duplicate, or triplicate if necessary. This procedure is adequate for a routine screen for a plus or minus answer on irreversible inhibition. As many as three inhibitor tubes can be run with one enzyme control in less than 1 working day.

With a positive compound, a larger amount of inhibitor–enzyme mixture can be set up, then a number of aliquots removed at varying times in order to obtain the half-life and extent of irreversible inhibition. For irreversible inhibitors having a fast initial inactivation, a zero-time tube can be set up as previously described for dihydrofolic reductase.[13]

α-Chymotrypsin-Catalyzed Hydrolysis of N-(3-Chlorophenoxyacetyl)metanilyl Fluoride (12). A.—To 1 l. of 10% H_2O–DMSO were added with stirring 700 mg (0.028 mmole) of α-chymotrypsin and 160 mg (0.465 mmole) of **12** (in 10 ml of DMSO); the additions were made in six equal aliquots over a period of 9 hr, then stirring was continued an additional 1 hr. The pH was maintained at 7.2-8.0 by addition of 0.01 *N* NaOH, and the temperature was maintained at 30-35°; a total of 78 ml (0.78 mmole) of base was consumed.

The mixture was filtered and the recovered **12** was washed with H_2O; 10 mg. The filtrate was extracted with four 200-ml portions of $CHCl_3$. The combined $CHCl_3$ extracts were washed with 400 ml of H_2O, then evaporated *in vacuo*. The residue was extracted with 25 ml of EtOH. Quantitative uv analyses showed the presence of 11 mg of **12** for a total recovery of 21 mg (13%).

The original aqueous solution was concentrated to about 200 ml by freeze evaporation, then was spin evaporated to residue *in vacuo*. The residue was suspended in 20 ml of H_2O, then adjusted to pH 1–2 with HCl. The separated protein was removed by centrifugation. To the supernatant was added 250 mg (2.5 mmoles) of guanidine hydrochloride. Since the guanidine salt of **12** failed to crystallize from solution, the solution was spin evaporated *in vacuo*. The residue was dissolved in 5 ml of H_2O and applied to four plates (20 × 20 cm) coated with a total of 50 g of silica gel GF. The plates were developed with 2:1 EtOH–petroleum ether (bp 60–110°). The zones of the guanidine salt of **15** were scraped off, combined, and extracted with 20% aqueous Me_2CO. The solution was clarified with carbon, then evaporated *in vacuo*. Crystallization of the residue from 2 ml of H_2O gave 30 mg (16%) of the guanidinium salt of **15**; this material was identical with an authentic sample as shown by ir, uv, and tlc. The filtrate contained additional product as shown by tlc.

B.—A run identical with **A**, except the α-chymotrypsin was omitted, gave an 81% recovery of **12**, and only traces of **15** could be detected by tlc; only 5.6 ml (0.056 mmole) of 0.01 *N* NaOH was consumed.

C.—A run identical with **A**, except that lyophilized bovine serum albumin was used in place of α-chymotrypsin, gave a 74% recovery of **12** without $CHCl_3$ extraction; only 5.0 ml (0.05 mmole) of 0.01 *N* NaOH was consumed.

Irreversible Enzyme Inhibitors. CXIV.[1,2] Proteolytic Enzymes. IV.[2] Additional Active-Site-Directed Irreversible Inhibitors of α-Chymotrypsin Derived from Phenoxyacetamides Bearing a Terminal Sulfonyl Fluoride

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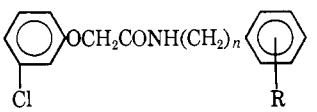
Fifteen candidate irreversible inhibitors of α-chymotrypsin derived from N-phenyl- or N-benzyl-3-chloro- or 3,4-dichlorophenoxyacetamide were synthesized that contained a fluorosulfonylbenzamido or fluorosulfonylphenylureido group on the N-aryl ring. Of these, ten showed irreversible inhibition of α-chymotrypsin; due to lack of solubility compared to their reversible binding constants (K_i), none of these compounds could completely inactivate α-chymotrypsin at their maximum solubility. The kinetics of inactivation indicated that these compounds were being enzymatically hydrolyzed to the corresponding sulfonic acids as well as causing inactivation of α-chymotrypsin by the active-site-directed mechanism.

