stirring to 100 ml of Ac_2O at 100–120°. After the exothermic reaction subsided, the dark reaction mixture was stirred and refluxed for 0.5–1.0 hr. EtOH was cautiously added until the excess Ac_2O was converted to EtOAc and AcOH. The resultant solutions were concd by flash evaporation to dark oils except in those cases where they were suspected of being highly volatile. The reaction mixture was then cooled and neutralized with $KHCO_3$ solution. The organic layer was extracted with $CHCl_3$, dried ($MgSO_4$), and distd *in vacuo*. Some of the acetates (IIId, IIIe, IIIi) crystallized on standing and were recrystd from petroleum ether or EtOH.

Substituted 2-Pyridylmethanols (IVa–i). **A.**—Concd HCl (50 ml) was added to 0.1 mole of a substituted 2-pyridylmethanol acetate and refluxed for 1 hr. The solution was evapd to dryness *in vacuo* to give the HCl salt of the substituted 2-pyridylmethanol. Some of the HCl salts were neutralized with $KHCO_3$ solution and the organic material was extracted with $CHCl_3$, dried ($MgSO_4$), and distilled. In some cases the free base crystallized on standing (IVd and IVe). Some of the HCl salts were neutralized without further purification and utilized in the oxidn reaction (C).

B.—NaOH (1.2 equiv wt) and a substituted 2-pyridylmethanol acetate (1.0 equiv wt) were added to H_2O and refluxed for 1 hr. The substituted methanol solidified on cooling and was filtered and dried or (if it did not solidify) extracted with $CHCl_3$, dried ($MgSO_4$), and distd.

Substituted 2-Formylpyridines (Va–i). **C.**—To a substituted 2-pyridylmethanol dissolved in $CHCl_3$ was added 2 to 3 times its wt of active MnO_2. The reaction mixture was allowed to stir and reflux for 2 hr and then filtered, and the MnO_2 cake washed well with boiling $CHCl_3$. The $CHCl_3$ extracts were combined, dried ($MgSO_4$), and distd to furnish the aldehydes. In the cases where the aldehydes were solids after evaporation of the $CHCl_3$, they were crystallized from petroleum ether. IVj was oxidized with MnO_2 in *i*-PrOH because of its poor solubility in $CHCl_3$.

D.—$Pb(OAc)_4$ (1.1 equiv wt) was added portionwise to the 2-pyridylmethanol (1.0 equiv wt) dissolved in dry $CHCl_3$. The yellow solution was allowed to stand 3 days at room temp, then treated with excess $KHCO_3$ solution, and filtered. The organic layer was sepd from the aq layer, dried ($MgSO_4$), and distd to give the pure aldehyde.

Acknowledgments.—We thank Dr. Harry B. Wood, Jr., Professor John Broomhead, Dr. R. Wallace Brockman, Professor Alan C. Sartorelli, and Dr. T. W. Brooks for helpful consultations and encouragement. We express our appreciation to Arvia Hosking and June French for the antitumor evaluations and to Dee J. Glasby and David Chu for excellent technical assistance.

Irreversible Enzyme Inhibitors. CLXXIII.[1,2] Cure of Walker 256 Ascites by Reversible and Irreversible Inhibitors of Dihydrofolic Reductase[3] Derived from 1-(Substituted-phenyl)-4,6-diamino-1,2-dihydro-2,2-dimethyl-*s*-triazine

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Nine active-site directed irreversible inhibitors and seven potent reversible inhibitors of dihydrofolic reductase were assayed against Walker 256 ascites, Dunning leukemia ascites, and intramuscular Walker 256 in the rat. Some of the irreversible and reversible inhibitors were remarkably effective in promoting cures of the 2 ascitic tumors; however, there was no correlation between tissue specificity of irreversible inhibition and *in vivo* activity, indicating that other unknown factors were playing important roles in these cures. The best compounds against Walker 256 ascites were the 1-phenyl-4,6-diamino-1,2-dihydro-2,2-dimethyl-*s*-triazines substituted on the Ph group with *p*-$(CH_2)_2CONHC_6H_3$-3-Me-4-SO_2F (**1**), *m*-$(CH_2)_4C_6H_4SO_2F$-*p*, (**7**), *p*-$(CH_2)_2CONHC_6H_4$-3-Me (**15**), 3-Cl-4-$O(CH_2)_3OC_6H_5$ (**16**), 3-Cl-4-$(CH_2)_2C_6H_5$ (**18**), or *p*-$(CH_2)_4C_6H_5$ (**19**) moieties. The best compounds against the Dunning leukemia ascites were **18**, **19**, and the phenyltriazine substituted by the 3-Cl-4-$(CH_2)_2C_6H_4$-SO_2F-*p* (**9**) and 3-Cl-4-$OCH_2CONHC_6H_5$ (**17**) moieties.

The design of enzyme inhibitors as possible chemotherapeutic agents has the advantage that direct answers on inhibition of the target can be obtained by assay with the isolated enzyme. With this approach complications such as transport through membranes and metabolism are avoided in order to gain insight on selectivity of attack of the target enzyme.[6] Concepts emerged over a period of 10 years[7] that allowed design of enzyme inhibitors so highly specific that they could differentiate between isozymes[8] or even the same enzyme (such as dihydrofolic reductase) from two or more different tissues in the same animal.[9]

Once this specificity at the isolated enzyme level had been achieved,[10] it was time to return to assay of these inhibitors in whole animals bearing a tumor; if these highly specific enzyme inhibitors failed to work *in vivo*, there was some assurance that the difficulty was not in failure to attack the target enzyme, but was due to the other *in vivo* factors such as transport and metabolism that had been deliberately avoided to this point. The first studies on these dihydrofolic reductase inhibitors were done with L1210 mouse leukemia;[11] although significant life extensions were

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

(2) For the previous paper in this series see R. Cardinaud and B. R. Baker, *J. Med. Chem.*, **13**, 467 (1970).

(3) For the previous paper on this enzyme see B. R. Baker, N. M. J. Vermeulen, and A. J. Ryan, *ibid.*, **13**, 281 (1970).

(4) (a) To whom correspondence should be addressed. (b) N. M. J. V. wishes to thank, the Council of Scientific and Industrial Research, Republic of South Africa, for a tuition fellowship.

(5) On sabbatical leave from the University of Sydney, Australia.

(6) B. R. Baker, "Design of Active-Site-Directed Irreversible Enzyme Inhibitors," Wiley, New York, N. Y., 1967.

(7) B. R. Baker, *Cancer Chemother. Rep.*, **4**, 1 (1959).

(8) B. R. Baker and R. P. Patel, *J. Pharm. Sci.*, **53**, 714 (1964).

