Irreversible Enzyme Inhibitors. CLXXVI.[1,2] Active-Site-Directed Irreversible Inhibitors of Dihydrofolate Reductase Derived from 4,6-Diamino-1,2-dihydro-2,2-dimethyl-1-phenyl-s-triazine by Ether Bridging to a Terminal Sulfonyl Fluoride

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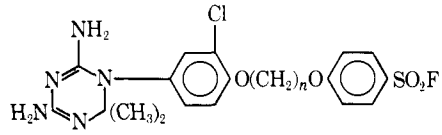
Sixteen candidate active-site-directed irreversible inhibitors of dihydrofolate reductase were synthesized and evaluated for inactivation of the enzyme from L1210 mouse leukemia, Walker 256 rat tumor, rat and mouse liver, and in some instances rat spleen, kidney, and intestine. Thirteen of the sixteen compounds were good to excellent irreversible inhibitors of the enzyme from either L1210 or Walker 256 or both. Tissue specificity with crude enzyme preparations was seen with nine of the compounds; when 6 of these 9 compounds were measured with purified enzymes, inactivation was essentially the same as with the tumor enzyme. Thus the observed tissue specificity is due to rapid detoxification of the SO_2F group of inhibitors to SO_3H by the "sulfonyl fluoridase" present in normal tissues.

In our search for active-site-directed irreversible inhibitors[3] of dihydrofolate reductase that showed good cell wall transport, **1** and **2**[4] emerged as particularly potent compounds; **1** and **2** had $ED_{50} = 0.01\ \mu M$[5] and

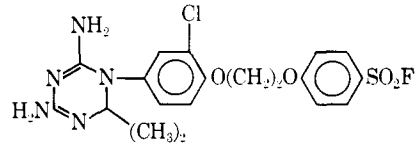


1, $n = 2$
2, $n = 3$

0.003 μM,[6] respectively, against L1210 mouse leukemic cells in culture, a number that gives a first approximation with compounds of this type of the rate of passive diffusion through the mammalian cell wall.[7] Since **1** and **2** showed little tissue specificity between tumor cell and liver dihydrofolate reductase,[4,6] but showed good inhibition of Walker 256 ascites *in vivo* (Table I and ref 6), a program on synthesis and evaluation of analogs of **1** and **2** was undertaken to determine if more tumor specific irreversible inhibitors of this enzyme could be found. Tissue specific inhibition of dihydrofolate reductase was examined with both crude and affinity column purified dihydrofolate reductase[2] in order to determine if the tissue specificity was due to rapid hydrolysis of the SO_2F group of the inhibitor by a "sulfonyl fluoridase" or was due to a difference in the structure of the dihydrofolate reductases. The results are the subject of this paper.

Biological Results.—Three series of compounds related to **1** and **2** were evaluated. In the first series the number of methylene groups in the bridge was extended to 4, 5 and 6; three diether bridges (**6-8**) were also evaluated (Table II). Compounds **1-8** were all excellent irreversible inhibitors of dihydrofolate reductase from the Walker 256 rat tumor; similarly, all but **4** were good to excellent irreversible inhibitors of the enzyme from L1210 mouse leukemia.

TABLE I
INHIBITION OF TUMORS *in Vivo* BY



NSC-123462

Tumor system	mg/kg per day[a]	Tumor control	Survivors No.	Survivors Day
W256[b]	200	(Toxic)		
	50	128	0/6	60
	25	742	3/6	60
	12.5	857	4/6	60
DL[c,d]	25	188	0/6	35
	12.5	399	4/6	35
	6.25	188	2/6	35
	3.13	213	0/6	35

[a] Single dose day 1-9. [b] Walker 256 ascites; controls survived 7 days. [c] Dunning leukemia ascites; controls survived 9 days. [d] Dihydrofolate reductase from this tumor was inactivated 96% when incubated with 0.072 μM inhibitor for 60 min at 37°, then assayed as previously described.[8]

