1-(2-Chloro-5-nitrophenoxy)-2-(4-fluorosulfonylphenoxy)ethane (29a) (Method B).—To 1.68 g (6.0 mmoles) of **25a** were added 1.06 g (6.0 mmoles) of **26**, 0.83 g (6.0 mmoles) of K_2CO_3, and 6 ml of DMF. The suspension was stirred at 95–100° for 22 hr, then processed as described in method A. The residual solid was recrystallized from EtOH; yield 682 mg (30%) of light yellow crystals, mp 126–127° (dc in C_6H_6). *Anal.* ($C_{14}H_{11}ClFNO_6S$) C, H, N.

For additional compounds prepared by this method see Table III.

α-(2-Chloro-4-nitrophenoxy)-α′-(p-fluorosulfonylphenoxy)-p-xylene (27g) (Method C).—A stirred mixture of 1.61 g (4.5 mmoles) of **23g**, 0.79 g (4.5 mmoles) of **26**, and 0.62 g (4.5 mmoles) of K_2CO_3 in 5 ml of DMF was kept at room temperature for 24 hr. Addition to 100 ml of H_2O caused the product to separate as a gummy oil which gradually solidified. The product was collected on a filter and washed with H_2O. Recrystallization once from *i*-PrOH and then twice from $CHCl_3$–MeOH yielded 1.15 g (57%) of white crystals, mp 128–129° (dc in C_6H_6). *Anal.* ($C_{20}H_{15}ClFNO_6S$) C, H, N.

For additional compounds prepared by this method see Table III.

***p*-Chlorophenyl *p*-[4-(2-Chloro-4-nitrophenoxy)butoxy]benzenesulfonate (32) (Method D).**—A mixture of 505 mg (1.25 mmoles) of **27c** and 205 mg (1.36 mmoles) of Na *p*-chlorophenoxide in 2 ml of DMF was stirred at room temperature for 4 hr and then added to 50 ml of H_2O. The initially gummy precipitate solidified rapidly upon standing. The crude product was collected on a filter and washed with H_2O. Recrystallization from MeOEtOH–H_2O with aid of charcoal yielded 452 mg (70%) of nearly white crystals, mp 133–134° (dc in C_6H_6). *Anal.* ($C_{22}H_{19}Cl_2NO_7S$) C, H, N.

***trans*-1,4-Bis(bromomethyl)cyclohexane (22h).**—In a 3-necked flask equipped with mechanical stirrer, condenser with drying tube, and addition funnel was placed 136 g (500 mmoles) of PBr_3. The reaction vessel was cooled in ice as a solution of 72.1 g (500 mmoles) of *trans*-1,4-cyclohexanedimethanol in 40 ml of pyridine and 40 ml of $CHCl_3$ was added dropwise with stirring to the PBr_3 over a period of 2 hr. The mixture was then stirred at 55–60° for an additional 18 hr, then filtered (twice to remove precipitated pyridine salts which were washed with C_6H_6. Additional C_6H_6 was added to the filtrate to bring the total volume to 500 ml. The C_6H_6 solution was washed successively with 750 ml of 5% HCl, 750 ml of 5% NaOH, and 1 l. of H_2O. After being dried ($MgSO_4$), the solution was spin-evaporated *in vacuo* giving a residual oil which solidified upon cooling and standing. Recrystallization from MeOH gave 80.5 g (60%) of colorless crystals, mp 53–55° (dc in petroleum ether), lit.[11] mp 55°.

Acknowledgment.—The technical assistance of Diane Shea, Janet Wood, and Julie Leseman with the enzyme assays is acknowledged.

Irreversible Enzyme Inhibitors. CLXXVII.[1,2] Active-Site-Directed Irreversible Inhibitors of Dihydrofolate Reductase Derived From 4,6-Diamino-1,2-dihydro-2,2-dimethyl-1-(phenylalkylphenyl)-*s*-triazines. II[3]

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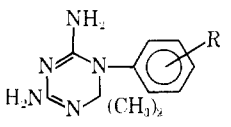
Twenty-eight analogs of the title compound bearing a terminal SO_2F group were investigated as irreversible inhibitors of dihydrofolate reductase from Walker 256 rat tumor, L1210 mouse leukemia, mouse liver, and several normal rat tissues. Only 4 of the irreversible inhibitors showed tissue specificity in the rat by giving good inactivation of the Walker 256 enzyme and poor inactivation of the crude rat liver enzyme, namely, the 1-phenyl-*s*-triazines with the following substituents on Ph: *p*-$(CH_2)_4C_6H_3SO_2F$-*p* (**5**), 3-Cl-4-$(CH_2)_2C_6H_3$-4-Cl-2-SO_2F (**8**), *p*-$(CH_2)_4OC_6H_4SO_2F$-*p* (**13**), and *p*-$(CH_2)_6C_6H_4SO_2F$-*p* (**14**); this tissue-specificity of inactivation was due to the rapid hydrolysis of the SO_2F group to SO_3H by a liver "sulfonyl fluoridase." Similarly the tissue specificity in enzyme inactivation of L1210 *vs.* mouse liver with 6 compounds (**4–7, 11, 14**) was due to the action of the "sulfonyl fluoridase." In contrast 6 other tissue specific irreversible inhibitors of L1210 dihydrofolic reductase owed their specificity to differences in the structure of the enzyme from L1210 and mouse liver; these were *m*-$(CH_2)_4C_6H_4SO_2F$-*m* (**3**), 3-Cl-4-$(CH_2)_2C_6H_3$-4-Cl-2-SO_2F (**8**), 3-Cl-4-$(CH_2)_3C_6H_3$-3-SO_2F (**17**), 3-Cl-4-$(CH_2)_5C_6H_3$-4-Cl-3-SO_2F (**18**), 3-$(CH_2)_4$-C_6H_3-2-Cl-4-SO_2F (**25**), and 3-$(CH_2)_4C_6H_3$-4-Cl-3-SO_2F (**28**).

