Inhibition of Human Leukocyte Elastase by Derivatives of *N*-Hydroxysuccinimide. A Structure-Activity-Relationship Study

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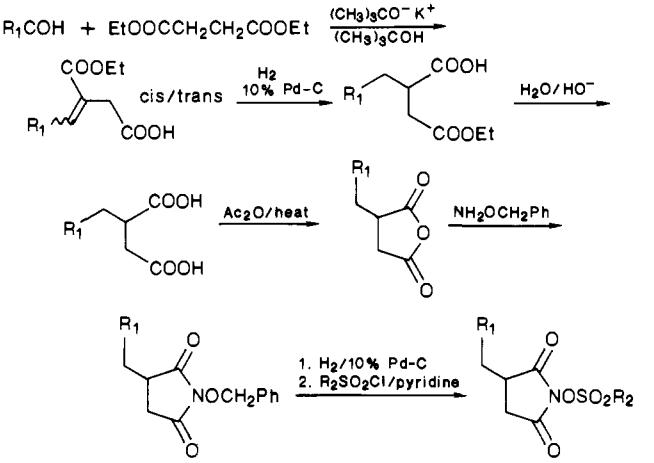
A series of compounds derived from 3-alkyl-*N*-hydroxysuccinimide have been synthesized and their inhibitory activity toward human leukocyte elastase has been investigated. Compounds having an isobutyl or isopropyl group at the C-3 position have been found to be particularly effective inactivators of the enzyme. The introduction of a *trans*-styryl group (as in compounds 16 and 18) results in a drastic enhancement in inhibitory activity indicative of a favorable interaction between the phenyl ring and the S_2' subsite of the enzyme. The compounds were found to be highly stable in buffer solution with no apparent change in structural integrity after 17 h (the period of observation). Studies with model compounds and high-field NMR indicate that these compounds function as mechanism-based inhibitors of the enzyme. Porcine pancreatic elastase is not inhibited by these compounds, while chymotrypsin and human leukocyte cathepsin G are also efficiently inactivated.

Human leukocyte elastase (HLE) has been the subject of intense investigation in recent years because of its likely involvement in the pathogenesis of pulmonary emphysema and other inflammatory ailments.[1] Modulation of the activity of HLE offers the promise of effective therapeutic intervention in the aforementioned disease states.[2-4] We have recently reported on the biochemical rationale behind the design and time-dependent, irreversible inactivation of HLE by *dl*-3-benzyl-*N*-[(methylsulfonyl)oxy]succinimide, **10**, a member of a new class of inhibitors of HLE and α-chymotrypsin (CT).[5] In continuing our studies in this area, we have attempted to delineate the structural features in this class of inhibitors that led to optimum inhibitory activity, probe further the mechanism of the inactivation process, and determine the stability and specificity of these compounds. The results of our studies are reported herein.

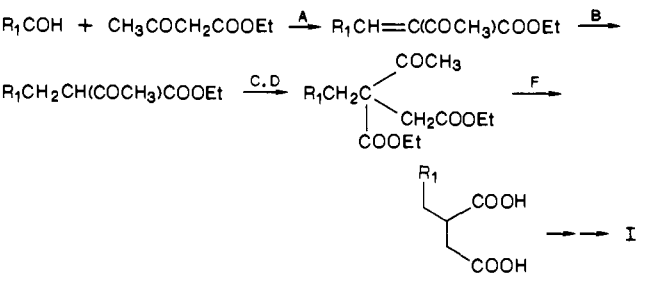
Chemistry

The compounds listed in Table I were prepared by starting with the appropriate substituted alkylsuccinic acids. The alkylsuccinic acids were synthesized by using the Stobbe condensation[6] (Scheme I), followed by reduction and hydrolysis. An equally satisfactory method involved the condensation of ethyl acetoacetate with the appropriate aldehyde, followed by reduction to yield the alkyl-substituted ethyl acetoacetate[7,8] (Scheme II). Alkylation with ethyl chloroacetate and subsequent hydrolysis yielded the desired alkyl succinic acids in good yields. The physical data of the synthesized compounds are listed in Table II.

Scheme I



Scheme II[a]



[a] (a) Pyrrolidine/HOAc/benzene, (b) H_2/Pd-C/THF, (c) NaOEt/EtOH, (d) $ClCH_2COOEt$, (e) H_2O/HO^-.

