Irreversible Enzyme Inhibitors. CXXXV.[1,2] Effect of Ring Substitution on the Selective Irreversible Inhibition of Dihydrofolic Reductase from L1210 Mouse Leukemia and Liver by 2,4-Diamino-5-(3,4-dichlorophenyl)-6-[*p*-(*p*-fluorosulfonylphenylureido)phenoxymethyl]pyrimidine

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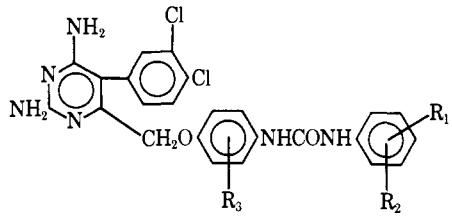
The title compound (**3**) was an active-site-directed irreversible inhibitor of the dihydrofolic reductase from L1210/0 and L1210/DF8 mouse leukemia. Since **3** could also appreciably inactivate mouse liver dihydrofolic reductase and since **3** was too poor a reversible inhibitor by a factor of 10, eight derivatives of **3** containing either one or two small substituents on the 6 side chain were synthesized and evaluated. The best compound (**6**) of the series was substituted with a 3-methyl group on the phenoxy moiety. This compound (**6**) had a satisfactory $K_I \simeq 10^{-8}$ *M* and showed 70% inactivation of the dihydrofolic reductase from L1210/DF8 at a K_I concentration; however, at $3K_i$ **6** still showed 25% inactivation of the dihydrofolic reductase from mouse liver.

The synthesis of **1** and its evaluation as an irreversible inhibitor of dihydrofolic reductase from L1210/FR8, L1210/DF8, and L1210/0 mouse leukemias, as well as mouse liver, was described in earlier papers of this series;[2,3] since **1** did not show irreversible specificity between the L1210 and mouse liver enzymes, a further study[2] was made on the effect of ring substitution (**2**)



1, $R_1 = m\text{-}SO_2F$; $R_2 = R_3 = H$
2, $R_1 = m\text{-}SO_2F$; R_2 or $R_3 \neq H$
3, $R_1 = p\text{-}SO_2F$; $R_3 = R_2 = H$
4, $R_1 = p\text{-}SO_2F$; R_2 or $R_3 \neq H$

on separation of irreversible inhibition of tumor *vs.* liver enzyme. It has now been found that the *p*-sulfonyl fluoride (**3**) is also an irreversible inhibitor of the dihydrofolic reductase from L1210 and mouse liver, being less effective on the enzyme from liver; therefore a study on the effect of ring substitution (**4**) on possible further separation of irreversible inhibition of the L1210 and liver enzymes has been performed. The results are the subject of this paper.

Enzyme Results.—The three criteria which should be met for a compound to be worthy of animal testing were discussed in detail in a previous paper:[2] (a) the $I_{50} \simeq 6K_I$ should be $\leq 10^{-7}$ *M*; (b) greater than 70% inactivation of the tumor enzyme should be seen at a $K_i \simeq I_{50}/6$ concentration; and (c) the liver enzyme should be inactivated <20% with the inhibitor at a $1\text{–}2I_{50}$ concentration.

Although **3** was an active-site-directed irreversible inhibitor[4] of dihydrofolic reductase from the three strains of L1210, **3** failed to meet any of three criteria for animal testing; however, at 10^{-6} *M* **3** did not show greater irreversible inhibition of the L1210/DF8 enzyme than the liver enzyme. Insertion of a 2-chloro atom (**5**) on the phenoxy moiety of **3** was not only detrimental to reversible binding, but irreversible inhibition was also less effective. In contrast, introduction of a 3-methyl group (**6**) on **3** gave a fivefold increment in reversible binding; furthermore, **6** at near K_i concentration gave good inactivation of the L1210/DF8 enzyme. Although the separation of effectiveness of inactivation on the L1210/DF8 *vs.* the liver enzyme was greater with **6** than in the case of **3**, the liver enzyme could be inactivated 68% by a concentration of **6** near $30K_i$.

