triazin-1-yl)phenoxyacetyl]sulfanilyl Fluoride Ethanesulfonate (2b) (Method D).—A mixture of 777 mg (2 mmoles) of **21a** (Table II), 65 mg of PtO_2, and 100 ml of EtOH was shaken with H_2 at 2–3 atm until reduction was complete. To the filtered mixture was added 225 mg of $EtSO_3H$ then the solution was evaporated *in vacuo*. To the residual **20a**·$EtSO_3H$ were added 20 ml of reagent Me_2CO and 177 mg (2.2 mmoles) of cyanoguanidine. After being refluxed with stirring for 18 hr, the cooled mixture was filtered and the product was washed (Me_2CO). Recrystallization from EtOH–H_2O gave 630 mg (53%) of white crystals, mp 224–226° dec. See Table III for additional data and compounds prepared by this method.

Method E was the same except Raney nickel was used as a catalyst.[5]

Irreversible Enzyme Inhibitors. CXLI.[1,2] Active-Site-Directed Irreversible Inhibitors of Dihydrofolic Reductase Derived from 1-[*p*-(*p*-Fluorosulfonylphenylureidomethyl)-phenyl]-4,6-diamino-1,2-dihydro-2,2-dimethyl-*s*-triazine

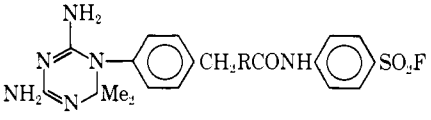
B. R. BAKER AND GERHARDUS J. LOURENS[3]

Department of Chemistry, University of California at Santa Barbara, Santa Barbara, California 93106

Received July 24, 1968

The title compound (**1**) showed a poor order of active-site-directed irreversible inhibition of dihydrofolic reductase from mouse L1210 leukemia, liver, spleen, or intestine since too high a concentration of inhibitor had to be used to show good inactivation. Substitution of a methyl group *meta* to the SO_2F moiety of **1** gave compound **6** that was still a relatively poor irreversible inhibitor of the L1210 enzyme, but now showed tissue specificity by its failure to inactivate the liver enzyme. More effective irreversible inhibitors (**5**, **8**, **9**) were obtained by substitution of a chloro atom *meta* to the junction of the 1-phenyl to the *s*-triazine ring; however, these compounds did not show specificity since the liver enzyme was still inactivated.

The title compound (**1**)[4] was found to be an active-site-directed irreversible inhibitor[5] of the dihydrofolic reductase from L1210/0 and L1210/DF8 mouse leukemia.[6] However, **1** was not as good an irreversible inhibitor as the prototype irreversible inhibitor **2**[6,7] since the total amount of inactivation by **1** was lower than **2** (Table I). Furthermore, neither **1** nor **2** showed specificity toward the L1210 enzyme with minimal effect on the enzyme from normal tissues such as liver, spleen, and intestine.[6] Therefore, additional synthetics related to **1** were made and evaluated to see if a better



1, R = NH
2, R = CH_2

and more selective irreversible inhibitor could be designed. The results are the subject of this paper.

Enzyme Results.—As pointed out in the earlier summary paper,[6] assay of **1** for irreversible inhibition of dihydrofolic reductase was difficult due to a medium order of total, but fast, irreversible inhibition that sometimes gave low zero-time points. Similar difficulties were encountered with **3**,[4] which showed 0–30% irreversible inhibition of the L1210 enzyme depending upon how low the zero-time point for enzyme concentration was; in repeated runs **3** failed to show any irreversible inhibition of mouse liver dihydrofolic reductase. Thus, **3** showed selectivity, but a poor order of irreversible inhibition of the L1210 enzyme; the latter was most probably due to extensive enzyme-catalyzed hydrolysis of the SO_2F moiety.[7,8] When the side chain was moved to the *meta* position (**4**), irreversible inhibition was lost.[9]

The effect of substituents on either or both benzene rings of **1** on irreversible inhibition was then studied. There is only one position on the phenyl group next to the triazine that can be substituted without loss of binding, and that is the position *meta* to the triazine junction; *ortho* substitution leads to a large loss in reversible binding.[10,11] The 3-chloro atom was selected since this could have a beneficial effect on both reversible binding[10] and irreversible inhibition.[2] The 3-chloro substituent **5** on **1** gave a fourfold increment in reversible binding. Furthermore, **5** was a better irreversible inhibitor of the L1210 enzyme than **1** when compared at a similar concentration of reversible EI complex;[12] however, **5** still lacked specificity since poor irreversible inhibition of the mouse liver enzyme was still observed.

