Irreversible Enzyme Inhibitors. CXXXIV.[1-4] Effect of Ring Substitution on the Selective Irreversible Inhibition of Dihydrofolic Reductase from L1210 Mouse Leukemia and Liver by 2,4-Diamino-5-(3,4-dichlorophenyl)-6-[*p*-(*m*-fluorosulfonylphenylureido)phenoxymethyl]pyrimidine

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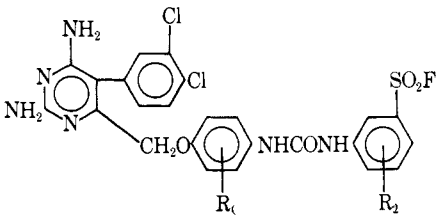
The dihydrofolic reductase from three different strains of L1210 mouse leukemia was inactivated by the title compound (**1**) by the active-site-directed mechanism. However, **1** also showed appreciable irreversible inhibition of the mouse liver enzyme; furthermore, **1** was too poor a reversible inhibitor by a factor of 5–15-fold. Therefore, thirteen derivatives of **1** containing a methyl, alkoxy, or chloro substituent on one or both of the benzene rings of the 6 side chain were synthesized for enzymic evaluation. The best compound (**3**) in the series was substituted with a 3-methyl group on the phenoxy moiety. This compound (**3**) had a good $K_i \simeq 10^{-8}$ *M* and showed excellent irreversible inhibition of the L1210 enzyme from the three strains at 6×10^{-8} *M* $\simeq 6K_i$ concentration; this latter concentration gave only 16% irreversible inhibition of mouse liver dihydrofolic reductase.

In an earlier paper of this series,[3a] the synthesis of **1** and its irreversible inhibition of the dihydrofolic reductase from L1210/FR8 mouse leukemia and mouse liver were described; although **1** could inactivate the L1210/FR8 enzyme, it also inactivated the liver enzyme somewhat less effectively (Table I). Since this earlier

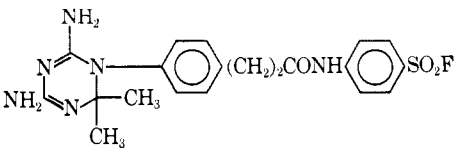


1, $R_1 = R_2 = H$
2, R_1 or $R_2 \neq H$

study, a new development has shifted the main emphasis from L1210/FR8[5] to two other strains of this mouse leukemia, namely L1210/DFS which is resistant to amethopterin due to a high dihydrofolic reductase level,[6] and L1210/0 which is the parent wild strain of this leukemia. This development was the current unavailability of L1210/FR8 leukemia for whole animal testing.[7] It has now been observed that **1** could also inactivate the dihydrofolic reductase from the L1210/0 and L1210/DFS strains (Table I). Therefore, a study of the effect of substitution on one or both benzene rings in the 6 side chain (**2**) on the selectivity of irreversible inhibition of the dihydrofolic reductases from mouse liver, L1210/DFS, and L1210/0 have been made; the results are the subject of this paper.

Enzyme Results.—The following criteria have been arbitrarily set[4] to establish whether or not a candidate irreversible inhibitor is worthy of studies with tumor-bearing animals: (1) the $I_{50} \simeq 6K_i$ should be $\leq 10^{-7}$ *M*;[8,9] (2) greater than 70% inactivation of the tumor enzyme should be seen at a $K_i = I_{50}/6$ concentration;[10,11] and (3) the liver enzyme should be inactivated <20% with the inhibitor at a $1\text{–}2I_{50} = 6\text{–}12K_i$ concentration.[11]

These criteria are partially based on the animal studies[4] with the dihydro-*s*-triazine irreversible type of inhibitor **16**.[12] Of the three criteria for an irreversible



16

inhibitor, **16** could only meet (1); **16** had $I_{50} = 1 \times 10^{-8}$ *M* with the dihydrofolic reductase from L1210/0 or mouse liver, but showed effective irreversible inhibition of L1210/0 only at $5I_{50} = 30K_i$. At this concentration, less irreversible inhibition of the mouse liver enzyme was seen, but the amount of inactivation was an appreciable 38%. Nevertheless, at optimum dosage, **16** gave a 70% life extension over L1210/0 controls in the mouse; however, the optimum dose range was fairly narrow.[4] Therefore, more specific compounds are needed that meet the above criteria.