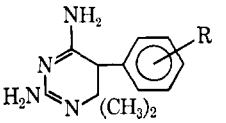
(9) (a) For a summary of this approach to highly selective inhibitors of dihydrofolic reductase see B. R. Baker, *Accounts Chem. Res.*, **2**, 129 (1969). (b) The tissue selectivity with these crude enzyme preparations could be due to selective irreversible inhibition of isozymes or to the rapid destruction of the irreversible inhibitor in normal tissue or both.

(10) (a) B. R. Baker and R. B. Meyer, Jr., *J. Med. Chem.*, **11**, 489 (1968), paper CXIX; (b) B. R. Baker and P. C. Huang, *ibid.*, **11**, 495 (1968), paper CXX.

(11) B. R. Baker, G. J. Lourens, R. B. Meyer, Jr., and N. M. J. Vermeulen, *ibid.*, **12**, 67 (1969), paper CXXIII.

TABLE I

In Vitro INHIBITION[a] OF DIHYDROFOLIC REDUCTASE WITH

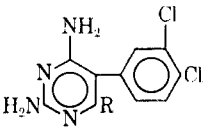


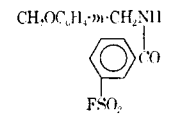
No.	R	Rat tissue	I_{50},[b] μM	Inhib, μM	Time, min	% inactvn[c]	ED_{50},[d] μM	Rating[e]
1[f]	4-$(CH_2)_2CONH$-(3-CH_3-4-SO_2F)	W256	0.013	0.065	60	100	0.001	8+
		DL[k]		0.065	20	78		
		Liver	0.012	0.065	60	28		
		Spleen		0.065	20	27		
		Kidney		0.065	20	7		
		Intestine		0.065	20	53		
5[g]	3-Cl-4-$O(CH_2)_4O$-SO_2F	W256	0.057	0.18	60	94	0.003	5+
		DL[k]		0.18	20	98		
		Liver	0.052	0.18	60	52		
		Spleen		0.18	20	66		
		Kidney		0.18	20	38		
		Intestine		0.18	20	70		
6[h]	3-Cl-4-$O(CH_2)_2NHCO$-(2-NH-5-FO_2S)	W256	0.012	0.050	60	77	0.003	7+
		DL[k]		0.050	20	56		
		Liver	0.014	0.050	60	0		
		Spleen		0.050	20	8		
		Kidney		0.050	20	16		
		Intestine		0.050	20	28		
7[i]	3-$(CH_2)_4$-SO_2F	W256	0.014	0.050	60	85	0.0002	8+
		DL[k]		0.050	20	21		
		Liver	0.018	0.050	60	95		
		Spleen		0.050	20	7		
		Kidney		0.050	20	15		
		Intestine		0.050	20	0		
8[g]	3-Cl-4-OCH_2CONH-SO_2F	W256	0.025	0.074	60	64	0.4	2+
		DL[k]		0.074	20	72		
		Liver	0.027	0.11	60	50		
		Spleen		0.11	20	79		
		Kidney		0.11	20	11		
		Intestine		0.11	20	65		
9[g]	3-Cl-4-$(CH_2)_2$-SO_2F	W256	0.008	0.050	60	86	0.03	6+
		DL[k]		0.050	20	34		
		Liver	0.018	0.050	60	86		
		Spleen		0.050	20	61		
		Kidney		0.050	20	43		
		Intestine		0.050	20	9		
10[g]	4-$(CH_2)_4$-SO_2F	W256	0.014	0.050	60	62	0.2	4+
		DL[k]		0.050	20	5		
		Liver	0.022	0.060	60	12		
		Spleen		0.060	20	36		
		Kidney		0.060	20	8		
		Intestine		0.060	20	5		
11[g]	3-Cl-4-$(CH_2)_2CONH$-SO_2F	W256	0.015	0.050	60	86	0.001	8+
		DL[k]		0.050	20	70		
		Liver	0.015	0.050	60	16		
		Spleen		0.050	20	36		
		Kidney		0.050	20	25		
		Intestine		0.050	20	8		
12[j,l]	3,4-Cl_2	W256	0.0029				0.0002	0
		Liver	0.010					

[a] The technical assistance of Diane Shea with the enzyme assays is acknowledged. [b] I_{50} = concn for 50% inhibn when assayed with 6 μM dihydrofolate in pH 7.4 Tris buffer contg 0.15 M KCl as previously described.[11] [c] A 45–90% $(NH_4)_2SO_4$ fraction of enzyme incubated with inhibitor at 37° in pH 7.4 Tris buffer contg 60 μM TPNH, then the remaining enzyme assayed as previously described;[11] the 20-min runs were done at 24° due to the thermal instab lity of the enzyme. [d] Concn for 50% inhibn of growth of L1210 cell culture. [e] Arbitrary rating for selectivity of enzyme inhibn. Irreversible inhibn of W256 enzyme: 5+, 85–100; 3+, 70–84; 1+, 60–69. Specificity: 1+ for each normal tissue showing <30% irreversible inhibn. [f] Synthesis.[23a] [g] Synthesis.[23b] [h] Synthesis.[12b] [i] Synthesis.[12c] [j] NSC-3077 obtd from CCNSC, National Cancer Institute. [k] DL = Dunning leukemia was grown as a solid tumor and supplied by Dr. Florence White. A 45–90% $(NH_4)_2SO_4$ fraction was prepd and assayed by the usual methods.[11] [l] Data from B. R. Baker, *J. Med. Chem.*, **11**, 483 (1968).

TABLE II

In Vitro INHIBITION[a] OF DIHYDROFOLIC REDUCTASE WITH

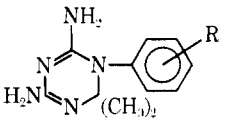


No.	R	Rat tissue	I_{50},[b] μM	Inhib, μM	Time, min	% inactvn[c]	ED_{50},[d] μM	Rating[e]
13[f]		W256	0.088	0.11	60	68	6	2+
		Liver	0.046	0.11	60	33		
		Spleen		0.11	20	46		
		Kidney		0.11	20	9		
		Intestine		0.11	20	46		
14[g]	CH_3		0.010[h]				0.00002	0

[a–e] See corresponding footnotes in Table I. [f] Synthesis.[12c] [g] NSC-19494 from CCNSC, National Cancer Institute. [h] Pigeon liver enzyme.

