

gates 1 and 2 display different growth curves compared to Loracarbef seems to rule out the possibility that siderophore hydrolysis is occurring and releasing Loracarbef prior to cellular absorption. Use of two isogenic *Escherichia coli* strains (RW193, *fhuA* positive; AN193, *fhuA* negative) differing only in the presence or absence of the hydroxamate ferrichrome receptor system suggests uptake by the iron-transport system.¹⁴ In any event, this greater killing effect, use of isogenic strains, and other evidence¹⁴ suggest that it may be entirely possible to smuggle other toxic moieties into microbes via the ferrichrome iron transport system. The possibility of expanding this mode of drug delivery is currently being investigated.

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E. Kurt Dolence, Albert A. Minnick, Marvin J. Miller*

Department of Chemistry
University of Notre Dame
Notre Dame, Indiana 46556

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Articles

Design and Synthesis of 4*H*-3,1-Benzoxazin-4-ones as Potent Alternate Substrate Inhibitors of Human Leukocyte Elastase¹

Allen Krantz,* Robin W. Spencer,[†] Tim F. Tam, Teng Jiam Liak, Leslie J. Copp, Everton M. Thomas, and Steven P. Rafferty

Syntex Research (Canada), 2100 Syntex Court, Mississauga, Ontario L5N 3X4, Canada. Received March 28, 1989

4*H*-3,1-Benzoxazin-4-ones are alternate substrate inhibitors of the serine proteinase human leukocyte elastase (HL elastase) and form acyl enzyme intermediates during enzyme catalysis. We have synthesized a large variety of benzoxazinones using specific methods that have been adapted to achieve the pattern of ring substitution dictated by theoretical considerations. The results of the inhibition of HL elastase by 175 benzoxazinones are reported herein with reference to hydrophobicity constants *D*, alkaline hydrolysis rates k_{OH^-} , inhibition constants K_i , and their component acylation and deacylation rate constants, k_{on} and k_{off} , respectively. The ranges for the compounds are considerable; alkaline hydrolysis rates and k_{on} span 6, k_{off} covers 5, and K_i spans 8 orders of magnitude. Multiple regression on this large data set has been used to isolate the contributions of electronic and steric effects, as well as other factors specific to compound stability and elastase inhibition. Essentially, a simple electronic parameter is sufficient to account for almost all the variance in the alkaline hydrolysis data, indicating that electronic factors are the major determinants of this type of benzoxazinone reactivity. Factors that significantly enhance the potency of benzoxazinones I are R_5 alkyl groups and electron withdrawal by R_2 . Bulk in R_7 and R_8 and compound hydrophobicity are not significant, but substitution in R_6 is highly unfavorable as are substituents linked via carbon to C_2 . The physicochemical factors that underlie these trends in K_i are further analyzed in terms of equations that describe k_{on} and k_{off} . A conclusion that emerges is that chemically stable, potent benzoxazinone inhibitors of HL elastase with inhibition constants in the nanomolar range can be designed with (1) R_5 alkyl groups to inhibit enzyme-catalyzed deacylation, (2) small alkyl substituents linked via heteroatoms to C_2 to enhance acylation and limit deacylation rates, and (3) strongly electron-donating groups at C_7 to stabilize the oxazinone ring to nucleophilic attack. Thus, 2-(isopropylamino)-5-*n*-propyl-7-(dimethylamino)benzoxazinone **95** has $k_{OH^-} = 0.01 \text{ M}^{-1} \text{ s}^{-1}$, which extrapolates to a half-life at pH 7.4 of over 8.5 years, and 2-ethoxy-5-ethylbenzoxazinone **38** has $K_i = 42 \text{ pM}$.

Serine proteinases are attractive targets for medicinal chemists² engaged in the design of enzyme inhibitors since the catalytic mechanisms of this class of enzymes have been extensively investigated over the past few decades.³ A rather compelling picture of enzyme catalyzed hydrolysis of amides and esters has emerged featuring formation and breakdown of tetrahedral and acyl enzyme intermediates.³ This mechanistic framework, centered on carbonyl chemistry, has provided the basis for the design of a growing number of active site directed reagents conceived as affinity labels,⁴ transition-state analogues,⁵ and suicide inhibitors.⁶ In addition to the broad range of compounds that can serve as substrates for serine proteinases and form acyl enzyme ester intermediates, a great deal is known from physical organic chemistry about ester reactivity and

the means of controlling it,⁷ which makes the acyl enzyme a natural focal point for rational drug design.

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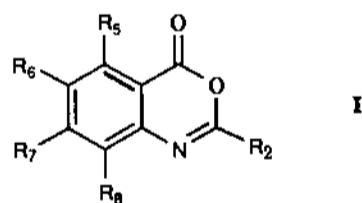
[†] Present address: Pfizer Central Research, Eastern Point Road, Groton, CT 06340.

Table I. 2-Carbabenzoxazinones^a

no.	R ₂	R ₅	R ₆	R ₇	R ₈	log D	log k _{OH⁻}	pK _i	log k _{on}	log k _{off}
1	H	Me	H	H	H		1.38	6.66	2.64	-4.02
2	Me	H	H	H	H	1.3	1.69	5.32	3.94	-1.38
3	Me	Me	H	H	H		0.85	6.00	3.48	-2.52
4	Me	H	Me	H	H	1.8	1.36	4.46		
5	Me	H	H	H	Me	2.1	1.18	5.13	3.50 ^b	-1.63
6	Me	Et	H	H	H		0.73	6.92	4.81	-2.11
7	Me	OMe	H	H	H		0.83	4.84	3.70 ^c	
8	Me	H	OMe	OMe	H		0.53	3.08 ^d		
9	Me	H	H	H	N		2.61	5.10	3.30 ^e	
10	Me	H	-CH=CHCH=CH-		H	2.8	2.19	5.14	4.20 ^e	
11	nPr	H	H	H	H			5.54	3.62	
12	tBu	H	H	H	H		1.38	5.05	4.00 ^e	
13	tBu	H	H	H	Me	4.0	0.82	4.26	3.70 ^e	
14	iBu	H	H	H	H			5.59	4.30 ^e	
15	PhCH ₂	H	H	H	H			5.44 ^e		
16	Ph	H	H	H	H		1.79	5.02	4.00 ^e	
17	3-NO ₂ Ph	H	H	H	H		1.82	5.36		
18	PhCH=CH	H	H	H	H	3.9	1.57	5.09	2.59	-2.50
19	2-furyl	H	H	H	H		1.94	5.75		
20	CH ₂ NHCOCH ₃	H	H	H	H		1.86	5.12	2.24	-2.88
21	Z-L-Ala	H	H	H	H		1.76	6.12	4.04	-2.08
22	Z-L-Pro	H	H	H	H		1.69	5.95	2.95	-3.00
23	CH ₂ Br	H	H	H	H		2.06	6.02		
24	CH ₂ Br	Me	H	H	H		1.58	6.82	4.44	-2.38
25	CF ₃	H	H	H	H	1.9	3.52	6.77	5.00 ^c	
26	CF ₃	Me	H	H	H	2.9	3.14	7.54	5.11	-2.43
27	CF ₃	H	Me	H	H		3.23	6.29		
28	CF ₈	H	H	H	Me		3.09	6.27	4.00 ^c	
29	CF ₃	OMe	H	H	H		3.54	6.38	5.30 ^c	
30	C ₂ F ₅	H	H	H	H			6.96 ^e		
31	nC ₃ F ₇	H	H	H	H		3.27	7.04	4.00 ^c	
32	nC ₈ F ₇	Me	H	H	H	2.9	2.75	7.51 ^d	3.41	-4.01

^aData presentation: alkaline hydrolysis as log k_{OH⁻} (M⁻¹ s⁻¹), elastase inhibition as pK_i = -log K_i (M), acylation rate as log k_{on} (M⁻¹ s⁻¹), deacylation rate as log k_{off} (s⁻¹). ^bDetermined at a single concentration. ^cLower limit. ^dStandard error > 20%. ^eData from ref 10b.

In this article we report structure-activity relationships (SAR) between variously substituted 4H-3,1-benzoxazin-4-ones (I) and human leukocyte elastase (EC 3.4.21.37, HLE),⁸ a serine proteinase that has attracted considerable interest as a putative agent of tissue destruction in the pathogenesis of several diseases.⁹ A number of benzoxa-



zinones of low chemical stability¹⁰ have been reported to

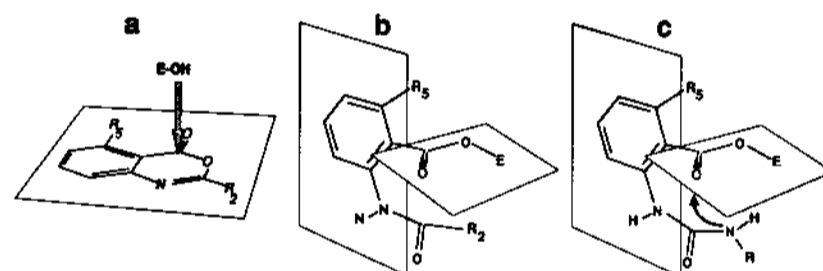
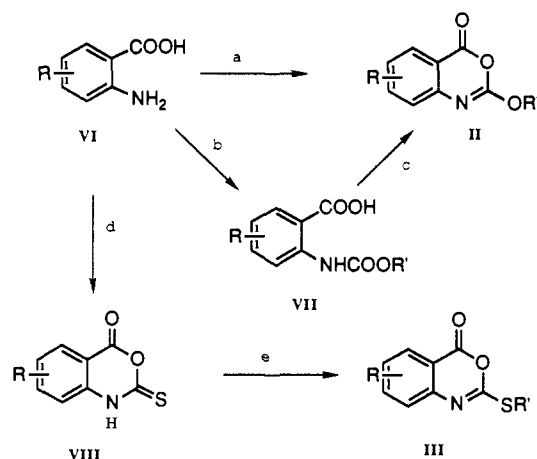


Figure 1. (a) Steric hindrance to attack at the benzoxazinone carbonyl should not be severe with simple alkyl substituents R₅; E-OH = enzyme. (b) Upon ring opening the scissile carbonyl twists out of the plane of the benzene ring and is shielded by the flanking ortho substituents from attack by external nucleophiles. (c) Deacylation by facile cyclization of 2-ureidobenzoyl elastases to give quinazolinone and regenerate free enzyme is profoundly inhibited by simple branched alkyl groups, R = iPr or sBu.

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be alternate substrate inhibitors that proceed to products via acyl enzyme intermediates.^{10c-e} We envisaged that the chemical stability and potency of benzoxazinones could be controlled by judicious choice of substituents which are known to influence carbonyl reactivity through well-established electronic and steric effects.¹¹

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Scheme I^a

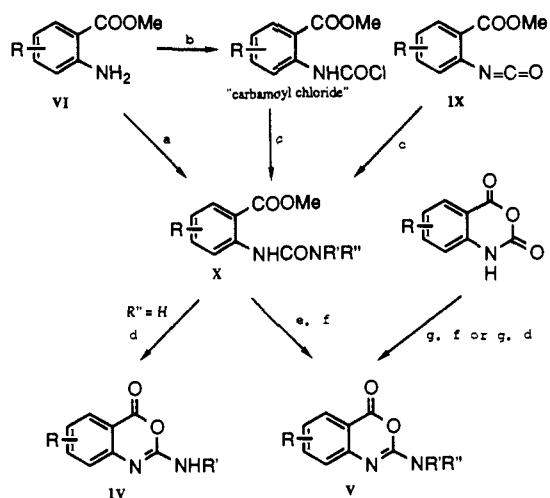
^a (a) R'OCOCI, pyridine; (b) R'OCOCI; (c) EDCI, CH₂Cl₂, or SOCl₂, ether; (d) CCl₄; (e) K₂CO₃, acetone, R'X. Method A: a. Method B: b, c. Method C: d, e.

Two major premises underlie our strategy for the design of benzoxazinone inhibitors of serine proteinases. The first is that conventional electron-donating groups can be used to enhance the stability of benzoxazinones by modulating the reactivity of the oxazinone carbonyl to nucleophilic attack. The second, more subtle point is that small alkyl groups, if properly deployed on the benzene ring¹² and side chain of benzoxazinones, can profoundly inhibit deacylation rates through steric effects on acyl enzyme intermediates (Figure 1). The net result of applying these principles has led to the development of hydrolytically stable benzoxazinones with potency in the nanomolar range.

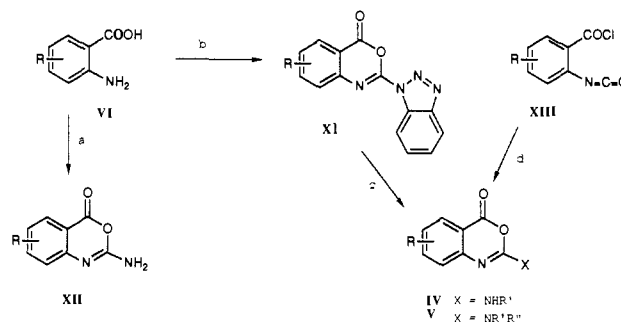
In line with our emphasis on mechanistic considerations and to determine the extent to which the data reveal patterns consistent with known chemical principles, we have attempted to evaluate quantitatively the contribution of physicochemical factors germane to benzoxazinone inhibition of human leukocyte elastase. Specifically, multiple regression on a large data set has been used to isolate the contributions of (1) electron donating and withdrawal, (2) steric effects at position 5, and (3) other factors specific to compound stability or elastase inhibition. Where possible, these effects are separately evaluated for enzyme acylation and deacylation. In addition, we have examined several trends in subsets of compounds which regression does not address.

Chemistry

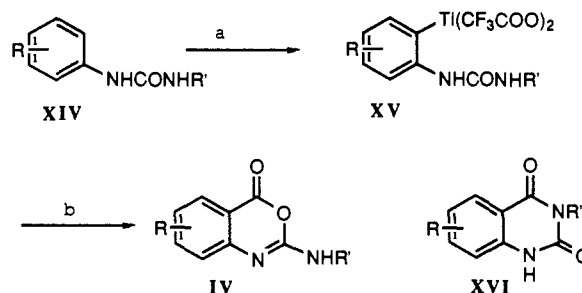
A variety of 4*H*-3,1-benzoxazin-4-one analogues has been synthesized and tested against HLE (Tables I-III). The general methods leading to the preparation of 2-alkyl derivatives (1-32), 2-alkoxy derivatives II, 2-alkylthio derivatives III, 2-alkylamino derivatives IV and 2-dialkylamino derivatives V of 4*H*-3,1-benzoxazin-4-ones are described below. The 2-alkyl-4*H*-3,1-benzoxazin-4-ones (1-32) were prepared by standard published methods.^{10b,c,13} The syntheses of 2-alkoxy-4*H*-3,1-benzoxazin-4-ones II (Scheme I) were generally accomplished by the reaction of 3.5 equiv of alkyl chloroformate with the appropriate, substituted 2-aminobenzoic acid VI in pyridine as a solvent (method A).¹⁴ Alternatively, reaction of 2-[(alkoxy-

Scheme II^a

^a (a) R'' = H, R'-N=C=O; (b) CCl₃OCOCI, R'R''NH, THF, or DMF; (c) R'R''NH; (d) concentrated H₂SO₄; (e) NaOH; (f) EDCI, CH₂Cl₂, or THF or DMF; (g) R'R''NH, NaOH. Method D: a, d or c, d. Method E: b, c, d. Method F: c, e, f or g, f.

