isomers was recrystallized from EtOH to give yellow crystals, mp 216–218°. *Anal.* ($C_{14}H_{10}FNO_4S$) C, H, N.

1-(*p*-Fluorosulfonylphenoxy)-2-(*p*-nitrophenoxy)ethane (53).—To a stirred solution of 10 g of **51**[25] (39 mmoles) in 60 ml of $CHCl_3$ cooled in ice bath was added dropwise 40 ml of $ClSO_3H$ over a period of 30 min. After being stirred an additional 30 min in the ice bath, the solution was poured into a stirred mixture of 500 g of crushed ice and 140 ml of $CHCl_3$. The separated organic layer was washed twice with cold H_2O, then dried with $MgSO_4$, and evaporated *in vacuo*. The residual crude **52** was stirred with 4.0 g of KF and 25 ml of DMF at 100° for 45 min. The cooled mixture was diluted with 200 ml of cold H_2O. The product was collected on a filter, washed with H_2O, then recrystallized from EtOH with the aid of charcoal. A second recrystallization from EtOH gave 3.8 g (29%) of white crystals, mp 117–118°, that moved as a single spot on the tlc (in C_6H_6). *Anal.* ($C_{14}H_{12}FNO_6S$) C, H, N.

***p*-Aminophenoxyacetylsulfanilyl Fluoride (27).**—A mixture of 0.88 g (2.5 mmoles) of **25**, 100 ml of MeOEtOH, and 5 ml of Raney Ni was shaken with H_2 at 2–3 atm for 45 min when reduction was complete. The mixture was filtered through a Celite pad, then the filtrate was evaporated *in vacuo*. Recrystallization of the residue from EtOH gave 0.27 g (33%) of nearly white crystals, mp 161–163° dec. *Anal.* ($C_{14}H_{13}FN_2O_4S$) C, H, N.

N-(*p*-Aminobenzyl)-N'-(*p*-fluorosulfonylphenyl)urea (35) was prepared from **32** in EtOH as described for **27**. Recrystallization from absolute EtOH gave 0.81 g (50%) of nearly white crystals, mp 191–193°. *Anal.* ($C_{14}H_{14}FN_3O_3S$) C, H, N.

4-Amino-4'-fluorosulfonyldiphenylethane (49) was prepared from **43** as described for **35**.[1] Recrystallization from EtOH–H_2O gave 340 mg (61%), mp 109–111°. *Anal.* ($C_{14}H_{14}FNO_2S$) C, H, N.

N-[*p*-(4,6-Diamino-1,2-dihydro-2,2-dimethyl-*s*-triazin-1-yl)-cinnamoyl]sulfanilyl Fluoride Ethanesulfonate (6).—A mixture of 400 mg (1.25 mmoles) of **24**, 140 mg (1.25 mmoles) of $EtSO_3H$, 110 mg (1.3 mmoles) of cyanoguanidine, and 20 ml of reagent Me_2CO was refluxed with stirring for 20 hr, then cooled. The product was collected on a filter, washed with Me_2CO, and recrystallized from *i*-PrOH–H_2O; yield 420 mg (61%), mp 220–222° dec. See Table II for additional data and other compounds prepared by this method.

Irreversible Enzyme Inhibitors. CXXVIII.[1,2] *p*-(4,6-Diamino-1,2-dihydro-2,2-dimethyl-*s*-triazin-1-yl)phenylpropionylsulfanilyl Fluoride, an Active-Site-Directed Irreversible Inhibitor of Dihydrofolic Reductase. IV.[2] Effects of Substitution on the Propionamide Bridge on Isozyme Specificity

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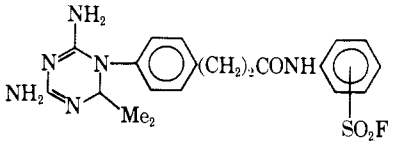
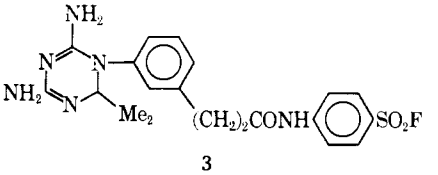
Substitution on the propionamido group of the title compound (**1**) by β-methyl (**4**), α-methyl (**6**), or N-methyl (**12**) gave little change in reversible binding to the dihydrofolic reductase from Walker 256 rat tumor or L1210/FR8 mouse leukemia, but irreversible inhibition was lost; this loss with **4** was attributed to the change in the staggered ground-state conformation of the ethane moiety to a skew conformation. Substitution of a β-phenyl (**5**) or α-phenethyl (**7**) led to a large loss in reversible binding due to a steric interaction within the enzyme–inhibitor complex which also could shift the position of the sulfonyl fluoride to account for the loss of irreversible inhibition. Substitution of an α-phenyl group (**8**) still allows the ethane moiety to have a staggered ground-state conformation and gives little change in reversible binding; thus **8** could still inactivate the Walker 256 enzyme and, less effectively, the L1210/FR8 enzyme. In contrast to **1**, **8** was a more effective irreversible inhibitor of the rat liver enzyme than the Walker 256 enzyme. Replacement of the α-phenyl group of **8** by *o*- (**9**), *m*- (**10**), or *p*-tolyl (**11**) gave little change in reversible binding to the Walker 256 enzyme, but irreversible inhibition by the *p*-tolyl derivative (**11**) was lost. The *o*- (**9**) and *m*-tolyl (**10**) derivatives inactivated the Walker 256 enzyme somewhat more effectively than the rat liver enzyme, a crossover from the α-phenyl derivative (**8**).