Substitution of CH_3 *meta* (**6**) to the SO_2F moiety of **1** also gave about a fourfold increment in reversible binding. Although **6** was not a better irreversible inhibitor of the L1210 enzyme at equal EI concentration,[12] **6** was more specific than **1** since **6** did not inactivate the liver enzyme. Introduction of CH_3 (**7**) *ortho* to the SO_2F moiety of **1** gave about a twofold increment in reversible binding; however, **7** was

(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 of this series see B. R. Baker and G. J. Lourens, *J. Med. Chem.*, **12**, 95 (1969), paper CXL of this series.

(3) G. J. L. wishes to thank the Council of Scientific and Industrial Research, Republic of South Africa, for a tuition fellowship.

(4) B. R. Baker and G. J. Lourens, *J. Med. Chem.*, **11**, 666 (1968), paper CXXVII 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) B. R. Baker, G. J. Lourens, R. B. Meyer, Jr., and N. M. J. Vermeulen, *J. Med. Chem.*, **12**, 67 (1969), paper CXXXIII of this series.

(7) B. R. Baker and G. J. Lourens, *ibid.*, **10**, 1123 (1967), paper CV of this series.

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

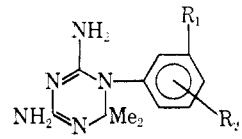
(9) B. R. Baker and G. J. Lourens, *ibid.*, **11**, 39 (1968), paper CXII of this series.

(10) (a) B. R. Baker, *ibid.*, **11**, 483 (1968); (b) B. R. Baker and M. A. Johnson, *ibid.*, **11**, 486 (1968); (c) B. R. Baker and B.-T. Ho, *J. Pharm. Sci.*, **53**, 1137 (1964).

(11) E. J. Modest, *J. Org. Chem.*, **21**, 1 (1956).

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

Table I
Inhibition[a] of Dihydrofolic Reductase by



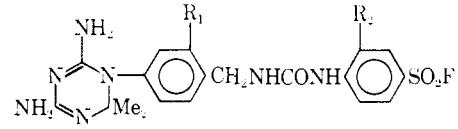
No.	R_1	R_2	Enzyme source[b]	Reversible[c] I_{50},[e] μM	Reversible[c] K_i,[f] μM	Irreversible[d] Inhib., μM	Irreversible[d] % [EI][g]	Irreversible[d] Time, min	Irreversible[d] % inactvn
1[h]	H	*p*-$CH_2NHCONHC_6H_4SO_2F$-*p*	L1210/0	0.052	0.0087	0.16	95	60	51[i]
			L1210/DF8	0.045	0.0075	0.16	95	60	42[i]
			Liver	0.023	0.0039	0.16	98	60	31[i]
			Spleen			0.16		60	45[i]
			Intestine			0.16		20	53[i]
2[h]	H	*p*-$(CH_2)_2CONHC_6H_4SO_2F$-*p*	L1210/0	0.012	0.0020	0.070	98	60	53[i]
						0.035	94	60	30[i]
			L1210/DF8	0.025	0.0041	0.14	97	60	92[i]
						0.070	94	60	73[i]
			Liver	0.015	0.0025	0.40	99	<2, 60	59, 59[i]
						0.070	96	<2, 60	38, 38[i]
			Spleen			0.10		60	73[i]
			Intestine			0.10		20	85[i]
3	H	*p*-$CH_2NHCONHC_6H_4SO_2F$-*m*	L1210/0			0.056		60	0–30[i,k]
			L1210/DF8			0.056		60	0–24[i,k]
			L1210/FR8[l]	0.028	0.0047	0.14	97	60	0[i]
4	H	*m*-$CH_2NHCONHC_6H_4SO_2F$-*m*	L1210/FR8[m]	0.066	0.011	0.33	97	60	0
			L1210/0			0.13		60	0[i]
			L1210/DF8			0.13		60	0[i]
5	Cl	*p*-$CH_2NHCONHC_6H_4SO_2F$-*p*	L1210/0	0.012	0.0020	0.060	97	60	75[i]
						0.012	87	60	10[i]
			L1210/DF8			0.060		60	86[i]
						0.012		60	0
			Liver			0.060		60	20[i]
			Spleen			0.06		60	68[i]
6	H	*p*-$CH_2NHCONHC_6H_3$-2-Me-4-SO_2F	L1210/0	0.012	0.0020	0.060	97	60	54[i]
			L1210/DF8			0.060		60	53[i]
			Liver			0.060		60	0[i]
7	H	*p*-$CH_2NHCONHC_6H_3$-3-Me-4-SO_2F	L1210/DF8	0.019	0.0031	0.038	92	60	70[i]
			L1210/0			0.038		60	53[i]
			Liver			0.038			23[i]
8	Cl	*p*-$CH_2NHCONHC_6H_3$-3-Me-4-SO_2F	L1210/0	0.011	0.0018	0.080	98	60	89[i]
						0.032	94	60	41[i]
			L1210/DF8	0.016	0.0026	0.032	92	60	57[i]
			Liver			0.080		60	25[i]
9	Cl	*p*-$CH_2NHCONHC_6H_3$-3-Cl-4-SO_2F	L1210/0	0.020	0.0033	0.040	92	60	71[i]
			L1210/DF8			0.040		60	65[i]
						0.0060			0[i]
			Liver			0.040		60	31[i]