It was demonstrated in a previous paper in this series[3] that **1** and **2** were excellent active-site-directed irreversible inhibitors[5] of the dihydrofolate reductase from L1210 mouse leukemia and Walker 256 rat tumor. Furthermore, **1** and **2** showed good cell wall transport—presumably by passive diffusion—since the compounds had ED_{50} 2×10^{-10} *M* and 9×10^{-10} *M*, respectively, against L1210 in cell culture. However, **1** and **2** showed little tissue specificity since they could also inactivate the crude enzyme prepared from mouse or rat liver; even so, **1** was highly effective *in vivo* against Walker 256 ascites in the rat.[6] Although **3** was as good a reversible inhibitor of L1210 dihydrofolate reductase as **1**, this simple structural change made **3** far less effective than **1** against L1210 cell culture,[3] indicating that diffusion through the cell wall was sensitive to structural change. More recently we have observed[7] that the tissue specificity for irreversible inhibition of dihydrofolate reductase is due to a "sulfonyl fluoridase" in



1, R = *m*-$(CH_2)_4C_6H_4SO_2F$-*p*
2, R = 3-Cl-4-$(CH_2)_4C_6H_4SO_2F$-*p*
3, R = *m*-$(CH_2)_4C_6H_4SO_2F$-*m*

(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 W. T. Ashton, *J. Med. Chem.*, **13**, 1149 (1970).

(3) For the previous paper on this subject see B. R. Baker, E. E. Janson, and N. M. J. Vermeulen, *ibid.*, **12**, 898 (1969), paper CLX of this series.

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

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

(6) 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.

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

normal tissue that can rapidly convert the SO_2F group to SO_3H; this "fluoridase" is apparently absent in Walker 256 and L1210 cells. We therefore embarked on design and synthesis of analogs of **1** and **2** in a search for more tissue-specific irreversible inhibitors that still would show good cell wall transport;[3,8,9] these inhibitors were measured for inactivation of both crude and purified dihydrofolate reductase in order to determine whether the specificity was due to a structural difference in the enzyme or due to rapid hydrolysis of the SO_2F group by the "sulfonyl fluoridase." The results are the subject of this paper.

Biological Results.—The compounds in Table I can be divided into 4 subclasses, namely, analogs of the 4-phenylbutyl series (**5**, **12–14**), analogs of the 3-chloro-4-phenylbutyl series (**2**, **15–22**), analogs of the 3-chloro-4-phenethyl series (**4**, **6–11**), and analogs of the *m*-phenylbutyl series (**1**, **23–28**).

The parent *p*-phenylbutylphenyl-*s*-triazine with a terminal SO_2F group on the para position (**5**) was previously shown to be a good active-site-directed irreversible inhibitor of the dihydrofolate reductase from L1210 mouse leukemia[3] and a fair inhibitor of this enzyme from Walker 256 rat tumor;[6] it was also active *in vivo*.[6] Tissue specificity was shown with crude enzyme from mouse liver[3] and several rat tissues;[6] this poor inhibition of the enzyme from normal tissues was shown to be due to rapid hydrolysis of the SO_2F group of **5** to SO_3H.[7] Introduction of 2-Cl (**12**) on the benzenesulfonyl fluoride moiety resulted in loss of tissue specificity, *i.e.*, **12** was an excellent irreversible inhibitor of the crude enzyme from both tumor and liver.

When the bridge was changed from Bu (**5**) to BuO (**13**) in order to increase the conformational flexibility of the terminal phenyl group, irreversible inhibition of the Walker 256 enzyme was not changed; however, the effectiveness of **13** as an irreversible inhibitor on the L1210 enzyme was considerably impaired. Good tissue specificity was seen with **13** between Walker 256 and rat liver or intestine; in the case of the rat liver enzyme the specificity was due to the action of the "fluoridase" as shown by the good inactivation of purified rat liver dihydrofolate reductase. The conformational flexibility of **13** compared with **5** apparently aided transport through the L1210 cell wall since **13** was 200-times as effective as **5** against L1210 cell culture by comparison of ED_{50}/I_{50} ratios.[9] When the bridge of **5** was increased from Bu to Hex (**14**), little change in irreversible inhibition, specificity, or inhibition of L1210 cell culture occurred.

The parent 3-chloro-4-phenylbutylphenyl-*s*-triazine (**2**) showed excellent irreversible inhibition of the dihydrofolate reductase from both L1210 and Walker 256, but tissue specificity was poor to fair. Insertion of a 2-Cl group (**15**) on the benzenesulfonyl moiety of **2** led to decreased selectivity between mouse tissues, but little apparent change in specificity between rat tissues. A 3-Cl group (**16**) was detrimental to irreversible inhibition of the enzyme from the two tumors.

When the SO_2F moiety of **2** was moved to the 3 position, the resultant **17** still showed strong inactivation of the enzyme from Walker 256, rat liver, and L1210, and 35% inactivation of the crude mouse liver enzyme. With purified mouse liver enzyme, the inactivation was about the same, 23%; thus **17** is not attacked by the "sulfonyl fluoridase" and **17** still shows a difference between inactivation of the L1210 and purified mouse liver enzyme—indicating that the enzymes from these two tissues are structurally different. Similar results were observed when a 4-Cl atom (**18**) was introduced on **17**; **17** is considered superior to **18** since **17** is 10-times as effective against L1210 cell culture, indicating better transport of **17**.

When the SO_2F group of **2** was moved to the 2 position, the resultant **19** was a poor irreversible inhibitor of the enzyme from Walker 256 or L1210. Introduction of a 5-Cl (**20**) or 4-Cl (**22**) atom gave compounds with excellent irreversible inhibition of the enzyme from Walker 256 and L1210; unfortunately tissue specificity was poor. In contrast, introduction of a 3-Cl gave a compound (**21**) that was even less effective than **19**.