Results and Discussion

Structure-Activity-Relationship Studies. (a) Nature of R_1. Compounds represented by structure I are time-dependent irreversible inhibitors of HLE (Figure 1). The inhibitory activity of the synthesized compounds is expressed in terms of the bimolecular rate constant $k_{obs}/[I]$, M^{-1} s^{-1},[9] and the results are summarized in Table I. The preference of HLE for small hydrophobic side chains is reflected clearly in the high inhibitory activity observed with compounds **15-18**. This finding is in accord with the known substrate specificity of HLE for a valine residue at the S_1 subsite.[10] Stein has shown[11] that P_1 specificity is regulated by peptide length and that HLE can accommodate bulky P_1 residues in reactive *monomeric* substrates. Thus, unsubstituted benzyl groups (**1-14**) and bulky alkyl groups at R_1 (**17-20**) are also tolerated by the

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(2) Groutas, W. C. *Med. Res. Rev.* **1987**, *7*, 227.
(3) Trainor, D. A. *Trends Pharmacol. Sci.* **1987**, *8*, 303.
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(6) Overberger, C. G.; Roberts, C. W. *J. Am. Chem. Soc.* **1949**, *71*, 3618.
(7) Al-Hassan, S. S.; Kulick, R. J.; Livingstone, D. B.; Suckling, C. J.; Wood, H. C. S.; Wriggleworth, R.; Ferone, R. *J. Chem. Soc., Perkin Trans. 1* **1980**, 2645.
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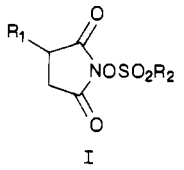
(9) Yoshimura, T.; Barker, L. N.; Powers, J. C. *J. Biol. Chem.* **1982**, *257*, 5077.
(10) Stein, R. L.; Trainor, D. A.; Wildonger, R. A. *Annu. Rep. Med. Chem.* **1985**, *20*, 237.
(11) (a) Stein, R. L. *Arch. Biochem. Biophys.* **1985**, *236*, 677. (b) Stein, R. L.; Strimpler, A. M. *Biochemistry* **1987**, *26*, 1301 and references cited therein.

0022-2623/89/1832-1607$01.50/0

Table I. Derivatives of 3-Alkyl-*n*-hydroxysuccinimide



I

compd	R_1	R_2	$k_{obs}/[I]$, M^{-1} s^{-1}
1	benzyl	phenyl	1171
2	benzyl	*p*-bromophenyl	1648
3	benzyl	*p*-fluorophenyl	1110
4	benzyl	*p*-tolyl	1608
5	benzyl	*m*-(trifluoromethyl)phenyl	316
6	benzyl	*trans*-styryl	9428
7	benzyl	1-naphthyl	620
8	benzyl	2-naphthyl	1580
9	benzyl	benzyl	770
10	benzyl	methyl	1050
11	benzyl	*N*-butyl	1680
12	benzyl	isopropyl	1100
13	benzyl	*N*-octyl	1273
14	benzyl	*N,N*-dimethylamino	323
15	isopropyl	methyl	3817
16	isopropyl	*trans*-styryl	>100000[a]
17	isobutyl	methyl	49338
18	isobutyl	*trans*-styryl	>100000[a]
19	isopentyl	methyl	361
20	isopentyl	*trans*-styryl	2251
21	*o*-(trifluoromethyl)benzyl	methyl	189
22	*o*-(trifluoromethyl)benzyl	*trans*-styryl	1860
23	*m*-(trifluoromethyl)benzyl	methyl	217
24	*p*-(trifluoromethyl)benzyl	methyl	84
25	*m*-fluorobenzyl	methyl	373
26	H	*trans*-styryl	inactive

[a] Inactivation was too fast to measure by ordinary sampling techniques.

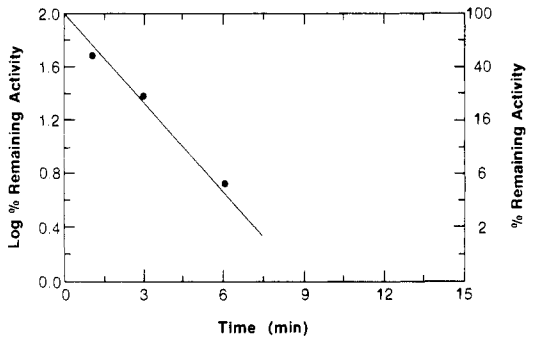
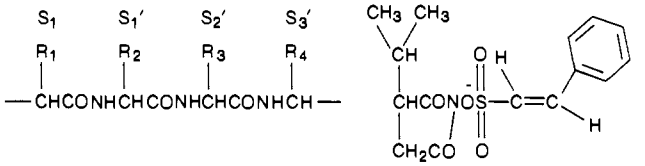


Figure 1. Kinetics of inactivation of HLE by compound 17. HLE (300 nm) was incubated with 17 (1.5 μM) in 0.1 M HEPES buffer, pH 7.2, and 0.5% DMSO. Aliquots were removed periodically and assayed for enzymatic activity using methoxysuccinyl-Ala-Ala-Pro-Val-*p*-nitroanilide.

enzyme. The introduction of a CF_3 group leads to compounds with diminished activity (**21**, **23**, and **24**), presumably because of steric reasons. Likewise, compounds with long straight alkyl chains exhibit greatly diminished activity. For example, when R_1 = *n*-dodecyl, $k_{obs}/[I]$ = 59 M^{-1} s^{-1} (unpublished observation).

