Irreversible Enzyme Inhibitors. CLXXVIII.[1,2] Active-Site-Directed Irreversible Inhibitors of Dihydrofolate Reductase Derived from 1-(4-Benzyloxy-3-chlorophenyl)-4,6-diamino-1,2-dihydro-2,2-dimethyl-*s*-triazine with a Terminal Sulfonyl Fluoride

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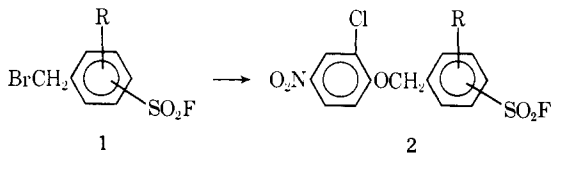
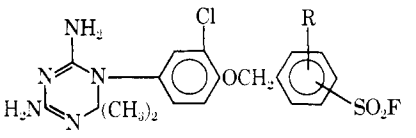
Thirteen candidate active-site-directed irreversible inhibitors of dihydrofolate reductase derived from the title compound were synthesized; these had an *o*-, *m*-, or *p*-SO_2F group on the benzyloxy moiety, and 10 of the inhibitors also contained Cl on the benzyloxy moiety. Of these 13 compounds, 9 were good to excellent irreversible inhibitors of dihydrofolate reductase from either Walker 256 rat tumor or L1210 mouse leukemia or both. Of the 9 irreversible inhibitors, 8 showed 0–40% inactivation of the crude dihydrofolate reductase from mouse or rat liver; this tissue specificity was due to rapid hydrolysis of the SO_2F group to SO_3H by a "sulfonyl fluoridase" in the crude liver enzyme preparation. The 2 best compounds, considering also their ability to inhibit L1210 cell culture, were the 3-Cl-4-SO_2F (**6**) and 3-SO_2F (**8**) derivatives; these showed 50% inhibition of L1210 cell culture at 2×10^{-10} *M* and 10×10^{-10} *M*, respectively.

The availability of a series of α-bromomethylbenzenesulfonyl fluorides (**1**; R = H, Cl) from another study in this laboratory[3] prompted an investigation of the synthesis of a series of 1-benzyloxyphenyl-*s*-triazines (**3**) for evaluation as irreversible inhibitors of dihydrofolate reductase. The key synthetic step would be alkylation of 2-chloro-4-nitrophenol with **1** to give **2**, without destruction of the SO_2F by the basic conditions of the displacement reaction; this reaction was successful as anticipated by the fact that *p*-hydroxybenzenesulfonyl fluoride can be alkylated under alkaline conditions.[4,5]



1 2



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The 13 candidate irreversible inhibitors of structure (**3**; R = H, Cl) were evaluated as irreversible inhibitors of the dihydrofolate reductase from L1210/DF8 mouse leukemia and Walker 256 rat tumor.[6] A number of the compounds showing good irreversible inhibition of the tumor enzymes were also investigated for tissue specificity of inhibition of the dihydrofolate reductase from normal rat and mouse tissues. Where good irreversible inhibition of a tumor enzyme and poor inhibition of a liver enzyme was observed, the irreversible inhibition of the affinity column purified enzyme[7] was investigated to see if the tissue specificity was due to hydrolysis of the SO_2F moiety by a "sulfonyl fluoridase" in liver[8] or was due to a difference in enzyme structure from the two tissues.

The ability of the compounds to penetrate a mammalian cell wall was approximated by the concentration necessary to inhibit L1210 cell culture.[6,9,10] The results of these *in vitro* assays are the subject of this paper.

