Irreversible Enzyme Inhibitors. CLV.[1,2] Active-Site-Directed Irreversible Inhibitors of Dihydrofolic Reductase Derived from 1-[4-(ω-Aminoalkoxy)-3-chlorophenyl]-4,6-diamino-1,2-dihydro-2,2-dimethyl-s-triazines Bearing a Terminal Sulfonyl Fluoride

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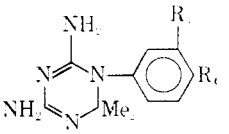
A series of 22 candidate active-site-directed irreversible inhibitors of dihydrofolic reductase derived from 1-[4-(ω-aminoalkoxy)-3-chlorophenyl]-4,6-diamino-1,2-dihydro-2,2-dimethyl-s-triazines by acylation of the terminal amino group by a fluorosulfonylbenzoyl, fluorosulfonylphenylsulfonyl, or fluorosulfonylphenylcarbamido group were synthesized and evaluated on the enzyme from L1210 mouse leukemia and mouse liver as well as L1210 cell culture; the alkoxy unit was varied between two and four carbons. Of these 22 compounds, 14 were excellent irreversible inhibitors of L1210 dihydrofolic reductase that at $5\text{–}10 \times 10^{-8}$ *M* gave 84–100% inactivation of the enzyme; the presence of the 3-Cl atom was necessary for good irreversible inhibition. Of these 14 compounds, none showed complete specificity since the liver enzyme could also be inactivated 18–83%. The three best compounds showing <25% inactivation of the liver enzyme were *m*- or *p*-fluorophenylcarbamido derivatives containing two (**16**) or three (**3**, **6**) CH_2 groups. When the efficiency of kill of L1210 cell culture was measured, the best six compounds (**11**, **13**, **16**, **21**–**23**) showed ED_{50}'s in the range of 0.003–0.15 μ*M*. The best compound of the series was, therefore, 1-[4-(*p*-fluorosulfonylphenylureidoethoxy)-3-chlorophenyl]-4,6-diamino-1,2-dihydro-2,2-dimethyl-s-triazine (**16**) which had ED_{50} = 0.003 μ*M* and at 0.072 μ*M* showed 86% inactivation of L1210 dihydrofolic reductase in <2 min at 37°, but at 0.16 μ*M* showed only 18% inactivation of the liver enzyme in 60 min.

In a previous paper from this laboratory, it was reported[3] that **1** was an active-site-directed irreversible inhibitor[4] of the dihydrofolic reductase from L1210 mouse leukemia and that **1** showed no irreversible inhibition of this enzyme from mouse liver. When Cl was removed from **1**, the resultant **2** showed no irreversible inhibition of the L1210 enzyme; this difference between **1** and **2** was attributed to the effect of the Cl on limiting allowable binding conformations of **1** for reversible complexing with dihydrofolic reductase.[3] Therefore, a study was initiated to see if the acetamido bridge of **1** could be replaced by an alkyleneurea bridge



1, R_3 = Cl; R_4 = $OCH_2CONHC_6H_4SO_2F$-*p*
2, R_3 = H; R_4 = $OCH_2CONHC_6H_4SO_2F$-*p*
3, R_3 = Cl; R_4 = $O(CH_2)_3NHCONHC_6H_4SO_2F$-*m*
4, R_3 = H; R_4 = $O(CH_2)_3NHCONHC_6H_4SO_2F$-*m*
5, R_3 = Cl; R_4 = $O(CH_2)_nNHR_1C_6H_3(R_2)SO_2F$

as in **3** and still maintain irreversible inhibition; such was indeed the case. Therefore a series of compounds (**5**) related to **3** have now been synthesized and evaluated for specific irreversible inhibition of L1210 dihydrofolic reductase and inhibition of intact L1210 cells in culture; the results are the subject of this paper.

Enzyme Results.—The inhibition of L1210 dihydrofolic reductase by the first member of the series (**3**) is shown in Table I; **3** was both an excellent reversible and irreversible inhibitor,[5] showing 83% inactivation in 2 min at 37°. The inactivation of the mouse liver enzyme by the same concentration of **3** was considerably less, presumably due to more enzyme-catalyzed hydrolysis of the SO_2F group[6] by the liver enzyme. That the chlorine atom of **3** was necessary for good irreversible inhibition[3] was shown with **4**. Reversible inhibition by **3** or **4** was essentially identical, but **4** showed only poor irreversible inhibition at twice the concentration used with **3**.