The selective inhibition of only one of the myriad of blood serum proteases[4] is not apt to be achieved with reversible inhibitors, but can theoretically be achieved with active-site-directed irreversible inhibitors[5] of the exo type that covalently link to the enzyme outside the active site.[6] Inhibitors that use a sulfonyl fluoride group for convalent linkage to enzymes are particularly well suited for exo-type irreversible inhibition since this functional group can rapidly attack the hydroxyl group of a serine or threonine within the reversible enzyme–inhibitor complex;[7] such hydroxyl groups are prevalent on the surface of proteins. In the previous paper of this series[2] it was shown that compounds of type **1** bearing a



1, $n = 0$
2, $n = 1$

sulfonyl fluoride (R = SO_2F) group could rapidly inactivate α-chymotrypsin by the active-site-directed mechanism. It was also established that chymotrypsin could catalyze the hydrolysis of these sulfonyl fluorides to the corresponding sulfonic acids. This paper reports additional studies where the sulfonyl fluoride group of the inhibitor is further removed from the carboxamide group of **1** since the latter is complexed within the active site; this further removal of the sulfonyl fluoride group in compounds of type **1** and **2** is more

(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 J. A. Hurlbut, *J. Med. Chem.*, **11**, 233 (1968).

(3) N.D.E.A. predoctoral fellow.

(4) For a discussion of the utility of such inhibitors in the cardiovascular and organ transplant areas see B. R. Baker and E. H. Erickson, *J. Med. Chem.*, **10**, 1123 (1967), paper CVI of this series.

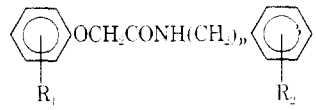
(5) B. R. Baker, "Design of Active-Site-Directed Irreversible Enzyme Inhibitors. The Organic Chemistry of the Enzymic Active-Site," John Wiley and Sons, Inc., New York, N. Y., 1967.

(6) (a) The exo type of irreversible inhibitor can have an extra dimension of specificity not present in reversible inhibitors by using the bridge principle of specificity; (b) see ref 5, Chapter IX, for a detailed discussion of this principle.

(7) B. R. Baker and G. J. Lourens, *J. Med. Chem.*, **10**, 1113 (1967), paper CV of this series.

Table I

Inhibition[a] of α-Chymotrypsin by



				Reversible[b]			Irreversible[c]		
No.	R_1	n	R_2	Concn, μM	% inhib	Est I_{50},[d] μM	Concn, μM	Time, min	% inacth
3[e]	3-Cl	0	*m*-SO_2F	50	50	50	90	60	100
							72	3, 12, 60	35, 50, 72
							36	3, 12, 60	15, 31, 52
							9	30, 60	17, 24
4	3,4-Cl_2	1	*m*-$NHCOCH_2Br$	200[f]	43	270	200	60	9
5	3,4-Cl_2	1	*m*-$NHCOC_6H_4SO_2F$-*m*	10[f]	23	35	10	60	0
6	3,4-Cl_2	1	*m*-$NHCOC_6H_4SO_2F$-*p*	3[f]	18	~14	2	60	13
7	3-Cl	1	*p*-$NHCOCH_2Br$	50[f]	35	90	50	60	0
8	3-Cl	1	*p*-$NHCOC_6H_4SO_2F$-*m*	18[f]	50	18	18	1, 60	38, 38
9	3-Cl	1	*p*-$NHCOC_6H_4SO_2F$-*p*	10[f]	14	~60	10	60	0
10	3-Cl	1	*p*-$NHCONHC_6H_4SO_2F$-*m*	5[f]	0	>25[g]	5	60	Near 0
11	3-Cl	1	*m*-$NHCOC_6H_4SO_2F$-*m*	10[f]	23	35	10	1, 60	44, 44
12	3-Cl	1	*m*-$NHCOC_6H_4SO_2F$-*p*	20[f]	23	67	20	5, 15, 60	25, 30, 30
13	3-Cl	1	*m*-$NHCONHC_6H_4SO_2F$-*m*	10[f]	13	~70	10	1, 60	23, 23
14	3-Cl	1	*o*-$NHCOCH_2Br$	160	50	160	160	130[h]	50
15	3-Cl	1	*o*-$NHCOC_6H_4SO_2F$-*m*	25[f]	29	53	20	10, 60, 120	24, 37, 37
16	3-Cl	1	*o*-$NHCOC_6H_4SO_2F$-*p*	20[f]	19	90	20	5, 15, 60	18, 34, 34
17	3-Cl	1	*o*-$NHCONHC_6H_4SO_2F$-*m*	10[f]	10	~90	10	5, 30, 60	22, 40, 40
18[e]	3-Cl	0	*m*-$NHCOCH_2Br$	100	50	100	155	60	0
19	3-Cl	0	*m*-$NHCOC_6H_4SO_2F$-*m*	10[f]	0	>50[g]	10	60	17
20	3-Cl	0	*m*-$NHCOC_6H_4SO_2F$-*p*	2.5[f]	0	>12[g]	2.5	60	0
21	3-Cl	0	*m*-$NHCONHC_6H_4SO_2F$-*m*	5[f]	0	>25[g]	4	60	19
22	3-Cl	0	*p*-$CH_2NHCONHC_6H_4SO_2F$-*m*	25[f]	30	60	20	1, 60	40, 40
23	3-Cl	0	*p*-$CH_2NH_2 \cdot HCl$	280	50	280			
24	3-Cl	0	*p*-CN	25[f]	13	~200			
25[e]	H	0	H	2000[f]	42	2600			
26	H	0	*p*-COOH	5200	50	5200			