Scheme III^a

^a (a) CNBr, NaOH; (b) benzotriazolylcarboxylic acid chloride, benzene; (c) NH₂R', pyr, CH₂Cl₂; (d) R'R''NH, THF. Method G: a. Method H: b, c. Method I: d.

Scheme IV^a

^a (a) Ti(CF₃COO)₃, CF₃COOH, THF; (b) cat. PdCl₂, LiCl, MgO, THF, 1 atm CO. Method J: a, b.

carbonyl)amino]benzoic acid VII with a dehydrating agent such as thionyl chloride or EDCI in methylene chloride or tetrahydrofuran (method B)¹⁵ produced II. Compound VII was in turn prepared from alkyl chloroformate and benzoic acid VI. Although method A has the obvious advantage over method B because it is a one-pot reaction process, method B is useful in cases where the corresponding alkyl chloroformate is either expensive or difficult to prepare. The syntheses of certain analogues of compound II, such as compounds 36, 39-41, and 44-45, have

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Table II. 2-Oxy- and 2-Thiobenzoxazinones^a

no.	R ₂	R ₅	R ₆	R ₇	R ₈	method	molecular formula	mp, °C	% yield	log <i>D</i>	log <i>k</i> _{OH} ⁻	HLE		
												p <i>K</i> ₁	log <i>k</i> _{on}	log <i>k</i> _{off}
33	MeO	Me	H	H	H	B	C ₁₀ H ₉ NO ₃	130-131	98			8.00 ^b	4.54	
34	EtO	H	H	H	H	A	C ₁₀ H ₉ NO ₃	88-89	89		1.91	8.19	5.20	-2.99
35	EtO	Me	H	H	H	A	C ₁₁ H ₁₁ NO ₃	104-105	83		1.31	9.94	5.01	-4.93
36	EtO	Me	H	MeO	H	c						9.40	4.66	-4.74
37	EtO	Me	H	H	Me	A	C ₁₂ H ₁₃ NO ₃	100-102	50			10.02 ^d	4.81	-5.21
38	EtO	Et	H	H	H	A	C ₁₂ H ₁₃ NO ₃	89-91	69		1.17	10.37	5.53	-4.84
39	EtO	Et	H	NH ₂	H	e				2.7	-0.54	8.00 ^b	3.81	
40	EtO	CH ₂ Br	H	H	H	e					1.93	10.17	6.05	-4.12
41	EtO	CHBr ₂	H	H	H	e					2.50	9.33	7.00 ^f	-2.33
42	EtO	Pr	H	H	H	e					1.17	9.64	5.49	-4.15
43	EtO	i-Pr	H	H	H	A	C ₁₃ H ₁₅ NO ₃ · 1/2H ₂ O	55-56	63		0.96 ^g	10.15	5.89	-4.26
44	EtO	CH=CHMe	H	H	H	e					1.41	9.72	5.54	-4.18
45	EtO	H	H	NH ₂	H	e	C ₁₀ H ₁₀ N ₂ O ₃	185-187	45		0.20	8.24	3.88	-4.36
46	EtO	H	H	NMe ₂	H	B	C ₁₂ H ₁₄ N ₂ O ₃	196-198	75		-0.10	8.31	3.46	-4.85
47	EtO	H	H	NHCOOEt	H	A	C ₁₃ H ₁₄ N ₂ O ₅	191-193	73		1.46	7.75	4.78	-2.97
48	iBuO	H	H	H	H	A	C ₁₂ H ₁₃ NO ₃	57-58	70		1.83	7.94	4.69	-3.25
49	iBuO	Me	H	H	H	A	C ₁₃ H ₁₅ NO ₃	41-43	65			8.80	3.85	-4.95
50	BnO	H	H	H	H	A	C ₁₆ H ₁₁ NO ₃	112-113	12		1.99	7.25	4.30	-2.95
51	BnO	H	OMe	OMe	H	B	C ₁₇ H ₁₅ NO ₅	152-154	54		0.97	6.03	2.82	-3.21
52	MeS	H	H	H	H	C	C ₉ H ₉ NO ₂ S	108-109	80		2.16	7.68	5.54	-2.14
53	MeS	Me	H	H	H	C	C ₁₀ H ₉ NO ₂ S	127-128	60		1.90	8.85	5.40	-3.45
54	EtS	H	H	H	H	C	C ₁₀ H ₉ NO ₂ S	57-58	57	3.0	2.19	8.09	6.30 ^b	
55	EtS	Me	H	H	H	C	C ₁₁ H ₁₁ NO ₂ S	65-66	93	3.6	1.58	9.03	6.18 ^d	-2.85
56	EtS	Et	H	H	H	C	C ₁₂ H ₁₃ NO ₂ S	60-61	54		1.37		5.41	
57	EtS	H	Me	H	H	C	C ₁₁ H ₁₁ NO ₂ S	90-91	84		1.95	6.77	6.00 ^b	
58	EtS	H	HNAc	H	H	C	C ₁₂ H ₁₂ N ₂ O ₂ S	194-195	80		2.24	5.84	4.00 ^b	
59	EtS	H	OMe	OMe	H	C	C ₁₂ H ₁₃ NO ₂ S	121-122	85		1.10	5.92	5.00 ^b	
60	EtS	H	NMe ₂	H	H	C	C ₁₂ H ₁₄ N ₂ O ₂ S	106-108	61		1.76	6.47	5.70 ^b	
61	EtS	H	H	Et	H	C	C ₁₂ H ₁₃ NO ₂ S	39-40	72		1.66	7.68	6.30 ^b	
62	EtS	H	H	H	Me	C	C ₁₁ H ₁₁ NO ₂ S	79-80	83		2.01	7.55	6.30 ^b	
63	EtS	H	-CH=CH-CH=CH-	H	H	C	C ₁₄ H ₁₁ NO ₂ S	121-122	80		2.54	7.14	6.30 ^b	
64	iPrS	H	H	H	H	C	C ₁₁ H ₁₁ NO ₂ S	62-63	33			8.11	6.46	-1.65
65	BnS	H	H	H	H	C	C ₁₆ H ₁₁ NO ₂ S	99-100	52	3.6	2.33	7.40	4.72	-2.88
66	BnS	H	H	H	Me	C	C ₁₆ H ₁₃ NO ₂ S	102-103	73			6.96	3.61	-3.35
67	BnS	H	OMe	OMe	H	C	C ₁₇ H ₁₆ NO ₂ S	135-136	82		0.96	5.67	3.76	-1.91
68	BnS	H	-CH=CH-CH=CH-	H	H	C	C ₁₉ H ₁₃ NO ₂ S	176-177	81			6.59	4.14	-2.45
69	SCH ₂ COOEt	H	H	H	H	C	C ₁₂ H ₁₁ NO ₄ S	83-84	68		2.93	7.14	4.75 ^f	-2.39
70	SCH ₂ CH=CHPh	H	OMe	OMe	H	C	C ₁₈ H ₁₇ NO ₄ S	129-130	79			5.16	4.70 ^b	
71	3-indolyl-CH ₂ S	H	OMe	OMe	H	C	C ₁₉ H ₁₆ N ₂ O ₄ S	195 dec	42			4.89	3.29	-1.60
72	4-imd-CH ₂ S	H	OMe	OMe	H	C	C ₁₄ H ₁₃ N ₃ O ₄ S	138-142	72		1.31	5.96	3.84	-2.12

^a Data presentation: alkaline hydrolysis as log *k*_{OH}⁻ (M⁻¹ s⁻¹), elastase inhibition as p*K*₁ = -log *K*(M), acylation rate as log *k*_{on} (M⁻¹ s⁻¹), deacylation rate as log *k*_{off} (s⁻¹). ^b Lower limit. ^c Reference 16. ^d Standard error >20%. ^e Reference 14. ^f Determined at a single concentration.

been described in recent publications^{14,16} and therefore their preparation will not be detailed here. 2-(Alkylthio)-4*H*-3,1-benzoxazin-4-ones III (52-72) were generally prepared by the S-alkylation of 1,2-dihydro-2-thio-4*H*-benzoxazin-4-one VIII with potassium carbonate and alkyl halide in acetone at room temperature (method C).¹⁷

Several synthetic routes (methods D-J) for the preparation of 2-amino-4*H*-3,1-benzoxazin-4-ones derivatives IV and V have been employed and are outlined in Schemes II-IV. In methods D and E^{18,19} benzoxazinones IV and V were formed by cyclizing the ureidobenzoate X in concentrated sulphuric acid. Compound X was in turn prepared from the condensation of the methyl 2-aminobenzoate VIa and an alkyl isocyanate or from isocyanate IX and R'NH₂. Where the isocyanate was unavailable commercially, the amino component VIa was converted with diphosgene to the corresponding carbamoyl chloride (Scheme II), which without isolation was reacted with the appropriate amine to yield urea X. Compounds 112-118 were synthesized by this route. The dialkylamino analogues V could also be prepared by hydrolyzing ester X to the free acid followed by cyclodehydration using method F.¹⁹ Method F is particularly useful in the preparation of

certain peptido analogues of compound V (169-174).

2-Aminobenzoxazinones XII (73-75) were available from the reaction of the corresponding 2-aminobenzoic acids with cyanogen bromide in sodium hydroxide (method G).²⁰ The products were insoluble in aqueous media and could easily be purified by filtration, column chromatography, and recrystallization. We have also prepared specific benzoxazinones by other literature methods as outlined in Scheme III. 2-Benzotriazolyl-4*H*-3,1-benzoxazin-4-ones XI were reacted with simple primary or secondary amines to give compounds IV and V via an addition-elimination reaction sequence (method H).^{19,21} An alternate method for the preparation of (dialkylamino)benzoxazinone V employed the procedure of Sayigh and co-workers.²² This involved the reaction of a secondary amine with commercially available 2-isocyanatobenzoyl chloride XIII to give benzoxazinones V in a one-pot reaction process (method I).

Certain 2-(alkylamino)- and 2-peptidylbenzoxazinones (86-87, 96, 103, 105, 157-158) were prepared by the thallation-carbonylation of the corresponding phenylurea (Scheme IV, method J).^{19,23} This method is particularly

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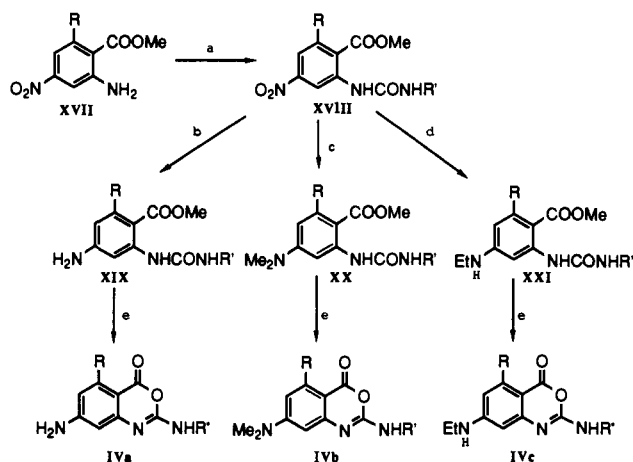
Table III. 2-Aminobenzoxazinones^a