The parent 3-chloro-4-phenethylphenyl-*s*-triazine (**4**) was an excellent irreversible inhibitor of the enzyme from Walker 256[6] and L1210.[3] There was poor tissue specificity in the rat,[6] but good tissue specificity between L1210 and mouse liver;[3] the latter was due to the action of the "sulfonyl fluoridase" as shown by excellent irreversible inhibition of the purified mouse liver enzyme.[7] In addition, **4** showed *in vivo* cures of Walker 256 ascites.[6] Introduction of a 2-Cl (**6**) or 3-Cl (**7**) did not change the specificity pattern; **7** was about as effective as **4** against L1210 cell culture, but **6** had greatly impaired cell wall transport.

When the SO_2F moiety of **4** was moved to the 2 position and a 4-Cl (**8**) or 5-Cl (**9**) substituent placed on the benzenesulfonyl fluoride moiety, the inactivation of the enzyme from Walker 256 or L1210 was somewhat impaired. However, the 4-Cl-2-SO_2F (**8**) showed an interesting irreversible inhibition pattern. Whereas the parent **4** showed no tissue specificity between Walker 256 and rat liver enzymes, **8** did; that this tissue specificity was due to the action of the liver "sulfonyl fluoridase" was shown by the extensive inactivation of the purified rat liver enzyme by **8**. In contrast, the tissue specificity between L1210 and mouse liver seen with **8** was unchanged when the purified mouse liver enzyme was inactivated by **8**; thus **8** apparently is not attacked by the "sulfonyl fluoridase" and the results indicate a difference in the primary structure of dihydrofolate reductase from L1210 and mouse liver. Unfortunately **8** was 35-fold less effective than **4** against L1210 cell culture.

The 4-Cl-3-SO_2F analog (**11**) of **4** showed tissue specificity between L1210 and mouse liver, the specificity being due to the hydrolysis of **11** by the "sulfonyl fluoridase."

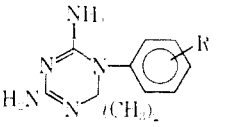
In the fourth subclass, the parent *m*-phenylbutyl-*s*-triazine (**1**) showed good irreversible inhibition of the enzyme from L1210 and Walker 256 with poor tissue specificity (Table I); **1** was effective *in vivo* against Walker 256.[6] Introduction of 4-Cl on the inside Ph (**23**) on **1** practically destroyed the irreversible inhibition. However, introduction of 2-Cl on the benzenesulfonyl fluoride moiety gave **25** which still showed excellent irreversible inhibition of the L1210 and Walker 256 enzymes. Although **25** showed no enzyme specificity between Walker 256 and rat liver, **25** did show good

(8) 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.

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

TABLE I

INHIBITION[a] OF DIHYDROFOLATE REDUCTASE BY



No.	R[b]	Enzyme source[c]	I_{50},[d] μM	Inhib, μM	Time, min	% inactvn[e]	ED_{50},[f] μM	ED_{50}/I_{50}
1	*m*-$(CH_2)_4C_6H_4SO_2F$-*p*	L1210/DF8 (A)[g]	0.0080	0.066	60	86	0.0002	0.03
		Mouse liver (A)[g]		0.066	60	54		
		W256 (A)[g]	0.014	0.050	60	95		
		Rat liver (A)[g]	0.018	0.050	60	95		
		Rat spleen (A)[h]		0.050	20	7		
		Rat kidney (A)[h]		0.050	20	15		
		Rat kidney (C)[i]		0.050	20	94		
		Rat intestine (A)[h]		0.050	20	0		
2	3-Cl-4-$(CH_2)_4C_6H_4SO_2F$-*p*	L1210/DF8 (A)[g]	0.0072	0.050	60	100	0.0009	0.1
		Mouse liver (A)[g]		0.050	60	41		
		W256 (A)[g]		0.050	60	95		
		Rat liver (A)[g]		0.050	60	97		
		Rat kidney (A)		0.050	20	0		
		Rat kidney (C)		0.050	20	64		
		Rat intestine (A)		0.050	20	15		
3	*m*-$(CH_2)_4C_6H_4SO_2F$-*m*	L1210/DF8 (A)[g]	0.0080	0.050	60	94	0.35	40
		Mouse liver (A)[g]		0.050	60	25		
		Mouse liver (C)		0.050	60	41		
		W256 (A)[g]		0.050	60	70		
		Rat liver (A)[g]		0.050	60	80		
4	3-Cl-4-$(CH_2)_2C_6H_4SO_2F$-*p*	L1210/DF8 (A)[g]	0.014	0.070	60	93	0.03	2
		Mouse liver (A)[g]		0.070	60	17		
		Mouse liver (C)[i]		0.070	60	92		
		W256 (A)[h]	0.0080	0.050	60	86		
		Rat liver (A)[h]	0.018	0.050	60	86		
		Rat intestine (A)[h]		0.050	20	9		
5	*p*-$(CH_2)_4C_6H_4SO_2F$-*p*	L1210/DF8 (A)[g]	0.020	0.10	60	75	0.18	9
		Mouse liver (A)[g]		0.10	60	27		
		Mouse liver (C)[i]		0.10	60	76		
		W256 (A)[h]	0.014	0.050	60	62		
		Rat liver (A)[h]	0.022	0.060	60	12		
		Rat liver (C)[i]		0.060	60	88		
		Rat kidney (A)[h]		0.060	20	8		
		Rat kidney (C)[i]		0.060	20	77		
		Rat intestine (A)[h]		0.060	20	5		
6	3-Cl-4-$(CH_2)_2C_6H_3$-2-Cl-4-SO_2F	L1210/DF8 (A)	0.0047	0.05	60	76	0.45	100
		Mouse liver (A)		0.05	60	9		
		Mouse liver (C)		0.05	60	52		
		W256 (A)		0.05	60	62		
		Rat liver (A)		0.05	60	46		
7	3-Cl-4-$(CH_2)_2C_6H_3$-3-Cl-4-SO_2F	L1210/DF8 (A)	0.014	0.05	60	83	0.05	4
		Mouse liver (A)		0.05	60	11		
		Mouse liver (C)		0.05	60	62		
		W256 (A)		0.05	60	88		
		Rat liver (A)		0.05	60	61		
		Rat intestine (A)		0.05	20	43		
8	3-Cl-4-$(CH_2)_2C_6H_3$-4-Cl-2-SO_2F	L1210/DF8 (A)	0.0080	0.05	60	65	0.6	70
		Mouse liver (A)		0.05	60	6		
		Mouse liver (C)		0.05	60	15		
		W256 (A)		0.05	60	81		
		Rat liver (A)		0.05	60	15		
		Rat liver (C)		0.05	60	79		
		Rat intestine (A)		0.05	20	20		
9	3-Cl-4-$(CH_2)_2C_6H_3$-5-Cl-2-SO_2F	L1210/DF8 (A)	0.014	0.05	60	31	0.8	60
		W256 (A)		0.05	60	51		
10	3-Cl-4-$(CH_2)_2C_6H_3$-3-Cl-2-SO_2F	L1210/DF8 (A)	0.0050	0.05	60	0	2.4	500
		W256 (A)		0.05	60	13		

