Irreversible Enzyme Inhibitors. CXXXVII.[1,2] Active-Site-Directed Irreversible Inhibitors of Dihydrofolic Reductase Derived from 6-(*p*-Aminomethylphenoxymethyl)-2,4-diamino-5-(3,4-dichlorophenyl)pyrimidine Bearing a Terminal Sulfonyl Fluoride

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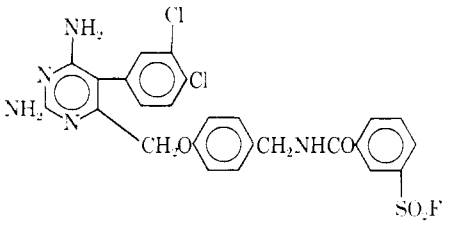
The *m*-fluorosulfonylbenzoyl derivative (**1**) of the title compound was previously shown to be an active-site-directed irreversible inhibitor of the dihydrofolic reductase from the L1210/FR8, L1210/DF8, and L1210/0 strains of mouse leukemia at a concentration of 10^{-7} *M*; good specificity was seen since **1** at 10^{-6} *M* failed to inactivate the enzyme from mouse liver. Since **1** showed good specificity, but probably required too high a concentration to be useful *in vivo*, 13 analogs were synthesized where the benzoyl moiety was varied to determine if more potent compounds with a similar specificity could be found. Some of the variants showed more potency such as the *p*-fluorosulfonylbenzoyl (**2**) and the 4-chloro-3-fluorosulfonylbenzoyl (**4**) derivatives, but specificity was lost since the dihydrofolic reductase from mouse liver could also be inactivated by **2** or **4**.

As a result of a search for active-site-directed irreversible inhibitors[3] of dihydrofolic reductase derived from 6-substituted 2,4-diamino-5-arylpyrimidines,[4-6] the sulfonyl fluoride **1** emerged; **1** could irreversibly inhibit the enzyme from L1210/FR8 mouse leukemia, but not mouse liver. This compound (**1**) was also shown to



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be an irreversible inhibitor of dihydrofolic reductase from the L1210/DF8 and L1210/0 strains.[7] Although **1** showed good tissue specificity, it did not meet the desired criteria arbitrarily set for animal evaluation, namely, (1) the compound should have a reversible $I_{50} \simeq 6K_i$ of <0.1 μM, (2) the compound should give greater than 70% inactivation of the enzyme at a $K_i \simeq I_{50}/6$ concentration, and (3) at $2I_{50}$ the compound should show less than 20% inactivation of the enzyme from liver.[8] Compound **1** failed to meet the first criterion by a factor of about 4 (Table I). Although it was not anticipated to be too difficult to tighten reversible binding by a factor of 4–10 by a variety of suitable simple structural modifications, such minor structural changes might be detrimental to the second or third criteria or both.[7,9,10] Some six approaches were investigated, one of which is described in this paper and one in the paper that follows. In this paper is described the effect on isozyme specificity of modification of the *m*-fluorosulfonylbenzoyl moiety of **1**.

Enzyme Results.—When the sulfonyl fluoride moiety of **1** was shifted to the *para* position to give **2**, the I_{50} was improved five- to tenfold, thus meeting the first criterion. Unfortunately, **2** was now not only less effective than **1** on the L1210 enzyme, but also quite effective on the liver enzyme. Insertion of a methyl group (**3**) *ortho* to the SO_2F moiety of **1** gave little change in I_{50}, but specificity was lost. A similar irreversible inhibition pattern was observed by insertion of an *o*-Cl group (**4**) although the I_{50} was improved four- to eightfold. Insertion of an *o*-*i*-Pr group (**5**) did not change the I_{50} compared to the parent **1**, but the irreversible inhibition pattern was dramatically changed. The enzymes from L1210/0 and L1210/DF8 were still inactivated, but in contrast to **1**, **5** could also inactivate the liver enzyme.

Similar results were seen by insertion of a methyl (**6**) or chlorine (**7**) on **1** where little change in I_{50} occurred, but the selectivity seen with **1** was destroyed; that is, the liver enzyme could now be inactivated by **6** or **7**.