[a] The (technical assistance of Maureen Baker with these assays is acknowledged. [b] Assayed with three-times recrystallized α-chymotrypsin in pH 7.4 Tris buffer containing 10% DMSO with 200 μM N-glutaryl-L-phenylalanine *p*-nitroanilide as previously described.[11] Incubated at 37° in pH 7.4 Tris buffer containing 10% DMSO as previously described.[2] [d] I_{50} = concentration of inhibitor required for 50% inhibition. [e] Data from ref 2. [f] Near maximum solubility. [g] Since 20% inhibition is readily detectable, the I_{50} is >5 times the concentration measured. [h] Half-life of inactivation. [i] Data from ref 14.

apt to give the desired exo type of irreversible inhibitor.

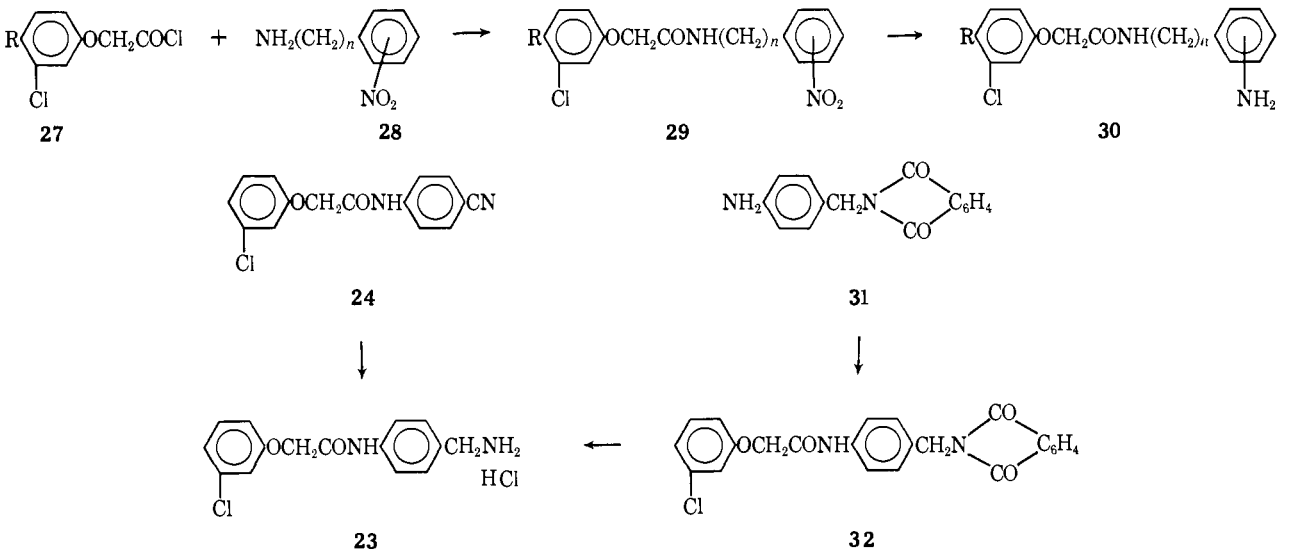
In Table I are listed the enzyme results of 18 new derivatives of **1** and **2**; the enzyme data with two previous compounds (**3**, **18**) are also reported for comparison. It was previously shown[2] that the *m*-sulfonyl fluoride could completely inactivate α-chymotrypsin at a concentration of 90 μM, which is about twice the I_{50} of 50 μM. As the concentration was reduced, the total amount of inactivation was decreased due to the simultaneous destruction of the inhibitor by enzyme-catalyzed hydrolysis of the sulfonyl fluoride group to the sulfonic acid; at 36 μM = $0.7I_{50}$, only about 50% total inactivation was seen which dropped to about 20% total inactivation at 9 μM = $0.2I_{50}$. Thus, a compound that has a maximum solubility below its I_{50} may only partially inactivate α-chymotrypsin due to concomitant enzyme-catalyzed hydrolysis of its sulfonyl fluoride.

