Irreversible Enzyme Inhibitors. CVII.[1] Proteolytic Enzymes. II.[2] Bulk Tolerance within Chymotrypsin–Inhibitor Complexes

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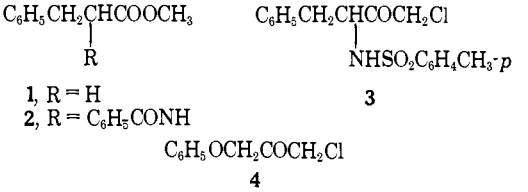
Fifty-eight compounds related to phenoxyacetone and phenoxyacetamide have been measured as inhibitors of α-chymotrypsin. Substitution of 3-chloro, 2,3-dichloro, or 3,4-dichloro groups on phenoxyacetone increased binding of phenoxyacetone 11–23-fold; bulky groups such as methyl or ethyl were detrimental to binding, but flat hydrocarbon substituents such as *o*-phenyl or benzo were beneficial to binding. Anilides and N-benzylamides of phenoxyacetic acid showed enhanced binding over N-methylphenoxyacetamide; this N-phenyl binding to the enzyme was also enhanced by nitro or chloro groups. N-(*p*-Chlorophenyl) derivatives of 3,4-dichlorophenoxyacetamide (**59**) and 3-chlorophenoxyacetamide (**58**) and N-(*m*-chlorobenzyl) (**61**) and N-(*p*-chlorobenzyl) (**60**) derivatives of 3,4-dichlorophenoxyacetamide were good inhibitors of chymotrypsin; **58–61** were complexed one to two times better than the substrate, N-glutaryl-L-phenylalanine-*p*-nitroanilide, and 55–110-fold better than phenoxyacetone, the compound with which this study was started. Since there is bulk tolerance for these N-phenyl moieties within the enzyme–inhibitor complex, placement of appropriately dimensioned leaving groups on these N-phenyl moieties make likely candidates for active-site-directed irreversible inhibitors of chymotrypsin that can attack outside of the active site.

A series of proteolytic enzymes in blood serum are involved in blood clotting, blood clot solution, histamine release, and antibody-augmented foreign-cell rejection; such areas of research[4] are important in the cardiovascular and organ transplant problems. At least two clear classes of these enzymes exist: (a) those that are "chymotryptic" and prefer to hydrolyze the polypeptide bonds of arylamino acids, and (b) those that are "tryptic" and prefer to hydrolyze the polypeptide bonds of basic amino acids. Selective reversible inhibition of one of these myriad of serum proteolytic enzymes for a given disease state is not apt to be achieved;[4] also irreversible inhibitors of the endo type[5,6] that attack within the active site are not sufficiently selective.[4] In contrast, active-site-directed irreversible inhibitors[5,6] of the exo type that operate by complexing to the active site, but covalently link to the enzyme outside of the active site,[7] have an extra dimension of specificity[8] that could lead to selective inhibition of only one of these many serum proteases.

The successful design of an active-site-directed irreversible inhibitor that operates by the exo mechanism is considerably more complicated than the design of a reversible inhibitor or an endo-type irreversible inhibitor. Success has so far only been achieved when a definite *modus operandi* is followed:[5] (a) the binding points necessary for reversible inhibition are determined (phase I), (b) bulky groups on the inhibitor that are tolerated by the enzyme (phase II), and (c) placement of a proper leaving group in the bulk tolerance area of the inhibitor (phase III). Since to our knowledge no exo-type irreversible inhibitor for a chymotryptic enzyme has yet been designed, we have initiated work on chymotrypsin toward this objective; the results of phase I and II studies with chymotrypsin are the subject of this paper.

Even though chymotrypsin has been one of the most extensively studied enzymes,[9] these studies did not have the continuity necessary for the design of an exo-type irreversible inhibitor.[10] The work described here is based on the following observations.[9,10] (a) Methyl hydrocinnamate (**1**)[11] binds to chymotrypsin as well as methyl N-benzoyl-L-phenylalaninate (**2**),[12] but the latter has a much superior velocity as a substrate. (b) Both 1-chloro-3-phenoxyacetone (**4**)[13] and L-1-chloro-4-phenyl-3-tosylamido-2-butanone (**3**) (TPCK)[14] inactivate chymotrypsin by the endo type of active-site-directed mechanism, each attacking histidine-57, but with **3** reacting considerably faster. Therefore, com-



1, R = H
2, R = C_6H_5CONH

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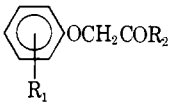

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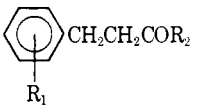

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pounds of types **5** and **6** were considered for phase I and II studies.

The first consideration was the nature of the bridge between the benzene ring and the carbonyl function. In addition to the $-OCH_2-$ bridge of **5** and the $-CH_2CH_2-$ bridge of **6**, the $-CH_2NH-$, $-CH{=}CH-$, $-SCH_2-$, and $-CH_2-$ bridges were investigated (Table I); in addition to optimum binding, it would be of

(1) This work was supported in part by Grant CA-08695 from the National Cancer Institute, U. S. Public Health Service.

