reaction accompanied each drop. After the addition, the reaction mixture was allowed to warm to room temperature and stirred 1 hr, 400 ml of dry C_6H_6 was added, and the ether was removed by heating on a water bath. Ice and then 200 ml of 10% HCl was added to the suspended salt, the organic layer was separated, the residue was washed with two 100-ml portions of benzene, and the combined organic layer was washed (100 ml of salt water, two 100-ml portions of 5% Na_2CO_3, salt water). After drying (Na_2SO_4), the solvent was removed and the product distilled to give 50.5 g (57%) of a clear yellow liquid: bp 133–135° (0.5 mm); ir (film), 3.0 (bonded OH), 3.3 (Ar-CH), 3.45, 3.50 (CH_2), 9.6 (C–O) μ. *Anal.* ($C_{10}H_{10}OS$) S.

This compound was identical with that prepared in 42% yield by $LiAlH_4$ reduction of IV.[19]

3-(β-Bromoethyl)benzo[*b*]thiophene (X).—Following the procedure of Cagniant,[19b] 30 g (0.168 mole) of IX was treated with 16 g of PBr_3 in 200 ml of dry $CHCl_3$ containing 1 g pyridine. After standing overnight, the mixture was heated to 50° for 1 hr, cooled, and poured into 200 ml of cold H_2O. The $CHCl_3$ layer was separated, washed (H_2O, 10% HCl, twice with 10% Na_2CO_3, H_2O), and dried (Na_2SO_4). Distillation gave 25 g (65%) of pale yellow oil: bp 134–137° (1 mm); ir (film), 3.27 (Ar-CH), 3.40 (CH_2), 14.85 (C–Br) μ. The oil was used without further purification, as it rapidly became cloudy and decomposed.

3-(β-t-Aminoethyl)benzo[*b*]thiophenes (XI).—All of the derivatives listed in Table I, except XIII, were prepared as follows: 5 g (0.021 mole) of X was added to each of five batches of 200 ml of anhydrous MeOH. To each flask was added 50 g of a secondary amine, di-*n*-propylamine, pyrrolidine, piperidine, morpholine, and N-methylpiperazine, respectively. Upon the addition of these amines, each reaction mixture became warm. The homogeneous solutions were allowed to remain at room temperature for 15 days, then solvent and excess amines were removed by distillation, and the residue was treated with 50 ml of 10% NaOH and extracted with three 50-ml portions of ether. The extracts were dried (Na_2SO_4), solvent was removed, and the amine derivatives were distilled.

3-(β-Dimethylaminoethyl)benzo[*b*]thiophene Hydrochloride (XIII).—The Eschweiler–Clarke variant of the Leuckart reaction was used in this preparation.[20] III (5 g, 0.0282 mole), 20 ml of 37% CH_2O, 20 ml of 99% HCOOH, and 16 ml of H_2O were heated gently on the steam bath for 4 hr, then 30 ml of 6 *N* HCl was added to the cool reaction mixture and the solvent was distilled at reduced pressure. The tan semisolid that remained was dried at 78° (1 mm), for 16 hr, washed with dry ether, and recrystallized twice from absolute EtOH, to yield 4.5 g (66%) of white crystals: mp 260–261°; ir (KBr), 3.41 (CH_2, CH_3), 3.73 ($N-CH_3$), 4.0 (R_3NH^+) μ.

Irreversible Enzyme Inhibitors. CXXXII.[1,2] Proteolytic Enzymes. VI.[3] Tolerance for Polar Groups on the Phenoxyacetanilide Type of Inhibitor of α-Chymotrypsin

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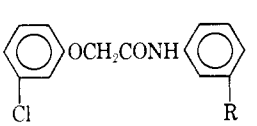
Candidate irreversible inhibitors derived from phenoxyacetanilide (**1**), such as N-[*m*-(*m*-fluorosulfonylphenylureido)phenyl]-3-chlorophenoxyacetamide (**3**), are too insoluble in water for enzymatic evaluation; therefore, a study was conducted on where to position polar groups on phenoxyacetanilide (**1**) that would not interfere with complex formation. Three useful classes of compounds emerged. The first class of compounds consisted of introduction of $RCOO^-$ or $CH_2NH_3^+$ groups on the N-phenyl moiety; this N-phenyl moiety is apparently complexed to a polar region of α-chymotrypsin since no binding was lost. The second class derived from **1** consisted of introduction of a COO^- group on the phenoxy moiety, which is complexed in a hydrophobic region. An *o*-COO^- group (**13**) was well tolerated in the complex, and inhibition could be further enhanced by introduction of a 4- or 5-chloro or 4-bromo atom. The third class consisted of a replacement of the phenoxymethyl moiety of **1** by a quaternized pyridylvinyl or pyridylethyl moiety; only N-methyl-2-pyridylacrylanilide (**28**) in this class was satisfactory, being complexed to the enzyme about one-third as well as **1**. The 2-carboxy-4-chlorophenoxy group of **19** was shown to be a suitable replacement for the 3-chlorophenoxy group of **3** in order to increase solubility; not only was **19** about 100 times as soluble as **3**, but irreversible inhibition was readily detected with **19** at 15% of its maximum solubility.