Some tissue specificity was observed with **1-8** when assayed with the dihydrofolate reductase purified only through the 45–90% $(NH_4)_2SO_4$ stage.[8] For example, **1** and **6** showed less than 22% irreversible inhibition of the crude rat liver enzyme; when the enzyme was purified through an affinity column,[2] **1** and **6** showed 100% inactivation of the rat liver enzyme. These results can be attributed[2] to the rapid hydrolysis of the SO_2F group of **1** and **6** to SO_3H by the "sulfonyl fluoridase" present in the 45–90% $(NH_4)_2SO_4$ fraction. Similarly, **2**, **4**, **5**, and **6** showed 28–42% inactivation of the crude enzyme from rat kidney, but 100% inactivation of the enzyme purified by the affinity column.[2] In the case of mouse liver enzyme, **5** and **8** showed only

(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.*, **13**, 1143 (1970).

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

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

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

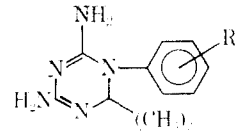
(6) B. R. Baker, N. M. J. Vermeulen, W. T. Ashton, and A. J. Ryan, *ibid.*, **13**, 1130 (1970), paper CLXXIII of this series.

(7) (a) B. R. Baker and R. B. Meyer, Jr., *ibid.*, **12**, 668 (1969), paper CLIV of this series; (b) B. R. Baker, E. E. Janson, and N. M. J. Vermeulen, *ibid.*, **12**, 898 (1969), paper CLX 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.

TABLE II

INHIBITION OF DIHYDROFOLATE REDUCTASE BY



No.	R[a]	Enzyme source[b]	I_{50},[c] μM	Inhibitor, μM	Time, min	% inactvn[d]	EO_{50},[e] μM	EO_{50}/C_{50}
1	3-Cl-4-O(CH$_2$)$_2$OC$_6$H$_4$SO$_2$F-*p*	L1210/DFS (A)	0.036[f]	0.12	60	97[f]	0.01[g]	0.3
		Mouse liver (A)	0.012[f]	0.12	60	41[f]		
		Walker 256 (A)[h]		0.072	60	86		
		Rat liver (A)[h]		0.072	60	22		
		Rat liver (C)[h]		0.072	60	100		
		Rat kidney (C)[h]		0.072	60	100		
2	3-Cl-4-O(CH$_2$)$_3$OC$_6$H$_4$SO$_2$F-*p*	L1210/DFS (A)	0.085[f]	0.085	60	81[f]	0.003[h]	0.04
		Mouse liver (A)		0.18	60	61[f]		
		Mouse spleen (A)		0.18	60	73[f]		
		Mouse intestine (A)		0.18	20	67[f]		
		Walker 256 (A)[f]	0.057	0.18	60	94		
		Rat liver (A)[f]	0.052	0.18	60	52		
		Rat liver (C)[h]		0.18	60	100		
		Rat kidney (A)[f]		0.18	20	38		
		Rat intestine (A)[f]		0.18	20	70		
3	3-Cl-4-O(CH$_2$)$_4$OC$_6$H$_4$SO$_2$F-*p*	L1210/DFS (A)	0.027	0.054	60	90	0.005	0.02
		Mouse liver (A)		0.054	60	38		
		W256 (A)		0.054	60	95		
		Rat liver (A)		0.054	60	94		
		Rat intestine (A)		0.054	20	62		
4	3-Cl-4-O(CH$_2$)$_5$OC$_6$H$_4$SO$_2$F-*p*	L1210/DFS (A)	0.027	0.050	60	25	0.02	0.7
		Mouse liver (A)		0.050	60	11		
		Mouse liver (C)		0.050	60	40		
		W256 (A)		0.050	60	95		
		Rat liver (A)		0.050	60	95		
		Rat kidney (A)		0.050	20	28		
		Rat kidney (C)		0.050	20	93		
		Rat intestine (A)		0.050	20	0		
		Rat spleen (A)		0.050	20	0		
5	3-Cl-4-O(CH$_2$)$_6$OC$_6$H$_4$SO$_2$F-*p*	L1210/DFS (A)	0.035	0.070	60	96	0.003	0.08
		Mouse liver (A)	0.10	0.10	60	18		
		Mouse liver (C)		0.10	60	84		
		W256 (A)		0.070	60	98		
		Rat liver (A)		0.10	60	94		
		Rat kidney (A)		0.10	20	29		
		Rat kidney (C)		0.10	20	100		
		Rat spleen (A)		0.10	20	24		
		Rat intestine (A)		0.10	20	7		
6	3-Cl-4-O(CH$_2$)$_2$O(CH$_2$)$_2$O	L1210/DFS (A)	0.073	0.15	60	76	0.001	0.01
		Mouse liver (A)		0.15	60	10		
	p-FSO$_2$C$_6$H$_4$	Mouse liver (C)		0.15	60	86		
		W256 (A)		0.15	60	95		
		Rat liver (A)		0.15	60	15		
		Rat liver (C)		0.15	60	100		
		Rat kidney (A)		0.15	20	42		
		Rat kidney (C)		0.15	20	100		
		Rat intestine (A)		0.15	20	79		
7	3-Cl-4-OCH$_2$ CH$_2$OC$_6$H$_4$SO$_2$F-*p*	L1210/DFS (A)	0.15	0.30	60	89	0.02	0.01
		Mouse liver (A)		0.30	60	32		
		Mouse liver (C)		0.30	60	100		
		W256 (A)		0.30	60	97		
		Rat liver (A)		0.30	60	68		
		Rat intestine (A)		0.30	20	63		
8	3-Cl-4-OCH$_2$ CH$_2$OC$_6$H$_4$SO$_2$F-*p* *trans*	L1210/DFS (A)	0.43	0.43	60	99	0.01	0.02
		Mouse liver (A)		0.43	60	17		
		Mouse liver (C)		0.43	60	94		
		W256 (A)		0.43	60	91		
		Rat liver (A)		0.43	60	38		

