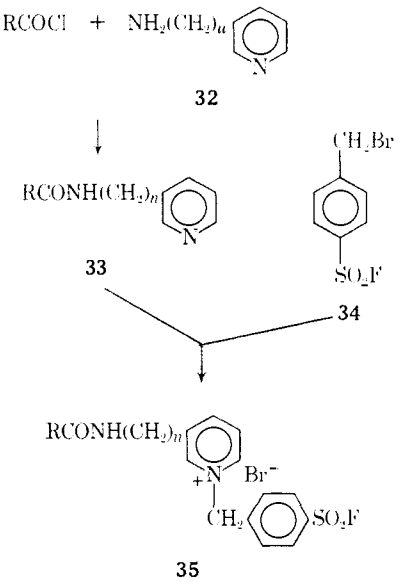
with **3** and **6**.[10] Thus, the 3,4-dichlorophenoxyacetyl group is equivalent to the N-tosyl-L-phenylalanyl group for reversible and irreversible inhibition of α-chymotrypsin, but the latter seems less effective for inhibition of complement.

The two most effective compounds in Tables I and II were **3** and **4**; however, these were less effective than **1**.[3] Many questions are raised by these initial studies that should be answered. (1) Can substituent effects on the C_6H_5 moiety of **21** and **22**, as well as additional substituent studies related to **24–29**, lead to enhanced activity? (2) What is the optimum number of methylenes between the amide and pyridyl moieties of **3** and **6**? With two or more methylenes the 2- and 4-pyridyl isomers can also be synthesized for evaluation, since these isomers of **3** and **6** cannot be synthesized due to instability.[10] (3) Could other bridges between the pyridinium N and the phenylsulfonyl fluoride moiety lead to enhanced activity? (4) Would a study of substituent effects on the phenylsulfonyl fluoride moiety be rewarding? (5) Would substitution on the amide N be beneficial?

Studies to answer some of these questions are currently proceeding in this laboratory.

Chemistry.—The synthesis of compounds in Table I have been described previously;[10] those in Table II were made by the same general route *via* **33** by quaternization with **34**.[12]



The physical properties of **36–45** and **20–22** and **24–31** are given in Tables III and IV, respectively.[13]

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

(13) Melting points were taken in capillary tubes on a Mel-Temp block and are uncorrected. Each analytical sample had the appropriate ir spectrum, moved as a single spot on tlc on Brinkmann silica gel GF (Table III) or polyamide MN (Table IV), and gave combustion values for C, H, and N or F within 0.4% of theory.

Irreversible Enzyme Inhibitors. CLVII.[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. I

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Received April 1, 1969

The title compound (**1**) is an active-site-directed irreversible inhibitor of the dihydrofolic reductase from L1210 mouse leukemia that shows specificity by not inactivating this enzyme from normal mouse liver; however, **1** had $K_i = 0.06$ μM which was considered too large for *in vivo* effectiveness. Twenty-eight related compounds with and without substituents on one of the two 6-phenyl moieties have now been investigated; six compounds had $K_i < 0.01$ μM and showed good irreversible inhibition of the L1210 enzyme, but specificity was decreased or lost. The ability of these 29 compounds to inhibit L1210 cell culture (ED_{50}) was investigated. When ED_{50}/I_{50} was used as an approximate estimation of transport through the cell wall, the best compound was 2,4-diamino-5-(3,4-dichlorophenyl)-6-[4-(*m*-fluorosulfonylphenylureido)-3-methylphenoxymethyl]pyrimidine (**16**) with $ED_{50} = 0.05$ μM and $ED_{50}/I_{50} = 2$. However, **16** was still two to three magnitudes less effective than 2,4-diamino-5-(3,4-dichlorophenyl)-6-methylpyrimidine with $ED_{50} = 2 \times 10^{-5}$ μM and $ED_{50}/I_{50} = 0.002$. From the differences in ED_{50}/I_{50} ratios, substitution of a 2-MeO or 3-Me group in the phenoxy moiety enhanced transport in half the cases; in most cases the compounds with a urea bridge showed better transport than the corresponding compounds with an amide bridge.

Several types of active-site-directed irreversible inhibitors,[4] bearing a terminal SO_2F moiety, have been found for dihydrofolic reductase that can inactivate the enzyme from L1210 mouse leukemia with no inactivation of this enzyme from normal mouse liver.[5-7] One of these isozyme-specific irreversible inhibitors was **1**.[7] However, **1** failed to meet the three criteria[5]

(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 J. A. Hurlbut, *J. Med. Chem.*, **12**, 677 (1969).