One of the goals in this laboratory has been the design and synthesis of active-site-directed irreversible inhibitors[5] of proteolytic enzymes[6] that operate by the exo mechanism, that is, the inhibitor forms a covalent bond outside of the active site of the enzyme.[7] α-Chymotrypsin is rapidly inactivated by the irreversible inhibitor **2**, and, in addition, α-chymotrypsin can catalytically hydrolyze the SO_2F group of **2** to the irreversible inert sulfonic acid;[8] neither reaction was seen between **2** and bovine serum albumin. A series of fifteen candidate irreversible inhibitors related to **2** were then synthesized which placed the SO_2F further from the CONH linkage of **2** which is believed to complex its CONH linkage to the catalytic part of the active site;[9] an example is **3**. Most of these compounds were too insoluble to be evaluated. Therefore a program was



1, R = H
2, R = SO_2F
3, R = $NHCONHC_6H_4SO_2F$-*m*

(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 in this series see B. R. Baker and J. L. Kelley, *J. Med. Chem.*, **11**, 686 (1968).

(3) For the previous paper on proteolytic enzymes see B. R. Baker and E. H. Erickson, *ibid.*, **11**, 245 (1968).

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

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

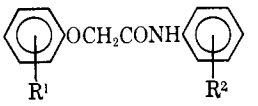
(6) For a discussion of the chemotherapeutic utility of selective irreversible inhibitors of serum proteases in the cardiovascular disease and organ transplantation area see B. R. Baker and E. H. Erickson, *J. Med. Chem.*, **10**, 1123 (1967).

(7) The exo type of irreversible inhibitor can have an extra dimension of specificity not present in reversible inhibitors, 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.*, **11**, 241 (1968), paper CXIV of this series.

TABLE I
INHIBITION[a,b] OF α-CHYMOTRYPSIN BY



Compd	R_1	R_2	Inhib conen, mM	% inhib	Estd I_{50},[c] mM
1	H	H	2.0[d]	42	2.6[e]
2	*m*-Cl	*m*-SO_2F	0.050	50	0.050[f]
3	*m*-Cl	*m*-$NHCONHC_6H_4SO_2F$-*m*	0.0050[d]	0	>0.025[g,h]
4	*m*-Cl	H	0.26	50	0.26
5	H	*p*-COO^-	5.2	50	5.2[g]
6	H	*m*-COO^-	5.2	50	5.2
7	H	*o*-COO^-	4.3	50	4.3
8	H	*p*-CH_2COO^-	4.5	50	4.5
9	H	*p*-OCH_2COO^-	4.8	50	4.8
10	3,4-Cl_2	*p*-OCH_2COO^-	0.51	50	0.51
11	*m*-Cl	*p*-$CH_2NH_3^+$	0.28	50	0.28[g]
12	*m*-Cl	*m*-SO_3^-	0.83	50	0.83[f]
13	*o*-COO^-	H	5.4	50	5.4
14	*m*-COO^-	H	13	50	13
15	4-Cl-2-COO^-	H	1.5	50	1.5
16	4-Br-2-COO^-	H	1.1	50	1.1
17	5-Cl-2-COO^-	H	1.5	50	1.5
18	4,6-Cl_2-2-COO^-	H	3.1	50	3.1
19	4-Cl-2-COO^-	*m*-$NHCONHC_6H_4SO_2F$-*m*	0.15[i]	50	0.15

[a] The technical assistance of Maureen Baker, Diane Shea, and Susan Black is acknowledged. [b] Assayed with crystalline α-chymotrypsin in pH 7.4 Tris buffer containing 10% DMSO with 0.2 mM N-glutaryl-L-phenylalanine-*p*-nitroanilide at 410 mμ as previously described.[10] [c] I_{50} = concentration for 50% inhibition. [d] Maximum solubility. [e] Data from ref 10. [f] Data from ref 8. [g] Data from ref 9. [h] Since 20% inhibition is readily detected, the I_{50} is at least five times greater than the concentration measured. [i] Solubility = 1 mM.

undertaken to determine where water-solubilizing polar groups could be placed on phenoxyacetanilide (**1**)[10] that would not interfere with complex formation with α-chymotrypsin; the candidate inhibitors could be divided into three classes: (a) a carboxylate or aliphatic amino group attached to the aniline ring, (b) a carboxyl group attached to the phenoxy ring, and (c) replacement of the phenoxy ring by a quaternized pyridine ring. Some members of all three classes met the desired criteria of increased solubility and little loss of complexing ability to the enzyme. The results are the subject of this paper.

Enzyme Results.—The first class of compounds consisted of a series of phenoxyacetanilides with ionic groups on the anilide moiety (Table I). Two baseline compounds should be kept in mind, phenoxyacetanilide (**1**)[10] with I_{50} = 2.6 mM and 3-chlorophenoxyacetanilide (**4**)[10] with I_{50} = 0.26 mM. Insertion of a carboxyl group, which would be fully ionized at pH 7.4, on the *para* (**5**), *meta* (**6**), or *ortho* position (**7**) gave only a twofold loss in binding compared to **1**. Similarly, insertion of a *p*-CH_2COOH (**8**) or *p*-OCH_2COOH (**9**) function was tolerated within the enzyme–inhibitor complex. 3,4-Dichloro substitution (**10**) on **9** gave a tenfold increment in binding as would be expected from previous studies on this mode of substitution.[10]

That a cationic group could be introduced with little loss in binding can be seen by comparison of **11** and **4**. However, a *m*-SO_3H group (**12**) gave a fourfold loss in binding compared to **4**; the fivefold enhancement in binding by the *m*-SO_2F group of **2** compared to **4** is due to the previously observed electron-withdrawing effect of a substituent on the binding of the anilide moiety.[10] Thus either an anionic carboxylate or cationic ammoniummethyl group can be inserted on the anilide moiety of **1** with good retention of binding and greatly increased solubility.

