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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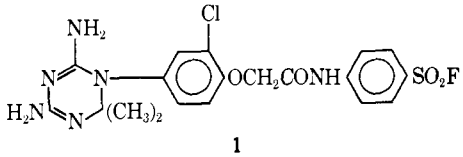
1-(3-Chlorophenyl)-4,6-diamino-1,2-dihydro-2,2-dimethyl-*s*-triazine bearing the 4-$OCH_2CONHC_6H_4SO_2F$-*p* (**1**) group on the Ph ring was previously shown to be a fair to good active-site-directed irreversible inhibitor of dihydrofolate reductase from L1210 mouse leukemia and Walker 256 rat tumor; **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

(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**, 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.

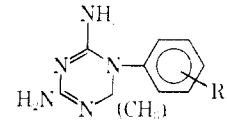
(6) B. R. Baker and N. M. J. Vermeulen, *ibid.*, **13**, 1143 (1970), paper CLXXV of this series.

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

(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 I

INHIBITION[a] OF DIHYDROFOLATE REDUCTASE BY



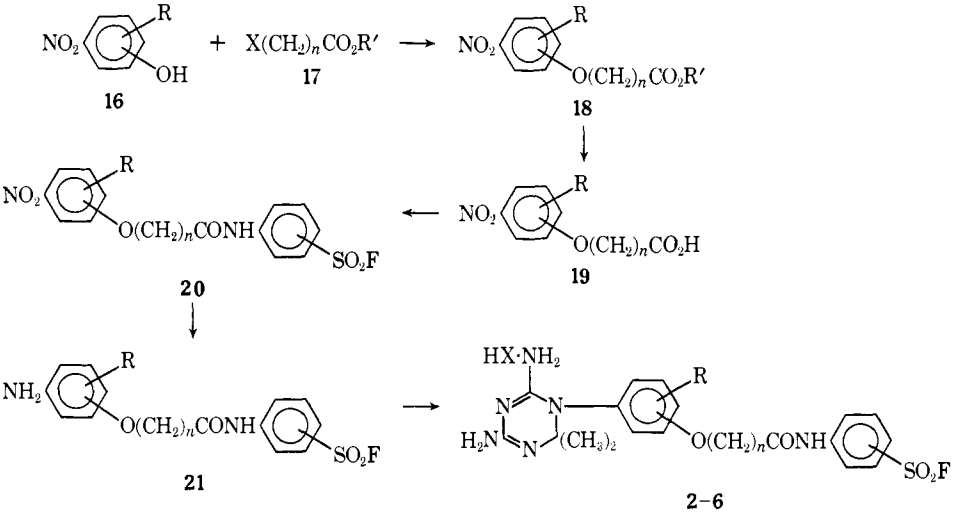
No.	R	Enzyme source[b]	I_{50},[d] μM	Inhibitor, μM	Time, min	% inactvn[e]	ED_{90},[f] μM	ED_{90}/I_{50}
1[g]	3-Cl-4-$OCH_2CONHC_6H_4SO_2F$-*p*	L1210/DF8 (A)	0.037	0.074	60	76	0.4	10
		Mouse liver (A)		0.16	60	0		
		Mouse liver (C)		0.16	60	68		
		W256 (A)	0.025	0.074	60	64		
		Rat liver (A)	0.027	0.11	60	50		
		Rat kidney (A)		0.11	20	11		
		Rat kidney (C)		0.11	20	72		
		Rat intestine (A)		0.11	20	65		
		Rat spleen (A)		0.11	20	79		
2	3-Cl-4-$O(CH_2)_3CONHC_6H_4SO_2F$-*p*	L1210/DF8 (A)	0.070	0.14	60	57	0.8	10
		W256 (A)		0.14	60	81		
		Rat liver (A)		0.14	60	9		
		Rat liver (C)		0.14	60	92		
3	3-Cl-4-$O(CH_2)_3CONHC_6H_4SO_2F$-*m*	L1210/DF8 (A)	0.067	0.13	60	48	2	30
		W256 (A)		0.13	60	54		
4	3-Cl-4-$OCH(CH_3)CONHC_6H_4SO_2F$-*p*	L1210/DF8 (A)	0.074	0.15	60	23	6	80
		W256 (A)		0.15	60	34		
5	3-Cl-4-$O(CH_2)_4CONHC_6H_4SO_2F$-*p*	L1210/DF8 (A)	0.058	0.11	60	89	0.5	9
		Mouse liver (A)		0.11	60	20		
		Mouse liver (C)		0.11	60	100		
		W256 (A)		0.11	60	95		
		Rat liver (A)		0.11	60	26		
		Rat liver (C)		0.11	60	100		
6	3-$OCH_2CONHC_6H_4SO_2F$-*p*	L1210/DF8 (A)	0.12	0.24	60	10	>2	>20
		W256 (A)		0.24	60	0		
7	3-Cl-4-OCH_2-C$_6$H$_4$-$CONHC_6H_4SO_2F$-*p*	L1210/DF8 (A)	0.12	0.24	60	96	0.1	0.8
		Mouse liver		0.24	60	55		
		W256 (A)		0.24	60	97		
		Rat liver (A)		0.24	60	47		
8	3-Cl-4-OCH_2-C$_6$H$_4$-$CONHC_6H_4SO_2F$-*m*	L1210/DF8 (A)	0.039	0.078	60	100	0.002	0.05
		Mouse liver (A)		0.078	60	16		
		Mouse liver (C)		0.078	60	100		
		W256 (A)		0.078	60	88		
		Rat liver (A)		0.078	60	100		
9	3-Cl-4-OCH_2-C$_6$H$_4$-$CONHC_6H_4SO_2F$-*p*	L1210/DF8 (A)	0.12	0.24	60	100	0.05	0.4
		Mouse liver (A)		0.24	60	63		
		W256 (A)		0.24	60	92		
		Rat liver (A)		0.24	60	96		
10	3-Cl-4-OCH_2-C$_6$H$_4$-$CONHC_6H_4SO_2F$-*m*	L1210/DF8 (A)	0.058	0.058	60	41	0.0007	0.01
		Mouse liver (A)		0.058	60	10		
		Mouse liver (C)		0.058	60	73		
		W256 (A)		0.058	60	77		
		Rat liver (A)		0.058	60	56		
11	3-Cl-4-OCH_2-C$_6$H$_4$-$CONHC_6H_4SO_2F$-*p*	L1210/DF8 (A)	0.057	0.11	60	12	4	70
		W256 (A)		0.11	60	6		
12	3-Cl-4-$SCH_2CONHC_6H_4SO_2F$-*p*	L1210/DF8	0.038	0.076	60	86	0.08	2
		Mouse liver (A)		0.076	60	0		
		Mouse liver (C)		0.076	60	70		
		W256 (A)		0.076	60	53		
		Rat liver (A)		0.076	60	60		
13	4-$SCH_2CONHC_6H_4SO_2F$-*p*	L1210/DF8 (A)	0.030	0.060	60	31	0.5	20
		W256 (A)		0.050	60	20		