(b) Nature of R_2. Potency appears to be unaffected by the nature of the R_2 group (compare, for example, compounds **1–4** and **10–13**), with the exception of the styryl group (vide infra). The fact that compound **13** is equipotent with other compounds with less bulky R_2 groups (**9–12**, for example) is indicative of the absence of any unfavorable steric interactions between R_2 and the S_n' subsites of the enzyme.

Scheme III



HLE is a glycoprotein with an extended binding site that functions as an induced-fit enzyme. Earlier studies[11] have shown that binding at remote subsites changes the size of the primary specificity site S_1 so that extended substrates with Phe at P_1 are unreactive. The diminished potency observed with compounds **5** and **14** suggests the involvement of such an unfavorable interaction. The most striking result exhibited in Table I is the observed drastic enhancement in inhibitory activity when R_2 is *trans*-styryl, irrespective of the nature of the R_1 group (**6**, **16**, **18**, **20**, and **22**). When R_1 = isopropyl or isobutyl and R_2 = *trans*-styryl, enzyme inactivation was too fast to measure with ordinary sampling techniques, even at an inhibitor to enzyme ratio of 5. It is conceivable that a favorable interaction involving the phenyl ring and the S_2' subsite of the enzyme may play a major role in the observed potency (Scheme III). Indeed, this is consistent with the observed K_i and k_2 values for compounds **10** (K_i = 22.8 μM, k_2 = 0.02 s^{-1}) and **6** (K_i = 4.24, μM, k_2 = 0.02 s^{-1}), where K_i and k_2 are the dissociation constant of the enzyme–inhibitor complex and the rate constant for inactivation, respectively. Thus, the *trans*-styryl group is affecting primarily the strength of binding. The replacement of a methyl group with a *trans*-styryl group increases the $k_{obs}/[I]$ ratio by a factor of 10 (compounds **21** and **22**). Clearly, such a favorable interaction is not possible in the case of compounds **1** and **9** since they do not have the requisite number of carbon atoms that would place the phenyl ring at the S_2' subsite (Scheme III). It should be noted that the presence of the *trans*-styryl group by itself is not sufficient to impart inhibitory activity to a compound (see compound **26**, for example), unless an appropriate P_1 residue is also present.

The S_n' subsites of HLE have been investigated by Bieth[12] using trifluoroacetyl peptides. He has shown that HLE has three S_n' subsites. The S_1' subsite is nonspecific, the preferred residue at S_2' is Leu, and the S_3' subsite is capable of providing favorable local interactions between the protein amino acid residues and the aromatic ring of the inhibitors. Our findings suggest that the development of potent inhibitors of HLE that interact with the S_n' domain of the enzyme can indeed be realized. The successful use of fluoromethyl inhibitors of CT that interact with the S_1'–S_3' subsites of the enzyme has been reported recently by Abeles.[13]

Mechanistic Studies. In a preliminary communication,[5] we demonstrated that the inactivation of HLE by compound **10** involves the active site (time-dependent inactivation is slowed down in the presence of substrate) and that fully inactivated enzyme regains its activity slowly ($k_{reactiv}$ = 1.9 × 10^{-4} s^{-1}) and incompletely (40–60%) via the hydrolysis of labile acyl or carbamate linkages. Furthermore, when buffered hydroxylamine was added to fully inactivated enzyme, rapid but incomplete reactivation of the enzyme was observed. We proposed the mechanism shown in Figure 2, whereby compound I manifests its inhibitory activity through an enzyme-activated mechanism.