Biological Results.—Of the 13 candidate irreversible inhibitors in Table I, six (**4–6**, **10**, **11**, and **15**) were good to excellent irreversible inhibitors of the dihydrofolate reductase from L1210/DF8 mouse leukemia. These 6 irreversible inhibitors were then measured as irreversible inhibitors of the crude dihydrofolate reductase in a 45–90% $(NH_4)_2SO_4$ fraction of mouse liver; all of these compounds showed 0–20% inactivation of the crude mouse liver enzyme, except **10** which showed 37% inactivation. When the inactivation of the affinity column purified dihydrofolate reductase[7] from mouse liver by the 6 compounds was measured, 63–98% irreversible inhibition was observed. Thus the tissue specificity observed with the six compounds was due to rapid hydrolysis of the SO_2F group to SO_3H by the sulfonyl fluoridase present in mouse liver,[7,8] and was not due to differences in the structure of dihydrofolate reductase from L1210 and mouse liver.

These 13 candidate irreversible inhibitors (Table I) were then investigated as irreversible inhibitors of the dihydrofolate reductase from a different tumor source, namely Walker 256 rat tumor; nine (**4–6**, **8**, **10–12**, **14**, and **15**) were good to excellent irreversible inhibitors. Six (**5**, **6**, **11**, **12**, **14**, and **15**) of these nine irreversible inhibitors showed less than 40% inactivation of the crude enzyme from rat liver. When the 6 compounds showing this tissue specificity in the rat were investigated as irreversible inhibitors of the affinity column purified dihydrofolate reductase[7] from rat liver, the inactivation was increased to 54–92%; thus the main reason for tis-

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

(2) (a) For the previous paper of this series, see B. R. Baker and N. M. J. Vermeulen, *J. Med. Chem.*, **13**, 1154 (1970). (b) To whom correspondence should be addressed.

(3) B. R. Baker and J. A. Hurlbut, *J. Med. Chem.*, **12**, 902 (1969), paper CLXI.

(4) B. R. Baker and N. M. J. Vermeulen, *ibid.*, **13**, 82 (1970), paper CLXVI.

(5) B. R. Baker and W. T. Ashton, *ibid.*, **13**, 1149 (1970), paper CLXXVI.

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

(7) B. R. Baker and N. M. J. Vermeulen, *ibid.*, **13**, 1143 (1970), paper CLXXV.

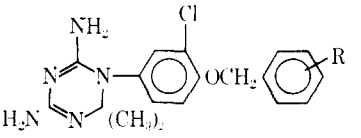
(8) A. J. Ryan, N. M. J. Vermeulen, and B. R. Baker, *ibid.*, **13**, 1140 (1970), paper CLXXIV.

(9) B. R. Baker and R. B. Meyer, Jr., *ibid.*, **12**, 668 (1969), paper CLIV.

(10) B. R. Baker, E. E. Janson, and N. M. J. Vermeulen, *ibid.*, **12**, 898 (1969), paper CLX.