(2) For the previous paper of this series see B. R. Baker and E. H. Erickson, *J. Med. Chem.*, **10**, 1123 (1967).

(3) NDEA predoctoral fellow.

(4) For a discussion and key references see ref 2.

(5) B. R. Baker, "Design of Active-Site-Directed Irreversible Enzyme Inhibitors. The Organic Chemistry of the Enzymic Active-Site," John Wiley and Sons, Inc., New York, N. Y., 1967.

(6) B. R. Baker, *J. Pharm. Sci.*, **53**, 347 (1964), a review.

(7) The active site is defined as that part of the enzyme necessary for complexing the substrate plus that part necessary for the catalytic action.

(8) See ref 5, Chapter IX.

(9) For a review of the literature on reversible binding to chymotrypsin pertinent to the design of active-site-directed irreversible inhibitors, see ref 5, Chapter III.

(10) Reference 5, Chapter VIII.

(11) J. E. Snoke and H. Neurath, *Arch. Biochem.*, **21**, 351 (1949).

(12) S. Kaufman and H. Neurath, *ibid.*, **21**, 437 (1949).

(13) K. J. Stevenson and L. B. Smillie, *J. Mol. Biol.*, **12**, 937 (1965).

(14) (a) G. Schoellman and E. Shaw, *Biochemistry*, **2**, 252 (1963); (b) E. B. Ong, E. Shaw, and G. Schoellman, *J. Biol. Chem.*, **240**, 694 (1965).

Table I

Inhibition[a] of Chymotrypsin by R_1COR_2

Compd	R_1	R_2	mM concn	% inhib	Est'd I_{50}[b]	$[I]/[S]_{50}$[c]	Source
7	$C_6H_5CH_2CH_2$	OC_2H_5	2[d]	33	4	20	Eastman
8	$C_6H_5CH{=}CH$	OC_2H_5	2[d]	36	4	20	Eastman
9	$C_6H_5CH_2NH$	OC_2H_5	15	50	15	75	*e*
10	$C_6H_5CH_2CH_2$	NEt_2	17	50	17	85	*f*
11	$C_6H_5CH_2CH_2$	$N(CH_3)C_6H_5$	3[d]	27	8	40	*g*
12	$C_6H_5OCH_2$	$N(CH_3)C_6H_5$	3.5	50	3.5	17	Expd
13	$C_6H_5CH_2CH_2$	CH_3	15	50	15	75	Columbia
14	$C_6H_5OCH_2$	CH_3	12	50	12	60	Eastman
15	$C_6H_5CH_2$	CH_3	12[d]	38	19	95	Eastman
16	p-$NO_2C_6H_4CH{=}CH$	CH_3	1.2[d]	25	3.6	18	*h*, *i*
17	p-$NO_2C_6H_4CH{=}CH$	H	0.75[d]	27	2.0	10	*j*
18	p-$NO_2C_6H_4OCH_2$	CH_3	4.0	50	4.0	20	*h*, *k*
19	$C_6H_5SCH_2$	CH_3	5.0	50	5.0	25	*l*

[a] Assayed with 0.2 m*M* N-glutaryl-L-phenylalanine *p*-nitroanilide (GPNA) in 0.05 *M* Tris buffer (pH 7.4) containing 10% DMSO. [b] I_{50} = m*M* concentration for 50% inhibition. [c] Ratio of concentration of inhibitor to 0.2 m*M* GPNA giving 50% inhibition. [d] Maximum solubility. [e] Prepared according to C. W. Whitehead and J. T. Traverso, *J. Am. Chem. Soc.*, **77**, 5872 (1955). [f] Prepared according to N. Maxim, *Bull. Soc. Chim. France*, **39**, 1024 (1926). [g] Prepared according to J. S. Pizey and R. L. Wain, *J. Sci. Food Agr.*, **10**, 577 (1959). [h] Prepared by M. A. Johnson in this laboratory. [i] Prepared according to S. S. Novikov and G. A. Shvekhgeimer, *Izv. Akad. Nauk SSSR, Otd. Khim. Nauk*, 2061 (1960). [j] Prepared according to S. G. Waley, *J. Chem. Soc.*, 2008 (1948). [k] Prepared according to ref 18a. [l] Prepared according to J. E. Banfield, W. Davies, N. W. Gamble, and S. Middleton, *J. Chem. Soc.*, 4791 (1956).

value to find the most easily synthesized bridge for the further structural variants of **5** and **6**.