When the SO_2F group of **3** was moved to the *para* position, the resultant **6** was a slightly better reversible inhibitor than **3**, but showed a similar irreversible inhibition pattern. Replacement of the NHCONH bridge of **3** or **6** by NHCO to give **7** or **8** resulted in little change in I_{50}, but irreversible inhibition of the L1210 enzyme was considerably less. In contrast, replacement of the urea bridge by $NHSO_2$ to give **9** or **10** showed just as effective irreversible inhibition of the L1210 enzyme, but the effectiveness on the liver enzyme was enhanced compared to **3** or **6**. Attempts to decrease irreversible inhibition of the liver enzyme without a corresponding decrease in irreversible inhibition of the L1210 enzyme by substitution on the C_6N_4-SO_2F moiety[7] were unsuccessful as shown with **11**–**14**; actually effectiveness of the liver enzyme was increased except with **13** where irreversible inhibition of the liver enzyme was about the same as with **6**.

Chain shortening of the propylene bridge of **3** to ethylene gave **15** which was a less effective irreversible inhibitor of the L1210 enzyme, but reversible inhibition was not changed. In contrast, the same structural change on the *p*-SO_2F isomer (**6**) gave an unchanged irreversible inhibition pattern with the resultant **16**. Again the corresponding benzamides (**17**, **18**) showed less effective irreversible inhibition. The sulfonamides (**19**, **20**) were also less effective irreversible inhibitors. Methyl substitution (**21**) on **16** did not change the

(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 R. B. Meyer, Jr., *J. Med. Chem.*, **12**, 668 (1969).

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

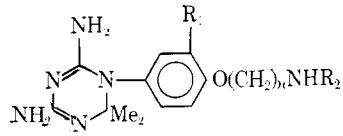
(4) B. R. Baker, "Design of Active-Site-Directed Irreversible Enzyme Inhibitors. The Organic Chemistry of the Enzymic Active-Site," John Wiley and Sons, Inc., New York, N. Y., 1967.

(5) For the assay methods see (a) 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; (b) B. R. Baker and G. J. Lourens, *ibid.*, **10**, 1113 (1967), paper CV of this series.

