Irreversible Enzyme Inhibitors. CXL.[1,2] Active-Site-Directed Irreversible Inhibitors Derived from 1-(3-Chlorophenyl)-4,6-diamino-1,2-dihydro-2,2-dimethyl-*s*-triazine

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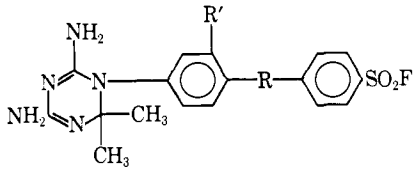
4,6-Diamino-1,2-dihydro-2,2-dimethyl-1-phenyl-*s*-triazines bearing the following substituents on the *para* position were not irreversible inhibitors of dihydrofolic reductase from L1210 mouse leukemia: $OCH_2CONHC_6H_4SO_2F$-*p* (**2a**), $O(CH_2)_2OC_6H_4SO_2F$-*p* (**3a**), $O(CH_2)_3OC_6H_4SO_2F$-*p* (**7**), $(CH_2)_2C_6H_4SO_2F$-*p* (**11**), and $OCH_2CONHC_6H_4SO_2F$-*m* (**16**). These results contrast with the $(CH_2)_2CONHC_6H_4SO_2F$-*p* derivative (**1a**) which is an active-site-directed irreversible inhibitor of L1210 dihydrofolic reductase, the difference being rationalized by the difference in allowable ground-state conformations. By limiting the number of ground-state conformations of **2a**, **3a**, **7**, **11**, and **16** by insertion of a chloro atom on the *meta* position of the 1-phenyl-*s*-triazine moiety, the resultant compounds (**2b**, **3b**, **8**, **12**, and **15**, respectively) were converted into irreversible inhibitors of the enzyme. Of these five irreversible inhibitors, **8** and **3b** could also inactivate the enzyme from mouse liver; since **2b**, **12**, and **15** did not inactivate the mouse liver enzyme appreciably, these three compounds showed the desired specificity pattern needed for chemotherapy.

The first active-site-directed irreversible inhibitor[4] of dihydrofolic reductase from L1210 mouse leukemia discovered in this laboratory was the 1-phenyl-*s*-triazine bearing a *p*-propionylsulfanilyl fluoride side chain (**1a**);[5] **1a** could also inactivate the dihydrofolic reductase from Walker 256 rat tumor[5] as well as a number of normal tissues from the rat[5] and mouse,[6] thus showing no specificity. In a study on R-bridged variants of **1a**, it was observed that the oxyacetamido bridge

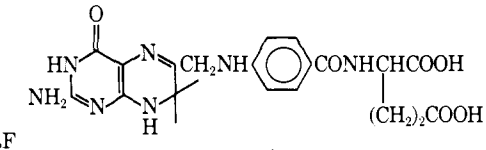


1a, R = $-(CH_2)_2CONH-$; R′ = H
b, R = $-(CH_2)_2CONH-$; R′ = Cl
2a, R = $-OCH_2CONH-$; R′ = H
b, R = $-OCH_2CONH-$; R′ = Cl
3a, R = $-O(CH_2)_2O-$; R′ = H
b, R = $-O(CH_2)_2O-$; R′ = Cl

(**2a**) and ethylenedioxy bridge (**3a**) gave compounds that were as good reversible inhibitors of dihydrofolic reductase as **1a**, but failed to show irreversible inhibition of the enzyme from L1210/FR8 mouse leukemia or Walker 256 rat tumor.[7]

The failure of **2a** and **3a** to inactivate the enzyme clearly showed that their SO_2F group was not positioned the same as **1a** within the rate-limiting reversible enzyme–inhibitor complex;[4] the difference in these complexes was rationalized on the basis that **2a** had one extra degree of free rotation (shown in **6**) and **3a** two extra degrees compared to the parent **1a** (shown in **5**); these extra degrees of freedom of rotation would allow **2a** and **3a** to assume a better conformation for reversible binding which could be different from the conformation **1a** needed for covalent bond formation within the enzyme–inhibitor complex. These different binding conformations can be illustrated with the following examples.

The substrate, dihydrofolate (**4**), can be assigned an arbitrary positioning of its pteridine ring as indicated in **4** by allowing the enzyme to position around this



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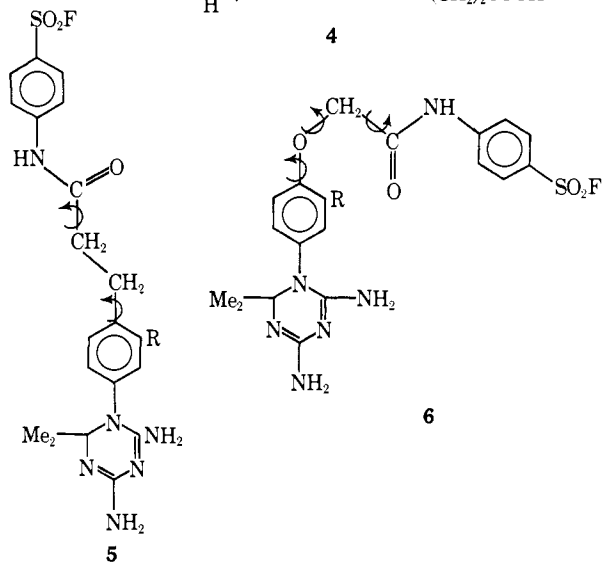


5 **6**

conformation.[8] The dihydro-*s*-triazine ring of **5** and **6** rotates in the active site in order to give optimum hydrophobic bonding of the 1-phenyl group to a hydrophobic bonding region on the enzyme that resides near the position assumed by the 4-oxo group (or 8-N) of dihydrofolate.[8] The substituent on the *para* position of the benzene ring will then take a conformation allowing maximum net reversible binding energy, that is, if an unfavorable conformation must be assumed by

(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 G. J. Lourens, *J. Med. Chem.*, **12**, 92 (1969).

(3) G. J. L. wishes to thank the Council for Scientific and Industrial Research, Republic of South Africa, for a tuition fellowship.