Since hydrocinnamic esters are slow substrates, they can be measured as inhibitors in the presence of a fast substrate. Ethyl hydrocinnamate (**7**) (Table I) was complexed 1/20 as well as the substrate, N-glutaryl-L-phenylalanine-*p*-nitroanilide (GPNA).[15] Since GPNA has $K_m = 6 \times 10^{-4}$ *M*,[15] **2** has $K_m = 46 \times 10^{-4}$ *M*,[12] and **1** has $K_m = 39 \times 10^{-4}$ *M*,[11] the observed binding of **7** is in reasonably good agreement. Since ethyl cinnamate (**8**) binds the same as **7**, it is clear that the $-CH{=}CH-$ bridge is as good as the $-CH_2CH_2-$ bridge; furthermore, the latter bridge has the staggered conformation when complexed to the enzyme. In contrast, the $-CH_2NH-$ bridge of **9** was only one-quarter as effective as the $-CH_2CH_2-$ bridge of **7**; this result can be attributed to either the decreased binding of the carbonyl group of **9** or that the NH group of **9** is forced into a hydrophobic region with resultant repulsion.

That tertiary amides of hydrocinnamate can complex to the enzyme is shown with **10** and **11**. Therefore, identical tertiary amide groups can be used for comparison of the bridges. Note that in the comparison of the pair, **11** and **12**, the $-OCH_2-$ bridge is about twice as effective as the $-CH_2CH_2-$ bridge; however, in comparison of the methyl ketones **13** and **14** or **16** and **18** the bridges are essentially equivalent.

Three other points emerge from Table I. The $-CH_2-$ bridge of **15** is about as effective as the $-CH_2CH_2-$ bridge of **13**. The second point is that the aldehyde carbonyl of **17** is about twice as effective as the ketone carbonyl of **16**; however, the synthesis of aldehydes of type **17** is less convenient than synthesis of methyl ketones of type **16**. The third point is that the $-SCH_2-$ bridge of **19** is about twice as effective as the $-OCH_2-$ bridge of **14**.

The most convenient bridge for study of binding and bulk tolerance is the $-OCH_2-$ bridge of compounds of types **12** and **14** since structural variants can be obtained merely by using one of many commercially available substituted phenols.

(15) B. E. Erlanger, F. Edel, and A. G. Cooper, *Arch. Biochem. Biophys.*, **115**, 206 (1966).

Table II

Inhibition[a] of Chymotrypsin by $RC_6H_4OCH_2COCH_3$

Compd	R	mM concn	% inhib	Est'd I_{50}[b]	$[I]/[S]_{50}$[c]
14	H	12	50	12	60
18	p-NO_2	4.0	50	4.0	20
20	m-NO_2	4.9	50	4.9	25
21	o-NO_2	3[d]	31	6.0	30
22	p-CN	10[d]	23	34	170
23	m-CN	22	50	22	110
24	p-COOH	10	0	>50[e]	>250
25	p-AcNH[f]	20[d]	0	>100[e]	>500
26	p-CH_3O	17	50	17	85
27	p-CH_3	19	50	19	95
28	m-CH_3[g]	12	50	12	60
29	p-Cl[h]	3.9	50	3.9	20
30	m-Cl	1.1	50	1.1	5.5
31	o-C_6H_5	0.75[d]	32	1.6	8.0
32	p-C_6H_5	0.10[d]	0	>0.5[e]	>2.5
33	m-C_6H_5	6[d]	0	>30[e]	>150
34	3,5-$(CH_3)_2$	5.0[d]	0	>25[e]	>120
35	3,4-Benzo[i]	1.8	50	1.8	9.0
36	2,3-Benzo[g]	1	18	~5	~25
37	3,4-Cl_2	0.75	50	0.75	3.7
38	2,3-Cl_2	0.52	50	0.52	2.6

[a] Assayed with 0.2 m*M* GPNA in 0.05 *M* Tris buffer (pH 7.4) containing 10% DMSO. [b] I_{50} = m*M* concentration for 50% inhibition. [c] Ratio of concentration of inhibitor to 0.2 m*M* substrate giving 50% inhibition. [d] Maximum solubility. [e] Since 15% inhibition is readily detectable, the I_{50} is greater than five times the concentration measured. [f] Prepared by M. A. Johnson in this laboratory. [g] Prepared according to ref 18b. [h] Prepared according to ref 18c. [i] Prepared according to ref 18d.

Phenoxyacetone (**14**) was selected for the next study, namely, the effect of substituents on the benzene ring on binding to chymotrypsin (Table II). Additional hydrocarbon interaction[16] was observed with the α-naphthyl (**36**) and β-naphthyl (**35**) ketones. The latter was complexed about seven times better than phenoxyacetone (**14**). A similar increment was seen with the *o*-phenyl substituent of **31**, but the *para* isomer (**32**) was too insoluble to measure. No further hydrocarbon interaction was seen with the *m*-methyl (**28**)

(16) For a review on the possible modes of complexing between inhibitors and enzymes, see ref 5, Chapter II.

