *Biochem. Biophys. Res. Commun.* **1986**, *141*, 741.
23. Groutas, W. C.; Stanga, M. A.; Brubaker, M. J. *J. Am. Chem. Soc.* **1989**, *111*, 1931.
24. Kerrigan, J. E.; Shirley, J. J. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 451.

25. **General procedure for the preparation of acylamino analogues (5–11 and 13–15)**. Triethylamine (0.20 mL, 1.6 mmol) was added to a stirred solution of compound **4** or **12** (200 mg, 0.8 mmol) and the appropriate acid chloride (1.6 mmol) in THF (5 mL) at room temperature. The mixture was allowed to stir overnight at room temperature. The mixture was diluted in ethyl acetate (15 mL), washed with 10% HCl (8 mL), saturated $NaHCO_3$ (8 mL), brine (8 mL), dried (Na_2SO_4) and concentrated. The crude solid obtained was recrystallized in the solvent indicated.

6-(Tosyl-L-phenylalanyl)amino-2-[(methylsulfonyl)oxy]-1*H*-isoindole-1,3-dione (5). Methanol (220 mg, 53%): mp 243–244 °C dec; ^{1}H NMR (DMSO, 300 MHz) δ 2.19 (s, 3H), 2.81 (dd, J = 13.6, 9.4 Hz, 1H), 2.93 (dd, J = 13.5, 9.4 Hz, 1H), 3.65 (s, 3H), 4.14 (q, J = 9.4 Hz, 1H), 7.13 (d, J = 8.0 Hz, 2H), 7.21 (m, 4H), 7.50 (d, J = 8.0 Hz, 2H), 7.63 (d, J = 8.8 Hz, 1H), 7.90 (d, J = 9.1 Hz, 2H), 8.33 (s, 1H), 8.45 (d, J = 9.3 Hz, 1H), 10.64 (s, 1H) ppm; FABHRMS calcd for $[C_{25}H_{23}N_3O_8S_2+H]^+$ 558.1005, found 558.0946.

6-(Dihydrocinnamyl)amino-2-[(methylsulfonyl)oxy]-1*H*-isoindole-1,3-dione (6). Ethyl acetate (130 mg, 43%); mp 194–196 °C; ^{1}H NMR (DMSO, 300 MHz) δ 2.73 (t, J = 7.7 Hz, 2H), 2.94 (t, J = 7.4 Hz, 2H), 3.63 (s, 3H), 7.27 (m, 6H), 7.92 (s, 1H), 8.29 (s, 1H), 10.75 (s, 1H) ppm; FABHRMS calcd for $[C_{18}H_{16}N_2O_6S+H]^+$ 389.0807, found 389.0814.

6-(*trans*-Cinnamyl)amino-2-[(methylsulfonyl)oxy]-1*H*-isoindole-1,3-dione (7). Ethyl acetate (60 mg, 30%); mp 243 °C dec; ^{1}H NMR (DMSO, 300 MHz) δ 3.65 (s, 3H), 6.85 (d, J = 15.9 Hz, 1H), 7.46 (m, 3H), 7.68 (m, 3H), 7.96 (d, J = 8.3 Hz, 1H), 8.04 (dd, J = 8.2, 1.7 Hz, 1H), 8.41 (d, J = 1.7 Hz, 1H), 10.99 (s, 1H) ppm; FABHRMS calcd for $[C_{18}H_{14}N_2O_6S+H]^+$ 387.0651, found 387.0709.

6-(Benzoyl)amino-2-[(methylsulfonyl)oxy]-1*H*-isoindole-1,3-dione (8). Ethanol (70 mg, 20%); mp 200–202 °C dec; ^{1}H NMR (DMSO, 300 MHz) δ 3.66 (s, 3H), 7.60 (m, 3H), 8.01 (m, 3H), 8.26 (dd, J = 8.3, 1.9 Hz, 1H), 8.46 (d, J = 1.7 Hz, 1H), 10.94 (s, 1H) ppm; FABHRMS calcd for $[C_{16}H_{12}N_2O_6S+H]^+$ 361.0494, found 361.0560.

