Pergamon

Bioorganic & Medicinal Chemistry, Vol. 4, No. 9, pp 1393–1400, 1996
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PII: S0968-0896(96)00133-2
0968-0896/96 $15.00+0.00

Design, Synthesis, and In Vitro Inhibitory Activity Toward Human Leukocyte Elastase, Cathepsin G, and Proteinase 3 of Saccharin-Derived Sulfones and Congeners

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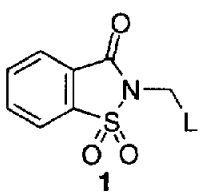
Abstract—The inhibitory activity toward human leukocyte elastase (HLE), cathepsin G (Cat G), and proteinase 3 (PR 3) of a series of saccharin derivatives having a sulfinate leaving group was investigated. The results of this study revealed that (a) inhibitory activity is dependent on the nature and pK_a of the leaving group, and (b) the synthesized saccharin derivatives exhibit selective inhibition toward HLE and PR 3, with low or no activity toward cathepsin G. The results of exploratory biochemical, HPLC and high-field ^{13}C NMR studies are also described. Copyright © 1996 Elsevier Science Ltd

Introduction

An array of inflammatory diseases, including pulmonary emphysema,[1,2] psoriasis and dermatitis,[3] and cystic fibrosis,[4] involve a massive influx of neutrophils, the subsequent production of reactive oxygen species, the extracellular release of the serine endopeptidases elastase, cathepsin G and proteinase 3, and elevated levels of inflammatory chemokines (interleukin-8, leukotriene B_4).[5–8] Inadequate control of the activity of these proteolytic enzymes due to depressed levels of their physiological inhibitors can lead to the destruction of the major components of the extracellular matrix.[9,10]



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A hallmark of inflammatory diseases is the existence of a compromised proteinase/antiproteinase screen.[11,12] Thus, in principle, the pathological processes associated with these diseases can be arrested by reestablishing a proteinase/antiproteinase balance via the use of potent and selective inhibitors of these enzymes.[13,14] This report describes the results of in vitro biochemical and mechanistic studies using saccharin derivatives (**1**) as inhibitors of human leukocyte elastase (HLE), cathepsin G (Cat G), and proteinase 3 (PR 3).

Key words: inhibitors, serine proteases, saccharin derivatives, elastase, cathepsin G, proteinase 3.

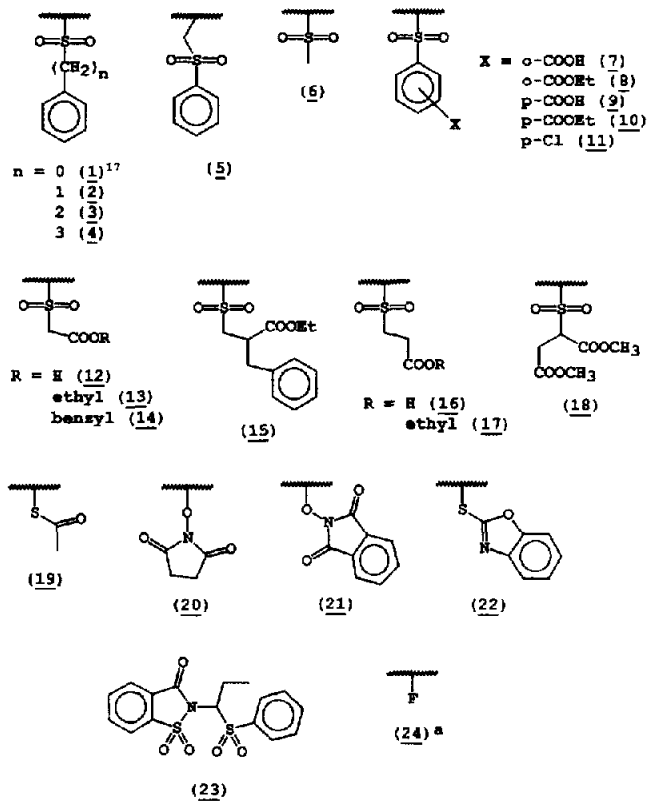
Chemistry

Compounds **1**, **6**, and **11** were readily synthesized by reacting the sodium salt of saccharin with chloromethyl phenyl sulfide, chloromethyl methyl sulfide or chloromethyl *p*-chlorophenyl sulfide, respectively, in DMF, followed by oxidation with *m*-chloroperbenzoic acid. Compounds **2–4**, **7–10**, and **12–18** were prepared by alkylating the appropriate mercaptan with *N*-(chloromethyl)saccharin in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU)/acetonitrile or triethylamine in THF,[15] followed by peracid oxidation of the resultant purified sulfide. Reaction of thiophenol with 2-(bromoethyl)saccharin in the presence of triethylamine, followed by peracid oxidation of the product gave compound **5**. Phenyl *n*-propyl sulfide was chlorinated with *N*-chlorosuccinimide (NCS) in methylene chloride[16] and the resulting alpha-chlorosulfide was alkylated with saccharin in the presence of triethylamine/acetonitrile. Subsequent peracid oxidation yielded compound **23**. Compounds **20** and **21** were readily obtained by stirring *N*-hydroxysuccinimide or *N*-hydroxyphthalimide with *N*-chloromethyl saccharin and triethylamine in dry acetonitrile at room temperature. Reaction of 2-mercaptobenzoxazole with sodium hydride in DMF, followed by the addition of 2-chloromethyl saccharin, yielded compound **22**. Compound **24** was synthesized as previously described.[17] The synthesized compounds and their physical and spectral data are listed in Tables 1 and 2, respectively.

Biochemical studies

Enzyme assays and inhibition studies with HLE, Cat G, and PR 3 were carried out as described in detail elsewhere.[18,19] Analysis of the data according to Kitz and Wilson yielded the apparent second-order inactivation rate constants $k_{obs}/[I]$ M^{-1} s^{-1} (listed in Table 3).