(12) Renaud, A.; Lestienne, P.; Hughes, D. L.; Bieth, J. G.; Dimicoli, J.-L. *J. Biol. Chem.* **1983**, *258*, 8312.
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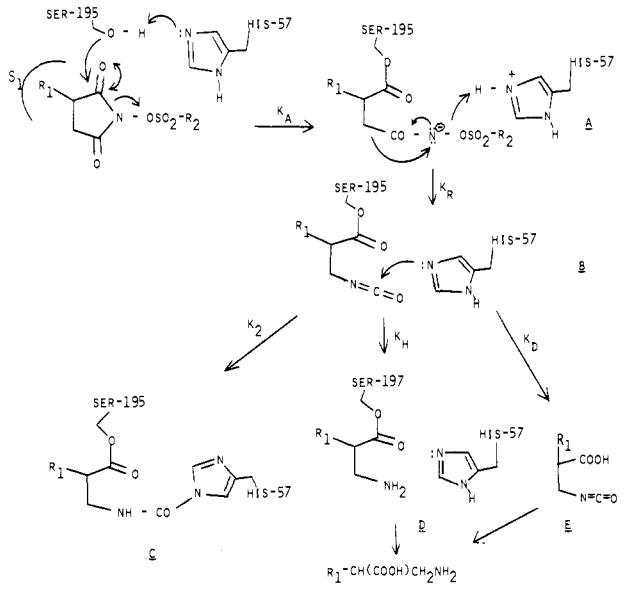
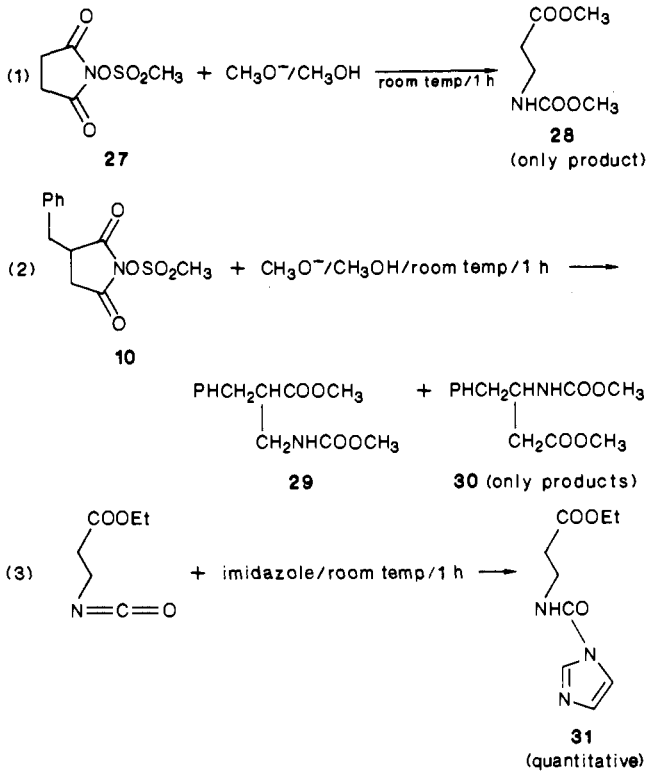


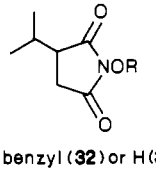
Figure 2. Proposed mechanism of inactivation of HLE by compound I.

Scheme IV



Acylation of the active-site serine generates an isocyanate, via an instantaneous Lossen rearrangement, that remains tethered at the active site until it reacts with a nucleophilic residue (presumably His-57). Prior to using ^{13}C-labeled inhibitor for the direct observation of enzyme–inhibitor adduct C by high-field NMR,[14] we investigated the chemical behavior of some model compounds in order to establish the chemical competence of the steps outlined in Figure 2. Thus, when compound **27** (3 mmol) was reacted with an equivalent amount of sodium methoxide in 20 mL of methanol at room temperature for 1 h, product **28**[15] was isolated as the only product. No *N*-hydroxysuccinimide could be detected by NMR analysis (Varian XL-300).

Likewise, when compound **10** was reacted with sodium methoxide under the same conditions, only the two isomeric Lossen rearrangement products **29** and **30** were produced.[15] The reaction of ethyl 3-isocyanatopropionate with imidazole in acetonitrile (25 °C/1 h) resulted in rapid and quantitative conversion to the corresponding azolide **31** (Scheme IV).[15] The absence of a good leaving group, as in compounds **32** and **33**, yields inactive compounds.



R = benzyl (**32**) or H (**33**)

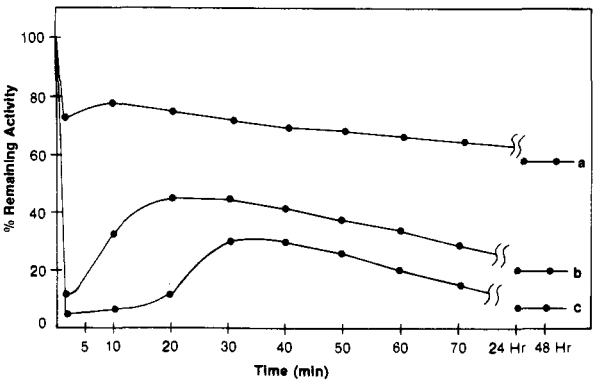


Figure 3. Time-dependent loss of enzymatic activity. HLE (300 nm) was incubated with compound **16** (a, 0.3 μM; b, 1.5 μM; c, 3.0 μM) in HEPES buffer, pH 7.2, 0.5 M NaCL, and 1% DMSO at 25 °C.

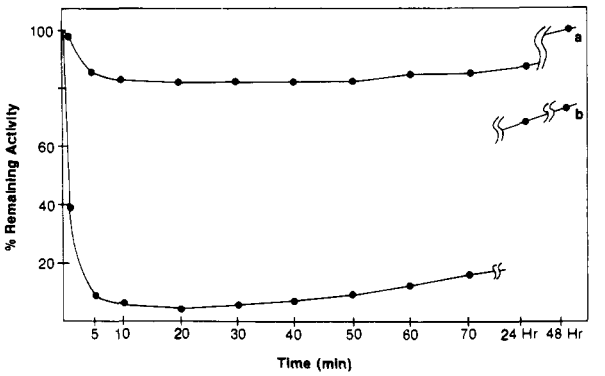


Figure 4. Time-dependent loss of enzymatic activity. HLE (300 nM) was incubated with compound **6** (a, 300 nM; b, 3.0 μM) in HEPES buffer, pH 7.2, 0.5 M NaCl, and 1% DMSO.