TABLE III

INHIBITION[a] OF CHYMOTRYPSIN BY $R_1C_6H_4OCH_2COR_2$

Compd	R_1	R_2	mM conen	% inhib	Estd I_{50}[b]	$([I]/[S])_{0.5}$[c]
14	H	CH_3	12	50	12	60
39	H	C_6H_5[d]	1.0	15	~5	~25
18	p-NO_2	CH_3	4.0	50	4.0	20
40	p-NO_2	C_6H_5	0.2[e]	0	>1.0[f]	>5
24	p-COOH	CH_3	10	0	>50[f]	>250
41	p-COOH	C_6H_5	4.0	0	>20[f]	>100
26	p-CH_3O	CH_3	17	50	17	85
42	p-CH_3O	C_6H_5	0.75[e]	29	1.9	9.5
29	p-Cl	CH_3	3.9	50	3.9	19
43	p-Cl	C_6H_5	0.1[e]	15	~0.5	~2.5
44	$HOOC(CH_2)_n$	C_6H_5				

[a] Assayed with 0.2 mM GPNA in 0.05 M Tris buffer (pH 7.4) containing 10% DMSO. [b] I_{50} = mM concentration for 50% inhibition. [c] Ratio of concentration of inhibitor to 0.2 mM substrate giving 50% inhibition. [d] Prepared according to ref 19a. [e] Maximum solubility. [f] Since 15% inhibition is readily detected, the I_{50} is greater than five times the concentration measured.

TABLE IV

INHIBITION[a] OF CHYMOTRYPSIN BY 

Compd	R_3	NR_1R_2	mM conen	% inhib	Estd I_{50}[b]	$([I]/[S])_{0.5}$[c]
12	H	$N(CH_3)C_6H_5$	3.5	50	3.5	50
45	H	NHC_6H_5	2.0[d]	42	2.6	13
46	H	$NHCH_2C_6H_5$	1.1	50	1.1	5.5
47	H	$NH(CH_2)_2C_6H_5$	1.0[d]	30	2.3	11
48	H	$NHC_6H_4NO_2$-p	0.030[d]	0	>0.15[e]	>0.7
49	H	$N(CH_3)C_6H_4NO_2$-p	0.60[d]	33	1.2	6.0
50	H	NHC_6H_4Cl-p	0.13[d]	31	0.30	1.5
51	H	NHC_6H_4Br-p	0.20[d]	36	0.35	1.7
52	H	NHC_6H_4Cl-m	0.30[d]	37	0.53	2.7
53	H	NHC_6H_4Cl-o	0.20[d]	0	>1.0[e]	>5
54	H	$NHCH_2C_6H_4Cl$-p	0.2[d]	28	0.44	2.2
55	H	$NHCH_2C_6H_4Cl$-m	0.24	50	0.24	1.2
56	H	$NHCH_2C_6H_4Cl$-o	0.65	50	0.65	3.2
57	m-Cl	$NHCH_2C_6H_5$	0.19	50	0.19	0.95
58	m-Cl	NHC_6H_4Cl-p	0.11	50	0.11	0.55
59	3,4-Cl_2	NHC_6H_4Cl-p	0.050[d]	20	0.21	1.1
60	3,4-Cl_2	$NHCH_2C_6H_4Cl$-p	0.050[d]	27	0.14	0.70
61	3,4-Cl_2	$NHCH_2C_6H_4Cl$-m	0.050[d]	28	0.13	0.65
62	H	$NHCH_2C_6H_4NO_2$-p	0.35	50	0.35	1.8
63	H	$NHCH_3$	25	50	25	125

[a] Assayed with 0.2 mM GPNA in 0.05 M Tris buffer (pH 7.4) containing 10% DMSO. [b] I_{50} = mM concentration for 50% inhibition. [c] Ratio of concentration of inhibitor to 0.2 mM substrate giving 50% inhibition. [d] Maximum solubility. [e] Since 15% inhibition is readily detectable, the I_{50} is greater than five times the concentration measured.

and *p*-methyl (**27**) substituents. When the *m*-methyl (**28**) group was increased to *m*-ethyl (**33**), a loss in binding occurred; similarly, the two *m*-methyl substituents of **34** caused a loss in binding. Thus bulky alkyl groups at the *meta* or *para* position are detrimental to binding, but flat planar phenyl groups of *o*-phenylphenyl (**31**), β-naphthyl (**35**), or α-naphthyl (**36**) give increased binding.

Whether or not there is some charge-transfer interaction[16] between these aryl groups and the enzyme is difficult to ascertain with the data in Table II, but it does not appear likely. The interpretation is clouded by the fact that the benzene ring is interacting with a hydrophobic area on the enzyme, but the substituents with varying Hammett σ constants (electronegativity) also have varying Hansch π constants (polarity). Perhaps a Hansch π–σ computer analysis of the data could resolve this issue,[17] although interpretation may be difficult due to steric hindrance of proper interaction of the benzene ring with the enzyme by such groups as *p*-acetamido. Polar groups such as cyano (**22** and **23**) and carboxylate (**24**) are detrimental to binding.