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

(2) For earlier papers on this type of irreversible inhibitor see B. R. Baker, P. C. Huang, and A. L. Pogolotti, *J. Med. Chem.*, **10**, 1134 (1967), and ref 3.

(3) (a) B. R. Baker and P. C. Huang, *ibid.*, **11**, 495 (1968), paper CXX of this series; (b) B. R. Baker, P. C. Huang, and R. B. Meyer, Jr., *ibid.*, **11**, 475 (1968), paper CXVI of this series.

(4) For the previous paper on dihydrofolic reductase see 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) M. Friedkin, E. Crawford, S. R. Humphreys, and A. Goldin, *Cancer Res.*, **22**, 600 (1962).

(6) L1210/DFS is a strain of L1210 resistant to amethopterin due to a 50-fold increase in dihydrofolic reductase that was isolated in the laboratory of Dr. A. Goldin, National Cancer Institute.

(7) Private communication from Dr. Florence White, CCNSC, National Cancer Institute.

(8) 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, pp 246–252.

(9) B. R. Baker and J. H. Jordaan, *J. Heterocycl. Chem.*, **4**, 31 (1967), paper LXXXIII of this series.

(10) (a) See ref 8, Chapter 8, for the kinetics of irreversible inhibition; (b) B. R. Baker, W. W. Lee, and E. Tong, *J. Theoret. Biol.*, **3**, 459 (1962).

(11) Two reactions can occur within the enzyme–inhibitor reversible complex with the SO_2F type of irreversible inhibitor; rapid covalent bond formation can take place, or the enzyme can catalyze the hydrolysis of the SO_2F group, or both reactions can occur. See B. R. Baker and J. A. Hurlbut, *J. Med. Chem.*, **11**, 233 (1968), paper CXIII of this series, for experimental evidence and more detailed discussion. If both reactions occur, a considerably greater than K_i concentration of the inhibitor will be needed to show good irreversible inhibition.

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

The parent compound (**1**) in this study also only met one of the three criteria: 0.27 μM **1** gave 85% inactivation of the L1210/DF8 enzyme, this concentration (K_i) being sufficient to complex 50% of the enzyme. However, the compound failed the other criteria, that is, the I_{50} was at least 16-fold too great, and the liver enzyme showed appreciable inactivation with **1**. Therefore, structural variants of **1** were synthesized for investigation with the hope a compound could be found in this series that would give better reversible inhibition of the tumor enzyme and less irreversible inhibition of the liver enzyme.

It cannot necessarily be assumed that a synthetic would emerge that would meet all three criteria without additional studies; therefore, the logistics of assaying one compound on three sources of enzyme becomes monumental. A protocol was developed that required less assays, but involved some calculated risks.

(1) Only one source of enzyme was measured for reversible inhibition, usually L1210/DF8; the I_{50} for a given compound seldom varied more than threefold for the enzyme from L1210/DF8, L1210/0, and mouse liver[4] (Table I).

(2) The extent of inactivation of the L1210/DF8 by $2I_{50} = 12K_i$ of the candidate compound in 60 min at 37° was measured; similarly, inactivation of L1210/0 was measured with the same concentration of inhibitor. If the compound showed >70% inactivation of either enzyme, it was advanced to step 3; if the compound showed <70% inactivation of both L1210 enzymes, studies were discontinued since previous experience showed that poor inactivation would occur at a K_i concentration of inhibitor.[11]

(3) The extent of inactivation of liver enzyme by a $2I_{50}$ concentration of inhibitor was measured. If >40% inactivation occurred, studies were discontinued; if <40% inactivation occurred then the compound was advanced to step 4.

(4) Higher and lower concentrations were then investigated for the extent of inactivation of the L1210 and mouse liver enzymes.

The first group of structural changes (**3–6**, Table I) had a chlorine or methyl group placed on the phenoxy moiety of **1**. The 3-methyl group of **3** gave a 17-fold increment in reversible binding to L1210/DF8 dihydrofolic reductase and an eightfold increment to the L1210/0 enzyme; thus **3** meets the first criterion above by having an $I_{50} < 10^{-7}$ M. Excellent inactivation of the dihydrofolic reductase from all three L1210 strains was seen with 0.06 μM inhibitor which was sufficient to complex about 90% of the enzyme; unfortunately, 0.31 μM **3** showed 38% inactivation of the mouse liver enzyme. At 6×10^{-8} M, irreversible inhibition of the L1210/DF8 enzymes was still good, in contrast to liver enzyme which showed only 16% inactivation.