(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.

(5) B. R. Baker and G. J. Lourens, *J. Med. Chem.*, **10**, 1123 (1967), paper CV of this series.

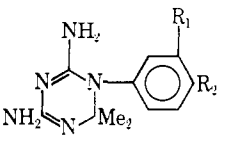
(6) B. R. Baker, G. J. Lourens, R. B. Meyer, Jr., and N. M. J. Vermeulen, *ibid.*, **12**, 67 (1969), paper CXXXIII of this series.

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

(8) For a review on the binding to dihydrofolic reductase and its hydrophobic region see ref 4, Chapter 10.

Table I

Inhibition[a] of Dihydrofolic Reductase by



No.	R_1	R_2	Enzyme source[b]	Reversible[c] I_{50},[e] μM	Reversible[c] K_I,[f] μM	Irreversible[d] Inhib, μM	Irreversible[d] % [EI][g]	Irreversible[d] Time, min	Irreversible[d] % inactvn
1a	H[h]	$(CH_2)_2CONHC_6H_4SO_2F$-*p*	L1210/FR8	0.080	0.013	0.070	86	<2, 10	77[i]
			L1210/0	0.012	0.0020	0.070	98	60	53[j]
						0.035	94	60	30[j]
			L1210/DF8	0.025	0.0041	0.070	94	60	73[j]
			Liver	0.015	0.0025	0.40	99	<2, 60	59[i,j]
						0.070	96	<2, 60	38[i,j]
			Spleen			0.10		60	73[j]
			Intestine			0.10		20	85[j]
1b	Cl	$(CH_2)_2CONHC_6H_4SO_2F$-*p*	L1210/FR8	0.048	0.0080	0.048	87	4, 60	85, 85[j]
						0.010	56	10, 60	10, 10[j]
			L1210/0	0.014	0.0023	0.096	98	60	95[j]
			L1210/DF8	0.024	0.0040	0.096	96	60	95[j]
						0.014	74	60	0[j]
			Liver	0.015	0.0025	0.096	98	60	30[j]
			Spleen			0.096		60	94[j]
			Intestine			0.13		20	75[j]
2a	H	$OCH_2CONHC_6H_4SO_2F$-*p*	L1210/FR8[k]	0.048	0.0080	0.24	97	60	0
			L1210/0			0.10		60	0–12[j]
			L1210/DF8	0.025	0.0042	0.10	96	60	0–17[j]
2b	Cl	$OCH_2CONHC_6H_4SO_2F$-*p*	L1210/DF8	0.037	0.0062	0.074	92	60	76[j]
			L1210/0			0.074		60	44[j]
			Liver			0.16		60	0[j]
3a	H	$O(CH_2)_2OC_6H_4SO_2F$-*p*	L1210/FR8[k]	0.052	0.0087	0.26	97	60	0
			L1210/0			0.10		60	0[j]
			L1210/DF8			0.10		60	0[j]
3b	Cl	$O(CH_2)_2OC_6H_4SO_2F$-*p*	L1210/FR8	0.062	0.013	0.31	97	60	95
						0.062	87	8, 60	50, 57[j]
			L1210/0	0.036	0.0060	0.12	96	60	80[j]
						0.036	87	3, 60	23, 23[j]
			L1210/DF8	0.036	0.0060	0.12	96	60	97[j]
			Liver	0.012	0.0020	0.12	99	60	41[j]
7	H	$O(CH_2)_3OC_6H_4SO_2F$-*p*	L1210/FR8	0.15	0.025	0.75	97	60	0
			L1210/0			0.30		60	6[j]
			L1210/DF8			0.75			18[j]
8	Cl	$O(CH_2)_3OC_6H_4SO_2F$-*p*	L1210/DF8	0.085	0.014	0.085	87	60	81[j]
			L1210/0			0.085		60	83[j]
			Liver			0.18		60	61[j]
			Spleen			0.18		60	73[j]
			Intestine			0.18		20	67[j]
9	H	$O(CH_2)_4OC_6H_4SO_2F$-*p*	L1210/FR8	0.068	0.011	0.34	97	60	0
			L1210/0			0.34		60	8–38[j]
			L1210/DF8			0.34		60	8–19[j]
10	H	$OC_6H_4SO_2F$-*p*	L1210/FR8	1.6	0.27	7.9	97	60	0
			L1210/0			3.2		60	0
			L1210/DF8			3.2		60	4[j]
11	H	$(CH_2)_2C_6H_4SO_2F$-*p*	L1210/FR8[k]	0.039	0.0065	0.19	97	60	0
			L1210/0			0.16		60	0[j]
			L1210/DF8			0.16		60	0[j]
12	Cl	$(CH_2)_2C_6H_4SO_2F$-*p*	L1210/DF8	0.014	0.0023	0.07	97	60	93[j]
						0.028	92	60	71[j]
			L1210/0			0.070		60	66[j]
						0.028		60	47[j]
			Liver			0.070		60	17[j]
13	H	$(CH_2)_4C_6H_4SO_2F$-*p*	L1210/DF8	0.020	0.0033	0.10	97	60	69[j]
			L1210/0			0.10		60	52[j]
			Liver			0.10		60	27[j]
14	Cl	$OCH_2CONC_6H_4SO_2F$-*p*	L1210/DF8	0.034	0.0057	0.068	92	60	47[j]
			L1210/0			0.068		60	35[j]
		CH_3	Liver			0.068		60	0[j]

TABLE I (*Continued*)

No.	R_1	R_2	Enzyme source[b]	Reversible[c] I_{50},[e] μM	Reversible[c] K_i,[f] μM	Irreversible[d] Inhib, μM	Irreversible[d] % [EI][g]	Irreversible[d] Time, min	Irreversible[d] % inactvn
15	Cl	$OCH_2CONHC_6H_4SO_2F$-*m*	L1210/DF8	0.019	0.0032	0.095	97	60	61[i]
						0.038	92	60	42[i]
			L1210/0			0.095			62[i]
			Liver			0.095			13[i]
16	H	$OCH_2CONHC_6H_4SO_2F$-*m*	L1210/FR8[k]	0.069	0.012	0.22	93	60	0
			L1210/0			0.14		60	0[i]