TABLE III

In Vivo INHIBITION OF WALKER 256 ASCITES BY



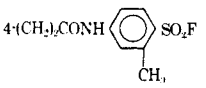
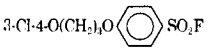
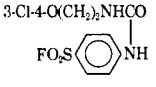
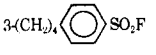
No.	R	Test group[a]	mg/kg per day[b]	Survivors Day 30	Survivors Day 60	Survivors Day 80	Chemother index[c]	Rating[d]
1		A[e]	200	Toxic			16	8+
		A	100	3/6[f]				
		A	50	6/6[f]				
		A	25	6/6[f]				
		B[e]	12.5	4/6	3/6	3/6		
		B	6.25	4/6	4/6	3/6		
		B	3.13	4/6	3/6	3/6		
		C[e]	1.56	1/6	1/6			
		C	0.78	2/6	2/6			
		C	0.39	1/6	1/6			
		C	0.19	0/6[g]				
5		A[e]	50	Toxic			4	5+
		A	25	1/6				
		G[e]	12.5	3/6	2/6	1/6		
		G	6.25	2/6	2/6	2/6		
		D[h]	3.13	4/6	4/6			
		D	1.56	4/6	1/6			
		D	0.78	6/6	3/6			
		D	0.39	3/6	1/6			
		D	0.19	2/6	0/6			
6		A[e]	100	Toxic			64	7+
		A	50	5/6[f]				
		A	25	4/6[f]				
		G[e]	12.5	4/6	2/6	2/6		
		G	6.25	4/6	3/6	3/6		
		E[h]	3.13	3/6	3/6			
		E	1.56	5/6	4/6			
		E	0.78	4/6	2/6			
		E	0.39	2/6	1/6			
		E	0.19	3/6	2/6			
7		A[e]	25	Toxic			32	8+
		G[e]	12.5	6/6	5/6	5/6		
		G	6.25	5/6	5/6	5/6		
		G	3.13	5/6	5/6	5/6		
		E[h]	1.56	4/6	4/6			
		E	0.78	5/6	3/6			
		E	0.39	6/6	4/6			
		E	0.19	3/6	2/6			

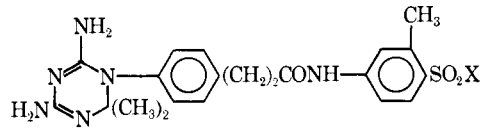
TABLE III (*Continued*)

No.	R	Test group[a]	mg/kg per day[b]	Survivors Day 30	Survivors Day 60	Survivors Day 80	Chemother index[c]	Rating[d]
8	3-Cl-4-OCH_2CONH-C$_6$H$_3$-SO_2F	A[e]	50	Toxic			16	2+
		A	25	2/6[f]				
		G[e]	12.5	5/6	3/6	1/6		
		G	6.25	3/6	3/6	3/6		
		E[h]	3.13	6/6	4/6			
		E	1.56	5/6	1/6			
		E	0.78	5/6	3/6			
		E	0.39	0/6[i]				
9	3-Cl-4-$(CH_2)_2$-C$_6$H$_3$-SO_2F	A[e]	100	Toxic			2	6+
		A	50	3/6[f]				
		A	25	1/6[f]				
		G[e]	12.5	3/6	1/6	1/6		
		G	6.25	5/6	1/6	1/6		
		E[h]	3.13	6/6	4/6	4/6		
		E	1.56	3/6	1/6			
		E	0.78	2/6	2/6			
		E	0.39	2/6	2/6			
		E	0.19	2/6	2/6			
10	4-$(CH_2)_4$-C$_6$H$_3$-SO_2F	A[e]	50	Toxic			1	4+
		A	25	3/6[f]				
		G[e]	12.5	5/6	5/6	5/6		
		G	6.25	2/6	2/6	2/6		
		G	3.13	3/6	0/6			
		E[h]	1.56	3/6	1/6			
		E	0.78	3/6	2/6			
		E	0.39	4/6	1/6			
		E	0.19	0/6[i]				
11	3-Cl-4-$(CH_2)_2CONH$-C$_6$H$_3$-SO_2F	A[e]	25	Toxic			>64	8+
		G[e]	12.5	5/6	4/6	3/6		
		G	6.25	3/6	2/6	2/6		
		F[e]	3.13	6/6	3/6			
		F	1.56	5/6	5/6			
		F	0.78	5/6	4/6			
		F	0.39	3/6	2/6			
		F	0.19	4/6	1/6			
12	3,4-Cl_2	G[e]	25	0/6			0	0
		G	12.5	2/6	0/6			
		G	6.25	0/6	0/6			
		G	3.13	2/6	2/6			
		D[h]	1.56	3/6	2/6			
		D	0.78	3/6	3/6			
		D	0.39	0/6	0/6			
		D	0.19	2/6	2/6			

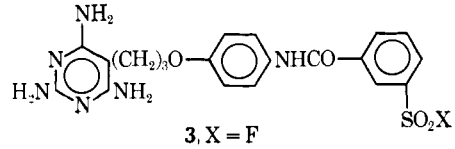
[a] Test group indicates which compds were run simultaneously with the same control group. [b] Single ip doses on days 1–9 after injection of 10^5 ascites cells on day 0.[16a] [c] The chemotherapeutic index was arbitrarily selected as the dose range giving 4/6 30-day survivors. [d] *In vitro* rating of selectivity of enzyme inhibn from Table I. [e] Controls survived a median of 8 days. [f] Animals sacrificed after 30 days and longer survival data not obtd. [g] Median survival time was 13.5 days; $T/C = 170$. [h] Controls survived median of 7.5 days. [i] Median survival time was 14 days; $T/C = 186$. [j] Median survival time was 12.5 days; $T/C = 166$.

achieved, the results with some of the more selective compounds indicated that an insufficient amount of the inhibitor was reaching the target enzyme. Part of the difficulty was traced to poor transport through the L1210 cell membrane by use of L1210 cell culture.[11] This difficulty was soon overcome by use of appropriate structures that allowed good transport.[12]

A second complication was found by Folsch and Bertino.[13] They observed that irreversible inhibitors of dihydrofolic reductase of the sulfonyl fluoride type, such as **1** and **3**, were rapidly converted by mouse



1, X = F
2, X = OH



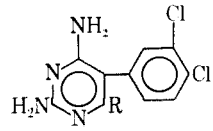
3, X = F
4, X = OH

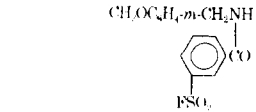
(12) (a) B. R. Baker and R. B. Meyer, Jr., *ibid.*, **12**, 668 (1969), paper CLIV. (b) B. R. Baker and E. E. Janson, *ibid.*, **12**, 672 (1969), paper CLV. (c) B. R. Baker and N. M. J. Vermeulen, *ibid.*, **12**, 680, 687 (1969), papers CLVII, CLVIII. (d) B. R. Baker and W. T. Ashton, *ibid.*, **12**, 894 (1969), paper CLIX. (e) B. R. Baker, E. E. Janson, and N. M. J. Vermeulen, *ibid.*, **12**, 898 (1969), paper CLX. (f) B. R. Baker and N. M. J. Vermeulen, *ibid.*, **13**, 82 (1970), paper CLXVI.