Introduction of a 3-chloro atom (**4**) on **1** gave a fivefold increment in reversible binding to the L1210/DF8 enzyme. A near K_i concentration (6×10^{-8} M) of **4** showed good irreversible inhibition of the L1210/DF8 and L1210/0 enzymes. The inactivation of the liver enzyme by this concentration was only 22%, but increased to 54% when the concentration was increased tenfold. Furthermore, **4** was just outside the desired range of $I_{50} < 10^{-7}$ M; thus **4** is just outside the limits of two of the criteria and is not as good a compound as **3**.

Introduction of a 2-chloro atom (**5**) had little influence on the I_{50}; unfortunately, the I_{50} of **5** was still 14-fold less as a reversible inhibitor than required by the criteria. However, **5** showed good inactivation of the L1210 enzymes at 0.23 μM, a K_i concentration; at twice this concentration, only 19% inactivation of the liver enzyme was seen. Thus **5** comes close to meeting two of the three criteria. Introduction of the 2,6-dimethyl groups (**6**) on **1** not only caused a twofold loss in reversible binding, but considerably impaired irreversible inhibition in the enzyme from both L1210/DF8 and L1210/0.

The second group of structural changes (**7–10**) consisted of changes on the benzene ring bearing the sulfonyl fluoride group. Introduction of a 4-OCH_3, 4-OEt, 6-OMe, or 6-Cl gave at best only a threefold increment in reversible binding; thus **7–10** failed to meet the first criterion of $I_{50} < 10^{-7}$ M by a factor of 2–5. Unfortunately, introduction of a 4-OMe (**7**), 6-OMe (**9**), or 6-Cl (**10**) on **1** enhanced irreversible inhibition of the liver enzyme. Introduction of a 4-OEt (**8**) group decreased irreversible inhibition of the L1210 enzymes to an unacceptable level.

The third class of structural changes consisted of a 3-chloro or 3-methyl group on the phenoxy moiety with a further substitution on the benzenesulfonyl fluoride moiety (**11–15**). In all five cases, the irreversible inhibition of the mouse liver enzyme was increased to the point where little differential irreversible inhibition was seen.

The best compound in Table I is **3**; it has a satisfactory I_{50} and at 6×10^{-8} M ($6K_i$ concentration) shows 75–80% inactivation of the L1210 enzymes with only 16% inactivation of the liver enzyme. Although this compound does not meet two of the three criteria, it comes close enough that animal studies may be warranted.

Chemistry.—All of the compounds in Table I can be generalized by structure **20** and were synthesized by the route previously described for **1** (= **20a,f**).[3a] The appropriate nitrophenol was condensed with **17**[3b] in DMF in the presence of K_2CO_3 at 70° to **18** (Scheme I). The nitro group of **18** was reduced catalytically with PtO_2 catalyst. Commercially available *m*-fluorosulfonylphenyl isocyanate (**21f**) was condensed with the arylamines (**19b–e**) in DMF to give the candidate irreversible inhibitors **3–6.**

The second class of irreversible inhibitors (**7–15**, Table I) had an extra substituent on the benzenesulfonyl moiety. The mixed urea synthesis of Crosby,[13] which involves the condensation of an O-phenylurethan such as **24**[14] with an aliphatic amine, proceeded poorly with arylamines such as **19**. Therefore, the more highly activated O-(*p*-nitrophenyl)urethans (**23**) were employed. Condensation of **23** with **19** proceeded smoothly in DMF at room temperature to give the desired irreversible inhibitors (**20**).

Experimental Section

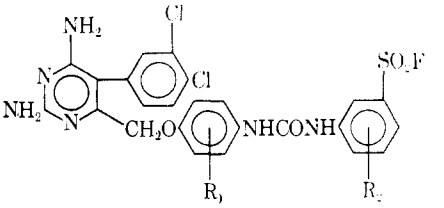
All analytical samples had proper uv and ir spectra; each moved as a single spot on tlc on Brinkmann silica gel GF and gave

(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).

(14) B. R. Baker and G. J. Lourens, *J. Med. Chem.*, **11**, 666 (1968), paper CXXVII of this series.