no.	R ₂	R ₅	R ₇	R ₈	method	molecular formula	mp, °C	%	yield	log D	log k _{OH}	HLE		
												pK _i	log k _{on}	log k _{off}
73	NH ₂	H	H	H	b	C ₈ H ₈ N ₂ O ₂	197-199				0.53	4.20	2.91 ^c	-1.29
74	NH ₂	Me	H	H	G	C ₉ H ₈ N ₂ O ₂	218-220	90			-0.05			-0.33
75	NH ₂	Et	H	H	G	C ₁₀ H ₁₀ N ₂ O ₂	224-226	54			-0.08	5.29	1.18	-4.11
76	MeNH	H	H	H	d	C ₉ H ₈ N ₂ O ₂	203-204		1.7	1.38	5.16	3.75 ^c	-1.41	
77	MeNH	Me	H	H	D ^e	C ₁₀ H ₁₀ N ₂ O ₂	192-193	91	2.3	0.54	4.51	3.69 ^c	-0.82	
78	MeNH	H	H	Me	D	C ₁₀ H ₁₀ N ₂ O ₂	179-180	76			4.01			
79	EtNH	H	H	H	d	C ₁₀ H ₁₀ N ₂ O ₂	169-171				7.28	5.81	-1.47	
80	EtNH	Me	H	H	D	C ₁₁ H ₁₂ N ₂ O ₂	170-172	71			7.47	4.72	-2.75	
81	nPrNH	H	H	H	D ^e	C ₁₁ H ₁₂ N ₂ O ₂	170-172	68	2.4		7.04	5.65	-1.39	
82	nPrNH	Me	H	H	D	C ₁₂ H ₁₄ N ₂ O ₂	163-164	51			8.22	4.75	-3.47	
83	iPrNH	H	H	H	D ^e	C ₁₁ H ₁₂ N ₂ O ₂	145-146	70	2.4	1.21	7.28	4.77	-2.51	
84	iPrNH	Me	H	H	D ^e	C ₁₂ H ₁₄ N ₂ O ₂	197-199	49	3.0	0.41	8.82	4.07	-4.75	
85	iPrNH	Et	H	H	D ^e	C ₁₃ H ₁₆ N ₂ O ₂	138-139	39		0.31	9.03	4.85	-4.18	
86	iPrNH	Me	Me	H	J ^e	C ₁₃ H ₁₆ N ₂ O ₂	215-217	40		-0.23	8.23	3.41	-4.82	
87	iPrNH	Me	H	Me	J ^e	C ₁₃ H ₁₆ N ₂ O ₂	206-208	45		0.20	8.18	3.12	-5.06	
88	iPrNH	Me	OMe	H	f	C ₁₃ H ₁₆ N ₂ O ₃	186-188			-0.42	7.42	4.32	-3.10	
89	iPrNH	Et	OnPr	H	f	C ₁₆ H ₂₂ N ₂ O ₃					7.67	4.63	-3.04	
90	iPrNH	Me	NH ₂	H	e	C ₁₂ H ₁₅ N ₃ O ₂	>300			-1.09	7.11	2.36	-4.75	
91	iPrNH	Me	N(Me) ₂	H	g	C ₁₄ H ₁₉ N ₃ O ₂				-1.61	5.90	2.01	-3.89	
92	iPrNH	Et	OMe	H	f	C ₁₄ H ₁₈ N ₂ O ₃	166-167			-0.60	7.96	5.35	-2.61	
93	iPrNH	Et	NH ₂	H	e	C ₁₃ H ₁₇ N ₃ O ₂	>250		1.6	-1.26	7.77	3.22	-4.55	
94	iPrNH	Et	N(Me) ₂	H	g	C ₁₅ H ₂₁ N ₃ O ₂	207-208			-1.88	6.96	3.17	-3.79	
95	iPrNH	nPr	N(Me) ₂	H	g	C ₁₆ H ₂₃ N ₃ O ₂	174-176			-2.00	6.55 ^h	2.81	-3.74	
96	iPrNH	H	Et	H	D ^e	C ₁₃ H ₁₆ N ₂ O ₂	149-150	60	3.2	0.69	6.74	4.23	-2.51	
97	iPrNH	H	NH ₂	H	e	C ₁₁ H ₁₃ N ₃ O ₂	144-145			-0.46	6.11	2.85 ^h	-3.26	
98	iPrNH	H	NHEt	H	I	C ₁₃ H ₁₇ N ₃ O ₂	175-176		2.4		6.16	2.65	-3.51	
99	iPrNH	H	NHAc	H	e	C ₁₃ H ₁₅ N ₃ O ₃	151-153		1.2	0.92	6.83	4.35	-2.48	
100	iPrNH	H	N(Me) ₂	H	g	C ₁₃ H ₁₇ N ₃ O ₂	204-206			-0.74	6.32	3.00	-3.32	
101	iPrNH	H	N(Et) ₂	H	g	C ₁₅ H ₂₁ N ₃ O ₂	182-184			-0.99	5.26	2.48	-2.78	
102	iPrNH	H	NHCONHnPr	H	e	C ₁₅ H ₂₀ N ₄ O ₃	238-240			0.39	5.87	4.10	-1.77	
103	iPrNH	H	Me	Me	J ^e	C ₁₃ H ₁₆ N ₂ O ₂	203-206		3.8	0.46	6.15	3.85	-2.30	
104	iPrNH	H	H	Me	D	C ₁₂ H ₁₄ N ₂ O ₂	185-187	45		0.69	6.23 ⁱ			
105	iPrNH	H	H	Et	J	C ₁₃ H ₁₆ N ₂ O ₂	148-149	35			6.00	4.38 ^h	-1.62	
106	nBuNH	H	H	H	d	C ₁₂ H ₁₄ N ₂ O ₂	129-131	78	3.1	1.09	7.08	4.82	-2.26	
107	nBuNH	Me	H	H	D ^e	C ₁₃ H ₁₆ N ₂ O ₂	130-132	72		0.41	7.64	3.88	-3.76	
108	nBuNH	Et	H	H	D ^e	C ₁₄ H ₁₈ N ₂ O ₂	136-137	45	3.8	0.38	8.38	5.11	-3.27	
109	nBuNH	H	NH ₂	H	e	C ₁₂ H ₁₅ N ₃ O ₂			1.7	-0.44	6.19	3.45	-2.74	
110	nBuNH	H	H	Me	D ^e	C ₁₃ H ₁₆ N ₂ O ₂	107-109	68		0.86	6.39	3.85 ^h	-2.54	
111	nPentNH	Me	H	H	E	C ₁₄ H ₁₉ N ₂ O ₂	98-99	44			5.47	2.62	-2.85	
112	(1-MeBu)NH	Me	H	H	E	C ₁₄ H ₁₉ N ₂ O ₂	109-110	86			6.13	3.70	-2.43	
113	(2-MeBu)NH	Me	H	H	E	C ₁₄ H ₁₈ N ₂ O ₂	109-110	30			6.18	2.05	-4.13	
114	(3-MeBu)NH	Me	H	H	E	C ₁₄ H ₁₈ N ₂ O ₂	159-160	31			5.81	2.52	-3.29	
115	(1,1-diMePr)NH	H	H	H	E	C ₁₃ H ₁₆ N ₂ O ₂	90-91	50			6.81	3.19	-3.62	
116	cPentNH	Me	H	H	E	C ₁₄ H ₁₈ N ₂ O ₂	174-176	48			6.70	2.61	-4.09	
117	(2,2-diMe-Pr)NH	Me	H	H	E	C ₁₄ H ₁₈ N ₂ O ₂	159-160	21			6.62	1.88 ^j	-4.74	
118	(1-EtPr)NH	Me	H	H	E	C ₁₄ H ₁₈ N ₂ O ₂	135-137	62			6.14	3.89	-2.25	
119	sBuNH	H	H	H	D ^e	C ₁₂ H ₁₄ N ₂ O ₂	122-123	48		0.92	7.36	5.38	-1.98	
120	nHxNH	H	H	H	D ^e	C ₁₄ H ₁₈ N ₂ O ₂	133-135	86	3.8	0.96	5.44 ^h	3.18	-2.26	
121	cHxNH	H	H	H	d	C ₁₄ H ₁₈ N ₂ O ₂	208-210		3.5	0.89	6.95 ^j	3.44 ^h	-3.51	
122	OctNH	H	H	H	D ^e	C ₁₆ H ₂₂ N ₂ O ₂	117-120	75		0.03	4.50 ^j	3.13 ^j	-1.37	
123	PhNH	H	H	H	d	C ₁₄ H ₁₀ N ₂ O ₂	190-192		3.5	1.18	4.96 ⁱ			
124	PhNH	Me	H	H	D ^e	C ₁₅ H ₁₂ N ₂ O ₂	222-223	50			6.96 ⁱ	3.03	-3.93	
125	PhNH	OMe	H	H	D	C ₁₅ H ₁₂ N ₂ O ₃	218-220	97	3.1	0.41	3.80 ^{i,k}			
126	PhCH ₂ NH	H	H	H	D	C ₁₅ H ₁₂ N ₂ O ₂	178-179	70	2.8	1.12	6.02 ^h	3.18	-2.84	
127	(3-NH ₂ Ph)CH ₂ NH	H	H	H	e	C ₁₅ H ₁₃ N ₃ O ₂	205-206	74		1.18	5.80	3.27	-2.53	
128	(4-NH ₂ Ph)CH ₂ NH	H	H	H	e	C ₁₅ H ₁₃ N ₃ O ₂	220-221	68	2.0	1.36	5.91	3.17	-2.74	
129	PhCH ₂ NH	H	H	Me	D ^e	C ₁₆ H ₁₄ N ₂ O ₂	164-165	40		0.92	5.70	2.49	-3.21	
130	(2-MePh)CH ₂ NH	H	H	H	D	C ₁₆ H ₁₄ N ₂ O ₂	208-209	65		1.01	5.55			
131	(4-NMe ₂ Ph)CH ₂ NH	H	H	H	D	C ₁₇ H ₁₇ N ₃ O ₂	172-174			1.81	5.70 ⁱ	3.44	-2.26	
132	PhEtNH	H	H	H	D	C ₁₆ H ₁₄ N ₂ O ₂	163-164	58	3.2	1.15	5.77	3.05	-2.72	
133	N(Et) ₂	H	H	H	I	C ₁₂ H ₁₄ N ₂ O ₂	46-47	57			6.43	3.51	-2.92	
134	N(Et) ₂	Me	H	Me	E	C ₁₄ H ₁₈ N ₂ O ₂	62-63	40			6.98	2.12	-4.86	
135	N(iPr) ₂	H	H	H	I	C ₁₄ H ₁₈ N ₂ O ₂	84-86	81	3.7	0.79	5.59	3.01	-2.58	
136	N(iPr) ₂	Me	H	H	E	C ₁₅ H ₂₀ N ₂ O ₂	143-144	40			6.40 ^h	1.99	-4.41	
137	N(iPr) ₂	Et	H	H	E	C ₁₆ H ₂₂ N ₂ O ₂	87-88	33			7.15	3.05	-4.10	
138	N(nBu) ₂	H	H	H	I	C ₁₈ H ₂₂ N ₂ O ₂	oil	76		-0.57	5.34 ⁱ	2.43 ^h	-2.91	
139	N(nBu) ₂	Me	H	H	E	C ₁₇ H ₂₄ N ₂ O ₂	oil	85			6.44	1.82	-4.62	
140	N(nBu) ₂	Et	H	H	E	C ₁₈ H ₂₆ N ₂ O ₂	oil	70			6.75	3.02	-3.73	
141	N(iBu) ₂	H	H	H	I	C ₁₆ H ₂₂ N ₂ O ₂	48-49	68		0.68	5.19 ⁱ	2.18 ^h	-3.01	
142	1-pyrrolidinyl	H	H	H	I	C ₁₂ H ₁₂ N ₂ O ₂	116-118	12		0.97	5.72 ^{h,i}	3.28 ^h	-2.44	
143	1-piperidinyl	H	H	H	I	C ₁₃ H ₁₄ N ₂ O ₂	101-102	32	3.0		6.19 ^{h,i}	3.41	-2.78	
144	1-morpholinyl	H	H	H	I	C ₁₂ H ₁₂ N ₂ O ₃	151-152	33		1.50	6.15 ⁱ	2.65	-3.50	
145	N(Me)(Ac)	H	H	H	e	C ₁₁ H ₁₀ N ₂ O ₃	113-115	65		2.57	8.24 ^h	6.04	-2.20	
146	N(nBu)(Ac)	H	H	H	e	C ₁₄ H ₁₆ N ₂ O ₃	47-48	52		2.48	8.00			
147	N(Me)(COOiBu)	H	H	H	g	C ₁₄ H ₁₆ N ₂ O ₄	44-45	10		2.21	8.57	5.48	-3.09	

Table III (Continued)

no.	R ₂	R ₅	R ₇	R ₈	method	molecular formula	mp, °C	%	log <i>D</i>	log <i>k</i> _{OH⁻}	HLE		
											p <i>K</i> ₁	log <i>k</i> _{on}	log <i>k</i> _{off}
148	GlyOEt	H	H	H	D ^{d,e}	C ₁₂ H ₁₂ N ₂ O ₄	147-148			1.40	4.19 ⁱ		
149	L-AlaOEt	H	H	H	D ^e	C ₁₃ H ₁₄ N ₂ O ₄	132-134	56		1.31	6.06 ⁱ	3.41	-2.65
150	AlaValOMe	H	H	H	D	C ₁₇ H ₂₁ N ₃ O ₅	84-86	39			6.74	3.45	-3.29
151	DL-LeuOMe	H	H	H	D ^e	C ₁₆ H ₁₆ N ₂ O ₄	80-91			1.32	7.18	4.32	-2.86
152	L-LeuOMe	H	H	H	D ^e	C ₁₆ H ₁₆ N ₂ O ₄	82-83	58	3.2	1.13	6.77	3.36	-3.41
153	D-LeuOMe	H	H	H	D ^e	C ₁₆ H ₁₆ N ₂ O ₄	90-92	26	3.2	1.32	7.43	4.58	-2.85
154	L-LeuOMe	Me	H	H	H ^e	C ₁₆ H ₂₀ N ₂ O ₄	127-128	34	3.7	0.59	6.60 ⁱ	2.57	-4.00
155	L-LeuOMe	Et	H	H	H ^e	C ₁₇ H ₂₂ N ₂ O ₄	74-75	38		0.48	7.92 ^h	3.67	-4.25
156	LeuOMe	Me	Me	H	D ^e	C ₁₇ H ₂₂ N ₂ O ₄	170-172	27	4.0	-0.18	5.90	1.20	-4.70
157	L-Leu-L-LeuOMe	Me	Me	H	J	C ₂₃ H ₃₃ N ₃ O ₅	56-60	44			7.40 ⁱ	3.10	
158	D-Leu-L-LeuOMe	Me	Me	H	J	C ₂₃ H ₃₃ N ₃ O ₅	55-65	20			6.99 ^h	2.56	-4.43
159	L-IleOMe	H	H	H	D ^e	C ₁₅ H ₁₈ N ₂ O ₄	86-87	42	2.6	1.32	6.67 ^{h,i}	2.88	-3.79
160	DL-PhGlyOMe	H	H	H	D	C ₁₇ H ₁₄ N ₂ O ₄	108-110	75	2.6	1.39	7.33	3.79	-3.54
161	D-PhGlyOMe	H	H	H	D	C ₁₇ H ₁₄ N ₂ O ₄	100-101	75	2.6	1.39	7.56	4.18	-3.38
162	DL-PheOEt	H	H	H	D	C ₁₉ H ₁₈ N ₂ O ₄	oil	50			5.92 ⁱ	2.81	-3.11
163	PheNH ₂	H	H	H	D ^e	C ₁₇ H ₁₆ N ₃ O ₃	215-216	60		1.26	6.50	3.08	-3.42
162	L-TyrOMe	H	H	H	D	C ₁₈ H ₁₈ N ₂ O ₅	210-211	45	0.3	1.28	6.35	3.28	-3.07
165	AsnNH ₂ Pr	H	H	H	D	C ₁₅ H ₁₈ N ₄ O ₄	221-222	38	0.2	1.45	5.67	2.72	-2.95
166	GlnNH ₂ Pr	H	H	H	D	C ₁₈ H ₂₀ N ₄ O ₄	219-220	40	-0.3	1.30	6.26	3.04	-3.22
167	DL-ProNH ₂	H	H	H	F	C ₁₃ H ₁₃ N ₃ O ₃	174-176	46	0.4	1.21	6.75	3.66	-3.09
168	L-ProNH ₂	H	H	H	D	C ₁₃ H ₁₃ N ₃ O ₃	193-194	49	0.4	1.21	6.62	3.61	-3.01
169	ProAlaNH ₂	H	H	H	F	C ₁₆ H ₁₆ N ₄ O ₄	245-246	50			6.63	3.54	-3.09
170	ProValNH ₂	H	H	H	F	C ₁₈ H ₂₂ N ₄ O ₄	234-235	25			6.33	3.17	-3.16
171	ProLeuNH ₂	H	H	H	F ^e	C ₁₉ H ₂₄ N ₄ O ₄	205-208	25	1.0	1.27	7.67	4.18	-3.49
172	ProLeuNH ₂	Et	NH ₂	H	F	C ₂₁ H ₂₆ N ₅ O ₄	148-150	71	1.6	-1.08	6.94	4.98	-1.96
173	ProLeuGlyNH ₂	H	H	H	F ^e	C ₂₁ H ₂₇ N ₅ O ₅	199-210		0.8	1.26	7.68 ⁱ	4.04	-3.64
174	ProPheNH ₂	H	H	H	F ^e	C ₂₂ H ₂₂ N ₄ O ₄	226-227		1.2	1.23	7.43	4.16	-3.27
175	GABA-OEt	H	H	H	D	C ₁₄ H ₁₈ N ₂ O ₄	124-126	29	2.3	1.21	4.73 ⁱ		

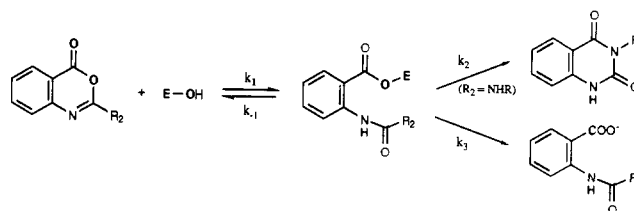
^a Data presentation: alkaline hydrolysis as log *k*_{OH⁻} (M⁻¹ s⁻¹), elastase inhibition as p*K*₁ = -log *K*₁ (M), acylation rate as log *k*_{on} (M⁻¹ s⁻¹), deacylation rate as log *k*_{off} (s⁻¹). ^b Reference 20. ^c *k*_{off} determined directly, and *k*_{on} calculated as *k*_{off}/*K*₁. ^d Reference 18. ^e Reference 19. ^f Reference 16. ^g Procedure described in the Experimental Section. ^h Standard error > 20%. ⁱ Determined with chromogenic substrate, see the Experimental Section. ^j Determined at a single concentration. ^k Upper limit. ^l Lower limit.

Scheme V^a

^a (a) CCl₃COCl, THF, R'NH₂; (b) H₂, 5% Pd/C, EtOH; (c) H₂, Raney Ni, HCHO, EtOH; (d) H₂, Raney Ni, CH₃CHO, NaOAc, EtOH; (e) H₂SO₄.

useful for the preparation of benzoxazinone IV in cases where the anthranilate precursor is not commercially available. A phenylurea XIV was first ortho-thallated with thallium(III) trifluoroacetate in 10% trifluoroacetic acid in tetrahydrofuran for 8 h. After solvent removal, the crude thallated derivative XV was immediately carbonylated with carbon monoxide in the presence of catalytic palladium(II) chloride, lithium chloride, and magnesium oxide to yield the benzoxazinone IV. In all the cases that we investigated, the potentially competing product, compound XVI, was not observed and benzoxazinone IV was formed as the only isolable product. The phenylureas studied tend to undergo thallation at the least hindered position, ortho to the urea. With 3-isopropyl(3-ethylphenyl)urea the 7-ethyl isomer 96 was formed exclusively.