When the carboxamido bridge of **1** was replaced by sulfonamido (**8**), reversible inhibition was enhanced fourfold; however, **8** was a considerably less effective irreversible inhibitor than **1** at an equal amount of enzyme–inhibitor reversible complex. With the sulfonamido bridge bearing a *p*-SO_2F moiety (**9**), the I_{50} was changed little compared to **1**; unfortunately, specificity was lost since **9** could now inactivate the liver enzyme.

Chain lengthening of the carboxamido bridge of **1** and **2** to ureido (**10** and **11**) gave little change in reversible inhibition; however, specificity with **10** and **11** was lost since the liver enzyme could also be inactivated. No additional specificity was seen when a methyl or chloro group was inserted (**12**–**14**) on the benzoyl moiety of **10** or **11**.

Studies on 2,4-diamino-5-(3,4-dichlorophenyl)py-

(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 N. M. J. Vermeulen, *J. Med. Chem.*, **12**, 82 (1969).

(3) 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.

(4) (a) B. R. Baker and J. H. Jordaan, *J. Heterocycl. Chem.*, **4**, 31 (1967), paper LXXXIII of this series; (b) B. R. Baker and J. H. Jordaan, *J. Pharm. Sci.*, **56**, 660 (1967), paper LXXXVIII of this series.

(5) (a) B. R. Baker, P. C. Huang, and A. L. Pogolotti, Jr., *J. Med. Chem.*, **10**, 1134 (1967), paper CVIII of this series; (b) B. R. Baker, P. C. Huang, and R. B. Meyer, Jr., *ibid.*, **11**, 475 (1968), paper CXVI of this series.

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

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

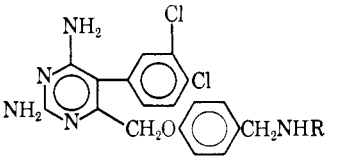
(8) For a more detailed discussion of these criteria, see ref 7.

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

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

TABLE I

INHIBITION[a] OF DIHYDROFOLIC REDUCTASE BY



			Reversible[b]			Irreversible[c]		
No.	R	Enzyme source	I_{50},[d] μM	K_i,[e] μM	Inhib, μM	% EI[f]	Time, min	% inactvn
1	$COC_6H_4SO_2F$-*m*	L1210/FR8[g]	0.70	0.1	0.70	87	2, 60	50, 97[h]
					0.12	50	8, 60	50, 90[h]
		L1210/0[i]	0.24	0.04	1.1	97	60	88[i]
					0.24	87	60	83[i]
					0.04	50	60	23[i]
		L1210/DF8[i]	0.37	0.06	1.4	96	60	100
					0.70	88	60	88
					0.12	66	60	75
		Liver[g]	0.29	0.05	3.5	99	2, 60	12, 12[h]
					0.7	95	60	0[h]
2[i]	$COC_6H_4SO_2F$-*p*	L1210/FR8	0.062	0.01	0.32	97	60	>95
					0.062	87	4, 60	67, 67[h]
		L1210/0	0.055	0.009	0.12	93	60	82
					0.055	87	2, 60	57, 57[h]
		L1210/DF8	0.035	0.006	0.12	95	60	96
					0.060	91	60	65
		Liver	0.018	0.003	0.12	98	60	56
3	COC_6H_3-3-SO_2F-4-CH_3	L1210/FR8	0.35	0.06	0.35	87	4, 60	70, 70[h]
		L1210/0	0.16	0.03	0.70	97	60	77
					0.16	87	2, 60	55, 55[h]
		L1210/DF8			0.16		60	95[i]
					0.030		60	0[i]
		Liver			0.35		60	87[i]
4	COC_6H_3-3-SO_2F-4-Cl	L1210/FR8	0.093	0.02	0.093	87	2, 60	90, 100[h]
		L1210/0	0.060	0.01	0.18	95	60	87
					0.060	87	2, 60	64, 64[h]
		L1210/DF8	0.047	0.008	0.18	97	60	100
					0.047	87	60	71
					0.010	59	60	20
		Liver	0.030	0.005	0.060	89	60	63[i]
5	COC_6H_3-3-SO_2F-4-$CH(CH_3)_2$	L1210/DF8	0.14	0.02	0.28	89	60	85[i]
		L1210/0			0.28		60	85[i]
		Liver			0.28		60	70[i]
6	COC_6H_3-2-CH_3-5-SO_2F	L1210/DF8	0.32	0.05	0.32	87	60	84
		L1210/0			0.64		60	90[i]
		Liver			0.64		60	80[i]
7	COC_6H_3-2-Cl-5-SO_2F	L1210/DF8	0.096	0.02	0.19	89	60	90
		L1210/0			0.19		60	83[i]
		Liver			0.10		60	58[i]
8	$SO_2C_6H_4SO_2F$-*m*	L1210/DF8	0.060	0.01	0.12	89	60	51[i]
		L1210/0			0.12		60	52[i]
9	$SO_2C_6H_4SO_2F$-*p*	L1210/DF8	0.15	0.03	0.30	89	60	85[i]
		L1210/0			0.30		60	85[i]
		Liver			0.30		60	73[i]
10	$CONHC_6H_4SO_2F$-*m*	L1210/DF8	0.15	0.03	0.30	89	60	100
		L1210/0			0.30		60	94
		Liver			0.30		60	88[i]
11	$CONHC_6H_4SO_2F$-*p*	L1210/FR8	0.17	0.03	0.17	87	23, 60	50, 79[h]
					0.03	50	60	0[h]
		L1210/0	0.20	0.03	0.20	87	2, 16, 60	54, 70, 76[h]
		L1210/DF8			0.20		60	85
		Liver			0.20		60	73[i]
12	$CONHC_6H_3$-3-SO_2F-4-CH_3	L1210/DF8	0.10	0.02	0.20		60	81[i]
		L1210/0			0.20		60	95[i]
		Liver			0.20		60	74[i]
13	$CONHC_6H_3$-2-Cl-5-SO_2F	L1210/DF8	0.43	0.07	0.43	87	60	100
		L1210/0			0.86		60	76
		Liver			0.86		60	93[i]