Introduction of a 3-chloro atom (**7**) on **3** appeared to give little change in reversible inhibition. Unfortunately, this substitution gave a compound with much more effectiveness as an irreversible inhibitor of the liver enzyme.

Further studies consisted of substitution of a Me, Cl, or OMe *ortho* to the sulfonyl fluoride group (**8–12**); this type of substitution was detrimental in all cases, but not for the same reasons in each case. Substitution of an *o*-Me group (**8**) on **3** gave no change in reversible binding, but irreversible inhibition on the tumor enzymes was considerably diminished. The same *o*-Me substitution (**9**) on **6** also gave no change in reversible binding, but inactivation of the mouse liver enzyme was increased. Substitution of an *o*-Me group (**10**) on **7** led to no change in the reversible or irreversible parameters; neither **7** nor **10** were satisfactory due to their efficiency in inactivating the mouse liver enzyme. As noted previously with **1**,[2] insertion of a methoxyl group (**12**) *ortho* to the sulfonyl fluoride of **7** was detrimental to irreversible inhibition.

These results again emphasize how sensitive an irreversible inhibitor can be to simple structural changes.[4] The best compound in Table I is **6**, which meets two of the three criteria for *in vivo* testing against L1210/DF8; **6** has $I_{50} = 10^{-7}$ *M* and gives 70% inactivation of enzymes at $I_{50}/6 \simeq K_i$. However, at a $3K_i$ concentration, **6** still shows 25% inactivation of the liver enzyme and thus fails the third criterion.

Studies on variation of the bridge between the benzenesulfonyl and pyrimidine moieties of **1** and **3** were then conducted which did lead to compounds meeting

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

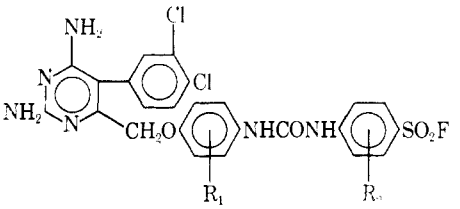
(2) For the previous paper of this series, see B. R. Baker and N. M. J. Vermeulen, *J. Med. Chem.*, **12**, 74 (1969).

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

(4) B. R. Baker, "Design of Active-Site-Directed Irreversible Enzyme Inhibitors. The Organic Chemistry of the Enzymic Active-Site," John Wiley and Sons, Inc., New York, N. Y., 1967.

TABLE I

INHIBITION[a] OF MOUSE DIHYDROFOLIC REDUCTASE BY

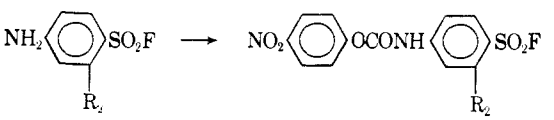


				Reversible[d]		Irreversible[e]			
No.	R_1[b]	R_2[c]	Enzyme source	I_{50},[f] μM	Estd $K_i \times 10^6$ M[g]	I, μM	EI,[h] %	Time, min	% inactvn
3	H	H	L1210/FR8	2.1	0.35	11	97	60	82
						2.1	87	25, 60	47, 47[i]
			L1210/0	1.1	0.18	4.2	96	60	74
						1.1	87	10, 60	21, 49[i]
			L1210/DF8			4.2		60	80
						1.1		60	72
			Liver			1.1		60	37
5	2-Cl	H	L1210/FR8	7.0	1.2	25	95	60	56
			L1210/DF8	3.3	0.55	3.3	87	60	58
			L1210/0			3.3		60	65
6	3-Me	H	L1210/FR8	0.31	0.052	0.31	87	8, 30, 60	50, 77, 92[i]
						0.050	50	20, 30, 60	50, 55, 71[i]
			L1210/0	0.19	0.032	0.62	95	60	86
						0.19	87	2, 60	63, 63[i]
			L1210/DF8	0.10	0.017	0.62	97	60	100
						0.050	75	60	84
						0.016	50	60	70
			Liver			0.62		60	68
						0.31		<30, 60	38, 38[i]
						0.050		60	25
7	3-Cl	H	L1210/DF8	0.98	0.16	2.0	92	60	90
			L1210/0			2.0		60	68
			Liver			2.0		60	87
8	H	3-Me	L1210/FR8	2.0	0.33	10	97	60	45
			L1210/0			2.0		60	29
			L1210/DF8			2.0		60	37
9	3-Me	3-Me	L1210/FR8	0.16	0.027	0.80	97	60	84
			L1210/0			0.32		60	61
			L1210/DF8			0.32		60	80
			Liver			0.32		60	81[i]
10	3-Cl	3-Me	L1210/DF8	0.98	0.16	2.0	92	60	87
			L1210/0			2.0		60	78[i]
			Liver			2.0		60	89
11	3-Cl	3-Cl	L1210/DF8	1.8	0.30	3.6	92	60	82[j]
			L1210/0			3.6		60	92[j]
			Liver			3.6		60	97[j]
12	3-Cl	3-OMe	L1210/DF8	0.52	0.087	1.0	92	60	63
			L1210/0			1.0		60	37