TABLE I (*Continued*)

No.	R[b]	Enzyme source[c]	I_{50},[d] μM	Inhib, μM	Time, min	% inactvn[e]	ED_{50},[f] μM	ED_{50}/I_{50}
11	3-Cl-4-$(CH_2)_2C_6H_3$-4-Cl-3-SO_2F	L1210/DF8 (A)	0.0054	0.05	60	76	0.16	30
		Mouse liver (A)		0.05	60	5		
		Mouse liver (C)		0.05	60	60		
		W256 (A)		0.05	60	94		
		Rat liver (A)		0.05	60	53		
		Rat intestine (A)		0.05	20	38		
12	*p*-$(CH_2)_4C_6H_3$-2-Cl-4-SO_2F	L1210/DF8 (A)	0.011	0.05	60	69	0.4	40
		Mouse liver (A)		0.05	60	98		
		W256 (A)		0.05	60	93		
		Rat liver (A)		0.05	60	99		
		Rat intestine (A)		0.05	20	60		
13	*p*-$(CH_2)_4OC_6H_4SO_2F$-*p*	L1210/DF8 (A)		0.05	20	45	0.0003	0.04
		Mouse liver (A)		0.05	20	0		
		W256 (A)	0.0073	0.05	60	66		
		Rat liver (A)		0.05	60	0		
		Rat liver (C)		0.05	60	79		
		Rat intestine (A)		0.05	20	4		
14	*p*-$(CH_2)_6C_6H_4SO_2F$-*p*	L1210/DF8 (A)	0.075	0.15	60	66	0.1	1
		Mouse liver (A)		0.15	60	10		
		Mouse liver (C)		0.15	60	84		
		W256 (A)		0.15	60	84		
		Rat liver (A)		0.15	60	23		
		Rat liver (C)		0.15	60	83		
15	3-Cl-4-$(CH_2)_4C_6H_3$-2-Cl-SO_2F	L1210/DF8 (A)	0.017	0.050	60	94	0.01	0.6
		Mouse liver (A)		0.050	60	95		
		W256 (A)		0.050	60	97		
		Rat liver (A)		0.050	60	100		
		Rat spleen (A)		0.050	20	5		
		Rat intestine (A)		0.050	20	22		
16	3-Cl-4-$(CH_2)_4C_6H_3$-3-Cl-4-SO_2F	L1210/DF8 (A)	0.020	0.050	60	35	0.007	0.4
		W256 (A)		0.050	60	31		
17	3-Cl-4-$(CH_2)_4C_6H_4$-3-SO_2F	L1210/DF8 (A)	0.0094	0.050	60	100	0.02	2
		Mouse liver (A)		0.050	60	35		
		Mouse liver (C)		0.050	60	23		
		W256 (A)		0.050	60	96		
		Rat liver (A)		0.050	60	97		
		Rat spleen (A)		0.050	20	25		
		Rat intestine (A)		0.050	20	0		
18	3-Cl-4-$(CH_2)_4C_6H_3$-4-Cl-3-SO_2F	L1210/DF8 (A)	0.0078	0.050	60	97	0.2	20
		Mouse liver (A)		0.050	60	37		
		Mouse liver (C)		0.050	60	33		
		W256 (A)		0.050	60	94		
		Rat liver (A)		0.050	60	98		
		Rat intestine (A)		0.050	20	46		
19	3-Cl-4-$(CH_2)_4C_6H_4$-2-SO_2F	L1210/DF8 (A)	0.017	0.050	60	46	0.0004	0.02
		W256 (A)		0.050	60	27		
20	3-Cl-4-$(CH_2)_4C_6H_3$-5-Cl-2-SO_2F	L1210/DF8 (A)	0.088	0.18	60	99	0.001	0.01
		Mouse liver (A)		0.18	60	41		
		W256 (A)		0.18	60	100		
		Rat liver (A)		0.18	60	82		
		Rat intestine (A)		0.18	20	85		
21	3-Cl-4-$(CH_2)_4C_6H_3$-3-Cl-2-SO_2F	L1210/DF8 (A)	0.0063	0.05	60	0	0.0003	0.05
		W256 (A)		0.05	60	30		
22	3-Cl-4-$(CH_2)_4C_6H_3$-4-Cl-2-SO_2F	L1210/DF8 (A)	0.015	0.050	60	93		
		Mouse liver (A)		0.050	60	56		
		W256 (A)		0.050	60	100		
		Rat liver (A)		0.050	60	97		
		Rat spleen (A)		0.050	20	7		
		Rat intestine (A)		0.050	20	0		
23	4-Cl-3-$(CH_2)_2C_6H_4SO_2F$-*p*	L1210/DF8 (A)	1.5	3.1	60	13	6	4
		W256 (A)		3.1	60	8		

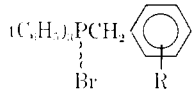
TABLE I (Continued)