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

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

TABLE I: INHIBITION[a] OF DIHYDROFOLIC REDUCTASE BY



No.	R_1	n	R_2	Mouse tissue	I_{50},[b] μM	Inhib., μM	Time, min	% inactivn[c]	ED_{50},[d] μM	ED_{50}/I_{50}
3	Cl	3	$CONHC_6H_4SO_2F$-*m*	L1210/DF8	0.053	0.10	2, 30	80, 87[e]	0.57	11
				L1210/0		0.10	60	82		
				Liver		0.10	60	25		
4	H	3	$CONHC_6H_4SO_2F$-*m*	L1210/DF8	0.048	0.24	60	23	7.0	150
				Liver	0.030	0.24	60	0		
6	Cl	3	$CONHC_6H_4SO_2F$-*p*	L1210/DF8	0.021	0.10	2, 8	93, 93[e]	0.55	26
						0.042	60	87		
				Liver		0.10	60	50		
						0.042	60	23		
7	Cl	3	$COC_6H_4SO_2F$-*m*	L1210/0	0.013	0.048	60	60	1.1	85
				L1210/DF8		0.048	60	72		
				Liver	0.024	0.12	60	18		
8	Cl	3	$COC_6H_4SO_2F$-*p*	L1210/DF8	0.046	0.092	60	69		
				Liver		0.092	60	47		
9	Cl	3	$SO_2C_6H_4SO_2F$-*m*	L1210/DF8		0.069	60	98	1.0	44
				Liver	0.023	0.069	60	69		
10	Cl	3	$SO_2C_6H_4SO_2F$-*p*	L1210/DF8		0.048	60	97	0.93	19
				Liver	0.048	0.096	60	73		
11	Cl	3	$CONHC_6H_3$-4-Me-3-SO_2F	L1210/DF8	0.0088	0.050	60	87	0.039	4.5
				Liver	0.018	0.050	60	44		
12	Cl	3	$CONHC_6H_3$-2-OMe-5-SO_2F	L1210/DF8		0.094	2, 30	94, 94[e]	0.54	11
				Liver	0.047	0.14	60	42		
13	Cl	3	$CONHC_6H_3$-3-Me-4-SO_2F	L1210/0		0.095	60	84	0.008	0.1
				L1210/DF8		0.095	60	97		
				Liver	0.057	0.38	60	64		
						0.19	60	30		
14	Cl	3	COC_6H_3-4-Me-3-SO_2F	L1210/DF8		0.090	60	84	4.3	95
				Liver	0.045	0.16	60	39		
15	Cl	2	$CONHC_6H_4SO_2F$-*m*	L1210/DF8		0.12	2, 4, 30	57, 64, 64[e]	6.0	150
						0.040	60	19		
				Liver	0.040	0.12	60	0		
16	Cl	2	$CONHC_6H_4SO_2F$-*p*	L1210/0	0.0095	0.066	60	86	0.0032	0.36
				L1210/DF8		0.072	2, 30	86, 86[e]		
				Liver		0.16	60	18		
17	Cl	2	$COC_6H_4SO_2F$-*m*	L1210/DF8		0.12	60	37	1.1	15
				Liver	0.075	0.18	60	9		
18	Cl	2	$COC_6H_4SO_2F$-*p*	L1210/DF8		0.082	2, 4, 30	65, 75, 75[e]	1.2	30
				Liver	0.041	0.082	60	25		
19	Cl	2	$SO_2C_6H_4SO_2F$-*m*	L1210/DF8		0.074	60	46	5.4	150
				Liver	0.037	0.074	60	24		
20	Cl	2	$SO_2C_6H_4SO_2F$-*p*	L1210/DF8		0.072	60	52	5.4	150
				Liver	0.036	0.072	60	27		
21	Cl	2	$CONHC_6H_3$-3-Me-4-SO_2F	L1210/DF8	0.015	0.072	2, 30	90, 90[e]	0.093	6.3
				Liver	0.036	0.11	60	33		
22	Cl	4	$CONHC_6H_4SO_2F$-*m*	L1210/DF8	0.020	0.050	60	98	0.059	3.0
				Liver		0.31	60	75		
						0.060	60	50		
23	Cl	4	$CONHC_6H_4SO_2F$-*p*	L1210/DF8		0.068	2, 30	81, 98[e]	0.15	4.4
				Liver	0.034	0.10	60	83		
24	Cl	4	$COC_6H_4SO_2F$-*m*	L1210/DF8		0.078	60	96	1.1	29
				Liver	0.039	0.12	60	67		
25	Cl	4	$COC_6H_4SO_2F$-*p*	L1210/DF8	0.022	0.050	60	100	1.5	68
				Liver		0.24	60	86		
						0.050	60	37		

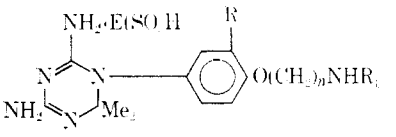
[a] The technical assistance of Diane Shea and Sharon Lafler with these assays is acknowledged. [b] Concentration for 50% inhibition when assayed with 6 μM dihydrofolate in 0.05 M Tris buffer (pH 7.4) containing 0.15 M KCl as previously described.[5] [c] Incubated at 37° in 0.05 M Tris buffer (pH 7.4) containing 60 μM TPNH, then the remaining enzyme was assayed with 12 μM dihydrofolate and 0.15 M KCl as previously described.[5] [d] Concentration for 50% inhibition of L1210 cell culture. [e] From a six-point time study.[5]

inhibition of L1210 enzyme, but **21** was more effective on the liver enzyme than **16**.

The two phenylureas (**22**, **23**) and two benzamides (**24**, **25**) with a butylene bridge were excellent irreversible inhibitors of L1210 dihydrofolic reductase; unfortunately irreversible inhibition of the liver enzyme was enhanced compared to **3** or **6**.