The better binding caused by the nitro substituent of **18**, **20**, and **21**, but slight loss in binding by *p*-methoxyl (**26**), suggested that the nonpolar, but electron-withdrawing, halogens be investigated. The *p*-chloro substituent of **29** gave a threefold increment in binding, but the *m*-chloro of **30** was even more effective and gave an 11-fold increment in binding. The effects of the *p*-chloro and *m*-chloro were not completely additive, but the 3,4-dichloro derivative (**37**) was only slightly more effective than the *m*-chloro derivative (**30**). Even more effective was the 2,3-dichloro derivative (**38**) which complexed 24 times more effectively than the parent phenoxyacetone (**14**). Thus maximum binding on Table II was seen with the 3-chlorophenyl (**30**), 3,4-dichlorophenyl (**37**), and 2,3-dichlorophenyl (**38**) moieties, these three groups differing only twofold in binding.

The next area studied was the replacement of the methyl group of phenoxyacetone (**14**) by phenyl (**39**) (Table III). Five pairs of compounds were synthesized for comparison; unfortunately, the ω-phenoxyacetophenones with *p*-nitro (**40**) and *p*-carboxy (**41**) groups could not be compared due to insolubility. The three remaining comparisons were subject to large error since at maximum solubility only 15% inhibition was seen; nevertheless, comparison of **14** and **39**, **26** and **42**, and **29** and **43** indicated that the phenyl group gave a three- to eightfold increment in binding. However, this series was considered to be too insoluble to be of further utility although it is possible that compounds such as **44** or its isomers could be useful for this study. Attention was turned to the more soluble anilides, since N-methylphenoxyacetanilide (**12**) was as good an inhibitor as phenoxyacetone (**14**) (Table I).

Replacement of the N-methyl group of **12** by H (**45**) gave a fourfold increment in binding (Table IV). Therefore, the higher analogs, **46** and **47**, were synthesized; the N-benzyl substituent of **46** was more than twice as effective as the N-phenyl substituent of **45**, but the N-phenyl and N-phenethyl (**47**) were about equivalent. Therefore, **45** and **46** were selected for study of substituent effects on the benzene ring.

Introduction of the *p*-nitro group (**48**) on **45** gave a compound too insoluble to measure. However, the more soluble N-methyl analogs, **12** and **49**, could be compared showing that the *p*-nitro group gave a ninefold increment in binding. Introduction of a *p*-chloro (**50**), *p*-bromo (**51**), or *m*-chloro (**52**) group gave a five- to tenfold increment in binding over the parent **45**; however, the *o*-chloro substituent of **53** did not give as good an increment in binding. If these effects by the electron-withdrawing nitro and halogen groups are identical in mechanism, they could be due to the effect on the polarizability of the carbonyl group (a binding point),[9] or could effect charge-transfer character if the benzene ring were binding partially to the enzyme as an electron acceptor, or both. These two effects are separable with the N-benzyl analogs where the methylene group insulates the carbonyl group against electronic effects of the benzene ring.

(17) T. Fujita, J. Iwasa, and C. Hansch, *J. Am. Chem. Soc.*, **86**, 5175 (1964).

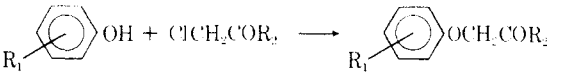
That the N-phenyl group of N-benzylphenoxyacetamide (**46**) is definitely complexed to the enzyme is shown by comparison with the N-methylamide (**63**); the former is 22 times as effective. That this binding to the enzyme by the N-phenyl group of **46** is influenced by an electron-withdrawing nitro group is shown with **62**; a threefold increment in binding between **46** and **62** was noted, which is less than the ninefold increment noted with **12** and **49**. Therefore, the nitro group of **49** has two almost equal effects: (a) increased binding by the benzene ring, and (b) increased binding of the carbonyl. That halogens had a similar beneficial effect was shown as follows.

The effect of halogen on binding of the N-benzylamides was also smaller than in the N-phenyl series. Note that in comparison with **46**, the *p*-chloro (**54**) substituent gave a 2.5-fold increment in binding and the *m*-chloro (**55**) a 4.5-fold increment in binding; the same substituents (**50** and **52**) on the N-phenyl group of **45** gave nine- and fivefold increments, respectively. Thus, the *meta* substituents have identical effects in both series, indicating that there is a direct fivefold effect on the binding of the 3-chlorophenyl group of both. The 3.6-fold difference in the *para* series with the anilide and benzylamide series can therefore be attributed to two effects. Since the 2.5-fold increment with **54** over the N-benzylamide (**46**) is due to a direct effect on chlorophenyl binding, the ninefold increment in binding by **50** over the anilide (**45**) is due to about equal contributions by chlorophenyl binding and by polarizability of the carbonyl group.

