Irreversible Enzyme Inhibitors. CXLV.[1,2] Proteolytic Enzymes. VIII. Active-Site-Directed Irreversible Inhibitors of α-Chymotrypsin Derived from α-(2-Carboxy-4-chlorophenoxy)acetamide Bearing a Terminal Sulfonyl Fluoride[3]

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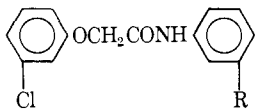
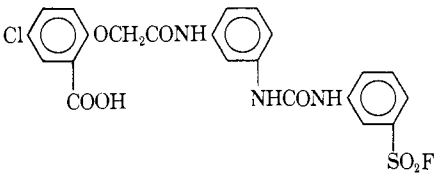
Thirteen candidate irreversible inhibitors derived from N-benzyl- or N-phenyl-α-(2-carboxy-4-chlorophenoxy)-acetamide with a terminal SO_2F group on the N substituent were synthesized and evaluated. A number of these at a K_i concentration gave essentially complete inactivation of α-chymotrypsin in 60 min at 37°. The best compound was α-(2-carboxy-4-chlorophenoxy)-N-[*m*-(*m*-fluorosulfonylphenylureido)benzyl]acetamide (**7**) which showed a half-life of inactivation of 2 min when a K_i = 81 μ*M* concentration was incubated with 1 μ*M* chymotrypsin.

The synthesis and enzymic evaluation of active-site-directed irreversible inhibitors[5] of proteolytic enzymes[6] that operate by covalent bond formation outside the active site, the so-called exo mechanism,[7] have been actively pursued in this laboratory. At a $2–3K_i$ concentration, **1** can rapidly inactivate α-chymotrypsin.[8] In order to place the SO_2F linkage



1, R = SO_2F

2, R = $NHCONHC_6H_4SO_2F$-*m*



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further from the CONH linkage of **1**, which is believed[9] to complex to the catalytic part of the active site,[10] 15 candidate irreversible inhibitors related to **2** were synthesized.[11] Although a number of these showed 15–45% irreversible inhibition, they could only be assayed at a $0.1–0.3K_i$ concentration due to insolubility.[11] A program was undertaken to determine where water-solubilizing groups could be placed on a molecule such as **2**. From this program emerged an irreversible inhibitor of type **3** with a COO^- group (at pH 7.4) on the *ortho* position of the phenoxy moiety; **3** was about 100 times more soluble than **2** in the aqueous medium used for assay.[3] In this paper is described the synthesis and enzymic evaluation of 12 analogs of **3** with variants in the anilide portion.

Enzyme Results.—The results with **3** and its 12 analogs (**4**–**15**) are recorded in Table I. The ability of the compounds to inactivate α-chymotrypsin was compared at concentrations sufficient to complex reversibly 50% of the enzyme, since the concentration of the reversible complex [E···I] is the rate-determining species with active-site-directed irreversible inhibitors.[12] When the parent **3** at an $I_{50} \simeq K_i$ concentration (0.15 m*M*) was incubated with chymotrypsin (~0.08 m*M*), only a total of 75% inactivation was observed. When the SO_2F moiety of **3** was moved to the *para* position, the resultant **4** was about a fivefold less effective reversible inhibitor; however, **4** and **3** were about equally effective irreversible inhibitors when each was assayed at a K_i concentration. Insertion of a Cl *para* to SO_2F moiety of **3** gave **5** which was about twice as effective as **3** as a reversible inhibitor, but equally effective is **3** as an irreversible inhibitor at K_i concentration. Insertion of an *o*-CH_3 group (**6**) on **3** gave about twofold less effective reversible inhibition, but **6** was considerably less effective than **3** as an irreversible inhibitor. The SO_2F type of irreversible inhibitor can not only form a covalent bond with chymotrypsin by the active-site-directed mechanism, but the enzyme can also catalyze hydrolysis of the SO_2F moiety.[8] It would appear that the *o*-CH_3 substituent of **6** has an unfavorable effect on the ratio of the rate of inactivation to the rate of enzyme-catalyzed hydrolysis.