The discovery that 1-phenyl-*s*-triazines bridged to a terminal sulfonyl fluoride such as **1** are active-site-directed irreversible inhibitors[4] of dihydrofolic reductase[5] has led to an intensive study on modification of **1** to give species- and tissue-specific irreversible inhibitors of this enzyme; **1** at a concentration of 0.05–0.1 μ*M* was an extremely rapidly acting irreversible inhibitor of dihydrofolic reductase from Walker 256 rat tumor, rat liver, L1210/FR8 mouse leukemia, mouse liver, and pigeon liver.[2,5] When the propionamide chain was moved to the *meta* position as in **3**, the latter could still rapidly inactivate pigeon liver dihydrofolic



1, *para*
2, *meta*



3

reductase, but not the dihydrofolic reductases from the other sources,[5] the first example of a species-specific irreversible inhibitor of dihydrofolic reductase among vertebrate sources.[6] When the sulfonyl fluoride group

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

(2) For the previous paper of this series see B. R. Baker and G. J. Lourens, *J. Med. Chem.*, **11**, 666 (1968).

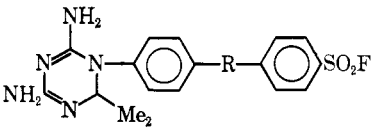
(3) G. J. L. wishes to thank the Council for Scientific and Industrial Research, Republic of South Africa, for a tuition fellowship.

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

(5) B. R. Baker and G. J. Lourens, *J. Med. Chem.*, **10**, 1113 (1967), paper CV of the series and paper I of the subseries.

(6) The selective irreversible inhibition of *E. coli* B dihydrofolic reductase with no inactivation of the pigeon liver enzyme was observed earlier; see B. R. Baker and J. H. Jordaan, *J. Pharm. Sci.*, **56**, 660 (1967), paper LXXXVIII of this series.

TABLE I
INHIBITION[a] OF DIHYDROFOLIC REDUCTASE BY



			Reversible[c]		Irreversible[d]			
No.	R	Enzyme source[b]	I_{50},[e] μM	K_i,[f] μM	Inhib concn, μM	% EI[g]	Time, min	% inactivn
1[h]	$(CH_2)_2CONH$	W256	0.020	0.003	0.050	95	<1, 10	90, 90[i]
					0.020	87	1, 3	50, 90[i]
		Rat liver	0.0060	0.001	0.050	98	<2, 60	70, 70[i]
					0.020		8	50[i]
		L1210/FR8	0.080	0.01	0.070	84	<2, 10	84, 84[i]
		Mouse liver			0.070		2, 60	38, 38[i]
					0.40		2, 60	59, 59[i]
4	$CHCH_2CONH$	W256	0.051	0.01	0.25	97	13, 60	50, 80[i]
	CH_3				0.050	87	8, 60	26, 43[i]
		L1210/FR8	0.081	0.01	0.081	87	2, 60	55, 55[i]
5	$CH_2CHCONH$	W256	1.8	0.3	9.0	97	60	0
	C_6H_5	L1210/FR8	13	2	25	92	60	0
6	$CH_2CHCONH$	W256	0.028	0.005	0.15	97	2, 60	32, 32[i]
	CH_3				0.074	93	60	0[i]
		L1210/FR8	0.050	0.01	0.25	97	60	0
7	$CH_2CHCONH$	W256	0.63	0.1	3.1	97	60	0
	$(CH_2)_2C_6H_5$	L1210/FR8	1.9	0.3	9.5	97	60	0
8	$CH_2CHCONH$	W256	0.074	0.01	0.37	97	<2	80
	C_6H_5				0.074	87	4, 9, 60	50, 71, 71[i]
		Rat liver	0.18	0.03	0.80	98	1, 3, 60	50, 93, 93[i]
					0.074	84	2, 8, 30	50, 88, 94[i]
		L1210/FR8	0.20	0.03	1.2	96	<2, 60	65, 65[i]
					0.16	86	60	27
9	$CH_2CHCONH$	W256	0.058	0.01	0.29	97	<2	80
	$C_6H_4CH_3$-*o*				0.058	87	25, 60	50, 66[i]
		Rat liver			0.058		4, 60	40, 40[i]
		L1210/FR8	0.35	0.06	1.7	97	60	43
10	$CH_2CHCONH$	W256	0.067	0.01	0.34	97	60	100
	$C_6H_4CH_3$-*m*				0.067	87	4, 60	61, 61[i]
		Rat liver			0.067		2, 60	44, 44[i]
		L1210/FR8	0.21	0.04	1.1	97	60	43
11	$CH_2CHCONH$	W256	0.058	0.01	0.29	97	60	0
	$C_6H_4CH_3$-*p*	L1210/FR8	0.63	0.1	3.1	97	60	13
12	$(CH_2)_2CON$	W256	0.0091	0.002	0.046	97	60	0
	CH_3	L1210/FR8	0.041	0.01	0.21	97	60	0