TABLE I (*Continued*)

No.	R'	Enzyme source[c]	I_{50},[d] μM	Inhibitor, μM	Time, min	% inactvn[e]	ED_{50},[f] μM	ED_{50}/I_{50}
14	3-Cl-4-$SCH_2CONHC_6H_4SO_2F$-*m*	L1210/DF8 (A)	0.022	0.050	60	27	6	300
		W256 (A)		0.050	60	33		
15	3-Cl-4-$S(CH_2)_2CONHC_6H_4SO_2F$-*p*	L1210/DF8 (A)	0.041	0.082	60	74	0.1	2
		Mouse liver (A)		0.082	60	13		
		Mouse liver (C)		0.082	60	59		
		W256 (A)		0.082	60	57		
		Rat liver (A)		0.082	60	68		

[a] The technical assistance of Julie Leseman, Janet Wood, and Diane Shea 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.[6] [d] Concn for 50% inhibn when assayed with 6 μM dihydrofolate and 0.15 *M* KCl in pH 7.4 Tris buffer as prveiously 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] 60-min incubns were run at 37° and 20-min incubns at 24°. [f] Concn for 50% inhibn of L1210 cell culture. [g] Data from ref 3, 5, and 6.

SCHEME I



L1210 mouse leukemia. The compounds were also evaluated for inhibition of L1210 cell culture;[9] when the ED_{50} for cell inhibition is normalized as ED_{50}/I_{50}, the resultant ratio gives a good estimate of the relative rates of penetration of the L1210 cell wall plus the irreversible inhibition of the target enzyme inside the cell.[7,10,11] Those compounds that were good irreversible inhibitors of a tumor enzyme were then investigated for inactivation of the dihydrofolate reductase from the liver to determine if tissue specificity had been achieved. The tissue specificity can be due to rapid hydrolysis of the SO_2F group to SO_3H by liver sulfonyl fluoridase[12] or due to a difference in the structure of the dihydrofolate reductase from tumor and liver.[13] These two mechanisms are differentiated by use of affinity column purified[4] dihydrofolate reductase from liver; when inactivation of crude enzyme is poor but inactivation of purified enzyme is good, the specificity with the crude enzyme is due to sulfonyl fluoridase catalyzed hydrolysis of the SO_2F group.[6]

Of the 15 compounds in Table I, seven (**1**, **2**, **5**, **7–10**) showed good irreversible inhibition of the enzyme from Walker 256 rat tumor. Of these 7 compounds, only two (**2**, **5**) showed tissue specificity with a low inactivation of the enzyme from rat liver; in both cases, inactivation of the affinity column purified enzyme from liver was high, indicating that specificity was due to the action of the sulfonyl fluoridase in liver.