In summary, **3**, **6**, **9**–**14**, **16**, and **21**–**25** were good to

Table II
Physical Constants of



No.	R_1	R_2	n	% yield[a]	Mp, °C dec	Formula[b]
3	$CONHC_6H_4SO_2F$-*m*	Cl	3	43[c]	176–177	$C_{21}H_{23}ClFN_7O_4S \cdot C_2H_5SO_3H$
4	$CONHC_6H_4SO_2F$-*m*	H	3	67[d]	195–196	$C_{21}H_{26}FN_7O_4S \cdot C_2H_5SO_3H$
6	$CONHC_6H_4SO_2F$-*p*	Cl	3	12[c]	198–200	$C_{21}H_{25}ClFN_7O_4S \cdot C_2H_5SO_3H$
7	$COC_6H_4SO_2F$-*m*	Cl	3	16[e]	150[f]	$C_{21}H_{24}ClFN_6O_4S \cdot C_2H_5SO_3H \cdot H_2O$
8	$COC_6H_4SO_2F$-*p*	Cl	3	21[g]	202–203	$C_{21}H_{24}ClFN_6O_4S \cdot C_2H_5SO_3H$
9	$SO_2C_6H_4SO_2F$-*m*	Cl	3	7[d]	140[f]	$C_{21}H_{24}ClFN_6O_5S_2 \cdot C_2H_5SO_3H$
10	$SO_2C_6H_4SO_2F$-*p*	Cl	3	66[d]	208–209	$C_{21}H_{24}ClFN_6O_5S_2 \cdot C_2H_5SO_3H$
11	$CONHC_6H_3$-4-Me-3-SO_2F	Cl	3	67[d]	212–213	$C_{22}H_{27}ClFN_7O_4S \cdot C_2H_5SO_3H$
12	$CONHC_6H_3$-2-OMe-5-SO_2F	Cl	3	58[d]	184–187	$C_{22}H_{27}ClFN_7O_5S \cdot C_2H_5SO_3H$
13	$CONHC_6H_3$-3-Me-4-SO_2F	Cl	3	44[e]	197–198	$C_{22}H_{27}ClFN_7O_4S \cdot C_2H_5SO_3H$
14	COC_6H_3-4-Me-3-SO_2F	Cl	3	75[h]	192–194	$C_{22}H_{26}ClFN_6O_4S \cdot C_2H_5SO_3H$
15	$CONHC_6H_4SO_2F$-*m*	Cl	2	36[e]	150[f]	$C_{20}H_{23}ClFN_7O_4S \cdot C_2H_5SO_3H$
16	$CONHC_6H_4SO_2F$-*p*	Cl	2	64[d]	206–207	$C_{20}H_{23}ClFN_7O_4S \cdot C_2H_5SO_3H$
17	$COC_6H_4SO_2F$-*m*	Cl	2	28[e]	212–214	$C_{20}H_{22}ClFN_6O_4S \cdot C_2H_5SO_3H$
18	$COC_6H_4SO_2F$-*p*	Cl	2	72[d]	224–226	$C_{20}H_{22}ClFN_6O_4S \cdot C_2H_5SO_3H$
19	$SO_2C_6H_4SO_2F$-*m*	Cl	2	78[d]	217–218	$C_{20}H_{22}ClFN_6O_5S_2 \cdot C_2H_5SO_3H$
20	$SO_2C_6H_4SO_2F$-*p*	Cl	2	56[d]	217–219	$C_{20}H_{22}ClFN_6O_5S_2 \cdot C_2H_5SO_3H$
21	$CONHC_6H_3$-3-Me-4-SO_2F	Cl	2	47[h]	150[f]	$C_{21}H_{25}ClFN_7O_4S \cdot C_2H_5SO_3H$
22	$CONHC_6H_4SO_2F$-*m*	Cl	4	52[i]	165[f]	$C_{22}H_{27}ClFN_7O_4S \cdot C_2H_5SO_3H$
23	$CONHC_6H_4SO_2F$-*p*	Cl	4	69[i]	173–175	$C_{22}H_{27}ClFN_7O_4S \cdot C_2H_5SO_3H$
24	$COC_6H_4SO_2F$	Cl	4	55[h]	176–178	$C_{22}H_{27}ClFN_6O_4S \cdot C_2H_5SO_3H$
25	$COC_6H_4SO_2F$-*p*	Cl	4	25[h]	158–160	$C_{22}H_{27}ClFN_6O_4S \cdot C_2H_5SO_3H$

[a] All compounds were made by H_2–PtO_2 reduction of **33**, then condensation of the resultant **32** with cyanoguanidine and acetone as described for compound **2b** in ref 3; yield is recorded for analytically pure material and is a minimum. [b] All analyzed for C, H, F. [c] Recrystallized from EtOH–H_2O. [d] Recrystallized from *i*-PrOH–H_2O. [e] Recrystallized from *i*-PrOH. [f] Beginning of wide decomposition range. [g] Recrystallized from Me_2CO. [h] Recrystallized from Me_2CO–EtOH. [i] Recrystallized from Me_2CO–H_2O.

excellent irreversible inhibitors of L1210 dihydrofolic reductase showing >80% inactivation at $2I_{50}$. Of these 14 compounds none showed complete specificity by giving no inactivation of the liver enzyme at 2–$3I_{50}$; only **3**, **6**, and **16** showed <25% inactivation of the liver enzyme and were therefore the best compounds in Table I.