[a] The technical assistance of Jean Reeder, Diane Shea, and Sharon Lafler is acknowledged. [b] W256 = Walker 256 rat tumor. [c] Assayed with 6 μM dihydrofolate and 30 μM TPNH in pH 7.4 Tris buffer as previously described.[5] [d] Incubated at 37° in pH 7.4 Tris buffer in the presence of 60 μM TPNH as previously described.[5] [e] I_{50} = concentration for 50% inhibition. [f] Estimated from $K_i = K_m[I]_{50}/[S]$ which is valid since $[S] = 6K_m = 6\ \mu M$ dihydrofolate; see ref 4, p 202. [g] Calculated from $[EI] = [E_t]/(1 + K_i/[I])$ where [EI] is the amount of total enzyme (E_t) reversibly complexed.[9] [h] Data from ref 2 and 5. [i] From time study plot; see ref 5.

of **1** was moved to the *meta* position (**2**) a separation of irreversible inhibition of two mammalian enzymes was achieved; **2** could still inactivate the dihydrofolic reductase from Walker 256 tumor and liver from the rat, but not L1210/FR8 mouse leukemia.[2,7] A further study on modification of the propionamide bridge of **1** showed that these structural changes were surprisingly much too severe to maintain irreversible inhibition; the irreversible inhibition was sensitive to the allowable ground-state conformations of the bridge.[2] Therefore a more subtle series of modifications was made to study their effects on tissue specificity; the effects of substitution on the propionamide bridge are the subject of this paper and the effects of ring substitution in the paper that follows.[8]

Enzyme Results.—The effects of substitution on the propionamide bridge of **1** are listed in Table I. In order to interpret these data the following points should be considered.

(1) The rate of irreversible inhibition by an active-site-directed irreversible inhibitor is dependent upon the concentration of reversible enzyme–inhibitor (EI) complex which in turn is dependent upon [I] and the dissociation constant, K_i.[9]

(2) A sulfonyl fluoride group juxtaposed to an enzymic nucleophile such as a serine hydroxyl can interact in two different ways. A covalent sulfonate ester can form between the enzyme and inhibitor leading to enzyme inactivation or the enzyme can catalyze hydrolysis of the sulfonyl fluoride to the irreversibly inert sulfonic acid;[10] either or both reactions can occur,[10] but if only enzyme-catalyzed hydrolysis occurs, it is not detectable by the methodology[5] used in Table I.

(3) In order to detect whether or not any inactivation of dihydrofolic reductase occurs, each compound is screened on a tumor enzyme at a concentration of $5I_{50}$ which is sufficient to reversibly complex 97% of the

(7) B. R. Baker and G. J. Lourens, *J. Med. Chem.*, **11**, 38 (1968), paper CXII of the series and paper II of the subseries.

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

(9) For the kinetics of irreversible inhibition see (a) ref 4, Chapter VIII; (b) B. R. Baker, W. W. Lee, and E. Tong, *J. Theor. Biol.*, **3**, 459 (1962).

(10) (a) B. R. Baker and J. A. Hurlbut, *J. Med. Chem.*, **11**, 233 (1968), paper CXIII of this series; (b) B. R. Baker and E. Erickson, *ibid.*, **11**, 245 (1968), paper CXV of this series.

enzyme. If greater than 70% inactivation is seen, then the inhibitor is subjected to a time study of the inactivation rate at a concentration of I_{50}; in cases where $5I_{50}$ shows less than 70% inactivation, an I_{50} concentration will show less than 40% inactivation due to complete destruction of the sulfonyl fluoride by enzyme-catalyzed hydrolysis. The I_{50} concentration varies little from one mammalian source of enzyme to another; if greater than an I_{50} concentration is needed for inactivation, little benefit in selectivity by the irreversible inhibition will be gained due to the pronounced reversible inhibition of the enzyme in all tissues.

(4) For an irreversible inhibitor to be worthy of chemotherapy studies on tumor inhibition in intact animals the following minimum arbitrary standards have been set: (a) the inhibitor should show >80% irreversible inhibition of the tumor enzyme when assayed at a concentration of $6K_i = I_{50}$ for dihydrofolic reductase, and (b) the inhibitor should show less than 20% irreversible inhibition of the liver enzyme at the same concentration used in a. It would be even more preferable if the inhibitor could effectively operate irreversibly on the tumor enzyme at a K_i concentration $= I_{50}/6$, but give less than 20% inactivation of the liver enzyme at $6K_i = I_{50}$; an irreversible inhibitor meeting these latter standards has been found in the 2,4-diamino-5-phenoxypropylpyrimidine series.[11]

It is clear that the prototype irreversible inhibitor **1** does not meet the criteria for animal evaluation on Walker 256 even though Walker 256 enzyme is inactivated eight times more rapidly than the liver enzyme and the total extent of inactivation at $2.5I_{50}$ is 90% with the tumor enzyme and 70% with liver enzyme. The prototype irreversible inhibitor (**1**) nearly meets the criteria for animal evaluation on L1210/FR8; an I_{50} concentration of **1** gives 84% inactivation of the L1210/FR8 enzyme in <2 min, but no further inactivation indicates that the sulfonyl fluoride has been hydrolyzed by the enzyme in <2 min; the same concentration of **1** shows 38% inactivation of the mouse liver enzyme before the simultaneous enzyme-catalyzed hydrolysis of the sulfonyl fluoride is complete.

Insertion of the β-methyl group (**4**) on the propionamide bridge of **1** led to little change in reversible binding but with both tumor enzymes led to a detrimental effect on the ratio of the rate of inactivation to the rate of enzyme-catalyzed hydrolysis at an I_{50} concentration; **4** gave only 43% inactivation of the Walker 256 enzyme before it was destroyed; at $5I_{50}$ it showed a total inactivation of 80% with a half-life of 13 min, considerably slower than the $5I_{50}$ of **1** with a half-life of <1 min. At $5I_{50}$, **4** was even less effective on the L1210/FR8 enzyme, only 55% inactivation having occurred before the sulfonyl fluoride was hydrolyzed by the enzyme.