The second class of compounds consisted of COO^- substitution on the phenoxy moiety (Table I). Although the phenoxy group of **1** is believed to complex to the same hydrophobic bonding region of α-chymotrypsin as the phenyl group of phenylalanine peptides,[10] no clear evidence exists that all of this phenyl group is buried in a hydrophobic region. That the *para* position of the benzene ring of phenoxyacetone was complexed in a hydrophobic bonding region was clearly indicated by the huge loss in binding when a *p*-COO^- was inserted on phenoxyacetone;[10] however, it was still possible that a polar group could be tolerated *ortho* or *meta* to the ether linkage of **1**. In fact, only a twofold loss in binding occurred when the *o*-COO^- group (**13**) was inserted on **1**; the loss in binding with a *m*-COO^- group (**14**) was larger, being fivefold compared to **1**.

Since an *o*-COO^- group (**13**) on the phenoxy moiety of **1** was tolerated in a complex with the enzyme, a study was made to see if halogen substitution could enhance binding as previously seen without the carboxylate group.[10] Insertion of a 4-chloro atom (**15**) on **14** gave a 3.6-fold increment in binding and a 4-bromo atom (**16**) gave a fivefold increment in binding; since there was less than a twofold difference between bromo and chloro, the chloro was preferred since less loss in solubility would occur. The same increment in binding was observed with the 5-chloro atom of **17** as with the 4-chloro atom of **15**. Although 3,4-dichloro substituents might be expected to give another threefold increment in binding,[10] the gain in binding would result

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

in a commensurate loss in solubility. 4,6-Dichloro substituents (**18**) gave a twofold loss in binding compared to **15**.

From synthetic, binding, and solubility standpoints, the best solubilizing combination in Table I is represented by **15**. Therefore, the 4-chloro-2-carboxy analog (**19**) of the candidate irreversible inhibitor, **3**, was synthesized; **19** had an I_{50} = 0.15 m*M* and was soluble in 10% DMSO to 1 m*M*, being about 100 times as soluble as **3** and solving the solubility problem proposed at the beginning. Furthermore, **19** was an irreversible inhibitor at 0.15 m*M*; these inactivation experiments will be reported in a future paper along with other analogs of **19**.

Since the *o*-COO^- group of **13** could be inserted on the phenoxy moiety of **1** with only a twofold loss in binding, a third class of compounds was investigated where the phenoxymethyl group of **1** was replaced by quaternized pyridylvinyl and pyridylethyl groups (Table II). The

Table II
Inhibition[a,b] of α-Chymotrypsin by $RCONHC_6H_5$

Compd	R	Inhib concn, m*M*	% inhibn	Est I_{50},[c] m*M*
1	$C_6H_5OCH_2$	2.0[d]	42	2.6[e]
20	2-Pyridyl-CH=CH	0.6[d]	17	~3
21	3-Pyridyl-CH=CH	3.0	50	3.0
22	4-Pyridyl-CH=CH	6.5	50	6.5
23	2-Pyridyl-$(CH_2)_2$	8.5	50	8.5
24	3-Pyridyl-$(CH_2)_2$	7.7	50	7.7
25	4-Pyridyl-$(CH_2)_2$	9.1	50	9.1
26	2-Quinolyl-$(CH_2)_2$	0.50[d]	29	1.4
27	2-Quinolyl-CH=CH	0.050[d]	0	>0.25[f]
28	N-Me-2-pyridyl-CH=CH	8.5	50	8.5
29	N-Et-2-pyridyl-CH=CH	15	50	15
30	N-Me-3-pyridyl-CH=CH	10	0	>50[f]
		20	−50[g]	
31	N-Me-4-pyridyl-CH=CH	2.0[h]	0	>10[f]
32	N-Me-2-pyridyl-$(CH_2)_2$	25	15	~140
33	N-Et-2-pyridyl-$(CH_2)_2$	20	20	~90
34	N-Me-3-pyridyl-$(CH_2)_2$	25	0	>120
35	N-Me-4-pyridyl-$(CH_2)_2$	20	0	>100[f]
36	N-Me-2-quinolyl-CH=CH	1.4	50	1.4
37	N-Me-3-isoquinolyl-CH=CH	0.62[a]	0	>3.1

[a-c] See corresponding footnotes in Table I. [d] Maximum solubility. [e] Data from ref 10. [f] Since 20% inhibition is readily detectable, the I_{50} is greater than five times the concentration measured. [g] 50% acceleration of the enzyme reaction; **30** was not a substrate at 410 mμ. [h] Maximum concentration allowing light transmission; solubility ≃15 m*M*.

inhibitor with the 2-pyridylvinyl group (**20**) was even less soluble than the phenoxymethyl inhibitor (**1**), but no loss in binding occurred. No loss in binding compared to **1** occurred with a 3-pyridylvinyl inhibitor (**21**), but solubility was increased only about twofold. A twofold loss in binding occurred with the 4-pyridylvinyl inhibitor (**22**), less loss than might be expected since the pyridine nitrogen is usually solvated with water,[11] and this *para* position does not tolerate polar groups.[10] The pyridylethyl analogs (**23–25**) were considerably more soluble than the pyridylvinyl analogs (**20–22**), but a three- to fourfold loss in binding occurred compared to **1**.