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

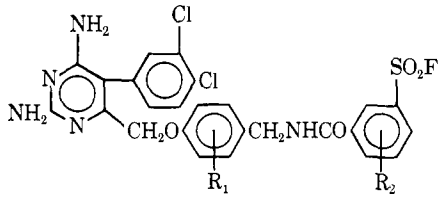
(4) B. R. Baker, "Design of Active-Site-Directed Irreversible Enzyme Inhibitors," John Wiley and Sons, Inc., New York, N. Y., 1967.

(5) (a) B. R. Baker and R. B. Meyer, Jr., *J. Med. Chem.*, **11**, 489 (1968), paper CXIX of this series; (b) B. R. Baker and R. B. Meyer, Jr., *ibid.*, **12**, 108 (1969), paper CXLIII of this series; (c) B. R. Baker and R. B. Meyer, Jr., *ibid.*, **12**, 224 (1969), paper CLI of this series.

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

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

(8) B. R. Baker, G. J. Lourens, R. B. Meyer, Jr., and N. M. J. Vermeulen, *ibid.*, **12**, 67 (1969), paper CXXXIII of this series.



1, $R_1 = R_2 = H$
2, $R_1 = H; R_2 \neq H$
3, $R_1 \neq H; R_2 = H$

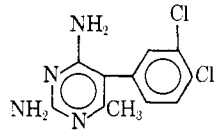
for *in vivo* testing; with its $I_{50} = 6K_i = 0.4\ \mu M$[6,7,9] it was not a sufficiently good reversible inhibitor, that is, too high a concentration of **1** is necessary to form a 50% reversible complex with the enzyme, the rate-determining species for active-site-directed irreversible inhibition.[10] A study was then made[9] where the amide linkage or the position of the SO_2F moiety was varied and where R_2 was a small substituent other than H (**2**) in order to try to increase the effectiveness of reversible inhibition; although inhibitors in this series with I_{50} as good as 0.03 μM were obtained, specificity was lost. A similar study has now been made where a substituent (R_1) was placed on the bridge to give structures related to **2**; the results are the subject of this paper. Other variants of the bridge are presented in papers to follow.[11,12]

Enzyme Results.—A number of the compounds in Table I (**4, 5, 9, 10, 16,** and **17**) had $I_{50} = 6K_i \leq 0.05\ \mu M$, which is in the desired range, and showed good irreversible inhibition of the dihydrofolic reductase from L1210; unfortunately, the liver enzyme was also inactivated, specificity being lost compared to **1**.

The best compounds in cell culture were **12**, **14**, **16**, and **20** with $ED_{50} = 0.5$, 0.2, 0.05, and 0.2 μM, respectively; the $ED_{50}/I_{50} \simeq 2$ for all four compounds compares them when the I_{50} is normalized. The following generalizations appear to be valid on the effect of structure on transport, as measured by ED_{50}/I_{50}.

(1) There is no correlation as to whether or not a *m*- or a *p*-SO_2F group was superior. (2) Comparison of urea *vs.* amide bridges showed four out of eight cases where the urea was better (**5** *vs.* **15**, **6** *vs.* **16**, **10** *vs.* **20**, and **11** *vs.* **21**). In no case was the amide bridge better, but in four out of eight cases the amide and urea were equal. Therefore a urea bridge in general is preferred to an amide for transport. (3) In three out of six cases the 2-MeO group gave better transport (**4** *vs.* **1**, **14** *vs.* **13**, and **20** *vs.* **19**), and in three out of six cases 2-MeO was equal to H. (4) Little change in transport was seen with the 2-Cl group in comparing six pairs. (5) In three out of six cases the 3-Me group was beneficial to transport (**12** *vs.* **9**, **16** *vs.* **13**, and **22** *vs.* **19**), and in the remaining three out of six cases transport was essentially equal. (6) In four out of five cases substitution on the $C_6H_4SO_2F$ moiety gave unchanged transport and in one out of five cases transport was less effective.

From these correlations one can conclude that a urea bridge to a *m*- or *p*-benzenesulfonyl fluoride and a 2-OMe or 3-Me group on the inside phenyl should give the best transport. Such a molecule was **16** which was the best in Table I with $ED_{50} = 0.05\ \mu M$ and $ED_{50}/I_{50} = 2$; however, these values were still two to three magnitudes higher than **32** with $ED_{50} = 2 \times 10^{-5}\ \mu M$ and $ED_{50}/I_{50} = 0.002$.[13] Furthermore, **16** showed poor specificity.