TABLE I

INHIBITION[a] OF DIHYDROFOLATE REDUCTASE BY



No.	R[b]	Enzyme source[c]	I_{50},[d] μM	Inhibitor, μM	Time, min	% inactvn[e]	ED_{50},[f] μM	ED_{50}/I_{50}
4	4-SO_2F	L1210/DF8 (A)	0.026	0.052	60	91	0.08	3
		Mouse liver (A)		0.078	60	8		
		Mouse liver (C)		0.078	60	80		
		W256 (A)		0.052	60	98		
		Rat liver (A)		0.078	60	92		
		Rat intestine (A)		0.078	20	79		
5	2-Cl-4-SO_2F	L1210/DF8 (A)	0.036	0.072	60	75	0.13	4
		Mouse liver (A)		0.11	60	11		
		Mouse liver (C)		0.11	60	76		
		W256 (A)		0.072	60	93		
		Rat liver (A)		0.11	60	27		
		Rat liver (C)		0.11	60	84		
		Rat spleen (A)		0.11	20	83		
		Rat intestine (A)		0.11	20	64		
6	3-Cl-4-SO_2F	L1210/DF8 (A)	0.037	0.074	60	97	0.0002	0.005
		Mouse liver (A)		0.11	60	20		
		Mouse liver (C)		0.11	60	83		
		W256 (A)		0.074	60	96		
		Rat liver (A)		0.11	60	40		
		Rat liver (C)		0.11	60	77		
		Rat spleen (A)		0.11	20	64		
		Rat intestine (A)		0.11	20	67		
7	α-CH_3-4-SO_2F	L1210/DF8 (A)	0.042	0.084	60	16	43	1000
		W256 (A)		0.084	60	33		
8	3-SO_2F	L1210/DF8 (A)	0.019	0.050	60	59	0.001	0.05
		Mouse liver (A)		0.050	60	0		
		Mouse liver (C)		0.050	60	58		
		W256 (A)		0.050	60	78		
		Rat liver (A)		0.050	60	50		
		Rat intestine (A)		0.050	20	60		
9	4-Cl-3-SO_2F	L1210/DF8 (A)	0.010	0.050	60	19	0.002	0.2
		Mouse liver (A)		0.050	60	18		
		W256 (A)		0.050	60	45		
		Rat liver (A)		0.050	60	19		
		Rat liver (C)		0.050	60	84		
10	6-Cl-3-SO_2F	L1210/DF8 (A)	0.042	0.084	60	87	0.2	5
		Mouse liver (A)		0.084	60	37		
		Mouse liver (C)		0.084	60	95		
		W256 (A)		0.084	60	95		
		Rat liver (A)		0.084	60	44		
		Rat intestine (A)		0.084	20	50		
11	2-Cl-3-SO_2F	L1210/DF8 (A)	0.032	0.064	60	79	0.4	10
		Mouse liver (A)		0.064	60	0		
		Mouse liver (C)		0.064	60	63		
		W256 (A)		0.064	60	82		
		Rat liver (A)		0.064	60	29		
		Rat liver (C)		0.064	60	64		
		Rat intestine (A)		0.064	20	60		
12	2-SO_2F	L1210/DF8 (A)	0.037	0.074	60	36	23	600
		Mouse liver (A)		0.11	60	0		
		W256 (A)		0.074	60	86		
		Rat liver (A)		0.11	60	31		
		Rat liver (C)		0.11	60	54		
13	3-Cl-2-SO_2F	L1210/DF8 (A)	0.038	0.11	60	19	16	400
		Mouse liver (A)		0.16	60	0		
		W256 (A)		0.11	60	8		
		Rat liver (A)		0.16	60	23		

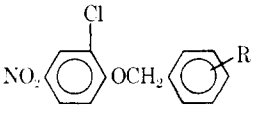
TABLE I (*Continued*)

No.	R[b]	Enzyme source[c]	I_{50},[d] μM	Inhibn, μM	Time, min	% inactvn[e]	ED_{50},[f] μM	ED_{50}/I_{50}
14	4-Cl-2-SO_2F	L1210/DF8 (A)	0.030	0.060	60	60	12	400
		Mouse liver (A)		0.090	60	0		
		Mouse liver (C)		0.090	60	82		
		W256 (A)		0.060	60	78		
		Rat liver (A)		0.090	60	0		
		Rat liver (C)		0.090	60	85		
15	5-Cl-2-SO_2F	L1210/DF8 (A)	0.054	0.11	60	73	11	200
		Mouse liver (A)		0.16	60	16		
		Mouse liver (C)		0.16	60	98		
		W256 (A)		0.11	60	80		
		Rat liver (A)		0.16	60	15		
		Rat liver (C)		0.16	60	92		
16	6-Cl-2-SO_2F	L1210/DF8 (A)	0.019	0.050	60	30	12	600
		Mouse liver (A)		0.057	60	0		
		W256 (A)		0.050	60	38		
		Rat liver (A)		0.057	60	5		

[a] The technical assistance of Diane Shea, Janet Wood, and Julie Lesenian with these assays is acknowledged. [b] Numbered from CH_2 junction. [c] W256 = Walker 256 rat tumor; L1210/DF8 = mouse leukemia resistant to methotrexate; A = 45–90% $(NH_4)_2SO_4$ fraction;[6] C = enzyme purified by affinity column.[7] [d] Concn for 50% reversible inhibn when assayed with 6 μM dihydrofolate and 0.15 M KCl in pH 7.4 Tris buffer as previously described.[6] [e] Incubated with enzyme in pH 7.4 Tris buffer containing 60 μM TPNH, then the remaining enzyme assayed as previously described;[6] 60-min incubations were run at 37° and 20-min incubations at 24°. [f] Concn for 50% inhibn of L1210 cell culture.