No.	R[b]	Enzyme source[c]	I_{50},[d] μM	Inhibitor, μM	Time, min	% reactivated[e]	ED_{50},[f] μM	ED_{50}/I_{50}
24	4-Cl-3-$(CH_2)_4C_6H_4SO_2F$-*p*	L1210/DF8 (A)	0.17	0.34	60	33	>2	>10
		W256 (A)		0.34	60	32		
25	3-$(CH_2)_4C_6H_3$-2-Cl-4-SO_2F	L1210/DF8 (A)	0.010	0.050	60	100	0.07	7
		Mouse liver (A)		0.050	60	31		
		Mouse liver (C)		0.050	60	23		
		W256 (A)		0.050	60	91		
		Rat liver (A)		0.050	60	96		
		Rat intestine (A)		0.050	20	43		
26	3-$(CH_2)_4C_6H_3$-3-Cl-4-SO_2F	L1210/DF8 (A)	0.020	0.050	60	33	0.02	1
		W256 (A)		0.050	60	52		
27	3-$(CH_2)_4C_6H_3$-5-Cl-2-SO_2F	L1210/DF8 (A)	0.011	0.050	60	16	0.02	2
		W256 (A)		0.050	60	35		
28	3-$(CH_2)_4C_6H_3$-4-Cl-3-SO_2F	L1210/DF8 (A)	0.0043	0.050	60	100	0.4	20
		Mouse liver (A)		0.050	60	33		
		Mouse liver (C)		0.050	60	45		
		W256 (A)		0.050	60	91		
		Rat liver (A)		0.050	60	76		
		Rat intestine (A)		0.050	20	30		

[a] The technical assistance of Diane Shea, Janet Wood, and Julie Leseman with these assays is acknowledged. [b] Numbered from triazine junction. [c] W256 = Walker 256 rat tumor; L1210/DF8 = mouse leukemia resistant to methotrexate; A, 45–90% $(NH_4)_2SO_4$ fraction;[8] C, enzyme purified by affinity column.[7] [d] Conen for 50% reversible inhibition when assayed with 6 μM dihydrofolate and 0.15 *M* KCl in pH 7.4 Tris buffer as previously described.[8] [e] Incubated with enzyme in pH 7.4 Tris buffer containing 60 μM TPNH, then the remaining enzyme assayed as previously described;[8] 20-min incubations were run at 24° and 60-min incubations at 37°. [f] Conen for 50% inhibition of L1210 cell culture. [g] Data from ref 3. [h] Data from ref 6. [i] Data from ref 7.

TABLE II

PHYSICAL PROPERTIES OF



No.[a]	R	Yield,[b] %	Mp, °C	Formula[c]
30a	3-Cl-4-SO_2F	90[d]	268–270	$C_{25}H_{20}BrClFO_2PS$
30b	2-Cl-4-SO_2F	70[e]	193–195	$C_{25}H_{20}BrClFO_2PS$[f]
30c	4-Cl-3-SO_2F	57[d]	261–263 dec	$C_{25}H_{20}BrClFO_2PS$
30d	4-Cl-2-SO_2F	45[d]	222–224	$C_{25}H_{20}BrClFO_2PS$
30e	3-Cl-2-SO_2F	52[d]	222–225 dec[g]	$C_{25}H_{20}BrClFO_2PS$[h]
30f	5-Cl-2-SO_2F	52[d]	264–266	$C_{25}H_{20}BrClFO_2PS$
30g	2-SO_2F	58[d]	228–230 dec	$C_{25}H_{21}BrFO_2PS$

[a] Compounds prepared by method A. [b] Yield of analytically pure material. [c] Anal., C, H. [d] Recrystd from EtOH–C_6H_6. [e] Recrystd from EtOH–H_2O. [f] Anal., C, H, F. [g] Mp 120°, resolidifies at 165°, then remelts. [h] Solvated with 0.5 C_6H_6.

specificity between L1210 and mouse liver; this specificity was apparently due to a difference in enzyme structure, since the purified liver enzyme was inactivated by **25** about the same extent as the crude preparation. Note that **25** was transported through the L1210 cell wall much less effectively than **1**, but still sufficient.

Introduction of 3-Cl (**26**) was detrimental to inactivation of the enzyme from L1210 and Walker 256. Similarly, when the SO_2F group was shifted to the ortho position, the resultant **27** was a very poor irreversible inhibitor. When the *p*-SO_2F group of **1** was moved to the meta position (**3**),[3] inactivation specificity between L1210 and mouse liver was seen that was apparently due to the difference in structure of the enzyme since the purified mouse liver enzyme was not inactivated appreciably more. Unfortunately **3** is transported about 1000-fold less effectively than **1**. Introduction of 4-Cl on **3** gave **28** with about the same specificity and transport pattern.

Discussion

Of the 28 compounds in Table I, only 4 (**5**, **8**, **13**, **14**) showed specificity in inactivation between the crude enzyme from Walker 256 and rat liver; in all 4 cases inactivation of the purified liver enzyme[7] was extensive, indicating that the specificity was due to rapid hydrolysis of SO_2F to SO_3H by "sulfonyl fluoridase" in liver.[10] In the case of L1210 mouse leukemia and mouse liver, 12 compounds (**3**–**8**, **11**, **14**, **17**, **18**, **25**, **28**) showed good specificity of irreversible inhibition; in 6 cases (**4**–**7**, **11**, **14**) the specificity was due to hydrolysis of SO_2F by the "sulfonyl fluoridase" and the remaining 6 were apparently due to differences in the structure of dihydrofolate reductase from L1210 and mouse liver.