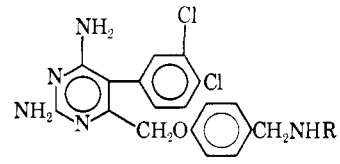
Table I (*Continued*)

			Reversible[b]			Irreversible[c]		
No.	R	Enzyme source	I_{50},[d] μM	K_i,[e] μM	Inhib, μM	% EI[f]	Time, min	% inactvn
14	$CONHC_6H_3$-3-CH_3-4-SO_2F	L1210/DFS	0.43	0.07	0.43		60	71
		L1210/0			0.86		60	80
		Liver			0.86			96[g]

[a] The technical assistance of Sharon Lafler, Diane Shea, and Carolyn Wade with these assays is acknowledged. [b] Assayed with 6 μM dihydrofolate and 30 μM TPNH in pH 7.4 Tris buffer containing 0.15 M KCl as previously described.[7] [c] Incubated at 37° in pH 7.4 Tris buffer in the presence of 60 μM TPNH as previously described.[7] [d] I_{50} = concentration for 50% inhibition. [e] Estimated from $K_i = K_m[I_{50}]/[S]$ which is valid since $[S] = 6K_m = 6\ \mu M$ dihydrofolate; see ref 3, p 202. [f] Calculated from $[EI] = [E_t]/(1 + K_i/[I])$ where [EI] is the amount of the total enzyme (E_t) reversibly complexed; see ref 3, Chapter 8. [g] Data from ref 6. [h] From time study plot.[9] [i] Zero point determined by adding inhibitor to cuvette.[7] [j] Data from ref 7.