Scheme VI



2-(Alkylamino)-7-amino derivatives of benzoxazinones IVa, IVb, and IVc (90, 91, 93-95, 97, 98, 100, 101, 109) were accessible via the route shown in Scheme V. The precursor 2-aminobenzoate XVII^{19,24} was treated with diphenylurea for 3 h. Quenching of the reaction mixture with amine R'NH₂ gave urea XVIII, which was reduced with H₂/Pd/C to give 4-amino derivative XIX.¹⁹ Reductive alkylation of XVIII with H₂/Raney Ni and the appropriate aldehyde afforded the [4-(dialkylamino)]urea XX.²⁵ Reductive alkylation of XVIII under controlled conditions H₂/Raney Ni/CH₃CHO/NaOAc²⁶ gave a moderate yield of the secondary amine XXI. Compounds XIX, XX, and XXI were converted to the corresponding benzoxazinones IVa, IVb, and IVc by cyclization with concentrated sulphuric acid.

Various general methods of synthesis of 2-alkoxy-, 2-alkylamino-, and 2-dialkylamino derivatives of benzoxazinones (III, IV, and V) have been discussed in our recent publications,^{14,16,19} which contain information relating to

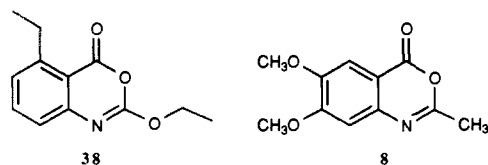
- (24) Takeski, M.; Junzo, T. *Jpn. Kokai Tokyo Koho*, 7,905,942, Jan 17, 1979; *Chem. Abstr.* 1979, 91, 56666t.
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the synthesis of individual benzoxazinones not described in this paper.

Results and Discussion

Tables I–III present the alkaline hydrolysis rates of benzoxazinones and inhibition data pertaining to HL elastase. The enzyme inhibition data include the acylation rate constants k_{on} ($=k_1$, Scheme VI) and k_{off} ($=k_{-1} + k_2 + k_3$), as well as the inhibition constants K_i . Because these are alternate substrate inhibitors which form acyl enzyme intermediates, identifying the factors which affect k_{on} and k_{off} is essential to interpreting the overall SAR for K_i .

The ranges of the compounds' activities are considerable: alkaline hydrolysis rates and k_{on} span 6 orders of magnitude, k_{off} covers 5 orders of magnitude, and K_i spans 8 orders of magnitude. This breadth is dramatically illustrated by comparing the least and most potent inhibitors. Compounds 38 and 8 have K_i values of 4.3×10^{-11} and 8.3



$\times 10^{-4}$ M, respectively, a range of 2×10^7 , despite their common benzoxazinone structure, size, and probable hydrophobicity and opportunities for hydrogen bonding. It is the goal of this paper to identify the factors which lead to such variation within the common mechanistic framework of Scheme VI and to emphasize the role that intrinsic chemical factors—as opposed to noncovalent interactions—play in determining inhibition constants.

Alkaline Hydrolysis

Selection of Parameters. Appropriate selection of molecular descriptors is the first step in data analysis. Alkaline hydrolysis data are useful here for two reasons. First, the factors affecting hydrolysis should be small in number and the regression should be good, because the chemistry does not involve any specific enzyme interactions. Secondly, since alkaline hydrolysis mimics the acylation sequence of enzyme inhibition (Scheme VI), the descriptors relevant to hydrolysis should be a minimal starting set for analyzing the factors responsible for enzyme inhibition. The mechanistic parallel between alkaline hydrolysis and target enzyme acylation has been previously established for β -lactams.²⁷

Since alkaline hydrolysis of benzoxazinones proceeds by hydroxide attack at C_4 ,^{28–30} a single electronic parameter should be sufficient to account for the variance in the data. A decision regarding which of the commonly tabulated parameters to choose (σ_p , σ_m , or σ^*) to represent ring substituents can be based on analysis of hydrolysis data versus **F** and **R**. This is especially important for position 2, because of uncertainties in the transmission of electronic effects through the oxazinone ring. The parameters **F** and **R** have the desirable property of being nearly orthogonal, but they are not widely tabulated. Preliminary regressions 1 and 2 were therefore carried out on published values³¹ in order to estimate **F** and **R** for the EtS, MeNH, and nBuNH substituents, so as to expand the range of com-

pounds included in regression 3.

$$\mathbf{F} = 0.021 - (0.43 \pm 0.07)\sigma_p + (0.31 \pm 0.02)\sigma^* \quad (1)$$

$$(n = 38, F = 98.3, r^2 = 0.849, s = 0.13)$$

$$\mathbf{R} = -0.028 + (3.83 \pm 0.17)\sigma_p - (0.46 \pm 0.05)\sigma^* \quad (2)$$

$$(n = 38, F = 295, r^2 = 0.944, s = 0.30)$$

Analysis 3 was then carried out for alkaline hydrolysis as a function of **F** and **R** for compounds 2, 12, 16, 23, 25, 34, 52, 54, 106, and 145, with the result that hydrolysis shows $25 \pm 5\%$ resonance contribution from the 2-substituent. Since σ_p , σ_m , and σ^* ³² show 38 ± 3 , 20 ± 2 , and $\log k_{OH^-} = 1.80 + (1.58 \pm 0.20)\mathbf{F} + (0.52 \pm 0.08)\mathbf{R}$ (3)

$$(n = 10, F = 51.1, r^2 = 0.936, s = 0.19)$$

$13 \pm 2\%$ resonance,³³ respectively, this result suggests that σ_m is the most appropriate single parameter to describe electron donation and withdrawal by R_2 . Correlation of the ^{13}C chemical shift of C_4 with **F** and **R** for R_2 leads to the same conclusion.³⁰

Parameterization of the benzene ring substituents is not as dependent on transmission through the oxazinone ring, and R_5 – R_8 were assigned a $\sum\sigma$ term, with σ_p for R_5 and R_7 , and σ_m for R_6 and R_8 , to reflect their positions relative to the carbonyl.

Molar refractivity (MR) was used as a measure of molecular volume,³⁴ with all values scaled such that MR for hydrogen = 0 and MR for methyl = 1.

Results of Regression

Equation 4 is the result of regression on 123 benzoxazinones with the maximum number of statistically significant parameters retained after stepwise removal.

$$\log k_{OH^-} = 1.89 + (2.65 \pm 0.11)\sigma_{R_2} + (2.70 \pm 0.10)\sigma_{ring} - (0.031 \pm 0.008)MR_2 - (0.20 \pm 0.03)MR_5 - (0.20 \pm 0.08)MR_8 - (0.22 \pm 0.05)MR_6 \quad (4)$$

$$(n = 123, F = 355, r^2 = 0.948, s = 0.24)$$

The only data from Tables I–III not included were compounds of uncertain parameterization (i.e. 9, 10, 63) and compounds which were outliers due to possible tautomeric variation (73–75) or poor solubility or steric interactions (29, 122, 138, 156).

Equation 4 shows that R_2 (using σ_m for σ_{R_2}) and R_5 – R_8 have identical ρ values and that steric influences from R_2 and R_7 are negligible. The fit also has a small but significant coefficient for MR_6 , which cannot plausibly represent steric hindrance but suggests that σ_m may slightly overestimate the electronic contribution from R_6 . Similarly, the coefficients of MR_5 and MR_8 may reflect either slight biases in parameterization or small steric interactions in the transition state for hydroxide attack on C_4 .³⁵ Removal of the MR_2 and MR_6 terms from equation 4 and

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(32) The resonance inherent in σ^* was determined by regression of tabulated σ^* vs. **F** and **R**.³³

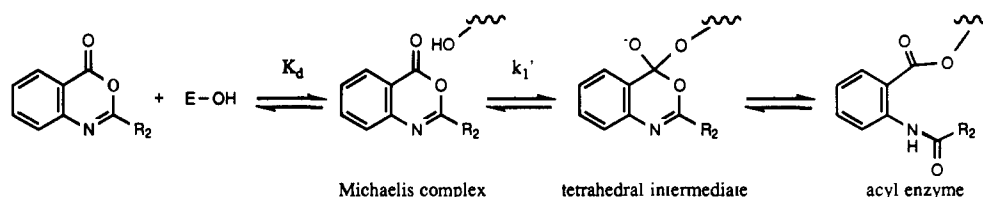
(33) Perrin, D. D.; Dempsey, B.; Serjeant, E. P. In *pK_a Prediction for Organic Acids and Bases*; Chapman and Hall: London, 1981.

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(35) The MR_5 term may reflect a small hindrance felt at C_4 , and the MR_8 term may reflect a disruption of a hydrogen bond from water to N_1 that assists attack at C_4 .³⁶

(36) Vicens, J.; Etter, M. C.; Errede, L. A. *Tetrahedron Lett.* **1983**, *24*, 723.

Scheme VII



merging of the electronic terms give eq 5, with the MR_3 term no longer significant.

$$\log k_{OH^-} = 1.64 + (2.64 \pm 0.07)\sigma_{total} - (0.15 \pm 0.03)MR_5 \quad (5)$$

$$(n = 123, F = 811, r^2 = 0.931, s = 0.27)$$

This impressively simple equation shows that electron donation and withdrawal are the major determinants of benzoxazinone reactivity over 6 orders of magnitude. The indication that R_5 may only slightly hinder attack at C_4 is consistent with perpendicular attack (Figure 1). The small size of the MR_5 coefficient and the lack of correlation between the MR_5 and MR_2 terms show that substitution at R_5 does not induce a change of mechanism, i.e. a shift to hydroxide attack at C_2 rather than C_4 .

Overall Trends in the Inhibition of HL Elastase

The complexity of serine proteinase inhibition by benzoxazinones limits what can be expected of traditional QSAR analysis. Even though Scheme VI is considerably more complex than a simple noncovalent binding scheme, it is a simplification. The acylation step (k_1) is expanded in Scheme VII to show the minimum of two intermediates and three transition states which it includes. First, there is the likely noncovalent "binding" to form a Michaelis complex, with the binding constant K_d . Second, serine 195 attacks at C_4 to form a tetrahedral intermediate (k_1'), and third, there must be collapse of the tetrahedral intermediate to the acyl enzyme. Of these three steps which make up k_1 , only the first noncovalent step is analogous to most simple drug-receptor interactions and might be expected to depend principally on $\log P$ and MR. Since the rate-determining step within k_1 is probably k_1' (because of the strong dependence of k_1 on electron withdrawal; vide infra) and since experimentally k_1 never saturates, the first adsorptive step is, in any case, not explicitly revealed by our data.

Similarly, deacylation is complex, with three demonstrated pathways for breakdown of the acyl enzyme (Scheme VI).

Results of Regression on pK_i . With the understanding that K_i is not a simple binding constant—because there are at least six transition states and five distinct intermediates in the full mechanism of inhibition—and despite the potential for multiple binding modes of comparatively small heterocycles at the active site of HLE, the QSAR is remarkably successful in accounting for the 2×10^7 -fold variation in K_i . The best overall regression is eq 6. Here σ_{R_2} , MR_5 , and MR_6 are defined as before. I_{C_2} is $pK_i = 7.21 + (3.46 \pm 0.30)\sigma_{R_2} - (1.66 \pm 0.15)I_{C_2} - (0.72 \pm 0.15)I_{2\phi} + (0.67 \pm 0.07)MR_5 - (0.77 \pm 0.12)MR_6$ (6)

$$(n = 162, F = 75.6, r^2 = 0.708, s = 0.71)$$

an indicator variable which equals 1 when the atom attached at C_2 is carbon, and 0 otherwise, and $I_{2\phi}$ equals 1 when R_2 contains an aryl group and 0 otherwise. These indicator variables were included after the examination of residuals from preliminary fits. The data set includes all the compounds of Tables I-III, with the exception of those with

5-methoxy substitution and several consistent outliers (73-78, 122, 148, 156, 175).

Electron withdrawal by R_2 is highly favorable in eq 6, while electron withdrawal or donation by R_5 - R_8 is not significant. Since $K_i = k_{off}/k_{on}$,^{10d,11a,37} these electronic effects are consistent with Scheme VI as well as with previous results from a more limited data set.^{10d} Because R_2 is part of the ring system in the benzoxazinone (Scheme VI), electron withdrawal by R_2 accelerates attack at C_4 and therefore increases $k_1 (=k_{on})$. However, in the acyl enzyme, R_2 is distal and cross-conjugated with respect to the serine ester carbonyl that is undergoing attack in the step corresponding to k_3 , and therefore electronic effects of R_2 , at least for hydrolysis, should be minimal. Conversely, electron withdrawal by R_5 - R_8 should affect acylation and deacylation equally (to a first approximation), and so σ_{ring} should have little effect on K_i .

The size of R_2 , as measured by MR, is not significant in regression 6. Only in specific subsets of the data does the importance of R_2 size and/or hydrophobicity become apparent (vide infra). The indicator variable I_{C_2} in eq 6 shows the strong general trend that heteroatom attachment at C_2 is highly favorable. This effect cannot be due to a simple electronic or steric factor, since I_{C_2} is not correlated with either MR_2 or σ_{R_2} . The parameter $I_{2\phi}$ shows that aryl functionality in R_2 is disfavored by a factor of 5.3 on K_i .

Steric and/or hydrophobic interactions of ring substituents R_5 - R_8 are clearly summarized by eq 6. The MR terms for R_7 and R_8 are not significant; this is especially noteworthy for R_7 , where the substituents range in size from H up to NHCONHPr. Substitution in R_6 is highly unfavorable; addition of one methylene unit to R_6 increases K_i 5.9-fold. Conversely, alkyl substitution in R_5 is very favorable, probably reflecting selective hindrance of deacylation (vide infra).^{10d,11}

It is noteworthy that compound hydrophobicity (as measured by $\log D$, an HPLC approximation to $\log P$,³⁸ Tables I-III) is not a significant determinant of potency. This emphasizes that these compounds owe their potency and diversity to the kinetics of acylation and deacylation and not to the noncovalent interactions of traditional drugs.

Results of Regression on $\log k_{on}$. Even though k_{on} is chemically simpler than K_i , the QSAR is less successful, perhaps because many of the most rapid acylating benzoxazinones have k_{on} values which are lower limits and were not included in regression. The best fit to $\log k_{on}$ which uses the same parameters as 6 is eq 7.

$$\log k_{on} = 4.36 + (2.98 \pm 0.42)\sigma_{R_2} + (1.30 \pm 0.34)\sigma_{ring} - (0.82 \pm 0.25)I_{C_2} - (0.85 \pm 0.22)I_{2\phi} + (0.26 \pm 0.10)MR_5 \quad (7)$$

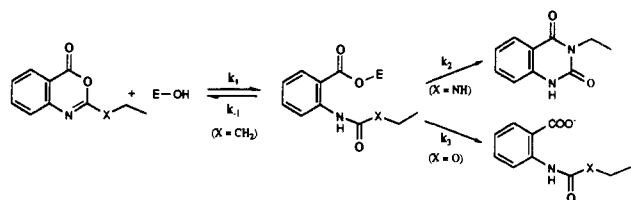
$$(n = 135, F = 16.6, r^2 = 0.391, s = 0.84)$$

Even though eq 7 accounts for only 39% of the variance in $\log k_{on}$, it identifies those substituent effects which affect

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Scheme VIII



K_i by modifying acylation rates. The σ_{R_2} and $I_{2\phi}$ terms are not significantly different between fits 6 and 7, showing that the electron withdrawal and 2-aryl effects on K_i are manifest in acylation; for the σ_{R_2} term this is as expected from Scheme VI. The large coefficients for σ_{R_2} and σ_{ring} are evidence that serine 195 attack at C_4 is the rate-determining step within acylation (Scheme VII).

