Experimental Section

All analytical samples had proper uv and ir spectra; each moved as a single spot on Brinkman silica gel GF 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. The physical properties of some compounds are given in Tables II and III.

4-Cyano-3-methylphenol.—A mixture of 35.5 g (0.25 mole) of 4-chloro-3-methylphenol, 31.3 g (0.35 mole) of CuCN,[18] and 100 ml of dry N-methylpyrrolidone was refluxed with stirring for 18 hr. The mixture was concentrated *in vacuo* to near dryness, then stirred with 50 ml of warm 6 *N* HCl for 30 min. After the addition of a solution of 100 g of $FeCl_3 \cdot 6H_2O$ in 100 ml of H_2O, the mixture was heated on a steam bath for 1 hr. The cooled mixture was extracted with Et_2O (four 200-ml portions). The combined extracts were washed with H_2O, then evaporated to a residue which was crystallized from 25 ml of CH_2Cl_2. One recrystallization from H_2O and three from C_6H_6 gave 15 g (43%) of product, mp 134–136° (lit.[19] mp 135–136° from an alternate process). This process has been previously employed for other benzonitriles.[20]

4-Cyano-2-methoxyphenol was synthesized in 80% yield, mp 87–89°, according to the general method of van Es;[21] lit.[22] mp 89–90° from an alternate method.

(18) H. J. Barber, *J. Chem. Soc.*, 79 (1943).

(19) R. J. S. Beer, K. Clark, H. G. Khorana, and A. Roberts, *ibid.*, 885 (1949).

(20) H. E. Harris and H. L. Herzog, U. S. Patent 3,259,646 (1966); *Chem. Abstr.*, **65**, 13621*f* (1966).

(21) T. van Es, *J. Chem. Soc.*, 1564 (1965).

(22) H. Rupe, *Ber.*, **30**, 2449 (1897).

Irreversible Enzyme Inhibitors. CLVIII.[1,2] Effect of Bridge Modification 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*-fluorosulfonylbenzamidomethyl)phenoxymethyl]pyrimidine. II

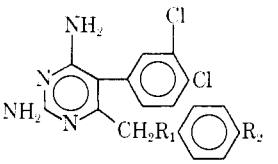
B. R. Baker and Nicolaas M. J. Vermeulen[3]

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

Received April 1, 1969

The title compound (**1**) is an active-site-directed irreversible inhibitor of the dihydrofolic reductase from L1210 mouse leukemia that also showed specificity by its failure to inactivate this enzyme from three normal mouse tissues. However, **1** still had two shortcomings; its $K_i = 0.06\ \mu M$ was considered too large to be effective *in vivo* and it showed poor transport through the L1210 cell wall. Thirty variants of the bridge between the pyrimidine and benzenesulfonyl fluoride moieties have now been investigated, such as (1) replacement of the oxymethyl group by thiomethyl, $(CH_2)_2$, or $(CH_2)_4$, (2) substituent effects on the phenoxy group, (3) variation of the CH_2NH moiety by NH and $(CH_2)_2NH$, and (4) variation of the amide linkage by CONH, NHCONH, and SO_2NH in the three previous classes. Sixteen of the compounds showed a predictable decrease in $I_{50} = 6K_i \leq 0.1\ \mu M$, but specificity was decreased or lost. The best five compounds showed inhibition of L1210 cell culture in the 0.5–1 μM range; this range is several magnitudes higher than that shown by the standard compound, 2,4-diamino-5-(3,4-dichlorophenyl)-6-methylpyrimidine (**35**).