[a] The technical assistance of Diane Shea, Sharon Lafler, and Carolyn Wade with these assays is acknowledged. [b] The L1210/0 is the parent wild strain, while /FR8 and /DF8 are strains resistant to amethopterin with a high level of dihydrofolic reductase. [c] Assayed with 6 μM dihydrofolate and 30 μM TPNH in pH 7.4 Tris buffer containing 0.15 M KCl as previously described.[6,7] [d] Incubated at 37° in pH 7.4 Tris buffer in the presence of 60 μM TPNH as previously described.[6,7] [e] I_{50} = concentration for 50% inhibition. [f] Estimated from $K_i = K_m[I_{50}]/[S]$ which is valid since $[S] = 6K_m = 6\ \mu M$ dihydrofolate; see ref 5, p 202. [g] Calculated from $[EI] = [E_t]/(1 + K_i/[I])$ where [EI] = amount of total enzyme (E_t) reversibly complexed.[12] [h] Data from ref 6. [i] Zero point determined by addition of inhibitor to assay cuvette.[6,7] [j] From six-point time study.[7] [k] Due to curvature in the assay for the zero point,[6] the zero-time enzyme concentration was quite variable. [l] Data from ref 4. [m] Data from ref 9.

neither a better nor more specific irreversible inhibitor than **1**.

The 3-chloro (**9**) and 3-methyl (**8**) derivatives of **7** were then synthesized for enzymic evaluation; little change in either reversible binding or irreversible inhibition was seen.

From these studies it would appear that **5**–**8** would be worthy of *in vivo* testing, even though the criteria arbitrarily set for *in vivo* testing[6] have not been met. Furthermore, since the CH_3 of **6** on **1** imparts specificity and the Cl atom of **5** imparts better irreversible inhibition, a combination of these substituents (**5a**) would be worthy of study where R_1 and R_2 are a combination of halogen, alkyl, or alkoxy; such a study is being pursued.



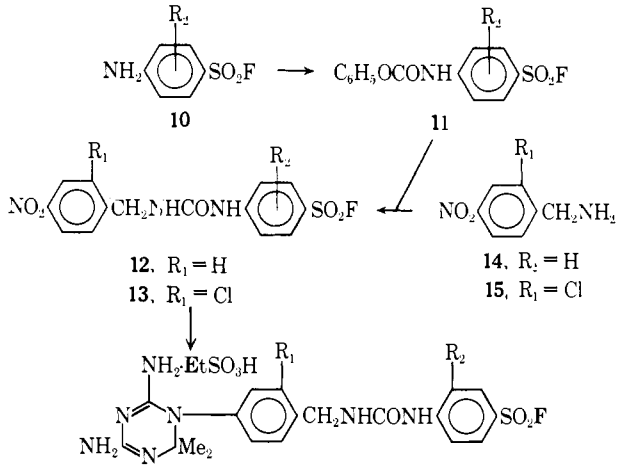
5a

Chemistry.—The key step was the synthesis of the mixed ureas (**12**, **13**) by the Crosby method.[13] The

(13) (a) D. G. Crosby and C. Niemann, *J. Am. Chem. Soc.*, **76**, 4458 (1954); (b) B. R. Baker and R. P. Patel, *J. Pharm. Sci.*, **52**, 927 (1963).

requisite O-phenylurethans (**11**) were prepared by reaction of the appropriately substituted sulfanilyl fluoride (**10**)[7,14] with phenyl chloroformate in boiling C_6H_6.[4] The urethans were then treated with 4-nitrobenzylamine (**14**)[4] or its 2-chloro derivative (**15**)[15] in DMF to give the mixed ureas (**12**, **13**) (Scheme I).