(13) E. Folsch and J. R. Bertino, *Mol. Pharmacol.*, **6**, 93 (1970).

TABLE IV

In Vivo INHIBITION OF WALKER 256 ASCITES BY

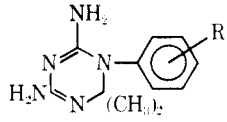


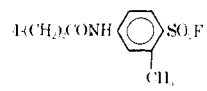
No.	R	Test group[a]	mg/kg per day[b]	Survivors, day 30	T/C	Chemother Index[c]	Rating[d]
13		A[e]	200[f]	5/6		<4	2+
		A	100	1/6			
		A	50	5/6			
		A	25	2/6			
		A	12.5	3/6			
		G[g]	6.25	0/6	125		
		G	3.12	0/6	113		
14	CH_3	G[h]	25	0/6	93	0	0
		G	12.5	0/6	139		
		G	6.25	0/6	146		
		D[g]	3.13	0/6	166		
		D	1.56	0/6	114		
		D	0.78	0/6	114		
		D	0.39	0/6	100		
		D	0.19	0/6	107		

[a–c] Same as corresponding footnotes in Table III. [d] *In vitro* rating of selectivity of enzyme inhibn from Table II. [e] Controls survived an average of 8 days.[16a] [f] Higher dose levels were not run. [g] Controls survived an average of 7.5 days.[16a]

TABLE V

In Vivo INHIBITION OF WALKER 256 ASCITES BY DELAYED TREATMENT WITH



No.	R	Test group[a]	mg/kg per day[b]	Survivors Day 30	Survivors Day 50	Survivors Day 80
1		J[c]	50	2/6	2/6	2/6
		J	25	4/6	4/6	1/6
		J	12.5	0/6		
		I[d]	50	2/6	2/6	
		I	25	5/6	5/6	
		I	12.5	3/6	3/6	
12	3,4-Cl_2	I	3.12	1/6	1/6	
		I	1.56	0/6		

[a] Test group indicates which compounds were run simultaneously with the same control group. [b] Single ip dose on days 5–13 after injection with 10^5 ascites cells on day 0; dose selected as optimum range from Table III. [c] Controls survived a median of 7 days.[16a] [d] Controls survived a median of 8 days.[16a]

serum into metabolic products which were most probably the corresponding sulfonic acids, **2** and **4**, respectively; this metabolic conversion was much less in rat serum and unobservable with human serum. Since it was clear from their results that the mouse was an unsatisfactory test animal, we turned to the rat bearing Walker 256 in the ascites form in the peritoneal cavity. Eight irreversible inhibitors of dihydrofolic reductase of the dihydro-*s*-triazine type such as **1** were selected for test only on the basis that they showed good to fair transport characteristics; one 2,4-diaminopyrimidine was also selected even though transport was less effective. The results are the subject of this paper.

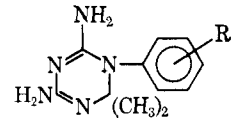
In Vitro **Assays.**—Assay of the 8 dihydro-*s*-triazines with the dihydrofolic reductase from Walker 256 rat tumor and 4 normal tissues are collated in Table I. The ED_{50} in L1210 cell culture[14] represents a first approximation of the ability of the compound to penetrate the L1210 cell membrane; a more exact approximation can be obtained by taking into account both the reversible and irreversible effect of the compounds on the target inside the cell, namely, dihydrofolic reductase.[12] The reversible I_{50}'s of the 8 compounds with the Walker 256 enzyme differed only sevenfold from **9** to **5**. Most of these inhibitors showed little difference in reversible inhibition of Walker 256 *vs.* liver enzyme, the maximum rpread for a single compound being twofold. Irseversible inhibition of the tumor enzyme varied from 100% for **1** down to 62% for **10**. A potent reversible inhibitor (**12**) is included for comparison; **12** is the most potent compound in Table I as a reversible inhibitor of the Walker 256 enzyme and as an inhibitor of L1201 cell culture.

In order to try to correlate enzyme activity in Table I with the effect of the compounds on Walker 256 ascites *in vivo* (Table III), each compound was assigned a rating for selectivity by their effectiveness of

(14) We wish to thank Dr. Florence White of the CCNSC for these data obtained by Drs. P. S. Thayer and P. Himmelfarb of Arthur D. Little, Inc.

TABLE VI

In Vivo INHIBITION OF WALKER 256 ASCITES BY REVERSIBLE INHIBITORS



		In vitro			*In vivo*			
		Rat	I_{50},[a]	ED_{50},[b]	Test	mg/kg	Survivors	
No.	R	tissue	μM	μM	group[c]	per day[d]	Day 30	Day 50
15	4-$(CH_2)_2CONHC_6H_4CH_3$-*m*	W256	0.022	0.01	K[e]	50	6/6	
		Liver	0.039		K	25	6/6	
					K	12.5	5/6	
					K	6.25	6/6	
					K	3.12	4/6	
16	3-Cl-4-$O(CH_2)_3OC_6H_5$	W256	0.048	0.0004	K[e]	50	2/6	
		Liver	0.043		K	25	5/6	
					K	12.5	6/6	
					K	6.25	4/6	
					K	3.12	4/6	
17	3-Cl-4-$OCH_2CONHC_6H_5$	W256	0.026	0.6	F[e]	100	Toxic	
		Liver	0.048		F	50	4/6	2/6
					F	25	5/6	2/6
					F	12.5	5/6	1/6
					F	6.25	4/6	1/6
					L[f]	3.12	4/6	
					L	1.56	1/6	
					L	0.78	0/6	
18	3-Cl-4-$(CH_2)_2C_6H_5$	W256	0.0086	0.003	F[e]	25	Toxic	
		Liver	0.021		F	12.5	4/6	4/6
					F	6.25	5/6	4/6
					F	3.12	3/6	3/6
					F	1.56	4/6	2/6
					L[f]	6.25	3/6	3/6
					L	3.12	4/6	3/6
					L	1.56	3/6	3/6
					L	0.78	4/6	4/6
					L	0.39	1/6	0/6
19	4-$(CH_2)_4C_6H_5$	W256	0.0078	0.01	F[e]	25	Toxic	
		Liver	0.0086		F	12.5	4/6	1/6
					F	6.25	6/6	6/6
					F	3.13	5/6	3/6
					F	1.56	5/6	4/6
					L[f]	6.25	2/6	2/6
					L	3.12	2/6	1/6
					L	1.56	2/6	2/6
					L	0.78	2/6	1/6
					L	0.39	2/6	2/6