When a phenyl group (**5**) was inserted on the β position of the propionamide bridge of **1**, a large loss in reversible binding occurred with both tumor enzymes; since either a *p*-benzyl group[12a] or the carboxamidophenyl moiety[2] of **1** gives added binding to the enzyme, it is clear that when both groups are present, one is not well tolerated within the enzyme–inhibitor complex. Furthermore even with sufficient inhibitor to reversibly complex over 90% of the enzyme, neither tumor enzyme was inactivated showing that the SO_2F group of **1** in the enzyme–inhibitor complex was positioned differently in the complex with **5**.

Substitution of an α-methyl group (**6**) on the propionamide bridge of **1** led to no change in reversible binding; however, the effect on inactivation was dramatic. At $5I_{50}$, **6** showed no irreversible inhibition of the L1210/FR8 enzyme. With the Walker 256 enzyme, **6** at $5I_{50}$ showed a rapid inactivation of 32% of the enzyme, but no further inactivation; when **6** was reduced to $2.6I_{50}$, no perceptible inactivation was seen apparently due to the rapid enzyme-catalyzed hydrolysis of the sulfonyl fluoride. When the α substituent was increased to phenethyl (**7**), not only was reversible binding less effective, but inactivation at $5I_{50}$ was lost with both tumor enzymes. Thus, the enzymes poorly tolerate both the phenylbutyl group and the carboxamide group even though each alone can lead to enhanced binding;[2,12b] furthermore, the position of the SO_2F group of **1** is shifted within in the enzyme–inhibitor complex when the α-phenethyl group (**7**) is introduced, since no inactivation is seen.

When an α-phenyl group (**8**) was introduced on the propionamide bridge of **1**, reversible binding was decreased about threefold. The inactivation of the L1210/FR8 enzyme still proceeded rapidly at a $5I_{50}$ concentration of **8**, but the relative rate of enzyme-catalyzed hydrolysis of the SO_2F was increased; at an I_{50} concentration only a total of 27% inactivation was observed, in contrast to **1** where 84% inactivation was seen. At an I_{50} concentration, **8** still showed 71% inactivation of the Walker 256 enzyme with a half-life of 4 min, somewhat slower than observed with the parent **1**. Since **8** showed >70% inactivation of the Walker 256 enzyme at an I_{50} concentration, its effect on the rat liver enzyme was investigated; in contrast to **1**, **8** was found to be more effective on the rat liver enzyme than on the Walker 256 enzyme, thus not being of chemotherapeutic utility.

The pronounced effects of the α- and β-methyl groups (**4**, **6**) on irreversible inhibition can be readily attributed to the change in ground-state conformation; **1** has a staggered ethane group, but **4** and **6** would have a skewed ethyl group. This skew would change considerably the positioning of the benzenesulfonyl fluoride moiety in the enzyme–inhibitor complex. In contrast, an α- or β-phenyl group should still allow the ethane group to be staggered. Therefore the loss in irreversible inhibition caused by the β-phenyl group of **5** is most probably caused by a steric effect on proper positioning of the sulfonyl fluoride, as shown by the large loss in reversible binding in **5** compared to **1**. In contrast the α-phenyl group of **8** gives only a threefold loss in binding, indicating no unfavorable steric interaction within the enzyme–inhibitor complex; since the ethane moiety is still staggered, irreversible inhibition by **8** could still be expected to occur. The loss in irreversible inhibition caused by the α-phenethyl group **7** can be attributed to both effects; reversible binding is considerably decreased due to a steric interaction and the ethane moiety of the propionamide bridge would

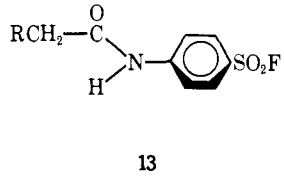
(11) B. R. Baker and R. B. Meyer, Jr., *J. Med. Chem.*, **11**, 489 (1968); paper CXIX of this series.

(12) (a) B. R. Baker and B.-T. Ho, *J. Heterocycl. Chem.*, **2**, 335 (1965); (b) B. R. Baker, B.-T. Ho, and G. J. Lourens, *J. Pharm. Sci.*, **56**, 737 (1967); paper LXXXVI of this series.

also have the unfavorable skew in the ground state.

In order to establish whether or not a separation of activity against the Walker 256 enzyme compared to the rat liver enzyme could be achieved, the phenyl group of **8** was substituted by a methyl group at the *ortho* (**9**), *meta* (**10**), or *para* (**11**) positions. The I_{50} was essentially unchanged, but the effect on irreversible inhibition was more dramatic. Both the *o*- (**9**) and *m*-methyl (**10**) derivatives were more effective on the tumor enzyme than on the rat liver enzyme, but not sufficiently different to be useful; the effect of substituents larger than methyl on tissue specificity would be worthy of study. Surprisingly, the *p*-methyl derivative (**11**) leads to a loss of irreversible inhibition at $I_{50} \times 5$, again showing the sensitivity of slight structural change on irreversible inhibition.