SCHEME I

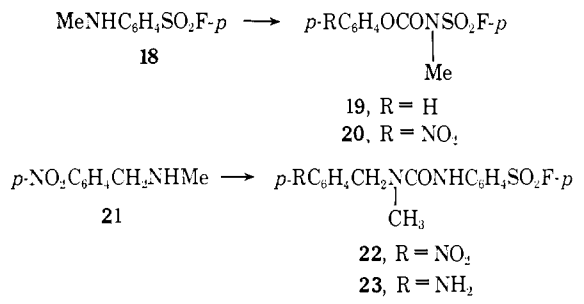


7, $R_1 = H$
8, $R_1 = Cl$

a, $R_2 = H$ **c**, R_2 = 3-Me **e**, R_2 = 2-OMe
b, R_2 = 2-Me **d**, R_2 = 3-Cl

The nitro group of **12** and **13** was reduced catalytically with a Raney Ni and PtO_2 catalyst, respectively; the resultant amines were condensed with cyanoguanidine and acetone by the method of Modest[11] to give the candidate irreversible inhibitors **5–9**.

The N-methyl-O-phenylurethans (**19**, **20**) were prepared from N-methylsulfanilyl fluoride (**18**);[16] unfortunately, condensation of **19** with *p*-nitrobenzylamine (**14**) failed to take place and the more reactive O-nitrophenylurethan (**20**)[17] gave mixtures.



19, R = H
20, R = NO_2

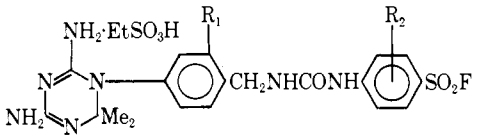
22, R = NO_2
23, R = NH_2

N-Methyl-4-nitrobenzylamine (**21**) condensed smoothly with **11a** to give **22** which was reduced catalytically to **23**; a crystalline triazine could not be prepared from **23** and cyanoguanidine.

Experimental Section

Melting points were taken in capillary tubes on a Mel-Temp block and are uncorrected. Each analytical sample moved as a single spot on tlc on Brinkmann silica gel GF and had ir and uv spectra in agreement with its assigned structure; each gave combustion values for C, H, and N or F within 0.4% of theoretical. The physical properties of **5–9** are given in Table II.

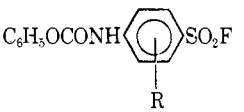
TABLE II
PHYSICAL PROPERTIES OF



No.	R_1	R_2[a]	Method[b]	% yield[c]	Mp, °C dec	Formula
5	Cl	H	D	36[d]	225–227	$C_{19}H_{21}ClFN_7O_3S \cdot C_2H_6SO_3H$
6	H	2-Me	E	53[d]	165–167	$C_{20}H_{24}FN_7O_3S \cdot C_2H_6SO_3H$
7	H	3-Me	E	46[d]	221–223	$C_{20}H_{24}FN_7O_3S \cdot C_2H_6SO_3H$
8	Cl	3-Me	D	26[e]	221–223	$C_{20}H_{23}ClFN_7O_3S \cdot C_2H_6SO_3H$
9	Cl	3-Cl	D	32[d]	213–215	$C_{19}H_{20}Cl_2FN_7O_3S \cdot C_2H_6SO_3H$

[a] Numbered from NH. [b] See ref 2 for methods. [c] Yields of analytically pure material analyzed for C, H, F. [d] Recrystallized from EtOH–H_2O. [e] Recrystallized from MeOEtOH–H_2O.

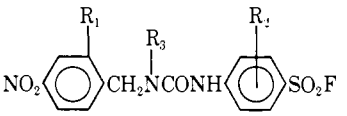
TABLE III
PHYSICAL PROPERTIES OF



No.	R[a]	% yield[b]	Mp, °C	Formula
11b	2-Me	76	127–129	$C_{14}H_{12}FNO_4S$
11c	3-Me	83	120–121	$C_{14}H_{12}FNO_4S$
11d	3-Cl	64	123–124	$C_{14}H_9ClFNO_4S$
11e	2-OMe	73	131–132	$C_{14}H_{12}FNO_5S$
19	N-Me	58	70–71	$C_{14}H_{12}FNO_4S$

[a] Numbered from NH. [b] Yield of analytically pure material recrystallized from C_6H_6–petroleum ether (bp 60–110°) and analyzed for C, H, N except **11e**.