TABLE II

PHYSICAL CONSTANTS OF



No.	R	Yield,[a,b] %	Mp, °C	Formula[c]
23	4-SO_2F	55[d]	173	$C_{13}H_9ClFNO_5S$
24	2-Cl-4-SO_2F	80[d]	202–204	$C_{13}H_8Cl_2FNO_5S$
25	3-Cl-4-SO_2F	28[d,e]	199–202	$C_{13}H_8Cl_2FNO_5S$
26	α-CH_3-4-SO_2F	78[f]	Oil	
27	3-SO_2F	27[d]	142–144	$C_{13}H_9ClFNO_5S$
28	4-Cl-3-SO_2F	33[g]	189–191	$C_{13}H_8Cl_2FNO_5S$
29	6-Cl-3-SO_2F	40[d,e]	183–184	$C_{13}H_8Cl_2FNO_5S$
30	2-Cl-3-SO_2F	40[d,e]	201–202	$C_{13}H_8Cl_2FNO_5S$
31	2-SO_2F	63[d]	182–184	$C_{13}H_9ClFNO_5S$
32	3-Cl-2-SO_2F	61[d]	196–197	$C_{13}H_8Cl_2FNO_5S$
33	4-Cl-2-SO_2F	25[d,e,h]	169–171	$C_{13}H_8Cl_2FNO_5S$
34	5-Cl-2-SO_2F	23[i]	190–192	$C_{13}H_8Cl_2FNO_5S$
35	6-Cl-2-SO_2F	31[d]	149–150	$C_{13}H_8Cl_2FNO_5S$

[a] Yield of analytically pure material; yield from α-bromo intermediate, except where indicated. [b] All compds prepared by method A. [c] Anal. C, H, N. [d] Recrystd from MeOEtOH–H_2O. [e] Overall yield for bromination and alkylation. [f] Yield of crude product. [g] Recrystd from *i*-PrOH–H_2O. [h] Reaction mixture added to pyridine; product pptd with H_2O. [i] Recrystd from MeOEtOH.

sue specificity in the rat was again due to selective detoxification of the SO_2F group by the sulfonyl fluoridase present in liver[7,8] as seen above in the mouse.

When the concentrations (ED_{50}) necessary for inhibition of L1210 cell culture are normalized as ED_{50}/I_{50}, the resultant numbers allow a comparison of efficiency of transport plus irreversible inhibition of the target enzyme;[6,9,10] an ED_{50}/I_{50} of less than 0.1 is considered a highly effective compound. Only **6** and **8** showed values in the desired range, the ED_{50}/I_{50} being 0.005 and 0.05, respectively; these two compounds are worthy of *in vivo* evaluation, particularly **6**.

It is noteworthy that when the SO_2F group was ortho to the OCH_2 bridge (**12–16**), transport was greatly impaired.

Chemistry.—Most of the α-bromomethylbenzenesulfonyl fluoride intermediates (**1**) used in the synthesis of the candidate irreversible inhibitors (**3**; R = H, Cl) had previously been prepared in this laboratory.[3,11,12] The synthesis of two new chloro-substituted α-bromomethylbenzenesulfonyl fluorides (**1a,b**) and the general sequence for the conversion of **1** to **3** are diagrammed in Scheme I. (Substituents are numbered from CH_3.)