The *o*-bromoacetamidobenzyl amide (**14**) could inactivate chymotrypsin at 37° with a half-life of about 2 hr; this rate of inactivation was linear when the per cent inactivation on a log scale was plotted against time on a linear scale, as previously observed with other bromoacetamides on other enzymes,[6b,8-13] since the enzymes do not hydrolyze these bromoacetamides. Three related sulfonyl fluorides (**15**–**17**) derived from the same *o*-aminobenzyl amide also showed some inactivation of chymotrypsin; the initial rate in the first 5–10 min was rapid, but then inactivation ceased at 35–40% of initial enzyme level. The maximum concentration that **15** could be assayed was $0.4I_{50}$; when compared to the data obtained with **3** at $0.2\text{–}0.7I_{50}$, it can be rationalized why inactivation did not go to completion. Similar results were seen with **16** and **17** at their maximum solubility of $0.2I_{50}$ and $0.1I_{50}$, respectively. Since these *ortho* compounds (**14**–**17**) can project their leaving group either back toward or away from the active site, it is possible that any one of these compounds can operate by either the exo or the endo mechanism, but probably not both.

Similarly, the sulfonyl fluorides (**11**–**13**) derived from the *m*-aminobenzyl amide showed a rapid initial inactivation in the first 1–5 min, then no further inactivation since the maximum attainable solubility was again only $0.1\text{–}0.3I_{50}$. The corresponding sulfonyl fluorides (**19**–**21**) were even less soluble and showed

(8) B. R. Baker and J. H. Jordan, *J. Pharm. Sci.*, **55**, 1417 (1966).
(9) See ref 5, Chapter X.
(10) B. R. Baker, W. W. Lee, and E. Tong, *J. Theor. Biol.*, **3**, 459 (1962).
(11) See ref 5, Chapter XII.
(12) H. J. Schaeffer, M. A. Schwartz, and E. Odin, *J. Med. Chem.*, **10**, 686 (1967).
(13) B. R. Baker and J. A. Kozma, *ibid.*, **10**, 682 (1967), paper XCV of this series.

SCHEME I



neither reversible nor irreversible inhibition at their maximum solubility.

Of the three sulfonyl fluorides (**8**–**10**) derived from the *p*-aminobenzyl amide only one (**8**) showed irreversible inhibition at its maximum solubility (I_{50}); it showed about 40% inactivation in the first minute, then no further inactivation. The other two compounds (**9**, **10**) could only be tested considerably below their I_{50} concentrations. An interesting contrast exists between **8** and the corresponding *p*-bromoacetamide (**7**); the latter failed to show any inactivation at a concentration $\simeq 0.6I_{50}$.

A last compound (**22**) was derived from the *p*-aminomethylanilide of 3-chlorophenoxyacetic acid; **22** showed a rapid 40% inactivation of chymotrypsin in 1 min, then no further inactivation similar to that observed with **8**. It is possible that **8** and **22** inactivate by the exo mechanism since the *m*-fluorosulfonylbenzamido function may be held in a direction away from the active site.

Even though the ten sulfonyl fluorides in Table I showing inactivation of chymotrypsin are not sufficiently soluble to give complete inactivation, several useful observations emerge from this study. (1) It was previously shown[2] that the initial 65–70% inactivation by **3** at 72 μM could be increased to 95% by treatment with a second portion of the inhibitor. Thus, two or more treatments of chymotrypsin by **8**, **11**–**13**, **15**–**17**, **19**, or **22** could be expected to give >90% inactivation; the resultant inactivated chymotrypsins could then be studied structurally to see which amino acid had become covalently linked.

(2) The inactivations of chymotrypsin by **8** and **22** could probably operate by the exo mechanism, that is, covalent linkage takes place outside the active site; it is also possible that the *meta* isomers (**11**–**13**) operate by the exo mechanism. Thus one of the early goals of this program, namely, that it may be possible to attack chymotrypsin by an active-site-directed irreversible inhibitor outside the active-site,[2] may be feasible. If solubility could be increased by introduction of a group such as a carboxyl or aliphatic amine that is completely ionized or protonated, respectively, at pH 7.4, then useful inhibitors from a chemotherapeutic standpoint could emerge. When the polar carboxylate group was placed on the *para* position of the phenoxy group, binding was ineffective, presumably since the phenoxy group is complexed to the hydrophobic region within the active site.[14] However, it might be possible to place a polar group on the N-phenyl substituent of **1**. That such a possibility was feasible was indicated by comparison of **24** and the related aminomethyl derivative (**23**), and by **25**[14] compared to its *p*-carboxy derivative (**26**); the change in binding between these pairs was minimal indicating tolerance for a polar group.