TABLE IV
PHYSICAL PROPERTIES OF



No.	R_1	R_2[a]	R_3	% yield[b]	Mp, °C	Formula
12b	H	2-Me	H	76	224–225	$C_{15}H_{14}FN_3O_5S$
12c	H	3-Me	H	80	205–206	$C_{15}H_{14}FN_3O_5S$
12e	H	2-OMe	H	70	172–173 dec	$C_{15}H_{14}FN_3O_6S$
13a	Cl	H	H	77	214–215 dec	$C_{14}H_{11}ClFN_3O_5S$
13b	Cl	2-Me	H	77	250–252 dec	$C_{15}H_{13}ClFN_3O_5S$
13c	Cl	3-Me	H	72	235–236	$C_{15}H_{13}ClFN_3O_5S$
13d	Cl	3-Cl	H	77	dec >235	$C_{14}H_{10}Cl_2FN_3O_5S$
22[c,d]	H	H	Me	80	166–167	$C_{15}H_{14}FN_3O_5S$

[a] Numbered from NH. [b] Yield of analytically pure material that was recrystallized from MeOEtOH–H_2O and analyzed for C, H, N. [c] The starting **21** was prepared by $NaBH_4$ reduction of *p*-nitrobenzaldehyde and $MeNH_2$ in 40% MeOH, then isolated as its HCl salt; see C. Paal and H. Springer, *Ber.*, **30**, 61 (1897). [d] Catalytic reduction with a Raney Ni catalyst in EtOH gave **23** in 72% yield, mp 155–157°. *Anal.* ($C_{15}H_{16}FN_3O_3S$) C, H, N.

***p*-Nitrophenyl-N-(*p*-fluorosulfonylphenyl)-N-methylurethan (20) (Method A).**—A mixture of 3.78 g (20 mmoles) of **18**,[16] 4.03 g (20 mmoles) of *p*-nitrophenyl chloroformate, and 50 ml of C_6H_6 was refluxed with stirring for 2 hr. The mixture was spin evaporated *in vacuo* until the product began to separate, then cooled. The product was collected on a filter, washed with petroleum ether (bp 60–110°), then recrystallized from C_6H_6;

(14) B. R. Baker and G. J. Lourens, *J. Med. Chem.*, **11**, 677 (1968), paper CXXIX of this series.
(15) Synthesized from 2-chloro-4-nitrotoluene by W. Rzeszotarski in this laboratory, unpublished.
(16) B. R. Baker and G. J. Lourens, *J. Med. Chem.*, **11**, 672 (1968), paper CXXVIII of this series.
(17) B. R. Baker and N. M. J. Vermeulen, *ibid.*, **12**, 74 (1969), paper CXXXIV of this series.

yield 3.00 g (43%), mp 139–140°. *Anal.* ($C_{14}H_{11}FN_2O_6S$) C, H, N. See Table III for other compounds prepared by this method.

N-(4-Fluorosulfonyl-3-methylphenyl)-N′-(4-nitrobenzyl)urea (12c) (Method B).—To a stirred mixture of 0.95 g (5 mmoles) of **14**·HCl,[16] 1.55 g (5 mmoles) of **11c** (Table III), and 4 ml of DMF was added 0.51 g (5 mmoles) of Et_3N. After 17 hr the mixture was diluted with 15 ml of H_2O. The product was collected on a filter, washed with 50% MeOH, then recrystallized from MeOEtOH–H_2O; yield 1.47 g (80%), mp 205–206°; tlc in 1:1 EtOAc–petroleum ether showed one spot. See Table IV for additional compounds prepared by this method.

Irreversible Enzyme Inhibitors. CXLII.[1,2] Further Studies on Active-Site-Directed Irreversible Inhibitors of Dihydrofolic Reductase Derived from 5-(*p*-Aminophenoxypropyl)-2,4,6-triaminopyrimidine Bearing a Terminal Sulfonyl Fluoride

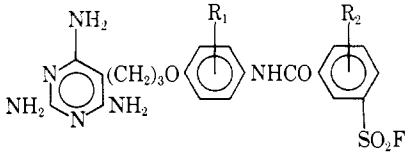
B. R. Baker and Rich B. Meyer, Jr.

