Irreversible Enzyme Inhibitors. CLV.^{1.2} Active-Site-Directed Irreversible Inhibitors of Dihydrofolic Reductase Derived from 1-[4-(ω-Aminoalkoxy)-3-chlorophenyl]-4,6diamino-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 5-10 × 10⁻⁵ M gave 84-100% inactivate to of the enzyme; the presence of the 3-Cl atom was necessary for good irreversible inhibitor. Of these 14 compounds, none showed complete specificity since the liver enzyme were *m*- or *p*-fluorophenylcarbamido derivatives containing two (**16**) or three (**3**, **6**) CH₂ groups. When the efficiency of kill of L1210 cell culture was measured, the best six compounds (**11**, **13**, **16**, **21-23**) showed ED₃₀'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 ED₅₀ = 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 reported³ that 1 was an active-site-directed irreversible inhibitor⁴ 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



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 SO₂F group⁶ by the liver enzyme. That the chlorine atom of **3** was necessary for good irreversible inhibition³ 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 molety⁷ 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-SO₂F 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, ihid. ${\bf 12},$ 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. Hurlbut, *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.	\mathbf{R}_1	к	\mathbf{R}_2	Mouse tissue	$_{\mu M}^{\mathrm{I}_{50},b}$	Inhib, μM	Time, min	% inactivn ^c	$\mathrm{ED}_{\mathfrak{s0}}, {}^d$ μM	E D50/ I50
3	Cl	3	$\operatorname{CONHC_6H_4SO_2F}$ -m	L1210/DF8 L1210/0	0.053	$\begin{array}{c} 0.10 \\ 0.10 \end{array}$	2, 30 60	80, 87° 82	0.57	11
				Liver		0.10	60	25		
4	H	3	$\text{CONHC}_6\text{H}_4\text{SO}_2\text{F}-m$	L1210/DF8	0.048	0.24	60	23	7.0	150
				Liver	0.030	0.24	60	0		
6	Cl	3	$\mathrm{CONHC_6H_4SO_2F}$ - p	L1210/DF8	0.021	0.10	2, 8	93, 93°	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	0.004	0.048	60	72		
0			COCH SO F	Liver	0.024	0.12	60	18		
8	CI	3	$COC_6H_4SO_2F-p$	L1210/DF8	0.046	0.092	60	69		
0				Liver		0.092	60	47	1 0	
9	CI	3	$SO_2C_6H_4SO_2F-m$	L1210/DF8	0.000	0.069	60	98	1.0	44
10	CI			Liver	0.023	0.069	60	69	0.00	10
10	CI	3	$50_{2}0_{6}H_{4}50_{2}F-p$	L1210/DF8	0.040	0.048	60	97	0.93	18
4.1			CONTRATA MERICE	Liver	0.048	0.090	60	73	0.020	4 -
11	CI.	3	$CON HC_6 H_3$ -4-Me-3- $SO_2 F$	L1210/DF8	0.0088	0.050	60 CO	87	0.039	4.5
10	CI	0	CONTRATE SOF	Liver	0.018	0.000	00	44	0.54	1 1
12	CI	3	$CONHC_6H_3$ -2- OMe -5- SO_2F	L1210/DF8	0.047	0.094	2, 30	94, 94	0.54	11
19	CI	0	CONHCIL 2 Ma 4 SO F	Liver	0.047	0.14	60 60	42	0.000	0.1
19	CI	ა	$CON HC_6 H_3 - 3 - M e - 4 - 5 O_2 r$	11210/0 T 1910/DF9		0.095	00 60	04 07	0.008	0.1
				LI2I0/DF8	0.057	0.095	60	91 64		
				Liver	0.037	0.00	60 60	20		
14	CI	9	COC.H. 4 Ma 2 SO F	T 1910/DF8		0.19	60	30 84	12	05
14	UI	0	0006113 - 4-MIC-3-6021	Liver	0.045	0.090	00 60	50 04	4.0	90
15	CI	0	CONHC H SO F-m	1910/DF8	0.040	0.10	2 4 30	57 64 64e	6.0	150
1.)	UI	2	00111061143021-11	11210/1018		0.12	2, 4, 50	10	0.0	100
				Liver	0.040	0.040	60	19		
16	CI	2	CONHC.H.SO.F-2	L1210/0	0.040	0.12	60	86	0.0032	0.36
10	C1	2	()()(()(()()()()()()()()()()()()()()()	L1210/DF8	0.0000	0.000	2 30	86 86	0.0002	0.00
				Liver		0.012	2, 00 60	18		
17	CI	2	$COC_{2}H_{2}SO_{2}F_{-m}$	L1210/DF8		0.10	60	37	1 1	15
11	01	-	00001140021 //	Liver	0.075	0.12	60	9	1.1	10
18	Cl	2	$COC_{*}H_{*}SO_{*}F_{-n}$	L1210/DF8	0.010	0.082	2 4 30	65 75 75	12	30
10	01	-	0000110021 p	Liver	0.041	0.082	2 , 1 , 00	25	1	00
19	Cl	2	$SO_{2}C_{6}H_{4}SO_{2}F-m$	L1210/DF8	01022	0.074	60	46	5.4	150
20	0.	-		Liver	0.037	0.074	60	24		200
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 ₂ F	L1210/DF8	0.015	0.072	2, 30	90, 90*	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	$\text{CONHC}_6\text{H}_4\text{SO}_2\text{F}$ -p	L1210/DF8		0.068	2, 30	81, 98*	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	\mathbf{Cl}	4	$\mathrm{COC}_{6}\mathrm{H}_{4}\mathrm{SO}_{2}\mathrm{F}$ - 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 $\theta \ \mu M$ dihydrofolate in 0.05 *M* Tris buffer (pH 7.4) containing 0.15 *M* KCl as previously described.^a ^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.^a ^c 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 irrevers-