Table II

Physical Properties of



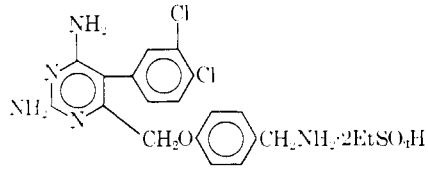
No.	R	Method[a]	% yield	Mp, °C dec[g]	Formula[h]
2	$COC_6H_4SO_2F$-*p*	A	16[b]	170	$C_{25}H_{20}Cl_2FN_5O_4S \cdot C_7H_8SO_3H \cdot 0.5H_2O$[c]
3	COC_6H_3-4-Me-3-SO_2F	A	26[d]	153	$C_{26}H_{22}Cl_2FN_5O_4S \cdot 0.5H_2SO_4$
4	COC_6H_3-4-Cl-3-SO_2F	A	23[b]	154	$C_{25}H_{19}Cl_3FN_5O_4S \cdot H_2SO_4$
5	COC_6H_3-4-Ip-3-SO_2F	A	20[d]	162	$C_{28}H_{26}Cl_2FN_5O_4S \cdot 0.5H_2SO_4$
6	COC_6H_3-2-Me-5-SO_2F	A	42[d]	153	$C_{26}H_{22}Cl_2FN_5O_4S \cdot 0.5H_2SO_4$
7	COC_6H_3-2-Cl-5-SO_2F	A	23[d]	148	$C_{25}H_{19}Cl_3FN_5O_4S \cdot 0.5H_2SO_4 \cdot H_2O$
8	$SO_2C_6H_4SO_2F$-*m*	A	11[d]	150	$C_{24}H_{20}Cl_2FN_5O_5S_2 \cdot 0.5H_2SO_4$
9	$SO_2C_6H_4SO_2F$-*p*	A	18[d]	181	$C_{24}H_{20}Cl_2FN_5O_5S_2 \cdot 0.5H_2SO_4$
10	$CONHC_6H_4SO_2F$-*m*	B	48[d]	185	$C_{25}H_{21}Cl_2FN_6O_4S \cdot 0.5H_2SO_4$
11	$CONHC_6H_4SO_2F$-*p*	B	12[d]	198	$C_{25}H_{21}Cl_2FN_6O_4S \cdot 0.5H_2SO_4$
12	$CONHC_6H_3$-4-Me-3-SO_2F	B[f]	32[d]	196	$C_{26}H_{23}Cl_2FN_6O_4S \cdot 0.5H_2SO_4$
13	$CONHC_6H_3$-2-Cl-5-SO_2F	B	40[d]	185	$C_{25}H_{20}Cl_3FN_6O_4S \cdot 0.5H_2SO_4$
14	$CONHC_6H_3$-3-Me-4-SO_2F	B	51[e]	200	$C_{26}H_{23}Cl_2FN_6O_4S \cdot 0.5H_2SO_4 \cdot 0.5H_2O$

[a] Method A was the same as method C previously described.[9] Method B was the same as method E previously described,[12] except 2 equiv of Et_3N was added to neutralize the $2EtSO_3H$ salt of **15**. [b] Recrystallized from EtOH–petroleum ether (bp 60–110°). [c] *p*-Toluenesulfonate. [d] Recrystallized from MeOEtOH–H_2O. [e] Recrystallized from MeOEtOH. [f] Intermediate *p*-nitrophenyl carbamate prepared by W. F. Wood in this laboratory, unpublished. [g] Melting gradually occurred over a wide range starting at the temperature indicated. [h] All compounds were analyzed for C, H, F.

rimidines bridged to a sulfonyl fluoride at the 6 position, in order to find compounds showing at low concentration a specific inactivation of L1210 dihydrofolic reductase, are continuing. One of the approaches is described in paper CXXXVIII.[11]

Experimental Section

The candidate irreversible inhibitors in Table I were synthesized from the key intermediate **15**; the preparation of dihydrochloride of **15** was previously described,[6] but it has now been found that reduction of the cyano precursor[6] proceeded more smoothly in the presence of $EtSO_3H$. Reaction of **15** with the appropriate benzoyl chloride or sulfonyl chloride in DMF with Et_3N as an acid acceptor gave **2–9**. The irreversible inhibitors with a urea bridge (**10–14**) were synthesized from **15** by reaction of the appropriately substituted O-(*p*-nitrophenyl)-N-phenylurethan[12] in DMF in the presence of 2 equiv of Et_3N.



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All analytical samples had ir and uv spectra in agreement with their assigned structures. Each moved as a single spot on tlc on Brinkmann silica gel GF and gave combustion values for C, H, and F within 0.4% of theoretical. Melting points were taken in capillary tubes on a Mel-Temp block and are uncorrected. The physical properties for **2–14** are listed in Table II.

(11) B. R. Baker and N. M. J. Vermeulen, *J. Med. Chem.*, **12**, 80 (1969), paper CXXXVIII of this series.

(12) B. R. Baker and N. M. J. Vermeulen, *ibid.*, **12**, 71 (1969), paper CXXXIV of this series.