2-Chloro-5-nitrotoluene (**18a**),[13] obtained from **17** *via* the Sandmeyer reaction, was reduced to the corresponding amine (**19a**).[13] Diazotization of **19a** followed by treatment with SO_2 in HOAc in the presence of $CuCl_2$[14] yielded the sulfonyl chloride (**20a**).[15] By reaction with KF according to the method of deCat and vanPoucke,[16] **20a** was converted into the sulfonyl fluoride (**21a**). Bromination with NBS afforded **1a**.

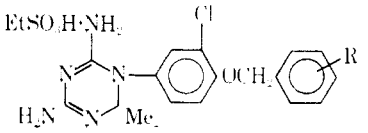
Similarly, the commercially available 2-chloro-3-nitrotoluene (**18b**) was reduced to **19b**,[17] which was converted into the sulfonyl fluoride (**21b**) without isolation of the intermediate sulfonyl chloride (**20b**). The brominated derivative **1b** was prepared in the same manner as **1a**, and both of these compounds were used without isolation.

Alkylation of 2-chloro-4-nitrophenol with **1** led to formation of the ether products **2**. When the NO_2 intermediates **2** were hydrogenated in the presence of Raney Ni catalyst in THF or EtOH, the amines **22** were obtained with little or no hydrogenolysis of either the benzyl ether group or the aromatic chloro substituents. The dihydro-*s*-triazines (**3**) were obtained by condensa-

(11) B. R. Baker and G. J. Lourens, *J. Med. Chem.*, **11**, 666 (1968), paper CXXVII.
(12) B. R. Baker and J. A. Hurlbut, *ibid.*, **12**, 221 (1969), paper CL.
(13) H. Goldschmidt and M. Hönig, *Chem. Ber.*, **20**, 199 (1887).
(14) H. Meerwein, G. Dittmar, R. Göllner, K. Hafner, F. Mensch, and O. Steinfort, *ibid.*, **90**, 841 (1957).
(15) W. P. Wynne, *J. Chem. Soc.*, **61**, 1036 (1892).
(16) A. H. deCat and R. K. vanPoucke, *J. Org. Chem.*, **28**, 3426 (1963).
(17) W. P. Wynne and A. Greeves, *Proc. Chem. Soc.*, 151 (1895).