[a] The technical assistance of Diane Shea, Sharon Lafler, and Carolyn Wade with the assays is acknowledged. [b] The L1210/0 is the parent wild strain, while the /FR8 and /DF8 are strains resistant to amethopterin with a high level of dihydrofolic reductase; liver, spleen, and intestine were from normal BDF_1 mice.[6] [c] Assayed with 6 μM dihydrofolate and 30 μM TPNH in pH 7.4 Tris buffer containing 0.15 M KCl as previously described.[5,6] [d] Incubated at 37° in pH 7.4 Tris buffer in the presence of 60 μM TPNH as previously described.[5,6] [e] I_{50} = concentration for 50% inhibition. [f] Estimated from $K_i = K_m[I_{50}]/[S]$ which is valid since $[S] = 6K_m = 6\ \mu M$ dihydrofolate; see ref 4, p 202. [g] Calculated from $[EI] = [E_t]/(1 + K_i/[I])$ where [EI] is the amount of total enzyme (E_t) reversibly complexed; see ref 4, Chapter 8. [h] Data from ref 5 and 6. [i] From time-study plot.[5] [j] The zero point was determined by adding the inhibitor to the assay cuvette.[5,6] [k] Data from ref 7.

the substituent, it does so at the expense of binding energy. Suppose the optimum reversible binding occurs in conformation **6**; the oxyacetamido bridge of **2a** can allow **2** to assume this conformation **6** with little or no loss of energy. In contrast, for **1a** to assume this **6** conformation, the ethane moiety would have to be eclipsed which would require about 2 kcal/mole of energy. Suppose the second best binding conformation is **5**; **1a** can assume this conformation **5** with no loss in binding since no change in conformation is required from the ground state. It is assumed that in this **5** conformation, **1a** can rapidly form a covalent bond with the enzyme. It follows that **2a** could assume conformation **5** for optimum binding if a proper substitution would no longer allow the ground-state conformation **6**. The proper substituent was chosen on the following basis.

A *m*-chloro substituent on the benzene ring of a 1-phenyl-1,2-dihydro-2-triazine complexes directly with the enzyme giving about a tenfold increment in binding.[9] That this binding by the Cl atom is toward the active site on the right as indicated by R = Cl in **5** and **6** has previously received experimental support.[10] Since the Cl atom binding to the enzyme on the right was indicated, the chlorophenyl moiety would prefer not to assume a conformation when binding to the enzyme that projects the Cl to the left. If the Cl atom is complexed to the right as in conformation **5** and **6**, the conformation **6** for the oxyacetamido bridge of **2b** will not be allowable due to an *ortho* steric effect; thus, the second most favorable conformation (**5**) for complexing the sulfanilyl fluoride will be assumed for complexing. Thus **2b** should be an irreversible inhibitor comparable to **1a**; furthermore, a similar Cl substitution (**1b**) on **1a** should still allow **1b** to be an irreversible inhibitor since both **1a** and **1b** can assume conformation **5** when complexing with the enzyme. The experimental support of this hypothesis on the effect of allowable ground-state conformations on irreversible inhibition[11] is the subject of this paper.

Enzyme Results.—It was previously reported that the phenoxyacetamidosulfanilyl fluoride (**2a**) was not an irreversible inhibitor of the dihydrofolic reductase from the L1210/FR8 strain of mouse leukemia;[7] it has now been observed that **3a** also does not show appreciable irreversible inhibition of the enzyme from L1210/0, the parent strain of mouse leukemia, nor the enzyme from mouse liver. Insertion of a Cl atom (**2b**) *ortho* to the oxyacetyl group of **2a** resulted in no change in reversible binding. As predicted by the hypothesis described above, **2b** was an irreversible inhibitor of the dihydrofolic reductase from L1210; however, **2b** still failed to inactivate this enzyme from mouse liver, thus showing specificity.

The hypothesis also predicts that substitution of a Cl atom (**1b**) on the parent **1a** should cause little change in the irreversible inhibition pattern. That both **1a** and **1b** were irreversible inhibitors of dihydrofolic reductase from three strains of L1210 and three normal tissues of the mouse is shown in Table I. Furthermore, there was no change in reversible binding between **1a** and **1b**. The fact that there is no change in reversible binding between **1a** and **1b** indicates that there is no *net* binding by the chlorine atom, in contrast to a tenfold increment that might be expected.[9] Thus, it is unlikely that **1a** assumes the extreme conformation **5**[11] when complexing to the enzyme, else a tenfold increment should have been seen with **1b**. It is likely that **1a** and **1b** assume a conformation when complexed to the enzyme that causes a slight steric interaction between the Cl and side chain of **1b**, which in turn causes a loss in binding of about 1 kcal/mole, thus resulting in no *net* binding by the chloro atom; such intermediate conformations between the extremes of **5** and **6** can be drawn.

The hypothesis also suggested that chloro substitution (**3b**) on the ethylenedioxysulfonyl fluoride (**3a**) might convert **3a** to an irreversible inhibitor of dihydrofolic reductase. It was previously reported that **3a** was not an irreversible inhibitor of the enzyme from L1210/FR8;[7] **3a** is also not an irreversible inhibitor of the enzyme from L1210/0 and mouse liver. In contrast, the chloro derivative (**3b**) was an irreversible inhibitor of the enzyme from all three sources; note that there was little change in reversible inhibition of the L1210/FR8 enzyme with **3a** and **3b**.

Since **3b** was an irreversible inhibitor of dihydrofolic reductase, but showed insufficient specificity toward the enzyme from L1210 and mouse liver, additional

(9) (a) B. R. Baker and B.-T. Ho, *J. Heterocycl. Chem.*, **2**, 335 (1965); (b) B. R. Baker, *J. Med. Chem.*, **11**, 483 (1968); (c) B. R. Baker and M. A. Johnson, *ibid.*, **11**, 486 (1968), paper CXVIII of this series.