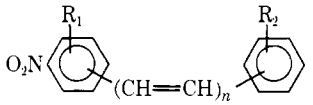
Folsch and Bertino[11] have observed that several of our irreversible inhibitors of dihydrofolate reductase of the SO_2F type are rapidly hydrolyzed to the sulfonic acids by mouse serum, slowly by rat serum,[10] and negligibly by human serum. They suggested that this destruction of the irreversible inhibitors by this "sulfonyl fluoridase" could account for the poor *in vivo* response of L1210 mouse leukemia to these inhibitors and suggested that the compounds be tested against rat tumors; this suggestion was followed with success.[6] The fact that 6 of the irreversible inhibitors (**3**, **8**, **17**, **18**, **25**, **28**) in Table I are not destroyed by the mouse liver "sulfonyl fluoridase" suggests that these 6 compounds might be effective on L1210 mouse leukemia *in vivo*, particularly if neither the mouse liver nor mouse serum "sulfonyl fluoridase" can destroy these 6 compounds. Furthermore, the 6 compounds have a sufficiently good ED_{50} (0.02–0.4 μM) against L1210 in cell culture that cell wall transport should not be a serious problem.

Chemistry.—All of the new compounds (**6**–**28**) in

(10) A. J. Ryan, N. M. J. Vermeulen, and B. R. Baker, *J. Med. Chem.*, **13**, 1140 (1970), paper CLXXIV of this series.

(11) E. Folsch and J. R. Bertino, *Mol. Pharmacol.*, **2**, 93 (1970).

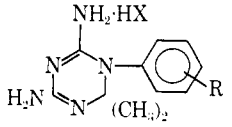
TABLE III: PHYSICAL PROPERTIES OF



No.[a]	R_1[b]	n	Position[b] of $(CH{=}CH)_n$	R_2[c]	Yield,[d] %	Mp, °C	Formula[e]
32a	3-Cl	1	4	2-Cl-4-SO_2F	61[f]	133–136	$C_{14}H_8Cl_2FNO_4S$
32b	3-Cl	1	4	3-Cl-4-SO_2F	72[g,h]	136–138	$C_{14}H_8Cl_2FNO_4S$
32c	3-Cl	1	4	4-Cl-2-SO_2F	65[g,h]	149–155	$C_{14}H_8Cl_2FNO_4S$
32d	3-Cl	1	4	5-Cl-2-SO_2F	62[i,f]	119–122	$C_{14}H_8Cl_2FNO_4S$
32e	3-Cl	1	4	3-Cl-2-SO_2F	31[h]	112–155[j]	$C_{14}H_8Cl_2FNO_4S$
32f	3-Cl	1	4	4-Cl-3-SO_2F	56[g,h]	150–156	$C_{14}H_8Cl_2FNO_4S$
32g	H	2	4	2-Cl-4-SO_2F[k]	54[f]	223–224	$C_{16}H_{11}ClFNO_4S$[l]
32h	H	3	4	4-SO_2F[m,n]	42[g,f]	157–160	$C_{18}H_{14}FNO_4S$
32i	3-Cl	2	4	2-Cl-4-SO_2F[o]	36[f]	274–276 dec	$C_{16}H_{10}Cl_2FNO_4S$[l]
32j	3-Cl	2	4	3-Cl-4-SO_2F[o]	60[f]	240–243	$C_{16}H_{10}Cl_2FNO_4S$
32k	3-Cl	2	4	3-SO_2F[o,p]	55[f]	208–210	$C_{16}H_{11}ClFNO_4S$[l]
32l	3-Cl	2	4	4-Cl-3-SO_2F[o]	42[f]	224–230	$C_{16}H_{10}Cl_2FNO_4S$
32m	3-Cl	2	4	2-SO_2F[o]	56[i,f]	216–218	$C_{16}H_{11}ClFNO_4S$
32n	3-Cl	2	4	5-Cl-2-SO_2F[o]	55[f]	228–230	$C_{16}H_{10}Cl_2FNO_4S$
32o	3-Cl	2	4	3-Cl-2-SO_2F[o]	41[h]	212–214	$C_{16}H_{10}Cl_2FNO_4S$
32p	3-Cl	2	4	4-Cl-2-SO_2F[o]	48[f]	245–247	$C_{16}H_{10}Cl_2FNO_4S$
32q	4-Cl	1	3	4-SO_2F[n]	77[g,f]	190–191	$C_{14}H_9ClFNO_4S$
32r	4-Cl	2	3	4-SO_2F[n]	67[f]	158–196[j]	$C_{16}H_{11}ClFNO_4S$
32s	H	2	3	2-Cl-4-SO_2F[k]	39[f]	175–180	$C_{16}H_{11}ClFNO_4S$
32t	H	2	3	3-Cl-4-SO_2F[k]	33[f]	201–205	$C_{16}H_{11}ClFNO_4S$
32u	H	2	3	5-Cl-2-SO_2F[k]	55[f]	218–220	$C_{16}H_{11}ClFNO_4S$
32v	H	2	3	4-Cl-3-SO_2F[k]	30[f]	184–186	$C_{16}H_{11}ClFNO_4S$

[a] All compounds prepared by method A in ref 14. [b] Numbered from 1-NO_2. [c] Numbered from 1-vinyl group. [d] Analytically pure material. [e] Anal., C, H, N unless otherwise indicated. [f] Recrystd from EtOH–THF. [g] Recrystd from EtOH–H_2O. [h] Recrystd from EtOH. [i] Recrystd from EtOH–THF–H_2O. [j] Two spots of cis–trans mixture on tlc. [k] For starting aldehyde see C. F. Göhring, *Ber.*, 18, 720 (1885). [l] Anal., C, H, F. [m] For starting aldehyde see ref 19. [n] For the starting Wittig reagent see ref 12. [o] For starting aldehyde see ref 3. [p] For the starting Wittig reagent see ref 3.