[a] The technical assistance of Sharon Lafler, Diane Shea, and Carolyn Wade with these assays is acknowledged. [b] Numbered from the ether linkage at the 1 position. [c] Numbered from the ureido linkage at the 1 position. [d] Assayed with 6 μM dihydrofolate and 30 μM TPNH in pH 7.4 Tris buffer containing 0.15 M KCl as previously described.[8,9] [e] Incubated at 37° in pH 7.4 Tris buffer in the presence of 60 μM TPNH as previously described;[9] the zero point was obtained by adding the inhibitor to zero-time aliquot at 0° unless otherwise indicated. [f] I_{50} = concentration for 50% inhibition. [g] Estimated from $K_i = K_m[I_{50}]/[S]$ which is valid since $[S] = 6K_m = 6\ \mu M$; see ref 4, p 202. [h] Estimated from $[EI] = [E_t]/(1 + K_i/[I])$ where [EI] is the fraction of total enzyme (E_t) reversibly complexed; see ref 4, Chapter 8. [i] From a time study plot; see ref 8. [j] Zero point modified by addition of inhibitor to cuvette.[8,9]

the three criteria for animal testing; these results will be presented in future papers.

Chemistry.—The irreversible inhibitors in Table I can be generalized by **4**. Such compounds are readily made by the Crosby[5] mixed-urea synthesis when modified by use[2] of a *p*-nitrophenyl leaving group as in **14**. Reaction of the substituted sulfanilyl fluoride (**13**)[6] with *p*-nitrophenyl chloroformate[7] in C_6H_6 gave the required urethans (**14**); these were condensed with the 6-amino-



13 **14**

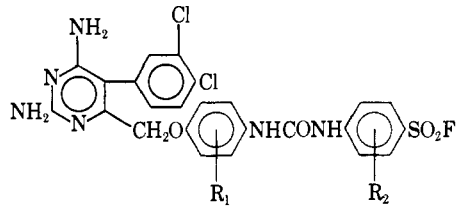
(5) (a) D. G. Crosby and C. Niemann, *J. Am. Chem. Soc.*, **76**, 4458 (1954); (b) B. R. Baker and R. P. Patel, *J. Pharm. Sci.*, **52**, 927 (1963).

(6) B. R. Baker and G. J. Lourens, *J. Med. Chem.*, **11**, 677 (1968), paper CXXIX of this series.

(7) G. W. Anderson and A. C. McGregor, *J. Am. Chem. Soc.*, **79**, 6180 (1957).