One of the types of active-site-directed irreversible inhibitors[4] that can inactivate dihydrofolic reductase from L1210 mouse leukemia with no inactivation of the enzyme from normal mouse liver, spleen, or intestine[5] is 1.[6] The latter with its $I_{50} = 6K_i = 0.4\ \mu M$ was not considered a sufficiently good reversible inhibitor for *in vivo* activity[5] since too high an intracellular concentration of 1 would be required to form 50% reversible enzyme complex, the rate-determining species for active-site-directed irreversible inhibition.[7] Furthermore, 1 required the relatively high concentration of 4 μM for 50% inhibition (ED_{50}) of L1210 cell culture,[2] showing insufficient cell wall penetration. Several types of studies, such as **2**–**4**, have been performed to try to increase the effectiveness of reversible inhibition without loss of irreversible specificity; compounds with $I_{50} = 6K_i$ as good as 0.03 μM were obtained, but either



1. $R_1 = O$; $R_2 = CH_2NHCOC_6H_4SO_2F$-*m*
2. $R_1 = CH_2$; $R_2 = CH_2NHCOC_6H_4SO_2F$-*m*
3. $R_1 = O$; $R_2 = NHSO_2C_6H_4SO_2F$-*m*
4. $R_1 = CH_2$; $R_2 = NHCOC_6H_4SO_2F$-*m*

irreversible inhibition or selectivity was decreased.[2,8-10]

Since specificity is most apt to be obtained by bridge modification,[9,11] the following types of compounds have now been made: (1) the R_2 group of **1** and **2** was moved to the *meta* position, (2) analogs of **3** were made with substituents on the phenyl bearing R_2, (3) the oxygen bridge was replaced by sulfur or $-(CH_2)_3-$, and (4) the R_2 bridge of 1 was extended to $-(CH_2)_2-$. The results are the subject of this paper.

Assay Results.—Of the compounds of type 1 in Table

(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 in this series, see B. R. Baker and N. M. J. Vermeulen, *J. Med. Chem.*, **12**, 680 (1969).

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

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

(5) 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.

(6) B. R. Baker and P. C. Huang, *ibid.*, **11**, 495 (1968), paper CXX of this series.

(7) See ref 4, pp 122–129, for the kinetics of irreversible inhibition.

(8) B. R. Baker and N. M. J. Vermeulen, *J. Med. Chem.*, **12**, 86 (1969), paper CXXXVII of this series.

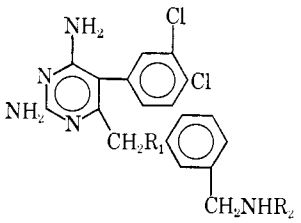
(9) B. R. Baker and N. M. J. Vermeulen, *ibid.*, **12**, 89 (1969), paper CXXXVIII of this series.

(10) B. R. Baker and N. M. J. Vermeulen, *ibid.*, **12**, 82 (1969), paper CXXXVI of this series.

(11) See ref 4, pp 172–184, for discussion of the bridge principle of specificity.

TABLE I

INHIBITION[a] OF DIHYDROFOLIC REDUCTASE BY

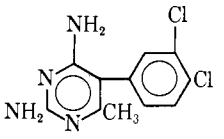


No.	R_1	R_2	Enzyme source	I_{50},[b] μM	Inhib, μM	Time, min	% inactvn[c]	ED_{50},[d] μM	ED_{50}/I_{50}
5	O	$COC_6H_4SO_2F$-*m*	L1210/DF8		0.11	60	88	6	100
			Liver	0.057	0.11	60	46		
6	O	$COC_6H_4SO_2F$-*p*	L1210/DF8	0.066	0.13	60	85	0.5	8
			Liver		0.13	60	72		
7	O	$CONHC_6H_4SO_2F$-*m*	L1210/DF8	0.068	0.068	60	93	0.6	9
			Liver		0.34	60	88		
					0.14	60	34		
8	O	$CONHC_6H_4SO_2F$-*p*	L1210/DF8	0.085	0.17	60	95	3	40
			Liver		0.17	60	68		
9	O	$CONHC_6H_3$-4-Me-3-SO_2F	L1210/DF8	0.054	0.11	60	93	1	20
			Liver		0.16	60	7		
					0.75	60	74		
10	O	$CONHC_6H_3$-2-Cl-5-SO_2F	L1210/DF8		0.66	60	97	6	20
			Liver	0.33	1.2	60	93		
11	O	$SO_2C_6H_4SO_2F$-*m*	L1210/DF8		0.22	60	22	2	20
			Liver	0.11	0.22	60	25		
12	O	$SO_2C_6H_4SO_2F$-*p*	L1210/DF8		0.12	60	54	6	100
			Liver	0.056	0.12	60	17–41[e]		
13	CH_2	$COC_6H_4SO_2F$-*p*	L1210/DF8		0.084	60	98	4	100
			Liver	0.042	0.13	60	82		
14	CH_2	$CONHC_6H_4SO_2F$-*p*	L1210/DF8		0.084	60	98	0.6	7
			Liver	0.084	0.25	60	93		
15	CH_2	$CONHC_6H_4SO_2F$-*m*	L1210/DF8		0.16	60	100	2	30
			Liver	0.080	0.24	60	92		
16	CH_2	$SO_2C_6H_4SO_2F$-*p*	L1210/DF8		0.11	60	83	5	90
			Liver	0.060	0.18	60	69		
17	CH_2	$SO_2C_6H_4SO_2F$-*m*	L1210/DF8		0.11	60	84	3	60
			Liver	0.056	0.17	60	73		