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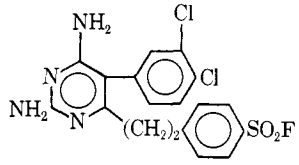
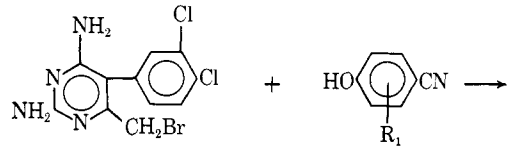


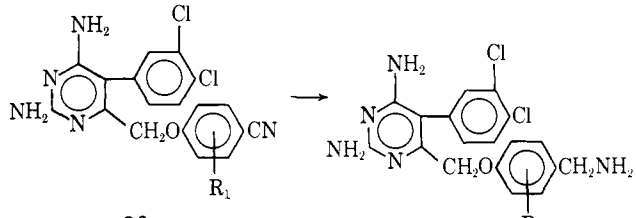
33

Further studies on modification of the bridge at the 6 position to overcome these difficulties are continuing. The specificity and reversible inhibition difficulties have been overcome with **33**[12] which shows rapid inactivation of the L1210 enzyme with no inactivation of the liver enzyme and has an acceptable $I_{50} = 6K_i = 0.04\ \mu M$; **33** still transports insufficiently with an $ED_{50} = 0.6\ \mu M$ and $ED_{50}/I_{50} = 15$.

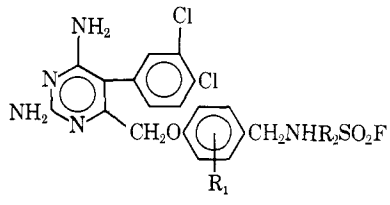
Chemistry.—The compounds in Table I were synthesized by appropriate modification of the route used for **1**[7,9]; these compounds can be generalized by structure **38**. Condensation of **34**[14] with the appropriate cyanophenol (**35**) in DMF in the presence of K_2CO_3 gave **36**.[9] Catalytic hydrogenation of **36** with a PtO_2 catalyst in the presence of acid afforded a salt of **37**[9] that was not purified but converted to **38** with the appropriate acid chloride[7,15] or O-(*p*-nitrophenyl)-*N*-arylurethan.[16,17]



34 **35**



36 **37**



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

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

(11) B. R. Baker and N. M. J. Vermeulen, *J. Med. Chem.*, **12**, 684 (1969), paper CLVIII of this series.

(12) B. R. Baker and N. M. J. Vermeulen, unpublished.

(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.

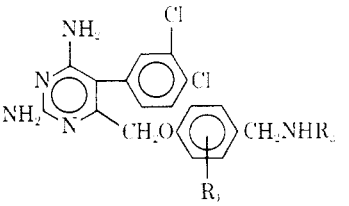
(15) B. R. Baker and R. B. Meyer, Jr., *ibid.*, **12**, 104 (1969), paper CXLII of this series.