Table 1. Derivatives of compound **1**, L =



Molecular modeling

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

Results and Discussion

Structure–activity relationship studies

During the course of some studies related to the development of mechanism-based inhibitors of the neutrophil-derived serine proteinases elastase, cathepsin G, and proteinase 3 based on the Gabriel–Colman rearrangement,[20–23] it became apparent that a phthalimide or saccharin ring system serving as a scaffold and embodying appropriate recognition and reactivity elements might function as an effective inhibitor of serine proteinases. Preliminary studies have shown that, irrespective of the nature of L, phthalimide derivatives are devoid of any inhibitory activity.[24] Furthermore, in the saccharin series, activity was found to be critically dependent on the nature of L. Only compounds with L = halogen, OOCR, OOCCHRNHCbz and SO_2R were found to be active.[17,25,26] These findings are in agreement with the work of Hlasta and co-workers[27–29] who have recently shown that 2,6-disubstituted aryl carboxylic acids, sulfides and related derivatives of benzisothiazolone are highly effective inhibitors of HLE.

In order to gain further insight into the interaction of **1** with serine proteinases, as well as probe the S_N' subsites of HLE, Cat G, and PR 3, a series of compounds represented by structure **1**, where $X = SO_2R$, were synthesized.

The interaction of sulfone ester **14** with HLE or PR 3, for example, led to a rapid and time-dependent loss of enzymatic activity (Fig. 1). Inactivated HLE or PR 3 regained activity slowly and incompletely after 24 h (75% and 80%, respectively). The behavior of the rest of the compounds toward HLE and PR 3 was similar to that of **14**, namely, rapid acylation of the enzyme was followed by very slow regain of enzymatic activity. The half-lives of reactivation varied between 10 and 15 h.

It is evident from Table 3 that saccharin-derived sulfone derivatives can be effective, time-dependent inhibitors of HLE and PR 3 (compounds **10–11**, **13–14** and **18**). Furthermore, the inhibitory activity of these compounds was greater toward HLE than PR 3. Most of the synthesized compounds were inactive or had very low inhibitory activity toward Cat G.

The position of the sulfone group was found to be critical for the manifestation of biological activity (Table 1, compound **5**), as dictated by the proposed mechanism of action of these compounds, namely, an absolute requirement for L to be a good leaving group (vide infra). This is in accord with earlier observations with *N*-alkyl substituted saccharins and related compounds which were found to be inactive.[17] It should be noted that the precursor sulfides and the corresponding sulfoxides of compounds **1–18** were *all* found to be inactive. While these observations suggest that the observed activity in this series of compounds is critically dependent on leaving group ability, nevertheless, hydrophobic binding interactions also play an important role. For instance, compound **3** is active, while **6** is inactive, despite the comparable pK_as of methanesulfinic and 3-phenyl-1-propanesulfinic acid (the pK_as of *n*-butyl sulfinic and 3-phenyl-1-propane sulfinic acids are 2.11 and 2.0, respectively).[30] This is also evident with compounds **19–22**. A noteworthy case is the observed difference in activity between **20** and **21**, despite the fact that the pK_a of *N*-hydroxysuccinimide is *lower* than that of *N*-hydroxyphthalimide (6.0 versus 7.0). This is probably due to a favorable lipophilic–lipophilic interaction between the aromatic ring in **21** and the lipophilic S_N' subsites of the enzyme. Although speculative, the specific protein residue involved in this interaction might be Phe-41. This favorable interaction is also evident in the case of compounds **14** and **15**. Indeed, molecular modeling studies using the X-ray crystal structures of the HLE–turkey ovomucoid inhibitor complex and compound **14** show the aromatic ring of the saccharin moiety nestled into the hydrophobic S_1 pocket, with the

—$CH_2SO_2CH_2COOCH_2Ph$ portion of the inhibitor extending into the S_N' hydrophobic subsites (Fig. 2).

The best inhibitors were found to be those combining both a better leaving group and enhanced hydrophobicity (Table 3, compounds **10–11**, **13–14**, **18** and **22**). The lack of activity of **23** is not intuitively obvious.

In order to enhance the aqueous solubility of these compounds, the corresponding carboxylic acids were also synthesized and screened for in vitro inhibitory activity toward HLE. It is clear from Table 1 that polar groups abolish or drastically reduce inhibitory activity (Table 3, compounds **7**, **9**, **12** and **16**). Molecular modeling studies (data not shown) suggest that while polar functionalities located near the S_1'–S_2' subsites are inimical to inhibitor potency, polar groups located beyond these subsites extend into the aqueous milieu and should be tolerated.

Recent studies by Hlasta and co-workers[27–29] using saccharin derivatives have shown that the introduction of an appropriate alkyl group at the C-4 position of the saccharin ring results in a significant enhancement in inhibitory activity. Thus, the inhibitory activity of the saccharin derivatives described herein should be augmented significantly via a similar structural manipulation.

Mechanistic studies

A tentative mechanism of action envisaged for **1** is illustrated in Figure 3, and is similar to the one previously proposed by Hlasta and co-workers in their studies with related compounds.[27] Thus, rapid acylation of the active site serine is followed by ring-opening and simultaneous departure of the leaving group, yielding a reactive electrophilic species (**A**). Subsequent reaction of **A** with an active site nucleophile (path a) or via path (b) yields inactive enzyme **C** or **D**, respectively. Spontaneous fragmentation of intermediate (**D**) would be expected to either generate formaldehyde (**F**), saccharin (**G**) and active enzyme, or acyl enzyme (**E**). Deacylation of **E** would also give active enzyme and other products.

The mechanism of action of **1** was explored using standard biochemical methodology, HPLC and high-field ^{13}C NMR using *N*-fluoromethylsaccharin **24** and ^{13}C-labeled derivative **25**.