The 2-quinolyl analog (**26**) of the 2-pyridyethyl inhibitor (**23**) was a sixfold better inhibitor than **23**, a result that might be expected since β-naphthoxyacetone is a sevenfold better inhibitor than phenoxyacetone;[10] however, solubility with **26** was greatly repressed as could be expected. Whether a similar increment in binding between the 2-quinolinylvinyl (**27**) and 2-pyridylvinyl (**20**) existed could not be determined due to the insolubility of **27**.

Conversion of the pyridines (**20–25**) to N-methyl quaternary derivatives (**28–35**) gave readily water-soluble products as could be expected. About a threefold loss in binding occurred when the 2-pyridylvinyl group of **20** was N-methylated (**28**); a fivefold loss in binding occurred with the N-ethyl group (**29**). A greater than 20-fold loss occurred in binding with the N-methyl-3-pyridylvinyl inhibitor (**30**) compared to **1**; this loss is considerably greater than the fivefold loss in binding when a 3-COO^- group (**14**) is inserted at this position (**1** *vs* **14**). Two factors can explain the difference between **14** and **30**: (a) **30** may have a larger water cage around the pyridinium ion that is repulsed by the hydrophobic region, and (b) the phenoxy group of **14** is more flexible in its conformation than the planar 3-pyridylvinyl group of **30**, thus allowing **14** to position in the complex more favorably with respect to repulsion of its polar group. At higher concentration, **30** was a consistent accelerator for enzymatic hydrolysis of the substrate, N-glutaryl-L-phenylalanine *p*-nitroanilide,[12] but **30** was not an observable substrate at 410 mμ.

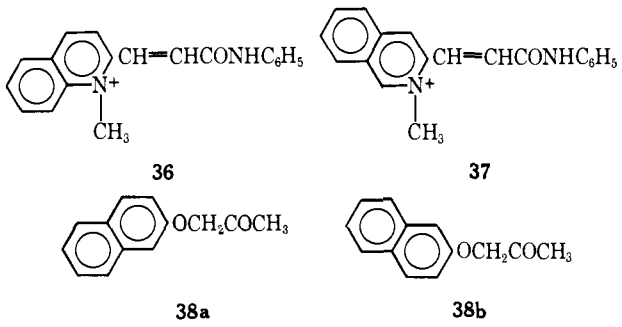
The N-methyl-4-pyridylvinyl analog (**31**) was sufficiently soluble, but its charge-transfer band in the 410-mμ region limited the concentration that could be used for inhibition studies; nevertheless, some binding was lost compared to **1** as would be expected.

The poor binding of the N-methyl-3-pyridylethyl (**34**) and N-methyl-4-pyridylethyl (**35**) analogs can be attributed to the repulsion of the polar quaternary group from a hydrophobic region on the enzyme. The N-methyl-2-pyridylethyl analog (**32**) ought to be at least one-third as good an inhibitor as the N-methyl-2-pyridylvinyl analog (**28**) by comparison of **23** and **20**; yet **28** was a 17-fold better inhibitor than **32**. This discrepancy may due to a shift in allowable ground-state conformations with **32** compared to **20**, **23**, and **28**. It is clear that the 2-pyridylvinyl group of **20** and **28** is planar, and this planar relationship between the pyridyl group and the vinyl or ethyl group may be optimum for binding to the enzyme. Thus, a threefold loss in binding with **23** compared to **20** may occur since the 2-pyridylethyl group might only require this little amount of energy to approach coplanarity. However, the solvated quaternary nitrogen of **32** may repulse the ethyl group of **32** even more from coplanarity in its ground-state conformation, thus accounting for the 47-fold loss in binding with **32** compared to **20**.

Since β-naphthoxyacetone (**38**) gives a sevenfold increment in binding compared to phenoxyacetone,[10] either **36** or **37** but not both should give a similar increment in binding depending upon whether **38** assumes conformation **38a** or **38b**. Either **36** or **37**, but not both, should be a poor inhibitor since the polar quaternary nitrogen can bind only in one conformation which projects the polar group away from the hydrophobic

(11) D. Pressman and M. Siegel, *J. Am. Chem. Soc.*, **79**, 994 (1957).

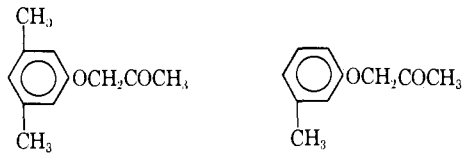
(12) B. E. Erlanger, F. Edel, and A. G. Cooper, *Arch. Biochem. Biophys.*, **115**, 296 (1966).



36 37

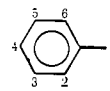
38a 38b

region. When the quaternary quinoline (**36**) was evaluated as an inhibitor of α-chymotrypsin, it gave a sixfold increment in binding compared to **28** due to a hydrophobic interaction of the benzo moiety of **36**. In contrast, **37** was less effective as an inhibitor than **36**; the exact amount of difference could not be determined for lack of solubility of **37**. When these results with **36–38** are combined with the previous observation that a 3,5-dimethylphenoxy group (**39**) gives a loss in binding compared to 3-methylphenoxy group (**40**),[10] a picture



39 40

of the hydrophobic region for the phenyl group of phenylalanine peptides begins to emerge, providing the assumption is correct that the aryl groups of **1–40** as well as phenylalanine are all complexed in the same ρ_2 area.[13] The 5 and 6 positions of the benzene ring (**41**) probably reside in a close-fitting crevice which has no bulk tolerance for a group larger than hydrogen. The