TABLE III

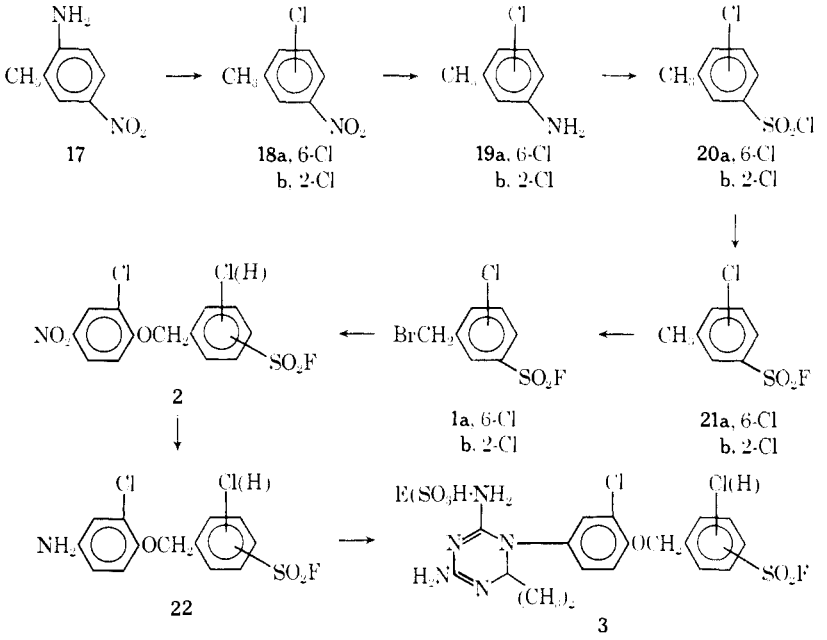
PHYSICAL CONSTANTS OF



No.	R	Yield,[a,b] %	Mp, °C dec	Formula[c]
4	4-SO_2F	51[d]	247–248	$C_{20}H_{25}ClFN_5O_6S_2$
5	2-Cl-4-SO_2F	40[d]	221–222	$C_{20}H_{24}Cl_2FN_5O_6S_2$
6	3-Cl-4-SO_2F	35[d]	230–232	$C_{20}H_{24}Cl_2FN_5O_6S_2$
7	α-CH_3-4-SO_2F	11[d]	211–213	$C_{21}H_{27}ClFN_5O_6S_2$
8	3-SO_2F	29[d]	218–219	$C_{20}H_{25}ClFN_5O_6S_2$
9	4-Cl-3-SO_2F	39[d]	232–233	$C_{20}H_{24}Cl_2FN_5O_6S_2$
10	6-Cl-3-SO_2F	56[d]	217–218	$C_{20}H_{24}Cl_2FN_5O_6S_2$
11	2-Cl-3-SO_2F	48[d]	225–226	$C_{20}H_{24}Cl_2FN_5O_6S_2$
12	2-SO_2F	48[d]	223–224	$C_{20}H_{25}ClFN_5O_6S_2$
13	5-Cl-2-SO_2F	40[d]	223	$C_{20}H_{24}Cl_2FN_5O_6S_2$
14	4-Cl-2-SO_2F	25[d]	211–212	$C_{20}H_{24}Cl_2FN_5O_6S_2$
15	5-Cl-2-SO_2F	15[d]	212–214	$C_{20}H_{24}Cl_2FN_5O_6S_2 \cdot 0.5C_3H_8O$
16	6-Cl-2-SO_2F	21[e]	221–222	$C_{20}H_{24}Cl_2FN_5O_6S_2$

[a] Yield of analytically pure material. [b] All compds prepared by method B. [c] Anal., C, H, F. [d] Recrystd from *i*-PrOH–H_2O. [e] Recrystd from *i*-PrOH.

SCHEME I



tion of the crude amines **22** with cyanoguanidine and acetone[18] in the presence of $EtSO_3H$.[19]

Experimental Section

Melting points were taken in capillary tubes on a Mel-Temp block and are uncorrected. All analytical samples gave combustion analyses within 0.3% of theory and had ir and uv spectra in agreement with their assigned structures. Analytical intermediates and dihydrotriazines were checked for purity on tlc with Brinkman silica gel GF or polyamide MN, respectively.

4-Chloro-3-methylbenzenesulfonyl Chloride (20a).—A stirred mixture of 15.5 g (110 mmoles) of 4-chloro-3-methylaniline (**19a**),[13] 45 ml of concd HCl, and 60 ml of H_2O was heated to boiling, then cooled to about 0°. To the suspension of the amine·HCl was added dropwise with vigorous stirring a soln of 9.3 g (135 mmoles) of $NaNO_2$ in 20 ml of H_2O while maintaining the temperature below 10°. The filtered soln of the diazonium salt was added slowly with stirring to a cold mixture of 4.5 g of $CuCl_2 \cdot 2H_2O$, 5 ml of H_2O, and 88 ml of 32% (v/v) SO_2 in HOAc. After being stirred at room temp for a few minutes, the mixture was warmed to 45°. When evoln of N_2 had ceased, 500 ml of ice–H_2O was added, causing the product to ppt. The yellow–orange solid was collected on a filter and washed with 5% HCl, then with a large vol of H_2O. The crude material was eluted from a silica gel column with petroleum ether (bp 65–110°). After evapn of solvent, the residual solid was washed with a small amount of cold petroleum ether (bp 30–60°) to give 10.6 g (42%) of colorless crystals, mp 65–66° (tlc in petroleum ether), lit.[15] mp 65° for prepn by another route.