Studies are in progress to increase solubility of some of the irreversible inhibitors in Table I.

Chemistry.—From a synthetic standpoint, the intermediate amines needed for the compounds in Table I could be divided into two classes (see Scheme I). The first class of amines were those with the amino group directly on a benzene ring (**30**). These were synthesized by condensation of the appropriate nitroarylamine (**28**) and appropriate phenoxyacetyl chloride (**27**) to **29** followed by catalytic hydrogenation with PtO_2 catalyst to **30**.

The second class of amine (**23**) had an aliphatic amino group on a side chain. Two routes to this compound were investigated. N-(*p*-Aminobenzyl)phthalimide (**31**)[15] was prepared in two steps from *p*-nitrobenzyl chloride using methods previously described for the *meta* isomer.[16] Conversion of **31** to **32** proceeded smoothly. However, hydrazinolysis of the phthalyl group of **32** was difficult due to its insolubility; the required benzylamine (**23**) was obtained in only 10% yield. An alternate route to **23** *via* hydrogenation of the cyano group of **24** with a PtO_2 catalyst gave a 60% yield, but the hydrogenation was relatively difficult.

The arylamines (**30**–**33**) were converted to three different types of irreversible inhibitors. Reaction with bromoacetic anhydride in Me_2CO[17] afforded the bromoacetamides (**36**) that gave a negative Bratton–Marshall test and a positive 4-(*p*-nitrobenzyl)pyridine test for activated halogen.[17] Reaction of **33** with *m*-fluoro-

(14) B. R. Baker and J. A. Hurlbut, *J. Med. Chem.*, **10**, 1129 (1967), paper CVII of this series.

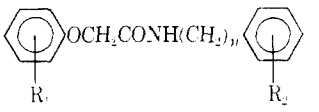
(15) N. Kornblum and D. C. Iffland, *J. Am. Chem. Soc.*, **71**, 2137 (1949).

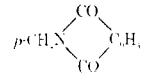
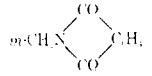
(16) B. R. Baker and J. K. Coward, *J. Heterocycl. Chem.*, **4**, 202 (1967), paper XCI of this series.

(17) B. R. Baker, D. V. Santi, J. K. Coward, H. S. Shapiro, and J. H. Jordan, *ibid.*, **3**, 425 (1966).