TABLE IV: PHYSICAL PROPERTIES OF



No.[a]	HX	R[b]	Yield,[c] %	Mp, °C dec	Formula[d]
6	$EtSO_3H$	3-Cl-4-$(CH_2)_2C_6H_3$-2-Cl-4-SO_2F	52	226–229	$C_{19}H_{20}Cl_2FN_5O_2S\cdot EtSO_3H$
7	$EtSO_3H$	3-Cl-4-$(CH_2)_2C_6H_3$-3-Cl-4-SO_2F	42	216–219	$C_{19}H_{20}Cl_2FN_5O_2S\cdot EtSO_3H$[e]
8	$EtSO_3H$	3-Cl-4-$(CH_2)_2C_6H_3$-4-Cl-2-SO_2F	44	209–211	$C_{19}H_{20}Cl_2FN_5O_2S\cdot EtSO_3H$
9	$EtSO_3H$	3-Cl-4-$(CH_2)_2C_6H_3$-5-Cl-2-SO_2F	52	210–212	$C_{19}H_{20}Cl_2FN_5O_2S\cdot EtSO_3H$
10	$EtSO_3H$	3-Cl-4-$(CH_2)_2C_6H_3$-3-Cl-2-SO_2F	40	224–226	$C_{19}H_{20}Cl_2FN_5O_2S\cdot EtSO_3H$
11	$EtSO_2H$	3-Cl-4-$(CH_2)_2C_6H_3$-4-Cl-3-SO_2F	52	215–216	$C_{19}H_{20}Cl_2FN_5O_2S\cdot EtSO_3H$
12	$EtSO_3H$	4-$(CH_2)_4C_6H_3$-2-Cl-4-SO_2F	32	204–205	$C_{21}H_{25}ClFN_5O_2S\cdot EtSO_3H$
13[f]	HCl	4-$(CH_2)_4OC_6H_4SO_2F$-*p*	39	223–225	$C_{21}H_{26}FN_5O_3S\cdot HCl$
14	$EtSO_3H$	*p*-$(CH_2)_6C_6H_4SO_2F$-*p*	34	205–207	$C_{23}H_{30}FN_5O_2S\cdot EtSO_3H$
15	$EtSO_3H$	3-Cl-4-$(CH_2)_4C_6H_3$-2-Cl-4-SO_2F	49	201–203	$C_{21}H_{24}Cl_2FN_5O_2S\cdot EtSO_3H$
16	$EtSO_3H$	3-Cl-4-$(CH_2)_4C_6H_3$-3-Cl-4-SO_2F	44	214–215	$C_{21}H_{24}Cl_2FN_5O_2S\cdot EtSO_3H$
17	$EtSO_3H$	3-Cl-4-$(CH_2)_4C_6H_4$-3-SO_2F	34	185–187	$C_{21}H_{25}ClFN_5O_2S\cdot EtSO_3H$
18	$EtSO_3H$	3-Cl-4-$(CH_2)_4C_6H_3$-4-Cl-3-SO_2F	44	198–200	$C_{21}H_{24}Cl_2FN_5O_2S\cdot EtSO_3H$
19	$EtSO_3H$	3-Cl-4-$(CH_2)_4C_6H_4$-2-SO_2F	42	194–197	$C_{21}H_{25}ClFN_5O_2S\cdot EtSO_3H$
20	$EtSO_3H$	3-Cl-4-$(CH_2)_4C_6H_3$-5-Cl-2-SO_2F	45	193–194	$C_{21}H_{24}Cl_2FN_5O_2S\cdot EtSO_3H$
21	$EtSO_3H$	3-Cl-4-$(CH_2)_4C_6H_3$-3-Cl-2-SO_2F	21	>215	$C_{21}H_{24}Cl_2FN_5O_2S\cdot EtSO_3H$
22	$EtSO_3H$	3-Cl-4-$(CH_2)_4C_6H_3$-4-Cl-2-SO_2F	29	200–202	$C_{21}H_{24}Cl_2FN_5O_2S\cdot EtSO_3H$
23	HCl	4-Cl-3-$(CH_2)_2C_6H_4$-4-SO_2F	37	218–220	$C_{19}H_{21}ClFNO_2S\cdot HCl$
24	$EtSO_3H$	4-Cl-3-$(CH_2)_4C_6H_4$-4-SO_2F	24	205–206	$C_{21}H_{25}ClFN_5O_2S\cdot EtSO_3H$
25	$EtSO_3H$	3-$(CH_2)_4C_6H_3$-2-Cl-4-SO_2F	32	168–170	$C_{21}H_{25}ClFN_5O_2S\cdot EtSO_3H$
26	$EtSO_3H$	3-$(CH_2)_4C_6H_3$-3-Cl-4-SO_2F	43	185–187	$C_{21}H_{25}ClFN_5O_2S\cdot EtSO_3H$
27	HCl	3-$(CH_2)_4C_6H_3$-5-Cl-2-SO_2F	16[g]	190–192	$C_{21}H_{25}ClFN_5O_2S\cdot HCl$
28	$EtSO_3H$	3-$(CH_2)_4C_6H_3$-4-Cl-3-SO_2F	28[h]	176–178	$C_{21}H_{25}ClFN_5O_2S\cdot EtSO_3H$

[a] Prepared by catalytic reduction of compounds in Table III in MeOEtOH with a PtO_2 catalyst in the presence of 1 equiv of HX, followed by condensation with cyanoguanidine,[3] unless otherwise indicated. [b] Numbered from triazine at position 1. [c] Analytically pure material recrystallized from *i*-PrOH–H_2O, unless otherwise indicated. [d] Anal., C, H, F unless otherwise indicated. [e] Anal., C, H, N. [f] Reduction performed with Raney Ni catalyst, then HCl added. [g] Recrystd from Me_2CO. [h] Recrystd once from EtOH and twice from Me_2CO.