(10) B. R. Baker, P. C. Huang, and R. B. Meyer, Jr., *ibid.*, **11**, 475 (1968), paper CXVI of this series.

(11) Conformations **5** and **6** are extremes to demonstrate the hypothesis; conformations intermediate between **5** and **6** are allowable with **1a**, but the same argument on a decrease in allowable binding conformations of **2b** compared to **2a** still holds.

homologs of **3a** and **3b** with varying oxygenated bridges were investigated. The higher trimethylene homolog (**7**) gave no inhibition of the enzyme from L1210, but showed a low order of irreversible inhibition of the mouse liver enzyme. Insertion of the Cl atom (**8**) gave a compound showing irreversible inhibition of the dihydrofolic reductase from L1210, but unfortunately **8** was also an irreversible inhibitor of the enzyme from mouse liver, spleen, and intestine.

The tetramethylenedioxy homolog (**9**) was a fast, but poor, irreversible inhibitor of the enzyme from L1210; the total irreversible inhibition by such a fast, poor inhibitor is difficult to measure accurately due to the variation in zero point.[6] Unfortunately the chloro derivative of **9** could not be measured due to failure of the synthetic route. When only an oxy bridge was present (**10**), the compound was too poor a reversible inhibitor to warrant synthesis of its chloro derivative; **10** was not an irreversible inhibitor. It was previously reported that the ethane bridge gave a compound (**11**) that was not an irreversible inhibitor of the enzyme from L1210/FR8;[7] as shown in Table I, **11** was also not an irreversible inhibitor of the enzyme from the other two strains of L1210. However, when a chloro substituent was introduced, the resultant **12** was a good irreversible inhibitor of the L1210 enzyme, but a barely detectable irreversible inhibitor of the mouse liver enzyme.

The candidate irreversible inhibitor with a butane bridge (**13**) was an irreversible inhibitor of the L1210 enzyme when assayed at a concentration of about $30K_i$; however, at this concentration it also showed a lower amount of irreversible inhibition of the mouse liver enzyme. The effectiveness of **13** as an irreversible inhibitor of dihydrofolic reductase compared to **3a** again demonstrates that the SO_2F group of **13** and **3a** is not positioned identically within their reversible enzyme–inhibitor complexes. The butane analog of **3b** would be worthy of synthesis and evaluation.

Some studies were then performed on variation of the structure of the oxyacetamidosulfanilyl fluoride irreversible inhibitor (**2**). When the SO_2F moiety of **2a** was moved to the *meta* position, the resultant **16**[7] was still not an irreversible inhibitor of dihydrofolic reductase from L1210. However, insertion of a chloro atom gave **15** that was an irreversible inhibitor of the enzyme from L1210, but not mouse liver.

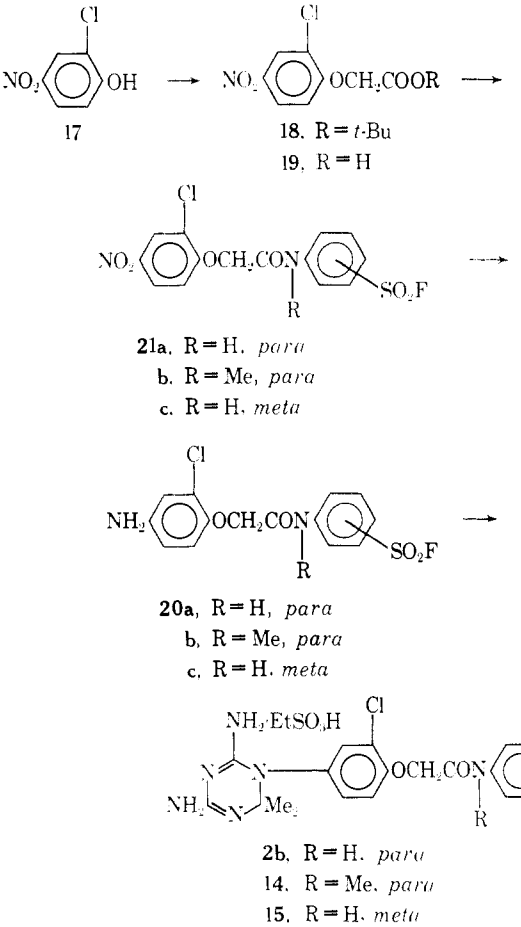
When an N-methyl group was inserted on **2b**, the resultant **14** was still an irreversible inhibitor, but was less effective than the parent **2b**. This N-methylation is known to make a profound change in the ground-state conformation of acetanilide[12] and presumably the same change between **2b** and **14**;[7] thus, it is possible that **2b** and **14** do not form a covalent bond with the same amino acid of the enzyme.

None of the compounds in Table I meet the three arbitrary enzyme criteria set for whether or not a compound is worthy of *in vivo* studies in mice.[6] Several of the compounds (**2b**, **12**, and **14**) meet all but the second criterion. Since these criteria are arbitrary, these compounds will be tested *in vivo* to check on the criteria.

Further bridge variants of **2b**, **3b**, and **12** with additional substituents on the benzenesulfonyl fluoride moiety are worthy of study to see if compounds can be found that meet all of the arbitrary criteria.

Chemistry.—Alkylation of 2-chloro-4-nitrophenol (**17**) with *t*-butyl chloroacetate in DMF in the presence of K_2CO_3 afforded the crystalline ester (**18**) in 67% yield of analytical purity (Scheme I). When **18** was

Scheme I



refluxed in toluene containing a trace of TsOH, isobutylene was evolved and the desired acid (**19**) was obtained in 92% yield. Conversion of **19** to the acid chloride with $SOCl_2$, then condensation with sulfanilyl fluoride in boiling toluene,[5,7] gave the desired amide (**21a**) in 71% yield. Similarly, **19** was converted to **21b** and **21c** by condensation with N-methylsulfanilyl fluoride[13] and metanilyl fluoride, respectively, in good yield. The NO_2 group of **21** was reduced catalytically to **20** with a PtO_2 catalyst since Raney Ni[5,14] caused considerable dehalogenation; the crude amines (**20**) were condensed[15] with cyanoguanidine in acetone in the presence of ethanesulfonic acid to give the triazines (**2b**, **14**, **15**).