4-Chloro-3-methylbenzenesulfonyl Fluoride (21a).—To a refluxing soln of 10.2 g (45 mmoles) of **20a** in 20 ml of dioxane was added slowly with stirring a soln of 5.8 g (100 mmoles) of KF in 5 ml of H_2O, followed by 2 ml of DMF. The mixture was refluxed with stirring for 80 min, then cooled, and added to 250 ml of ice–H_2O, causing the product to precipitate. Recrystallization from petroleum ether (bp 60–110°) gave 4.50 g (48%) of colorless crystals, mp 60–61° (tlc in 1:1 C_6H_6–petroleum ether). *Anal.* ($C_7H_6ClFO_2S$) C, H, F.

2-Chloro-3-methylbenzenesulfonyl Fluoride (21b).—2-Chloro-

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

(19) B. R. Baker and G. J. Lourens, *J. Med. Chem.*, **12**, 95 (1969), paper CXL.

3-methylaniline (**19b**)[17] was diazotized and converted into the intermediate sulfonyl chloride **20b** as described for **20a**, except that **20b** was obtained as an oil, which was extracted with C_6H_6. The crude sulfonyl chloride was converted without isolation into the sulfonyl fluoride **21b** according to the procedure used for **21a**. Two recrystns from MeOH furnished 1.28 g (10% overall) of pale orange crystals, mp 47–48° (tlc in 1:1 C_6H_6–petroleum ether). *Anal.* ($C_7H_6ClFO_2S$) C, H.

2-Chloro-4-nitrophenyl 2-Chloro-5-fluorosulfonylbenzyl Ether (29) (Method A).—A mixture of 1.62 g (8.0 mmoles) of **21a**, 1.42 g (8.0 mmoles) of NBS, 25 mg of benzoyl peroxide, and 8 ml of CCl_4 was refluxed with stirring under irradiation from a uv lamp for 18 hr. The mixture was then cooled in ice and filtered to remove the pptd succinimide, which was washed with CCl_4. The combined filtrate and washings were spin-evapd *in vacuo* to give 2.24 g (97%) of yellow–orange oil (**1a**). To the residue of crude **1a** were added 1.34 g (7.8 mmoles) of 2-chloro-4-nitrophenol, 1.08 g (7.8 mmoles) of K_2CO_3, and 7 ml of DMF. The mixture was stirred at room temp with protection from moisture for 22 hr and then added to 100 ml of 10% Na_2CO_3. The pptd product was collected on a filter and washed with 10% Na_2CO_3, then H_2O, and finally a large vol of petroleum ether. Recrystallization from MeOEtOH–H_2O with aid of charcoal yielded 1.17 g (40% overall) of very light tan needles, mp 183–184° (tlc in C_6H_6). *Anal.* ($C_{13}H_8Cl_2FNO_5S$) C, H, N.

See Table II for additional compounds prepared by this method.

1-[3-Chloro-4-(2-chloro-5-fluorosulfonylbenzyloxy)]phenyl-4,-6-diamino-1,2-dihydro-2,2-dimethyl-*s*-triazine Ethanesulfonate (10) (Method B).—A mixture of 760 mg (2.0 mmoles) of **29**, about 1 g of Raney Ni, and 100 ml of THF was shaken with H_2 at 1–3 atm until tlc showed that the reaction was complete. The filtered soln was spin-evapd *in vacuo*. To the residue of the crude amine were added 225 mg (2.05 mmol) of $EtSO_3H$, 176 mg (2.1 mmol) of cyanoguanidine, and 15 ml of Me_2CO. The mixture was refluxed with stirring for 20 hr, then cooled, and filtered. The crude product was washed with Me_2CO and recrystd from *i*-PrOH–H_2O giving 656 mg (56%) of white crystals, mp 217–218° dec (tlc in 5:1 Me_2CO–*i*-PrOH). *Anal.* ($C_{20}H_{24}Cl_2FN_5O_6S_2$) C, H, F.

See table III for additional compounds prepared by this method.