[a] The technical assistance of Diane Shea and Sharon Lafler with these assays is acknowledged. [b] I_{50} = concentration for 50% inhibition when measured with 6 μM dihydrofolate and 0.15 M KCl at pH 7.4 as previously described.[5] [c] Enzyme was incubated with inhibitor at 37° in pH 7.4 Tris buffer containing 60 μM TPNH, then the remaining enzyme was assayed as previously described.[5] [d] Concentration for 50% inhibition of L1210 cell culture. [e] Difficulty was encountered in determining the zero-time point.[5]

I, ten (**5, 6–9, 13–17**) showed the desired $I_{50} = 6K_i \leq 0.1$ μM and gave good irreversible inhibition of the dihydrofolic reductase from L1210. Unfortunately, none showed complete specificity by failure to inactivate the mouse liver enzyme; the greatest selectivity was shown by **9**. When measured for their ability to inhibit L1210 cells in culture,[12] the best ED_{50}'s (0.5–1 μM) were shown by **6**, **7**, and **9**, although the range in the whole table was only about tenfold.

Of the compounds of types **2–4** in Table II, six (**22**, **25–29**) showed the desired $I_{50} = 6K_i \leq 0.1$ μM as expected and gave good irreversible inhibition of the L1210 enzyme; however, all six also showed extensive inactivation of the mouse liver enzyme. The best compounds for inhibition of L1210 cell culture were **25**, **27**, and **29**, which showed ED_{50}'s in the range of 0.5–0.7 μM. Since this range is several magnitudes less effective than **35** with $ED_{50} = 2 \times 10^{-5}$ μM,[13] the compounds in Tables I and II are transported too inefficiently through the L1210 cell wall to be effective. No



35

generalizations on the best structures for transport were apparent from the data in Tables I and II.

Studies of other bridge modifications on the 6 position of the pyrimidine ring are continuing to see if irreversible inhibitors with good specificity and good L1210 cell wall transport can be obtained.

Chemistry.—Alkylation of **36**[14] with *m*-cyanophenol in DMF in the presence of K_2CO_3[15] afforded **37** (Scheme I). Hydrogenation of **37** in acid solution with Adams catalyst gave **39**.[9] Reaction of **39** with the appropriate acid chloride[9] or O-(*p*-nitrophenyl)-N-phenylurethan[15,16] gave irreversible inhibitors of type **42** (**5–12** in

(12) We wish to thank Dr. Florence White of the CCNSC for these data obtained by Dr. Philip Himmelfarb of Arthur D. Little, Inc.

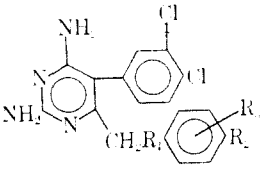
(13) B. R. Baker and R. B. Meyer, Jr., *J. Med. Chem.*, **12**, 668 (1969), paper CLIV of this series.