ible 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



^{*a*} All compounds were made by H_2 -PtO₂ 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-H₂O. ^{*d*} Recrystallized from *i*-PrOH-H₂O. ^{*e*} Recrystallized from *i*-PrOH. ^{*f*} Beginning of wide decomposition range. ^{*a*} Recrystallized from Me₂CO. ^{*b*} Recrystallized from Me₂CO-H₂O.

excellent irreversible inhibitors of L1210 dihydrofolic reductase showing >80% inactivation at 2I₅₀. Of these 14 compounds none showed complete specificity by giving no inactivation of the liver enzyme at 2–3I₅₀; 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 $ED_{50} =$ $0.003-0.15 \ \mu M; \ (2)$ 3, 6, 7, 9, 10, 12, 17, 18, 24, and 25 with $ED_{50} = 0.5-1.5 \ \mu M$; and (3) 4, 14, 15, 19, and 20 with $ED_{50} = 4-7 \ \mu M$, a total spread of about 2000-fold. The ED₅₀ 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 synthesis⁹ 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, P. I. Almaule, and W. C. Werkheisher, J. Med. Chem., 7, 24 (1964).

TABLE III PHYSICAL CONSTANTS OF R_2 $NO_2 \bigcirc O(CH_2)_n NHR_1$

No.	Rı	\mathbf{R}_2	n	Method ^a	yield	Mp, °C	Formula
30b	$\rm CO_2 CH_2 C_6 H_5$	Cl	3	A	375	85 - 86	$C_{17}H_{17}ClN_2O_5$
31a	H·HBr	Cl	2	\mathbf{C}	36°	226 - 227	$C_8H_9ClN_2O_3\cdot HBr$
31b	H•HBr	\mathbf{Cl}	3	в	99	208^{d}	$C_9H_{11}ClN_2O_3\cdot HBr$
31c	H·HCl	Cl	4	D	49^d	162 - 163	$C_{10}H_{13}CIN_2O_3 \cdot HCl$
31d	H · HBr	н	3	С	28^{d}	134 - 135	$C_9H_{12}N_2O_3\cdot HBr$
39	$\text{CONHC}_{6}\text{H}_{4}\text{SO}_{2}\text{F-}m$	Cl	2	\mathbf{F}	72°	168 - 170	$\mathrm{C}_{15}\mathrm{H}_{13}\mathrm{ClFN}_{3}\mathrm{O}_{6}\mathrm{S}$
40	$\text{CONHC}_6\text{H}_4\text{SO}_2\text{F}$ -p	\mathbf{Cl}	2	\mathbf{E}^{m}	7 37	194 - 197	$C_{15}H_{13}ClFN_3O_6S$
41	$COC_6H_4SO_2F$ -m	Cl	2	G	746	178 - 180	$C_{15}H_{12}ClFN_2O_6S$
42	$COC_6H_4SO_2F-p$	Cl	2	G	69 ^b	200-202	$\mathrm{C_{15}H_{12}ClFN_2O_6S}$