When a CH_2 group was inserted in the anilide moiety of **3**, the resultant benzylamide (**7**) was a twofold better reversible inhibitor than **3**; furthermore, **7** was a better irreversible inhibitor than **3** since **7** at a K_i concentra-

(1) This work was generously supported by Grant CA-08695 from the National Cancer Institute, U. S. Public Health Service; some of the enzyme assays were performed on a Gilford 2400 spectrophotometer purchased on Grant FR07099, U. S. Public Health Service.

(2) For the previous paper in this series and on proteolytic enzymes see B. R. Baker and E. H. Erickson, *J. Med. Chem.*, **12**, 112 (1969).

(3) For the previous paper on chymotrypsin see B. R. Baker and J. A. Hurlbut, *ibid.*, **11**, 1054 (1968), paper CXXXII of this series.

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

(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) For a discussion of the chemotherapeutic utility of selective irreversible inhibitors of serum proteases in the cardiovascular disease and organ transplantation area see B. R. Baker and E. H. Erickson, *J. Med. Chem.*, **10**, 1123 (1967), paper CVI of this series. α-Chymotrypsin serves as a model for these studies.

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

(8) B. R. Baker and J. A. Hurlbut, *J. Med. Chem.*, **11**, 233 (1968), paper CXIII of this series.

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

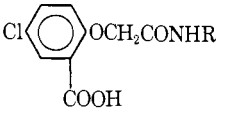
(10) The active site is defined to include those amino acid residues involved in complexing the substrate and those amino acids involved in the catalytic action. The dimensions of the active site of an enzyme using a protein as substrate are difficult to guess since it is not known how many amino acid residues of the substrate are in contact with the enzyme; however, the farther the SO_2F moiety of the inhibitor is removed from the catalytic site in the complex, the higher is the probability that two proteases would differ in primary and tertiary structure in the vicinity of the SO_2F group; see ref 5, p 188.

(11) B. R. Baker and J. A. Hurlbut, *J. Med. Chem.*, **11**, 241 (1968), paper CXIV of this series.

(12) For the kinetics of irreversible inhibition see ref 5, Chapter 8.

TABLE I

IRREVERSIBLE INHIBITION[a] OF α-CHYMOTRYPSIN BY



		Reversible		Irreversible				
No.	R	I_{50},[b] mM	Estd K_i,[c] mM	Assay method[d]	Inhib, mM	% E···I[e]	Time, min	% inactvn
3	*m*-$C_6H_4NHCONHC_6H_4SO_2F$-*m*	0.15	0.15	GPNA	0.15	50	60	75
4	*m*-$C_6H_4NHCONHC_6H_4SO_2F$-*p*	0.70	0.70	GPNA	0.62	48	60	82
5	*m*-$C_6H_4NHCONHC_6H_3$-2-Cl-5-SO_2F	0.080	0.080	GPNA	0.080	50	60	75
				BTEE	0.080	50	60	84
				BTEE	0.080	50	14	50[f]
6	*m*-$C_6H_4NHCONHC_6H_3$-4-Me-3-SO_2F	0.26	0.26	GPNA	0.26	50	60	35
7	*m*-$CH_2C_6H_4NHCONHC_6H_4SO_2F$-*m*	0.081	0.081	GPNA	0.081	50	60	100
				BTEE	0.081	50	60	96
				BTEE	0.040	33	60	89
				BTEE	0.010	11	60	62
				BTEE	0.081	50	2	50[f]
8	*m*-$CH_2C_6H_4NHCONHC_6H_4SO_2F$-*p*	0.59	0.59	GPNA	0.59	50	60	98
9	*p*-$CH_2C_6H_4NHCONHC_6H_4SO_2F$-*m*	0.30	0.30	GPNA	0.30	50	60	100
				BTEE	0.30	50	3	50[f]
10	*p*-$CH_2C_6H_4NHCONHC_6H_4SO_2F$-*p*	1.0	1.0	GPNA	1.0	50	60	72
11	*m*-$CH_2C_6H_4NHCONHC_6H_3$-4-Me-3-SO_2F	0.48	0.48	GPNA	0.48	50	60	79
12	*m*-$CH_2C_6H_4NHCONHC_6H_3$-2-Cl-5-SO_2F	0.16	0.16	GPNA	0.16	50	60	100
				BTEE	0.020	11	60	100
				BTEE	0.16	50	1	50[f]
13	*m*-$CH_2C_6H_4NHCONHC_6H_3$-3-Cl-4-SO_2F	0.30	0.30	GPNA	0.30	50	60	100
14	*p*-$CH_2C_6H_4NHCONHC_6H_3$-4-Me-3-SO_2F	0.40	0.40	GPNA	0.40	50	60	43
15	*p*-$CH_2C_6H_4NHCONHC_6H_3$-2-Cl-5-SO_2F	0.23	0.23	GPNA	0.23	50	60	97