Cell Culture Assays.[8]—With L1210 cell culture the concentration of inhibitor (ED_{50}) for 50% cell kill can be measured. The compounds can be divided into three groups: (1) **11**, **13**, **16**, and **21**–**23** with ED_{50} = 0.003–0.15 μM; (2) **3**, **6**, **7**, **9**, **10**, **12**, **17**, **18**, **24**, and **25** with ED_{50} = 0.5–1.5 μM; and (3) **4**, **14**, **15**, **19**, and **20** with ED_{50} = 4–7 μM, a total spread of about 2000-fold. The ED_{50} of an inhibitor is dependent upon such factors as cell wall transport, effectiveness on the target receptor in the cell, and rate of metabolism of the inhibitor in the cell compared to rate of inactivation of the target receptor by the inhibitor. With a reversible inhibitor, the latter factor reduces to rate of metabolism as there is no inactivation of the target enzyme. Since the compounds in Table I are such potent inhibitors of dihydrofolic reductase, it is improbable that L1210 cell death is due to inhibition of some other enzyme; the blockade of dihydrofolic reductase will in turn lead to a block of DNA synthesis[9] due to the inability of thymidylate synthetase to operate in the absence of dihydrofolic reductase.[10] Metabolism of the inhibitor is unlikely to be important with inhibitors that give rapid inactivation of the target enzyme such as **16**. Therefore as a first approximation the ED_{50} is a measure of cell wall penetration of fast, potent irreversible inhibitors of dihydrofolic reductase particularly when normalized by use of ED_{50}/I_{50} to avoid differences in reversible binding. Furthermore, it is probable that the compounds in Table I enter a cell by passive diffusion.[11] With these assumptions the following factors in transport can be deduced as a working basis for the design of compounds with better transport properties. (1) Comparison of the ED_{50}/I_{50} ratios of ureidophenyl with the corresponding benzamido derivatives such as **3** *vs.* **7**, **11** *vs.* **14**, **16** *vs.* **18**, and **22** *vs.* **24** indicates that compounds with a phenylureido bridge are transported better than compounds with a benzamido bridge. (2) Compounds with a benzenesulfonamido bridge are not transported as well as compounds with a phenylureido bridge (**16** *vs.* **20**). (3) No consistent correlation with the number of methylenes in the bridge and transport could be made; compare **6**, **16**, and **23**.

Of the three potent irreversible inhibitors (**3**, **6**, **16**) of L1210 dihydrofolic reductase giving the most specificity, **16** has the lowest ED_{50}, 0.0032 μM. Therefore, **16** should show the best *in vivo* activity; such *in vivo* tests on the compounds in Table I with the lowest ED_{50} values are in progress.

One other point of interest in Table I is that **3** and its deschloro derivative (**4**) have nearly identical I_{50}'s but **3** is 12 times as effective as **4** in the cell culture assay;

(8) We wish to thank Dr. Florence White of the CCNSC for these assays performed by Dr. P. Himmelfarb, Arthur D. Little, Inc.

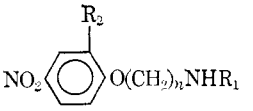
(9) D. Roberts and I. Wodinsky, *Cancer Res.*, **28**, 1955 (1968).

(10) T. H. Jukes and H. P. Broquist in "Metabolic Inhibitors," R. M. Hochster and J. H. Quastel, Ed., Academic Press Inc., New York, N. Y., 1963, pp 481–534.

(11) (a) See ref 4, pp 263–266; (b) R. C. Wood and G. H. Hitchings, *J. Biol. Chem.*, **234**, 2377, 2381 (1959); (c) B. R. Baker, D. V. Santi, F. I. Almaula, and W. C. Werkheisher, *J. Med. Chem.*, **7**, 24 (1964).