Table II (*Continued*)

No.	R[a]	Enzyme source[b]	I_{50},[c] μM	Inhibitor, μM	Time, min	% inactvn[d]	DD_{50},[e] μM	ED_{50}/I_{50}
		Rat liver (C)		0.43	60	100		
		Rat intestine (A)		0.43	20	66		
9	3-O$(CH_2)_2OC_6H_4SO_2F$-*p*	L1210/DF8 (A)	0.015	0.050	60	25	0.7	50
		W256 (A)		0.050	60	28		
10	3-O$(CH_2)_3OC_6H_4SO_2F$-*p*	L1210/DF8 (A)	0.011	0.050	60	18	0.18	20
		W256 (A)		0.050	60	28		
11	3-O$(CH_2)_4OC_6H_4SO_2F$-*p*	L1210/DF8 (A)	0.032	0.064	60	48	0.2	7
		W256 (A)		0.064	60	71		
		Rat liver (A)		0.096	60	60		
12	4-Cl-3-O$(CH_2)_2OC_6H_4SO_2F$-*p*	L1210/DF8 (A)	0.054	0.11	60	29	>2	>40
		W256 (A)		0.11	60	55		
		Rat liver (A)		0.16	60	31		
13	4-Cl-3-O$(CH_2)_3OC_6H_4SO_2F$-*p*	L1210/DF8 (A)	0.031	0.062	60	26	1	30
		W256 (A)		0.062	60	50		
		Rat liver (A)		0.062	60	9		
		Rat liver (C)		0.062	60	29		
14	4-Cl-3-O$(CH_2)_4OC_6H_4SO_2F$-*p*	L1210/DF8 (A)	0.067	0.14	60	51	0.2	3
		Mouse liver (A)		0.20	60	0		
		W256 (A)		0.14	60	69		
		Rat liver (A)		0.20	60	52		
15	4-Cl-3-O$(CH_2)_5OC_6H_4SO_2F$-*p*	L1210/DF8 (A)	0.19	0.48	60	49	0.2	1
		W256 (A)		0.48	60	61		
16	3-Cl-4-O$(CH_2)_4OC_6H_4(SO_3C_6H_4Cl$-*p*$)$-*p*	L1210/DF8 (A)	0.28	0.56	60	89	2	7
		W256 (A)		0.56	60	98		
		Rat liver (A)		0.56	60	98		
		Rat liver (C)		0.56	60	100		

[a] Numbered from triazinyl position = 1. [b] A, 45–90% ammonium sulfate fraction;[8] C, purified with affinity column.[2] [c] I_{50} = concentration for 50% inhibition when assayed with 6 μM dihydrofolate and 0.15 M KCl in pH 7.4 Tris buffer as previously described.[8] [d] Enzyme incubated with inhibitor in pH 7.4 Tris buffer containing 60 μM TPNH, then the remaining enzyme assayed as previously described;[8] 60-min runs were at 37° and 20-min runs a 24°. [e] Concentration for 50% inhibition of L1210 cell culture. [f] Data from ref 4. [g] Data from ref 5. [h] Data from ref 2. [i] Data from ref 6.