The inactivation of HLE by compound **16** is illustrated in Figure 3. The initial rapid loss of enzymatic activity is followed by a transient and partial regain in activity, followed by a gradual and irreversible loss of enzymatic activity. Compounds **17** and **18** behave similarly, while in the case of the less potent inhibitors the regain in activity is more gradual (Figure 4).

Taken together, these data are supportive of the cascade of events shown in Figure 2, namely, rapid acylation of the

(14) The synthesis of 3-benzyl-*N*-[methylsulfonyl)oxy]succinimide-5-^{13}C has been completed recently and subsequent NMR studies have established the enzyme-induced formation of isocyanate B and enzyme–inhibitor adduct C (Groutas, W C.; Stanga, M. A.; Brubaker, M. B. *J. Am. Chem. Soc.* **1989**, *111*, 1931–1932.

(15) The spectral data of the compounds were consistent with the assigned structures.

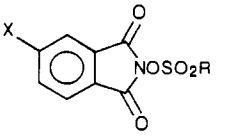
Table II. Physical Constants and Spectral Data of Compounds 1–26

compd	mp	IR	NMR	(MF) anal.
1	80–82	1735	2.46 (dd, 1 H), 2.75 (dd, 1 H), 2.97 (dd, 1 H), 3.13 (dd, 1 H), 3.27 (m, 1 H), 7.27 (m, 6 H), 7.75 (m, 4 H)	($C_{17}H_{15}NO_5S$) C, H, N
2	93–96	1735	2.43 (dd, 1 H), 2.65 (dd, 1 H), 2.83 (dd, 1 H), 3.10 (dd, 1 H), 3.30 (m, 1 H), 7.28 (m, 5 H), 7.55 (m, 2 H), 8.04 (m, 2 H)	($C_{17}H_{14}NO_5SBr$) C, H, N
3	107–9	1750	2.49 (dd, 1 H), 2.77 (dd, 1 H), 2.98 (dd, 1 H), 3.15 (dd, 1 H), 3.28 (dd, 1 H), 7.28 (m, 5 H), 7.75 (m, 4 H)	($C_{17}H_{14}NO_5SF$) C, H, N
4	90–94	1750	2.42 (s, 3 H), 2.44 (dd, 1 H), 2.69 (dd, 1 H), 2.90 (dd, 1 H), 3.16 (m, 2 H), 7.13 (d, 2 H), 7.29 (m 5 H), 7.80 (d, 2 H)	($C_{18}H_{17}NO_5S$) C, H, N
5	105–7	1735	2.38 (dd, 1 H), 2.68 (dd, 1 H), 2.85 (qd, 2 H), 3.23 (m, 1 H), 7.14 (d, 2 H), 7.23 (m, 3 H), 7.88 (m, 2 H), 8.17 (m, 2 H)	($C_{18}H_{14}NO_5SF_3$) C, H, N
6	87–88	1740	2.47 (dd, 1 H), 2.75 (dd, 1 H), 2.84 (dd, 1 H), 3.02 (dd, 1 H), 3.30 (m, 1 H), 7.23 (m, 5 H), 7.52 (m, 4 H), 7.80 (m, 3 H)	($C_{19}H_{17}NO_5S$) C, H, N
7	117–19	1735	2.34 (dd, 1 H), 2.62 (dd, 1 H), 2.73 (dd, 1 H), 2.89 (dd, 1 H), 3.18 (m, 1 H), 7.12 (m, 2 H), 7.24 (m, 3 H), 7.70 (m, 2 H), 7.81 (m, 1 H), 8.17 (m, 2 H), 8.48 (dd, 2 H)	($C_{21}H_{17}NO_5S$) C, H, N
8	99–101	1735	2.42 (dd, 1 H), 2.67 (dd, 1 H), 2.81 (dd, 1 H), 2.91 (dd, 1 H), 3.22 (m, 1 H), 7.19 (m, 5 H), 7.78 (m, 3 H), 8.15 (m, 3 H), 8.69 (s, 1 H)	($C_{21}H_{17}NO_5S$) C, H, N
9	67–70	1735	2.49 (dd, 1 H), 2.59 (dd, 1 H), 2.88 (dd, 1 H), 2.87 (dd, 1 H), 3.17 (dd, 1 H), 5.10 (s, 2 H), 7.30 (m, 6 H), 7.45 (m, 2 H), 7.60 (m, 2 H)	($C_{18}H_{17}NO_5S$) C, H, N