TABLE III

PHYSICAL CONSTANTS OF

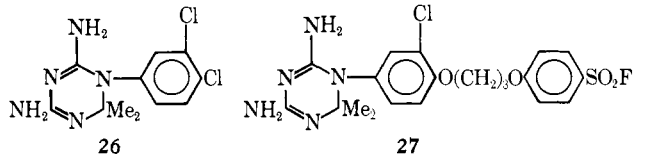


No.	R_1	R_2	n	Method[a]	% yield	Mp, °C	Formula
30b	$CO_2CH_2C_6H_5$	Cl	3	A	37[b]	85–86	$C_{17}H_{17}ClN_2O_5$
31a	H·HBr	Cl	2	C	36[c]	226–227	$C_8H_9ClN_2O_3 \cdot HBr$
31b	H·HBr	Cl	3	B	99	208[d]	$C_9H_{11}ClN_2O_3 \cdot HBr$
31c	H·HCl	Cl	4	D	49[d]	162–163	$C_{10}H_{13}ClN_2O_3 \cdot HCl$
31d	H·HBr	H	3	C	28[d]	134–135	$C_9H_{12}N_2O_3 \cdot HBr$
39	$CONHC_6H_4SO_2F$-*m*	Cl	2	F	72[e]	168–170	$C_{15}H_{13}ClFN_3O_6S$
40	$CONHC_6H_4SO_2F$-*p*	Cl	2	E[m]	73[f]	194–197	$C_{15}H_{13}ClFN_3O_6S$
41	$COC_6H_4SO_2F$-*m*	Cl	2	G	74[b]	178–180	$C_{15}H_{12}ClFN_2O_6S$
42	$COC_6H_4SO_2F$-*p*	Cl	2	G	69[b]	200–202	$C_{15}H_{12}ClFN_2O_6S$
43	$SO_2C_6H_4SO_2F$-*m*	Cl	2	G[g]	92[h]	143–145	$C_{14}H_{12}ClFN_2O_7S_2$
44	$SO_2C_6H_4SO_2F$-*p*	Cl	2	G[g]	88[h]	146–147	$C_{14}H_{12}ClFN_2O_7S_2$
45	$CONHC_6H_3$-3-Me-4-SO_2F	Cl	2	E[i]	44[j]	Amorphous[k]	
46	$CONHC_6H_4SO_2F$-*m*	Cl	3	F	37[l]	160–161	$C_{16}H_{15}ClFN_3O_6S$
47	$CONHC_6H_4SO_2F$-*m*	H	3	F	74[b]	162–163	$C_{16}H_{16}FN_3O_6S$
48	$CONHC_6H_4SO_2F$-*p*	Cl	3	E[m]	74[n]	147–148	$C_{16}H_{15}ClFN_3O_6S$
49	$COC_6H_4SO_2F$-*m*	Cl	3	G	71[b]	129–131	$C_{16}H_{14}ClFN_2O_6S$
50	$COC_6H_4SO_2F$-*p*	Cl	3	G	50[a,o]	160–165	$C_{16}H_{14}ClFN_2O_6S$
51	$SO_2C_6H_4SO_2F$-*m*	Cl	3	G[g]	63[h]	125–126	$C_{15}H_{14}ClFN_2O_7S_2$
52	$SO_2C_6H_4SO_2F$-*p*	Cl	3	G[g]	83[h]	125–126	$C_{15}H_{14}ClFN_2O_7S_2$
53	$CONHC_6H_3$-4-Me-3-SO_2F	Cl	3	E[i]	54[e]	147–150	$C_{17}H_{17}ClFN_3O_6S$
54	$CONHC_6H_3$-2-OMe-5-SO_2F	Cl	3	E	76[p]	206–208	$C_{17}H_{17}ClFN_3O_7S$
55	$CONHC_6H_3$-3-Me-4-SO_2F	Cl	3	E[i]	56[f]	161–163	$C_{17}H_{17}ClFN_3O_6S$
56	COC_6H_3-4-Me-3-SO_2F	Cl	3	G[q]	30[e]	Amorphous[k]	
57	$CONHC_6H_4SO_2F$-*m*	Cl	4	F	68[b]	163–165	$C_{17}H_{17}ClFN_3O_6S$
58	$CONHC_6H_4SO_2F$-*p*	Cl	4	E[m]	32[b]	175–177	$C_{17}H_{17}ClFN_3O_6S$
59	$COC_6H_4SO_2F$-*m*	Cl	4	G	14[b]	82–84	$C_{17}H_{16}ClFN_2O_6S$
60	$COC_6H_4SO_2F$-*p*	Cl	4	G	55[b,o]	115–116	$C_{17}H_{16}ClFN_2O_6S$