The candidate irreversible inhibitors with an alkylenedioxy bridge (**3**, **7**–**10**) can be generalized by structure **27**. The synthesis of one member of this series was previously described, namely **3a** (= **27a**).[7] The key reaction was the chlorosulfonation of an α-(nitrophenoxy)-ω-phenoxyalkane (**23**) in $CHCl_3$ to give **25** (Scheme II). That a phenyl ether chlorosulfonates

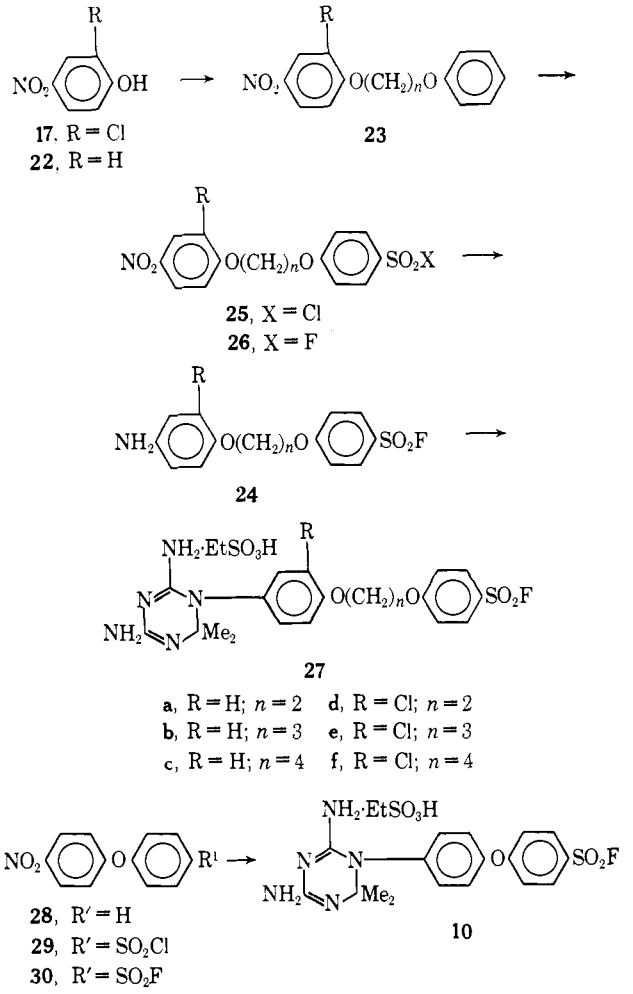
(12) B. F. Pedersen and B. Pedersen, *Tetrahedron Letters*, 2995 (1965).

(13) B. R. Baker and G. J. Lourens, *J. Med. Chem.*, **11**, 672 (1968), paper CXXVIII of this series.

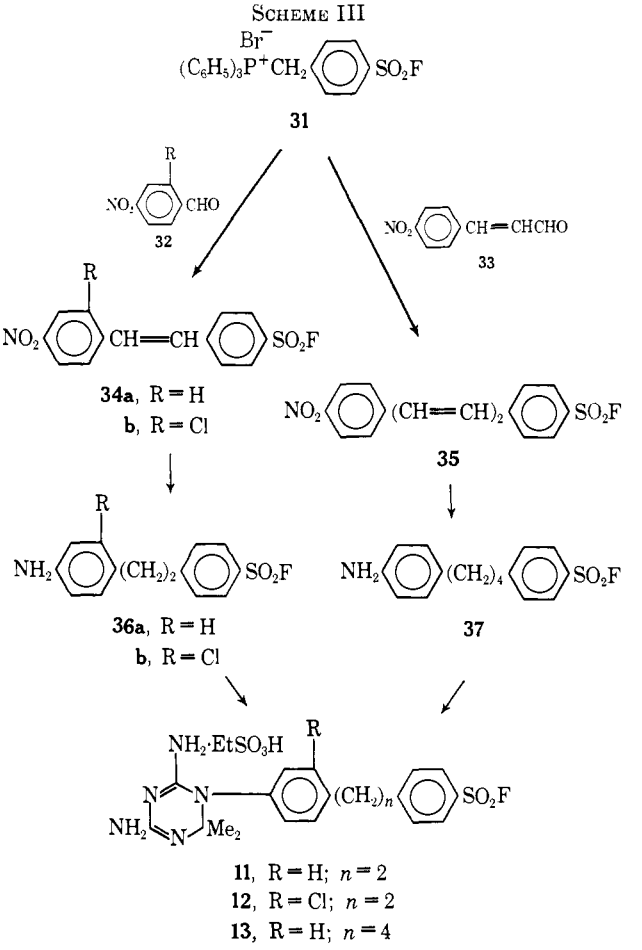
(14) A. H. deCat and R. K. vanPoucke, *J. Org. Chem.*, **28**, 3426 (1963).

(15) E. J. Modest, *ibid.*, **21**, 1 (1956).

SCHEME II



SCHEME III



para to the ether linkage has been previously demonstrated;[16] the nitrophenoxy ring would be considerably less reactive, due to the deactivation by the NO_2 group, than the other phenoxy ring. Similarly, **28** could be chlorosulfonated to **29**. The NO_2 group of **26** and **30** was reduced catalytically with a Raney nickel catalyst if a Cl atom was absent and with a PtO_2 catalyst if present.