Table 2. Physical constants and spectral data of inhibitors

Compd	mp (°C)	NMR δ	MF (anal.)
1	167–168	5.1 (s, 2H), 7.55 (m, 2H), 7.7 (m, 1H), 7.81–8.05 (m, 6H)	$C_{14}H_{11}NO_5S_2$ (C,H,N)
2	141–143	4.53 (s, 2H), 4.84 (s, 2H), 7.42 (m, 3H), 7.58 (m, 2H), 7.89–8.03 (m, 3H), 8.15 (m, 1H)	$C_{15}H_{13}NO_5S_2$ (C,H,N)
3	110–111	3.25 (m, 2H), 3.54 (m, 2H), 4.89 (s, 2H), 7.27–7.40 (m, 5H), 7.90–8.03 (m, 3H), 8.13 (m, 1H)	$C_{16}H_{15}NO_5S_2$ (C,H,N)
4	103–104	2.28 (m, 2H), 2.81 (t, 2H), 3.27 (t, 2H), 4.91 (s, 2H), 7.19–7.38 (m, 5H), 7.89–8.04 (m, 3H), 8.13 (d, 1H)	$C_{17}H_{17}NO_5S_2$ (C,H,N)
5	146–148	3.67 (t, 2H), 4.15 (t, 2H), 7.58–7.69 (m, 3H), 7.81–8.08 (m, 6H)	$C_{15}H_{13}NO_5S_2$ (C,H,N)
6	174–175	3.21 (s, 3H), 5.31 (s, 2H), 8.05–8.25 (m, 3H), 8.43 (d, 1H)	$C_9H_9NO_5S_2$ (C,H,N)
7	222–223	5.65 (s, 2H), 7.72–8.20 (m, 7H), 8.34 (d, 1H)	$C_{15}H_{15}NO_7S_2$ (C,H,N)
8	206–210	1.35 (t, 3H), 4.40 (q, 2H), 5.60 (s, 2H), 7.77–8.20 (m, 7H), 8.40 (m, 1H)	$C_{15}H_{11}NO_7S_2$ (C,H,N)
9	250–252	5.49 (s, 2H), 8.00–8.22 (m, 7H), 8.38 (d, 1H)	$C_{15}H_{11}NO_7S_2$ (C,H,N)
10	166–168	1.42 (t, 3H), 4.43 (q, 2H), 5.09 (s, 2H), 7.88–8.08 (m, 3H), 8.10 (d, 2H), 8.25 (d, 2H)	$C_{17}H_{15}NO_7S_2$ (C,H,N)
11	166–167	5.09 (s, 2H), 7.56 (m, 2H), 7.87–7.99 (m, 5H), 8.05 (m, 1H),	$C_{16}H_{10}NO_5S_2Cl$ (C,H,N)
12	155–157	4.52 (s, 2H), 5.43 (s, 2H), 8.05–8.25 (m, 3H), 8.43 (d, 1H)	$C_{10}H_9NO_7S_2$ (C,H,N)
13	113–116	1.36 (t, 3H), 4.31 (s,2 H), 4.35 (q, 2H), 5.38 (s, 2H), 7.90–8.05 (m, 3H), 8.17 (m, 1H)	$C_{12}H_{13}NO_7S_2$ (C,H,N)
14	oil	4.35 (s, 2H), 5.27 (s, 2H), 5.33 (s, 2H), 7.37 (m, 5H), 7.88–8.00 (m, 3H), 8.10 (d, 1H)	$C_{17}H_{15}NO_7S_2$ (C,H,N)
15	120–121	1.18 (t, 3H), 2.96 (dd, 1H), 3.09 (dd, 1H), 3.28 (dd, 1H), 3.40 (m, 1H), 3.84 (dd, 1H), 4.15 (dq, 2H), 4.99 (dd, 2H), 7.16–7.32 (m, 5H), 7.88–8.01 (m, 3H), 8.12 (m, 1H)	$C_{20}H_{21}NO_7S_2$ (C,H,N)
16	190–191	2.78 (t, 2H), 3.60 (t, 2H), 5.37 (s, 2H), 8.06–8.26 (m, 3H), 8.43 (d, 1H)	$C_{11}H_{11}NO_7S_2$ (C,H,N)
17	136–137	1.28 (t, 3H), 2.94 (t, 2H), 3.61 (t, 2H), 4.20 (q, 2H), 5.07 (s, 2H), 7.90–8.05 (m, 3H), 8.15 (m, 1H)	$C_{13}H_{15}NO_7S_2$ (C,H,N)
18	151–152	3.18 (t, 2H), 3.73 (s, 3H), 3.92 (s, 3H), 5.04 (dd, 1H), 5.17 (d, 1H), 5.65 (d, 1H), 7.90–8.05 (m, 3H), 8.17 (m, 1H)	$C_{14}H_{15}NO_5S_2$ (C,H,N)
19	111–113	2.44 (s, 3H), 5.32 (s, 2H), 7.88–8.00 (m, 3H), 8.10 (m, 1H)	$C_{10}H_9NO_4S_2$ (C,H,N)
20	190	2.61 (s, 4H), 5.65 (s, 2H), 8.01–8.16 (m, 2H), 8.21 (m, 1H), 8.35 (m, 1H)	$C_{12}H_{10}N_2O_6S$ (C,H,N)
21	200–205	5.80 (s, 2H), 7.88 (s, 4H), 8.06–8.17 (m, 2H), 8.24 (m, 1H), 8.35 (m, 1H)	$C_{16}H_{10}N_2O_6S$ (C,H,N)
22	131–134	5.82 (s, 2H), 7.50 (m, 2H), 7.72 (m, 2H), 8.00–8.17 (m, 3H), 8.33 (m, 1H)	$C_{15}H_{10}N_2O_4S_2$ (C,H,N)
23	144–145	1.12 (t, 3H), 2.31–2.47 (m, 1H), 2.61–2.76 (m, 1H), 5.30 (br, 1H), 7.58 (m, 2H), 7.68 (m, 1H), 7.84–7.97 (m, 3H), 8.02 (m, 3H)	$C_{16}H_{15}NO_5S_2$ (C,H,N)

Table 3. Inhibitory activity of 1 toward human leukocyte elastase, cathepsin G and proteinase 3

Compd	$k_{obs}/[I]$ M^{-1} s^{-1} HLE	Cat G	PR 3
1	570	10	180
2	870	80	220
3	830	NA[a]	180
4	330	NA	NA
5	NA	ND[b]	ND
6	NA	NA	NA
7	NA	ND	ND
8	730	40	320
9	440	NA	270
10	4470	NA	900
11	3870	230	1090
12	NA	ND	ND
13	2090	70	1020
14	5190	130	1570
15	1160	NA	360
16	NA	ND	ND
17	700	NA	190
18	3690	NA	1600
19	NA	ND	ND
20	NA	ND	ND
21	340	ND	ND
22	7000	ND	ND
23	NA	ND	ND

[a]Compounds producing less than 50% inhibition when incubated with the enzyme for 10 min at an inhibitor to enzyme ratio of 200 were classified as inactive.
[b]Not determined.