43	$SO_2C_6H_4SO_2F$ -m	Cl	2	Gø	92^{h}	143 - 145	$\mathrm{C_{14}H_{12}ClFN_2O_7S_2}$
44	$SO_2C_6H_4SO_2F$ -p	Cl	2	\mathbf{G}^{g}	88 ^h	146 - 147	$\mathrm{C_{14}H_{12}ClFN_2O_7S_2}$
45	$CONHC_{6}H_{3}$ -3-Me-4- $SO_{2}F$	Cl	2	E^{i}	44 ⁱ	Amorphous ^k	
46	$\text{CONHC}_{6}\text{H}_{4}\text{SO}_{2}\text{F}-m$	Cl	3	\mathbf{F}	371	160 - 161	$C_{16}H_{15}ClFN_3O_6S$
47	$\text{CONHC}_{6}\text{H}_{4}\text{SO}_{2}\text{F-}m$	\mathbf{H}	3	\mathbf{F}	74^{b}	162 - 163	$\mathrm{C}_{16}\mathrm{H}_{16}\mathrm{FN}_{3}\mathrm{O}_{6}\mathrm{S}$
4 8	$\mathrm{CONHC_{6}H_{4}SO_{2}F}$ -p	Cl	3	\mathbf{E}^{m}	74^n	147 - 148	$C_{16}H_{15}ClFN_{8}O_{6}S$
49	$COC_6H_4SO_2F-m$	Cl	3	G	71 ⁵	129 - 131	$C_{16}H_{14}ClFN_2O_6S$
50	$\mathrm{COC}_6\mathrm{H}_4\mathrm{SO}_2\mathrm{F}$ - p	\mathbf{Cl}	3	G	500,0	160 - 165	$C_{16}H_{14}ClFN_2O_6S$
51	$SO_2C_6H_4SO_2F$ -m	Cl	3	\mathbf{G}^{g}	63^{h}	125 - 126	$\mathrm{C}_{15}\mathrm{H}_{14}\mathrm{ClFN}_{2}\mathrm{O}_{7}\mathrm{S}_{2}$
52	$\mathrm{SO_2C_6H_4SO_2F}$ -p	Cl	3	\mathbf{G}^{g}	83 ^h	125 - 126	$\mathrm{C}_{15}\mathrm{H}_{14}\mathrm{ClFN}_{2}\mathrm{O}_{7}\mathrm{S}_{2}$
53	$CONHC_{6}H_{3}$ -4-Me-3- $SO_{2}F$	Cl	3	E^{i}	54^{e}	147 - 150	$C_{17}H_{17}ClFN_3O_6S$
54	$CONHC_{6}H_{3}$ -2-OMe-5-SO ₂ F	Cl	3	\mathbf{E}	76^{p}	206-208	$C_{17}H_{17}ClFN_3O_7S$
55	$CONHC_{6}H_{3}$ -3-Me-4- $SO_{2}F$	Cl	3	E^{i}	56^{f}	161 - 163	$C_{17}H_{17}ClFN_3O_6S$
56	COC_6H_3 -4-Me-3- SO_2F	\mathbf{Cl}	3	\mathbf{G}^{q}	30°	$\operatorname{Amorphous}^{k}$	
5 7	$\mathrm{CONHC_6H_4SO_2F}$ -m	Cl	4	\mathbf{F}	68^{b}	163 - 165	$C_{17}H_{17}ClFN_3O_6S$
58	$\mathrm{CONHC_6H_4SO_2F}{-p}$	Cl	4	\mathbf{E}^{m}	32^{b}	175 - 177	$C_{17}H_{17}ClFN_3O_6S$
59	$\rm COC_6H_4SO_2F$ -m	CI	4	G	14^{b}	82 - 84	$\mathrm{C}_{17}\mathrm{H}_{16}\mathrm{ClFN}_{2}\mathrm{O}_{6}\mathrm{S}$
60	$\mathrm{COC}_{6}\mathrm{H}_{4}\mathrm{SO}_{2}\mathrm{F}$ - p	Cl	4	G	$55^{b,o}$	115 - 116	$\mathrm{C_{17}H_{16}ClFN_2O_6S}$