[a] The technical assistance of Marlene Dean and Jean Reeder is acknowledged. [b] I_{50} = concentration necessary for 50% inhibition when assayed with 0.2 m*M* N-glutaryl-L-phenylalanine *p*-nitroanilide (GPNA) in pH 7.4 Tris buffer as previously described.[9] [c] Estimated as $K_i = I_{50}$.[8] [d] GPNA: 40–80 μ*M* enzyme incubated with inhibitor at 37° in pH 7.4 Tris buffer containing 10% DMSO, then the remaining enzyme was determined by assay with 0.2 m*M* GPNA.[8] BTEE: 1 μ*M* enzyme incubated with inhibitor at 24° in pH 7.4 Tris buffer containing 10% DMSO, then assayed with BTEE in pH 8.1 Tris buffer + 0.1 *M* $CaCl_2$.[13] [e] Calculated from [EI] = $[E_t]/(1 + K_i/[I])$ where [EI] = amount of reversible complex expressed as a percentage of total enzyme (E_t);[12] this calculation is valid only when inhibitor is in excess of enzyme. [f] From a six-point time study.[8]

tion gave total inactivation of chymotrypsin. A shift of the SO_2F moiety of **7** from *meta* to *para* gave **8** which showed an eightfold loss in reversible binding, but was an equally effective irreversible inhibitor at K_i concentration. Similarly, the shift of the phenylurea moiety of **7** from *meta* to *para* (**9**) resulted in less effective reversible inhibition, but similar irreversible inhibition. However, when the SO_2F moiety of **9** was shifted from *meta* to *para*, the resultant **10** was less effective both reversibly and irreversibly. Insertion of a *o*-Me (**11**) or *p*-Cl (**12**) on **7** was detrimental to reversible binding, but irreversible inhibition was not changed in **12**. Similarly, insertion of an *o*-Me (**14**) on **9** was detrimental to irreversible inhibition. However, insertion of an *o*-Cl (**13**) on **8** gave twofold better reversible binding with no change in the excellent irreversible inhibition.

The two irreversible inhibitors with the lowest K_i's were then compared in a six-point time study;[8] **7** was a better irreversible inhibitor since it showed a half-life of inactivation of **2** min, in contrast to **5** which had a half-life of 14 min. Two other compounds (**9**, **12**) that showed total inactivation of chymotrypsin, but had less effective K_i's than **5**, were also subjected to a time study; both **9** and **12** were faster irreversible inhibitors than compound **5**, having half-lives of 3 and 1 min, respectively, when assayed at K_i concentration.

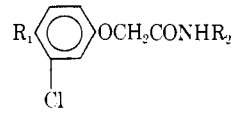
The best irreversible inhibitor in Table I was **7** since it rapidly and totally inactivated chymotrypsin at the lowest concentration due to its low K_i. Therefore **7** was studied in more detail. In order to study **7** at less than $K_i \simeq 0.08$ m*M*, it was necessary to devise an incubation that used less than 0.08 m*M* chymotrypsin; by use of N-benzoyl-L-tyrosine ethyl ester (BTEE)[13] instead of N-glutaryl-L-phenylalanine *p*-nitroaniline (GPNA),[14] it was possible to reduce the incubation concentration of chymotrypsin[15] to about 1 μ*M* since BTEE has a vastly superior V_{max}. When the irreversible inhibition by **7** at 0.081 m*M* concentration of 1 μ*M* chymotrypsin was reexamined with the BTEE assay, inactivation was again essentially total. When the concentration of **7** was reduced to $0.5K_i$ = 0.04 m*M*, which is sufficient to complex reversibly 33% of the enzyme, 89% inactivation was seen in 1 hr. Further reduction of the concentration of **7** to 0.01 m*M* = $K_i/8$, which reversibly complexed only 11% of the enzyme, gave 62% inactivation in 1 hr. That total inactivation of 1 μ*M* chymotrypsin was not seen by 10 μ*M* = $K_i/8$ of **7** indicates that enzyme-catalyzed hydrolysis of **7** becomes apparent as the inhibitor concentration approaches that of the enzyme. In contrast, the chloro derivative (**12**) of **7** at 20 μ*M* = $K_i/8$

(13) B. C. W. Hummel, *Can. J. Biochem. Physiol.*, **37**, 1393 (1959).