Table I

Inhibition[a] of Mouse Dihydrofolic Reductases by



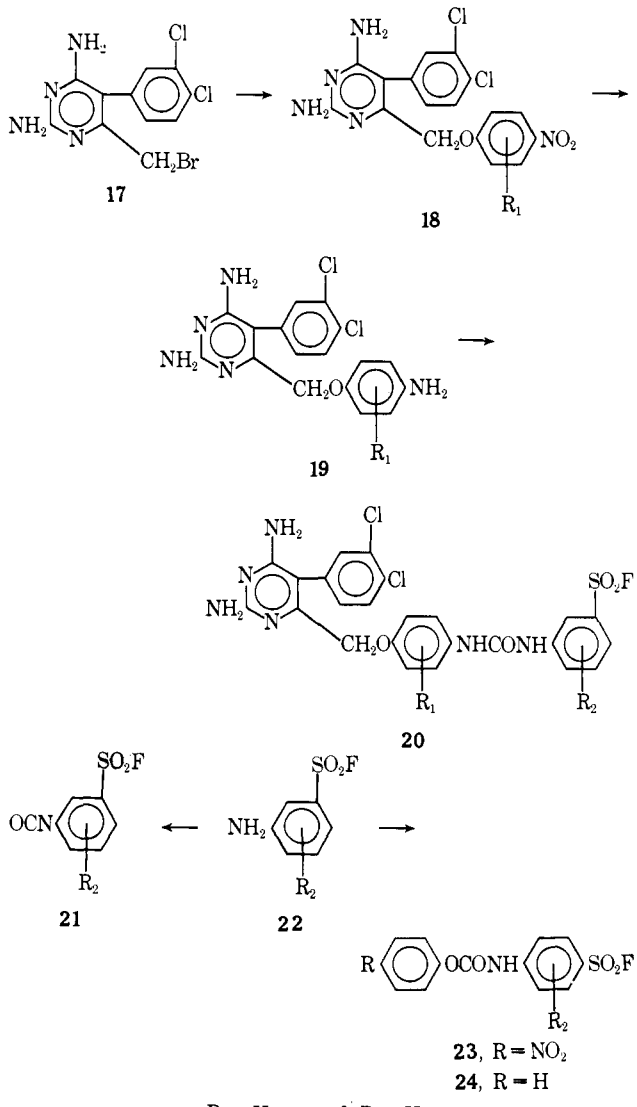
No.	R_1[b]	R_2[c]	Enzyme source	Reversible[d] I_{50}, μM[e]	Reversible[d] Estd $K_i \times 10^6$, M[f]	Irreversible[e] I, μM	Irreversible[e] % EI[h]	Irreversible[e] Time, min	Irreversible[e] % inactvn
1	H	H	L1210/FR8[g]	1.0	0.17	1.0	87	6, 30	50, 85[i]
						0.20	54	13, 18, 60	60, 65, 65[i]
			L1210/0	0.51	0.085	2.0	96	60	90
						0.51	87	10, 60	50, 75[i]
			L1210/DF8	1.6	0.27	2.0	88	60	90
						0.50	65	60	90
						0.27	50	60	85
			Liver			1.0	60	6, 60	43, 43[i,j]
3	3-Me	H	L1210/FR8	0.31	0.052	0.31	87	2, 8, 30	89, 93, 100[i]
						0.050	50	2, 8, 30	67, 82, 95[i]
			L1210/0	0.063	0.010	0.62	99	60	100
						0.063	87	2, 10, 60	50, 68, 80[i]
						0.01	50	60	60
			L1210/DF8	0.094	0.016	0.31	95	60	95
						0.050	76	60	75
						0.016	50	60	63
			Liver	0.056	0.0094	0.31	97	<30, 60	38, 38[i]
						0.060	87	60	16
4	3-Cl	H	L1210/DF8	0.34	0.057	0.68	93	60	100
						0.34	87	60	92
						0.060	50	60	86
			L1210/0			0.68		60	100
						0.34		60	91
						0.060		60	91
			Liver			0.68		60	54
						0.060		60	22[k]
5	2-Cl	H	L1210/FR8	2.2	0.37	2.2	87	8, 30, 60	50, 68, 79[i]
						0.40	52	11, 30, 60	50, 67, 85[i]
			L1210/0	1.4	0.23	2.2	91	60	79[k]
			L1210/DF8	1.4	0.23	2.2	91	60	98
						0.40	64	60	88
						0.23	50	60	80
			Liver			1.4		60	37
						0.4		60	19
6	2,6-Me_2	H	L1210/DF8	3.3	0.55	3.3	87	60	17
			L1210/0			3.3		60	33
7	H	4-OMe	L1210/0	0.17	0.030	2.0	99	60	80[k]
			L1210/DF8			2.0		60	82[k]
			Liver			2.0		60	75[k]
8	H	4-OEt	L1210/FR8	0.45	0.075	2.3	97	60	55
			L1210/0			0.90		60	30
			L1210/DF8			0.90		60	33[k]
9	H	6-OMe	L1210/FR8	0.48	0.080	0.48	87	7, 30, 60	50, 65, 76[i]
			L1210/0			0.96		60	53
			Liver			0.48		<30, 60	66, 66[i]
10	H	6-Cl	L1210/FR8	0.56	0.094	0.56	87	7, 30, 60	50, 65, 72[i]
			L1210/0	0.27	0.045	1.1	96	60	75
						0.27	87	5, 16, 60	50, 63, 63[i]
			L1210/DF8			0.56		60	82
			Liver			0.56		60	87[k]
11	3-Cl	4-OMe	L1210/DF8	0.21	0.035	0.42	93	60	95
			L1210/0			0.42		60	82
			Liver			0.42		60	95[k]
12	3-Cl	6-OMe	L1210/DF8	0.28	0.047	0.55	93	60	55
			L1210/0			0.55		60	76
			Liver			0.55		60	54
13	3-Me	6-OMe	L1210/FR8	0.14	0.023	0.73		60	100
			L1210/0			0.29		60	83
			L1210/DF8			0.29		60	100
			Liver			0.29		60	97[k]