(14) B. R. Baker, P. C. Huang, and R. B. Meyer, Jr., *ibid.*, **11**, 475 (1968), paper CXVI of this series.

(15) B. R. Baker and N. M. J. Vermeulen, *ibid.*, **12**, 74 (1969), paper CXXXIV of this series.

(16) B. R. Baker and N. M. J. Vermeulen, *ibid.*, **12**, 79 (1969), paper CXXXV of this series.

Table II: Inhibition[a] of Dihydrofolic Reductase by



No.	R	R_2	R_3	Enzyme source	I_{50},[b] μM	Inhib, μM	Time, min	% inactvn[c]	ED_{50},[d] μM	ED_{50}/I_{50}
18[e]	O	$NHCONHC_6H_4SO_2F$-*m*	H	L1210/DF8	1.6	0.5	60	99	80	50
				Liver		1.0	60	43		
19	S	$NHCONHC_6H_4SO_2F$-*m*	H	L1210/DF8		0.14	60	72	6	40
				Liver	0.14	0.42	60	58		
20	$(CH_2)_3$	$NHCOC_6H_4SO_2F$-*m*	H	L1210/DF8		0.30	60	98	10	80
				Liver	0.15	0.30	60	61		
21	$(CH_2)_3$	$NHCOC_6H_4SO_2F$-*p*	H	L1210/DF8		0.11	60	66		
				Liver	0.11	0.22	60	77		
22[f]	CH_2	$NHCOC_6H_4SO_2F$-*m*	H	L1210/DF8		0.19	60	75	3	90
				Liver	0.034	0.19	60	31		
23[f]	O	$NHSO_2C_6H_4SO_2F$-*m*	H	L1210/DF8	0.018	0.036	60	42	1	60
				Liver		0.090	60	58		
24	O	$NHSO_2C_6H_4SO_2F$-*m*	2-Cl	L1210/DF8		0.058	60	23	6	200
				Liver	0.029	0.087	60	23		
25	O	$NHSO_2C_6H_4SO_2F$-*m*	3-Me	L1210/DF8		0.06	60	96	0.5	20
				Liver	0.020	0.10	60	97		
26[f]	O	$NHSO_2C_6H_4SO_2F$-*p*	H	L1210/DF8	0.049	0.098	60	93	5	100
				Liver		0.098	60	73		
27	O	$NHSO_2C_6H_4SO_2F$-*p*	2-Cl	L1210/DF8		0.094	60	72	0.7	10
				Liver	0.047	0.14	60	57		
28	O	$NHSO_2C_6H_4SO_2F$-*p*	3-Me	L1210/DF8		0.11	60	97	5	100
				Liver	0.053	0.11	60	46		
29	O	$(CH_2)_2NHCOC_6H_4SO_2F$-*m*	H	L1210/DF8		0.11	60	91	0.6	10
				Liver	0.056	0.11	60	55		
30	O	$(CH_2)_2NHCOC_6H_4SO_2F$-*p*	H	L1210/DF8		0.32	60	87	1	6
				Liver	0.16	0.48	60	76		
31	O	$(CH_2)_2NHCONHC_6H_4SO_2F$-*m*	H	L1210/DF8		0.22	60	98	4	40
				Liver	0.11	0.33	60	48		
32	O	$(CH_2)_2NHCONHC_6H_4SO_2F$-*p*	H	L1210/DF8		0.30	60	84	6	40
				Liver	0.15	0.45	60	73		
33	O	$(CH_2)_2NHSO_2C_6H_4SO_2F$-*m*	H	L1210/DF8		0.098	60	60	6	100
				Liver	0.049	0.098	60	23		
34	O	$(CH_2)_2NHSO_2C_6H_4SO_2F$-*p*	H	L1210/DF8		0.22	60	82	5	50
				Liver	0.11	0.22	60	60		
1[g]	O	$CH_2NHCOC_6H_4SO_2F$-*m*	H	L1210/DF8	0.37	0.70	60	88	4	10
						0.12	60	75		
				Liver	0.29	3.5	60	12		
						0.7	60	0		

[a–d] See corresponding footnotes in Table I. [e] Enzyme data from ref 15. [f] Enzyme data from ref 10. [g] Enzyme data from ref 5.