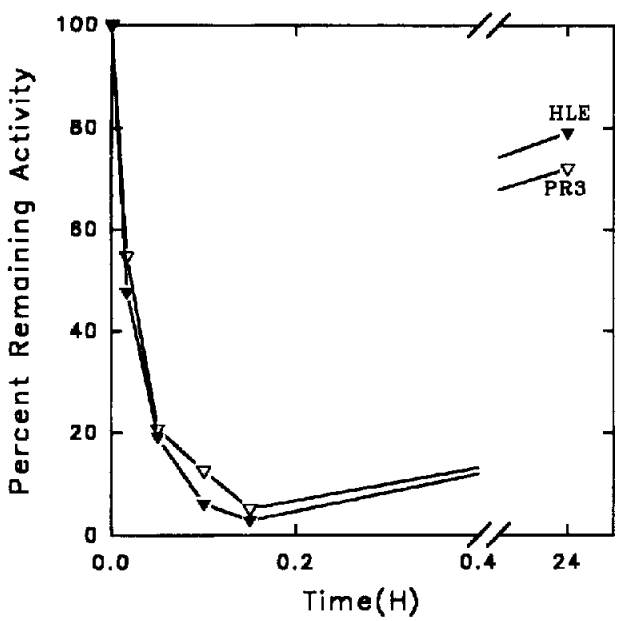


Figure 1. Conditions: HLE (138 nM) was incubated with inhibitor **14** (1.38 μM) in 0.1 M HEPES buffer, pH 7.25 and 1% dimethyl sulfoxide. Aliquots were withdrawn at different time intervals and assayed for enzymatic activity using methoxysuccinyl Ala-Ala-Pro-Val *p*-nitroanilide. In a separate experiment, PR 3 (373 nM) was incubated with inhibitor **14** (3.73 μM) in 0.1 M phosphate buffer, pH 6.51 and 1% dimethyl sulfoxide. Aliquots were withdrawn at different time intervals and assayed for enzymatic activity using BOC-Ala-*p*-nitrophenol ester.

Incubation of **24** with HLE was found to lead to rapid and time-dependent loss of enzymatic activity, followed by slow recovery of enzymatic activity. The regain in enzymatic activity after 24 h was near total, and the half-life of reactivation was determined to be 12 h.[17] In separate experiments, treatment of fully inactivated enzyme with excess hydroxylamine led to rapid recovery of enzymatic activity, suggesting that the interaction of the enzyme with **24** leads to the formation of labile acyl linkages.[31] Furthermore, the interaction of **24** with HLE involves the active site, as evidenced by an observed decrease in the $k_{obs}/[I]$ M^{-1} s^{-1} when the experiment was repeated in the presence of substrate (data not shown).

The inhibition of α-chymotrypsin by **24** was transient in nature, with a half-life of reactivation of ca. 1 h. Thus, reverse-phase HPLC was used to determine the nature of the product(s) arising from turned over inhibitor. α-Chymotrypsin was incubated with excess inhibitor **24** for 22 h. The excess inhibitor was removed by Centricon filtration at 5 °C and the filtrate was analyzed by HPLC. The major product formed was found to be saccharin by spiking with an authentic sample. *o*-Carboxybenzene-sulfonamide was not detected.

The interaction of **25** with chymotrypsin was studied using high-field NMR.[32] The ^{13}C NMR experiments established that processing of **25** by the enzyme leads to rapid formation of formaldehyde hydrate (δ 82.5). The formation of formaldehyde was detected within 30 min of incubation. A control experiment showed that inhibitor **25** retained its structural integrity under the same conditions. Further verification and quantitation of the generated formaldehyde was achieved using a standard spectrophotometric assay.[33] Enzymatic processing of **25** was found to yield phenyl sulfinic acid (**B**) and saccharin (**G**), as evidenced by reverse-phase HPLC using authentic samples of **B** and **G**. These findings are consistent with the mechanism shown in Figure 3, whereby the formation of the Michaelis–Menten complex is followed by acylation of the active site serine and the formation of **A** (not detected by ^{13}C NMR). The available evidence suggests that products **F** and **G** arise from **D** by a concerted process, with simultaneous formation of activated enzyme. The fact that the formation of formaldehyde can be detected by ^{13}C NMR within 30 min of incubating the enzyme and inhibitor solutions is in accord with this interpretation. However, the observed *slow* recovery of enzymatic activity is suggestive of the possible formation of one or more stable E–I complexes, such as **C**/**E**. Slow deacylation of **E** via a general base-catalysed (His-57) deacylation process, could presumably yield active enzyme and **G**. The limited aqueous solubility of **25** thwarted attempts at detecting any E–I′ complexes by NMR. Whether a 'double hit' mechanism leading to the formation of (**C**) (path a) is also operative remains to be established.

In summary, the inhibitory activity and mechanism of action of a series of saccharin-derived sulfones toward

the serine proteinases elastase, cathepsin G, and proteinase 3 have been described.

Experimental

Melting points were recorded on a Mel-Temp apparatus and are uncorrected. The IR and NMR spectra of the synthesized compounds were recorded on a Perkin–Elmer 1330 IR spectro-photometer and a Varian XL-300 NMR spectrometer, respectively. 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 Company, Owensville, Missouri. Human leukocyte cathepsin G and proteinase 3 were purchased from Athens Research and Technology Company, Athens, Georgia. Methoxysuccinyl Ala-Ala-Pro-Val *p*-nitroanilide, methoxysuccinyl Ala-Ala-Pro-Phe *p*-nitroanilide, and Boc-Ala-*p*-nitrophenol were purchased from Sigma Chemicals Co., St Louis, Missouri. The enzyme–inhibitor NMR studies were performed using a 500-MHz Varian Unity Plus NMR spectrometer consisting of a SPARC station computer, graphic pulsed field gradient and three-channel detection system.