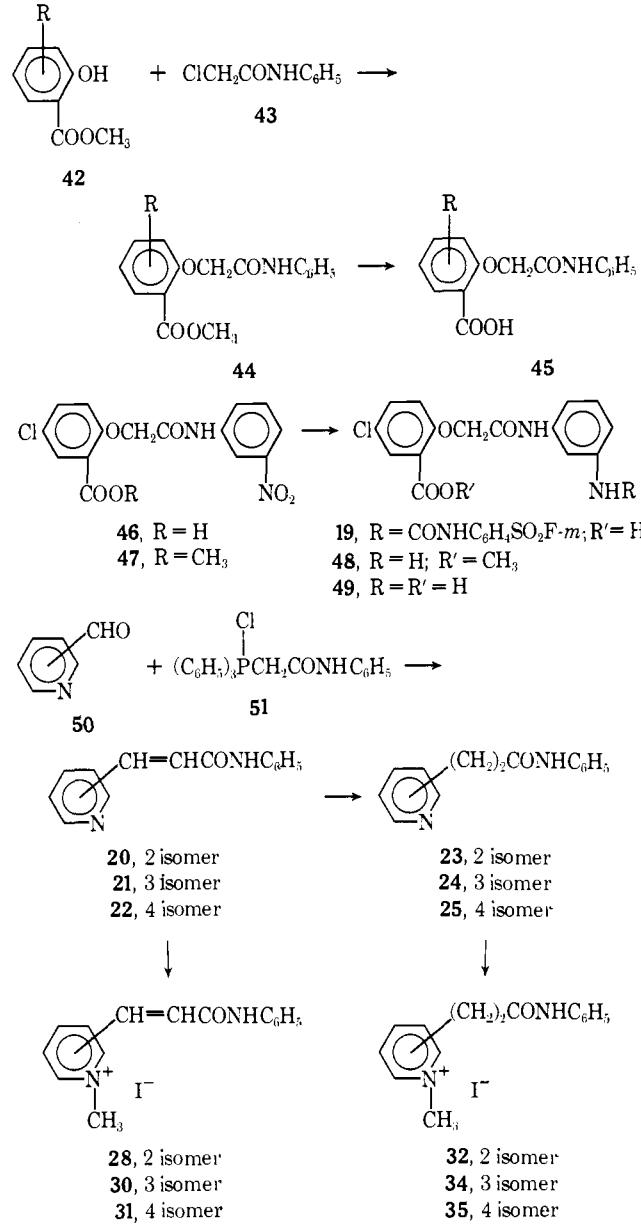
41

2 position is probably not in contact with enzyme and thus polar groups such as MeN^+ and COO^- can be tolerated in this area. Finally, additional hydrophobic bonding can be obtained by appropriate substituents at the 3 or 4 position; the size of this extra hydrophobic bonding area is as yet uncertain, but could be determined by appropriate additional compounds derived from **14** or **28**.

Chemistry.—Compound **6** (Table I) was prepared by acylation of *m*-aminobenzoic acid with phenoxyacetyl chloride in $CHCl_3$ with Et_3N as an acid acceptor; more strongly basic conditions were needed for synthesis of **7** and **8**. Since direct acylation of *p*-aminophenoxyacetic acid proceeded poorly, acylation was performed on the corresponding *t*-butyl ester; heating the latter in benzene in the presence of TsOH afforded **9** and **10** (Table I).

Compounds **13–18** (Table I) were prepared by alkylation of the appropriate derivative of methyl salicylate (**42**) with α-chloroacetanilide (**43**) to **44** followed by careful hydrolysis with NaOH in H_2O–MeOH (Scheme I). Similarly, **46** and **47** were prepared; two methods were investigated for conversion of **47** to the intermediate amine (**49**) needed for the irreversible inhibitor **19**. The intermediate amine (**49**) was better prepared by catalytic reduction of **47** to **48** followed by saponification than by saponification of **47** to **46** followed by catalytic hydrogenation. The amine (**49**) was smoothly converted to **19** by reaction with *m*-fluorosulfonylphenyl isocyanate in acetone.

SCHEME I



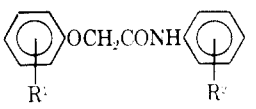
The pyridylacryanilides (**20–22**) (Table II) were synthesized by a Wittig condensation of the appropriate pyridine aldehyde (**50**) with the Wittig reagent (**51**)[14] from α-chloroacetanilide in DMF with Et_3N as the base; this route was considered superior to the more standard route of **50** to β-(2-pyridyl)acrylic acid to **20** *via* a mixed anhydride.

Reduction of **20–22** catalytically with a Pd–C catalyst proceeded smoothly to **23–25**. The quaternary salts **28–35** were prepared with the appropriate alkyl

(13) (a) G. E. Hein and C. Niemann, *J. Am. Chem. Soc.*, **84**, 4495 (1962); (b) J. B. Jones, C. Niemann, and G. E. Hein, *Biochemistry*, **4**, 1735 (1965); (c) see ref 5, Chapter 3.

(14) H. Fuerst, G. Wetzke, W. Berger, and W. Schubert, *J. Prakt. Chem.*, **17**, 299 (1962).