[a] The technical assistance of Diane Shea with the enzyme assays is acknowledged. I_{50} = concn for 50% inhibn of dihydrofolic reductase when assayed with 6 μM dihydrofolate in pH 7.4 Tris buffer contg 0.15 *M* KCl as previously described.[11] [b] Concn for 50% inhibn of growth of L1210 cell culture. [c] Test group indicates which compds were run simultaneously with the same control group. [d] Single ip dose on days 1–9 after injection of 10^5 ascites cells on day 0. [e] Median survival of controls = 8 days.[16a] [f] Median survival of controls = 8 days.[16b]

irreversible inhibition on the tumor enzyme and lack of effectiveness on the enzyme from 4 normal tissues. This rating system varied from 0 to 9+ as described in footnote *e* of Table I. The compounds with the highest rating (8+) were **1**, **7**, and **11**; the irreversible inhibitors with lowest rating were **8** (2+) and **10** (4+). Of course, a reversible inhibitor such as **12** would have a rating of zero since **12** has no selectivity in inhibition at the target level.

Corresponding data with a reversible and an irreversible inhibitor of the 2,4-diaminopyrimidine type are shown in Table II.

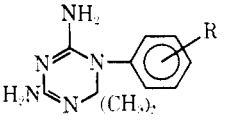
In Vivo **Inhibition of Walker 256 Ascites.**—When the compounds in Tables I and II were assayed against peritoneal Walker 256 ascites in the rat ip,[15] all showed some cures except the potent reversible inhibitor **14** (Tables III and IV).[16a] The irreversible inhibitors were clearly more effective *in vivo* than the reversible inhibitors, **12** and **14**, even though the latter 2 compounds were more potent *in vitro* against L1210 cell culture. The further superiority of one irreversible inhibitor (**1**) over the excellent reversible inhibitor (**12**)

(15) CCNSC, *Cancer Chemother. Rep.*, **25**, 1 (1962).

(16) We wish to thank Dr. Florence White of the CCNSC for these data obtained by (a) I. Wodinsky of Arthur D. Little, Inc. (b) Ann Smith of Southern Research Institute, (c) Hazelton Laboratories.

TABLE VII

In Vivo INHIBITION OF WALKER 256 ASCITES BY DELAYED TREATMENT WITH



No.	R	Test group[a]	mg/kg per day[b]	Survivors No.	Survivors Day	T/C
5	3-Cl-4-O(CH$_2$)$_3$O-C$_6$H$_3$-SO$_2$F	M[c]	25	1/6	60	265
		M	12.5	0/6	60	211
		M	6.25	1/6	60	224
7	3-(CH$_2$)$_4$-C$_6$H$_4$-SO$_2$F	N[d]	12.5	5/6	50	509
		N	6.25	0/6	50	164
		N	3.12	0/6	50	128
9	3-Cl-4-(CH$_2$)$_4$-C$_6$H$_3$-SO$_2$F	N[d]	6.25	2/6	50	289
		N	3.12	1/6	50	181
		N	1.56	1/6	50	187
10	4-(CH$_2$)$_4$-C$_6$H$_4$-SO$_2$F	N[d]	12.5	1/6	50	215
		N	6.25	1/6	50	185
		N	1.56	1/6	50	202
15	4-(CH$_2$)$_2$C(O)NHC$_6$H$_4$CH$_3$-*m*	M[c]	100	0/6	60	147
		M	50	3/6	60	497
		M	25	2/6	60	352
		M	12.5	1/6	60	215
16	3-Cl-4-O(CH$_2$)$_3$OC$_6$H$_5$	M[c]	25	3/6	60	484
		M	12.5	2/6	60	383
		M	6.25	0/6	60	182
18	3-Cl-4-(CH$_2$)$_2$C$_6$H$_5$	N	12.5	3/6	50	418
		N	6.25	3/6	50	382
		N	3.12	1/6	50	201
19	4-(CH$_2$)$_4$C$_6$H$_5$	N[d]	12.5	2/6	50	264
		N	6.25	3/6	50	346
		N	3.12	3/6	50	335

[a,b] See Table V. [c] Controls survived median of 8 days.[16b] [d] Controls survived median of 9 days.[16a]

was clearly demonstrated by delay of treatment until 2 days before death (Table V);[16a,b] a number of cures by **1** were still obtained, demonstrating the remarkable activity of **1** in this test system.

Attempts to correlate the chemotherapeutic indices with the *in vitro* ratings of the irreversible inhibitors in Table III were poor; other arbitrary assignments for the chemotherapeutic index and *in vitro* rating gave even poorer correlation. This lack of correlation indicated that one or more other unknown factors than those presented in Tables I and III were playing a role in the selectivity.

This lack of correlation between the irreversible inhibitors also cast some doubt on the conclusion that irreversible inhibitors such as **1** were more effective than any reversible inhibitor; for example, it was possible—though seemingly improbable—that the irreversible inhibitors, **1** and **5–11**, were more effective than the reversible inhibitor **12** due to the more complex side chains of the irreversible inhibitors. Therefore, 5 reversible inhibitors (**15–19**) having the structure of **1**, **5**, **8**, **9**, and **10** (Table III), respectively, where only the SO_2F group was removed, were synthesized for *in vivo* evaluation. Both *in vitro* and *in vivo* data are presented in Table VI.[16b] Comparisons of some of the reversible and irreversible inhibitors in delayed treatment are collated in Table VII;[16b] a number of the compounds were remarkably effective, particularly the reversible inhibitors. As could be expected, the compounds were less effective by ip injection against Walker 256 in the leg muscle, but still showed a positive response (Table VIII).

The Dunning leukemia in ascites form is responsive to amethopterin treatment, 60–70% extension of life being observed, but there were no 30-day survivors (Table IX). Several of the reversible and irreversible inhibitors were far more effective than amethopterin, the most effective being the reversible inhibitors **17–19**.

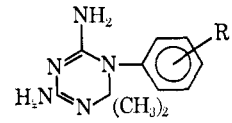
Discussion

A series of 9 active-site-directed irreversible inhibitors of dihydrofolic reductase were selected for extensive evaluation in the rat against Walker 256 ascites. This selection was based mainly on the ability of the compounds to be transported through a mammalian cell wall as approximated by inhibition of L1210 cell culture, regardless of whether a compound showed good tissue specificity in inhibition of dihydrofolic reductase (Tables I and II). Some of these irreversible inhibitors were remarkably effective in curing the Walker 256 ascites (Table III) even when treatment was delayed to 2 days before death (Table V).