18% inactivation of the crude enzyme, but 73 and 94% inactivation of the affinity column purified enzyme, respectively.

The ED_{50} against L1210 in cell culture gives an approximation of cell wall transport. When the results are normalized as ED_{50}/I_{50} ratios, the resultant number is then dependent upon cell wall transport plus the effectiveness of irreversible inhibition of the dihydrofolate reductase inside the cell.[7] An ED_{50}/I_{50} ratio <0.1 is considered highly effective; **2**, **3**, and **5–8** were in this range, the most potent being **6**.

The second series of compounds were bridged from the meta position of the 1-phenyltriazine (**9–11**). These were good reversible inhibitors, but poor irreversible inhibitors on the two tumor enzymes with the exception of **11** on the Walker 256 enzyme; **9–11** were poor against L1210 cell culture. Insertion of a Cl on the para position of the 1-phenyltriazine (**12–15**) to restrict the possible conformations failed to increase the effectiveness against L1210 in cell culture; furthermore these compounds were only fair irreversible inhibitors of the enzyme from Walker 256 and poor irreversible inhibitors of the enzyme from L1210.

A notable difference in the irreversible inhibition of dihydrofolate reductase from L1210 and Walker 256 is observed with **4**; only 25% irreversible inhibition of the L1210 enzyme is seen under conditions where the Walker 256 enzyme is inactivated 95%. Since these two sources of enzyme do not appear to contain the "sulfonyl fluoridase," the difference can likely be due to a difference in enzyme structure.[2] Similarly, **4** was a more effective irreversible inhibitor of the purified rat liver enzyme than the purified mouse liver enzyme, indicating a difference in the structure of the dihydrofolate reductases from these two rodents.

Finally, the leaving group of **3** was changed from F to *p*-chlorophenoxy (**16**); **16** was an excellent irreversible inhibitor of the enzyme from L1210, Walker 256, and rat and mouse liver. Unfortunately **16** was 100-fold less effective than **3** against L1210 cell culture indicating that transport was impaired with **16**; nevertheless, further studies on phenolate leaving groups would be worthwhile to see if isozyme specificity and good transport can be achieved.

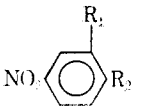
Further searches for isozyme specific irreversible inhibitors of dihydrofolate reductase are continuing.

Chemistry.—The key intermediates (**18**) in the synthesis of **1** and **2** has been prepared[4] by the chlorosulfonation of an α-(nitrophenoxy-ω-phenoxyalkane (**17**) (Scheme I). The chloro- or fluorosulfonation of **17** with $n > 3$ proceeded very poorly, however, and a more generally useful route was undertaken to prepare the compounds in this series (Scheme II).

Alkylation of the appropriate nitrophenol (**19–21**) with excess α,ω-dibromoalkane (**22a–e**) yielded the nitrophenoxyalkyl bromide (**23–25**). The modified