TABLE I (*Continued*)

No.	R_1[b]	R_2[c]	Enzyme source	Reversible[d] I_{50}, M[f]	Reversible[d] Estd $K_i \times 10^6$ M[g]	Irreversible[e] 1, μM	Irreversible[e] % EI[h]	Irreversible[e] Time, min	Irreversible[e] % inactvn
14	3-Cl	6-Cl	L1210/DF8	0.67	0.11	1.3	97	60	100
			L1210/0			1.3		60	86
			Liver			1.3		60	95
15	3-Me	6-Cl	L1210/FR8	0.13	0.021	0.67	97	60	100
			L1210/0			0.27		60	94
			L1210/DF8			0.27		60	100
			Liver			0.27		60	97[k]

[a] The technical assistance of Sharon Lafler, Diane Shea, and Carolyn Wade with these assays is acknowledged. [b] Numbered from ether linkage at the 1 position. [c] Numbered from ureido linkage at the 1 position *via* 3-SO_2F. [d] Assayed with 6 μM dihydrofolate and 30 μM TPNH in pH 7.4 Tris buffer containing 0.15 M KCl as previously described.[4,12] [e] Incubated at 37° in pH 7.4 Tris buffer in the presence of 60 μM TPNH as previously described;[4,12] the inhibitor was added to the zero-time aliquot at 0° unless otherwise indicated. [f] I_{50} = concentration for 50% inhibition. [g] Estimated from $K_i = K_m[I_{50}]/[S]$ which is valid since $[S] = 6K_m = 6\ \mu M$; see ref 8, p 202. [h] Estimated from $[EI] = [E_t]/(1 + K_i/[I])$ where [EI] is the amount of total enzyme (E_t) reversibly complexed.[10] [i] Data from ref 4. [j] From time study plot; see ref 12. [k] Zero point modified by addition of inhibitor to cuvette.[4,12]

SCHEME I

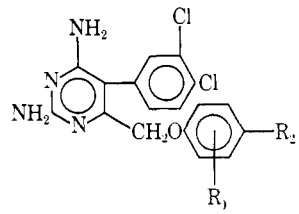


a, R_1 = H; b, R_1 = 3-Me; c, R_1 = 3-Cl; d, R_1 = 2-Cl; e, R_1 = 2,6-Me_2; f, R_2 = H; g, R_2 = 4-OMe; h, R_2 = 4-OEt; i, R_2 = 6-OMe; j, R_2 = 6-Cl

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.

6-(3-Chloro-4-nitrophenoxymethyl)-2,4-diamino-5-(3,4-dichlorophenyl)pyrimidine (18c) (Method A).—A mixture of 3.00 g (8.6 mmoles) of **17**,[3b] 1.24 g (9.0 mmoles) of K_2CO_3, 1.56 g (9 mmoles) of 3-chloro-4-nitrophenol,[15] and 20 ml of DMF was stirred in a bath at 70° for 2 hr when a test for activated halogen[16] was negative and tlc showed the reaction was complete. The mixture was diluted with 50 ml of H_2O, then the product was collected on a filter and washed with H_2O. Two recrystallizations from EtOH–THF gave 2.0 g (53%) of light yellow crystals, mp 207–209°. See Table II for additional data and other compounds made by this method.