10	70–71	1730	2.53 (dd, 1 H), 2.86 (dd, 1 H), 3.01 (dd, 1 H), 3.32 (m, 2 H), 3.43 (s, 3 H), 7.47 (m, 5 H)	($C_{12}H_{13}NO_5S$) C, H, N
11	oil	1730	0.91 (t, 3 H), 1.45 (m, 2 H), 1.96 (m, 2 H), 2.46 (dd, 1 H), 2.72 (dd, 1 H), 2.85 (dd, 1 H), 3.19 (m, 2 H), 3.45 (m, 2 H), 7.12 (d, 2 H), 7.24 (m, 3 H)	($C_{12}H_{19}NO_5S$) C, H, N
12	58–59	1725	1.47 (d, 6 H), 2.54 (dd, 1 H), 2.80 (dd, 1 H), 2.88 (dd, 1 H), 3.13 (dd, 1 H), 3.16 (m, 1 H), 3.83 (m, 1 H), 7.25 (m, 5 H)	($C_{14}H_{17}NO_5S$) C, H, N
13	47–49	1730	0.89 (t, 3 H), 1.30 (m, 8 H), 1.48 (m, 2 H), 2.00 (m, 2 H), 2.54 (dd, 1 H), 2.79 (dd, 1 H), 2.92 (dd, 1 H), 3.26 (m, 2 H), 3.51 (m, 2 H), 7.25 (m, 5 H)	($C_{19}H_{27}NO_5S$) C, H, N
14	103–5	1740	2.51 (dd, 1 H), 2.80 (dd, 1 H), 2.97 (s, 6 H), 3.0 (dd, 1 H), 3.21 (dd, 1 H), 3.29 (m, 1 H), 7.25 (m, 5 H)	($C_{13}H_{16}N_2O_5S$) C, H, N
15	71–73	1725	0.97 (dd, 6 H), 2.13 (m, 1 H), 2.70 (dd, 1 H), 2.85 (dd, 1 H), 3.18 (m, 1 H), 3.60 (s, 3 H)	($C_8H_{13}NO_5S$) C, H, N
16	oil	1735	0.97 (dd, 6 H), 2.30 (m, 1 H), 2.55 (dd, 1 H), 2.82 (m, 2 H), 6.98 (d, 1 H), 7.5 (m, 5 H), 7.72 (d, 1 H)	($C_{15}H_{17}NO_5S$) C, H, N
17	57–58	1735	0.93 (dd, 6 H), 1.40 (m, 1 H), 1.6 (m, 2 H), 2.55 (m, 1 H), 2.9 (m, 2 H), 3.5 (s, 3 H)	($C_9H_{15}NO_5S$) C, H, N
18	oil	1740	0.93 (dd, 6 H), 1.43 (m, 1 H), 1.77 (m, 2 H), 2.75 (m, 1 H), 2.94 (m, 2 H), 6.97 (d, 1 H), 7.52 (m, 5 H), 7.76 (d, 1 H)	($C_{16}H_{19}NO_5S$) C, H, N
19	38–40	1725	0.85 (dd, 6 H), 1.21 (m, 2 H), 1.5 (m, 2 H), 1.73 (m, 1 H), 2.55 (dd, 1 H), 2.95 (m, 2 H), 3.58 (s, 3 H)	($C_{10}H_{17}NO_5S$) C, H, N
20	64–66	1745	0.85 (dd, 6 H), 1.21 (m, 2 H), 1.5 (m, 2 H), 1.7 (m, 1 H), 2.55 (dd, 1 H), 2.95 (dd, 2 H), 7.5 (m, 4 H), 7.8 (m, 3 H)	($C_{17}H_{21}NO_5S$) C, H, N
21	124–5	1740	2.64 (dd, 1 H), 2.86 (dd, 1 H), 3.05 (dd, 1 H), 3.4 (m, 2 H), 3.57 (s, 3 H), 7.45–7.73 (m, 4 H)	($C_{13}H_{12}NO_5SF_3$) C, H, N
22	88–90	1735	2.60 (dd, 1 H), 2.83 (dd, 1 H), 3.00 (dd, 1 H), 3.35 (m, 2 H), 7.67 (m, 11 H)	($C_{20}H_{16}NO_5SF_3$) C, H, N
23	62–64	1735	2.53 (dd, 1 H), 2.86 (dd, 1 H), 3.01 (dd, 1 H), 3.32 (m, 2 H), 3.43 (s, 3 H), 7.47 (m, 4 H)	($C_{13}H_{12}NO_5SF_3$) C, H, N
24	78–79	1745	2.57 (dd, 1 H), 2.84 (dd, 1 H), 3.0 (dd, 1 H), 3.4 (m, 2 H), 3.55 (s, 3 H), 7.57 (dd, 4 H)	($C_{13}H_{12}NO_5SF_3$) C, H, N
25	75–76	1735	2.56 (dd, 1 H), 2.82 (dd, 1 H), 2.90 (dd, 1 H), 3.14 (dd, 1 H), 3.3 (dd, 1 H), 3.5 (m, 3 H), 7.12 (m, 3 H), 7.65 (m, 1 H)	($C_{12}H_{12}NO_5SF$) C, H, N
26	161–2	1735	2.72 (s, 4 H), 7.48–7.80 (m, 7 H)	($C_{12}H_{11}NO_5S$) C, H, N