Comparison of the I_{C_2} terms in 6 and 7 show that 2-carba substitution is unfavorable because it both slows acylation and speeds deacylation. This result for acylation is reasonable. Acylation may be slower for 2-carbabenzoxazinones than for the corresponding heteroatom-linked systems for either of two reasons related to hydrogen-bonding interactions. First, direct hydrogen bonding or other electrostatic interactions between heteroatom and enzyme may facilitate binding and acylation. Secondly, electron density at N_1 is increased by heteroatom substitution for carbon at C_2 , which may promote intrinsic binding and catalysis through more efficient hydrogen bonding of N_1 to the enzyme.

The MR_5 term is positive, a surprising result that is due to the rapid acylation by 5-ethyl benzoxazinones. The dependence of acylation and deacylation on the 5-substituent is examined in detail with a 2-ethoxy-5-R subset of compounds (vide infra).

Factors Affecting Deacylation. Since $k_{off} = k_{on}K_i$, factors which affect deacylation can be inferred from eq 6 and 7. Qualitatively, this suggests that electron withdrawal by R_5 - R_8 and substitution at R_6 accelerate deacylation, while bulk at R_5 and heteroatom substitution at R_2 slow deacylation. The reason why 2-carbabenzoxazinones generally deacylate faster than their 2-hetero counterparts may be connected with the observation that O-nucleophilicity is generally greater for amides than for more encumbered groups such as carbamates,^{39,40} thus providing the 2-amidobenzoyl elastases with a relatively facile reversion route to substrate and free enzyme.

Beyond these general kinetic effects, the substituent R_2 profoundly affects the products of enzyme deacylation. For example, the major deacylation pathway differs for each analogue in the series 2-(ethylamino)-, 2-ethoxy-, and 2-*n*-propylbenzoxazinone, **79**, **34**, and **11**, respectively (Scheme VIII). N-Cyclization yielding a 1*H*,3*H*-quinazolin-2,4-dione has been established as the dominant mode of deacylation stemming from the reaction of HLE with 2-(ethylamino)benzoxazinone **79**,¹¹ (i.e. k_2 in Scheme VIII), whereas hydrolysis is essentially exclusively observed for the 2-ethoxy system (**34**) (k_3 in Scheme VIII).⁴¹ Although Stein could find no evidence for a covalent interaction between 2-*n*-propylbenzoxazinone **11** and HLE,^{10e} by monitoring the difference spectrum of the interaction of benzoxazinone with human sputum elastase, we have detected an acyl enzyme intermediate with $\Delta\lambda_{max} = 309$ nm. The product of deacylation is exclusively benz-

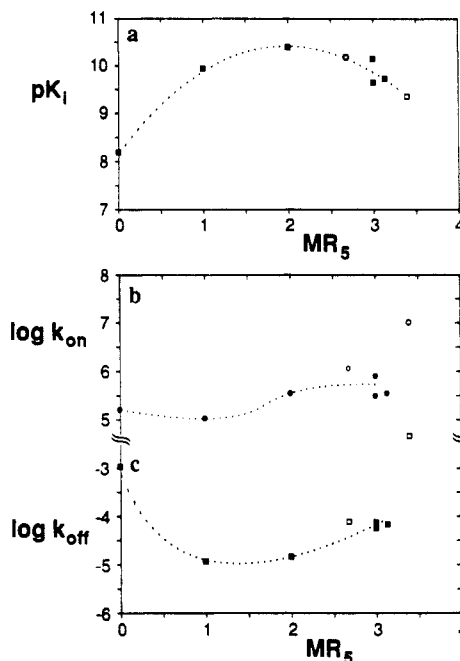
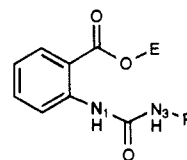


Figure 2. Plot of the molar refractivity of substituent R_5 versus pK_i (M), $\log k_{on}$ ($M^{-1} s^{-1}$), and $\log k_{off}$ (s^{-1}) for compounds $R_2 = EtO$, $R_6, R_7, R_8 = H$, where \bullet, \blacksquare , are for $R_5 = H, Me, Et, nPr, iPr$, and $CH=CHCH_3$; \circ, \square are for $R_5 = CH_2Br$ and $CHBr_2$.

oxazinone **11** (i.e. $k_{-1} \gg k_3$ in Scheme VI), as shown by single-turnover experiments (see the Experimental Section).

Additionally, acyl enzymes derived from 2-(alkylamino)benzoxazinones partition differently for different alkyl groups RNH. Benzoxazinones with linear R groups deacylate predominantly via N-cyclization, while those with branched R groups prefer hydrolysis or reversion to benzoxazinone.¹¹ It may be worth noting that O-cyclization of 2-ureidobenzoyl elastases can, in principle, proceed by two distinct transition states, one involving activation of the N_1 -H and the other involving activation of the N_3 -H. Considering the complex nature of deacylation, it is not surprising that the variance in the data is difficult to account for in simple terms.



Separate Trends in the Inhibition of HL Elastase

The overall trends in HL elastase inhibition are clear from the regression results. The data set is so large, however, and the ranges of inhibition constants so broad that other structure-activity relationships become apparent only within particular subsets of compounds. In many cases molecules were specifically synthesized to pursue some of these SAR's.

Optimum Size of R_5 . Regression on pK_i for the full data set (eq 6) consistently shows that 5-alkyl substitution is highly advantageous, and regression on $\log k_{on}$ (eq 7) shows that at least part of this benefit is due to a general acceleration of acylation. Details of the effects of R_5 are best examined within the most complete subset of 5-substituted benzoxazinones, with $R_2 = ethoxy$ (**34**, **35**, **38**, **40-44**). For these compounds the effects of the size of R_5 on K_i , acylation, and deacylation are shown in Figure 2.

The filled symbols in Figure 2 indicate 2-ethoxybenzoxazinones with $R_5 = H, Me, Et, nPr, iPr$, and $CH=C-$

(39) Scott, F. L.; Glick, R. E; Winstein, S. *Experientia* 1957, 13, 183.

(40) Determann, H.; Heuer, J.; Pfaender, P.; Reinartz, M.-L. *Justus Liebigs Ann. Chem.* 1966, 694, 190.

(41) Abeles and colleagues observe the same with chymotrypsin.^{10c}

HCH₃. The open symbols indicate R₅ = CH₂Br and CHBr₂, which are included for completeness but differ significantly in electronic character from the alkyl substituents. Specific substituent effects are first discussed for *k*_{on} and *k*_{off} separately, and then the corresponding effects on *K*₁ simply represent the combination of these effects.

Figure 2b shows how 5-methyl substitution slightly decreases *k*_{on} over the parent 5-unsubstituted compound, whereas 5-ethyl and -propyl substitutions result in an average 2.7-fold increase. Other than the possibility of relief of strain from peri interactions between R₅ and the carbonyl, there is no obvious basis in electronic theory for this acceleration.⁴² It may well be that the Michaelis binding and/or serine attack at C₄ is assisted by specific R₅-enzyme interactions only in instances when R₅ favorably competes with R₂ for binding to the enzyme at subsite S₁. Note that HL elastase prefers amino acids with small alkyl side chains like valine in the P₁ position of its peptidyl substrates and inhibitors.⁴³ Recent reports⁴⁴ have shown that with porcine pancreatic elastase and 2-tBOC-Val-5-Cl- or 2-tBOC-Val-5-methylbenzoxazinone the 5-substituent extends toward the S₁ site in the acyl enzyme.

The effect of 5-substitution on deacylation can largely be understood on physicochemical grounds. Figure 2c shows that 5-methyl vs 5-H substitution results in an 87-fold decrease in *k*_{off}. If the principal mode of deacylation is hydrolysis (*k*₃, Scheme VI), then a large effect is expected due to hindrance of water attack at the ester carbonyl (Figure 1).^{11,45} If the principle mode of deacylation is O-cyclization (*k*₋₁, Scheme VI), then the crowding and conformational restriction imposed on the acyl enzyme by diortho substitution might also be expected to slow deacylation. The slight recovery of deacylation rates for R₅ = propyl might be due to specific enzyme interactions; as suggested above, recognition of R₅ at the S₁ site might improve the geometries for both acylation and deacylation.

Combining the 5-effects on acylation and deacylation gives the effect on *K*₁. As shown in Figure 2a, this is an optimum for 2-ethoxy-5-ethylbenzoxazinone with its *K*₁ = 42 pM. The electron withdrawal by the bromoalkyl substituents (open symbols), which accelerates both acylation and deacylation, neatly compensates to give a deceptively simple parabolic relation embracing all of the 2-ethoxy-5-R compounds.

Effects of Size and α -Branching in 2-Alkylamino Substituents. 2-Aminobenzoxazinones are generally more stable than 2-carba-, oxy-, or thiobenzoxazinones, because of their greater R₂ electron donation. For this reason they are more attractive candidates for medicinal development, and so their SAR's have been pursued more completely.

- (42) On the contrary, a diminution in the rate of alkaline hydrolysis, the model reaction, was observed with R₅ substitution.
- (43) (a) Marossy, K.; Szabo, G. Cs.; Pozsgay, M.; Elodi, P. *Biochem. Biophys. Res. Commun.* 1980, 96, 762. (b) McRae, B.; Nakajima, K.; Travis, J.; Powers, J. C. *Biochemistry* 1980, 19, 3973. (c) Nakajima, K.; Powers, J. C.; Ashe, B. M.; Zimmerman, M. J. *Biol. Chem.* 1979, 254, 4027. (d) Harper, J. W.; Cook, R. R.; Roberts, C. J.; McLaughlin, B. J.; Powers, J. C. *Biochemistry* 1984, 23, 2995.
- (44) (a) Meyer, E. J., Jr.; Bode, W. In *QSAR in Drug Design and Toxicology*; Proc. 6th European Symposium on Quantitative Structure-Activity Relationships; Hadzi D., Jerman-Blazic, B., Eds.; Elsevier: Amsterdam, 1987. (b) Presta, L. G.; Meyer, E. F., Jr. *J. Biopolymers* 1987, 26, 1207. (c) Bode, W.; Meyer, E. F., Jr.; Powers, J. C. *Biochemistry* 1989, 28, 1951. (d) Radhakrishnan, R.; Presta, L. G.; Meyer, E. F., Jr.; Wildonger, R. J. *Mol. Biol.* 1987, 198, 417.
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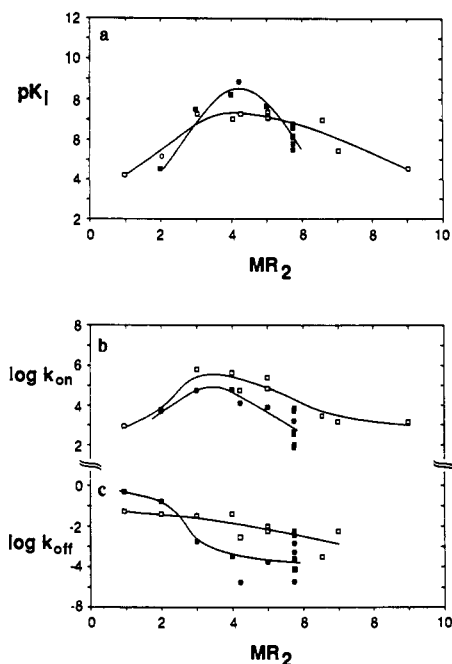


Figure 3. Plot of the molar refractivity of substituent R₂ versus (a) p*K*₁(M), (b) log *k*_{on}(M⁻¹ s⁻¹), and (c) log *k*_{off} (s⁻¹), for the simple 2-(alkylamino) compounds 73, 74, 76, 77, 79–84, 106, 107, 111 and 112 where □ is for R₅ = H; ■ is for R₅ = Me.

Simple 2-aminoalkyl compounds have been examined in the greatest detail.

As noted above, the substituent effects on *K*₁ are best understood as the sum of effects on acylation and deacylation. The effects of the size of R₂ on *k*_{on} are presented in Figure 3b, and show that for compounds with either R₅ = H (open squares) or R₅ = CH₃ (filled squares) acylation rates are maximal for R₂ = NH₂ and NH₂Pr and decline for larger and smaller groups. Beyond this generalization the SAR's are less clear, as demonstrated by the points clustered at MR₂ = 5.75. These are the isomers of R₂ = aminopentyl, R₅ = CH₃ (111–118), which span a 100-fold range in *k*_{on}. The only apparent trend for the aminopentyl compounds is that those with branching β to the amine (113,117) are substantially slower acylators than the linear or α-branched isomers, which must reflect a specific enzyme interaction.

The effects of R₂ on deacylation are more interesting. We have previously established that the preferred deacylation pathway when R₂ is a linear aminoalkyl group is N-cyclization to form a 1*H*,3*H*-quinazoline-2,4-dione (*k*₂, Scheme VI) and hydrolysis (*k*₃) or O-cyclization (*k*₋₁) when R₂ is an α-branched aminoalkyl.¹¹ Thus, one might expect subtle changes in the structure of NHR to have a marked effect on deacylation rate by virtue of their influence on the reaction pathway.

Synergistic Effects of R₂ and R₅ Substitution. Figure 3c shows that the deacylation rates are not parallel for R₅ = H and R₅ = CH₃. For R₅ = H (open squares), *k*_{off} is constant for R₂ = NH₂, NHMe, NH₂Et, and NH₂Pr, and then decreases slightly for larger R₂ groups. Within this trend the negative outliers are for α-branched groups, namely R₂ = NH₂Pr and NH-cyclohexyl (83, 121), consistent with a sterically induced change in mechanism.^{11b} For R₅ = methyl (filled squares), *k*_{off} falls dramatically for R₂ larger than NHMe, suggesting a synergy between R₂ and R₅: their combined substitution can restrict the acyl enzyme enough to lower *k*_{off} to rates of ca. 10⁻⁴ s⁻¹. The sensitivity of this system to even very small alkyl groups at R₂ and R₅ is noteworthy and deserves further comment.

If the effect of substitution at R_5 were totally independent from that of R_2 , as R_2 is varied the N-cyclization rate should follow the trend observed for $R_5 = H$ and neither deviate from $k_{off} \approx k_2$ nor drop until $R_2 > NHnPr$ or branched alkyl. In fact, a simple chemical model of N-cyclization suggests that an alkyl substituent of modest bulk at R_5 should not dramatically reduce this rate, since a favorable conformation for N-cyclization (Figure 1c) is still attainable. Indeed, the data (k_{off}) for $R_2 = NH_2$, $NHMe$ when $R_5 = Me$ are consistent with this hypothesis, as are the results of varying R_5 in a model ureidobenzoic acid ester.^{11b} But as indicated by the decline in k_{off} when $R_2 = NHEt$, $R_5 = Me$, even small alkyl groups can combine to bring specific effects into play that prevent the acyl enzyme from achieving an optimal transition state for N-cyclization. Note that the considerable variation among the $R_2 = NH$ -pentyl isomers is also a reminder that specific interactions can override generalizations based on simple chemical models.