TABLE III

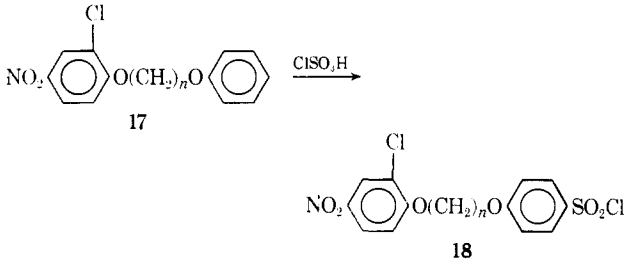
PHYSICAL CONSTANTS OF



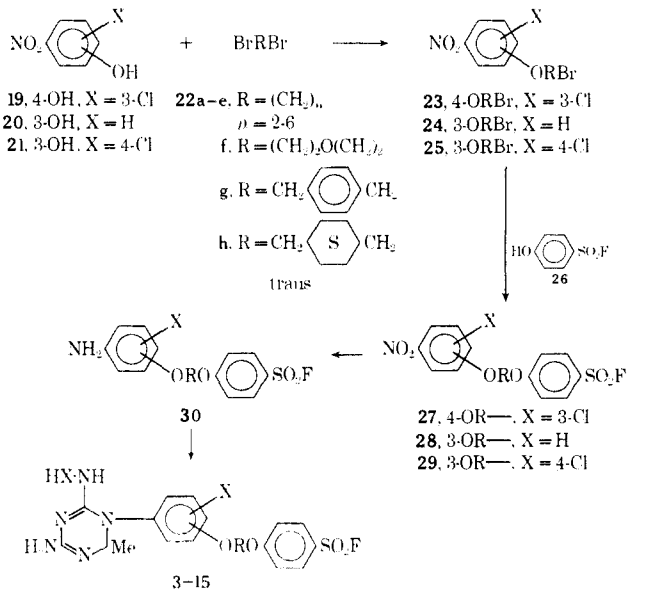
No.	R_1	R_2	Method	Yield, %	Mp, °C	Formula[a]
23c	Cl	$O(CH_2)_4Br$	A[b]	25[c,d]	43–44[e]	
23d	Cl	$O(CH_2)_5Br$	A[b]	26[c,d]	41–43	$C_{11}H_{13}BrClNO_3$
23e	Cl	$O(CH_2)_6Br$	A[f]	54	Oil	
23f	Cl	$O(CH_2)_2O(CH_2)_2Br$	A	59[c]	56–57	$C_{10}H_{11}BrClNO_3$
23g	Cl	$OCH_2C_6H_4CH_2Br$-*p*	A[f,g]	18[h]	129–130	$C_{14}H_{11}BrClNO_3$
23h	Cl	$OCH_2C_6H_{10}$-4-C_6H_2Br(*trans*)	A[f]	37[d]	93–94	$C_{14}H_{17}BrClNO_3$
24a	$O(CH_2)_2Br$	H	A[b]	24[c]	38–39[i]	
24b	$O(CH_2)_3Br$	H	A[f,j]	58	Oil[k]	
24c	$O(CH_2)_4Br$	H	A[b]	60[c]	31–33[l]	
25a	$O(CH_2)_2Br$	Cl	A	43[d]	73–75[m]	
25b	$O(CH_2)_3Br$	Cl	A	40[c,d]	61–62	$C_9H_9BrClNO_3$
25c	$O(CH_2)_4Br$	Cl	A	42[c,d]	42–43	$C_{10}H_{11}BrClNO_3$
25d	$O(CH_2)_5Br$	Cl	A[f]	33	Oil	
27c	Cl	$O(CH_2)_4OC_6H_4SO_2F$-*p*	B	50[n]	105–107	$C_{16}H_{15}ClFNO_6S$
27d	Cl	$O(CH_2)_5OC_6H_4SO_2F$-*p*	B	84[o]	Oil	
27e	Cl	$O(CH_2)_6OC_6H_4SO_2F$-*p*	B[o]	20[d]	58–59	$C_{18}H_{19}ClFNO_6S$
27f	Cl	$O(CH_2)_2O(CH_2)_2OC_6H_4SO_2F$-*p*	B[c]	42[d]	79–80	$C_{16}H_{15}ClFNO_7S$
27g	Cl	$OCH_2C_6H_4$-4-$CH_2OC_6H_4SO_2F$-*p*	C	57[p,q]	128–129	$C_{20}H_{15}ClFNO_6S$
27h	Cl	$OCH_2C_6H_{10}$-4-$CH_2OC_6H_4SO_2F$-*p* (*trans*)	B	30[r]	156–158	$C_{20}H_{21}ClFNO_6S$
28a	$O(CH_2)_2OC_6H_4SO_2F$-*p*	H	B	43[s]	92–93[s]	$C_{14}H_{12}FNO_6S$
28b	$O(CH_2)_3OC_6H_4SO_2F$-*p*	H	B	18[d,t]	86–88	$C_{15}H_{14}FNO_6S$
28c	$O(CH_2)_4OC_6H_4SO_2F$-*p*	H	B[t]	31[d]	76–78[u]	$C_{16}H_{16}FNO_6S$
29a	$O(CH_2)_2OC_6H_4SO_2F$-*p*	Cl	B	30[v]	126–127	$C_{14}H_{11}ClFNO_6S$
29b	$O(CH_2)_3OC_6H_4SO_2F$-*p*	Cl	B	26[w]	126–128	$C_{15}H_{13}ClFNO_6S$
29c	$O(CH_2)_4OC_6H_4SO_2F$-*p*	Cl	B	45[x]	131–133	$C_{16}H_{15}ClFNO_6S$
29d	$O(CH_2)_5OC_6H_4SO_2F$-*p*	Cl	B	18[x]	118–120	$C_{17}H_{17}ClFNO_6S$
32	Cl	$O(CH_2)_4OC_6H_4$-4-$SO_3C_6H_4Cl$-*p*	D	70[r]	133–134	$C_{22}H_{19}Cl_2NO_7S$