The fact that irreversible inhibitors such as **1** and **5-11** were much more effective *in vivo* than the potent

TABLE VIII

In Vivo INHIBITION OF INTRAMUSCULAR WALKER 256 BY



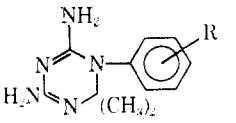
No.	R	Test group[a]	mg/kg day[b]	Survivors[d]	Animal wt change[c]	Tumor wt: Test	Tumor wt: Control	T/C
1	4-$(CH_2)_2CONH$-(3-CH_3)-SO_2F	P	100	3/6	−23	0.9	8.5	10
		P	50	5/6	−29	0.5	8.5	5
		P	25	6/6	−22	1.6	8.5	18
		Q	25	6/6	−2	4.4	7.2	61
		Q	12.5	6/6	−1	5.6	7.2	77
		Q	6.25	6/6	+5	7.1	7.2	98
5	3-Cl-4-$O(CH_2)_2O$-SO_2F	R	100	0/6				
		R	50	6/6	−26	1.2	3.9	30
		R	25	6/6	−10	2.0	3.9	51
		S	25	6/6	−9	2.6	4.9	53
		S	12.5	6/6	−3	3.7	4.9	75
		S	6.25	6/6	−1	4.7	4.9	95
6	3-Cl-4-$O(CH_2)_2NHCO$-(4-FO_2S-NH)	R	100	6/6	−19	0.9	3.9	23
		R	50	6/6	−6	1.6	3.9	41
		S	50	6/6	−9	1.3	4.9	26
		S	25	6/6	−8	2.5	4.9	51
		S	12.5	6/6	+2	3.5	4.9	71
7	3-$(CH_2)_4$-SO_2F	R	50	4/6	−19	0.7	3.9	17
		R	25	6/6	−3	1.4	3.9	35
		S	25	6/6	−5	1.7	4.9	34
		S	12.5	6/6	0	2.9	4.9	59
		S	6.25	6/6	+2	3.5	4.9	71
8	3-Cl-4-OCH_2CONH-SO_2F	R	100	6/6	−30	1.2	3.9	30
		R	50	6/6	−15	2.1	3.9	53
		R	25	6/6	−2	2.5	3.9	64
		S	25	6/6	0	3.2	4.9	65
		S	12.5	6/6	+3	2.8	4.9	57
		S	6.25	6/6	+1	3.1	4.9	63
9	3-Cl-4-$(CH_3)_2$-SO_2F	S	50	6/6	−16	0.4	4.9	8
		S	25	6/6	−4	0.9	4.9	18
		S	12.5	6/6	−1	2.8	4.9	57
10	4-$(CH_2)_4$-SO_2F	S	25	6/6	−3	1.0	4.9	20
		S	12.5	6/6	1	1.9	4.9	38
		S	6.25	6/6	0	3.2	4.9	65
11	3-Cl-4-$(CH_2)_2CONH$-SO_2F	S	12.5	6/6	−19	0.6	4.9	12
		S	6.25	6/6	−7	1.9	4.9	38
		S	3.13	6/6	−2	2.1	4.9	42
15	4-$(CH_2)_2CONHC_6H_4CH_3$-*m*	S	100	6/6	−2	0.1	4.9	2
		S	50	6/6	−4	1.7	4.9	34
		S	25	6/6	−4	2.6	4.9	53
19	4-$(CH_2)_4C_6H_5$	R	25	6/6	−14	1.1	3.9	28
		R	12.5	6/6	−5	3.3	3.9	84
		S	12.5	6/6	−7	1.3	4.9	26
		S	6.25	6/6	+1	2.9	4.9	59
		S	3.13	6/6	+4	4.8	4.9	97

[a] Test group indicates which compds were run simultaneously with the same control group.[16c] [b] Single ip dose on days 3–6. [c] From controls. [d] Day 7.

reversible inhibitors (Tables III and IV) with a 3,4-dichlorophenyl side chain (**12**, **14**) was initially exciting since it supported our concept that good irreversible inhibitors should be better agents than reversible inhibitors;[7] this concept was later formalized in the bridge principle of specificity.[8,17] However, it was disturbing that good correlation of *in vitro* irreversible specificity (Table I) with *in vivo* cures was not seen; therefore a series of reversible inhibitors (**15–19**) differing only from the irreversible inhibitors by replacement of the SO_2F group with H were synthesized for comparison.

The reversible inhibitors (**15–19**) with more complex

(17) Reference 6, pp 173–184.

TABLE IX: *In Vivo* INHIBITION OF DUNNING LEUKEMIA ASCITES BY 

No.	R	Test group[a]	mg/kg per day[b]	Survivors, day 35	T/C
1	4-(CH_3)$_2$CONH, 3-SO_2F, 4-CH_3	T	25	0/6	162
		T	12.5	0/6	156
		T	6.25	0/6	118
		T	3.13	0/6	131
		T	1.56	0/6	125
		T	0.78	0/6	125
5	3-Cl-4-O(CH_2)$_3$O, SO_2F	T	25	1/6	175
		T	12.5	2/6	250
		T	6.25	1/6	175
		T	3.13	0/6	175
		T	1.56	0/6	162
		T	0.78	0/6	137
6	3-Cl-4-O(CH_2)$_2$NHCO, FO_2S, NH	T	25	0/6	162
		T	12.5	0/6	137
		T	6.25	0/6	137
		T	3.13	0/6	137
		T	1.56	0/6	125
		T	0.78	0/6	118
7	3-(CH_2)$_4$, SO_2F	V	12.5	2/6	296
		V	6.25	4/6	399
		V	3.13	0/6	144
8	3-Cl-4-OCH_2CONH, SO_2F	U	12.5	2/6	237
		U	6.25	0/6	155
		U	3.13	0/6	172
		W	1.56	0/6	127
9	3-Cl-4-(CH_2)$_2$, SO_2F	U	50	0/6	127
		U	25	3/6	262
		U	12.5	2/6	172
		U	6.25	4/6	399
		W	3.13	0/6	200
10	4-(CH_2)$_4$, SO_2F	U	25	3/6	324
		U	12.5	2/6	302
		U	6.25	0/6	177
		W	3.13	3/6	383
		W	1.56	0/6	205
11	3-Cl-4-(CH_2)$_2$CONH, SO_2F	V	12.5	0/6	91
		V	6.25	1/6	216
		W	3.13	3/6	397
		W	1.56	2/6	284
		W	0.78	0/6	200
17	3-Cl-4-OCH_2CONHC_6H_5	U	50	5/6	399
		U	25	4/6	399
		U	12.5	3/6	290
		U	6.25	3/6	273
		W	3.13	0/6	177
18[c]	3-Cl-4-$(CH_2)_2C_6H_5$	U	12.5	4/6	399
		U	6.25	2/6	285
		U	3.13	5/6	399
		W	1.56	2/6	284
		W	0.78	0/6	205
19[d]	4-$(CH_2)_4C_6H_5$	U	12.5	4/6	399
		U	6.25	5/6	399
		U	3.13	3/6	324
20	[Amethopterin]	T	1.5	0/6	170
		T	0.75	0/6	163
		T	0.38	0/6	163
		T	0.19	0/6	137
	[Cytoxan]	W	5	2/6	247