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

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

Table I

Inhibition[a] of Dihydrofolic Reductase by



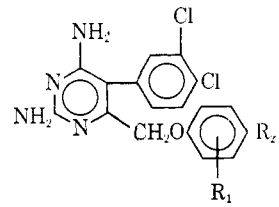
No.	R_1[b]	R_2	Enzyme source	I_{50},[c] μM	Inhib., μM	Time, min	% inactvn[d]	ED_{50},[e] μM	ED_{50}/[f] I_{50}
1[f]	H	$COC_6H_4SO_2F$-*m*	L1210/DF8	0.37	0.70	60	88	4	10
					0.12	60	75		
			Liver	0.29	3.5	60	12		
					0.7	60	0		
4	2-OMe	$COC_6H_4SO_2F$-*m*	L1210/DF8		0.052	60	91	0.6	10
			Liver	0.052	0.16	60	64		
5	2-Cl	$COC_6H_4SO_2F$-*m*	L1210/DF8		0.096	60	87	10	200
			Liver	0.048	0.096	60	33		
6	3-Me	$COC_6H_4SO_2F$-*m*	L1210/DF8		0.18	2, 30	97, 97[g]	4	40
			Liver	0.092	0.28	60	100		
7	2-Cl	COC_6H_3-2-Cl-5-SO_2F	L1210/DF8		0.18	60	85	3	40
			Liver	0.089	0.27	60	65		
8	2-Cl	COC_6H_3-4-Me-3-SO_2F	L1210/DF8		0.24	60	88	6	50
			Liver	0.12	0.36	60	77		
9[f]	H	$COC_6H_4SO_2F$-*p*	L1210/DF8	0.035	0.12	60	96	5	100
			Liver		0.12	60	56		
10	2-OMe	$COC_6H_4SO_2F$-*p*	L1210/DF8		0.090	2, 30	73, 90[g]	5	100
			Liver	0.047	0.14	60	68		
11	2-Cl	$COC_6H_4SO_2F$-*p*	L1210/DF8		0.10	60	79	5	100
			Liver	0.046	0.10	60	56		
12	3-Me	$COC_6H_4SO_2F$-*p*	L1210/DF8		0.32	60	100	0.5	3
			Liver	0.16	0.48	60	98		
13[f]	H	$CONHC_6H_4SO_2F$-*m*	L1210/DF8	0.15	0.30	60	100	3	20
			Liver		0.30	60	88		
14	2-OMe	$CONHC_6H_4SO_2F$-*m*	L1210/DF8		0.22	2, 30	93, 93[g]	0.2	2
			Liver	0.076	0.22	60	83		
15	2-Cl	$CONHC_6H_4SO_2F$-*m*	L1210/DF8		0.13	60	51	0.6	10
			Liver	0.063	0.13	60	88		
16	3-Me	$CONHC_6H_4SO_2F$-*m*	L1210/DF8	0.030	0.060	60	100	0.05	2
			Liver		0.090	60	37		
17	2-Cl	$CONHC_6H_3$-2-Cl-5-SO_2F	L1210/DF8		0.072	60	89	4	100
			Liver	0.036	0.11	60	60		
18	2-Cl	$CONHC_6H_3$-4-Me-3-SO_2F	L1210/DF8		0.30	60	86	2	10
			Liver	0.15	0.45	60	62		
19[f]	H	$CONHC_6H_4SO_2F$-*p*	L1210/0	0.20	0.20	2, 60	54, 76[g]	8	40
			L1210/DF8		0.20	60	85		
			Liver		0.20	60	73		
20	2-OMe	$CONHC_6H_4SO_2F$-*p*	L1210/DF8		0.20	2, 30	77, 77[g]	0.2	2
			Liver	0.10	0.30	60	85		
21	2-Cl	$CONHC_6H_4SO_2F$-*p*	L1210/DF8		0.12	60	83	0.6	10
			Liver	0.062	0.12	60	46		
22	3-Me	$CONHC_6H_4SO_2F$-*p*	L1210/DF8		0.17	2, 30	99, 99[g]	0.5	6
			Liver	0.086	0.26	60	68		
23	2-Cl	$CONHC_6H_3$-3-Me-4-SO_2F	L1210/DF8		0.24	60	73	6	50
			Liver	0.12	0.36	60	78		
24[f]	H	$SO_2C_6H_4SO_2F$-*m*	L1210/DF8	0.060	0.12	60	51	5	80
25	2-OMe	$SO_2C_6H_4SO_2F$-*m*	L1210/DF8		0.078	60	46	6	200
			Liver	0.039	0.12	60	37		
26	2-Cl	$SO_2C_6H_4SO_2F$-*m*	L1210/DF8		0.041	60	32	2	50
			Liver	0.041	0.12	60	20		
27	3-Me	$SO_2C_6H_4SO_2F$-*m*	L1210/DF8		0.12	60	92	4	70
			Liver	0.060	0.18	60	78		
28[f]	H	$SO_2C_6H_4SO_2F$-*p*	L1210/DF8	0.15	0.30	60	85	4	30
			Liver		0.30	60	73		
29	2-OMe	$SO_2C_6H_4SO_2F$-*p*	L1210/DF8		0.17	60	74	4	50
			Liver	0.085	0.26	60	37		
30	2-Cl	$SO_2C_6H_4SO_2F$-*p*	L1210/DF8		0.13	60	48	0.9	10
			Liver	0.13	0.26	60	12–41[h]		

TABLE I (*Continued*)