Table III
Physical Properties of



No.	R_2	R_1	Method	Yield, %	Mp, °C	Formula[m]
6	H	*m*-COOH	A	27[a,b]	221–224	$C_{15}H_{13}NO_5$
7	H	*o*-COOH	B	18[c]	198–201	$C_{15}H_{13}NO_5$
8	H	*p*-CH_2COOH	B	31[c,d]	142–145	$C_{16}H_{15}NO_5$
9	H	*p*-OCH_2COOH	D	55[e]	143–147	$C_{16}H_{15}NO_6$
10	3,4-Cl_2	*p*-OCH_2COOH	D	72[e]	192–195	$C_{16}H_{13}Cl_2NO_6 \cdot C_2H_5OH$[f]
13	*o*-COOH	H	F	62[c]	198–201	$C_{15}H_{13}NO_5$
14	*m*-COOH	H	F	91[d]	185–189	$C_{15}H_{13}NO_5$
15	4-Cl-2-COOH	H	F	76[g]	228–231	$C_{15}H_{12}ClNO_5$
16	4-Br-2-COOH	H	F	66[g]	227–231	$C_{15}H_{12}BrNO_5$
17	5-Cl-2-COOH	H	F[h]	52[e]	250–257 dec	$C_{15}H_{12}ClNO_5$
18	4,6-Cl_2-2-COOH	H	F	21[c]	188–191	$C_{15}H_{11}Cl_2NO_5$
19	4-Cl-2-COOH	*m*-$NHCONHC_6H_4SO_2F$-*m*	H[i]	73[a]	257–265 dec	$C_{22}H_{17}ClFN_3O_7S$
44a	*o*-$COOCH_3$	H	E[j]	14[a]	103–105	$C_{16}H_{15}NO_5$
44b	*m*-$COOCH_3$	H	E	84[a]	96–98	$C_{16}H_{15}NO_5$
44c	4-Cl-2-$COOCH_3$	H	E	83[a]	129–131	$C_{16}H_{14}ClNO_5$
44d	4-Br-2-$COOCH_3$	H	E	63[a]	145–148	$C_{16}H_{14}BrNO_5$
44e	5-Cl-2-$COOCH_3$	H	E[k]	33[g]	136–146	$C_{16}H_{14}ClNO_5$
44f	4,6-Cl_2-2-$COOCH_3$	H	E	14[a]	97–100	$C_{16}H_{13}Cl_2NO_5$
46	4-Cl-2-COOH	*m*-NO_2	F	51[b]	260–263	$C_{15}H_{11}ClN_2O_6$
47	4-Cl-2-$COOCH_3$	*m*-NO_2	E	82[b]	208–211	$C_{16}H_{13}ClN_2O_6$
48	4-Cl-2-$COOCH_3$	*m*-NH_2	G[l]	72[c]	166–168	$C_{16}H_{15}ClN_2O_4$
49	4-Cl-2-COOH	*m*-NH_2	F	38[d]	211–213 dec	$C_{15}H_{13}ClN_2O_4$
			G	32		
53	H	*p*-OCH_2CO_2Bu-*t*	C	31[a]	90–93	$C_{20}H_{23}NO_5$
54	3,4-Cl_2	*p*-OCH_2CO_2Bu-*t*	C	86[f,g]	147–150	$C_{20}H_{21}Cl_2NO_5$

[a] Recrystallized from EtOH. [b] Recrystallized from Me_2CO. [c] Recrystallized from $CHCl_3$. [d] Recrystallized from toluene. [e] The presence of EtOH was verified by nmr; integration showed a 1:1 ratio. [f] Recrystallized from $CHCl_3$–EtOH. [g] Recrystallized from MeOH. [h] 4-Chlorosalicylic acid prepared in 38% yield according to R. Kuhn and H. R. Hensel, *Chem. Ber.*, **84**, 557 (1961).[16] [i] See ref 9, method G; Me_2CO was used as solvent. [j] Reaction run in MeOH. [k] HCl omitted in reaction. [l] Recrystallized from EtOH–petroleum ether (bp 60–110°). [m] All compounds were analyzed for C, H, N, except **19**, which was analyzed for C, H, F.

iodide in boiling Me_2CO. The quinoline and isoquinoline derivatives (**26**, **27**, **36**, **37**, Table II) were prepared by similar routes from the appropriate carboxaldehydes.

Experimental Section

Each analytical sample had a proper ir spectrum and moved as a single spot on tlc on Brinkmann silica gel GF or polyamide MN. Melting points were taken in capillary tubes on a Mel-Temp block and are uncorrected. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

N-(Phenoxyacetyl)-*m*-aminobenzoic Acid (6) (Method A).—To a stirred solution of 1.37 g (10 mmoles) of *m*-aminobenzoic acid in 50 ml of $CHCl_3$ and 3.5 ml (25 mmoles) of Et_3N was added dropwise a solution of 1.54 ml (11 mmoles) of phenoxyacetyl chloride in 30 ml of $CHCl_3$ over a period of 15 min at ambient temperature. After being stirred an additional 15 min, the mixture was heated to boiling for 5 min. The hot mixture was extracted with two 100-ml portions of 5% $NaHCO_3$. Acidification with 6 *N* HCl gave the product which was recrystallized once from EtOH and once from Me_2CO; yield, 0.73 g (27%) of white crystals, mp 221–224°, that gave a negative Bratton-Marshall test for aromatic amines.[15] No attempt was made to recover additional material from the filtrates. See Table III for additional data.

N-(Phenoxyacetyl)anthranilic Acid (7) (Method B).—To a stirred solution of 1.37 g (10 mmoles) of anthranilic acid in 20 ml of DMF and 5 ml of aqueous 2 *N* NaOH (10 mmoles) cooled in an ice bath was added dropwise a solution of 1.68 ml (12 mmoles) of phenoxyacetyl chloride in 15 ml of DMF over a period of 15 min. The mixture was treated with 5 ml of 2 *N* NaOH, then stirred 5 min more and finally heated on a steam bath for 5 min. The mixture was cooled, then treated with 10 g of ice and 30 ml of 5% HCl. The product was collected on a filter and then washed with H_2O. Recrystallization from $CHCl_3$ gave 0.50 g (18%) of white needles, mp 198–201°, that gave a negative Bratton-Marshall test for aromatic amine.[15] See Table III for additional data.