Representative syntheses

2-[(4-Carboxyphenylsulfonyl)methyl]-1,2-benzisothiazol-3(2*H*)-one 1,1 dioxide (9). A mixture of *N*-chloromethylsaccharin (1.39 g, 6 mmol) and 4-mercaptobenzoic acid (0.93 g, 6 mmol) in 20 mL dry acetonitrile was treated with DBU (1.83 g, 12 mmol). The reaction mixture was stirred overnight at room temperature. The solvent was removed in vacuo and the residue was taken up in 5% aq sodium bicarbonate (50 mL) and extracted with ethyl acetate (2 × 30 mL). The aq layer was acidified (to pH 1) with 6 N HCl. The precipitated solid was collected by suction filtration and purified, yielding 1.02 g (49% yield) pure sulfide.

The sulfide (0.35 g, 1 mmol) in 2 mL dry DMF was treated with *m*-chloroperbenzoic acid (0.50 g of 57–86% peracid) and stirred at room temperature overnight. The solvent was removed under vacuum and the residue was purified by flash chromatography using silica gel (hexane:ethyl acetate), yielding 0.20 g (53% yield) of **9**, mp 250–252 °C.

2-[(4-Carboxyphenylsulfonyl)methyl]-1,2-benzisothiazol-3(2*H*)-one 1,1 dioxide ethyl ester (10). The sulfide (0.7 g, 2 mmol) obtained from the reaction of *N*-chloro-

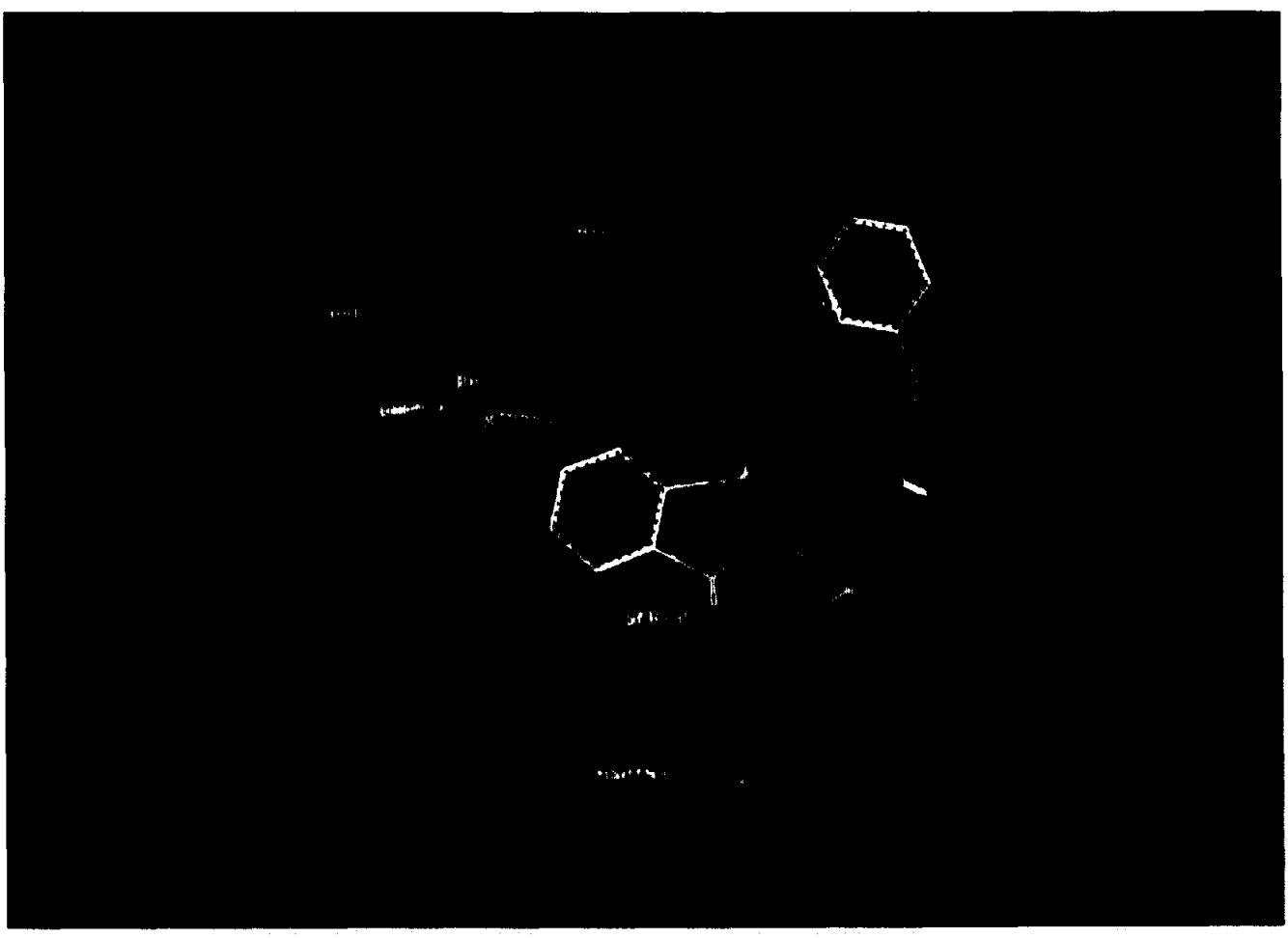


Figure 2. Energy-minimized structure of compound **14** with HLE detailing the binding and catalytic sites. Hydrogen bonds formed between HLE (blue) and **14** and indicated by dotted lines.