No.	R_1[b]	R_2	Enzyme source	I_{50},[c] μM	Inhib, μM	Time, min	% inactvn[d]	ED_{50},[e] μM	ED_{50}/I_{50}
31	3-Me	$SO_2C_6H_4SO_2F$-*p*	L1210/DF8		0.22	60	98	4	40
			Liver	0.11	0.33	60	94		

[a] The technical assistance of Diane Shea and Sharon Lafler with these assays is acknowledged. [b] Numbered from phenoxy oxygen. [c] I_{50} = concentration for 50% inhibition when measured with 6 μM dihydrofolate and 0.15 M KCl at pH 7.4 as previously described.[8] [d] Enzyme incubated with inhibitor at 37° in pH 7.4 Tris buffer containing 60 μM TPNH, then the remaining enzyme was assayed as previously described.[8] [e] Concentration for 50% inhibition of L1210 cell culture. We wish to thank Dr. Florence White of the CCNSC and Dr. Philip Himmelfarb of Arthur D. Little, Inc., for these data. [f] Enzyme data from ref 9. [g] From a six-point time study.[8] [h] Difficulty was encountered with the zero-time point.[8]

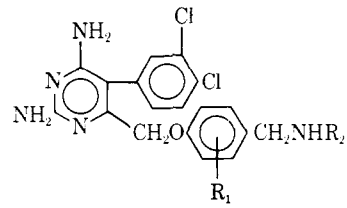
TABLE II
PHYSICAL PROPERTIES OF



No.	R_1[a]	R_2	Method[b]	% yield	Mp, °C	Formula	Analyses
36a	2-Cl[c]	CN	A	83[d]	200–203	$C_{18}H_{12}Cl_3N_5O$	C, H, N
36b	2-OMe	CN	A	60[d]	216–218	$C_{19}H_{15}Cl_2N_5O_2$	C, H, N
36c	3-Me	CN	A	66[e]	199–201	$C_{19}H_{15}Cl_2N_5O$	C, H, N
37a	2-Cl	$CH_2NH_2 \cdot HCl$	B	50[f]	Indef	$C_{18}H_{14}Cl_3N_5O \cdot 2HCl$	
37b	2-OMe	$CH_2NH_2 \cdot HCl$	B	88[f]	Indef	$C_{19}H_{17}Cl_2N_5O_2 \cdot 2HCl$	
37c	3-Me	$CH_2NH_2 \cdot HCl$	B	46[f]	Indef	$C_{19}H_{17}Cl_2N_5O \cdot 2HCl$	

[a] Numbered from CH_2O group. [b] See ref 9 for method A. Method B[9] was modified by conversion of the $EtSO_3H$ salt to the free base, then conversion to the HCl salt with dry HCl in EtOH–THF. [c] For starting phenol see S. S. Berg and J. Newbery, *J. Chem. Soc.*, 642 (1949). [d] Recrystallized from EtOH–THF. [e] Recrystallized from EtOH. [f] Uniform on tlc in EtOH, but contained variable solvation.