[a] Additional methods: E, from the appropriate O-phenylurethan and amine as described for compound **32** in ref 16; F, from *m*-fluorosulfonylphenyl isocyanate and the appropriate amine as described for compound **31** in ref 16; G, from the appropriate acid chloride and amine as described for compound **43** in ref 16; all compounds were analyzed for C, H, N. [b] Recrystallized from EtOH. [c] Recrystallized from EtOH–THF. [d] Recrystallized from EtOH–Et_2O. [e] Recrystallized from *i*-PrOH–petroleum ether (bp 30–60°). [f] Recrystallized from *i*-PrOH. [g] Reaction run in THF. [h] Recrystallized from C_6H_6. [i] See ref 7 for starting sulfonyl fluoride. [j] Recrystallized from EtOAc. [k] Amorphous product not obtained analytically pure. [l] Recrystallized from EtOH–H_2O. [m] See ref 16 for starting sulfonyl fluoride. [n] Recrystallized from EtOH–petroleum ether (bp 30–60°). [o] Used for further transformation, but showed several spots on tlc; analytical sample was obtained by preparative tlc. [p] Recrystallized from *i*-PrOH–EtOAc. [q] See B. R. Baker and R. B. Meyer, Jr., *J. Med. Chem.*, **12**, 104 (1969), for starting sulfonyl fluoride.

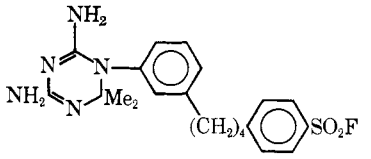
although the greater effectiveness of **3** is probably due to its potency as an irreversible inhibitor, in contrast to **4** which is not, this deduction is equivocal until it can be established whether or not the Cl atom of **3** has enhanced the transport.

Finally, it should be emphasized that the compounds in group **1** with ED_{50}'s in the range of 0.003–0.15 μM are still considerably less potent than the reversible inhibitor **26** with its $ED_{50} = 2 \times 10^{-4}$ μM. With **27**,[3]



26

27



28

an $ED_{50} = 0.003$ μM has now been observed; thus the replacement of the NHCONH moiety of **6** by –O– increases potency about 200-fold. Potency is further increased with the completely hydrocarbon bridge of **28**, which is a potent irreversible inhibitor of L1210 dihydrofolic reductase;[12] **28** has an ED_{50} of 2×10^{-4} μM, and ED_{50}/I_{50} ratio of 0.009, and has the most potency yet observed in L1210 cell culture with an irreversible inhibitor of dihydrofolic reductase.

The modification of molecules such as **27** and **28** is being vigorously pursued in order to impart isozyme specific irreversible inhibition to these classes of compounds that show such good cell wall transport.

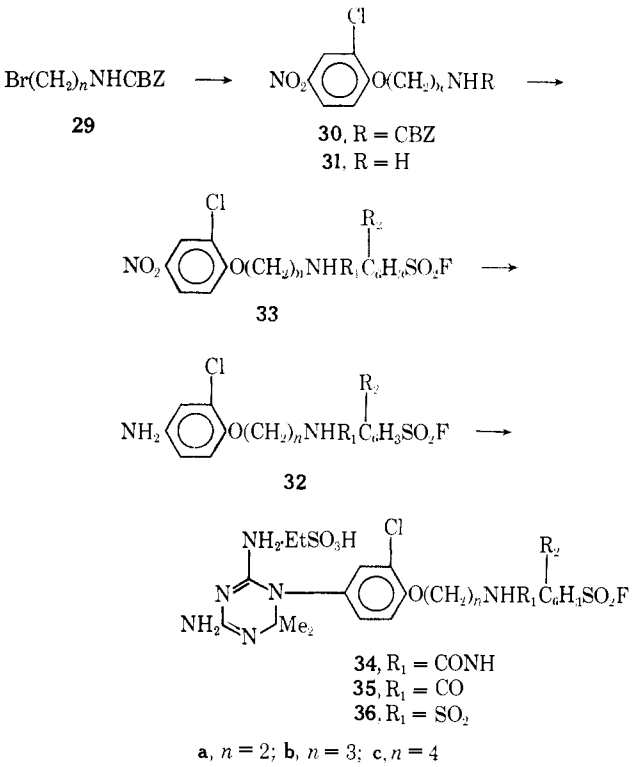
Chemistry.—The irreversible inhibitors in Table I, except for **4**, can be generalized by structures **34–36** (Scheme I); these were made from the appropriate arylamines (**32**) by condensation with cyanoguanidine and acetone.[13] The arylamines were made by the following sequence. The amino function of an ω-bromoalkylamine was blocked by reaction with carbobenzoxy chloride;[14] the resultant **29** was used to

(12) E. E. Janson, M.S. Thesis, University of California at Santa Barbara, Dec 1968; to be published.

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

(14) (a) E. Katchalski and D. Ben-Ishai, *ibid.*, **15**, 1067 (1950); (b) B. R. Baker and J. K. Coward, *J. Heterocycl. Chem.*, **4**, 202 (1967), paper XCI of this series.