Seven compounds (**1**, **5**, **7–9**, **12**, **15**) in Table I showed good irreversible inhibition of the enzyme from L1210 mouse leukemia. Of these 7 irreversible inhibitors, five (**1**, **5**, **8**, **12**, **15**) showed tissue specificity by giving a low inactivation of the mouse liver enzyme; this specificity was due to the action of the sulfonyl fluoridase.

Of the 6 compounds showing inactivation of one of the two tumor enzymes and showing tissue specificity, the order of effectiveness on L1210 cell culture was **8** ≫ **12** = **15** > **1** = **2** = **5**; since **8** is 100-fold more effective than **1** in L1210 cell culture and since **1** shows good *in vivo* activity against Walker 256 ascites[5] and fair *in vivo* activity against L1210 (confirmed 30% life extension),[14] **8** would be particularly worthy of *in vivo* evaluation.

Chemistry.—The general route used for the synthesis of compounds **2–6** is shown in Scheme I. This pathway has been previously described[3] for the synthesis of **1**. Alkylation of the nitrophenols (**16**, R = Cl, H) with the appropriate bromo- or chloro-substituted esters (**17**, X = Br, Cl) yielded ester derivatives of type **18**, which were hydrolyzed to the acids **19**. The Me and

(9) We wish to thank Dr. Florence White of the CCNSC for the L1210 cell culture data obtained by Dr. P. Thayer of Arthur D. Little, Inc.

(10) B. R. Baker and R. B. Meyer, Jr., *J. Med. Chem.*, **12**, 668 (1969), paper CLIV of this series.

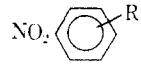
(11) B. R. Baker, E. E. Janson, and N. M. J. Vermeulen, *ibid.*, **12**, 898 (1969), paper CLX of this series.

(12) A. J. Ryan, N. M. J. Vermeulen, and B. R. Baker, *ibid.*, **13**, 000 (1970), paper CLXXIV of this series.

(13) B. R. Baker and N. M. J. Vermeulen *ibid.*, **13**, 1154 (1970), paper CLXXVII of this series.

(14) Unpublished data from this laboratory.