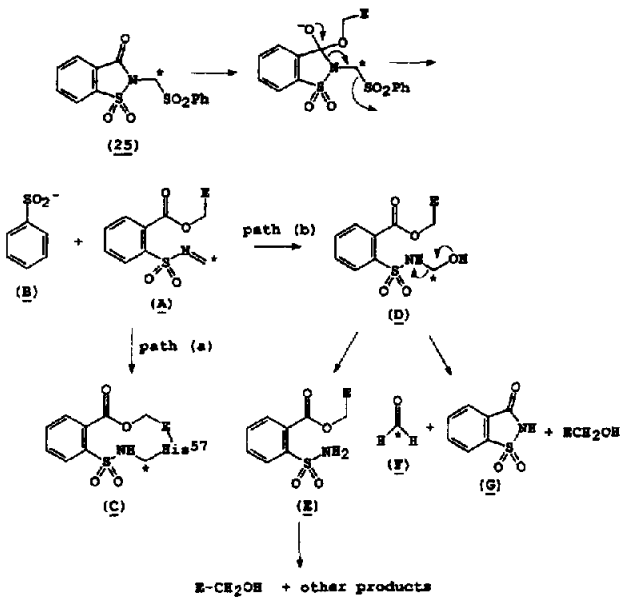
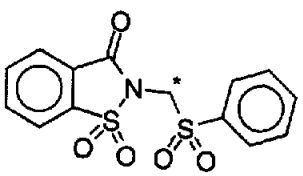


Figure 3. Postulated mechanism of action of 1.



25

methylsaccharin and 4-mercaptobenzoic acid (*vide supra*) was esterified by stirring overnight a mixture of dry ethanol (1 mL), dimethyl formamide (2.5 mL), dicyclohexylcarbodiimide (0.42 g, 2 mmol) and 4-dimethylaminopyridine (0.03 g, 0.25 mmol). The sulfide ester was isolated by pouring the reaction mixture into 40 mL water and extracting with ethyl ether (2 × 40 mL). The organic layer was dried over anhydrous sodium sulfate, filtered, and the solvent evaporated to yield a crude product which was purified by flash chromatography on silica gel (hexane:methylene chloride; 0.39 g; 52% yield).

The sulfide ester (0.23 g, 0.61 mmol) was mixed with *m*-chloroperbenzoic acid (0.35 g of 60% peracid) in 10 mL methylene chloride and stirred overnight. The reaction mixture was diluted with methylene chloride (5 mL) and extracted with 5% aq sodium bicarbonate (5 mL). Evaporation of the solvent left 0.22 g (88% yield) of sulfone ester **10**.

2-[(4-Chlorophenylsulfonyl)methyl]-1,2-benzisothiazol-3(2*H*)-one 1,1 dioxide (11). A solution of saccharin (0.96 g, 5.25 mmol) and chloromethyl 4-chlorophenyl sulfide (0.97 g, 5 mmol) in dry acetonitrile (20 mL) was treated with triethylamine (0.51 g, 5 mmol) and refluxed for 16 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 5% sodium bicarbonate. The organic layer was dried over anhydrous sodium sulfate and the solvent removed under vacuum. The crude product was purified by flash chromatography using silica gel (hexane:methylene chloride), yielding 1.0 g (59% yield) of pure sulfide. The sulfide (0.34 g, 1 mmol) was dissolved in methylene chloride (10 mL) and oxidized with *m*-chloroperbenzoic acid (0.50 g of 57–88% peracid) by stirring at room temperature for 10 h. The solution was extracted with 5% sodium bisulfite (15 mL) and 5% sodium bicarbonate. The organic layer was dried and the solvent evaporated in vacuo to yield 0.36 g (97% yield) of pure **11**.

2-[(Thioacetate)methyl]-1,2-benzisothiazol-3(2*H*)-one 1,1 dioxide (19). A solution of *N*-chloromethylsaccharin (2.31 g, 10 mmol), thiolacetic acid (0.80 g, 10.5 mmol) and triethylamine (1.01 g, 10 mmol) in 25 mL dry THF was stirred overnight. The solvent was removed in vacuo and the residue was taken up in ethyl acetate (85 mL) and extracted with 5% hydrochloric acid (2 × 50 mL). The organic layer was dried over anhydrous sodium sulfate, filtered, and evaporated. The crude product was purified by recrystalization from hexane:ethyl acetate (2.2 g, 92% yield), mp 111–113 °C.

2-[(Phenylsulfonyl)methyl]-1,2-benzisothiazol-3(2*H*)-one 1,1 dioxide (2-methyl-^{13}C) (25). 1,8-Diazabicyclo[5.4.0]undecen-7-ene (DBU); 4.26 g; 28 mmol) was added to a solution of thiophenol (3.1 g, 28 mmol) in benzene (80 mL) and the solution was placed in an ice-bath. ^{13}C Iodomethane (4.0 g, 28 mmol) was added and stirring was continued for 2 h. The reaction mixture was washed with 5% HCl, saturated sodium bicarbonate and satd sodium chloride. After drying, the solvent was evaporated to give 3.0 g (85% yield) of product.

^{13}C-labeled methyl phenyl sulfide (3.0 g; 24 mmol) in 40 mL of benzene was mixed with *N*-chlorosuccinimide (3.53 g, 26.4 mmol). The reaction mixture was stirred for 20 h at room temperature. The precipitate was filtered off and the organic layer was washed with 10% sodium bicarbonate (20 mL), brine (20 mL), and dried over anhydrous sodium sulfate. Evaporation of the solvent left 3.7 g (97% yield) of pure product.

A mixture of ^{13}C-labeled chloromethyl phenyl sulfide (1.27 g, 8 mmol), saccharin (1.47 g, 8 mmol), triethylamine (0.8 g, 8 mmol) in dry acetonitrile (16 mL) was refluxed for 24 h. The solvent was removed in vacuo and the residue was taken up in ethyl acetate (35 mL) and washed with water (15 mL), 5% HCl (15 mL), 5% $NaHCO_3$ (15 mL) and dried over anhydrous sodium sulfate. The solvent was evaporated off and the crude product was purified by flash chromatography using silica gel (hexane:methylene chloride) to yield 1.08 g (44% yield) of pure sulfide.

The ^{13}C-labeled sulfide (0.92 g, 3 mmol) in dry methylene chloride (20 mL) was treated with *m*-chloroperbenzoic acid (1.73 g) and the mixture was stirred overnight at room temperature. The solution was

extracted with 5% sodium sulfite (15 mL), 5% sodium bicarbonate (15 mL) and dried. The solvent was removed and the crude product was purified by dissolving it in methylene chloride and passing it through a pad of silica gel. Evaporation of the solvent left 0.80 g (80% yield) of pure **25**.