The final substitution study was introduction of an N-methyl (**12**) on **1**. According to Pedersen and Pedersen,[13] an N-methylacetanilide derivative (**14**) has a significantly different ground-state conformation than a derivative of acetanilide (**13**); thus N-methylation (**12**) of the parent **1** could be expected to have a pronounced effect on either reversible or irreversible inhibition or both. Actually N-methylation (**12**) increased the efficiency of reversible binding about twofold, but completely negated irreversible inhibition of both tumor enzymes.



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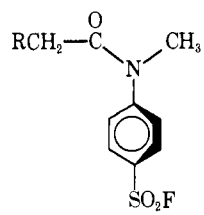

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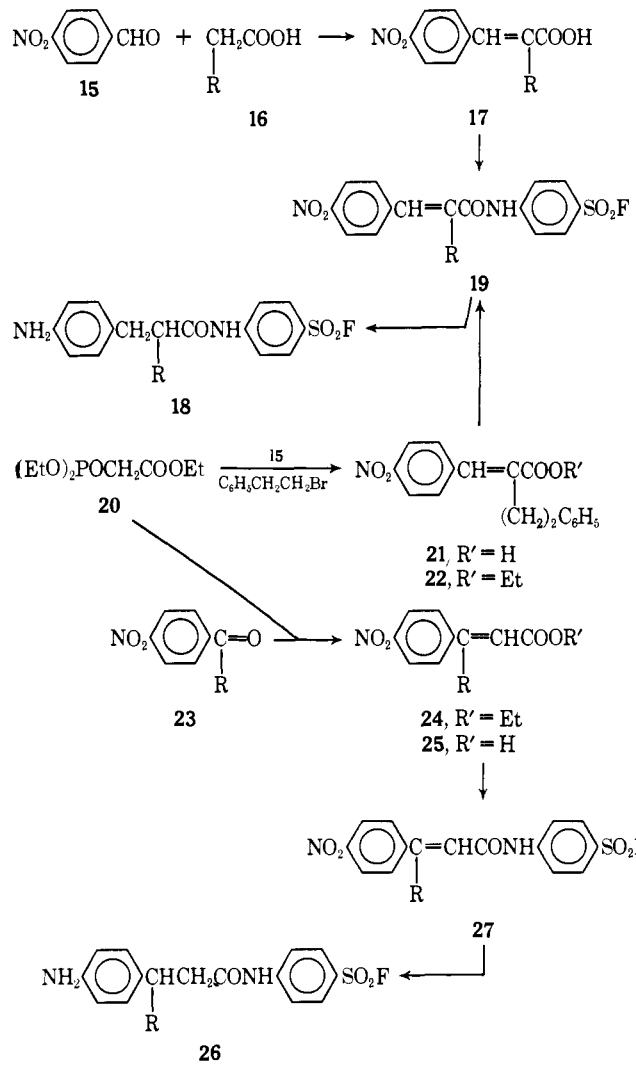
It is apparent from this study on bridge substitution and the preceding study on bridge types[2] that the positioning of the sulfonyl fluoride to a nucleophilic group on the enzyme surface in an enzyme–inhibitor complex is greatly affected by relatively small changes in ground-state conformation to the extent that irreversible inhibition can be completely lost in some cases. A less dramatic effect with possibly more utility for tissue specificity would be substitution on one of the benzene rings where ground-state effects are less pronounced, but steric differences between isozymes may still be achieved; such a study resulting in essentially unchanged irreversible inhibition of the L1210/FR8 mouse leukemia enzyme, but with pronounced diminished effect on the mouse liver enzyme is described in the paper that follows.[8]

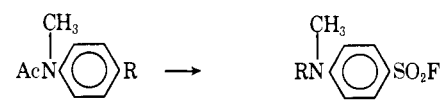
Chemistry.—The dihydro-*s*-triazines (**4**–**12**) in Table I were synthesized by the three-component method of Modest[14] by condensation of an appropriate arylamine such as **18** or **26** with cyanoguanidine and acetone in the presence of ethanesulfonic acid. These arylamines (**18**, **26**) were prepared in turn from the appropriately substituted *p*-nitrocinnamic acids (**17**, **21**, **25**, **33**) followed by catalytic reduction with a Raney Ni catalyst, as previously described for the parent unsubstituted *p*-nitrocinnamic acid (**19**, R = H).[5]

(13) B. F. Pedersen and B. Pedersen, *Tetrahedron Letters*, 2995 (1965).
(14) E. J. Modest, *J. Org. Chem.*, **21**, 1 (1956).

SCHEME I



a, R = Me
b, R = $C_6H_5(CH_2)_2$
c, R = C_6H_5
d, R = $C_6H_4CH_3$-*o*
e, R = $C_6H_4CH_3$-*m*
f, R = $C_6H_4CH_3$-*p*



28, R = H
29, R = SO_2Cl
30, R = SO_2F
31, R = H
32, R = *p*-$NO_2C_6H_4CH{=}CHCO$
33, R = *p*-$NH_2C_6H_4(CH_2)_2CO$

Two routes (Scheme I) for the α-substituted *p*-nitrocinnamic acids were employed. Where the appropriate α-substituted acetic acids (**16**) such as propionic acid or phenylacetic acids were available, Perkin condensation[15] with *p*-nitrobenzaldehyde (**15**) was employed to give **17**. The remaining α-phenethylcinnamic acid (**21**) was prepared by alkylation of the phosphonate (**20**) with phenethyl bromide and NaH in DMF, followed by Wittig reaction with *p*-nitrobenzaldehyde (**15**);[16] the intermediate ester was not purified but was hydrolyzed to the desired **21** in 19% over-all yield.