Since halogen substitutions on both the phenoxy group (Table II) and the arylamide group (Table IV) are beneficial to binding, a study was made to see if these were additive. Substitution of a *m*-chloro group (**57**) on the phenoxy moiety of an N-benzylamide was also beneficial to binding, a sixfold increment between **57** and **46** being observed; the *m*-chlorophenoxy of **58** gave a threefold increment in binding over the phenoxy group of **50**. The increment in the *p*-chloroanilide series between **59** and **50** was even smaller. The effect of the 3,4-dichloro substituents was threefold in the *p*-chlorobenzylamide series (**54** *vs.* **60**) and twofold in the *m*-chlorobenzylamide series (**55** *vs.* **61**). Thus, the effects (1.5–6-fold) of substitution in both benzene rings were only partially additive since an 11-fold difference between *m*-chlorophenoxyacetone (**30**) and phenoxyacetone (**14**) and an 18-fold difference between 3,4-dichlorophenoxyacetone (**37**) and **14** was seen in Table I.

Compounds **58–61** are quite effective inhibitors of chymotrypsin, being complexed one- to twofold better than the substrate GPNA and 55–110-fold better than the phenoxyacetone (**14**), the compound with which this study was initiated. The *m*-chlorophenoxy derivatives are considered to have superior utility over the 3,4-dichlorophenoxy derivatives due to the better aqueous solubility of the *m*-chloro series. The N-aryl group of N-benzyl- (**57**) and N-phenyl-3-chlorophenoxyacetamide is tolerated within the enzyme–inhibitor complex; therefore, a logical place to position leaving groups for potential exo-type active-site-directed irreversible inhibitors of chymotrypsin is on these N-aryl groups. Such studies are currently being pursued.

Chemistry.—All of the phenoxyacetones (**65**) were prepared by alkylation of the appropriate substituted phenol with chloroacetone in DMF or acetone in the



64 **65**, $R_2 = CH_3$; **66**, $R_2 = C_6H_5$

presence of sodium carbonate and sodium iodide;[18] the ω-phenoxyacetophenones (**66**) were prepared similarly from phenacyl chloride.[19] The only difficulties encountered were in synthesis of the *p*-carboxyphenoxy ketones. The chloro ketones smoothly alkylated ethyl *p*-hydroxybenzoate, but saponification of **65** or **66** with hot base led to resinification; at room temperature the hydrolysis was slow, but the appropriate *p*-carboxyphenoxy ketones could be isolated in low yields.

The amides were made by reaction of the appropriate acid chlorides and amines in chloroform. Pyridine was used as an acid acceptor with the aromatic amines, but triethylamine was used with the more basic aliphatic amines; in three cases an excess of amine was used as the acid acceptor.

Experimental Section[20]

Method A. 2,3-Dichlorophenoxyacetone (38).—A mixture of 45 ml of DMF, 1.61 ml (20 mmoles) of chloroacetone, 0.15 g of NaI, 2.65 (25 mmoles) of Na_2CO_3, and 3.26 g (20 mmoles) of 2,3-dichlorophenol was magnetically stirred in a bath at 70° for 15–18 hr. The cooled mixture was diluted with 200 ml of H_2O, then extracted three times with $CHCl_3$. The combined extracts were washed with 10% NaOH (two 15-ml portions) and H_2O (two 15-ml portions). The solvent was removed *in vacuo* and the residual oil was distilled at 114–115° (0.35 mm); the crystalline distillate was twice recrystallized from petroleum ether (bp 60–110°) to give 2.5 g (57%) of white crystals, mp 58–59°. See Table V for additional data.

In some cases, the $CHCl_3$ residue readily crystallized and was directly recrystallized; if the compound was distilled, both the melting point and boiling point were recorded in Table V.

Method B was the same as method A, except boiling acetone was used as the solvent; salts were removed by filtration. The filtrate was evaporated, $CHCl_3$ was added, and the solution was washed as in method A.

Method C. α-(*m*-Chlorophenoxy)-*p*-chloroacetanilide (58).—A mixture of 1.87 g (10 mmoles) of *m*-chlorophenoxyacetic acid[21] and 5 ml of $SOCl_2$ was refluxed until gas evolution was complete (about 30 min). Excess $SOCl_2$ was removed *in vacuo*. The residual acid chloride in 25 ml of $CHCl_3$ was added dropwise to a magnetically stirred solution of 1.28 g (10 mmoles) of *p*-chloroaniline and 0.82 ml (10 mmoles) of pyridine in 50 ml of $CHCl_3$; no cooling was employed and the addition required about 15 min. The mixture was refluxed for 5 min, then washed successively with H_2O, 5% HCl, 5% Na_2CO_3, and H_2O. Solvent was removed *in vacuo*. Three recrystallizations of the residue from absolute EtOH gave 1.4 g (47%) of white needles, mp 120–122°. See Table V for additional data.