6-(1-Naphthoyl)amino-2-[(methylsulfonyl)oxy]-1*H*-isoindole-1,3-dione (9). Ethanol (350 mg, 88%); mp 250–252 °C dec; ^{1}H NMR (DMSO, 300 MHz) δ 3.67 (s, 3H), 7.67 (m, 3H), 8.07 (m, 4H), 8.30 (dd, J = 8.2, 1.9 Hz, 1H), 8.50 (d, J = 1.9 Hz, 1H), 8.66 (s, 1H), 11.10 (s, 1H) ppm; FABHRMS calcd for $[C_{20}H_{14}N_2O_6S+H]^+$ 411.0651, found 411.0704.

6-(Oxalyl)amino-2-[(methylsulfonyl)oxy]-1*H*-isoindole-1,3-dione (10). Ethanol (213 mg, 80%); mp 232–235 °C; ^{1}H NMR (DMSO, 400 MHz) δ 3.64 (s, 3H), 3.89 (s, 3H), 7.99 (d, J = 8.3 Hz, 1H), 8.24 (dd, J = 8.3, 1.7 Hz, 1H), 8.37 (d, J = 1.6 Hz, 1H), 11.45 (s, 1H) ppm; FABHRMS calcd for $[C_{12}H_{10}N_2O_8S+H]^+$ 343.0236, found 343.0291.

6-(Methylglutaryl)amino-2-[(methylsulfonyl)oxy]-1*H*-isoindole-1,3-dione (11). Ethanol (160 mg, 53%); mp 166–167 °C; ^{1}H NMR (DMSO, 400 MHz) δ 1.86 (m, 2H), 2.39 (t, J = 7.3 Hz, 2H), 2.46 (t, J = 7.4 Hz, 2H), 3.60 (s, 3H), 3.63 (3H), 7.91 (m, 2H), 8.26 (d, J = 0.8 Hz, 1H), 10.63 (s, 1H) ppm; FABHRMS calcd for $[C_{15}H_{16}N_2O_8S+H]^+$ 385.0706, found 385.0715.

6-(Oxalyl)amino-2-[(ethylsulfonyl)oxy]-1*H*-isoindole-1,3-dione (13). Ethanol (250 mg, 69%); mp 210–215 °C dec; ^{1}H NMR (DMSO, 400 MHz) δ 1.47 (t, J = 7.2 Hz, 3H), 3.79 (q, J = 7.2 Hz, 2H), 3.88 (s, 3H), 7.98 (d, J = 8.3 Hz, 1H), 8.24 (dd, J = 8.3, 1.8 Hz, 1H), 8.36 (d, J = 1.6 Hz, 1H), 11.45 (s, 1H) ppm; FABHRMS calcd for $[C_{13}H_{12}N_2O_8S+H]^+$ 357.0393, found 357.0470.

6-(Methylsuccinyl)amino-2-[(ethylsulfonyl)oxy]-1*H*-isoindole-1,3-dione (14). Ethanol (196 mg, 69%); mp 198–200 °C; ^{1}H NMR (DMSO, 400 MHz) δ 1.46 (t, J = 6.4 Hz, 3H), 2.64 (t, J = 5.5 Hz, 2H), 2.79 (t, J = 5.6 Hz, 2H), 3.60 (s, 3H), 3.77 (q, J = 6.3 Hz, 2H), 7.92 (m, 2H), 8.24 (s, 1H), 10.74 (s, 1H) ppm; FABHRMS calcd for $[C_{15}H_{16}N_2O_8S+H]^+$ 385.0706, found 385.0684.

6-(Methylglutaryl)amino-2-[(ethylsulfonyl)oxy]-1*H*-isoindole-1,3-dione (15). Ethanol (26 mg, 9%); mp 114–116 °C; ^{1}H NMR (DMSO, 400 MHz) δ 1.47 (t, J = 7.3 Hz, 3H), 1.86 (m, 2H), 2.39 (t, J = 7.3 Hz, 2H), 2.46 (t, J = 7.4 Hz, 2H), 3.59 (s, 3H), 3.78 (q, J = 7.3 Hz, 2H), 7.93 (m, 2H), 8.26 (s, 1H), 10.64 (s, 1H) ppm; FABHRMS calcd for $[C_{16}H_{18}N_2O_8S+H]^+$ 399.0862, found 399.0943.