The last candidate irreversible inhibitor (**12**) in Table I was synthesized from N-methylacetanilide (**28**) by

(15) C. F. Koelsch and P. R. Johnson, *J. Am. Chem. Soc.*, **65**, 565 (1943).
(16) This type of reaction sequence has been described by W. S. Wadsworth and W. D. Emmons, *ibid.*, **83**, 1733 (1961).

chlorosulfonation to **29** followed by F^- displacement[17] to **30**, then acid hydrolysis to **31**; condensation with *p*-nitrocinnamoyl chloride to **32**, then catalytic hydrogenation, afforded the desired arylamine (**33**).

Experimental Section[18]

***p*-Nitro-α-(*p*-tolyl)cinnamic Acid (17f).**—A mixture of 11.4 g (75 mmoles) of **15**, 8.4 g of anhydrous NaOAc, 36 ml of Ac_2O, and 10.5 g (70 mmoles) of *p*-tolylacetic acid was refluxed with stirring for 19 hr, then cooled and poured into 150 ml of H_2O. The solution was decanted from the gummy solid and the latter was triturated with three 200-ml portions of H_2O. The residue was dissolved in 100 ml of 7% of NaOH, then the solution was filtered through a Celite pad and acidified with 40 ml of HOAc. The product was collected on a filter, washed with H_2O, and recrystallized from EtOH with the aid of charcoal; yield 4.50 g (23%) of yellow needles, mp 217–219° dec, which moved as a single spot on tlc with MeOH. *Anal.* ($C_{16}H_{13}NO_4$) C, H, N.

Similarly, **17e** was prepared in 35% yield of analytically pure material, mp 205–206°. *Anal.* ($C_{16}H_{13}NO_4$) C, H, N. Also **17d** was prepared in 24% yield, mp 188–190°. *Anal.* ($C_{16}H_{13}NO_4$) C, H, N.

Known compounds prepared by this method were **17a** in 56% yield, mp 206–209° (lit.[19] mp 205–207), **17c** in 34% yield, mp 210–214° (lit.[20] mp 208–210).

***p*-Nitro-α-phenethylcinnamic Acid (21).**—To a stirred mixture of 2.20 g of NaH (60% in mineral oil, 55 mmoles) and 10 ml of DMF protected from moisture and cooled in an ice bath was added 12.3 g (55 mmoles) of **20** over a period of 10 min. After being stirred at ambient temperature for 30 min, 11.1 g (60 mmoles) of phenethyl bromide was added. The mixture was stirred 30 min at ambient temperature and 1 hr at 75–80°, then cooled in an ice bath. After the addition of 2.20 g of NaH (60% in mineral oil, 55 mmoles), the mixture was stirred at ambient temperature for 20 min. The mixture was cooled in an ice bath, then a solution of 7.55 g (50 mmoles) of **15** in 35 ml of DMF was added over about 6 min. The mixture was stirred at ambient temperature for 2.2 hr, then diluted with 150 ml of H_2O. The mixture was extracted with 150 ml, then 50 ml, of C_6H_6. The combined extracts were washed with H_2O, dried with $MgSO_4$, and evaporated *in vacuo*. Tlc of the remaining oil (20 g) with 1:1 C_6H_6–petroleum ether (bp 60–110°) showed a major spot of **22** and a minor spot of ethyl *p*-nitrocinnamate.

To the oily **22** was added a solution of 5.6 g of KOH in 100 ml of 75% EtOH. After being refluxed with stirring for 90 min, the mixture was poured into 400 ml of 3 *N* HCl. The brown solid was collected on a filter, washed with H_2O, and dissolved in a mixture of 25 ml of EtOH and 25 ml of 20% KOH. The solution was clarified with charcoal, then acidified with HOAc. The solid was collected on a filter and washed with H_2O. Three recrystallizations from absolute EtOH gave 3.0 g (19%) of analytically pure, yellow crystals, mp 197–200°. *Anal.* ($C_{17}H_{15}NO_4$) C, H, N.

Ethyl *p*-Nitro-β-phenylcinnamate (24c).—To a stirred suspension of 4.21 g of NaH (60% in mineral oil, 105 mmoles) and 200 ml of 1,2-dimethoxyethane protected from moisture and cooled in an ice bath was added 24.7 g (110 mmoles) of **20** over a period of 10 min. After being stirred at ambient temperature for 45 min, the mixture was treated dropwise with a solution of 22.7 g of **23c** in 200 ml of 1,2-dimethoxyethane over 10 min. The mixture was stirred 2 hr more, carefully treated dropwise with 100 ml of H_2O, then poured into a stirred mixture of 1.2 l. of H_2O and 100 ml of petroleum ether (bp 60–110°). After being stirred 1 hr, the mixture was filtered and the product was washed successively with H_2O and petroleum ether. Recrystallization from absolute EtOH gave 13.3 g (45%) of pure product as yellow crystals, mp 82–85°. *Anal.* ($C_{17}H_{15}NO_4$) C, H, N.

Similarly, **24a** was prepared in 31% yield, mp 73–74° (lit.[21] mp 74°).

***p*-Nitro-β-phenylcinnamic Acid (25c).**—A mixture of 2.97 g (10 mmoles) of **24c** and 50 ml of 2.2% KOH was refluxed for 35 min. The solution was poured into 200 ml of 1.5 *N* HCl. The product was collected on a filter and washed with H_2O. Recrystallization from EtOH–H_2O gave 2.27 g (84%) of yellow crystals, mp 183–185° dec. *Anal.* ($C_{15}H_{11}NO_4$) C, H, N.