[a] Test group indicates which compds were run simultaneously with the same control group.[16a] [b] Single ip doses on days 1–9. [c] I_{50} = 0.0076 μM with Dunning leukemia dihydrofolic reductase. [d] I_{50} = 0.0066 μM with Dunning leukemia dihydrofolic reductase.

side chains were much more effective *in vivo* (Table VI) than the 3,4-dichlorophenyl analog **12**, even though the latter was more effective as a reversible enzyme inhibitor and in inhibition of L1210 cell culture (Table I). When the reversible inhibitor—irreversible inhibitor pairs (**1** *vs.* **15**, **5** *vs.* **16**, **8** *vs.* **17**, **9** *vs.* **18**, and **10** *vs.* **19**) were compared *in vivo* against Walker 256 ascites (Tables III, VI, VII) the reversible inhibitors were not only as effective cures, but in some cases were better than the corresponding irreversible inhibitors.

That a potent reversible inhibitor of dihydrofolic reductase could be effective against Walker 256 was anticipated;[18] Walker 256 cells are dependent upon folic acid–folic reductase for growth, but are deficient in the active transport of folic acid[19] and therefore would be inhibited by a reversible inhibitor that enters the cell by passive diffusion.[18] However, this borderline folic acid deficiency would not explain the difference in *in vivo* activity between the simpler reversible inhibitors (**12**, **14**) and the more complex reversible inhibitors (**15–19**).

Some of the compounds were then assayed by ip injection against Walker 256 in the leg muscle (Table VIII). A number showed inhibition, but were much less effective than ip treatment of peritoneal Walker 256 ascites as might be expected; first, the local ip concentration would be much higher initially than the concentration achievable in the leg by ip injection and, second, a compound would have to go through the blood stream from the ip cavity to the leg with the possibility for destruction of the compound. Again the reversible inhibitors were as good as the corresponding irreversible inhibitors.

Since Walker 256 cells are on the verge of folic acid deficiency due to impaired transport a folic acid, Walker 256 does not respond to amethopterin which presumably must enter a cell by the folic acid transport mechanism.[18,19] Therefore a rat tumor with an adequate active transport system for folic acid that was responsive to amethopterin—namely the Dunning leukemia ascites—was selected for evaluation of the folic reductase inhibitors (Table IX). There was no correlation between irreversible inhibition of the Dunning leukemia dihydrofolic reductase (Table I) and *in vivo* activity; in fact some of the poorest irreversible inhibitors (**9**, **10**) were the most effective. Surprisingly, reversible inhibitors were definitely more effective than the corresponding irreversible inhibitors.

Even though some of these inhibitors are sufficiently active *in vivo* to warrant pharmacologic studies prior to study in man, the lack of correlation of *in vivo* and *in vitro* data is indeed disconcerting for a so-called program in rational design of enzyme inhibitors for cancer chemotherapy. Therefore, an ancillary study on the metabolic fate of **1** was undertaken.[20] The only metabolic product detectable in the urine and feces was the sulfonic acid **2** which accounted for 77% of the radioactivity injected; no sulfonyl fluoride (**1**) was excreted. Since rabbit liver, kidney, intestine, and plasma contain an enzyme that can hydrolyze DFP to diisopropyl hydrogen phosphate[21] and since mouse serum contains an enzyme that can hydrolyze **1** to the sulfonic acid **2**,[13] rat liver and serum were investigated for hydrolysis of **1** to **2**. Hydrolysis by rat serum was slow,[13] requiring 3 hr for 65% completion. Hydrolysis by a rat liver extract (either 0–45% or 45–90% $(NH_4)_2SO_4$ fractions) was nearly complete in 1 hr; it was clear that **1** was being rapidly detoxified to **2**.[20] Although **2** was a good reversible inhibitor, this zwitterion penetrated the membrane cell wall very poorly (ED_{50} = 18 μM; compare **1**) and was therefore a detoxification product.

The occurrence of this "fluoridase" in liver brought up the possibility that the tissue specificity of inactivation of dihydrofolic reductase by a number of inhibitors was not due to differences in enzyme structure from tissue to tissue, but was due to the relative rate of hydrolysis of sulfonyl fluorides by this "fluoridase." In another ancillary study, dihydrofolic reductase from mouse and rat liver was purified by an affinity column, then the irreversible inhibition reinvestigated.[22] All of the tumor specific dihydrofolic reductase inhibitors now showed good irreversible inhibition of the purified liver enzyme.

Even though these sulfonyl fluorides effective on L1210 or Walker 256 dihydrofolic reductase were ineffective on the liver enzyme due to the presence of the "fluoridase" in liver, the tissue specificity is still real and still should be usable in chemotherapy in the same manner as isozyme specificity, a possibility previously demonstrated.[8] With this new insight on a major biochemical difference between at least some tumors and a normal tissue such as liver—namely, the presence of the detoxifying "fluoridase" in liver and its absence in several tumors—further investigations on irreversible enzyme inhibitors of the sulfonyl fluoride type will be on a firmer basis. It is now less likely that tumor and liver dihydrofolic reductase are isozymes, since no isozyme specificity has been seen with at least 100 active-site-directed irreversible inhibitors of the sulfonyl fluoride type. However, it is still possible that other enzymes will have isozyme forms—particularly those of large molecular weight that are dissociable into subunits; selective irreversible inhibition of lactic dehydrogenase, which has 2 different subunits, was achieved early in this program.[8] With enzymes of lower molecular weight such as dihydrofolic reductase (20,000), or even dissociable enzymes of higher molecular weight, the desired tumor specificity for chemotherapy still may be achievable by use of selective detoxification of sulfonyl fluorides by "sulfonyl fluoridase" in normal tissue.

Finally, the question can be asked why are the more complex reversible inhibitors (**15–19**) more effective against Walker 256 *in vivo* than the simpler reversible inhibitor **12**; if and when this question is answered, perhaps another new and usable concept for specificity will arise.