TABLE II
PHYSICAL PROPERTIES OF



No.	R_1	R_2	Method	% yield	Mp, °C	Formula[a]
18b	3-Me	4-NO_2	A	91	194–196	$C_{18}H_{15}Cl_2N_5O_3$
18c	3-Cl	4-NO_2	A	53	207–209	$C_{17}H_{12}Cl_3N_5O_3$
18d	2-Cl	4-NO_2	A	72	208–209	$C_{17}H_{12}Cl_3N_5O_3$
18e	2,6-Me_2	4-NO_2	A	90	221–224	$C_{19}H_{17}Cl_2N_5O_3$
19b	3-Me	4-NH_2	B	85[b]		$C_{18}H_{17}Cl_2N_5O$
19c	3-Cl	4-NH_2	B	96[b]		$C_{17}H_{14}Cl_3N_5O$
19d	2-Cl	4-NH_2	B	96[b]		$C_{17}H_{14}Cl_3N_5O$
19e	2,6-Me_2	4-NH_2	B	90[b]		$C_{19}H_{19}Cl_2N_5O$

[a] All compounds analyzed for C, H, and N unless otherwise indicated. [b] Not further purified nor analyzed, but uniform on tlc and positive Bratton–Marshall test for aromatic amine.[17]

6-(4-Amino-3-chlorophenoxymethyl)-2,4-diamino-5-(3,4-dichlorophenyl)pyrimidine (19c) (Method B).—A mixture of 1.8 g (4.1 mmoles) of **18c**, 50 ml of EtOH, 50 ml of MeOEtOH, and 60 mg of PtO_2 was shaken with H_2 at 2–3 atm for 2 hr when reduction was complete. The filtered solution was evaporated to dryness *in vacuo* leaving 1.63 g (96%) of product as an amorphous solid that was not further purified. The compound gave a positive Bratton–Marshall test for aromatic amine[16] and moved as a single spot on tlc with 1:9 EtOH–$CHCl_3$. For additional amines prepared by this method see Table II.

***p*-Nitrophenyl N-(3-Fluorosulfonylphenyl)carbamate (23f) (Method C).**—A mixture of 1.1 g (5.2 mmoles) of **22f·HCl**, 1.1 g (5.5 mmoles) of *p*-nitrophenyl chloroformate,[17] and 25 ml of C_6H_6 was refluxed with magnetic stirring for 4 hr when no more HCl was evolved. The mixture was cooled, then the product was collected on a filter and washed with C_6H_6. Recrystallization from CH_2Cl_2, then C_6H_6, gave 1.1 g of white crystals, mp 139–142°.

(15) H. E. Ungnade and I. Ortega, *J. Org. Chem.*, **17**, 1475 (1952).
(16) B. R. Baker, D. V. Santi, J. K. Coward, H. S. Shapiro, and J. H. Jordaan, *J. Heterocycl. Chem.*, **3**, 425 (1966).
(17) G. W. Anderson and A. C. McGregor, *J. Am. Chem. Soc.*, **79**, 6180 (1957).

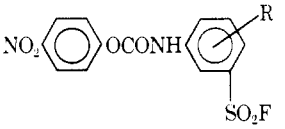
See Table III for additional data and other compounds prepared by this route; in some cases the free amine was used in the same manner.

2,4-Diamino-5-(3,4-dichlorophenyl)-6-[2-chloro-4-(3-fluorosulfonylphenylureido)phenoxymethyl]pyrimidine (5) Hemisulfate (Method D).—To a stirred solution of 300 mg (0.75 mmole) of **19d** in 1.1 ml of DMF cooled in an ice bath was added 150 mg (0.75 mmole) of **21f** (Aldrich Chemical Co.). After being stirred for 5 min, the solution was removed from the ice bath and stirred an additional 35 min. The solution was diluted with 10 ml of H_2O then the base was collected on a filter and washed with H_2O. The crude base was dissolved in 2 ml of MeOEtOH and added to 6 ml of 2 *N* H_2SO_4. The salt was collected on a filter and washed with H_2O. Two recrystallizations from Me EtOH–EtOH gave 110 mg (21%) of white crystals, mp 245° dec. See Table IV for additional compounds prepared by this method.