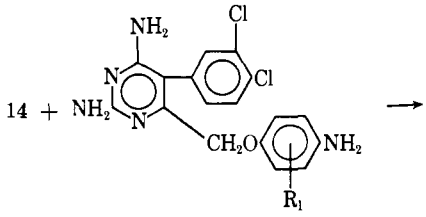
TABLE II

PHYSICAL PROPERTIES OF



No.[a]	R_1	R_2	Yield, %	Mp, °C dec[g]	Formula	Analyses
3	H	H	15[b]	210	$C_{24}H_{19}Cl_2FN_6O_4S \cdot 0.5H_2SO_4 \cdot 0.5H_2O$	C, H, F
5	2-Cl	H	49[b]	210	$C_{24}H_{18}Cl_3FN_6O_4S \cdot 0.5H_2SO_4 \cdot H_2O$	C, H, F
6	3-Me	H	21[c]	203	$C_{25}H_{21}Cl_2FN_6O_4S \cdot 0.5H_2SO_4 \cdot 0.5H_2O$	C, H, F
7	3-Cl	H	45[b]	201	$C_{24}H_{18}Cl_3FN_6O_4S \cdot 0.5H_2SO_4$	C, H, F
8	H	3-Me	22[d]	180	$C_{25}H_{21}Cl_2FN_6O_4S \cdot C_2H_5SO_3H \cdot 0.5H_2O$	C, H, F
9	3-Me	3-Me	52[e]	201	$C_{26}H_{23}Cl_2FN_6O_4S \cdot 0.5H_2SO_4 \cdot 0.5H_2O$	C, H, F
10	3-Cl	3-Me	42[b]	188	$C_{25}H_{20}Cl_3FN_6O_4S \cdot 0.5H_2SO_4 \cdot 2H_2O$	C, H, F
11	3-Cl	3-Cl	38[f]	203	$C_{24}H_{17}Cl_4FN_6O_4S \cdot 0.5H_2SO_4$	C, H, F
12	3-Cl	3-OMe	54[d]	176	$C_{25}H_{20}Cl_3FN_6O_5S \cdot 0.5C_2H_5OH$	C, H, F

[a] Prepared by method E as previously described.[2] [b] Recrystallized from MeOEtOH–H_2O. [c] Recrystallized by MeOH–MeOEtOH. [d] Recrystallized from EtOH. [e] Recrystallized from MeOEtOH–EtOH. [f] Recrystallized from MeOEtOH. [g] Melting gradually occurred over a wide range starting at the temperature indicated.



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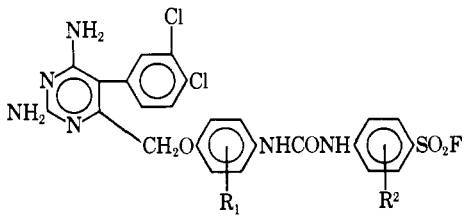

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a, R_2 = H e, R_1 = H
b, R_2 = Me f, R_1 = 2-Cl
c, R_2 = Cl g, R_1 = 3-Me
d, R_2 = OMe h, R_1 = 3-Cl

phenoxymethylpyrimidines (**15**)[2] to give the irreversible inhibitors (**4**).

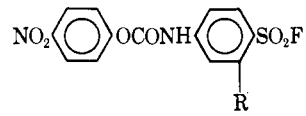
Experimental Section

Melting points were taken in capillary tubes on a Mel-Temp block and are uncorrected. All analytical samples had uv and ir spectra compatible with their assigned structures and each moved as a single spot on tlc. All analytical samples gave combustion values for C, H, and N or F within 0.4% of theoretical. The urethans (**14**) in Table III were prepared by method C and the irreversible inhibitors in Table II were prepared by method E, as described in the previous paper.[2]

The enzyme assays were performed as previously described.[8,9]

TABLE III

PHYSICAL PROPERTIES OF



No.[a]	R	Yield, %	Mp, °C	Formula	Analyses
14a	H	58[b]	169–170	$C_{13}H_9FN_2O_6S$	C, H, F
14b	Me	53[c]	171–173	$C_{14}H_{11}FN_2O_6S$	C, H, N
14c[d]	Cl	44[b]	181–187	$C_{13}H_8ClFN_2O_6S$	C, H, N
14d	OMe	53[e]	175–178	$C_{14}H_{11}FN_2O_7S$	C, H, N

[a] Prepared by method C as previously described;[2] see ref 6 for starting amines. [b] Recrystallized from EtOAc–petroleum ether (bp 60–110°). [c] Recrystallized from CH_2Cl_2. [d] Prepared by E. H. Erickson of this laboratory. [e] Recrystallized from C_6H_6.

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

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