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

(15) We wish to thank Professor Howard J. Schaeffer for pointing out this stoichiometry in previous papers.[8,11]

Table II
Irreversible Inhibition[a] of α-Chymotrypsin by



No.	R_1	R_2	Estd K_i,[b] μM	Irreversible[c] Inhib, μM	% Enz inactd[d]	Time, min	% inactvn
1	H	$C_6H_4SO_2F$-*m*	36	45	55	60	86
2	H	*m*-$C_6H_4NHCONHC_6H_4SO_2F$-*m*	>25	10[e]	<29	60	72
16	H	*m*-$CH_2C_6H_4NHCONHC_6H_4SO_2F$-*m*	70	10[e]	13	60	62
17	H	*p*-$CH_2C_6H_4NHCONHC_6H_4SO_2F$-*p*	>25	5[e]	<17	60	44
18	H	*m*-$C_6H_4NHCOC_6H_4SO_2F$-*m*	>50	10[e]	<17	60	66
19	H	*m*-$C_6H_4NHCOC_6H_4SO_2F$-*p*	>12	2.5[e]	<17	60	10
20	H	*p*-$C_6H_4CH_2NHCONHC_6H_4SO_2F$-*m*	60	25[e]	30	60	89
				25[e]	30	5	50[f]
21	H	*m*-$CH_2C_6H_4NHCOC_6H_4SO_2F$-*m*	35	20[e]	36	60	85
				20[e]	36	5	50[e]
22	H	*m*-$CH_2C_6H_4NHCOC_6H_4SO_2F$-*p*	67	20[e]	23	60	79
23	H	*p*-$CH_2C_6H_4NHCOC_6H_4SO_2F$-*m*	18	18[e]	50	60	76
24	H	*p*-$CH_2C_6H_4NHCOC_6H_4SO_2F$-*p*	60	10[e]	14	60	62
25	H	*o*-$CH_2C_6H_4NHCONHC_6H_4SO_2F$-*m*	90	10[e]	10	60	65
26	H	*o*-$CH_2C_6H_4NHCOC_6H_4SO_2F$-*m*	53	20[e]	28	60	68
27	H	*o*-$CH_2C_6H_4NHCOC_6H_4SO_2F$-*p*	90	20[e]	18	60	87
				20[e]	18	2, 10, 60	50, 78, 85[f]
28	Cl	*m*-$CH_2C_6H_4NHCOC_6H_4SO_2F$-*m*	35	10[e]	22	60	63
29	H	$C_6H_4SO_2F$-*o*	42	42	50	60	95
30	Cl	$C_6H_4SO_2F$-*o*	20	20	50	60	86
31	Cl	$C_6H_4SO_2F$-*m*	>50	10[e]	<17	60	57
32[g]	H	C_6H_4-2-OMe-5-SO_2F	92	13[e]	12	60	0

[a] See Table I; see ref 11 for synthesis. [b] Estimated as $K_i = I_{50}$.[8,11] [c] Enzyme (~1 μM) incubated with inhibitor at 24° in pH 7.4 Tris buffer containing 10% DMSO, then assayed with BTEE in pH 8.1 Tris buffer containing 0.1 *M* $CaCl_2$.[13] [d] See footnote *e*, Table I. [e] Near maximum solubility. [f] From six-point time study.[8] [g] Prepared from 3-NH_2-4-$OMeC_6H_4SO_2F$ by method E[9] in 69% yield, mp 208–211°. *Anal.* ($C_{15}H_{13}ClFNO_5S$) C, H, N.

concentration still showed total inactivation; thus, this Cl substitution (**12**) has a favorable effect on the ratio of the rate of enzyme inactivation to the rate of enzyme-catalyzed hydrolysis.[8]