***t*-Butyl *p*-Nitrophenoxyacetate (52).**—A solution of 6.00 g (40 mmoles) of *t*-butyl chloroacetate and 5.91 g (30 mmoles) of sodium *p*-nitrophenoxide dihydrate in 50 ml of DMF was stirred in a bath at 60–70° for 18 hr, then diluted with 400 ml of cold H_2O. The product was collected on a filter, washed with H_2O, and dissolved in 200 ml of $CHCl_3$. The solution was washed with two 100-ml portions of ice-cold 10% NaOH, then with H_2O. Dried with $MgSO_4$, the $CHCl_3$ solution was evaporated *in vacuo*. Crystallization from petroleum ether (bp 60–110°) gave 6.66 g (88%) of nearly colorless plates, mp 83–86°. *Anal.* ($C_{12}H_{15}NO_5$) C, H, N.

***t*-Butyl *p*-(Phenoxyacetamido)phenoxyacetate (53) (Method C).**—A solution of 2.53 g (11 mmoles) of **52** in 100 ml of EtOH was shaken with H_2 at 2–3 atm in the presence of 0.2 g of 5% Pd–C for 1 hr when reduction was complete. The filtered solution was evaporated *in vacuo* leaving 2.30 g (92%) of *t*-butyl *p*-aminophenoxyacetate as an oil which darkened on standing.

To a stirred solution of 1.12 g (5 mmoles) of the amine in 25 ml of $CHCl_3$ and 0.61 g (6 mmoles) of Et_3N cooled in an ice bath was added dropwise a solution of 0.85 ml (6 mmoles) of phenoxyacetyl chloride in 5 ml of $CHCl_3$ over a period of 15 min. The mixture was warmed on a steam bath for 5 min, then washed successively with H_2O (two 50-ml portions), 50 ml of 5% Na_2CO_3, and 50 ml of H_2O. Dried with $MgSO_4$, the solution was evaporated *in vacuo*. Two recrystallizations from toluene gave 0.55 g (31%) of white plates, mp 90–93°, which gave a negative Bratton-Marshall test for aromatic amines.[15] See Table III for additional data.

[15] B. R. Baker, D. V. Santi, J. K. Coward, H. S. Shapiro, and J. H. Jordaan, *J. Heterocycl. Chem.*, **3**, 425 (1966).

TABLE IV
PHYSICAL PROPERTIES OF $RCONHC_6H_5$

No.	R	Method	Yield, %	Mp, °C	Formula[l]
20	2-Pyridyl-CH=CH	I	63[a]	147–150	$C_{14}H_{12}N_2O$
21	3-Pyridyl-CH=CH	I	63[b,c]	163–166	$C_{14}H_{12}N_2O$
22	4-Pyridyl-CH=CH	I	63[b]	166–169	$C_{14}H_{12}N_2O$
23	2-Pyridyl-$(CH_2)_2$	J	89[d]	108–111[e]	$C_{14}H_{14}N_2O$
24	3-Pyridyl-$(CH_2)_2$	J	71[b]	124–126	$C_{14}H_{14}N_2O$
25	4-Pyridyl-$(CH_2)_2$	J	84[b]	136–137	$C_{14}H_{14}N_2O$
26	2-Quinolyl-$(CH_2)_2$	J	59[b]	136–138	$C_{18}H_{16}N_2O$
27	2-Quinolyl-CH=CH	I[f]	54[b,g]	140–144	$C_{18}H_{14}N_2O$
28	N-Me-2-pyridyl$^+$-CH=CH, I^-	K (3)	14[h]	234–236 dec	$C_{15}H_{15}IN_2O$
29	N-Et-2-pyridyl$^+$-CH=CH, I^-	K (72)	26[i]	213–225 dec	$C_{16}H_{17}IN_2O$
30	N-Me-3-pyridyl$^+$-CH=CH, I^-	K (2)	77[h]	220–250 dec	$C_{15}H_{15}IN_2O$
31	N-Me-4-pyridyl$^+$-CH=CH, I^-	K (2)	79[h]	220–285 dec	$C_{15}H_{15}IN_2O$
32	N-Me-2-pyridyl$^+$-$(CH_2)_2$, I^-	K (3)	83[i]	165–167	$C_{15}H_{17}IN_2O$
33	N-Et-2-pyridyl$^+$-$(CH_2)_2$, I^-	K (24)	56[j]	148–151	$C_{16}H_{19}IN_2O$
34	N-Me-3-pyridyl$^+$-$(CH_2)_2$, I^-	K (12)	75[i]	158–161	$C_{15}H_{17}IN_2O$
35	N-Me-4-pyridyl$^+$-$(CH_2)_2$, I^-	K (12)	71[i]	192–195	$C_{15}H_{17}IN_2O$
36	N-Me-2-quinolyl$^+$-CH=CH, I^-	K (72)	13[h]	215–225 dec	$C_{19}H_{17}IN_2O$
37	N-Me-3-isoquinolyl$^+$-CH=CH, I^-	K (12)	47[h]	262–263 dec	$C_{19}H_{17}IN_2O$
55	3-Isoquinolyl-CH=CH	I[k]	41[b,l]	163–166	$C_{18}H_{14}N_2O$
56	2-Quinolyl$^+$-$(CH_2)_2$, I^-	K (12)	15[h]	219–220	$C_{19}H_{19}IN_2O$