Table I). By a similar route employing *p*-hydroxyphenylacetonitrile and **36** *via* **46** and **47**, inhibitors of type **48** (**29–34** in Table II) were prepared.

When **36** was alkylated with a 4-nitrophenol bearing either a 2-Cl or 3-Me group, **38** was obtained;[6,15] reduction of **38** to **40** was followed by conversion to inhibitors of type **41** (**24–28** in Table II). Similarly, alkylation of **36** with *p*-acetamidothiophenol afforded **43** which was hydrolyzed to **44** with base, then converted to **45** = **19** with *m*-fluorosulfonylphenyl isocyanate.

Wittig condensation of **49**[10] with *p*-nitrocinnamaldehyde[17] in DMF in the presence of DBN (1,5-diazabicyclo[4.3.0]nonene)[18] afforded **50**[9] (Scheme II). Hydrogenation[9] to **52** was followed by conversion to inhibitors of type **53** (**20**, **21** in Table II). Similarly, Wittig condensation of **49** with *m*-cyanobenzaldehyde afforded **51** which was reduced to **54** and converted to inhibitors of type **55** (**13–17** in Table I).

(17) B. R. Baker and J. H. Jordaan, *J. Med. Chem.*, **8**, 35 (1965).

(18) H. Oediger, H. Kabbe, F. Möller, and K. Eiter, *Chem. Ber.*, **99**, 2012 (1966).

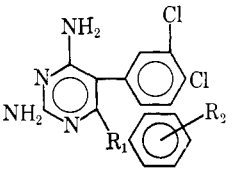
Experimental Section

All analytical samples had proper uv and ir spectra; each moved as a single spot on tlc with Brinkmann silica gel GF 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. The physical properties of some compounds are listed in Tables III–V.

6-(*p*-Acetamidophenylthiomethyl)-2,4-diamino-5-(3,4-dichlorophenyl)pyrimidine (43) Hydrochloride.—A mixture of 1.74 g (5 mmoles) of **36**,[14] 0.90 g (5.4 mmoles) of *p*-acetamidothiophenol, 0.69 g (5 mmoles) of K_2CO_3, and 20 ml of DMF was stirred at ambient temperature for 14 hr. The mixture was diluted with several volumes of H_2O; the solid was collected on a filter, washed (H_2O), dried, and dissolved in THF. The solution was treated with HCl gas, then the product was collected by filtration. Two recrystallizations from EtOH gave 1.2 g (50%) of white crystals which gradually decomposed over 192°. *Anal.* ($C_{19}H_{17}Cl_2N_5OS \cdot HCl \cdot 0.5H_2O$) C, H, N.

6-(*p*-Aminophenylthiomethyl)-2,4-diamino-5-(3,4-dichlorophenyl)pyrimidine (44) Dihydrochloride.—A mixture of 0.53 g