[a] Analyzed for C, H, N. [b] Crude product crystallized from petroleum ether (bp 30–60°) after removal of insoluble material. [c] Recrystallized from petroleum ether (bp 30–60°). [d] Recrystallized from MeOH. [e] B. R. Baker and E. H. Erickson [*J. Med. Chem.*, **12**, 112 (1969), paper CXLIV of this series] reported mp 41–43°. [f] Crude product purified by silica gel column chromatography. [g] Reaction run at room temperature. [h] Recrystallized from C_6H_6–petroleum ether (bp 60–110°). [i] A. Weddige [*J. Prakt. Chem.*, [2] **24**, 241 (1881)] reported mp 39°. [j] Crude product partially purified by distillation. [k] Bp 149–152° (0.8 mm); W. C. Wilson and R. Adams [*J. Amer. Chem. Soc.*, **45**, 528 (1923)] reported by 186–188° (7 mm). [l] J. P. Mason, H. L. Hennessy, and R. G. McInnis [*U. S. Dep. Com., Office Tech. Serv., AD* **268214** (1961); *Chem. Abstr.*, **59**, 3803 (1963)] reported mp 34–35°. [m] Lit.[14] mp 77–78°. [n] Recrystallized from EtOH. [o] Crude product used without further purification. [p] Recrystallized from *i*-PrOH. [q] Recrystallized from $CHCl_3$–MeOH. [r] Recrystallized from MeOEtOH–H_2O. [s] Analytical sample, mp 92–94°, prepared in this laboratory by Eunice E. Janson, M. S. Thesis, 1968. [t] Recrystallized from petroleum ether (bp 60–110°). [u] Analytical sample had mp 73–75°.

SCHEME I



SCHEME II

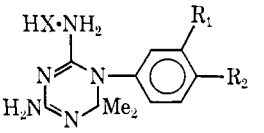


analogs (**23f–h**) were obtained in a similar manner. By use of the method of Meldola,[9] 2-chloro-5-nitrophenol (**21**) was obtained from 2-amino-5-nitrophenol *via* the Sandmeyer reaction. Treatment of diethylene glycol with PBr_3 afforded a high yield of 2,2′-dibromodiethyl ether[10] (**22f**). Similar treatment of *trans*-1,4-

(9) R. Meldola, G. H. Woolcott, and E. Wray, *J. Chem. Soc.*, **69**, 1321 (1896).

(10) P. A. McCusker and J. W. Kroeger, *J. Amer. Chem. Soc.*, **59**, 213 (1937).