The next group of candidate irreversible inhibitors (**11–13**) contained an alkane bridge. The synthesis of one member (**11**) of this series had been described previously;[7] the key reaction was a Wittig condensation between *p*-nitrobenzaldehyde (**32a**) and the *p*-fluorosulfonylphosphonium salt (**31**)[7] to give **34a** (Scheme III). In a similar fashion, **34b** and **35** were synthesized from **31** by Wittig condensation with **32b** and **33**, respectively. The remainder of the sequence was the same as for the synthesis of **11**.[7]

The last candidate irreversible inhibitor (**1b**) was synthesized[17] by the conversion of **32b** diacetate to 2-chloro-4-nitrocinnamic acid; the remainder of the sequence was the same as the conversion of **19a** to **2b**.

Experimental Section

Melting points were taken in capillary tubes on a Mel-Temp block and are uncorrected. All analytical samples gave a single spot on tlc and had ir and uv spectra in agreement with their assigned structures; each gave combustion values for C, H, and N or F within 0.4% of theoretical.

***t*-Butyl 2-Chloro-4-nitrophenoxyacetate (18).**—A mixture of 8.7 g (50 mmoles) of **17**, 8.28 g (55 mmoles) of *t*-butyl chloroacetate, 6.9 g (50 mmoles) of K_2CO_3, and 30 ml of DMF was stirred at 55–60° for 24 hr, then poured into a stirred mixture of 500 ml of ice–water and 50 ml of petroleum ether (bp 60–110°). The product was collected on a filter, washed (H_2O), and dried. Recrystallization from petroleum ether gave 9.65 g (67%) of beige crystals, mp 95–96°. *Anal.* ($C_{12}H_{14}ClNO_5$) C, H, N.

2-Chloro-4-nitrophenoxyacetic Acid (19).—A solution of 8.5 g (30 mmoles) of **18** and 50 mg of TsOH in 25 ml of toluene was refluxed with stirring for 1 hr during which time part of the product separated. The cooled mixture was filtered and the product was washed with toluene. Recrystallization from EtOH–H_2O gave 6.3 g (92%) of crystals, mp 177–179°; mp 175–176 was recorded for this compound prepared by a different route.[15]

2-Chloro-4-nitrophenyl Phenoxypropyl Ether (23e) (Method A).—A mixture of 17.4 g (0.1 mole) of **17**, 23.8 g (0.11 mole) of 3-bromopropyl phenyl ether, 13.6 g (0.1 mole) of K_2CO_3, and 90 ml of DMSO was stirred in a bath at 95–100° for 48 hr. The mixture was poured with stirring into 800 ml of H_2O and 100 ml of petroleum ether. The product was collected on a filter and washed (H_2O). Recrystallization (EtOH) gave 25.8 g (84%) of product, mp 89–90°. See Table II for additional data and compounds prepared by this method.

1-(*p*-Fluorosulfonylphenoxy)-3-(2-chloro-4-nitrophenoxy)propane (26e) (Method B).—To a stirred mixture of 12 g of **23e** in 100 ml of $CHCl_3$ cooled to −5 to 0° was added dropwise 48 ml of $ClSO_3H$ over 30 min with protection from moisture. After being stirred for an additional 90 min at 0°, the mixture was poured into 500 g of crushed ice. The mixture was diluted with 150 ml of $CHCl_3$, then the separated organic layer was washed (300 ml of ice-cold H_2O, 200 ml of ice-cold 5% $NaHCO_3$, 300 ml

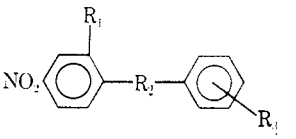
(16) E. H. Huntress and F. H. Carter, *J. Am. Chem. Soc.*, **62**, 603 (1940).

(17) This synthesis was performed in this laboratory by M. A. Johnson, M.S. thesis, 1967.

(18) Ilford, Ltd., Belgian Patent 662,316 (1962); *Chem. Abstr.*, **59**, P14146e (1963).

Table II

Physical Properties of

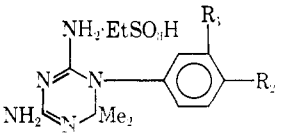


No.	R_1	R_2	R_3	Method	% yield[b]	Mp, °C	Formula
21a	Cl	OCH_2CONH	*p*-SO_2F	C[a]	71[c]	198–199 dec	$C_{14}H_{10}ClFN_2O_6S$
21b	Cl	$OCH_2CON(CH_3)$	*p*-SO_2F	C	82[d]	178–179	$C_{15}H_{12}ClFN_2O_6S$
21c	Cl	OCH_2CONH	*m*-SO_2F	C	66[d]	202–204	$C_{14}H_{10}ClFN_2O_6S$
23b	H	$O(CH_2)_3O$	H	A	66[e]	71–72	$C_{17}H_{15}NO_4$
23c	H	$O(CH_2)_4O$	H	A	87[e]	86–87	$C_{18}H_{17}NO_4$
23d	Cl	$O(CH_2)_2O$	H	A	27[d]	124–126	$C_{14}H_{12}ClNO_4$
23e	Cl	$O(CH_2)_3O$	H	A	84[e]	89–90	$C_{15}H_{14}ClNO_4$
23f[f]	Cl	$O(CH_2)_4O$	H	A	62[d]	86–87	$C_{16}H_{16}ClNO_4$
26b	H	$O(CH_2)_3O$	*p*-SO_2F	B	25[e]	115–116	$C_{17}H_{14}FNO_6S$
26c	H	$O(CH_2)_4O$	*p*-SO_2F	B	21[e]	100–101	$C_{18}H_{16}FNO_6S$
26d	Cl	$O(CH_2)_2O$	*p*-SO_2F	B	27[d]	124–126	$C_{14}H_{11}ClFNO_6S$
26e	Cl	$O(CH_2)_3O$	*p*-SO_2F	B	16[d]	116–118	$C_{15}H_{13}ClFNO_6S$
30	H	O	*p*-SO_2F	B	48[e,g]	101–102	$C_{12}H_8FNO_5S$
39	Cl	$(CH_2)_2CONH$	*p*-SO_2F	C	80[d]	257–260 dec	$C_{15}H_{10}ClFN_2O_5S$
40[c]	H	CH_2O	*p*-SO_2F	B	16[g]	121–122	$C_{13}H_{10}FNO_2S$

[a] C: by condensation of the appropriate acid chloride and aminobenzenesulfonyl fluoride by the method previously described.[5] [b] Yield of analytically pure material analyzed for C, H, N. [c] Recrystallized from MeOEtOH. [d] Recrystallized from MeOEtOH–H_2O. [e] Recrystallized from EtOH. [f] Attempts to chlorosulfonate **23f** caused cleavage to **17**. [g] Recrystallized from EtOH–H_2O. [h] Yield from corresponding sulfonyl chloride prepared according to V. H. Dermer and O. C. Dermer, *J. Am. Chem. Soc.*, **64**, 3056 (1942). [i] Attempts to reduce the NO_2 group led to mixtures.