Since **7** and **12** at a concentration of $K_i/8$ showed good inactivation of 1 μM chymotrypsin by the BTEE assay, some of the previously reported compounds[11] that were soluble only in 0.1-0.5K_i region were reassayed by this new method (Table II). Of these compounds, four (**20**–**22**, **27**) were good irreversible inhibitors at their maximum solubilities which were sufficient to complex reversibly 18–36% of the enzyme. The best three were compared for half-life of inactivation; even though 20 μM of **27** complexed only 18% of the enzyme, the half-life of inactivation was 2 min compared to 5 min for **20** and **21** at a concentration of 25 and 20 μM, sufficient to complex 30 and 36% of the enzyme, respectively. Benzenesulfonyl fluoride can inactivate α-chymotrypsin by covalent bond formation with serine-195.[16] Presumably *p*-tolylsulfonyl fluoride attacks this same amino acid when 100 μM inactivates α-chymotrypsin with a half-life of 32 min.[8] The inactivation of α-chymotrypsin by **7**, **12**, and **27** is considerably more rapid than inactivation by *p*-tolylsulfonyl fluoride.

Three inhibitors (**1**, **29**, **30**) that gave excellent irreversible inhibition of ~80 μM chymotrypsin when assayed above 80 μM with GPNA also gave excellent irreversible inhibition at their K_i concentration (20–40 μM) when assayed with 1 μM chymotrypsin and BTEE.

(16) D. E. Fahrney and A. M. Gold, *J. Am. Chem. Soc.*, **85**, 997 (1963).

It is unlikely that the same amino acid moiety of chymotrypsin is covalently linked by all the irreversible inhibitors in Tables I and II; proof of such an assumption is a worthy pursuit that could give additional insight into the tertiary structure of chymotrypsin. Inhibition of the "chymotryptic" component of the complement system[17] has been observed with some members of Table I; these studies are being pursued vigorously.

Chemistry.—The irreversible inhibitors, **3**–**6**, can be generalized by **37**. The synthesis of **3** *via* **34** by condensation of **35** with *m*-fluorosulfonylphenyl isocyanate was described previously.[3] The remainder (**4**–**6**) were synthesized from **35** by condensation with the appropriate O-(*p*-nitrophenyl) carbamate (**36**) (Scheme I).[18] Attempts to convert **35** to **39b** by acylation with *m*-fluorosulfonylbenzoyl chloride gave mixtures which could not be purified. The corresponding ester (**38**) could be acylated to **39a**, but attempted acid hydrolysis to **39b** was not sufficiently selective over the amide linkages. Attempts to prepare the *p*-amino isomer of **35** were unsuccessful due to the insolubility of the nitro precursors.

The irreversible inhibitors **7**, **8**, **11**–**13** can be generalized by structure **45**, synthesized from the amine (**44**) by reaction with appropriate carbamate (**36**); the amine (**44**) was synthesized *via* compounds **40**–**43** as previously described for **35**;[3] an attempt to condense

(17) (a) Ciba Foundation Symposium, Complement, G. E. W. Wolstenholme and J. Knight, Ed., Little, Brown and Co., Boston, Mass., 1965; (b) H. J. Müller-Eberhard, *Adcon. Immunol.*, **8**, 1 (1968).

(18) B. R. Baker and N. M. J. Vermeulen, *J. Med. Chem.*, **12**, 71 (1969), paper CXXXIV of this series.