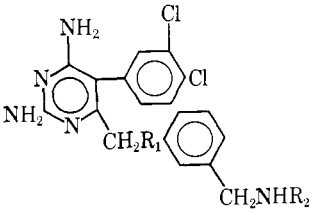
TABLE III: PHYSICAL PROPERTIES OF



No.	R_1	R_2	Method[a]	% yield	Mp °C	Formula	Analyses
37	CH_2O	*m*-CN	A	62[b]	191–193	$C_{18}H_{13}Cl_2N_5O$	C, H, N
39	CH_2O	*m*-$CH_2NH_2 \cdot 2HCl$	C	57	Indef[c]	$C_{18}H_{17}Cl_2N_5O \cdot 2HCl$	
46	CH_2O	*p*-CH_2CN	A	55	176–178	$C_{19}H_{15}Cl_2N_5O$	C, H, N
47	CH_2O	*p*-$(CH_2)_2NH_2 \cdot 2EtSO_3H$	C	95	Indef[c]	$C_{19}H_{19}Cl_2N_5O \cdot 2C_2H_5SO_3H$	
50	$(CH{=}CH)_2$	*p*-NO_2	B	71[b]	>172[d]	$C_{20}H_{15}Cl_2N_5O_2$	C, H, N
51	CH=CH	*m*-CN	B	52[e]	279–284	$C_{19}H_{13}Cl_2N_5$	C, H, N
52	$(CH_2)_4$	*p*-$NH_2 \cdot 2EtSO_3H$	C	93	Indef[c]	$C_{20}H_{21}Cl_2N_5 \cdot 2C_2H_5SO_3H$	
54	$(CH_2)_2$	*m*-$CH_2NH_2 \cdot 2HCl$	C	65	Indef[c]	$C_{19}H_{17}Cl_2N_5 \cdot 2HCl$	

[a] Method A: see method A in ref 15; B: for Wittig conditions see ref 9; C: for hydrogenation conditions see ref 9 and 2. [b] Recrystallized from EtOH–THF. [c] One spot on tlc in EtOH, but solvation varied. [d] Gradually decomposes starting at this temperature. [e] Recrystallized from MeOEtOH.

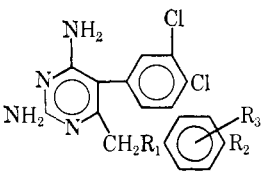
TABLE IV: PHYSICAL PROPERTIES OF



No.	R_1	R_2	Method[a]	% yield	Mp, °C, dec[b]	Formula[c]
5	O	$COC_6H_4SO_2F$-*m*	D	28[d]	127	$C_{25}H_{20}Cl_2FN_5O_4S \cdot 0.5H_2SO_4$
6	O	$COC_6H_4SO_2F$-*p*	D	27[d]	150	$C_{25}H_{20}Cl_2FN_5O_4S \cdot 0.5H_2SO_4$
7	O	$CONHC_6H_4SO_2F$-*m*	E	36[d]	210	$C_{25}H_{21}Cl_2FN_6O_4S \cdot 0.5H_2SO_4$
8	O	$CONHC_6H_4SO_2F$-*p*	E	17[d]	213	$C_{25}H_{21}Cl_2FN_6O_4S \cdot 0.5H_2SO_4$
9	O	$CONHC_6H_3$-4-Me-3-SO_2F	E	51[d]	236	$C_{26}H_{23}Cl_2FN_6O_4 \cdot 0.5H_2SO_4$
10	O	$CONHC_6H_3$-2-Cl-5-SO_2F	E	26	156	$C_{25}H_{20}Cl_3FN_6O_4 \cdot 0.5H_2SO_4$
11	O	$SO_2C_6H_4SO_2F$-*m*	F	39[d]	125	$C_{24}H_{20}Cl_2FN_5O_5S_2 \cdot 0.5H_2SO_4$
12	O	$SO_2C_6H_4SO_2F$-*p*	F	45[d]	127	$C_{24}H_{20}Cl_2FN_5O_5S_2 \cdot 0.5H_2SO_4$
13	CH_2	$COC_6H_4SO_2F$-*p*	D	36[d]	130	$C_{26}H_{22}Cl_2FN_5O_3S \cdot 0.5H_2SO_4 \cdot$ MeOEtOH
14	CH_2	$CONHC_6H_4SO_2F$-*p*	E	42[d]	170	$C_{26}H_{23}Cl_2FN_6O_3S \cdot 0.5H_2SO_4$
15	CH_2	$CONHC_6H_4SO_2F$-*m*	E	25[e]	182	$C_{26}H_{23}Cl_2FN_6O_3S \cdot 0.5H_2SO_4$
16	CH_2	$SO_2C_6H_4SO_2F$-*p*	F	32[e]	165	$C_{25}H_{22}Cl_2FN_5O_4S_2 \cdot 0.5H_2SO_4$
17	CH_2	$SO_2C_6H_4SO_2F$-*m*	F	20[e]	163	$C_{25}H_{22}Cl_2FN_5O_4S_2 \cdot 0.5H_2SO_4$

[a] Method D: the same as method C in ref 6; E: the same as method E in ref 15; F: the same as method C in ref 15. [b] Decomposition gradually occurred over a wide range starting at the temperature indicated. [c] *Anal.* C, H, F. [d] Recrystallized from MeOEtOH–H_2O. [e] Recrystallized from EtOH.