TABLE II
PHYSICAL CONSTANTS OF



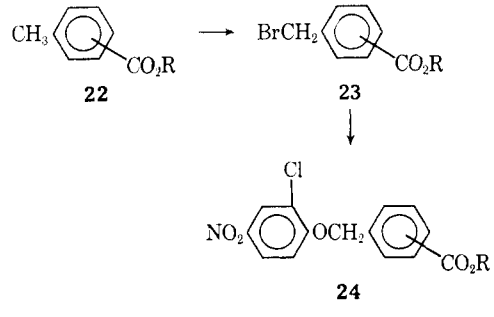
No.	R	Method	Yield,[a] %	Mp, °C	Formula[b]
18a	3-Cl-4-O(CH$_2$)$_3$CO$_2$Et	A	92[c]	Oil	
18b	3-Cl-4-OCH(CH$_3$)CO$_2$Et	A[d]	83[e]	116–117	$C_{11}H_{12}ClNO_5$
18c	3-Cl-4-O(CH$_2$)$_4$CO$_2$Et	A	93[c]	Oil	
18d	3-OCH$_2$CO$_2$Bu-*t*	A	85[f]	53	$C_{12}H_{15}NO_5$
19a	3-Cl-4-O(CH$_2$)$_3$CO$_2$H	B	73[g,h]	152–153[i]	
19b	3-Cl-4-OCH(CH$_3$)CO$_2$H	B	75[e]	153–154[j]	
19c	3-Cl-4-O(CH$_2$)$_4$CO$_2$H	B	63[g,k]	103–104	$C_{11}H_{12}ClNO_5$
19d	3-OCH$_2$CO$_2$H	C	99	156[l]	
20a	3-Cl-4-O(CH$_2$)$_3$CONHC$_6$H$_4$SO$_2$F-*p*	D	76[c]	181–182	$C_{16}H_{14}ClFN_2O_6S$
20b	3-Cl-4-O(CH$_2$)$_3$CONHC$_6$H$_4$SO$_2$F-*m*	D	61[h]	122–123	$C_{16}H_{14}ClFN_2O_6S$
20c	3-Cl-4-OCH(CH$_3$)CONHC$_6$H$_4$SO$_2$F-*p*	D	77[c]	208–209	$C_{15}H_{12}ClFN_2O_6S$
20d	3-Cl-4-O(CH$_2$)$_4$CONHC$_6$H$_4$SO$_2$F-*p*	D	78[c]	150–151	$C_{17}H_{16}ClFN_2O_6S$
20e	3-OCH$_2$CONHC$_6$H$_4$SO$_2$F-*p*	D	84[c]	200–201	$C_{14}H_{11}FN_2O_6S$
24a	3-Cl-4-OCH$_2$C$_6$H$_4$CO$_2$Me-*p*	E	62[m]	207–208	$C_{15}H_{12}ClNO_5$
24b	3-Cl-4-OCH$_2$C$_6$H$_4$CO$_2$Et-*m*	E	54[m]	135–136	$C_{16}H_{14}ClNO_5$
24c	3-Cl-4-OCH$_2$C$_6$H$_4$CO$_2$Et-*o*	E	58[m]	116–117	$C_{16}H_{14}ClNO_5$
25a	3-Cl-4-OCH$_2$C$_6$H$_4$CO$_2$H-*p*	B	82[c]	254	$C_{14}H_{10}ClNO_5$
25b	3-Cl-4-OCH$_2$C$_6$H$_4$CO$_2$H-*m*	B	67[n]	215–217[o]	$C_{14}H_{10}ClNO_5$
25c	3-Cl-4-OCH$_2$C$_6$H$_4$CO$_2$H-*o*	B	51[c]	189	$C_{14}H_{10}ClNO_5$
26a	3-Cl-4-OCH$_2$C$_6$H$_4$-4-CONHC$_6$H$_4$SO$_2$F-*p*	D	83[p]	222–223	$C_{20}H_{14}ClFN_2O_6S$
26b	3-Cl-4-OCH$_2$C$_6$H$_4$-4-CONHC$_6$H$_4$SO$_2$F-*m*	D	80[c]	231–232	$C_{20}H_{14}ClFN_2O_6S$
26c	3-Cl-4-OCH$_2$C$_6$H$_4$-3-CONHC$_6$H$_4$SO$_2$F-*p*	D	76[c]	237	$C_{20}H_{14}ClFN_2O_6S$
26d	3-Cl-4-OCH$_2$C$_6$H$_4$-3-CONHC$_6$H$_4$SO$_2$F-*m*	D	67[c]	191–192	$C_{20}H_{14}ClFN_2O_6S$
26e	3-Cl-4-OCH$_2$C$_6$H$_4$-2-CONHC$_6$H$_4$SO$_2$F-*p*	D	71[c]	226	$C_{20}H_{14}ClFN_2O_6S$
29a	3-Cl-4-SCH$_2$CO$_2$Et	F	78[q]	72–73	$C_{10}H_{10}ClNO_4S$
29b	4-SCH$_2$CO$_2$Et	A	36[q]	46–48[r]	
29c	3-Cl-4-S(CH$_2$)$_2$CO$_2$Me	F	69[s]	92–93	$C_{10}H_{10}ClNO_4S$
30a	3-Cl-4-SCH$_2$CO$_2$H	B	87[s]	127	$C_8H_6ClNO_4S$
30b	4-SCH$_2$CO$_2$H	B	92	155–157[t]	
30c	3-Cl-4-S(CH$_2$)$_2$CO$_2$H	G	64[u]	117–118	$C_9H_8ClNO_4S \cdot 0.25C_6H_6$
31a	3-Cl-4-SCH$_2$CONHC$_6$H$_4$SO$_2$F-*p*	D	77[c]	204	$C_{14}H_{10}ClFN_2O_5S_2$
31b	4-SCH$_2$CONHC$_6$H$_4$SO$_2$F-*p*	D	70[c]	192–193	$C_{14}H_{11}FN_2O_5S_2$
31c	3-Cl-4-SCH$_2$CONHC$_6$H$_4$SO$_2$F-*m*	D	76[c]	192–194	$C_{14}H_{10}ClFN_2O_5S_2$
31d	3-Cl-4-S(CH$_2$)$_2$CONHC$_6$H$_4$SO$_2$F-*p*	D	73[c]	195–196	$C_{15}H_{12}ClFN_2O_5S_2$