SCHEME I

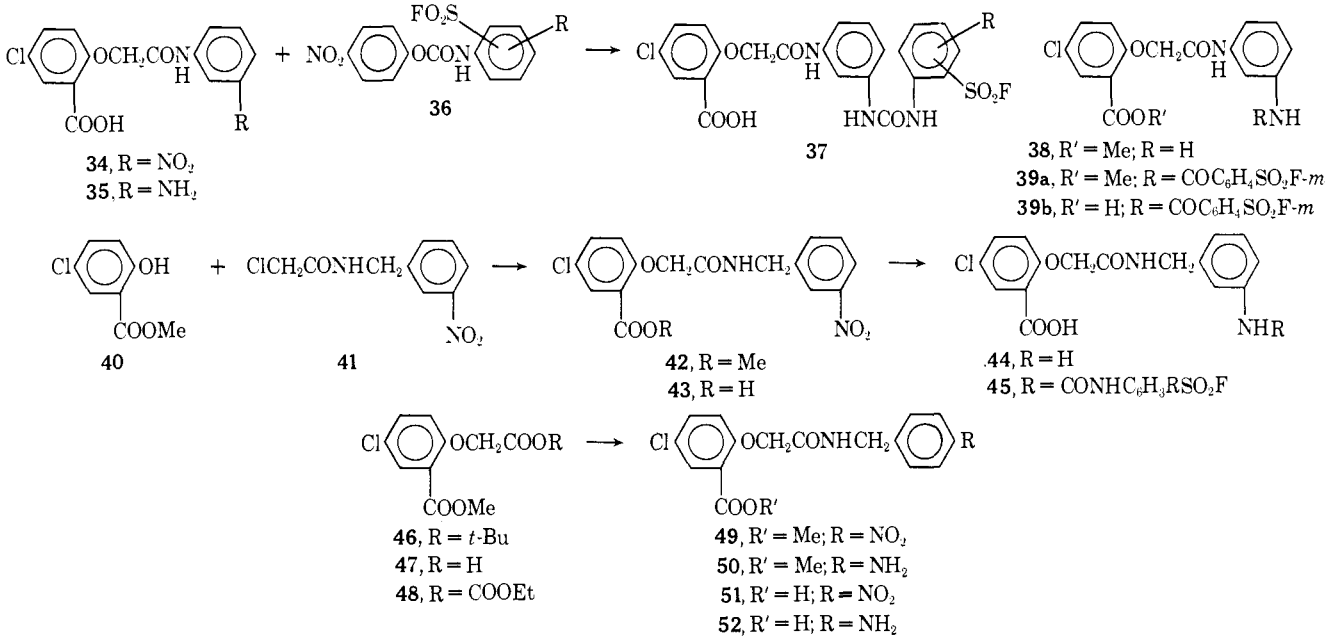
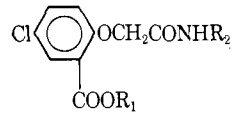


TABLE III

PHYSICAL PROPERTIES OF



No.	R_1	R_2	Method[a]	Yield, %	Mp, °C	Formula
42	Me	$CH_2C_6H_4NO_2$-*m*	E[b]	66[c]	156–159	$C_{17}H_{13}ClN_2O_6$
43	H	$CH_2C_6H_4NO_2$-*m*	F	96[d]	158–160	$C_{16}H_{13}ClN_2O_6$
44	H	$CH_2C_6H_4NH_2 \cdot HCl$-*m*	G	72[e]	224–226 dec	$C_{16}H_{13}ClN_2O_4 \cdot HCl$
50	Me	$CH_2C_6H_4NH_2 \cdot HCl$-*p*	G[f]	88[d]	214–215 dec	$C_{17}H_{17}ClN_2O_4 \cdot HCl$
51	H	$CH_2C_6H_4NO_2$-*p*	F	36[d]	223–228	$C_{16}H_{13}ClN_2O_6$
52	H	$CH_2C_6H_4NH_2$-*p*	F	90[d]	175–177	$C_{16}H_{13}ClN_2O_4$
53	Me	$C_6H_4NO_2$-*p*	E	81[g]	246–248	$C_{16}H_{13}ClN_2O_6$

[a] For methods E–G, see ref 3. All samples were analyzed for C, H, N. [b] Intermediate **41** prepared from *m*-nitrobenzylamine hydrochloride,[19] chloroacetyl chloride, and Et_3N in $CHCl_3$ in 91% yield, mp 132–135°. *Anal.* ($C_9H_9ClN_2O_3$) C, H, N. [c] Recrystallized from Me_2CO. [d] Recrystallized from EtOH. [e] Recrystallized from EtOH–petroleum ether (bp 60–110°). [f] Hydrogenation was performed in EtOAc, then HCl gas was added to precipitate the HCl salt. [g] Recrystallized from MeOEtOH.

40 with the *p*-nitro isomer of **41** to form **49** gave a black polymer, presumably initiated through the benzyl carbanion. Therefore, **49** was synthesized by an alternate route. Alkylation of methyl 5-chlorosalicylate (**40**) with *t*-butyl chloroacetate gave **46** as an oil which was readily converted to **47** by acid-catalyzed elimination of isobutylene. The amide (**49**) was then prepared from **47** *via* **48** and *p*-nitrobenzylamine.[19] Of the two routes to **52**, the route *via* **50** was preferred over the route *via* **51**. The irreversible inhibitors, **9**, **10**, **14**, and **15**, were prepared from **52** and the appropriate carbamate (**36**).