2-[(2-Thioacetic acid)methyl]-1,2-benzisothiazol-3(2*H*)-one 1,1 dioxide (26). *N*-chloromethylsaccharin (1.39 g, 6 mmol) and 2-mercapto-acetic acid (0.56 g, 6 mmol) were dissolved in dry THF (20 mL) and treated with triethylamine (1.21 g, 12 mmol). The reaction mixture was stirred overnight at room temperature. The solvent was then removed in vacuo and the residue taken up in 5% aq sodium bicarbonate (50 mL) and washed with ethyl acetate (2 × 25 mL). The aq phase was removed and acidified to pH 1 with 6 N HCl. Extraction with ethyl acetate (3 × 40 mL) and removal of the solvent left 1.3 g (75% yield) of pure product.

2-[(2-Thioacetic acid)methyl]-1,2-benzisothiazol-3(2*H*)-one 1,1 dioxide ethyl ester (27). Compound **26** (0.86 g, 3 mmol) was mixed with dry ethanol (2 mL) and methylene chloride (6 mL). 4-Dimethylaminopyridine (60 mg) was added and the solution was cooled to 0 °C. Dicyclohexylcarbodiimide (0.62 g, 3 mmol) was added and the reaction mixture was stirred for 5 min at 0 °C at room temperature overnight. The mixture was filtered and the filtrate was evaporated in vacuo. The crude product was purified by flash chromatography (0.40 g, 42% yield).

2-[(Sulfonylacetic acid)methyl]-1,2-benzisothiazol-3(2*H*)-one 1,1 dioxide ethyl ester (13). Compound **27** (0.32 g, 1 mmol) was dissolved in 10 mL methylene chloride and treated with *m*-chloroperbenzoic acid (0.58 g of 60% peracid). The mixture was stirred at room temperature overnight, diluted with 5 mL methylene chloride, and then extracted with 5% aq sodium sulfite (5 mL) and 5% aq sodium bicarbonate (5 mL). The organic phase was dried over anhydrous sodium sulfate, filtered, and evaporated in vacuo, leaving 0.28 g (81% yield) of product.

2-[(*N*-Oxysuccimide)methyl]-1,2-benzisothiazol-3(2*H*)-one 1,1 dioxide (20). *N*-Hydroxysuccinimide (0.58 g, 5 mmol) and *N*-chloromethylsaccharin (0.58 g, 2.5 mmol) were dissolved in 5 mL acetonitrile. Triethylamine (0.51 g, 5 mmol) was added and the mixture stirred for 2 h at room temperature. The solvent was removed in vacuo and the residue taken up in ethyl acetate (40 mL). The organic layer was washed with 5% aq hydrochloric acid (2 × 15 mL) and 5% aq sodium bicarbonate (2 × 15 mL). The organic layer was dried over anhydrous sodium sulfate, filtered, and evaporated in vacuo to yield 0.52 g (67% yield) of pure product.

2-[(Ethyl phenylsulfonyl)methyl]-1,2-benzisothiazol-3(2*H*)-one 1,1 dioxide (23). A solution of phenyl *n*-propyl sulfide (1.52 g, 10 mmol) in methylene chloride (20 mL) was treated with *N*-chlorosuccinimide (1.34 g, 10 mmol) and stirred at room temperature overnight. The precipitate was filtered off and the solvent removed under vacuum. The crude α-chlorosulfide (1.47 g, 7.8 mmol) was dissolved in acetonitrile (20 mL) and treated with saccharin (1.83 g, 10 mmol) and triethylamine (1.01 g, 10 mmol). The reaction mixture was refluxed for 5 h, cooled to room temperature, and the solvent was removed under vacuum. The residue was taken up in ethyl acetate (100 mL) and extracted with 5% hydrochloric acid (2 × 35 mL) and 5% sodium bicarbonate (2 × 35 mL). The organic layer was dried over anhydrous sodium sulfate, filtered, and the solvent was removed under vacuum. The crude product was purified by flash chromatography (hexane:ethyl acetate) to yield 1.29 g (39% yield) of pure **23**.

^{13}C NMR studies

Inhibitor **25** (200 μL, 2.01 mM in deuterated dimethyl sulfoxide) was incubated with α-chymotrypsin (10 μL of a 2.0 mM solution) in 690 μL 0.1 M phosphate buffer, pH 7.5, and 300 μL deuterium oxide. 125.70 MHz ^{13}C DEPT-45 NMR spectra were recorded at different time intervals on a 500 MHz Varian UNITY Plus NMR spectrometer using the following conditions: 1.0 s repetition time, 1000 scans, 27 K sweep-width (SW), 32 K data points, 45° variable pulse (^{1}H) to equally excite all protonated carbons, and broad-band ^{1}H decoupling. A control consisting of 200 μL inhibitor (2.01 mM in deuterated dimethyl sulfoxide), 690 μL 0.1 M phosphate buffer, pH 7.5, and 310 μL deuterium oxide was also used.

Product analysis

HPLC analysis of the interaction of α-chymotrypsin with *N*-(fluoromethyl)saccharin was conducted on a C-18 column using an elution system consisting of 80% water, 10% acetonitrile, 10% methanol, and 0.15% acetic acid, and a flow rate of 1.5 mL min^{-1}. The eluants were monitored at 265 nm. An aq solution of α-chymotrypsin (25 μL, 2.01 mM) were mixed with 100 μL of inhibitor **24** (12.2 mM in acetonitrile) and 875 μL distilled water. After a 22 h incubation period, the solution was centrifuged with centricon filter at 5000 × *g* for 1.5 h at 5 °C. The recovered filtrate was analysed by C-18 reverse-phase HPLC. An enzyme control solution prepared by mixing 100 μL acetonitrile, 875 μL distilled water and 25 μL enzyme (2.01 mM in water) was also used. A similar inhibitor control solution was prepared by mixing 100 μL inhibitor **24** (12.2 mM) and 900 μL was used.

Acknowledgment

Support of this work by the National Institutes of Health (HL 38048) and the Kansas Health Foundation is gratefully acknowledged.