[a] Yield of anal. pure material, except where indicated. [b] Anal. C, H, N. [c] Yield of crude product. [d] Product crystd directly upon addn of reaction mixture to H_2O. [e] Recrystd from 2-methoxyethanol–H_2O. [f] Recrystd from petroleum ether (bp 30–60°). [g] Overall yield for alkylation and hydrolysis. [h] Recrystd from EtOH–H_2O. [i] Lit. [J. K. Faulkner and D. Woodcock, *J. Chem. Soc. C*, 884 (1966)] mp 150–151° for prepn by a different route. [j] Lit.[16] mp 150–152°. [k] Recrystd from C_6H_6–petroleum ether (bp 65–110°). [l] Lit.[17] mp 154–155°. [m] Recrystd from DMF–H_2O. [n] Recrystd from EtOAc–petroleum ether (bp 65–110°). [o] Anal. sample, apparently a different cryst form, had mp 194–195°. Subsequent prepn. gave the higher melting point. [p] Recrystd from 2-methoxyethanol. [q] Recrystd from MeOH. [r] Lit. [P. Friedländer and A. Chwala, *Monatsh. Chem.*, **28**, 247 (1907)]. mp 46–47° for prepn by another route. [s] Recrystd from PhMe. [t] Lit.[7] mp 156–158° for prepn by a different method. [u] Recrystd from C_6H_6.

Et esters were hydrolyzed by $^-$OH in aq EtOH, whereas the *t*-Bu ester (**18d**) was converted into the acid (**19d**) by elimination of isobutylene when refluxed in PhMe containing a catalytic amount of TsOH.[3] Treatment of **19** with $SOCl_2$ followed by reaction with sulfanilyl or metanilyl fluoride afforded the amides **20**. Catalytic hydrogenation of **20** in the presence of PtO_2 yielded the crude amines **21**, which were reacted with cyanoguanidine and acetone in the presence of acid[15] to give the dihydrotriazines (**2–6**). When a crystalline ethanesulfonate could not be obtained, the triazine was isolated as the HCl salt.

Inhibitors **7–11** were prepared in much the same manner as that described above. In this case the isomeric toluic acid esters **22** were treated with NBS to give the α-bromo derivatives **23**, which were converted without isolation into **24** by reaction with 2-chloro-4-nitrophenol at room temperature (Scheme II). Basic

SCHEME II



hydrolysis of **24** to the acids (**25**, see Table II) proceeded under conditions similar to those employed for **18** except that a longer reaction time was required owing to the relative insolubility of **24** in aqueous alcohol. Aque-

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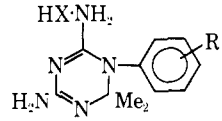
TABLE III
PHYSICAL CONSTANTS OF



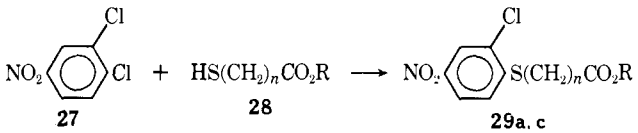
No.	R	HX	Method	Yield,[a] %	Mp, °C dec	Formula[b]
2	3-Cl-4-O(CH$_2$)$_3$CONHC$_6$H$_4$SO$_2$F-*p*	HCl	H	49[c]	242–243	$C_{21}H_{25}Cl_2FN_6O_4S$
3	3-Cl-4-O(CH$_2$)$_3$CONHC$_6$H$_4$SO$_2$F-*m*	HCl	H	21[d]	198–201	$C_{21}H_{25}Cl_2FN_6O_4S$
4	3-Cl-4-OCH(CH$_3$)CONHC$_6$H$_4$SO$_2$F-*p*	EtSO$_3$H	H	37[c]	216	$C_{22}H_{28}ClFN_6O_7S_2$
5	3-Cl-4-O(CH$_2$)$_4$CONHC$_6$H$_4$SO$_2$F-*p*	HCl	H	23[c]	224–226	$C_{22}H_{27}ClFN_6O_4S$
6	3-OCH$_2$CONHC$_6$H$_4$SO$_2$F-*p*	EtSO$_3$H	H	35[c]	194–196	$C_{21}H_{27}FN_6O_7S_2$
7	3-Cl-4-OCH$_2$C$_6$H$_4$-4-CONHC$_6$H$_4$SO$_2$F-*p*	EtSO$_3$H	I[e]	22[c]	213–215	$C_{27}H_{30}ClFN_6O_7S_2$
8	3-Cl-4-OCH$_2$C$_6$H$_4$-4-CONHC$_6$H$_4$SO$_2$F-*m*	EtSO$_3$H	I[f]	49[c]	193–195	$C_{27}H_{30}ClFN_6O_7S_2$
9	3-Cl-4-OCH$_2$C$_6$H$_4$-3-CONHC$_6$H$_4$SO$_2$F-*p*	EtSO$_3$H	I	57[c]	188–189	$C_{27}H_{30}ClFN_6O_7S_2$
10	3-Cl-4-OCH$_2$C$_6$H$_4$-3-CONHC$_6$H$_4$SO$_2$F-*m*	EtSO$_3$H	I	11[c]	178–179	$C_{27}H_{30}ClFN_6O_7S_2$
11	3-Cl-4-OCH$_2$C$_6$H$_4$-2-CONHC$_6$H$_4$SO$_2$F-*p*	EtSO$_3$H	I	33[c]	174–177	$C_{27}H_{30}ClFN_6O_7S_2$
12	3-Cl-4-SCH$_2$CONHC$_6$H$_4$SO$_2$F-*p*	EtSO$_3$H	J[g]	33[c]	196–197	$C_{21}H_{26}ClFN_6O_6S_3$
13	4-SCH$_2$CONHC$_6$H$_4$SO$_2$F-*p*	EtSO$_3$H	J	62[c]	228–230	$C_{21}H_{27}FN_6O_6S_3$
14	3-Cl-4-SCH$_2$CONHC$_6$H$_4$SO$_2$F-*m*	EtSO$_3$H	J[g]	27[c]	202–203	$C_{21}H_{26}ClFN_6O_6S_3$
15	3-Cl-4-S(CH$_2$)$_2$CONHC$_6$H$_4$SO$_2$F-*p*	EtSO$_3$H	J[g]	51[c]	179–181	$C_{22}H_{28}ClFN_6O_6S_3$