Table III

Physical Properties of



No.	R_1	R_2	Method	% yield[a]	Mp, °C dec	Formula	Analyses
1b	Cl	$(CH_2)_2CONHC_6H_4SO_2F$-*p*	D	59[b]	213–214	$C_{29}H_{32}ClFN_8O_9S \cdot EtSO_3H$	C, H, N
2b	Cl	$OCH_2CONHC_6H_4SO_2F$-*p*	D	53[c]	224–226	$C_{19}H_{20}ClFN_6O_9S \cdot EtSO_3H$	C, H, F
3b	Cl	$O(CH_2)_2OC_6H_4SO_2F$-*p*	D	58[d]	218–220	$C_{19}H_{21}ClFN_5O_8S \cdot EtSO_3H$	C, H, F
7	H	$O(CH_2)_3OC_6H_4SO_2F$-*p*	E	60[d]	216–217	$C_{20}H_{24}FN_5O_8S \cdot EtSO_3H$	C, H, F
8	Cl	$O(CH_2)_3OC_6H_4SO_2F$-*p*	D	51[d]	211–212	$C_{20}H_{23}ClFN_5O_8S \cdot EtSO_3H$	C, H, F
9	H	$O(CH_2)_4OC_6H_4SO_2F$-*p*	E	52[d]	218–219	$C_{21}H_{26}FN_5O_8S \cdot EtSO_3H$	C, H, F
10	H	$OC_6H_4SO_2F$-*p*	E	32[d]	202–203	$C_{17}H_{18}FN_5O_6S \cdot EtSO_3H$	C, H, F
12	Cl	$(CH_2)_2C_6H_4SO_2F$-*p*	D	64[d]	226–227	$C_{20}H_{24}ClFN_5O_7S \cdot EtSO_3H$	C, H, F
13	H	$(CH_2)_4C_6H_4SO_2F$-*p*	E	43[e]	210–212	$C_{21}H_{26}FN_5O_7S \cdot EtSO_3H$	C, H, F
14	Cl	$OCH_2CON(CH_3)C_6H_4SO_2F$-*p*	D	54[c]	201–203	$C_{20}H_{22}ClFN_6O_8S \cdot EtSO_3H$	C, H, F
15	Cl	$OCH_2CONHC_6H_4SO_2F$-*m*	D	65[e]	208–210	$C_{19}H_{20}ClFN_6O_9S \cdot EtSO_3H$	C, H, F

[a] Yield of analytically pure material. [b] Recrystallized from EtOH. [c] Recrystallized from EtOH–H_2O. [d] Recrystallized from *i*-PrOH–H_2O. [e] Recrystallized from MeOEtOH.

of ice-cold H_2O). Dried with $MgSO_4$, the $CHCl_3$ solution was evaporated *in vacuo*.

To the residual sulfonyl chloride (**25e**) were added 25 ml of DMF and 4.8 g of KF. After being stirred at 90–95° for 45 min, the cooled reaction mixture was poured into 500 ml of H_2O. The mixture was extracted with 250 ml of $CHCl_3$. Dried with $MgSO_4$, the $CHCl_3$ solution was evaporated *in vacuo*. Recrystallization of the residue from MeOEtOH–H_2O gave 2.4 g (16%) of product, mp 116–118°. See Table II for additional data and compounds prepared by this method.

2-Chloro-4′-fluorosulfonyl-4-nitrostilbene (34b).—To a stirred mixture of 1.95 g (10 mmoles) of **32b**,[19] 5.16 g (10 mmoles) of **31**,[5] and 6 ml of reagent MeOH cooled in an ice bath and protected from moisture was added 1.22 g (10 mmoles) of Et_3N dropwise over a period of 10 min. After being stirred 30 min in an ice bath and 3.5 hr at ambient temperature, the mixture was filtered and the product was washed (cold MeOH). The small amount of unchanged **32b**, the presence of which was indicated by tlc with C_6H_6, was removed by leaching the product with hot 50% EtOH; yield 1.89 g (55%), mp 118–190°, which was a mixture of *cis-trans* isomers suitable for the next step. Recrystallization of a sample from MeOEtOH gave the analytical sample, mp 238–239°. *Anal.* ($C_{14}H_9ClFNO_4S$) C, H, N.

2-Chloro-4-nitrocinnamic Acid (38).[5]—A stirred mixture of 14.7 g of 2-chloro-4-nitrobenzaldehyde diacetate (prepared[20] by the method of Spalding, *et al.*,[20] for an isomer), 5.30 g of NaOAc, and 15 ml of HOAc was refluxed for 20 hr. The cooled mixture was diluted with 75 ml of H_2O, then the product was collected on a filter and washed (H_2O). The crude product was dissolved in 150 ml of H_2O by adding sufficient concentrated NaOH to bring to pH 10–11. The solution was clarified by filtration, then acidified with HOAc. The product was collected on a filter and washed with H_2O; yield 7.46 g (64%), mp 193–196°. *Anal.* ($C_9H_6ClNO_4$) C, H, N.

N-[2-Chloro-4-(4,6-diamino-1,2-dihydro-2,2-dimethyl-s-

(19) B. R. Baker, B.-T. Ho, and G. J. Loubens *J. Pharm. Sci.*, **56**, 737 (1967), paper LXXXVI of this series.