References

1. Bernstein, P. R.; Edwards, P. D.; Williams, J. C. *Prog. Med. Chem.* **1994**, *31*, 59.

2. Crystal, R. G. *J. Clin. Invest.* **1990**, *85*, 1343.

3. Wiedow, O.; Wiese, F.; Streit, V.; Kalm, C.; Christophers, E. *J. Invest. Dermatol.* **1992**, *99*, 306.

4. Meyer, K. C.; Lewandoski, J. R.; Zimmerman, J. J.; Nunley, D.; Calhoun, W. J.; Dopico, G. A. *Am. Rev. Resp. Dis.* **1991**, *144*, 580.

5. Edwards, S. W. *Biochemistry and Physiology of the Neutrophil*; Cambridge University: New York, 1994.

6. Baggiolini, M.; Clarke-Lewis, I. *FEBS Lett.* **1993**, *307*, 97.

7. McElvaney, N. G.; Nakamura, H.; Birrer, P.; Hebert, C. A.; Wong, W. L.; Alphonso, M.; Baker, J. B.; Catalano, M. A.; Crystal, R. G. *J. Clin. Invest.* **1992**, *90*, 1296.

8. Constan, M. W.; Walenga, R. W.; Hilliard, K. A.; Hilliard, J. B. *Am. Rev. Resp. Dis.* **1993**, *148*, 896.

9. Kao, R. C.; Wehner, N. G.; Skubitz, K. M.; Gray, B. H.; Hoidal, J. R. *J. Clin. Invest.* **1988**, *82*, 1963.

10. Janusz, M. J.; Doherty, N. S. *J. Immunol.* **1991**, *146*, 3922.

11. Janoff, A. *Am. Rev. Resp. Dis.* **1985**, *133*, 149.

12. Birrer, P.; McElvaney, N. G.; Rudeberg, A.; Sommer, C. W.; Liechti-Gallati, S.; Kraemer, R.; Hubbard, R.; Crystal, R. G. *Am. J. Resp. Crit. Care Med.* **1994**, *150*, 207.

13. Edwards, P. D.; Bernstein, P. R. *Med. Res. Rev.* **1994**, *14*, 127.

14. Weinbaum, G.; Groutas, G. C. In *Focus on Pulmonary Pharmacology and Toxicology*; Hollinger, M. A., Ed.; CRC: Boca Raton, 1991.

15. Ono, N.; Miyake, H.; Saito, T.; Kar, A. *Synthesis* **1980**, 952.

16. Tuleen, D. L.; Stephens, T. B. *J. Org. Chem.* **1969**, *34*, 31.

17. Groutas, W. C.; Houser-Archield, N.; Chong, L. S.; Venkataraman, R.; Epp, J. B.; Huang, H.; McClenahan, J. J. *J. Med. Chem.* **1993**, *36*, 3178.

18. Groutas, W. C.; Brubaker, M. J.; Stanga, M. A.; Castrisos, J. C.; Crowley, J. P.; Schatz, E. J. *J. Med. Chem.* **1989**, *32*, 1607.

19. (a) Groutas, W. C.; Venkataraman, R.; Brubaker, M. J.; Stanga, M. A. *Biochemistry* **1991**, *30*, 4132; (b) Groutas, W. C.; Brubaker, M. J.; Venkataraman, R.; Epp, J. B.; Stanga, M. A.; McClenahan, J. J. *Arch. Biochem. Biophys.* **1992**, *294*, 144.

20. Hill, J. H. M. *J. Org. Chem.* **1965**, *30*, 620.

21. Zinnes, H.; Comes, R. A.; Shavel, J. *J. Org. Chem.* **1964**, *29*, 2068.

22. Gabriel, S.; Colman, J. *Chem. Ber.* **1900**, *33*, 980.

23. (a) Groutas, W. C.; Chong, L. S.; Venkataraman, R.; Epp, J. B.; Kuang, R.; Brubaker, M. J.; Houser-Archield, N.; Huang, H.; McClenahan, J. J. *Biochem. Biophys. Res. Comm.* **1993**, *194*, 1491; (b) Groutas, W. C.; Brubaker, M. J.; Venkataraman, R.; Epp, J. B.; Houser-Archield, N.; Chong, L. S.; McClenahan, J. J. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 175;

24. For an exception, see Groutas, W. C.; Chong, L. S.; Venkataraman, R.; Huang, H.; Epp, J. B.; Kuang, R. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 2745.

25. Groutas, W. C.; Houser-Archield, N.; Chong, L. S.; Venkataraman, R.; Epp, J. B.; Huang, H.; McClenahan, J. J. *J. Med. Chem.* **1993**, *36*, 3178.

26. Groutas, W. C.; Huang, H.; Venkataraman, R.; Houser-Archield, N.; Epp, J. B. *Bioorg. Med. Chem.* **1993**, *1*, 273.

27. (a) Dunlap, R. P.; Boaz, N. W.; Mura, A. J.; Hlasta, D. J. *Chem. Abstr.* **1991**, *114*, 228897f; (b) Hlasta, D. J.; Bell, M.; Boaz, N.; Court, J.; Desai, R.; Eiff, S.; Franke, C.; Mura, A.; Subramanyam, C.; Dunlap, R. 202nd ACS National Meeting, 28 August, 1991, New York.

28. Hlasta, D. J.; Bell, M. R.; Boaz, N. W.; Court, J. J.; Desai, R. C.; Franke, C. A.; Mura, A. J.; Subramanyam, C.; Dunlap, R. P. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1801.

29. Subramanyam, C.; Bell, M. R.; Carabateas, P.; Court, J. J.; Dority, J. A.; Ferguson, E.; Gordon, R.; Hlasta, D. J.; Kumar, V.; Saindane, M. *J. Med. Chem.* **1994**, *37*, 2623, and ref cited therein.

30. Oae, S. *Organic Sulfur Chemistry: Structure and Mechanism*; CRC: Boca Raton, 1991.

31. Kam, C.-H.; Fujikawa, K.; Powers, J. C. *Biochemistry* **1988**, *27*, 2547.

32. The high expense of HLE necessitated the use of α-chymotrypsin.

33. Nash, T. *Biochem. J.* **1953**, *55*, 416.

(*Received in U.S.A.* 9 *July* 1995)