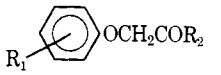
(18) (a) D. S. Tarbell, *J. Org. Chem.*, **7**, 251 (1942); (b) A. M. Dowell, Jr., H. S. McCullough, and P. K. Calloway, *J. Am. Chem. Soc.*, **70**, 226 (1948); (c) H. Suter and H. Zutter, *Ann.*, **576**, 215 (1952); (d) C. D. Hurd and P. Perletz, *J. Am. Chem. Soc.*, **68**, 38 (1946).

(19) (a) P. Yates, D. Farnum, and G. Stout, *ibid.*, **80**, 196 (1958); (b) F. Kroehnke, G. Kroehnke, and G. Abrenholz, *J. Prakt. Chem.*, **11**, 239 (1960).

(20) All analytical samples had infrared spectra compatible with their assigned structures and moved as a single spot on tlc on Brinkmann silica gel GF with ethyl acetate–petroleum ether (bp 60–110°); spots were detected by visual examination under uv light. Melting points were determined in capillary tubes on a Mel-Temp block and are corrected.

(21) B. R. Baker, W. W. Lee, W. A. Skinner, A. P. Martinez, and E. Tong, *J. Med. Pharm. Chem.*, **2**, 633 (1960).

TABLE V

PHYSICAL CONSTANTS OF 

Compd	R_1	R_2	Method	% yield	Bp (mm) or mp, °C	Calcd, % C	Calcd, % H	Calcd, % N	Found, % C	Found, % H	Found, % N
12	H	$N(CH_3)C_6H_5$	D	71[a]	89–93	74.7	6.27	5.81	74.6	6.22	5.72
20	*m*-NO_2	CH_3	B	33[b]	82–84[c]						
21	*o*-NO_2	CH_3	B	67[d]	65–68[e]						
22	*p*-CN	CH_3	B	9[d,a]	61–62	68.6	5.18	8.00	68.4	5.25	8.15
23	*m*-CN	CH_3	B	17[f]	68–70	68.6	5.18	8.00	68.3	5.32	8.30
24	*p*-COOH	CH_3	F	5[g]	158–171 dec	61.8	5.19		61.7	5.08	
26	*p*-CH_3O	CH_3	B	36[a]	88–90 (0.18), 47–49	66.6	6.71		66.5	6.57	
27	*p*-CH_3	CH_3	B	41	61–63 (0.15)[h]						
30	*m*-Cl	CH_3	B	43	94–96 (0.6)	58.5	4.91		58.4	5.10	
31	*o*-C_6H_5	CH_3	A	33[f]	120–130 (0.3), 65–67	79.6	6.24		79.5	6.22	
32	*p*-C_6H_5	CH_3	A	31[b]	103–105	79.6	6.24		79.4	6.23	
33	*m*-C_2H_5	CH_3	B	22	61–64 (0.05)	74.1	7.92		73.9	7.86	
34	3,5-$(CH_3)_2$	CH_3	B	34	78–80 (0.3), 37–41	74.1	7.92		74.3	8.09	
37	3,4-Cl_2	CH_3	B	41[a]	104–108 (0.3), 49–51	49.3	3.68		49.5	3.70	
38	2,3-Cl_2	CH_3	A	57[a]	114–115 (0.4), 58–59	49.3	3.68		49.4	3.00	
40	*p*-NO_2	C_6H_5	B	47[i,j]	148–150[k]						
41	*p*-COOH	C_6H_5	F	31[l]	171–177 dec	70.3	4.72		70.1	4.61	
42	*p*-CH_3O	C_6H_5	B	37[d,m]	67–69	74.4	5.82		74.3	5.90	
43	*p*-Cl	C_6H_5	B	57[b]	95–96[n]						
45	H	NHC_6H_5	C	90[a]	98–101	74.0	5.76	6.16	73.7	5.61	6.36
46	H	$NHCH_2C_6H_5$	E	46[o]	84–86[b]						
47	H	$NH(CH_2)_2C_6H_5$	E	23[f]	72–75	75.3	6.71	5.49	75.5	6.84	5.26
48	H	$NHC_6H_4NO_2$-*p*	C	33[i]	189–191	61.8	4.44	10.3	61.9	4.48	10.1
49	H	$N(CH_3)C_6H_4NO_2$-*p*	C	88[b]	119–122	62.9	4.93	9.79	62.9	5.15	9.57
50	H	NHC_6H_4Cl-*p*	C	69[b]	136–139	64.2	4.62	5.35	64.0	4.72	5.37
51	H	NHC_6H_4Br-*p*	C	82[b]	130–132	54.9	3.95	4.57	54.7	4.11	4.43
52	H	NHC_6H_4Cl-*m*	C	88[b]	94–95	64.2	4.62	5.35	64.3	4.75	5.49
53	H	NHC_6H_4Cl-*o*	C	67[b]	88–91	64.2	4.62	5.35	64.0	4.73	5.50
54	H	$NHCH_2C_6H_4Cl$-*p*	E	44[p]	124–126	65.3	5.12	5.08	65.3	5.21	5.07
55	H	$NHCH_2C_6H_4Cl$-*m*	E	94[p]	84–87	65.3	5.12	5.08	65.6	5.17	5.00
56	H	$NHCH_2C_6H_4Cl$-*o*	E	58[a,d]	91–93	65.3	5.12	5.08	65.5	5.29	5.12
57	*m*-Cl	$NHCH_2C_6H_5$	E	92[a,d]	84–87	65.3	5.12	5.08	65.5	5.25	5.18
58	*m*-Cl	NHC_6H_4Cl-*p*	C	47[b]	120–122	56.8	3.74	4.73	56.9	3.80	4.78
59	3,4-Cl_2	NHC_6H_4Cl-*p*	C	36[b]	153–156	50.9	3.05	4.24	50.6	3.20	4.21
60	3,4-Cl_2	$NHCH_2C_6H_4Cl$-*p*	E	64[b]	127–130	52.3	3.51	4.06	52.4	3.70	3.99
61	3,4-Cl_2	$NHCH_2C_6H_4Cl$-*m*	E	46[b]	120–122	52.3	3.51	4.06	52.3	3.44	4.15
62	H	$NHCH_2C_6H_4NO_2$-*p*	E	17[a]	82-84	62.9	4.93	9.79	62.9	5.09	9.65
63	H	$NHCH_3$	E	18[q]	66–68[r]						