Similarly, **25a** was prepared in 75% yield, mp 168–169° (lit.[21] mp 168–169°).

N-Acetyl-N-methylsulfanilyl Fluoride (30).—To a stirred mixture of 8.65 g (35 mmoles) of **29**[22] and 10 ml of dioxane heated under reflux in a bath at 120–125° was added a solution of 3.2 g (55 mmoles) of KF in 3.5 ml of H_2O.[17] After 30 min, the mixture was cooled and diluted with 150 ml of H_2O. The product was collected on a filter, washed with H_2O, and recrystallized from EtOH–H_2O; yield 5.20 g (75%) of white crystals, mp 154–155°, that moved as a single spot on tlc in EtOAc. *Anal.* ($C_9H_{10}FNO_3S$) C, H, N.

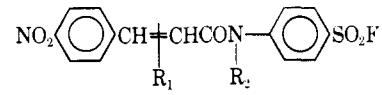
N-Methylsulfanilyl Fluoride (31).—To a stirred solution of 4.63 g (20 mmoles) of **30** in 20 ml of EtOH heated under reflux was added 20 ml of 12 *N* HCl. After being refluxed and stirred for 50 min, the solution was poured into a stirred solution of 22 g of $NaHCO_3$ in 200 ml of ice-cold H_2O. The product was collected on a filter, washed with H_2O, then recrystallized from EtOH–H_2O; yield 2.17 g (57%) of white crystals, mp 63–64°. *Anal.* ($C_7H_8FNO_2S$) C, H, N.

N-[*p*-Nitro-α-(*p*-tolyl)cinnamoyl]sulfanilyl Fluoride (19f) (Method A).—A mixture of 2.83 g (10 mmoles) of **17f**, 3.5 ml of $SOCl_2$, and 15 ml of C_6H_6 was refluxed with stirring for 2.8 hr. The solution was evaporated *in vacuo*, then 20 ml of C_6H_6 was added and the evaporation was repeated. To the residual acid chloride were added 70 ml of $CH_3C_6H_5$ and 1.75 g (10 mmoles) of sulfanilyl fluoride. The mixture was refluxed for 1.2 hr during which time about 15 ml of solvent was allowed to distil. The mixture was cooled to room temperature, then the product was collected on a filter and washed with $CH_3C_6H_5$. Recrystallization from MeOEtOH with the aid of charcoal gave 2.58 g (54%) of nearly white crystals, mp 243–244°, that moved as a single spot on tlc in 1:1 C_6H_6–petroleum ether (bp 60–110°). See Table II for additional data and compounds made by method A.

N-[*p*-(4,6-Diamino-1,2-dihydro-2,2-dimethyl-*s*-triazin-1-yl)-α-(*p*-tolyl)hydrocinnamoyl]sulfanilyl Fluoride (11) Ethanesulfonate (Method B).—A mixture of 881 mg (2 mmoles) of **19f**, 10 ml of Raney Ni, and 100 ml of EtOH was shaken with H_2 at 2–3 atm for 1 hr when reduction was complete. The mixture was filtered through a Celite pad, then the filtrate was evaporated *in vacuo*; the residual **18f** moved as a single spot on tlc in 1:1 EtOAc–petroleum ether (bp 60–110°) and had λ_{max} 245, 273 mμ

Table II

Physical Properties of



No.[a]	R_1	R_2	% yield	Mp, °C	Formula[b]
19a	α-CH_3	H	53	215–216	$C_{16}H_{13}FN_2O_5S$
19b	α-$C_6H_5(CH_2)_2$	H	63	183–184	$C_{23}H_{19}FN_2O_5S$
19c	α-C_6H_5	H	66	193–194	$C_{21}H_{15}FN_2O_5S$
19d	α-$C_6H_4CH_3$-*o*	H	46[c]	207–209 dec	$C_{22}H_{17}FN_2O_5S$
19e	α-$C_6H_4CH_3$-*m*	H	66[c]	190–191	$C_{22}H_{17}FN_2O_5S$
19f	α-$C_6H_4CH_3$-*p*	H	54	243–244	$C_{22}H_{17}FN_2O_5S$
27a	β-CH_3	H	42[c]	215–216	$C_{16}H_{13}FN_2O_5S$
27c	β-C_6H_5	H	13	237–239 dec	$C_{21}H_{15}FN_2O_5S$
32	H	CH_3	67[c]	174–175	$C_{16}H_{13}FN_2O_5S$

[a] Prepared by method A and recrystallized from MeOEtOH unless otherwise indicated. [b] All compounds showed a correct analysis for C, H, N. [c] Recrystallized from MeOEtOH–H_2O.

(17) A. H. deCat and R. K. van Poucke, *J. Org. Chem.*, **28**, 3426 (1963).

(18) All analytical samples gave combustion values within 0.4% of theoretical; each had uv and ir spectra compatible with their assigned structures. Each intermediate to the dihydro-*s*-triazines moved as a single spot on tlc on Brinkmann silica gel GF when detected under uv light. Melting points were determined in capillary tubes on a Mel-Temp block and are uncorrected.

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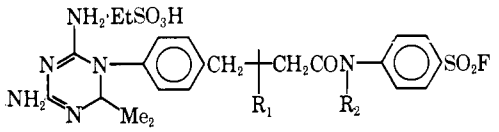
(20) T. R. Lewis, M. G. Pratt, E. D. Horniller, B. F. Tullar, and S. Archer, *J. Am. Chem. Soc.*, **71**, 3749 (1949).