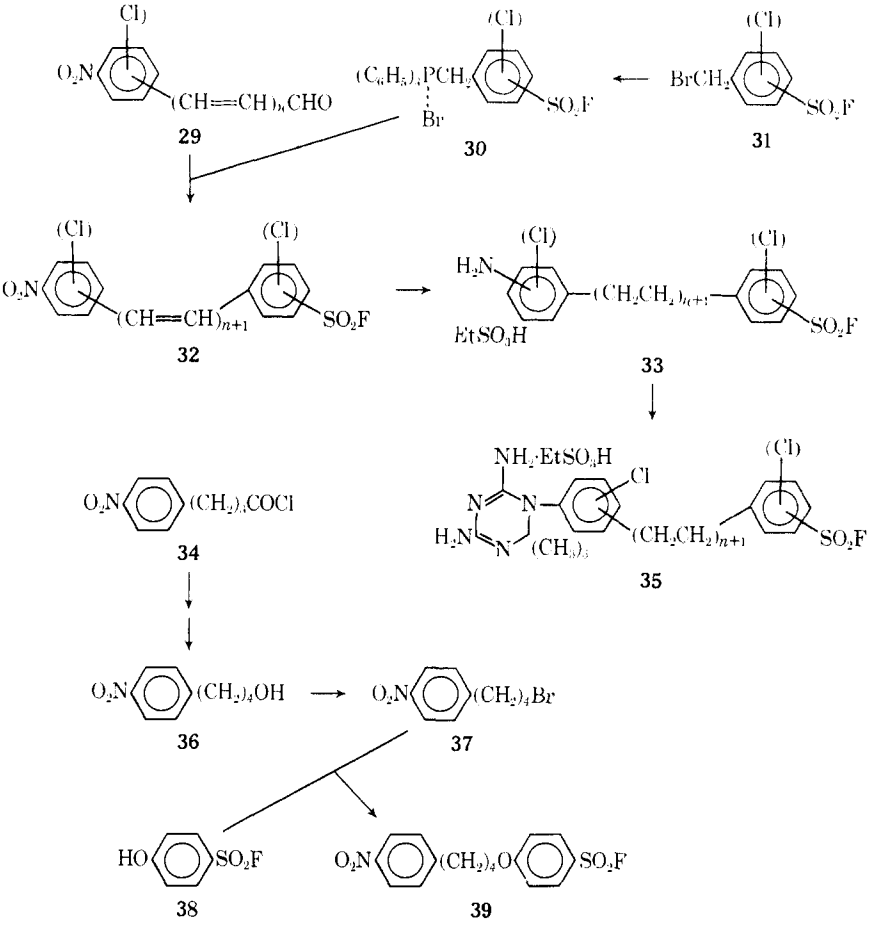


Table I, except **13**, were prepared by the previously described general methods used for **1–5**.[3,12–14] The appropriate fluorosulfonylbenzyl bromide (**31**) was converted into the Wittig reagents (**30**) with Ph_3P; the chloro derivatives of **30** were available from another study.[15] Wittig condensation of **29** and **30** afforded **32**; these were catalytically reduced in the presence of $EtSO_3H$ and PtO_2 to **33** which were condensed with cyanoguanidine and acetone by the method of Modest[16] to give the requisite dihydro-*s*-triazines (**35**).

Reduction of *p*-nitrophenylbutyryl chloride (**34**) to the alcohol **36** was accomplished with $NaBH_4$ in dioxane. Conversion of **36** into **37** with PBr_3, then alkylation of *p*-hydroxybenzenesulfonyl fluoride[17] afforded **39**; the latter was converted into **13** by reduction, then condensation with cyanoguanidine.

Experimental Section

Melting points were taken in capillary tubes on a Mel-Temp block and are uncorrected. All analytical samples had proper ir spectra and moved as a single spot on tlc; Brinkmann silica gel GF was used for all compounds except **35** where Brinkmann polyamide MN was employed. All analytical samples gave combustion values for C, H, N, or F within 0.4% of theoretical.

2-Chloro-5-nitrocinnamaldehyde (29a).—Condensation of 2-chloro-5-nitrobenzaldehyde[18] with MeCHO, as described[19] for the condensation of 4-nitrocinnamaldehyde with MeCHO, gave a crude product that was recrystd from C_6H_6; yield, 40% of analytically pure material, mp 141–143°. *Anal.* ($C_9H_6ClNO_3$) C, H, N.

3-Chloro-4-fluorosulfonylbenzyltriphenylphosphonium Bromide (30a). Method A.—A soln of 2.0 g (7 mmoles) of 3-chloro-4-fluorosulfonylbenzyl bromide (**31a**)[15] and 1.9 g (7.3 mmoles) of Ph_3P in 100 ml of C_6H_6 was refluxed 16 hr; during this time the product separated. The cooled reaction mixture was filtered; the solid was washed with C_6H_6 and recrystd from EtOH-C_6H_6 to give white crystals, mp 268–270°. See Table II for additional data and other compounds prepared by this method.

4-(*p*-Nitrophenylbutoxy)benzenesulfonyl Fluoride (39).—Reduction of the crude acid chloride (**34**) prepared from 6.3 g (60 mmoles) of *p*-nitrophenylbutyric acid with $NaBH_4$ in dioxane[20] gave 5.3 g (45%) of **36** which was converted into **37**[21] with PBr_3 in CCl_4 in 75% yield. A mixture of 2.58 g (10 mmoles) of **37**, 1.93 g (11 mmoles) of **38**,[22] 1.38 g (10 mmoles) of K_2CO_3, and 25 ml of DMF was stirred in a bath at 80° for 14 hr.[17] The cooled reaction mixture was dild with 50 ml of H_2O and extracted with $CHCl_3$ (three 50-ml portions). The combined extracts were washed successively with 25 ml of 10% Na_2CO_3 and 25 ml of H_2O, then dried ($MgSO_4$). Evapn *in vacuo* gave 3.4 g (97%) of an oil which showed one major spot and one trace component on tlc with 4:1 $CHCl_3$–petroleum ether (bp 60–110°). The compound had an appropriate ir spectrum and was used without further purification for conversion into **13**; see Table IV.

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

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

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

(15) B. R. Baker and J. A. Hurlbut, *ibid.*, **12**, 902 (1969), paper CLXI of this series.

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(20) E. Walton, A. F. Wagner, F. W. Bachelor, L. H. Peterson, F. W. Holley, and K. Folkers, *J. Amer. Chem. Soc.*, **77**, 5144 (1955).

(21) B. Elpern, L. N. Gardner, and L. Grumbach, *ibid.*, **79**, 1951 (1957).

(22) W. Steinkopf, *J. Prakt. Chem.*, **117**, 1 (1927).