Experimental Section

Each analytical sample had an appropriate ir spectrum, moved as a single spot on tlc on Brinkmann silica gel GF or Brinkmann MN–polyamide UV_{254}, and gave combustion values for C, H, and N or F within 0.4% of theoretical. Melting points were taken in capillary tubes on a Mel-Temp block and are uncorrected. Methods E–G have been previously described.[3] Physical properties of **42–44** and **50–53** are listed in Table III.

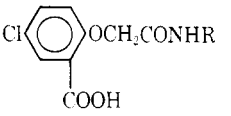
N-[*m*-(*m*-Fluorosulfonylbenzamido)phenyl]-2-carbomethoxy-4-chlorophenoxyacetamide (39a).—To a stirred solution of 1.01 g (3 mmoles) of **38**[3] in 20 ml of DMF and 0.80 g (10 mmoles) of pyridine was added at ambient temperature 0.72 g (3.2 mmoles) of *m*-fluorosulfonylbenzoyl chloride in 10 ml of DMF over 5 min. After an additional 10 min the solution was treated with 50 ml of 2% HCl. The product was collected on a filter and washed with H_2O, then EtOH. Recrystallization from Me_2CO gave 1.15 g (74%) of white crystals, mp 232–234°. *Anal.* ($C_{23}H_{18}ClFN_2O_7S$) C, H, F.

2-Carbomethoxy-4-chlorophenoxyacetic Acid (47).—Reaction of 39.2 g (0.21 mole) of **40**[3] with 30.2 g (0.20 mole) of *t*-butyl chloroacetate by method E[3] gave **46** as an oil isolated by CH_2Cl_2 extraction, then removal of any unchanged **40** by extraction with two 200-ml portions of 10% NaOH. The residual **46** (must be free of DMF) was refluxed in 200 ml of toluene containing 1 g of TsOH for 3 hr when isobutylene evolution was complete, then diluted with 100 ml of petroleum ether (bp 60–110°). The product was collected and recrystallized twice from toluene; yield 32.6 g (76% over-all), mp 135–138°. *Anal.* ($C_{10}H_9ClO_5$) C, H.

2-Carbomethoxy-4-chloro-N-(*p*-nitrobenzyl)phenoxyacetamide (49).—To a stirred solution of 12.3 g (50 mmoles) of **47** in 250 ml of THF containing 5.05 g (50 mmoles) of Et_3N was added 5.43 g (50 mmoles) of ethyl chloroformate over a period of 5 min, maintaining the temperature at −5 to 0°. After stirring for an additional 45 min at −5 to 0°, the mixture was treated with a cold solution of 9.45 g (50 mmoles) of *p*-nitrobenzylamine hydrochloride[19] and 10.1 g (0.1 mole) of Et_3N in 250 ml of THF; stirring was continued at −5 to 0° for 1 hr, then 3 hr at ambient