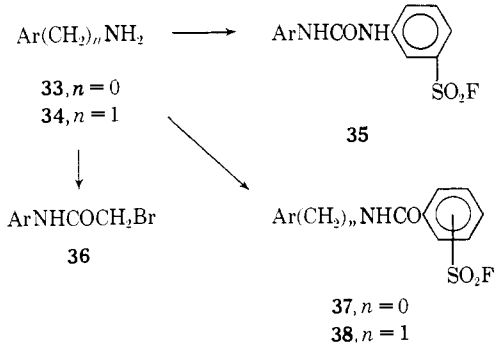
Table II

Physical Properties of



No.	n	R_1	R_2	Method	Yield, %	Mp, °C	Formula	Analyses
4	1	3,4-Cl_2	*m*-$NHCOCH_2Br$	F	67[a]	155–157	$C_{17}H_{15}BrCl_2N_2O_3$	C, H, N
5	1	3,4-Cl_2	*m*-$NHCOC_6H_4SO_2F$-*m*	B	39[a]	183–185	$C_{22}H_{17}Cl_2FN_2O_5S$	C, H, N
6	1	3,4-Cl_2	*m*-$NHCOC_6H_4SO_2F$-*p*	B	63[a]	199–200	$C_{22}H_{17}Cl_2FN_2O_5S$	C, H, N
7	1	3-Cl	*p*-$NHCOCH_2Br$	F	71[a]	189–192 dec	$C_{17}H_{16}BrClN_2O_3$	C, H, N
8	1	3-Cl	*p*-$NHCOC_6H_4SO_2F$-*m*	B	22[a]	176–178	$C_{22}H_{18}ClFN_2O_5S$	C, H, N
9	1	3-Cl	*p*-$NHCOC_6H_4SO_2F$-*p*	B	65[a]	198–201	$C_{22}H_{18}ClFN_2O_5S$	C, H, N
10	1	3-Cl	*p*-$NHCONHC_6H_4SO_2F$-*m*	G	60[a]	200–203	$C_{22}H_{19}ClFN_3O_5S$	C, H, N
11	1	3-Cl	*m*-$NHCOC_6H_4SO_2F$-*m*	B	47[a]	138–140	$C_{22}H_{18}ClFN_2O_5S$	C, H, N
12	1	3-Cl	*m*-$NHCOC_6H_4SO_2F$-*p*	B	47[a]	181–183	$C_{22}H_{18}ClFN_2O_5S$	C, H, N
13	1	3-Cl	*m*-$NHCONHC_6H_4SO_2F$-*m*	G	63[a]	178–181	$C_{22}H_{19}ClN_3O_5S$	C, H, N
14	1	3-Cl	*o*-$NHCOCH_2Br$	F	48[b,c]	125–128	$C_{17}H_{16}BrClN_2O_3$	C, H, N
15	1	3-Cl	*o*-$NHCOC_6H_4SO_2F$-*m*	B	51[c,d]	164–166	$C_{22}H_{18}ClFN_2O_5S$	C, H, N
16	1	3-Cl	*o*-$NHCOC_6H_4SO_2F$-*p*	B	65[a]	188–191	$C_{22}H_{18}ClFN_2O_5S$	C, H, N
17	1	3-Cl	*o*-$NHCONHC_6H_4SO_2F$-*m*	G	59[a]	194–197	$C_{22}H_{19}ClFN_3O_5S$	C, H, N
19	0	3-Cl	*m*-$NHCOC_6H_4SO_2F$-*m*	B	68[e]	220–222	$C_{21}H_{16}ClFN_2O_5S$	C, H, N
20	0	3-Cl	*m*-$NHCOC_6H_4SO_2F$-*p*	B	63[d,e]	241–243	$C_{21}H_{16}ClFN_2O_5S$	C, H, N
21	0	3-Cl	*m*-$NHCONHC_6H_4SO_2F$-*m*	G	68[e]	198–200	$C_{21}H_{17}ClFN_3O_5S$	C, H, N
22	0	3-Cl	*p*-$CH_2NHCONHC_6H_4SO_2F$-*m*	G[f]	36[g,e]	198–201	$C_{22}H_{19}ClFN_3O_5S$	C, H, N
23	0	3-Cl	*p*-$CH_2NH_2 \cdot HCl$	C	60[a]	253–256 dec	$C_{15}H_{16}Cl_2N_2O_2$	C, H, N
				D	10[a]	251–256 dec		
24	0	3-Cl	*p*-CN	A	50[a]	186–189	$C_{15}H_{11}ClN_2O_2$	C, H, N
26	0	H	*p*-COOH	B	22[h]	226–229	$C_{15}H_{12}ClNO_4$	C, H, N
32	0	3-Cl	*p*- 	A	40[a]	188–191	$C_{23}H_{17}ClN_2O_4$	C, H, N
39	1	3,4-Cl_2	*m*-NO_2	B	70[a]	138–140	$C_{15}H_{12}Cl_2N_2O_4$	C, H, N
40	1	3,4-Cl_2	*m*-NH_2	E	86[g,h]	108–110	$C_{15}H_{14}Cl_2N_2O_2$	C, H, N
41	1	3-Cl	*p*-NO_2	B	69[a]	123–125	$C_{15}H_{13}ClN_2O_4$	C, H, N
42	1	3-Cl	*p*-NH_2	E	71[i,e]	99–101	$C_{15}H_{15}ClN_2O_2$	C, H, N
43	1	3-Cl	*m*-NO_2	B	67[a]	93–95	$C_{15}H_{13}ClN_2O_4$	C, H, N
44	1	3-Cl	*m*-NH_2	E	85[e,g]	81–83	$C_{15}H_{15}ClN_2O_2$	C, H, N
45	1	3-Cl	*o*-NO_2	B	60[g]	85–88	$C_{15}H_{13}ClN_2O_4$	C, H, N
46	1	3-Cl	*o*-NH_2	E	86[g,e]	113–114	$C_{15}H_{15}ClN_2O_2$	C, H, N
47	0	3-Cl	*m*-NO_2	B	39[d]	141–143	$C_{14}H_{11}ClN_2O_4$	C, H, N
48	0	3-Cl	*m*-NH_2	E	83[j,k]	117–119	$C_{14}H_{13}ClN_2O_2$	C, H, N
49	0	3-Cl	*m*-CN	A	65[a]	145–148	$C_{15}H_{11}ClN_2O_2$	C, H, N
50	0	3-Cl	*m*- 	B	75[f]	224–226	$C_{23}H_{17}ClN_2O_4$	C, H, N
51	0	3-Cl	*m*-$CH_2NH_2 \cdot HCl$	C	8[a]	217–220 dec	$C_{15}H_{16}Cl_2N_2O_2$	C, H, N
52	1	3,4-Cl_2	*p*-NO_2	B	25[a]	172–174	$C_{15}H_{12}Cl_2N_2O_4$	C, H, N