[a] Yield of anal. pure material. [b] Anal. C, H, F. [c] Recrystd from *i*-PrOH–H$_2$O. [d] Recrystd from Me$_2$CO–*i*-PrOH. [e] MeOH added to reaction mixture as cosolvent. [f] DMF added to reaction mixture as cosolvent, then removed by evapn *in vacuo* when reaction was complete; after addn of fresh Me$_2$CO, mixture was refluxed until product pptd. [g] Hydrogenation run in HOAc in presence of EtSO$_3$H.

ous DMSO has subsequently been found to be a useful solvent for the hydrolysis of esters of this type.[14] Hydrogenation of the NO$_2$ group of the corresponding amides **26** in the presence of Raney Ni proceeded smoothly with little or no hydrogenolysis.

The esters **29** which served as initial intermediates in the synthesis of **12–15**, the thio analogs of **1**, were prepared by one of two methods. Compound **29b** was obtained by alkylation of commercial *p*-nitrothiophenol with BrCH$_2$CO$_2$Et at room temp. This method is analogous to that shown for **18** in Scheme I. Since 2-chloro-4-nitrothiophenol was unavailable, a different procedure was utilized to prepare the remaining esters (Scheme III). The strongly activated 4-chloro group

SCHEME III



of 3,4-dichloronitrobenzene (**27**) readily underwent nucleophilic displacement by the mercapto esters **28** in the presence of K$_2$CO$_3$ in DMF to give **29a,c**.

The remainder of the sequence leading to triazines **12–15** generally corresponded to Scheme I. Because ester **29c** (n = 2; R = Me) is subject to facile base-catalyzed β elimination, acid hydrolysis was employed for the conversion of **29c** into its acid (**30c**). To prevent hydrogenolysis of the thioether linkage during the reduction of the NO$_2$ group of the amides **31**, Pd appeared to be the preferable catalyst. Since Pd can catalyze the hydrogenolysis of aromatic chloro substituents, however, compounds **31a,c,d** presented a special problem. An attempt to hydrogenate **31a** using PtO$_2$ as catalyst led to the apparent partial destruction of the thioether. Fortunately, when 10% Pd–C was used as catalyst, the hydrogenation of **31a,c,d** proceeded in HOAc in the presence of EtSO$_3$H with little or no hydrogenolysis.

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 consistent with their assigned structures; each gave combustion analyses for C, H, and N, or F within 0.4% of theory. The purity of analytical intermediates and dihydrotriazines was confirmed by tlc with Brinkman silica gel GF or polyamide MN, respectively.

tert-**Butyl *m*-Nitrophenoxyacetate (18d) (Method A).**—A mixture of 13.9 g (100 mmoles) of *m*-nitrophenol, 16.6 g (110 mmoles) of *tert*-butyl chloroacetate, 13.8 g (100 mmoles) of K$_2$CO$_3$, and 80 ml of DMF was stirred at 55° for 23 hr, then cooled, and added to 500 ml of H$_2$O. The product was extd with 300 ml of EtOAc and washed with 200 ml of 10% Na$_2$CO$_3$, then with three 300-ml portions of H$_2$O. The EtOAc soln was dried (MgSO$_4$), decolorized with charcoal, and spin-evaporated *in vacuo*. Recrystallization of the residue from petroleum ether (bp 30–60°) gave 21.5 g (85%) of nearly colorless crystals, mp 53° (tlc in C$_6$H$_6$). *Anal.* (C$_{12}$H$_{15}$NO$_5$) C, H, N.