TABLE III
PHYSICAL PROPERTIES OF



No.	R_1[a]	R_2	Method[b]	% yield	Mp, °C[c]	Formula[d]
4	2-OMe	$COC_6H_4SO_2F$-*m*	C	19[e]	161	$C_{26}H_{22}Cl_2FN_5O_5 \cdot 0.5H_2SO_4$
5	2-Cl	$COC_6H_4SO_2F$-*m*	C	33[e]	160	$C_{25}H_{19}Cl_3FN_5O_4S \cdot 0.5H_2SO_4$
6	3-Me	$COC_6H_4SO_2F$-*m*	C	47[f]	178	$C_{26}H_{22}Cl_2FN_5O_4S \cdot 0.5H_2SO_4 \cdot H_2O$
7	2-Cl	COC_6H_3-2-Cl-5-SO_2F	C	34[g]	152	$C_{25}H_{18}Cl_4FN_5O_4S \cdot 0.5H_2SO_4$
8	2-Cl	COC_6H_3-4-Me-3-SO_2F	C	57[g]	163	$C_{26}H_{21}Cl_3FN_5O_4S \cdot 0.5H_2SO_4$
10	2-OMe	$COC_6H_4SO_2F$-*p*	C	49[e]	178	$C_{26}H_{22}Cl_2FN_5O_5S \cdot 0.5H_2SO_4 \cdot 0.5H_2O$
11	2-Cl	$COC_6H_4SO_2F$-*p*	C	30[e]	172	$C_{25}H_{19}Cl_3FN_5O_4S \cdot 0.5H_2SO_4$
12	3-Me	$COC_6H_4SO_2F$-*p*	C	49[g]	182	$C_{26}H_{22}Cl_2FN_5O_4S \cdot 0.5H_2SO_4 \cdot H_2O$
14	2-OMe	$CONHC_6H_4SO_2F$-*m*	D	39[e,g]	189	$C_{26}H_{23}Cl_2FN_6O_5S \cdot 0.5H_2SO_4$
15	2-Cl	$CONHC_6H_4SO_2F$-*m*	D	47[e]	186	$C_{25}H_{20}Cl_3FN_6O_4S \cdot 0.5H_2SO_4$
16	3-Me	$CONHC_6H_4SO_2F$-*m*	D	41[h]	169	$C_{26}H_{23}Cl_2FN_6O_4S \cdot 0.5H_2SO_4 \cdot 2H_2O$
17	2-Cl	$CONHC_6H_3$-2-Cl-5-SO_2F	D	47[i]	165	$C_{25}H_{19}Cl_4FN_6O_4S \cdot C_2H_5SO_3H$[j]
18	2-Cl	$CONHC_6H_3$-4-Me-3-SO_2F	D	48[g]	172	$C_{26}H_{22}Cl_3FN_6O_4S \cdot 0.5H_2SO_4$
20	2-OMe	$CONHC_6H_4SO_2F$-*p*	D	58[e]	237	$C_{26}H_{23}Cl_2FN_6O_5S \cdot 0.5H_2SO_4$
21	2-Cl	$CONHC_6H_4SO_2F$-*p*	D	21[e]	220	$C_{25}H_{20}Cl_3FN_6O_4S \cdot 0.5H_2SO_4$
22	3-Me	$CONHC_6H_4SO_2F$-*p*	D	37[h]	181	$C_{26}H_{23}Cl_2FN_6O_4S \cdot 0.5H_2SO_4$
23	2-Cl	$CONHC_6H_3$-3-Me-4-SO_2F	D	40[g]	179	$C_{26}H_{22}Cl_3FN_6O_4S \cdot 0.5H_2SO_4$
25	2-OMe	$SO_2C_6H_4SO_2F$-*m*	E	22[f]	164	$C_{25}H_{22}Cl_2FN_5O_6S_2 \cdot 0.5H_2SO_4$
26	2-Cl	$SO_2C_6H_4SO_2F$-*m*	E	29[i]	167	$C_{24}H_{19}Cl_3FN_5O_5S_2 \cdot 0.5H_2SO_4$
27	3-Me	$SO_2C_6H_4SO_2F$-*m*	E	20[e]	163	$C_{25}H_{22}Cl_2FN_5O_5S_2 \cdot 0.5H_2SO_4$
29	2-OMe	$SO_2C_6H_4SO_2F$-*p*	E	25[e,g]	177	$C_{25}H_{22}Cl_2FN_5O_6S_2 \cdot 0.5H_2SO_4$
30	2-Cl	$SO_2C_6H_4SO_2F$-*p*	E	33[e]	167	$C_{24}H_{19}Cl_3FN_5O_5S_2 \cdot 0.5H_2SO_4 \cdot$ MeOEtOH
31	3-Me	$SO_2C_6H_4SO_2F$-*p*	E	21[e]	165	$C_{25}H_{22}Cl_2FN_5O_5S_2 \cdot 0.5H_2SO_4$

[a] Numbered from phenoxy oxygen. [b] Methods C and E have been previously described.[7] Method D was the same as method E in ref 16. [c] Decomposition gradually occurred over a wide temperature range starting at the temperature indicated. [d] *Anal.* C, H, F. [e] Recrystallized from MeOEtOH–H_2O. [f] Recrystallized from EtOH–THF. [g] Recrystallized from HOAc–H_2O. [h] Recrystallized from HOAc. [i] Recrystallized from *i*-PrOH. [j] C: calcd, 42.1; found, 43.1.

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.

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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

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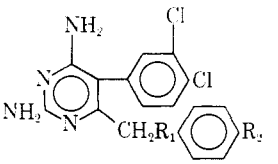
The title compound (**1**) is an active-site-directed irreversible inhibitor of the dihydrofolic reductase from L1210 mouse leukemia that also showed specificity by its failure to inactivate this enzyme from three normal mouse tissues. However, **1** still had two shortcomings; its $K_i = 0.06\ \mu M$ 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).

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

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