[a] Recrystallized from EtOH. [b] Recrystallized from Me_2CO–H_2O. [c] Recrystallized from C_6H_6–petroleum ether (bp 60–110°). [d] Recrystallized from $CHCl_3$. [e] Recrystallized from EtOH–Me_2CO. [f] Et_3N added to neutralize HCl salt of **23**. [g] Recrystallized from EtOH–H_2O. [h] Recrystallized from EtOAc–petroleum ether. [i] Recrystallized from MeOEtOH–H_2O. [j] Recrystallized from Me_2CO. [k] Recrystallized from C_7H_8–Me_2CO.



sulfonylphenyl isocyanate in chloroform afforded the urea-bridged sulfonyl fluorides (**35**). The amide-bridged sulfonyl fluorides (**37**, **38**) were prepared by acylation of the appropriate amine (**33**, **34**) with *m*- or *p*-fluorosulfonylbenzoyl chloride in $CHCl_3$ containing Et_3N.

Experimental Section

All analytical samples had proper ir spectra, moved as a single spot on tlc on Brinkmann silica gel GF, and gave combustion values for CHN within 0.3% of the theoretical percentage. Melting points were taken in capillary tubes on a Mel-Temp block and those below 230° are corrected. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

Method A.—Acylation of the appropriate arylamine (**28**, **31**) with the appropriate substituted phenoxyacetyl chloride was performed in $CHCl_3$ with C_5H_5N as the acid acceptor by the previously described method.[14]

Method B.—The benzylamines (**28**, $n = 1$) were acylated by

method A at −5 to 0° except Et_3N was used as the acid acceptor.

Method C. N-(*p*-Aminomethylphenyl)-3-chlorophenoxyacetamide Hydrochloride (23).—A solution of 2.86 g (10 mmoles) of **24** and 1.5 g (15 mmoles) of $EtSO_3H$ in 200 ml of 95% aqueous methoxyethanol was stirred with 0.10 g of decolorizing carbon, then filtered. After the addition of 50 mg of PtO_2, the mixture was shaken with H_2 at 2–3 atm. When about one-fourth of the calculated amount of H_2 had been consumed, hydrogenation ceased. The mixture was re-treated with decolorizing carbon and filtered, and then 50 mg of fresh PtO_2 was added. After being shaken with H_2 at 2–3 atm for an additional 24 hr, hydrogenation was essentially complete. The filtered solution was diluted with 600 ml of H_2O and a small amount of **24** was removed by filtration. The filtrate was adjusted to pH 11–13 with 10% NaOH, then extracted with three 50-ml portions of $CHCl_3$. The combined extracts were dried ($MgSO_4$), then saturated with HCl gas. The product was collected on a filter and recrystallized from EtOH; yield, 1.97 g (60%) of white crystals, mp 253–256 dec. See Table II for additional data.

Method D.—A mixture of 2.11 g (5 mmoles) of **32**, 50 ml of MeOEtOH, and 1.0 ml (26 mmoles) of 85% $N_2H_4 \cdot H_2O$ was refluxed with stirring for 20 min when solution was complete. Solvent was removed by spin evaporation *in vacuo*. The residue was stirred with 60 ml of 1 *N* HCl at about 85° for 15 min; the insoluble phthalhydrazide containing some **32** was removed by filtration and washed with H_2O. The phthalhydrazide containing some **32** was again treated with N_2H_4 for 20 min as above. The combined HCl solutions were brought to about pH 13 with 50% NaOH. The mixture was extracted with two 100-ml portions of Et_2O. The combined extracts were dried ($MgSO_4$), then treated with HCl gas until no more product separated. The product was collected and recrystallized from EtOH; yield 0.15 g (10%), mp 251–256° dec.

Method E was the same as method C except no H_2O or $EtSO_3H$ was added to the reduction mixture.