2-(2-Chloro-4-nitrophenoxy)propionic Acid (19b) (Method B).—A mixture of 9.10 g (35 mmoles) of **18b** and 40 ml of 1.0 *N* NaOH (40 mmoles) in 90% MeOH was refluxed with swirling for 5 min, then cooled. The product which pptd upon addn of excess 5% HCl was collected on a filter and washed with H$_2$O. Recrystallization from 2-methoxyethanol–H$_2$O yielded 6.42 g (75%) of very light tan crystals: mp 153–154° (tlc in MeOH); lit.[16] mp 150–152°.

m-**Nitrophenoxyacetic Acid (19d) (Method C).**—A soln of 19.0 g (75 mmoles) of **18d** and 100 mg of TsOH·H$_2$O in 80 ml of PhMe was refluxed with stirring for 1.5 hr during which time pptn of product occurred. The cooled mixture was filtered and the product washed with C$_6$H$_6$ to give 14.6 g (99%) of nearly white crystals: mp 156° (tlc in MeOH); lit.[17] mp 154–155° for prepn by a different method.

N-(*m*-**Nitrophenoxyacetyl)sulfanilyl Fluoride (20e) (Method D).**—A mixture of 1.97 g (10 mmoles) of **19d**, 4 ml of SOCl$_2$, and 12 ml of C$_6$H$_6$ was refluxed with stirring with protection from moisture. After 2.5 hr, when evoln of gas had ceased, the soln was spin-evaporated *in vacuo*. To the residual oil were added 1.75 g (10 mmoles) of sulfanilyl fluoride and 30 ml of PhMe. The stirred mixture was refluxed for 16 hr, then cooled in ice. The product was collected by filtration and washed with C$_6$H$_6$. Recrystallization from 2-methoxyethanol–H$_2$O gave 2.96 g

(84%) of very light tan crystals: mp 200–201° (tlc in 1:1 EtOAc–petroleum ether). *Anal.* ($C_{14}H_{11}FN_2O_6S$) C, H, N.

Methyl α-(2-Chloro-4-nitrophenoxy)-*p*-toluate (24a) (Method E).—A mixture of 30.0 g (200 mmoles) of methyl *p*-toluate, 35.6 g (200 mmoles) of NBS, 300 mg of BzO_2, and 200 ml of CCl_4 was refluxed with stirring for 25 hr, then cooled in ice. The ppted succinimide was removed by filtration and washed with CCl_4. The combined filtrate and washings were spin-evapd under vacuum. To the residue were added 34.8 g (200 mmoles) of 2-chloro-4-nitrophenol, 27.6 g (200 mmoles) of K_2CO_3, and 200 ml of DMF. The mixture was stirred at room temp for 26 hr and then added to 1500 ml of H_2O. The ppted product was collected on a filter and washed with a large vol of H_2O, then with petroleum ether. Recrystn from DMF–H_2O afforded 40.0 g (62%) of light tan needles: mp 207–208° (tlc in 1:1 EtOAc–petroleum ether). *Anal.* ($C_{15}H_{12}ClNO_5$) C, H, N.

Ethyl 2-[(2-Chloro-4-nitrophenyl)thio]acetate (29a) (Method F).—A mixture of 9.60 g (50 mmoles) of 3,4-dichloronitrobenzene, 6.0 g (50 mmoles) of ethyl 2-mercaptoacetate, 6.9 g (50 mmoles) of K_2CO_3, and 50 ml of DMF was stirred at 75–80° for 45 min, then cooled, and added to 750 ml of H_2O. The product was collected on a filter, washed with H_2O, and recrystd from MeOH to give 10.8 g (78%) of light yellow crystals: mp 72–73° (tlc in C_6H_6). *Anal.* ($C_{10}H_{10}ClNO_4S$) C, H, N.

3-[(2-Chloro-4-nitrophenyl)thio]propionic Acid (30c) (Method G).—A stirred mixture of 5.50 g (20 mmoles) of **29c**, 100 ml of 6 *N* HCl, and 50 ml of dioxane was refluxed for 75 min, then cooled, and added to 500 ml of H_2O. The oil, which sepd, crystd readily upon scratching. The crude solid was dissolved as completely as possible in 100 ml of 5% $NaHCO_3$. The soln was filtered, washed with three 100-ml portions of $CHCl_3$, and finally acidified with 5% HCl. The product was collected on a filter and washed with H_2O. Recrystallization from C_6H_6 yielded 3.60 g (64%) of light yellow crystals: mp 117–118° (tlc in MeOH). *Anal.* ($C_9H_8ClNO_4S \cdot 0.25C_6H_6$) C, H, N.