The combination of the effects of R_2 on k_{on} and k_{off} is manifest in K_i , shown in Figure 3a. Here the independence of k_{off} on MR_2 for $R_5 = H$ means that the broad optimum in k_{on} is reflected in K_i (open squares), and the pattern of decline in k_{off} for $R_5 = methyl$ results in a much narrower optimum for K_i (filled squares), peaking for $R_2 = NHnPr$ and $NHiPr$, $R_5 = CH_3$.

Trends in Amino Acyl Substitution. The benzoxazinones are excellent HL elastase inhibitors and yet have no obvious homology to the natural, peptidyl substrates and inhibitors of this enzyme. It is reasonable to expect that the acylation and deacylation rates of benzoxazinones (or any other xenobiotic lead structure) could be improved beyond what simple principles of chemical reactivity would suggest by taking advantage of specific interactions that mimic those during normal catalysis. To probe for such interactions a number of acyl 2-amino- and 2-peptidyl-benzoxazinones were made (Table III, 148 ff). Except for compounds with 5- and/or 7-substitution, they have comparable alkaline hydrolysis rates, demonstrating the similar electron donation of the R_2 amino acids and peptides. This leaves specific hydrophobic, steric, and H-bond interactions to account for their 3000-fold ranges in K_i (compound 173 vs 148).

The most obvious trend seen in the acyl 2-aminobenzoxazinones is that none of them is significantly more potent than the simple 2-(alkylamino) compounds, and several (148, 149, 156, 162, 165, 175) are orders of magnitude less potent. For the most part this is due to large decreases in k_{on} . Thus one or more of the steps in acylation can be severely compromised by unfavorable interactions with R_2 , which may be related to the decrease in k_{on} seen with larger 2-(alkylamino) substitution (Figure 3a, open squares, $MR_2 > 5$). The 2-amino acyl compounds do have lower deacylation rates than those with 2-(alkylamino) substitution, but this is overridden by the even lower acylation rates.

One of the hallmarks of specific enzyme interaction is a strong dependence on chirality. In the three cases represented in Table III, the D-amino acyl isomers are better inhibitors than the L isomers. In the one case for which the separate enantiomers and the racemic mixture were all available (151–153), the K_i and k_{on} values calculated for the racemate agree well with those observed,⁴⁶ so that in the other cases (160, 161 and 167, 168) the values for the missing isomer can be inferred with confidence. The effect

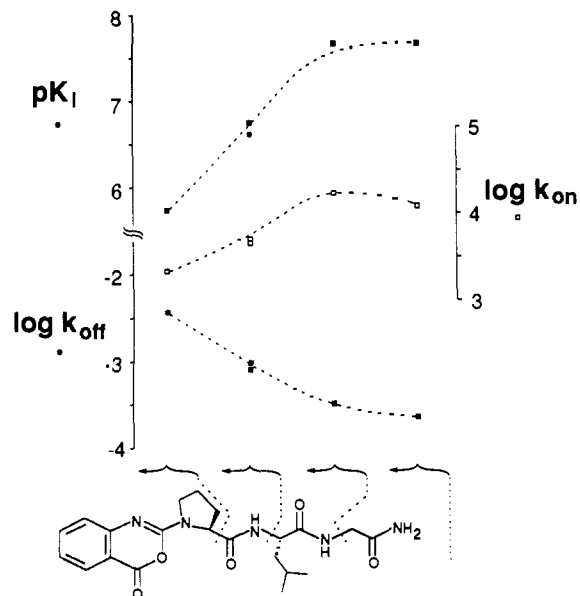


Figure 4. Effect of R_2 peptidyl substitution of compounds 142, 167, 168, 171, and 173 on $pK_i(M)$, $\log k_{on}$ ($M^{-1} s^{-1}$), and $\log k_{off}$ (s^{-1}).

of chirality varies; expressed as the ratio $K_{i,L}/K_{i,D}$ the D isomers are better inhibitors by factors of ~ 5.3 (2-phenylglycyl methyl ester), 4.6 (2-leucyl methyl ester), and only ~ 1.7 (2-prolinamido). In all cases the principal difference is due to slower acylation by the L isomers.

Trends in Peptidyl Substitution. Like the acyl 2-aminobenzoxazinones, the 2-di- and -tripeptidyl compounds (150, 157, 158, 169–174) are generally no better than 2-(alkylamino)benzoxazinones; i.e., peptidyl substitution generally spoils inhibition (by lowering k_{on}) rather than enhancing it. However, there is one subset of compounds which demonstrates that beneficial interactions are available.

Figure 4 shows the effects of R_2 extension from 1-pyrrolidinyl (142) to prolylleucylglycinamide (173). The 100-fold improvement in K_i is due to comparable increases in k_{on} and decreases in k_{off} as the peptide is extended to prolylleucinamide; the C-terminal glycine in 173 gives no further improvement.

Geometric Speculations. The structure of HL elastase has recently been published.⁴⁷ Other than the substitution of phenylalanine for glutamine at residue 192, the active site is quite similar to that of pancreatic elastase.⁴⁸ In addition, the structure of the acyl enzyme of porcine pancreatic elastase and 5-substituted benzoxazinones have recently been reported.⁴⁴ These findings and our data prompt some speculation on specific interactions which might pertain in HLE inhibition.

Given the 1:1 stoichiometry,^{10c} acyl enzyme crystal structure,⁴⁴ and pH dependence of acylation and deacylation,^{10d,11a} we conclude that inhibition begins with the histidine 57 catalyzed attack of serine 195 O_γ at the benzoxazinone C_4 while the carbonyl oxygen occupies the "oxyanion hole" formed by glycine 194 and serine 195. This in turn implies that there are two general geometries available for the Michaelis complex (Scheme IX); these involve O_γ attack at either the *re* or *si* face of C_4 .

(46) It can be shown that for K_i , $K_{DL} = 2K_LK_D/(K_L + K_D)$ and for k_{on} , $k_{DL} = k_L/2 + k_D/2$.

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Scheme IX^o

^a (a) Hypothetical Michaelis complex of an acyl 2-D-aminobenzoxazinone. Serine 195 is behind the plane of the benzoxazinone and is about to attack at the *re* face of the carbonyl. Note the possible amino acyl side chain recognition in the P₁ site and the room for extended R₂ substituents toward the P₂, P₃ sites. (b) Hypothetical complex of a 2-L-peptidylbenzoxazinone. Here attack will be at the *si* face and the R₂ substituent extends down the P₁', P₂' channel. Now the R₆ substituent may interact favorably at the P₁ site.

If O_γ attacks the *re* face (Scheme IXa), then the R₂ substituent extends into solvent and/or the P₁, P₂ domains—those sites which are occupied by the acyl enzyme residues in normal catalysis. In particular, R₂ occupancy of the hydrophobic but sterically limited P₁ site might be the cause of the distinct optimum in *k*_{on} shown by aminoalkyl R₂ (Figure 3b). The preference for D- over L-amino acyl R₂ (vide supra) would also be consistent with *re* face attack: Scheme IXa shows that any R₂ peptide must be inverted (in the N- to C-terminal sense) from the normal substrates, which might lead to the D stereochemical preference. Powers et al.⁴⁹ suggest *re* face attack for their sterically similar 3-alkoxy-7-amino-7-chloroiso-coumarins, on the basis of the enzyme specificity for various 3-OR groups, and X-ray crystal structure data supports this.⁵⁰

Equally valid arguments suggest *si* attack (Scheme IXb). First, the porcine pancreatic acyl enzyme structure⁴⁴ shows the R₂ substituent extending down the P₁', P₂' channel, which makes *si* attack seem more reasonable than the *re* attack, followed by extensive rotation and rearrangement to the observed acyl enzyme. Secondly, the 2-peptidyl results of Figure 4 imply favorable interactions with a longer LL moiety, which might be expected from substrate mimicry at the P₁', P₂' sites. Thirdly, the observed R₅ = Et, Pr optimum in acylation may suggest a favorable R₅/P₁ interaction.

It is appropriate to conclude these speculations by noting that *both re* and *si* attack may pertain, depending on substitution. Naruto et al.⁵¹ have recently shown, in a molecular mechanics study of chymotrypsin acylation by chiral haloenol lactones, that the energy differences between very different Michaelis geometries may be very small, as confirmed experimentally.

Conclusions

We present here the results of inhibition of HL elastase by 175 benzoxazinones. Given the complexity of the acylation-deacylation mechanism (Schemes VI and VII) and the 7 log order range of *K*_i, a five-parameter QSAR is remarkably good at accounting for the data. This QSAR (eq 6) shows that physicochemical terms (σ_{R_2} , MR₅) and

enzyme-specific interactions (*I*_{C₂}, *I*_{2φ}, MR₆) are all important in determining inhibition. Some of the determinants of the remaining variance in *K*_i can be understood by examining specific subsets of compounds.

Potency and stability are obviously desirable for any potential therapeutic agent. In addition, several of these mechanism-based inhibitors form acyl enzymes with lifetimes over 10 h. This very long period in which the enzyme remains inactive may give these compounds especially favorable pharmacokinetic properties. We address optimization of each of these properties—stability, potency, and acyl enzyme lifetime—below.

Stability, as measured by alkaline hydrolysis rates, is achieved simply by electron donation. For example, 2-(isopropylamino)-5-propyl-7-(dimethylamino)benzoxazinone **95** has *k*_{OH⁻} = 0.01 M⁻¹ s⁻¹, which extrapolates to a half-life at pH 7.4 of over 8.5 years.

Potency is achieved by rapid acylation combined with slow deacylation. To this end, the best 2-substituents are small alkyl groups linked via NH, O, or S. Electron withdrawal by R₂ is very favorable, as is alkyl R₅ substitution. For example, 2-ethoxy-5-ethylbenzoxazinone **38** has *K*_i = 42 pM, remarkably potent for a nonpeptidyl inhibitor of molecular weight 219.

Acyl enzyme stability is obtained by a combination of steric and electronic effects, principally electron donation by R₅–R₈ and small alkyl substitution at R₅. Additional synergistic stabilization occurs with dual R₅, branched-R₂ substitution. For example, 2-(isopropylamino)-5-methylbenzoxazinone **84** forms an acyl enzyme which deacylates with a half-life of 10.8 h.

Experimental Section

General Procedures. Analytical thin-layer chromatography (TLC) was performed with Merck silica gel 60 F-254 aluminum-backed plates. Compounds were visualized by ultraviolet light or phosphomolybdic acid spray reagent. Preparative TLC was carried out with 1-mm Whatman 4861-480 plates. Flash column chromatography was carried out with Whatman WPS-II silica gel.

¹H NMR spectra were recorded on a Bruker WP-80 SY spectrometer (80 MHz). Chemical shifts (ppm) are given downfield from tetramethylsilane, and coupling constants are given in hertz. Infrared spectra were obtained with a Perkin-Elmer Model 298 spectrometer. Mass spectra were recorded with a Finnigan MAT-112S instrument in electron-impact mode and high-resolution spectra were measured with a Finnigan MAT-311A. Ultraviolet absorption spectra and kinetics were monitored with a Perkin-Elmer 559A spectrophotometer at 25 ± 1 °C. Melting points were determined with a Büchi 510 melting point apparatus and are uncorrected.

Syntheses. Procedures for the preparation of 4H-3,1-benzoxazin-4-ones, described below as methods A–J, are general for categories of benzoxazinones as indicated in the text.

Method A. 2-Ethoxy-4H-3,1-benzoxazin-4-one (34). To a solution of anthranilic acid (13.71 g, 0.1 mol) in dry pyridine (100 mL) at room temperature under anhydrous conditions was added ethyl chloroformate (38.3 mL, 0.4 mol) in a dropwise manner over 15 min. After stirring for 2 h, the solvent was removed under reduced pressure and the residue was stirred vigorously in 250 mL of ice-cold water. The insoluble solid was filtered, washed with water, and air-dried. The crude product was dissolved in ethyl acetate, treated with charcoal, and recrystallized from ethyl acetate–petroleum ether to give the title compound as a white solid (17 g, 89%): mp 88–90 °C; IR 1760, 1630 cm⁻¹; ¹H NMR (CDCl₃) 1.46 (t, 3 H, CH₃CH₂), 4.53 (q, 2 H, OCH₂), 7.30–8.20 (m, 4 H, ArH). Anal. (C₁₀H₉NO₃) C, H, N.

Method B. 7-(Dimethylamino)-2-ethoxy-4H-3,1-benzoxazin-4-one (46). 4-(Dimethylamino)anthranilic acid⁵² (300 mg,

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1.66 mmol), ethyl chloroformate (0.313 mL, 0.32 mmol), and anhydrous potassium carbonate (0.9 g, 6.52 mmol) were stirred in dry THF (20 mL) at room temperature overnight. The reaction mixture was diluted with ethyl acetate (40 mL) and the insoluble material was filtered. The filtrate was washed with 0.5 N HCl (3 × 20 mL) and brine (20 mL) and dried over MgSO₄. Solvent evaporation gave a solid residue, which was recrystallized from ether to give 2-(carboethoxyamino)-4-(dimethylamino)benzoic acid (285 mg, 72%) as a white solid: mp 188–190 °C; IR 3600–2700, 3400, 1730 cm⁻¹; ¹H NMR (CDCl₃) 1.37 (t, 3 H, CH₃CH₂, *J* = 7.1), 3.10 (s, 6 H, N(CH₃)₂), 4.25 (q, 2 H, CH₂CH₃), 6.25–8.10 (m, 3 H, ArH), 10.5 (br s, 1 H, NH). A mixture of this benzoic acid (280 mg, 1.17 mmol) and EDCI (247 mg, 1.29 mmol) was stirred at room temperature for 1 h. The reaction mixture was diluted with methylene chloride and washed with water. The organic layer was dried over MgSO₄ and evaporated under reduced pressure to give a yellow solid. This solid was recrystallized from 20% methylene chloride/ether to give 46 (0.29 g, 75%): mp 196–198 °C; IR 1740 cm⁻¹; ¹H NMR (CDCl₃) 1.43 (t, 3 H, CH₃CH₂), 3.10 (s, 6 H, N(CH₃)₂), 4.45 (q, 2 H, CH₂CH₃), 6.45–7.96 (m, 3 H, ArH). Anal. (C₁₂H₁₄N₂O₃) C, H, N.

Method C. 2-(Ethylthio)-5-methyl-4H-3,1-benzoxazin-4-one (55). To a solution of 2-amino-6-methylbenzoic acid (25 g, 0.165 mol) and triethylamine (47.26 mL) in dry CH₂Cl₂ (250 mL) was added chlorotrimethylsilane (43 mL, 0.34 mol) in small portions, followed by thiophosgene (13.23 mL, 0.174 mol) added in a dropwise manner over a period of 0.5 h. After stirring for an additional 0.5 h, methanol was added and the mixture was evaporated to dryness. The residue was stirred in ice/water (500 mL) and the resulting yellow powder was collected by filtration. The solid was dissolved in methanol, treated with charcoal, and then with petroleum ether to give 1,2-dehydro-5-methyl-2-thio-4H-3,1-benzoxazin-4-one⁵³ as a white powder: mp 218–219 °C; IR 3600–3400 cm⁻¹; ¹H NMR (DMSO-*d*₆) 2.71 (s, 3 H, CH₃), 7.05–7.77 (m, 3 H, ArH), 13.2 (b, 1 H, NH). A solution of 1,2-dehydro-5-methyl-2-thio-4H-3,1-benzoxazin-4-one (19.3 g, 0.1 mol), iodoethane (16 mL, 0.2 mol), and potassium carbonate (27.46 g, 0.2 mol) in 200 mL of acetone was then refluxed for 2 h. The solid was precipitated by the addition of 400 mL of ice-cold water and was filtered. The insoluble solid was recrystallized from ether/pentane and gave 55 as a colorless solid (19.7 g, 93%): mp 65–66 °C; IR 1765 cm⁻¹; ¹H NMR (CDCl₃) 1.44 (t, 3 H, SCH₂CH₃), 2.75 (s, 3 H, CH₃), 3.15 (q, 2 H, SCH₂), 7.10–7.70 (m, 3 H, ArH). Anal. (C₁₁H₁₁NO₂S) C, H, N, S.