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

TABLE IV
PHYSICAL PROPERTIES OF



No.[a]	R	Yield, %	Mp, °C dec	Formula
4	*m*-$C_6H_4NHCONHC_6H_4SO_2F$-*p*	63[b]	245–247	$C_{22}H_{17}ClFN_3O_5S$
5	*m*-$C_6H_4NHCONHC_6H_3$-2-Cl-5-SO_2F	65[c,d]	268–270	$C_{22}H_{16}Cl_2FN_3O_5S$
6	*m*-$C_6H_4NHCONHC_6H_3$-4-Me-3-SO_2F	62[b,e]	255–257	$C_{23}H_{19}ClFN_3O_5S$
7	*m*-$CH_2C_6H_4NHCONHC_6H_4SO_2F$-*m*	73[e]	225–227	$C_{23}H_{19}ClFN_3O_5S$
8	*m*-$CH_2C_6H_4NHCONHC_6H_4SO_2F$-*p*	78[d,e]	230–232	$C_{23}H_{19}ClFN_3O_5S$
9	*p*-$CH_2C_6H_4NHCONHC_6H_4SO_2F$-*m*	72[b,c,e]	227–229	$C_{23}H_{19}ClFN_3O_5S$
10	*p*-$CH_2C_6H_4NHCONHC_6H_4SO_2F$-*p*	73[f]	231–234	$C_{23}H_{19}ClFN_3O_5S$
11	*m*-$CH_2C_6H_4NHCONHC_6H_3$-4-Me-3-SO_2F	87[b]	238–241	$C_{24}H_{21}ClFN_3O_5S$
12	*m*-$CH_2C_6H_4NHCONHC_6H_3$-2-Cl-5-SO_2F	51[b]	232–234	$C_{23}H_{18}Cl_2FN_3O_5S$
13	*m*-$CH_2C_6H_4NHCONHC_6H_3$-3-Cl-4-SO_2F	35[c,d]	227–229	$C_{23}H_{18}Cl_2FN_3O_5S$
14	*p*-$CH_2C_6H_4NHCONHC_6H_3$-4-Me-3-SO_2F	80[c,d]	186–220	$C_{24}H_{21}ClFN_3O_5S$
15	*p*-$CH_2C_6H_4NHCONHC_6H_3$-2-Cl-5-SO_2F	71[c,e]	229–231	$C_{23}H_{18}Cl_2FN_3O_5S$

[a] All prepared by method A and analyzed for C, H, and F; each moved as a single spot on polyamide MN. [b] Precipitated from MeOEtOH with H_2O, then washed with hot EtOH and hot acetone. [c] Reprecipitated from MeOEtOH with H_2O. [d] Recrystallized from Me_2CO–H_2O. [e] Recrystallized from EtOH. [f] Recrystallized from MeOH.

temperature. The mixture was spin evaporated *in vacuo*. The residue was crystallized from DMF–H_2O, then MeOEtOH; yield 11.9 g (63%) of white needles, mp 182–184°. *Anal.* ($C_{17}H_{15}ClN_2O_6$) C, H, N.

2-Carboxy-4-chloro-N-[*m*-(3-fluorosulfonyl-4-methylphenylureido)benzyl]phenoxyacetamide (11) (Method A).—A solution of 371 mg (1 mmole) of **44**, 354 mg (1 mmole) of O-(*p*-nitrophenyl) N-(3-fluorosulfonyl-4-methylphenyl)carbamate (**36**),[20] and 100 mg (1.3 mmoles) of pyridine in 5 ml of DMF was allowed to stand for 12 hr, then diluted with 20 ml of 5% HCl. The product was collected on a filter and washed with H_2O, Me_2CO, and hot EtOH. The product was reprecipitated from MeOEtOH with H_2O, then washed again with hot EtOH; yield 480 mg (87%) of white powder, mp 238–241° dec, negative Bratton–Marshall test for aromatic amine.[21] See Table IV for additional data and compounds prepared by this method.

(20) Prepared in this laboratory by W. F. Wood by the previously described general method.[18]

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Derivatives of Imidazole. III. Synthesis and Pharmacological Activities of Nitriles, Amides, and Carboxylic Acid Derivatives of Imidazo[1,2-*a*]pyridine

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A number of improved laboratory procedures for the synthesis of imidazo[1,2-*a*]pyridine derivatives are reported. The resulting compounds, which are structurally related to indole derivatives, have been screened for analgetic, antipyretic, anticonvulsant, and antiinflammatory activity. Phytopharmacological tests have also been performed on the derivatives structurally related to indoleacetic acid.

Previous publications from this laboratory[2–4] described some aspects of the chemistry of imidazo[1,2-*a*]pyridines and showed that these and related compounds react easily with electrophilic reagents, similarly to indole, in the 3 position. π-Electron density calculations[5] for imidazo[1,2-*a*]pyridines further confirmed that electrophilic substitution (on carbon) should occur at the 3 position; other chemical similarities between imidazo[1,2-*a*]pyridines and indole derivatives were recently shown.[6–9]

In accord with a continuing program designed for the pharmacological screening of new imidazo[1,2-*a*]pyridines, we thought it would be interesting to synthesize and test a number of derivatives that may be considered analogs of indoleacetic acid and indomethacin.[10]

Pharmacological tests on the analgetic, antiinflammatory, antipyretic, and muscle relaxant activity were

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