***N*-[*m*-(4,6-Diamino-1,2-dihydro-2,2-dimethyl-*s*-triazin-1-yl)-phenoxyacetyl]sulfanilyl Fluoride Ethanesulfonate (6) (Method H).**—A mixture of 1.06 g (3.0 mmoles) of **20e**, 100 mg of PtO_2, and 100 ml of EtOH was shaken with H_2 at 1–3 atm until the reaction was complete (21 hr). THF was added to dissolve some precipitated product and the filtered soln was evapd *in vacuo*. To the residue were added 335 mg (3.05 mmoles) of $EtSO_2H$, 260 mg (3.1 mmoles) of cyanoguanidine, and 30 ml of Me_2CO. The mixture was refluxed with stirring for 24 hr, then cooled, and filtered. The crude product was washed with Me_2CO and recrystd twice from *i*-PrOH–H_2O to give 586 mg (35%) of white crystals: mp 194–196° dec (tlc in *i*-PrOH). *Anal.* ($C_{21}H_{25}FN_8O_5S_2$) C, H, F.

Method I was the same as method H except that Raney Ni was used as catalyst.

Method J was the same as method H except that 10% Pd-C was used as catalyst.

Synthesis and Biological Activity of Some 5-(1-Adamantyl)pyrimidines. I[1]

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Preparation of several 2-amino-4-hydroxy-5-(1-adamantyl)pyrimidines (**1–4**) and 5-(1-adamantylamino)uracil (**5**) is described. 2-Amino-4-hydroxy-5-(1-adamantyl)pyrimidine (**1**) and 2-amino-4-hydroxy-5-(1-adamantyl)-6-methylpyrimidine (**3**) were found to be moderately inhibitory to several lines of mouse sarcoma 180 cells (S-180) and to mouse mammary adenocarcinoma (TA3) in culture. Neither of these pyrimidines inhibited the enzyme folate reductase.

Diaminopteridines and pyrimidines play an important role as chemotherapeutic agents. Methotrexate is widely used in the treatment of acute childhood leukemia and choriocarcinoma[2] while pyrimethamine is effective in the treatment of malaria.[3] The chemotherapeutic activity of these drugs is due to the inhibition of the enzyme dihydrofolate reductase (also known as folate reductase and tetrahydrofolate dehydrogenase, EC 1.5.1.3).[4,5] Whereas methotrexate, one of the most potent inhibitors of this class of compounds, is distinguished by its lack of species specificity, diaminopyrimidines with 5-phenyl substituents exhibit highly specific inhibitory effects for dihydrofolate reductases from different species. Thus, for instance, pyrimethamine is 4000 and 50,000 times more inhibitory for plasmodial dihydrofolate reductase[6] than for the corresponding enzymes from human tissue or *Escherichia coli*, respectively.[5] On the other hand, trimethoprim (2,4-diamino-5-trimethoxyphenylpyrimidine) is 60,000 times more inhibitory for *E. coli* dihydrofolate reductase than for that of human origin.[5]

It was of interest to investigate the biochemical and biological activity of pyrimidines having in position 5 a highly lipophilic and bulky adamantyl group. The ultimate aim of this work was to prepare 2,4-diaminopyrimidines substituted with adamantane and its derivatives in position 5. However, preparation of such compounds was much more difficult than that of corresponding 2-amino-4-hydroxypyrimidines.[7] While the work on 2,4-diaminopyrimidines continues, a series of 2-amino-4-hydroxypyrimidines was prepared and tested for their biological activity.[8]

Synthesis.—Pyrimidines **1–3** (Table I) were prepared by condensing the appropriate β-carbonyl ester derivatives (**9–11**) with guanidine (Scheme I). The ester derivatives were synthesized by adaptation and modification of the procedures of Lunn, *et al.*,[9] who reported the preparation of ethyl (1-adamantyl)malonate (**10**) by condensation of ethyl malonate with 1-adamantanol as catalyzed by BF_3.

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(7) The term "hydroxypyrimidine" will be used throughout this paper for convenience even though it is realized that these compounds actually exist in the lactam, rather than the lactim, form.

(8) Recently, preparation of some 6-(1-adamantyl)pyrimidines as potential antiviral agents has been reported. M. Kuchar, J. Strof, and J. Vachek, *Coll. Czech. Chem. Commun.*, **34**, 2278 (1969).

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