Method E was the same as method D with the following changes: (a) the *p*-nitrophenylurethans (**23**) dissolved in DMF were employed in place of **21**, (b) the reaction was run at ambient temperature for 2 hr, and (c) the sulfate salt was isolated directly from the reaction mixture by addition to 4 vol of 2 *N* H_2SO_4.

Table III

Physical Properties of

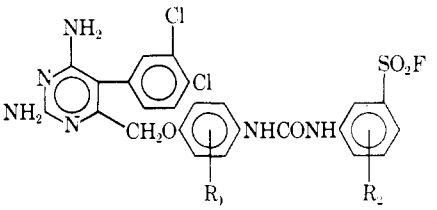


No.[a]	R[b]	% yield	Ref to amine	Mp, °C	Formula	Analyses
23f	H	62[c,d]	*e*	139–142	$C_{13}H_9FN_2O_6S$	C, H, N
23g	4-OMe	92[c]	*e*	162–164	$C_{14}H_{11}FN_2O_7S$	C, H, F
23h	4-OEt	81[f]	*f*	154–155		
23i	6-OMe	92[c]	*g*	160–162	$C_{14}H_{11}FN_2O_7S$	C, H, F
23j	6-Cl	67[c]	*g*	168–171	$C_{13}H_8ClFN_2O_6S$	C, H, N

[a] All compounds were prepared by method C. [b] Position with reference to 3-SO_2F. [c] Recrystallized from CH_2Cl_2. [d] Recrystallized from C_6H_6. [e] Aldrich Chemical Co. [f] Prepared in this laboratory by E. H. Erickson; to be published. [g] A. H. deCat and R. K. van Poucke, *J. Org. Chem.*, **28**, 3426 (1963).

Table IV

Physical Properties of



No.	R_1	R_2	Method	% yield	Mp, °C dec[g]	Formula[h]
3	3-Me	H	D	34[a]	200	$C_{25}H_{21}Cl_2FN_6O_4S\cdot 0.5H_2SO_4\cdot 0.5H_2O$
4	3-Cl	H	E	35[a]	198	$C_{24}H_{18}Cl_3FN_6O_4S\cdot 0.5H_2SO_4$
5	2-Cl	H	D	21[b]	245	$C_{24}H_{18}Cl_3FN_6O_4S\cdot 0.5H_2SO_4\cdot 0.5EtOH$
6	2,6-Me_2	H	E	55[c]	220	$C_{26}H_{23}Cl_2FN_6O_4S\cdot 0.5H_2SO_4$
7	H	4-OMe	E	25[c]	210	$C_{25}H_{21}Cl_2FN_6O_5S\cdot 0.5H_2SO_4$
8	H	4-OEt	E	25[c]	202	$C_{26}H_{23}Cl_2FN_6O_5S\cdot 0.5H_2SO_4$
9	H	6-OMe	E	23[a]	219	$C_{25}H_{21}Cl_2FN_6O_5S\cdot 0.5H_2SO_4$
10	H	6-Cl	E[d]	22[c]	212	$C_{24}H_{18}Cl_3FN_6O_4S$
11	3-Cl	4-OMe	E	27[a]	205	$C_{25}H_{20}Cl_3FN_6O_5S\cdot 0.5H_2SO_4$
12	3-Cl	6-OMe	E	48[b]	190	$C_{25}H_{20}Cl_3FN_6O_5S\cdot 0.5H_2SO_4$
13	3-Me	6-OMe	E	36[f]	214	$C_{26}H_{23}Cl_2FN_6O_5S\cdot H_2SO_4$
14	3-Cl	6-Cl	E[d]	48[e]	205	$C_{24}H_{17}Cl_4FN_6O_4S$
15	3-Me	6-Cl	E	29[e]	195	$C_{25}H_{20}Cl_3FN_6O_4S\cdot 0.5H_2SO_4$

[a] Recrystallized from MeOEtOH–H_2O. [b] Recrystallized from MeOEtOH–EtOH. [c] Recrystallized from MeOEtOH. [d] H_2SO_4 omitted in procedure. [e] Recrystallized from EtOH. [f] Recrystallized from EtOH–MeOEtOH–petroleum ether (bp 60–110°). [g] Melting gradually occurred over a wide range starting at the temperature indicated. [h] All compounds were analyzed for C, H, F.