Department of Chemistry, University of California at Santa Barbara, Santa Barbara, California 93106

Received July 24, 1968

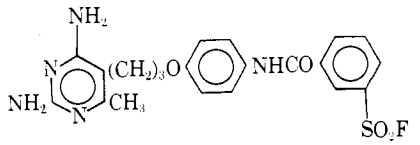
5-[*p*-(*m*-Fluorosulfonylbenzamido)phenoxypropyl]-2,4,6-triaminopyrimidine (**1**) at a K_i concentration is an active-site-directed irreversible inhibitor of the dihydrofolic reductase from three strains of L1210 mouse leukemia; furthermore, **1** at $40K_i$ concentration showed no irreversible inhibition of enzyme from mouse liver. The lack of effective *in vivo* action of **1** has been attributed to its relatively high $K_i \simeq 1\ \mu M$. The maximum enhancement in reversible binding that could be achieved by substitution of chloro, methyl, or isopropyl on one of the two benzene rings was only about twofold, as seen with the 2-chlorophenoxy analog (**9**) of **1**; this was still insufficient for effective *in vivo* activity. The specificity patterns of irreversible inhibition with the four analogs of **1** are presented and discussed.

The 5-phenoxypropylpyrimidine **1** was observed[3,4] to be an active-site-directed irreversible inhibitor[5] of dihydrofolic reductase from Walker 256 rat tumor as well as three strains of L1210 mouse leukemia; furthermore, **1** showed good tissue specificity since it failed to inactivate the enzyme from rat liver or mouse liver. However, **1** failed to show activity against



1, $R_1 = R_2 = H$

2, R_1 or $R_2 \neq H$



3

L1210 *in vivo*;[4] the failure of **1** to work *in vivo* could be attributed to a combination of poor solubility of the sulfate salt of **1** used in the assay and the relatively poor reversible complexing of **1** to dihydrofolic reductase. The latter is an important factor since the rate of active-site-directed irreversible inhibition of an enzyme is dependent upon the concentration of the reversible enzyme–inhibitor complex which in turn is dependent upon the binding constant (K_i) and the concentration of inhibitor;[6] that is, the lower the K_i, the less inhibitor is needed to convert 50% of the enzyme to the rate-limiting reversible complex. Furthermore, the lower the K_i, the less soluble a compound must be to be effective. Therefore two approaches were investigated to seek compounds with a better $I_{50} \simeq 6K_i$. In this paper is described the effect of substitution (**2**) on the benzene rings of **1** on the I_{50}; in the paper that follows[7] is described the synthesis of **3** which would be expected to show a 10–150-fold increment in reversible binding,[8] but may or may not still be an irreversible inhibitor.

Enzyme Results.—5-Phenoxypropyl-2,4,6-triaminopyrimidine (**4**) was synthesized and evaluated as a reversible inhibitor of the dihydrofolic reductase from L1210/0 in order to establish a base line. Note that **4** had $I_{50} = 31\mu M$ (Table I) which is about tenfold less than the parent irreversible inhibitor **1** (Table II); thus when the *m*-fluorosulfonylbenzamido moiety (**1**) is attached to **4** about a tenfold increment in binding emerged. The positions open for study on the phenoxy ring of **1** in order to increase binding were 2, 3, 5, and 6.

Insertion of a 2-chloro (**5**) or 3-chloro (**6**) atom gave a twofold increment in binding over the parent **1**. When a 3-chloro atom was inserted on 2-chlorophenoxy group of **5** to give **7**, another twofold gain in binding was observed indicating that the effect of the 2- and 3-chloro atoms was additive. No change in binding occurred when a 5-chloro atom (**8**) was inserted on **5**. Whether or not 2,6-dichloro substituents would have given better binding was not investigated.

(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 of this series see B. R. Baker and G. J. Lourens, *J. Med. Chem.*, **12**, 101 (1969).

(3) B. R. Baker and R. B. Meyer, Jr., *ibid.*, **11**, 489 (1968), paper CXIX of this series.

(4) B. R. Baker, G. J. Lourens, R. B. Meyer, Jr., and N. M. J. Vermeulen, *ibid.*, **12**, 67 (1969), paper CXXXIII 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) For the kinetics of irreversible inhibition see (a) ref 5, Chapter 8; (b) B. R. Baker, W. W. Lee, and E. Tong, *J. Theoret. Biol.*, **3**, 459 (1962).

(7) B. R. Baker and R. B. Meyer, Jr., *J. Med. Chem.*, **12**, 108 (1969), paper CXLIII of this series.

(8) B. R. Baker, B.-T. Ho, and D. V. Santi, *J. Pharm. Sci.*, **54**, 1115 (1965).