TABLE IV
PHYSICAL CONSTANTS OF

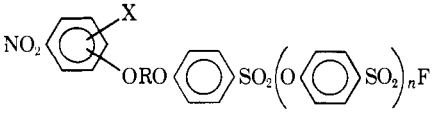


No.	R_1	R_2	HX	Yield,[a] %	Mp, °C dec	Formula[b]
3	Cl	$O(CH_2)_4OC_6H_4SO_2F$-*p*	$EtSO_3H$	52[c]	213–215	$C_{23}H_{31}ClFN_5O_7S_2$
4	Cl	$O(CH_2)_5OC_6H_4SO_2F$-*p*	$EtSO_3H$	40[d]	190–191	$C_{24}H_{33}ClFN_5O_7S_2$
5	Cl	$O(CH_2)_6OC_6H_4SO_2F$-*p*	$EtSO_3H$	48[c]	205–206	$C_{25}H_{35}ClFN_5O_7S_2$
6	Cl	$O(CH_2)_2O(CH_2)_2OC_6H_4SO_2F$-*p*	$EtSO_3H$	56[c]	200–201	$C_{23}H_{31}ClFN_5O_8S_2$
7	Cl	$OCH_2C_6H_4$-4-$CH_2OC_6H_4SO_2F$-*p*	$EtSO_3H$	49[c,e]	229	$C_{27}H_{31}ClFN_5O_7S_2$
8	Cl	$OCH_2C_6H_{10}$-4-$CH_2OC_6H_4SO_2F$-*p* trans	$EtSO_3H$	56[c]	223–225	$C_{27}H_{37}ClFN_5O_7S_2$
9	$O(CH_2)_2OC_6H_4SO_2F$-*p*	H	HCl	35[f]	206–207	$C_{19}H_{23}ClFN_5O_4S$
10	$O(CH_2)_3OC_6H_4SO_2F$-*p*	H	HCl	60[c]	190–192[g]	$C_{20}H_{25}ClFN_5O_4S$
11	$O(CH_2)_4OC_6H_4SO_2F$-*p*	H	TsOH	19[h]	180–182	$C_{28}H_{34}FN_5O_7S_2$
12	$O(CH_2)_2OC_6H_4SO_2F$-*p*	Cl	$EtSO_3H$	34[c]	204–205	$C_{21}H_{27}ClFN_5O_7S_2$
13	$O(CH_2)_3OC_6H_4SO_2F$-*p*	Cl	HCl	19[c]	202–203	$C_{20}H_{24}Cl_2FN_5O_4S$
14	$O(CH_2)_4OC_6H_4SO_2F$-*p*	Cl	HCl	31[c]	211–212	$C_{21}H_{26}Cl_2FN_5O_4S$
15	$O(CH_2)_5OC_6H_4SO_2F$-*p*	Cl	HCl	18[c]	209–210	$C_{22}H_{28}Cl_2FN_5O_4S$
16	Cl	$O(CH_2)_4OC_6H_4$-4-$SO_3C_6H_4Cl$-*p*	$EtSO_3H$	70[c]	210–212	$C_{29}H_{35}Cl_2N_5O_8S_2$[i]

[a] Prepared by Pt-catalyzed hydrogenation of nitro intermediate followed by condensation of amine salt with cyanoguanidine in acetone;[4] yield of analytically pure material. [b] Analyzed for C, H, F. [c] Recrystallized from *i*-PrOH–H_2O. [d] Recrystallized from *i*-PrOH. [e] Nitro intermediate hydrogenated with Raney nickel catalyst in THF. [f] Reprecipitated from EtOH at room temperature with petroleum ether. [g] Became amorphous 143–144°. [h] Reprecipitated from *i*-PrOH at room temperature with petroleum ether. [i] Analyzed for C, H, N.

cyclohexanedimethanol gave **22h**, which had previously been prepared[11] by a different procedure.

The key step in Scheme II is the alkylation of *p*-hydroxybenzenesulfonyl fluoride (**26**) with the nitrophenoxyalkyl bromides (**23–25**). Compound **26** was obtained by the fluorosulfonation of phenol as described by Steinkopf.[12a] The fact that this phenol could be alkylated under relatively vigorous conditions[12b] once again affirms the remarkable stability of the SO_2F group. Actually, some apparent polymerization did take place, giving rise to a train of spots which moved more slowly on tlc than the product. The spacings and relative intensities suggested that these spots correspond to derivatives of type **31**. These side products were usually destroyed to a large extent (without undue decomposition of the product) by running the reaction at relatively high temperature (90–110°) for 1 to 3 days.