Method D. 2-sec-Butylamino-4H-3,1-benzoxazin-4-one (119). 2-Carbomethoxyphenyl isocyanate (250 mg, 1.41 mmol) was added to a solution of *sec*-butylamine (0.15 mL, 1.48 mmol) in 10 mL of dry THF. The resulting mixture was stirred for 8 h at room temperature and then evaporated to dryness. The resulting solid, after recrystallization from ether/petroleum ether yielded methyl 2-(3-*sec*-butylureido)benzoate (240 mg, 64%), mp 124–125 °C. This material was dissolved in concentrated sulphuric acid (2 mL) and stirred for 3 h. The reaction mixture was added slowly to an ice-cold stirred mixture of saturated sodium bicarbonate solution and ethyl acetate. After neutralization, the ethyl acetate layer was extracted, separated, and washed further with brine solution. The organic layer was dried over MgSO₄ and evaporated to give the title compound as a solid which was recrystallized from ether/petroleum ether (147 mg, 48%): mp 122–123 °C; IR 3290, 1740, 1635, 1600 cm⁻¹; ¹H NMR (CDCl₃) 1.0 (t, 3 H, CH₃CH₂), 1.3 (d, 3 H, CH₃CH); 1.6 (m, 2 H, CH₂), 4.0 (m, 1 H, CHN), 7.2, 7.68 (3 m, 4 H, ArH). Anal. (C₁₂H₁₄N₂O₂) C, H, N.

Method E. 5-Methyl-2-[(3-methylbutyl)amino]-4H-3,1-benzoxazin-4-one (114). A solution of methyl 2-amino-6-methylbenzoate (1.04 g, 6.25 mmol) in THF was added slowly to a solution of trichloromethyl chloroformate (1.01 g, 5.14 mmol) in THF. The solution was stirred under argon at room temperature for 24 h and then concentrated under reduced pressure in a well-ventilated fume-hood. A solution of 3-methylbutylamine (2 mL, 17.2 mmol) in THF (10 mL) was added and the mixture was stirred for 24 h, concentrated under reduced pressure, extracted with ethyl acetate and 5% HCl solution, and then washed

with water. The ethyl acetate layer was dried over MgSO₄ and evaporated, leaving a white solid, which upon purification by column chromatography (25% ethyl acetate/petroleum ether) gave methyl 2-[3-(3-methylbutyl)ureido]-6-methylbenzoate (0.75 g): mp 118–119 °C; IR 1712, 1658, 1650, 1610 cm⁻¹; ¹H NMR (DMSO-*d*₆) 0.90 (d, 6 H, 2CH₃), 1.40 (m, 3 H, CH₂CH), 2.20 (s, 3 H, ArCH₃), 3.13 (q, 2 H, CH₂N), 6.55 (t, 1 H, NH), 7.30 (m, 3 H, ArH), 8.05 (s, 1 H, NH); *R*_f = 0.45 (40% EtOAc/petroleum ether 30/60). 3-(3-methylbutyl)-5-methyl-1*H*,3*H*-quinazolin-2,4-dione (0.24 g) was also formed as a byproduct: mp 191–193 °C; ¹H NMR (DMSO-*d*₆) 0.92 (d, 6 H, 2 CH₃), 1.45 (m, 3 H, CH₂CH), 2.35 (s, 3 H, CH₃), 3.90 (t, 2 H, CH₂N), 7.50 (m, 3 H, ArH); *R*_f = 0.55 (40% EtOAc/petroleum ether 30/60). Concentrated H₂SO₄ was added to the benzoate and the resulting mixture was stirred for 2.5 h. The solution was added dropwise to a rapidly stirred mixture of ethyl acetate and supersaturated sodium bicarbonate solution. After neutralization, the mixture was extracted with ethyl acetate and washed further with water and brine solution. Solvent evaporation gave a solid which, when recrystallized from 3% ethyl acetate/petroleum ether, provided 0.4 g (31%) of 114: mp 99–100 °C; IR 3300, 1730, 1632, 1603 cm⁻¹; ¹H NMR (CDCl₃) 0.87 (d, 6 H, CH₃CHCH₃), 1.30–1.70 (m, 3 H, CH₂CH), 2.30 (s, 3 H, CH₃), 3.30 (m, 2 H, CH₂N), 7.00 (t, 1 H, ArH), 7.65 (m, 2 H, ArH), 7.93 (m, 1 H, NH). Anal. (C₁₄H₁₈N₂O₂) C, H, N.

Method F. *N*-(4-oxo-4H-3,1-benzoxazin-2-yl)-L-prolyl-phenylalaninamide (174). L-prolyl-L-phenylalaninamide (148 mg, 0.50 mmol) was treated with 2-carbomethoxyphenyl isocyanate (88.5 mg, 0.50 mmol), in a manner similar to that described under method D, to give *N*-[(2-carbomethoxyphenyl)carbamoyl]-L-prolylphenylalaninimide (170 mg, 78%), mp 75–77 °C. A solution of the urea (170 mg, 0.39 mmol) in methanol (2 mL) and 1 N NaOH (10 mL) was stirred for 2 days at room temperature. Methanol was evaporated and the resulting solution was partitioned between ethyl acetate and water. The aqueous layer was acidified with 6 M HCl to pH 2 and *N*-[(2-carboxyphenyl)carbamoyl]-L-prolyl-L-phenylalaninimide (140 mg, 85%), mp 191–192 °C, was isolated upon filtration. A solution of *N*-[(2-carboxyphenyl)carbamoyl]-L-prolyl-L-phenylalaninimide (140 mg, 0.33 mmol) in THF (50 mL) was mixed with 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide (98 mg, 0.50 mmol) and stirred for 48 h at room temperature. After evaporation of the solvent under reduced pressure, the residue was partitioned between ethyl acetate and water. The ethyl acetate layer was then dried over MgSO₄ and evaporated under reduced pressure to give a white solid, which upon recrystallization from ethyl acetate yielded 98 mg of *N*-[4-oxo-4H-3,1-benzoxazin-2-yl]-L-prolylphenylalaninamide (72%): mp 226–227 °C; IR 3200–3400, 3200, 3280, 1770, 1650, 1600 cm⁻¹; ¹H NMR (DMSO-*d*₆) 1.80 (m, 4 H, CH₂CH₂), 3.0 (m, 2 H, PhCH₂), 3.6 (m, 2 H, CH₂), 4.4 (m, 2 H, 2 NCHCO), 7.20 (m, 9 H, Ph, 2 H(ArH), CONH₂), 7.70, 7.90 (2 m, 2 H, ArH), 8.20 (d, 1 H, NH). Anal. (C₂₂H₂₂N₄O₄) C, H, N.

Method G. 2-Amino-5-ethyl-4H-3,1-benzoxazin-4-one (75). A solution of 6-ethylanthranilic acid (120 mg, 0.73 mmol) and sodium hydroxide (70 mg, 1.75 mmol) in 10 mL of water was stirred at room temperature. A solution of CNBr (150 mg, 1.37 mmol, 97% pure) in 10 mL of water was then added. After 16 h, the insoluble solid was filtered and washed vigorously with water. The resulting brown solid was dried under vacuum and further purified by column chromatography (ethyl acetate) to give a yellow solid. This material was mixed with ether and the insoluble white solid that resulted upon filtration yielded 74 mg of 75 (54%): mp 224–226 °C; IR 3400–3600, 1750, 1700, 1590, 1570 cm⁻¹; ¹H NMR (DMSO-*d*₆) 1.30 (t, 3 H, CH₃), 3.05 (q, 2 H, CH₂), 7.20 (d, 1 H, ArH), 7.50 (m, 2 H, ArH). Anal. (C₁₀H₁₀N₂O₂) C, H, N.

Method H. *N*-(5-Methyl-4-oxo-4H-3,1-benzoxazin-2-yl)-L-leucine Methyl Ester (154). A solution of 6-methylanthranilic acid (900 mg, 6.35 mmol) in toluene (30 mL) and triethylamine (1.77 mL, 12.6 mmol) was added to a solution of 1-benzotriazolecarboxylic acid chloride (2.1 g, 12.7 mmol) in toluene (30 mL) over a period of 30 min. The precipitate was filtered and the filtrate was reduced to one-half of its original volume, resulting in a solid, which was isolated by suction filtration. The combined residues were washed with water, dried under high vacuum, and recrystallized from chloroform to give 2-(1-benzotriazolyl)-5-

methyl-4*H*-3,1-benzoxazin-4-one (400 mg, 23%), mp 214–215 °C dec.¹⁹ This material could be used without further purification. To a solution of L-leucine methyl ester hydrochloride (130 mg, 0.72 mmol) in methylene chloride (30 mL) was added triethylamine (0.1 mL, 0.72 mmol). The mixture was stirred for 30 min, whereupon a solution of 2-(1-benzotriazolyl)-5-methyl-4*H*-3,1-benzoxazin-4-one (200 mg, 0.72 mmol) in methylene chloride (150 mL) was added. The reaction mixture was stirred at room temperature for 16 h, concentrated, and partitioned between ethyl acetate and water. The ethyl acetate layer was dried over MgSO₄ and evaporated to give a solid, which was further purified by thick-layer plate chromatography (20% ethyl acetate/petroleum ether 30/60, *R_f* = 0.8). Recrystallization from pentane gave 75 mg (34%) of 154: mp 127–128 °C; IR 3300, 2960, 1750, 1730, 1640, 1595 cm⁻¹; ¹H NMR (CDCl₃) 1.0 (d, 6 H, 2 CH₃), 1.7 (m, 3 H, CH₂CH), 2.7 (s, 3 H, Ar-CH₃), 3.8 (s, 3 H, OMe), 4.7 (m, 1 H, CHCOOMe), 5.1 (m, 1 H, NH), 6.9–7.6 (m, 3 H, ArH). Anal. (C₁₆H₂₀N₂O₄) C, H, N.

Method I. 2-(Diisopropylamino)-4*H*-3,1-benzoxazin-4-one (135). Isatoic anhydride (2 g, 12.26 mmol) was added to a solution of diisopropylamine (30.65 mL, 4 M aqueous solution) and the mixture was stirred for 2 days and then diluted with water (50 mL). The solution was acidified with 6 M H₂SO₄, whereupon a yellowish precipitate formed, which was filtered. This material was dissolved in ethyl acetate and dried over MgSO₄. Following evaporation of solvent, a solid was obtained that was cyclized in concentrated H₂SO₄ (in the same manner as that described under method D) over a period of 5 h at room temperature to give 540 mg (18%) of the title compound after workup: mp 84–86 °C; IR 1750, 1585, 1560 cm⁻¹; ¹H NMR (CDCl₃) 1.36 (d, 12 H, 2CH₂CHCH₃), 4.29 (h, 2 H, 2 CH), 7.10 (m, 2 H, ArH), 7.59 (m, 1 H, ArH), 8.0 (m, 1 H, ArH). Anal. (C₁₄H₁₈N₂O₂) C, H, N.

Method J. 5,7-Dimethyl-2-(isopropylamino)-4*H*-3,1-benzoxazin-4-one (86). To a solution of thallium trifluoroacetate (2.28 g) in trifluoroacetic acid (2 mL) and THF (10 mL) was added a solution of 3,5-dimethyl-1-(3-isopropylureido)benzene (0.99 g). The solution was stirred in the dark for 16 h and evaporated to dryness. The residual oil was azeotroped with 1,2-dichloroethane and evaporated to dryness. The residue was pumped under high vacuum for 1 h. A solution of this residue in THF (25 mL) was added to a suspension of lithium chloride (0.365 g), palladium chloride (124 mg, 60% pure from Alfa Chemicals), and magnesium oxide (0.338 g) in dry THF (20 mL). The flask and its contents were flushed with carbon monoxide. The solution was stirred in the dark under 1 atm of carbon monoxide for 16 h, filtered through Celite, and evaporated to dryness. The residue was chromatographed on silica gel twice (20% ethyl acetate/petroleum ether 30/60, *R_f* = 0.7). The product was recrystallized from ethyl acetate and petroleum ether: mp 215–218 °C; IR (KBr) 3280, 1730, 1640, 1605 cm⁻¹; ¹H NMR (DMSO-*d*₆) 1.19 (d, 6 H, CH₃CHCH₃), 2.30 (s, 3 H, CH₃Ar), 2.60 (s, 3 H, CH₃Ar), 3.90 (m, 1 H, CHNH), 6.80 (d, 2 H, H-6, H-8, *J* = 4), 7.75 (d, 1 H, NH). Anal. (C₁₃H₁₈N₂O₂) C, H, N.

2-Ethoxy-5-propyl-4*H*-3,1-benzoxazin-4-one (42). A solution of 44 (270 mg, 1.17 mmol) in 5 mL of THF and 5 mL of 2% NaOH was stirred at room temperature for 6 h. THF was removed under reduced pressure. The aqueous solution was acidified to pH 2 with 6 M HCl and extracted with ethyl acetate. The organic layer was dried over MgSO₄ and evaporated to an oil. A solution of this oil in ethanol was hydrogenated at 35 psi H₂ to yield 194 mg of 2-[(ethoxycarbonyl)amino]-6-propylbenzoic acid. A solution of the above acid in CH₂Cl₂ was mixed with EDCI (184 mg, 0.96 mmol) and stirred at room temperature for 24 h. The reaction mixture was extracted with water and the organic layer was then separated, dried over Na₂SO₄, and evaporated to an oil. Purification of the oil by column chromatography yielded 150 mg (87%) of 42 as an oil: IR 1765, 1640, 1600, 1570 cm⁻¹; ¹H NMR (CDCl₃) 0.89 (t, 3 H, CH₃), 1.43 (t, 3 H, CH₃), 1.60 (m, 2 H, CH₂), 3.11 (t, 2 H, CH₂), 4.94 (q, 2 H, CH₂CH₂O), 7.16 (m, 2 H, ArH), 7.55 (m, 1 H, ArH); MS 233 (M⁺), 205, 187, 172, 159.