active site serine is followed by the formation of a reactive species that is tethered to the active site. Subsequent partitioning of the reactive species is determined by the relative magnitudes of the rate constants for inactivation (k_2), deacylation (k_d), and k_H. K_D leads to the release of the electrophilic species into the surrounding milieu, as indicated by the partial enzyme protection afforded by the addition of the external nucleophile dithiothreitol.[5]

Stability Studies. Using compound **6** as a representative member of this class of compounds, the stability of this compound in buffer solution was examined by high-pressure liquid chromatography. No change in the structural integrity of compound **6** was observed after stirring the compound for 17 h in buffer solution. Thus, these compounds embody two highly advantageous features, namely, high stability and high inhibitory activity, the former being an attribute that is not shared by many of the hitherto reported enzyme-activated inhibitors of proteolytic enzymes.

The high stability of these compounds contrasts sharply with that of the corresponding derivatives of *N*-hydroxyphthalimide (**34** and **35**). These compounds inhibit HLE



R = alkyl or phenyl. X = H (**34**) or NH_2 (**35**)

reversibly and are highly labile in HEPES buffer, pH 7.2, with the introduction of an amino group yielding inhibitors with half-lives of about 7 h.

Specificity. Compounds represented by structure I did not inhibit porcine pancreatic elastase. However, these compounds were found to be highly efficient inhibitors of chymotrypsin and cathepsin G, leading to total loss of enzymatic activity following a 5-min incubation period (inhibitor:enzyme ratio = 100).

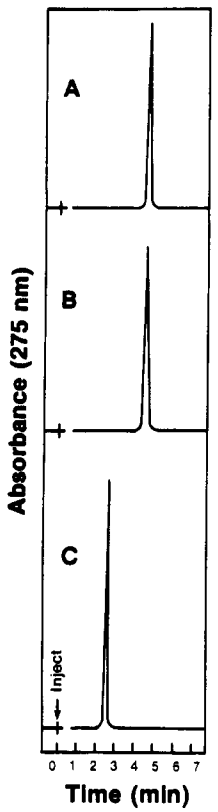


Figure 5. Stability of compound **6** in 0.1 M HEPES buffer, pH 7.2 and 1% DMSO. Aliquots were withdrawn at different time intervals and analyzed by HPLC (15 cm × 4.6 mm MC-18 column, 85% acetonitrile/water, 275 nm). A and B are traces of compound **6** immediately after mixing **6** with buffer and after 17 h, respectively (4.5 min). Trace C is authentic 3-benzyl-*N*-hydroxysuccinimide (2.5 min).

In conclusion, a series of compounds derived from 3-alkyl-*N*-hydroxysuccinimide have been synthesized and shown to be highly effective inhibitors of proteolytic enzymes. The results of in vivo studies using an animal model of emphysema and related structural and mechanistic studies will be reported at a later time.

Experimental Section

Melting points were recorded on a Mel-Temp apparatus and are uncorrected. The infrared and NMR spectra were recorded on a Perkin-Elmer 1330 infrared spectrophotometer and a Varian XL-300 NMR spectrometer, respectively. A Gilford UV/vis spectrophotometer was used in the enzyme assays and inhibition studies. Human leukocyte elastase was purchased from Elastin Products Co., St. Louis, MO. Methoxysuccinyl-Ala-Ala-Pro-Val-*p*-nitroanilide, *N*-succinyl-Ala-Ala-Pro-Phe-*p*-nitroanilide, and chymotrypsin were purchased from Sigma Chemical Co., St. Louis, MO. Human neutrophil cathepsin G was obtained from Athens Research and Technology, Inc., Athens, GA.

Representative Syntheses. 3-Isopropylsuccinic Anhydride. 2-Isopropylsuccinic acid (16.0 g, 0.1 mol), obtained by catalytic hydrogenation (10% Pd–C) and subsequent hydrolysis of the known monoester arising from the Stobbe condensation of dry acetone with diethyl succinate,[6] was mixed with 15 mL of acetic anhydride and held at reflux for 1 h. Removal of the excess anhydride in vacuo, followed by vacuum distillation of the residue, yielded 13.5 g (95%) of a colorless oil, $bp_{0.35}$ 95–98 °C. IR (neat): 1850 and 1770 (C=O) cm^{-1}. NMR ($CDCl_3$): δ 1.5 (dd, 6 H), 2.30 (m, 1 H), 2.75 (dd, 1 H), 3.0 (dd, 1 H), 3.1 (m, 1 H). Anal. ($C_7H_{10}O_3$) C, H.