[a] Recrystallized from petroleum ether (bp 60–110°). [b] Recrystallized from absolute EtOH. [c] Lit.[18a] mp 79–81°. [d] Recrystallized from aqueous MeOH. [e] Lit.[18a] mp 67–69°. [f] Recrystallized from absolute EtOH–petroleum ether. [g] Recrystallized from EtOH–C_6H_6. [h] Lit.[18a] bp 53° (0.15 mm). [i] Recrystallized from acetone. [j] Recrystallized from 2-methoxyethanol. [k] Lit.[19b] mp 149–150°. [l] Recrystallized from $CHCl_3$–EtOH. [m] Recrystallized from aqueous 2-methoxyethanol. [n] Lit.[19b] mp 98–100°. [o] O. C. Dermer and J. King, *J. Org. Chem.*, **8**, 168 (1943), reported mp 84.5–86.0°. [p] Recrystallized from C_6H_6–petroleum ether. [q] Recrystallized from EtOAc–hexane. [r] Mp 69–70° recorded by J. L. Shapiro, V. A. Parrino, and L. Freedman, *J. Am. Chem. Soc.*, **81**, 3728 (1959).

Method D was the same as method C except an excess of the amine was used in place of pyridine.

Method E was the same as method C except that triethylamine was used in place of pyridine and the reaction was run at 0°.

Method F. *p*-Carboxyphenoxyacetone (24).—The crude ethyl ester of **24** was prepared by method B; then it was allowed to stand at ambient temperature in excess 1 *N* NaOH in 70% EtOH for 3 days. The solution was acidified and the tarry product was collected. It was dissolved in cold 5% NaOH; then the solution was washed ($CHCl_3$). The basic solution was acidified, and the product was collected on a filter, then washed with water. Recrystallization from EtOH–C_6H_6 gave white crystals, mp 158–171° dec. See Table V for additional data.

Enzyme Assays.[22]—Three-times recrystallized, salt-free, α-chymotrypsin from bovine pancreas and N-glutaryl-L-phenylalanine *p*-nitroanilide (GPNA) were purchased from Mann Research Laboratories. The buffer employed was 0.05 *M* Tris, pH 7.4. The assay was a modification of the method of Erlanger, *et al.*[15] The enzyme was dissolved in 1 m*M* HCl at 3 mg/ml; the solution was stored at 0° and could be used for 1 week. A stock solution of 3.1 m*M* GPNA was prepared by dissolving 20 mg in 1.00 ml of DMSO, then dilution with 15 ml of buffer; it was stored in a brown bottle. Inhibitors were dissolved in DMSO.

In a 3-ml glass cuvette were placed 200 μl of 3.1 m*M* GPNA, 2.50 ml of buffer, and 300 μl of DMSO (with or without inhibitor). When the system had balanced at 410 mμ, 100 μl of enzyme was added and the rate of increase in OD at 410 mμ was followed on a 0–0.1 OD slide wire of a Gilford recording spectrophotometer. The velocity without inhibitor (V_0) was about 0.01 OD unit/min. Sufficient inhibitor was employed to give V_I's in the range of 30–70% inhibition. Then V_0/V_I was plotted against [I]; the I_{50} is where $V_0/V_I = 2$, the concentration of inhibitor required for 50% inhibition.[21] The cuvette concentration of GPNA was 0.2 m*M*.

(22) The technical assistance of Maureen Baker and Pepper Caseria with these assays is acknowledged.