Irreversible Enzyme Inhibitors. CLXXIX.[1,2] Active-Site-Directed Irreversible Enzyme Inhibitors of Dihydrofolate Reductase from 1-(3-Chlorophenyl)-4,6-diamino-1,2-dihydro-2,2-dimethyl-*s*-triazine with Oxyamide Bridges to a Terminal Sulfonyl Fluoride

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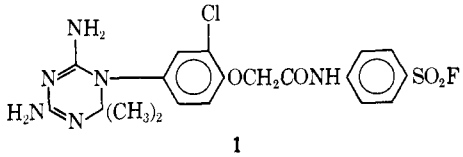
1-(3-Chlorophenyl)-4,6-diamino-1,2-dihydro-2,2-dimethyl-*s*-triazine bearing the 4-$OCH_2CONHC_6H_4SO_2F$-*p* (**1**) group on the Ph ring was previously shown to be a fair to good active-site-directed irreversible inhibitor of dihydrofolate reductase from L1210 mouse leukemia and Walker 256 rat tumor; **1** was also effective against the tumors *in vivo*. Therefore 14 analogs were synthesized to see if specificity and cell wall transport could be increased. The most effective analog for L1210 mouse leukemia had the 4-$OCH_2C_6H_4$-*p*-$CONHC_6H_4SO_2F$-*m* (**8**) side chain; **8** was an excellent irreversible inhibitor of the L1210 enzyme showing little inactivation of the crude enzyme from mouse liver and was 200 times more effective than **1** against L1210 cell culture. The most effective compounds for Walker 256 had the 4-$O(CH_2)_nCONHC_6H_4SO_2F$-*p* side chains where $n = 3$ or 4 (**2**, **5**); these two compounds showed greater inactivation of tumor enzyme than **1** and more specificity, and were transported through the cell wall as effectively as **1**.

Subsequent to the synthesis of **1**,[3] reports on various *in vitro* and *in vivo* biological activities related to antitumor activity have been reported for **1** from this laboratory. It was observed that **1** was a good active-site-directed irreversible enzyme inhibitor[4] of dihydrofolate reductase from L1210 mouse leukemia[3] and Walker 256 rat tumor.[5] Furthermore, **1** showed some tissue specificity in inactivation of dihydrofolate reductase, that is, **1** failed to inactivate appreciably the crude enzyme from mouse liver[3] or rat kidney.[5] Although this selectivity in inactivation was attributed to differences in the structure of the dihydrofolate reductase from the different tissues,[3,5] this difference has recently been shown to be due to the rapid conversion of the SO_2F group into SO_3H by a "sulfonyl fluoridase" present in some normal tissues, but apparently absent in L1210 and Walker 256 tissues.[6] Finally, **1** was highly effective against Walker 256 ascites *in vivo* in the rat,[5] although it was questionable whether inactivation of the tumor dihydrofolate reductase *in vivo* was a major contributing factor;[5] Therefore, we embarked on synthesis and evaluation of analogs of **1**; in one case the benzenesulfonyl fluoride moiety was varied.[7] We now wish to report the synthesis and *in vitro* evaluation of two new series of analogs of **1**, the first where the oxyamide bridge length has been varied, and the second where the ether O in the bridge was replaced by S.



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Biological Results.—The 14 analogs of **1** were evaluated as reversible and irreversible inhibitors[8] of the dihydrofolate reductase from Walker 256 rat tumor and

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(2) For the previous paper of this series see B. R. Baker and W. T. Ashton, *J. Med. Chem.*, **13**, 1161 (1970).

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

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

(5) B. R. Baker, N. M. J. Vermeulen, W. T. Ashton, and A. J. Ryan, *J. Med. Chem.*, **13**, 1130 (1970), paper CLXXIII of this series.

(6) B. R. Baker and N. M. J. Vermeulen, *ibid.*, **13**, 1143 (1970), paper CLXXV of this series.

(7) B. R. Baker and W. T. Ashton, *ibid.*, **12**, 894 (1969), paper CLIX of this series.

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