[a] Recrystallized from Me_2CO–H_2O. [b] Recrystallized from toluene. [c] Recrystallized from $CHCl_3$–petroleum ether (bp 60–110°). [d] Recrystallized from C_6H_6–petroleum ether (bp 60–110°). [e] Mp 109–110° reported by F. H. McMillan and J. A. King, *J. Am. Chem. Soc.*, **73**, 3165 (1951) for **23** prepared by a different route. [f] Quinoline-2-carboxaldehyde prepared in 17% yield according to H. Kaplan, *J. Am. Chem. Soc.*, **63**, 2654 (1941). [g] Recrystallized from EtOH–H_2O. [h] Recrystallized from MeOH. [i] Recrystallized from EtOH. [j] Recrystallized from $CHCl_3$–Me_2CO. [k] Isoquinoline-3-carboxaldehyde prepared in 8% yield according to C. E. Teague, Jr., and A. Roe, *J. Am. Chem. Soc.*, **73**, 688 (1951). [l] All compounds were analyzed for C, H, N except for **23** which was not analyzed.

***p*-(Phenoxyacetamido)phenoxacetic Acid (9) (Method D).**—A solution of 452 mg (1.5 mmoles) of **53** and 50 mg of TsOH in 25 ml of C_6H_6 was refluxed for 2 hr, then cooled. The product was collected on a filter and recrystallized from $CHCl_3$; yield, 247 mg (55%) of white crystals, mp 143–147°. See Table III for additional data.

α-(2-Carbomethoxy-4-chlorophenoxy)acetanilide (44c) (Method E).—To a solution of 1.68 g (9 mmoles) of methyl 5-chlorosalicylate[16] and 0.46 g (8.5 mmoles) of NaOMe in 25 ml of dry DMF protected from moisture was added a solution of 1.35 g (8 mmoles) of **43** in 10 ml of dry DMF. After being stirred in a bath at 50–60° for 18 hr, the mixture was diluted with 200 ml of 4% aqueous $NaHCO_3$. The product was collected on a filter, washed with H_2O, then recrystallized from EtOH; yield, 2.10 g (83%) of white needles, mp 129–131°. See Table III for additional data.

α-(2-Carboxy-4-chlorophenoxy)acetanilide (15) (Method F).—A mixture of 1.28 g (4 mmoles) of **44c** and 30 ml of 80% aqueous MeOH containing 200 mg (5 mmoles) of NaOH was heated on a steam bath for 30 min, then diluted with 60 ml of H_2O. The filtered solution was acidified with 5% HCl, then the product was collected on a filter and washed with H_2O. Recrystallization from MeOH gave 0.94 g (76%) of white crystals, mp 228–231°. See Table III for additional data.

α-(2-Carboxy-4-chlorophenoxy)-*m*-aminoacetanilide (49) (Method G).—A solution of 0.73 g (2.1 mmoles) of **46** in 100 ml of 90% aqueous MeOEtOH containing 0.4 ml of 12 *N* HCl (4.8 mmoles) was shaken with H_2 at 2–3 atm in the presence of 50 mg of PtO_2 for 35 min when reduction was complete. The filtered solution was neutralized with 2 *N* NaOH, then diluted with 200 ml of H_2O. The product was collected on a filter, washed with H_2O, then recrystallized from EtOH–petroleum ether (bp 60–110°); yield, 0.26 g (38%) of white crystals, mp 211–213° dec. See Table III for additional data.

β-(2-Pyridyl)acrylanilide (20) (Method I).—To a stirred solution of 2.16 g (5 mmoles) of 51[14] and 0.61 g (6 mmoles) of Et_3N in 10 ml of DMF was added a solution of 0.54 g (5 mmoles) of 2-pyridinecarboxaldehyde in 10 ml of DMF. After being stirred at ambient temperature of 16 hr, the mixture was clarified by filtration, then diluted with 100 ml of H_2O. The product was collected on a filter and washed with H_2O.

The hydrochloride salt was crystallized from 5% HCl. The salt was stirred with excess aqueous Na_2CO_3, then collected on a filter and washed with H_2O. The free base was recrystallized from Me_2CO–H_2O; yield, 0.70 g (63%) of yellow crystals, mp 147–150°. See Table IV for additional data.

β-(3-Pyridyl)propionanilide (24) (Method J).—A solution of 1.60 g (7.1 mmoles) of **21** in 100 ml of EtOH was shaken with H_2 at 2–3 atm in the presence of 0.2 g of 5% Pd–C until 1 equiv was consumed (about 2 hr). The filtered solution was evaporated *in vacuo* and the residue recrystallized from toluene; yield, 1.13 g (71%), mp 124–126°. See Table IV for additional data.

β-(2-Pyridyl)acrylanilide Methiodide (28) (Method K).—A stirred solution of 0.450 g (2 mmoles) of **20** in 10 ml of Me_2CO and 2.1 g (15 mmoles) of MeI was refluxed for 3 hr, then cooled. The product was collected on a filter, washed with acetone, then recrystallized from MeOH; yield, 0.103 g (14%) of yellow crystals, mp 234–236° dec. See Table IV for additional data. Variable reaction times were used with method K for related pyridines and quinolines, these times being indicated in parentheses in Table IV.

(16) J. Klosa, *Arch. Pharm.*, **289**, 143 (1956); other salicylic acids in this study were also esterified by this $POCl_3$–MeOH method.