Scheme I



alkylate 2-chloro-4-nitrophenol to give to ethers (**30**). The CBZ protecting group of **30** was then removed with HBr–HOAc[15] to give **31**; for $n = 4$, the phthalyl blocking group was employed. Reaction of **31** with the appropriate O-phenylurethan,[16] acid chloride, or sulfonyl chloride gave **33** with R_1 = CONH, CO, and SO_2, respectively. Reduction of the NO_2 group of **33** to **32** without hydrogenolysis of the Cl atom was achieved with PtO_2 catalyst;[3] the amines (**32**) were converted directly to **34–36** without purification.

Experimental Section

Melting points were taken in capillary tubes on a Mel-Temp block and are uncorrected. Each analytical sample had ir and uv spectra compatible with its structure, moved as a single spot on tlc on Brinkman silica gel GF_{254}, and gave combustion analyses for C, H, and N or F within 0.4% of theory. The physical constants of **3**, **4**, **6–25** are given in Table II.

N-(Carbobenzoxy)-3-(2-chloro-4-nitrophenoxy)propylamine (30b) (Method A).—A mixture of 38.5 g (0.22 mole) of 2-chloro-4-nitrophenol, 60.4 g (0.22 mole) of **29b**,[14b] 24 g (0.22 mole) of K_2CO_3, and 150 ml of DMF was stirred at 85° for 36 hr. The mixture was poured into 1 l. of H_2O and extracted with three 300-ml portions of $CHCl_3$. The combined extracts were washed successively with three 500-ml portions of H_2O, two 200-ml portions of 1 *N* NaOH, and two 500-ml portions of H_2O. Dried with $MgSO_4$, the $CHCl_3$ solution was evaporated *in vacuo*. Two recrystallizations from EtOH with the aid of a charcoal gave 29.7 g (37%) of product, mp 85–86°. See Table III for additional data.

3-(2-Chloro-4-nitrophenoxy)propylamine (31b) Hydrobromide (Method B).—A mixture of 5.9 g (16 mmoles) of **30b** and 100 g of 15% anhydrous HBr in HOAc was stirred for 25 min when solution took place and CO_2 evolution ceased. Dilution with several volumes of Et_2O gave 5.0 g (97%) of product, mp 205–208°, that was suitable for further transformation. Recrystallization of a sample from EtOH-Et_2O gave the analytical sample, mp 208°. See Table III for additional data and compounds prepared by this method.

Method C was the same as B except the intermediate **30** was an oil and the yield is over-all for the two steps from **29**.

N-[4-(2-Chloro-4-nitrophenoxy)butyl]phthalimide (37) was prepared from commercial N-(4-bromobutyl)phthalimide by method A by reaction at 40–45° for 72 hr. One recrystallization from EtOH with the aid of charcoal gave a 54% yield of material, mp 101–105°, that was suitable for further transformation. An analytical sample, mp 105–107°, was obtained by one more recrystallization from EtOH. *Anal.* ($C_{18}H_{15}ClN_2O_5$) C, H, N.

N-(2-Chloro-4-nitrophenoxy)butylamine (31c) Hydrochloride (Method D).—A mixture of 12.3 g (32.6 mmoles) of **37**, 120 ml of EtOH, and 4.8 ml of 85% $N_2H_4 \cdot H_2O$ was refluxed for 30 min when a solid had separated. The mixture was strongly acidified with 12 *N* HCl, then diluted with several volumes of H_2O. The filtered solution was evaporated *in vacuo*. The residual amine hydrochloride was dissolved in H_2O, then the solution was filtered from insoluble phthalhydrazide. The filtrate was made basic with Et_3N and extracted twice with $CHCl_3$. The combined extracts were washed with H_2O, dried ($MgSO_4$), and evaporated *in vacuo*. The residual amine was dissolved in EtOH and acidified with 12 *N* HCl, then the HCl salt was precipitated with Et_2O. Recrystallization from EtOH–Et_2O gave 4.5 g (49%) of the product, mp 162–163°. See Table III for additional data.

5-Carbophenoxyamino-2-methylbenzenesulfonyl fluoride (38) was prepared from 5-amino-2-methylbenzenesulfonyl fluoride[7] and phenyl chloroformate by the method previously described[16] for similar compounds; yield 77%, mp 120–122°. *Anal.* ($C_{14}H_{12}FNO_4S$) C, H, N.

(15) D. Ben-Ishai, *J. Org. Chem.*, **19**, 112 (1954).

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