(21) C. Schroeter, *Ber.*, **40**, 1589 (1907).

(22) H. Hassan and L. M. Srivastava, *Curr. Sci.* (India), **14**, 107 (1945).

TABLE III

PHYSICAL CONSTANTS OF



No.[a]	R_1	R_2	% yield	Mp, °C	Formula	Analyses
4	β-CH_3	H	67	212–214	$C_{21}H_{25}FN_6O_3S \cdot C_2H_5SO_3H$	C, H, N
5	β-C_6H_5	H	59	218–219	$C_{26}H_{27}FN_6O_3S \cdot C_2H_5SO_3H$	C, H, N
6	α-CH_3	H	67	206–207	$C_{21}H_{25}FN_6O_3S \cdot C_2H_5SO_3H$	C, H, N
7	α-$C_6H_5(CH_2)_2$	H	53	226–227	$C_{28}H_{31}FN_6O_3S \cdot C_2H_5SO_3H$	C, H, N
8	α-C_6H_5	H	56	230–231 dec	$C_{26}H_{27}FN_6O_3S \cdot C_2H_5SO_3H$	C, H, N
9	α-$C_6H_4CH_3$-*o*	H	49	213–215 dec	$C_{27}H_{29}FN_6O_3S \cdot C_2H_5SO_3H$	C, H, F
10	α-$C_6H_4CH_3$-*m*	H	38	232–233 dec	$C_{27}H_{29}FN_6O_3S \cdot C_2H_5SO_3H$	C, H, N
11	α-$C_6H_4CH_3$-*p*	H	53	225–226	$C_{27}H_{29}FN_6O_3S \cdot C_2H_5SO_3H$	C, H, N
12	H	CH_3	28	199–200 dec	$C_{21}H_{25}FN_6O_3S \cdot C_2H_5SO_3H$	C, H, F

[a] All compounds were prepared by method B and recrystallized from *i*-PrOH–H_2O; each had an ir band at 1395–1405 cm^{-1} characteristic of SO_2F.

showing the reduction of the C=C. To the **18f** were added 25 ml of Me_2CO, 225 mg (2.2 mmoles) of $EtSO_3H$, and 177 mg (2.2 mmoles) of cyanoguanidine. The mixture was refluxed for 21 hr with stirring, then cooled. The product was collected on a filter, washed with Me_2CO, and recrystallized from *i*-PrOH–H_2O; yield 680 mg (53%) of white crystals, mp 225–226°. See Table III for additional data and other compounds prepared by method B.

Irreversible Enzyme Inhibitors. CXXIX.[1,2] *p*-(4,6-Diamino-1,2-dihydro-2,2-dimethyl-*s*-triazin-1-yl)phenylpropionylsulfanilyl Fluoride, an Active-Site-Directed Irreversible Inhibitor of Dihydrofolic Reductase. V.[2] Effects of Substitution on the Benzenesulfonyl Fluoride Moiety on Isozyme Specificity

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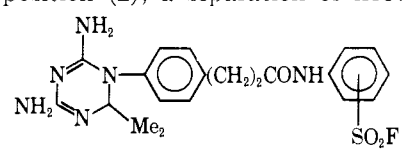
The title compound (**1**) is a fairly general irreversible inhibitor of dihydrofolic reductase, being able to rapidly inactivate this enzyme from Walker 256 tumor and liver from the rat and L1210/FR8 leukemia and liver from the mouse. Furthermore, the enzyme could catalyze the hydrolysis of the sulfonyl fluoride to the irreversibly ineffective sulfonic acid; the efficiency of inactivation of the enzyme by an inhibitor such as **1** was dependent on the ratio of these two rates. Substitution of a methyl group (**4**) *ortho* to the sulfonyl fluoride group of **1** gave little change in the ratio of these two rates with the L1210/FR8 enzyme, but increased the ratio of the rate of enzyme-catalyzed hydrolysis by the liver enzyme to enzyme inactivation; thus **4** at 5×10^{-8} *M* gave 78% inactivation of L1210/FR8 enzyme, but only 15% inactivation of the liver enzyme, a more favorable chemotherapeutic situation than with the parent **1**. With the Walker 256 rat tumor enzyme, this substitution (**4**) was detrimental since the ratio of the rates of inactivation to hydrolysis was decreased; similarly, substitution of an *o*-methoxyl group (**5**) on **1** was detrimental to the inactivation of both tumor enzymes. Other patterns, including total loss of irreversible inhibition, were seen depending upon the type of substitution.

It was previously reported that the title compound (**1**) was an active-site-directed irreversible inhibitor[4] of dihydrofolic reductase; **1** could inactivate the dihydrofolic reductase from Walker 256 rat tumor, rat liver, L1213/FR8 mouse leukemia, mouse liver, and pigeon liver, but showed insufficient separation of irreversible inhibition.[5] When the sulfonyl fluoride was moved to the *meta* position (**2**), a separation of irreversible inhibition on the enzymes from mouse and rat tissues was observed, that is, the enzyme from Walker 256 rat tumor and rat liver was still inactivated, but the en-



1, *para*
2, *meta*

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

(2) For the previous paper of this series see B. R. Baker and G. J. Lourens, *J. Med. Chem.*, **11**, 672 (1968).

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

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

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