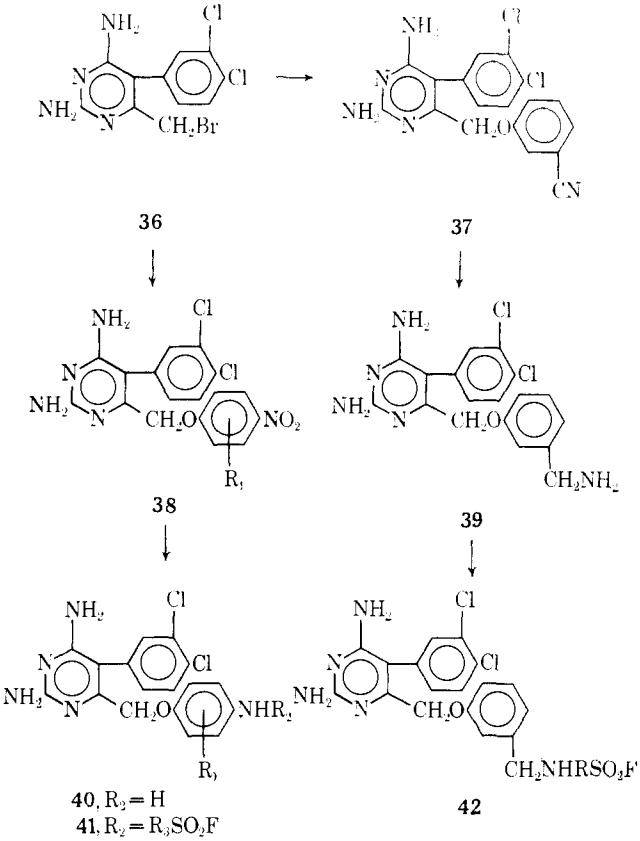
TABLE V: PHYSICAL PROPERTIES OF



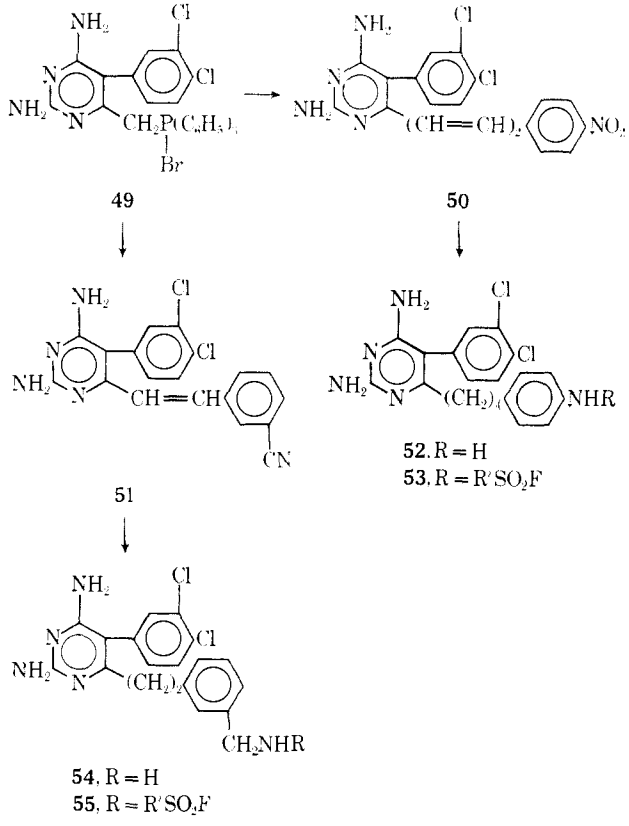
No.	R_1	R_2	R_3	Method[a]	% yield	Mp, °C dec[b]	Formula[c]
19	S	$NHCONHC_6H_4SO_2F$-*m*	H	E	36[d]	198	$C_{24}H_{19}Cl_2FN_6O_3S_2 \cdot 0.5H_2SO_4$
20	$(CH_2)_3$	$NHCOC_6H_4SO_2F$-*m*	H	D	58[e]	140	$C_{27}H_{24}Cl_2FN_5O_3S \cdot 0.5H_2SO_4$
21	$(CH_2)_3$	$NHCOC_6H_4SO_2F$-*p*	H	D	49[e]	181	$C_{27}H_{24}Cl_2FN_5O_3S \cdot 0.5H_2SO_4 \cdot 0.5H_2O$
24	O	$NHSO_2C_6H_4SO_2F$-*m*	2-Cl	F	33[e]	159	$C_{23}H_{17}Cl_3FN_5O_5S_2 \cdot 0.5H_2SO_4$
25	O	$NHSO_2C_6H_4SO_2F$-*m*	3-Me	F	30[e]	153	$C_{24}H_{20}Cl_2FN_5O_5S_2 \cdot 0.5H_2SO_4$
27	O	$NHSO_2C_6H_4SO_2F$-*p*	2-Cl	F	16[e]	186	$C_{23}H_{17}Cl_3FN_5O_5S_2 \cdot 0.5H_2SO_4$
28	O	$NHSO_2C_6H_4SO_2F$-*p*	3-Me	F	22[e]	171	$C_{24}H_{20}Cl_2FN_5O_5S_2 \cdot 0.5H_2SO_4$
29	O	$(CH_2)_2NHCOC_6H_4SO_2F$-*m*	H	D	25[e]	150	$C_{26}H_{22}Cl_2FN_5O_4S \cdot 0.5H_2SO_4$
30	O	$(CH_2)_2NHCOC_6H_4SO_2F$-*p*	H	D	27[e]	167	$C_{26}H_{22}Cl_2FN_5O_4S \cdot 0.5H_2SO_4$
31	O	$(CH_2)_2NHCONHC_6H_4SO_2F$-*m*	H	E	61[e]	165	$C_{26}H_{23}Cl_2FN_6O_4S \cdot 0.5H_2SO_4 \cdot H_2O$