Method F. N-(*m*-Bromoacetamidobenzyl)-3,4-dichlorophenoxyacetamide (4).—To a solution of 325 mg (1 mmole) of **48** in 10 ml of Me_2CO was added 390 mg (1.5 mmoles) of bromoacetic anhydride.[17] The mixture was stirred for 4 hr, then diluted (H_2O) if the product had not separated. The product was collected on a filter, then washed (Me_2CO–H_2O, 5% $NaHCO_3$, H_2O). Recrystallization from EtOH afforded 300 mg (67%) of white needles, mp 155–157°, that gave a negative Bratton–Marshall test for arylamine and a positive 4-(*p*-nitrobenzyl)pyridine test for activated halogen.[17] See Table II for additional data.

Method G. 3-(*m*-Chlorophenoxyacetamidomethyl)-3'-fluorosulfonyldiphenylurea (13).—To a solution of 291 mg (1 mmole) of **48** in 15 ml of $CHCl_3$ was added 220 mg (1.1 mmoles) of *m*-fluorosulfonylphenyl isocyanate in 15 ml of $CHCl_3$. After 20 min, the mixture was filtered and the product was washed with $CHCl_3$. Recrystallization from EtOH gave 319 mg (63%) of white crystals, mp 178–181°, which gave a negative Bratton–Marshall test for arylamine.[17] See Table II for additional data.

Irreversible Enzyme Inhibitors. CXV.[1,2] Proteolytic Enzymes. V. Active-Site-Directed Irreversible Inhibitors of Trypsin Derived from *p*-(Phenoxyalkoxy)benzamidines with a Terminal Sulfonyl Fluoride

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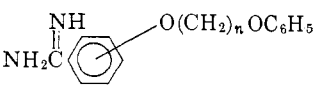
Eight candidate irreversible inhibitors of trypsin have been synthesized from *p*-phenoxypropoxybenzamidine (**3**) and *p*-phenoxybutoxybenzamidine by insertion of a leaving group on the *para* position of the phenoxy moiety. The *p*-bromoacetamido derivative (**6**) of **3** was a good reversible inhibitor of trypsin, but failed to show any irreversible inhibition. When the methylene bridge of **6** was increased to butyl, the resultant bromoacetamide (**11**) showed slow irreversible inhibition of trypsin with a half-life of about 5 hr. Much more dramatic results were obtained by insertion of a *m*- or *p*-fluorosulfonylbenzamido or *m*-fluorosulfonylphenylureido group on the phenoxy moiety. Five irreversible inhibitors of this type could inactivate trypsin with a half-life of 3–6 min. The kinetics indicated that not only did these sulfonyl fluorides rapidly inactivate trypsin, but trypsin could catalyze the hydrolysis of the sulfonyl fluorides to the irreversibly ineffective sulfonic acids.

Selective inhibitors for serum proteases could have utility for chemotherapy of cardiovascular diseases and of organ transplantation.[3] Since a number of these enzymes are "tryptic" in character, our initial studies have concentrated on trypsin as a model. Furthermore, active-site-directed irreversible inhibitors[4] of the exo type could be expected to have the necessary selectivity in inhibition of these closely related serum proteases. The design of such exo-type irreversible inhibitors is best performed stepwise: (a) the binding points of an inhibitor should be determined; (b) a position on the inhibitor should be found where large groups can be tolerated without interfering with formation of a reversible enzyme–inhibitor complex, a necessary intermediate in enzyme inactivation by the active-site-directed mechanism; (c) a properly positioned leaving group should be placed on this large moiety which becomes juxtaposed to a nucleophilic group when complexed to the enzyme and has the proper electrophilicity to react with this enzymic nucleophile; (d) the irreversible inhibitor is further modified to give selective inhibition of closely related enzymes or isozymes.

Since benzamidine is an excellent reversible inhibitor of trypsin,[5] it was selected for further study in order to convert it to an active-site-directed irreversible inhibitor of the exo type. In our first paper on trypsin,[3] we established that phenoxyalkoxy groups could be substituted on benzamidine at either the *para* (**1**) or *meta* positions (**2**) with retention of binding; in fact



1, *para* isomers
2, *meta* isomers

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

(2) For the previous paper see B. R. Baker and J. A. Hurlbut, *J. Med. Chem.*, **11**, 241 (1968).

(3) For a more detailed discussion of inhibition of these enzymes see B. R. Baker and E. H. Erickson, *ibid.*, **10**, 1123 (1967), paper CVI of this series.

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

(5) M. Mares-Guia and E. Shaw, *J. Biol. Chem.*, **240**, 1579 (1965).