3-Isopropyl-*N*-(benzyloxy)succinimide. A solution of the above anhydride (6.11 g, 0.043 mol) in 36 mL of dry toluene was brought to reflux. (Benzyloxy)amine (5.41 g, 0.044 mol) in 18 mL of toluene was then added and the refluxing was continued for 30 min. The hot solution was filtered through anhydrous sodium sulfate and the solvent was removed on a rotary evaporator. The residue was taken up in ethyl acetate (100 mL) and washed with 10% sodium bicarbonate. Removal of the solvent in vacuo gave a solid which was recrystallized from toluene/hexane to yield 8.5 g (80%) of product, mp 74–76 °C. IR (Nujol mull): 1720 cm^{-1}. NMR (DMSO-d_6): δ 0.8 (dd, 6 H), 2.1 (m, 1 H), 2.4 (dd, 1 H), 2.6 (dd, 1 H), 2.75 (m, 1 H), 5.0 (s, 2 H), 7.4 (m, 5 H). Anal. ($C_{14}H_{17}NO_3$) C, H, N.

3-Isopropyl-*N*-hydroxysuccinimide. 3-Isopropyl-*N*-(benzyloxy)succinimide (7.41 g, 0.03 mol) in 125 mL of dry THF was hydrogenolyzed with 10% Pd–C (1 g) for 3 h. Addition of Celite followed by filtration and removal of the solvent in vacuo left a colorless oil (4.5 g, 95% yield). IR (neat): 3200 (OH), 1785 and 1710 (C=O) cm^{-1}. NMR ($CDCl_3$): 0.8 (d, 3 H), 1.0 (d, 3 H), 2.3 (m, 1 H), 2.45 (dd, 1 H), 2.7 (dd, 1 H), 2.8 (m, 1 H), 7.3 (br s, 1 H).

3-Isopropyl-*N*-[(methylsulfonyl)oxy]succinimide, 15. 3-Isopropyl-*N*-hydroxysuccinimide (0.79 g, 5 mmol) in 8 mL of dry toluene was treated with dry pyridine (1.86 g, 10 mmol) and freshly distilled methanesulfonyl chloride (0.57 g, 5 mmol). After the reaction was stirred overnight at room temperature in an inert atmosphere, 50 mL of ethyl acetate and 20 mL of dilute HCl were added. The two layers were separated, and the aqueous layer was extracted once with ethyl acetate (40 mL). The combined organic extracts were dried over anhydrous sodium sulfate. Filtration and removal of the solvent in vacuo left a solid which was triturated with hexane and collected by suction filtration (0.92 g, 78% yield), mp 71–73 °C. IR (Nujol mull): 1730 cm^{-1}. NMR (DMSO-d_6): 1.10 (dd, 6 H), 2.3 (m, 1 H), 2.8 (dd, 1 H), 2.95 (dd, 1 H), 3.1 (m, 1 H), 3.75 (s, 3 H). Anal. ($C_8H_{13}NSO_5$) C, H, N.

The rest of the compounds listed in Table II were prepared in an analogous manner.

Enzyme Assays and Inhibition Studies. Human leukocyte elastase was assayed by mixing 10 μL of a 2.23×10^{-5} M enzyme solution (in 0.05 M sodium acetate buffer, pH 5.5), 10 μL of DMSO, and 980 μL of HEPES buffer, pH 7.2, in a thermostated test tube. A 100-μL aliquot was transferred to a thermostated cuvette containing 880 μL of HEPES buffer and 20 μL of a 3.06×10^{-3} M solution of methoxysuccinyl-Ala-Ala-Pro-Val-*p*-nitroanilide and the change in absorbance was monitored at 410 nm for 2 min.

In a typical inhibition run, 20 μL of a 2.23×10^{-2} M solution of the inhibitor in DMSO was mixed with 10 μL of 2.23×10^{-5} M enzyme solution and 970 μL of HEPES buffer, pH 7.2, and placed in a constant temperature bath. 100-μL aliquots were withdrawn at different time intervals and transferred to a cuvette containing substrate, 20 μL of a 3.06×10^{-3} M solution, and HEPES buffer (880 μL). After incubating for 30 s, the absorbance was monitored at 410 nm for 2 min. The pseudo-first-order inactivation rate constants (k_{obs}) were obtained from plots of ln (v_t/v_0) vs t and expressed in terms of the apparent second-order inactivation rate constants $k_{obs}/[I]$, M^{-1} s^{-1}. These are the average of two determinations.

The k_2/K_i values for inhibitors **6** and **10** were computed by determining the k_{obs} values at various inhibitor concentrations and replotting the data according to the equation shown below:

$$1/k_{obs} = (K_i/k_2)(1/[I]) + 1/k_2$$

Chymotrypsin was assayed as described by Abeles[16] and cathepsin G was assayed by using a procedure similar to the one used for HLE.

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