(20) D. P. Spalding, G. W. Moersch, H. S. Mosher, and F. C. Whitmore, *J. Am. Chem. Soc.*, **68**, 1595 (1946).

triazin-1-yl)phenoxyacetyl]sulfanilyl Fluoride Ethanesulfonate (2b) (Method D).—A mixture of 777 mg (2 mmoles) of **21a** (Table II), 65 mg of PtO_2, and 100 ml of EtOH was shaken with H_2 at 2–3 atm until reduction was complete. To the filtered mixture was added 225 mg of $EtSO_3H$ then the solution was evaporated *in vacuo*. To the residual **20a**·$EtSO_3H$ were added 20 ml of reagent Me_2CO and 177 mg (2.2 mmoles) of cyanoguanidine. After being refluxed with stirring for 18 hr, the cooled mixture was filtered and the product was washed (Me_2CO). Recrystallization from EtOH–H_2O gave 630 mg (53%) of white crystals, mp 224–226° dec. See Table III for additional data and compounds prepared by this method.

Method E was the same except Raney nickel was used as a catalyst.[5]

Irreversible Enzyme Inhibitors. CXLI.[1,2] Active-Site-Directed Irreversible Inhibitors of Dihydrofolic Reductase Derived from 1-[*p*-(*p*-Fluorosulfonylphenylureidomethyl)-phenyl]-4,6-diamino-1,2-dihydro-2,2-dimethyl-*s*-triazine

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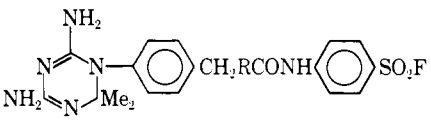
The title compound (1) showed a poor order of active-site-directed irreversible inhibition of dihydrofolic reductase from mouse L1210 leukemia, liver, spleen, or intestine since too high a concentration of inhibitor had to be used to show good inactivation. Substitution of a methyl group *meta* to the SO_2F moiety of **1** gave compound **6** that was still a relatively poor irreversible inhibitor of the L1210 enzyme, but now showed tissue specificity by its failure to inactivate the liver enzyme. More effective irreversible inhibitors (**5**, **8**, **9**) were obtained by substitution of a chloro atom *meta* to the junction of the 1-phenyl to the *s*-triazine ring; however, these compounds did not show specificity since the liver enzyme was still inactivated.

The title compound (**1**)[4] was found to be an active-site-directed irreversible inhibitor[5] of the dihydrofolic reductase from L1210/0 and L1210/DF8 mouse leukemia.[6] However, **1** was not as good an irreversible inhibitor as the prototype irreversible inhibitor **2**[6,7] since the total amount of inactivation by **1** was lower than **2** (Table I). Furthermore, neither **1** nor **2** showed specificity toward the L1210 enzyme with minimal effect on the enzyme from normal tissues such as liver, spleen, and intestine.[6] Therefore, additional synthetics related to **1** were made and evaluated to see if a better



1, R = NH
2, R = CH_2

and more selective irreversible inhibitor could be designed. The results are the subject of this paper.

Enzyme Results.—As pointed out in the earlier summary paper,[6] assay of **1** for irreversible inhibition of dihydrofolic reductase was difficult due to a medium order of total, but fast, irreversible inhibition that sometimes gave low zero-time points. Similar difficulties were encountered with **3**,[4] which showed 0–30% irreversible inhibition of the L1210 enzyme depending upon how low the zero-time point for enzyme concentration was; in repeated runs **3** failed to show any irreversible inhibition of mouse liver dihydrofolic reductase. Thus, **3** showed selectivity, but a poor order of irreversible inhibition of the L1210 enzyme; the latter was most probably due to extensive enzyme-catalyzed hydrolysis of the SO_2F moiety.[7,8] When the side chain was moved to the *meta* position (**4**), irreversible inhibition was lost.[9]

The effect of substituents on either or both benzene rings of **1** on irreversible inhibition was then studied. There is only one position on the phenyl group next to the triazine that can be substituted without loss of binding, and that is the position *meta* to the triazine junction; *ortho* substitution leads to a large loss in reversible binding.[10,11] The 3-chloro atom was selected since this could have a beneficial effect on both reversible binding[10] and irreversible inhibition.[2] The 3-chloro substituent **5** on **1** gave a fourfold increment in reversible binding. Furthermore, **5** was a better irreversible inhibitor of the L1210 enzyme than **1** when compared at a similar concentration of reversible EI complex;[12] however, **5** still lacked specificity since poor irreversible inhibition of the mouse liver enzyme was still observed.

Substitution of CH_3 *meta* (**6**) to the SO_2F moiety of **1** also gave about a fourfold increment in reversible binding. Although **6** was not a better irreversible inhibitor of the L1210 enzyme at equal EI concentration,[12] **6** was more specific than **1** since **6** did not inactivate the liver enzyme. Introduction of CH_3 (**7**) *ortho* to the SO_2F moiety of **1** gave about a twofold increment in reversible binding; however, **7** was

(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 G. J. Lourens, *J. Med. Chem.*, **12**, 95 (1969), paper CXL of this series.

(3) G. J. L. wishes to thank the Council of Scientific and Industrial Research, Republic of South Africa, for a tuition fellowship.

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

(5) 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.

(6) 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.

(7) B. R. Baker and G. J. Lourens, *ibid.*, **10**, 1123 (1967), paper CV of this series.

(8) B. R. Baker and J. A. Hurlbut, *ibid.*, **11**, 233 (1968), paper CXIII of this series.

(9) B. R. Baker and G. J. Lourens, *ibid.*, **11**, 39 (1968), paper CXII of this series.

(10) (a) B. R. Baker, *ibid.*, **11**, 483 (1968); (b) B. R. Baker and M. A. Johnson, *ibid.*, **11**, 486 (1968); (c) B. R. Baker and B.-T. Ho, *J. Pharm. Sci.*, **53**, 1137 (1964).

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

(12) For the kinetics of irreversible inhibition see ref 5, Chapter 8.