6-(Acetylamino)-2-(ethylthio)-4*H*-3,1-benzoxazin-4-one (58). 5-Amino-2-nitrobenzoic acid (1.27 g, 5.23 mmol, 75% pure from Fluka) was stirred in acetic anhydride (10 mL) in the presence of triethylamine (2 mL) at room temperature for 1 h. The precipitate was filtered and then washed with ether and methylene chloride to give 5-(acetylamino)-2-nitrobenzoic acid

triethylamine salt (0.91 g, 56%), mp 109–112 °C. This material (500 mg, 1.61 mmol) was dissolved in ethanol. Cyclohexene (1.25 mL) and 10% Pd/C (250 mg) were added, and the resulting mixture was refluxed for 1 h. The catalyst was removed by filtration through Celite. The combined filtrate upon evaporation provided 5-(acetylamino)anthranilic acid as a syrup (380 mg). This crude anthranilic acid was converted to 6-(acetylamino)-2-(ethylthio)-4*H*-3,1-benzoxazin-4-one (58) according to the procedure described in method C: mp 194–195 °C; IR (KBr) 3360, 1740, 1690 cm⁻¹; ¹H NMR (CDCl₃) 1.45 (t, 3 H, CH₃CH₂), 2.20 (s, 3 H, COCH₃), 3.18 (q, 2 H, CH₂CH₃), 7.37–8.28 (m, 3 H, ArH). Anal. (C₁₂H₁₂N₂O₃S) C, H, N, S.

7-(Ethylamino)-2-(isopropylamino)-4*H*-3,1-benzoxazin-4-one (98). Sodium acetate (2 g, 24.4 mmol) and acetaldehyde (15 mL, 0.27 mmol) were added to a solution of ethyl 2-(3-isopropylureido)-4-nitrobenzoate¹⁹ (220 mg, 0.75 mmol) in methanol. Raney Ni was added to this solution and the mixture was hydrogenated at 50 psi H₂ for 16 h. The catalyst was removed by suction filtration through Celite and destroyed with water. The filtrate was evaporated to give an oil, which was further extracted with ethyl acetate and 10% HCl (2 × 50 mL). The aqueous extract was basified with NaHCO₃ and reextracted with ethyl acetate. The ethyl acetate layer was dried over MgSO₄ and was then evaporated, leaving an oil, which upon purification by column chromatography (30% ethyl acetate/petroleum ether) gave ethyl 2-(3-isopropylureido)-4-(ethylamino)benzoate as an oil (152 mg, *R_f* = 0.5): IR 3300, 1660, 1620, 1585, 1530 cm⁻¹; ¹H NMR (CDCl₃) 1.10–1.40 (m, 12 H, CH₃CHCH₃, CH₂CH₂, CH₂CH₂O), 3.20 (q, 2 H, CH₂N), 3.75–4.20 (m, 3 H, NHCH, NH), 4.25 (q, 2 H, OCH₂CH₃), 4.80 (br d, 1 H, NH), 6.10 (dd, 1 H, ArH, *J* = 2.4, 8.9), 7.78 (d, 1 H, ArH, *J* = 8.9), 7.81 (d, 1 H, ArH, *J* = 2.4). This oil was mixed with concentrated H₂SO₄ (2 mL) and stirred at room temperature for 2 h. The mixture was worked up in the same manner as that described under method D to give the title compound (110 mg, 59%) as a white solid: mp 175–176 °C; IR 3420, 3280, 1710, 1630 cm⁻¹; ¹H NMR (CDCl₃) 1.26 (d, 6 H, CH₃CHCH₃), 1.28 (t, 3 H, CH₂CH₃), 3.22 (m, 2 H, CH₂N), 4.20 (m, 2 H, CHNH), 4.50 (m, 1 H, NH), 6.35 (m, 2 H, ArH), 7.77 (apparent d, 1 H, ArH). Anal. (C₁₃H₁₇N₃O₂) C, H, N.

7-(Diethylamino)-2-(isopropylamino)-4*H*-3,1-benzoxazin-4-one (101). A solution of methyl 2-(3-isopropylureido)-4-nitrobenzoate¹⁹ (300 mg, 1.07 mmol) in acetaldehyde (15 mL, 0.268 mol) and methanol was hydrogenated over Raney Ni for 16 h at 50 psi. The Raney Ni catalyst was removed by filtration through Celite and immediately destroyed with water. The filtrate was evaporated to an oil and extracted with ethyl acetate and 8% HCl (2 × 50 mL). The acid extract was neutralized with NaHCO₃ and extracted with ethyl acetate. The organic layer was dried over MgSO₄ and then evaporated to give an oil, which upon purification by column chromatography (35% ethyl acetate/petroleum ether) gave 180 mg (55%) of methyl 4-(diethylamino)-2-(3-isopropylureido)benzoate: mp 126–127 °C. Anal. (C₁₆H₂₅N₃O₃) C, H, N. This solid (90 mg, 0.29 mmol) was mixed with 2 mL of concentrated H₂SO₄ and stirred at room temperature for 2 h. The mixture was worked up in the same manner as that described under method D to give 60 mg (74%) of 101: mp 182–184 °C; ¹H NMR (CDCl₃) 1.22, 1.26 (d and t, 12 H, CH₃CHCH₃, 2 CH₃), 3.43 (q, 4 H, 2 CH₂N), 4.69 (m, 1 H, CH), 4.50 (m, 1 H, NH), 6.22 (d, 1 H, ArH, *J* = 2.5), 6.50 (dd, 1 H, ArH), 7.81 (d, 1 H, ArH, *J* = 8.9). Anal. (C₁₅H₂₁N₃O₂) C, H, N. In a similar manner, compounds 91, 94, 95, and 100 were prepared from their corresponding precursors.¹⁹

2-[(Isobutoxycarbonyl)methylamino]-4*H*-3,1-benzoxazin-4-one (147). Isobutyl chloroformate (0.64 mL, 4.68 mmol) was added to a stirred mixture of NaHCO₃ (0.2 g, 2.38 mmol), 4-(dimethylamino)pyridine (0.28 g, 2.29 mmol), and 2-(methylamino)-4*H*-3,1-benzoxazin-4-one (76; 0.40 g, 2.27 mmol) in 15 mL of THF and 20 mL of CH₂Cl₂. The mixture, after refluxing for 4 h, was cooled and the insoluble material was filtered. The filtrate was mixed with petroleum ether and once again filtered. Evaporation of the filtrate gave a solid, which was recrystallized from petroleum ether and yielded 0.23 mg (18%) of 147: mp 44–45 °C; IR 1775, 1722, 1600, 1570 cm⁻¹; ¹H NMR (CDCl₃) 1.00 (d, 6 H, CH₃CHCH₃), 2.02 (m, 1 H, CH₃CHCH₃), 3.45 (s, 3 H, NCH₃), 4.06 (d, 2 H, CH₂O), 7.20–7.80 (m, 3 H, ArH), 8.15 (m, 1 H, ArH). Anal. (C₁₄H₁₆N₂O₄) C, H, N.

N-(7-Amino-5-ethyl-4-oxo-4*H*-3,1-benzoxazin-2-yl)-*L*-prolyl-*L*-leucinamide (172). A solution of ethyl 2-amino-6-ethyl-4-nitrobenzoate (400 mg, 1.79 mmol) in 5 mL of ethyl acetate was added dropwise to a solution of trichloromethyl chloroformate (0.35 g, 1.78 mmol) in 10 mL of ethyl acetate. The solution was stirred at room temperature for 2 h, refluxed for 3 h, and then cooled. This solution was added slowly to a solution of *L*-prolyl-*L*-leucinamide (0.36 g, 1.78 mmol) and triethylamine (0.75 mL, 5.38 mmol) in 10 mL of THF. After 3 h, the solvent was evaporated under reduced pressure and the residue was extracted with ethyl acetate. The organic layer, which was washed in turn with water and 6 M HCl solution, was then dried over MgSO₄ and evaporated to give an oil. Purification of this oil by column chromatography (50% ethyl acetate/petroleum ether to ethyl acetate) gave 350 mg (40%) of *N*-[[2-(carboethoxy)-3-ethyl-5-nitrophenyl]carbamoyl]-*L*-prolyl-*L*-leucinamide. A solution of this urea was mixed with 10 mL of ethanol and 10 mL of 2.5% NaOH and stirred at room temperature for 6 h. The solution was acidified with 6 M HCl and extracted with ethyl acetate. The organic layer was dried over MgSO₄ and evaporated to give crude *N*-[(2-carboxy-3-ethyl-5-nitrophenyl)carbamoyl]-*L*-prolyl-*L*-leucinamide as an oily solid. Without further purification, a solution of this acid and EDCI (210 mg, 1.01 mmol) in 10 mL of dry THF was stirred at room temperature for 16 h. The solution was evaporated to dryness and the residue was partitioned between ethyl acetate and water. The organic layer, after drying over MgSO₄, was evaporated to a solid, which upon recrystallization gave 195 mg (61%) of *N*-(5-ethyl-7-nitro-4-oxo-4*H*-3,1-benzoxazin-2-yl)-*L*-prolyl-*L*-leucinamide as a yellow solid: mp 240–244 °C; IR 3410, 3300, 3200, 2960, 1765, 1665 cm⁻¹; ¹H NMR (DMSO-*d*₆) 0.81, 0.91 (2 d, 6 H, CH₃CHCH₃), 1.20 (t, 3 H, CH₃CH₂), 1.50 (m, 3 H, CH₂CH), 1.95 (m, 4 H, CH₂CH₂), 3.15 (q, 2 H, ArCH₂CH₃), 3.61 (m, 2 H, CH₂N), 4.10–4.60 (m, 2 H, NCH, NCHCO), 6.9–7.3 (m, 2 H, CONH₂), 7.53 (br s, 1 H, ArH), 7.72 (d, 1 H, ArH, *J* = 1.95), 8.20 (d, 1 H, CONH, *J* = 8.3); MS (70 eV) 445 (M⁺), 428, 415, 316, 288. A solution of *N*-(5-ethyl-7-nitro-4-oxo-4*H*-3,1-benzoxazin-2-yl)-*L*-prolyl-*L*-leucinamide (100 mg, 0.22 mmol) in 10 mL of THF and 100 mL of cyclohexane was stirred at room temperature under argon. After 150 mg of 10% Pd/C was added portionwise, the solution was refluxed for 2.5 h and then cooled. The catalyst was filtered and the filtrate was evaporated to give 66 mg (71%) of 172 as a white solid: mp 148–150 °C; IR 3480, 3420, 3360, 3230, 1740, 1690, 1600 cm⁻¹; ¹H NMR (CDCl₃) 0.86 (2 d, 6 H, CH₃CHCH₃), 1.18 (t, 3 H, CH₃CH₂), 1.50–1.80 (m, 3 H, CH₂CH), 2.00–2.40 (m, 4 H, CH₂CH₂), 3.00 (q, 2 H, ArCH₂CH₃), 3.60–3.85 (m, 2 H, CH₂N), 4.30–4.70 (m, 4 H, NCH, NCHCO, ArNH₂), 5.90 (br s, 1 H, NH), 6.20 (d, 1 H, ArH, *J* = 2.2), 6.28 (d, 1 H, ArH, *J* = 2.2), 6.90 (br s, 1 H, NH), 7.20 (d, 1 H, NHCO); MS (70 eV) 415 (M⁺), 372, 287, 259, 189.

Alkaline Hydrolysis. Alkaline hydrolysis rate constants of 4*H*-3,1-benzoxazin-4-ones were determined at several pH values at 25 °C by following the disappearance of the long-wavelength UV chromophore (λ_{max} typically 300–345 nm) in the manner described previously.⁵⁴

Enzymatic Studies. Human sputum elastase (HSE) was purchased from Elastin Products Co., Inc. (Pacific, MO). 7-[(Methoxysuccinyl)alanylalanylprolylvalinamido]-4-methylcoumarin was obtained from Peninsula Laboratories, Inc. (Belmont, CA). 7-[(Methoxysuccinyl)alanylalanylprolylvalinamido]-4-(trifluoromethyl)coumarin was purchased from Enzyme Systems Products (Livermore, CA). (Methoxysuccinyl)alanylalanylprolylvaline *p*-nitroanilide was obtained from Calbiochem (La Jolla, CA). Gel filtration media and PD-10 columns were acquired from Pharmacia (Dorval, Quebec). Soybean trypsin inhibitor (SBTI) was purchased from Sigma (St. Louis, MO). All methods of analysis were as indicated previously.^{6c}

HL elastase was prepared from human leukocytes as published previously.^{6c,6b,55} Enzyme inhibition was assayed by the progress curve method (pH 7.8, 25 °C) as described previously.^{6c,10d,11b} A few compounds, as noted in Table III, were assayed with the

chromogenic substrate (methoxysuccinyl)alanylalanylprolylvaline *p*-nitroanilide by monitoring Δ*A* at 410 nm. Data were fit to [product] = *Ae*^{-*kt*} + *B* + *Ct*. Linear regression of the observed *k* vs [I] gave *k*_{on}. Regression of the steady-state rates vs [I] with the program VKKI (a special case of the program COMP⁵⁶ for [S] ≪ *K*_m) gave *K*_i. The deacylation rate, *k*_{off}, was calculated as *k*_{on}*K*_i. In a few cases, *k*_{off} was measured directly by isolating the acyl enzyme using a PD-10 column at low pH.⁵⁷ Deacylation was monitored by the reappearance of enzyme activity upon dilution (1 in 40) of acyl enzyme into assay buffer containing fluorogenic substrate.

Deacylation products for 2-(ethylamino)benzoxazinone **79** with HLE were determined as previously described¹¹ by analysis of the fluorescence spectrum after exhaustive incubation of enzyme with inhibitor. Deacylation products for 2-*n*-propylbenzoxazinone **11** and 2-ethoxybenzoxazinone **34** with elastase were identified by their UV absorption spectra as determined from UV difference spectra and by HPLC analyses. Stoichiometric amounts of HSE and inhibitor, **11** or **34** (each at a final concentration of 12.5 μM, pH 7.8 and 25 °C), were placed in separate compartments of split cuvettes and then a base line difference spectrum was obtained. The sample cuvette was then mixed, and a difference spectrum and an HPLC analysis of the mixture were immediately obtained. The difference spectrum for **11** showed a Δλ_{max} = 309 nm, similar to the model base-hydrolysis difference spectrum where Δλ_{max} = 313 nm. The corresponding difference spectra for **34** showed Δλ_{max} = 319 nm for both the putative acyl enzyme and the base-hydrolysis product. Immediately following these determinations and before significant deacylation had occurred, 4 equiv of SBTI were added to trap the enzyme to approximate single-turnover conditions. Difference spectra and HPLC analyses were obtained 10–16 min later. Products were identified by comparison to spectral data and retention times of the authentic benzoxazinones **11** and **34** and their base-hydrolysis products, i.e. the amido- and carbamoylbenzoate, respectively. The product of deacylation of **34** was identified as the carbamoylbenzoate. 2-*n*-Propylbenzoxazinone itself appeared to be the exclusive product of deacylation for compound **11**, on the basis of the regeneration of the starting base line difference spectrum concomitant with the appearance of approximately 1 equivalent of benzoxazinone as determined by HPLC. To provide additional support for this result, HSE (25 μM) was mixed with 4 equiv of **11** to fully inhibit the enzyme. This mixture was chromatographed on a PD-10 column equilibrated and eluted with pH 4.0 buffer (50 mM NaOAc, 1 M NaCl, 0.1 % Brij) to separate the protein (presumably the acyl enzyme) from excess inhibitor. At pH 4.0 acyl enzyme is stable⁵⁷ and excess benzoxazinone is rapidly hydrolyzed (*t*_{1/2} = 3.6 min for **11** in elution buffer). An aliquot of the protein fraction brought to pH 7.8 with an equal volume of K₂HPO₄ solution (conditions which allow deacylation) and then analyzed by UV and HPLC showed that approximately 1 equiv of benzoxazinone **11** was thus formed clearly as a consequence of deacylation.

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