32	O	$(CH_2)_2NHCONHC_6H_4SO_2F$-*p*	H	E	52[e]	174	$C_{26}H_{23}Cl_2FN_6O_4S \cdot 0.5H_2SO_4 \cdot H_2O$
33	O	$(CH_2)_2NHSO_2C_6H_4SO_2F$-*m*	H	F	33[e]	115	$C_{25}H_{22}Cl_2FN_5O_5S_2 \cdot 0.5H_2SO_4 \cdot 0.5$MeOEtOH
34	O	$(CH_2)_2NHSO_2C_6H_4SO_2F$-*p*	H	F	31[e]	130	$C_{25}H_{22}Cl_2FN_5O_5S_2 \cdot 0.5H_2SO_4 \cdot 0.5$MeOEtOH

[a–c] See corresponding footnotes in Table IV. [d] Recrystallized once from EtOH–THF and once from MeOEtOH–H_2O. [e] Recrystallized from MeOEtOH–H_2O.

Scheme I



Scheme II



(1.1 mmoles) of **43**, 10 ml of EtOH, 2 ml of H_2O, and 2.5 ml of 50% aqueous NaOH was refluxed with stirring for 4 hr. Most of the solvent was removed *in vacuo* and the residue was stirred with 15 ml of H_2O. The solid was collected on a filter, dissolved in THF, and treated with HCl gas. The product was collected on a filter and washed with THF, yielding 0.48 g (95%) which showed one spot on tlc in 1:9 EtOH–$CHCl_3$ and gave a positive Bratton–Marshall test for aromatic amine;[19] satisfactory analytical results were not obtained due to variable solvation.

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