^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-Et₂O. ^e Recrystallized from *i*-PrOH-petroleum ether (bp 30-60°). ^f Recrystallized from *i*-PrOH. ^e Reaction run in THF. ^h Recrystallized from C₆H₆. ⁱ See ref 7 for starting sulfonyl fluoride. ^f Recrystallized from EtOH. ^e Recrystallized from EtOH-H₂O. ^m See ref 16 for starting sulfonyl fluoride. ⁿ Recrystallized from EtOH-H₂O. ^o Recrystallized from EtOH-H₂O. ^e 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. ^e 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 $\text{ED}_{50} = 2 \times 10^{-4} \, \mu M$. With **27**,³



an ED₅₀ = 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 ED₅₀ of $2 \times 10^{-4} \mu M$, and ED₅₀/I₅₀ 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

(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.

⁽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).



alkylate 2-chloro-4-nitrophenol to give to ethers (30). The CBZ protecting group of 30 was then removed with HBr-HOAc¹⁵ 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 $R_1 = \text{CONH}$, CO, and SO₂, respectively. Reduction of the NO₂ group of 33 to 32 without hydrogenolysis of the Cl atom was achieved with PtO₂ 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 the on Brinkman silica gel GF₂₅₆, 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-4nitrophenol, 60.4 g (0.22 mole) of **29b**,^{14b} 24 g (0.22 mole) of K₂CO₃, and 150 ml of DMF was stirred at 85° for 36 hr. The mixture was poured into 1 l, of H₂O and extracted with three 300ml portions of CHCl₈. The combined extracts were washed successively with three 500-ml portions of H₂O, two 200-ml portions of 1 N NaOH, and two 500-ml portions of H₂O. Dried with MgSO₄, the CHCl₈ solution was evaporated *in vacuo*. Two recrystallizations from EtOH with the aid of a charcoal gave 29.7 g (37 $_{C}$) 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 CO₂ evolution ceased. Dilution with several volumes of Et₂O gave 5.0 g (97%) of product, mp 205–208°, that was suitable for further transformation. Recrystallization of a sample from EtOH-Et₂O 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.* (C₁₅H₁₅CIN₂O₅) C, H, N. **N-(2-Chloro-4-nitrophenoxy)butylamine** (31c) Hydrochloride

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% N₂H₄·H₂O 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 H₂O. The filtered solution was evaporated *in vacuo*. The residual amine hydrochloride was dissolved in H₂O, then the solution was filtered from insoluble phthalhydrazide. The filtrate was made basic with Et₃N and extracted twice with CHCl₃. The combined extracts were washed with H₄O, dried (MgSO₄), and evaporated *in vacuo*. The residual amine was dissolved in EtOH and acidified with 12 N HCl, then the HCl salt was precipitated with Et₄O. Recrystallization from EtOH=Et₄O 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 fluoride⁷ and phenyl chloroformate by the method previously described¹⁶ for similar compounds; yield 77%, mp 120–122°. Anal. (C₁₄H₁₂FNO₄S) C. H. N.

⁽¹⁵⁾ D. Ben-Ishai, J. Org. Chem., 19, 62 (1954).

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