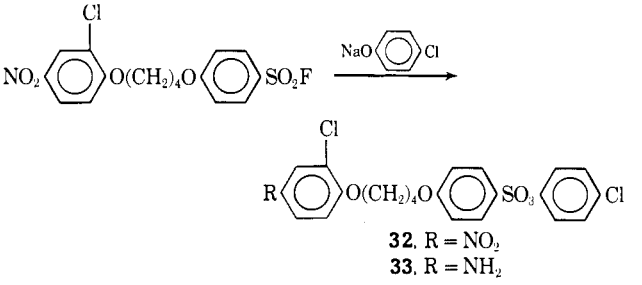


31

Intermediate **32** for the synthesis of **16** was prepared by treating the corresponding sulfonyl fluoride (**27c**) with sodium *p*-chlorophenoxide (Scheme III).

The nitro intermediates (**27–29**, **32**) were then reduced with H_2 and PtO_2 to the corresponding amines (**30**, **33**), which were not isolated. Condensation of the amines with cyanoguanidine and acetone in the presence of acid[13] furnished the dihydrotriazines (**3–16**) as described previously.[4] When a crystalline ethanesulfonate salt could not be obtained, the triazine was isolated as the hydrochloride. In the case of **11**, only the *p*-toluenesulfonate salt could be prepared in crystalline form.

SCHEME III



32, R = NO_2
33, R = NH_2

Experimental Section

Melting points were taken in capillary tubes on a Mel-Temp block and are uncorrected. All analytical samples had ir and uv spectra in accord with their assigned structures; each gave combustion analyses for C, H, and N or F within 0.4% of theory. Analytical intermediates were checked for purity on tlc with Brinkman silica gel GF; polyamide MN was used for triazines.

1-Bromo-2-(2-chloro-5-nitrophenoxy)ethane (25a) (Method A).—A mixture of 4.07 g (25 mmoles) of 2-chloro-5-nitrophenol (**21**), 21 ml (about 250 mmoles) of 1,2-dibromoethane, 3.45 g (25 mmoles) of K_2CO_3, and 25 ml of DMF was stirred at 95–100° for 2.5 hr, then cooled, and added to 250 ml of H_2O. The organic materials were extracted into 100 ml of $CHCl_3$ and washed with two 150-ml portions of 10% Na_2CO_3, then 200 ml of H_2O. The $CHCl_3$ solution was dried ($MgSO_4$), decolorized with charcoal, and evaporated *in vacuo* to remove solvent and unreacted dibromide. Upon cooling and standing, the residual oil solidified. Recrystallization from MeOH (after removal of some insoluble white solid) gave 3.04 g (43%) of light yellow-orange crystals, mp 73–75° (tlc in 1:1 C_6H_6–petroleum ether), lit.[14] mp 77–78°.

For additional compounds prepared by this method see Table III.

(11) R. Malachowski, J. J. Wasowska, and S. Jözkiewicz, *Chem. Ber.*, **71**, 759 (1938).

(12) (a) W. Steinkopf, *J. Prakt. Chem.*, **117**, 1 (1927); (b) B. R. Baker and N. M. J. Vermeulen, *J. Med. Chem.*, **12**, 82 (1970), paper CLXVI of this series.

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

(14) Kalle A.-G., French Patent 1,375,314 (1964); *Chem. Abstr.*, **62**, 5372 (1965).

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° (dec 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 of 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° (dec 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° (dec 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° (dec 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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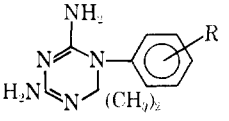
Twenty-eight analogs of the title compound bearing a terminal SO_2F group were investigated as irreversible inhibitors of dihydrofolate reductase from Walker 256 rat tumor, L1210 mouse leukemia, mouse liver, and several normal rat tissues. Only 4 of the irreversible inhibitors showed tissue specificity 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*-$(CH_2)_4C_6H_4SO_2F$-*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-$(CH_2)_6C_6H_3$-4-Cl-3-SO_2F (**18**), 3-$(CH_2)_4$-C_6H_3-2-Cl-4-SO_2F (**25**), and 3-$(CH_2)_3C_6H_3$-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.