Experimental Section

The reversible inhibitors (**15–19**) in Table VI were synthesized by the same routes used for the corresponding irreversible

(18) B. R. Baker, D. V. Santi, P. I. Almaula, and W. C. Werkheiser, *J. Med. Chem.*, **7**, 24 (1964).

(19) (a) W. C. Werkheiser, *Proc. Amer. Ass. Cancer Res.*, **3**, 371 (1962). (b) W. C. Werkheiser, L. W. Law, R. A. Roosa, and C. A. Nichol, *ibid.*, **4**, 71 (1963).

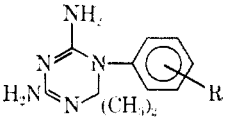
(20) A. J. Ryan, N. M. J. Vermeulen, and B. R. Baker, *J. Med. Chem.*, **13**, 1140 (1970), paper CLXXIV.

(21) A. Mazur, *J. Biol. Chem.*, **164**, 271 (1946).

(22) B. R. Baker and N. M. J. Vermeulen, *J. Med. Chem.*, **13**, 1143 (1970), paper CLXXV.

TABLE X

PHYSICAL PROPERTIES OF



No.[a]	R	Yield,[b] %	Mp, °C dec	Formula[c]
15	4-$(CH_2)_2CONHC_6H_4CH_3$-*m*	49[d]	210–211	$C_{29}H_{32}N_6O_4S$
16[e]	3-Cl-4-$O(CH_2)_3OC_6H_5$	20[d]	200–204	$C_{22}H_{20}ClN_5O_5S$
17	3-Cl-4-$OCH_2CONHC_6H_5$	28[d]	200–203	$C_{21}H_{27}ClN_6O_5S$
18	3-Cl-4-$(CH_2)_2C_6H_5$	52[d]	205–207	$C_{21}H_{28}ClN_5O_3S$
19[f]	4-$(CH_2)_4C_6H_5$	44[g]	199–200	$C_{23}H_{33}N_5O_3S$

[a] Synthesized by method D.[23b] [b] Yield of anal. pure material from nitro intermediate. [c] Analyzed for C, H, N. [d] Recrystd from *i*-PrOH–H_2O. [e] See ref 23b for NO_2 intermediate. [f] The synthesis of the HCl salt was previously described by B. R. Baker, B. T. Ho, and G. J. Lourens, *J. Pharm. Sci.*, **56**, 737 (1967). [g] Recrystd from *i*-PrOH.

TABLE XI

PHYSICAL PROPERTIES OF INTERMEDIATES

No.	Compound	Yield,[a] %	Mp, °C	Formula[b]
21[c]	*p*-$NO_2C_6H_4CH$=$CHCONHC_6H_4CH_3$-*m*	61	170–173[d]	$C_{16}H_{14}N_2O_3$
22	4-NO_2-2-$ClC_6H_3OCH_2CONHC_6H_5$	43[e,f]	157–158	$C_{14}H_{11}ClN_2O_4$
23	4-NO_2-2-ClC_6H_3CH=CHC_6H_5	72[g,h]	105–108[i]	

[a] Yield of anal. pure material. [b] *Anal.* C, H, N. [c] See method A in B. R. Baker and G. J. Lourens, *J. Med. Chem.*, **11**, 672 (1968). [d] Recrystd from EtOH–THF. [e] Recrystd from $CHCl_3$–EtOH. [f] Prepd from α-chloroacetanilide according to method A in ref 23b. [g] Prepd from 2-chloro-4-nitrobenzaldehyde according to method A in ref 12c. [h] Recrystd from EtOH–H_2O. [i] Lit. mp 111–112° prepared by an alternate route; see L. Chardonnens and P. Heinrich, *Helv. Chim. Acta*, **23**, 292 (1940).

inhibitors;[12,23] properties are listed in Table X and intermediates in Table XI. Melting points were taken in capillary tubes on a Mel-Temp block and are uncorrected. All analytical samples had proper uv and ir spectra and moved as a single spot on tlc with Brinkman silica gel GF; each gave combustion values for C, H, and N within 0.4% of theoretical.

(23) (a) B. R. Baker and G. J. Lourens, *J. Med. Chem.*, **11**, 677 (1968); (b) B. R. Baker and G. J. Lourens, *ibid.*, **12**, 95 (1969).

Irreversible Enzyme Inhibitors. CLXXIV.[1,2] Metabolism of 4-[*p*-(4,6-Diamino-1,2-dihydro-2,2-dimethyl-*s*-triazin-1-yl)hydrocinnamido]-*o*-toluenesulfonyl Fluoride (NSC-113423), an Active-Site-Directed Irreversible Inhibitor of Dihydrofolic Reductase

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The ^{14}C-labeled title compound (**1**) was synthesized from [^{14}C]cyanoguanidine, acetone, and *p*-(*p*-aminohydrocinnamido)-*o*-toluenesulfonyl fluoride (**4**). The sulfonic acid **2** corresponding to hydrolysis of the SO_2F group was the only radioactive excretion product, 20% of the radioactivity appearing in the urine and 58% in the feces. Investigation of serum and liver extract of the rat showed that the liver contained a "sulfonyl fluoridase" that rapidly converted **1** into **2**; hydrolysis by serum was much slower, but complete in 20 hr at 37°.

In the previous paper of this series,[2] 9 active-site-directed irreversible inhibitors of dihydrofolic reductase of the SO_2F type were tested against Walker 256 ascites and Dunning leukemia ascites in the rat; although cures could be achieved, no correlation between tissue specificity of irreversible inhibition of the enzyme and *in vivo* effectiveness was apparent. Furthermore, 5 reversible inhibitors—where the SO_2F group had been replaced by H—were as effective *in vivo* as the irreversible inhibitors. These results indicated that other factors such as transport and metabolism might be playing a role which negated correlation. Therefore one of the compounds (**1**) was labeled with ^{14}C in the triazine ring and subjected to a metabolic study in the rat. The results are the subject of this paper.

Chemistry.—The ^{14}C-labeled **1** was synthesized by condensation of [^{14}C]cyanoguanidine, the arylamine precursor **4**,[5] and acetone according to the general method of Modest.[6] Two obvious possibilities for metabolic change of the side chain of **1** were hydrolysis of

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(2) For the previous paper in this series see B. R. Baker, N. M. J. Vermeulen, W. A. Ashton, and A. J. Ryan, *J. Med. Chem.*, **13**, 1130 (1970).

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(5) B. R. Baker and G. J. Lourens, *J. Med. Chem.*, **11**, 677 (1968), paper CXXIX.

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