6-Chloro-9-(*m*-nitrophenyl)purine (73) (Method A).—To a solution of 600 mg (2.26 mmoles) of **70** in 15 ml of ethyl orthoformate was added 1 ml of 12 *N* HCl. Within 1 min a product began to separate. After being stirred 24 hr at ambient temperature, the mixture was filtered and the product was washed with 5 ml of H_2O; yield 500 mg (80%), mp 206–209°, that was suitable for further transformation. See Table II for additional data and other compounds prepared by this method.

9-(*m*-Nitrophenyl)hypoxanthine (11) (Method B).—A mixture of 170 mg (0.62 mmole) of **73** and 5 ml of 6 *N* HCl was refluxed for 3 hr during which time solution occurred, then the product separated. The cooled mixture was filtered and the product was washed with 5 ml of H_2O. Recrystallization from 75 ml of 2:1 EtOH–H_2O gave 115 mg (73%) of white crystals, mp >300°. See Table II for additional data and other compounds prepared by this method.

9-(*p*-Nitrophenyl)adenine (20) (Method C).—To 15 ml of absolute EtOH saturated with NH_3 at 0° was added 233 mg (0.85 mmole) of **74**. After being heated in a steel bomb at 115° for 24 hr, the cooled mixture was concentrated until the product began to separate; yield 188 mg (80%), mp 278–279°. See Table II for additional data.

Irreversible Enzyme Inhibitors. CXXVII.[1-3] *p*-(2,6-Diamino-1,2-dihydro-2,2-dimethyl-*s*-triazin-1-yl)phenylpropionylsulfanilyl Fluoride, an Active-Site-Directed Irreversible Inhibitor of Dihydrofolic Reductase. III. Effects of Modification of the Propionamide Bridge on Isozyme Specificity

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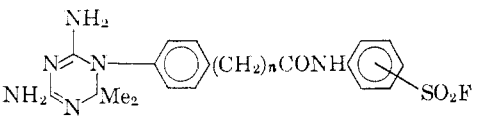
The title compound (1) is an active-site-directed irreversible inhibitor of the dihydrofolic reductase from Walker 256 rat tumor, rat liver, L1210/FR8 mouse leukemia, and mouse liver.[6] Six variants of the propionamide bridge, where the number of bridge atoms was held at four, were synthesized for enzymic evaluation, namely, acrylamido (**6**), methyleneureido (**8**), N-ethylsulfonamido (**10**), N-ethylcarboxamido (**12**), oxyacetamido (**13**), and oxyethyloxy (**15**). All but **6** were as good reversible inhibitors of dihydrofolic reductase as **1** with a K_i in the range of 1–6 × 10^{-9} *M* for Walker 256; **6** was 300-fold less effective. With the Walker 256 enzyme four showed no irreversible inhibition and two (**10**, **12**) only poor irreversible inhibition. Of these six compounds, none showed irreversible inhibition of the L1210/FR8 enzyme. These results were rationalized on the basis of allowable ground-state conformations of the compounds *vs.* the conformation needed for juxtaposition of the sulfonyl fluoride to an enzymic nucleophilic group within the enzyme–inhibitor complex. The metanilyl isomer (**3**) of 1 can inactivate the dihydrofolic reductase from Walker 256 and rat liver, but not L1210/FR8.[7] When the propionamide bridge of **3** was changed to acrylamido (**7**), methyleneureido (**9**), N-ethylsulfonamido (11), or oxyacetamido (**14**), irreversible inhibition was again lost. These results show the sensitivity of active-site-directed irreversible inhibition to proper positioning of the inhibitor's leaving group with an enzymic nucleophilic group within the reversible complex.

The first active-site-directed irreversible inhibitor[5] of the terminal sulfonyl fluoride type found to be effective on the dihydrofolic reductase from Walker 256 rat tumor and L1210/FR8 mouse leukemia was the title compound **1**;[6] the compound could also inactivate the dihydrofolic reductase from rat liver[6] and mouse liver (Table I). The sulfonyl fluoride with a shorter bridge (**2**) could also inactivate the tumor enzymes, but a much higher concentration of **2** was required since it was a considerably less effective reversible inhibitor than **1**. When the sulfonyl fluoride group of **1** was moved to the *meta* position (**3**), a separation of activity on the enzymes of the two species was observed;[7] **3** failed to inactivate the dihydrofolic reductase from L1210/FR8 mouse leukemia but could still inactivate the two rat tissue enzymes. Conversely, a cross-over specificity



1, $n = 2$, *para* isomer
2, $n = 0$, *para* isomer
3, $n = 2$, *meta* isomer
4, $n = 3$, *para* isomer
5, $n = 0$, *meta* isomer

was noted with **5** which could inactivate the L1210/FR8 mouse leukemia enzyme, but not the Walker 256 rat tumor enzyme. Furthermore, when the bridge length was increased to give **4**, the Walker 256 enzyme was still inactivated, but the L1210/FR8 enzyme was not.[7] A study was then initiated on the effect that the changes in the bridge between the two benzene rings of **1**–**3** would have on the active-site-directed irreversible inhibition. Such a study might answer the following questions. (1) Can other effective bridges be found that would give additional flexibility in synthesis of active analogs? (2) What is the relationship of ground-state conformation of the bridge to effective irreversible inhibition? (3) Can activity be further separated[8] between tumor and liver enzymes? 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 in this series see B. R. Baker, W. F. Wood, and J. A. Kozma, *J. Med. Chem.*, **11**, 661 (1968).

(3) For the previous paper on inhibitors of dihydrofolic reductase see B. R. Baker and P. C. Huang, *ibid.*, **11**, 495 (1968), paper CXX of the series.

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

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

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

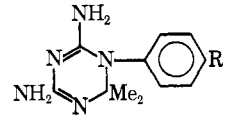
(7) B. R. Baker and G. J. Lourens, *ibid.*, **11**, 38 (1968), paper CXII of this series.

(8) The effective inhibition of a tumor enzyme with little or no effect on the corresponding liver enzyme has been previously reported with 5-phenoxypropylpyrimidines[9] and 6-phenoxymethylpyrimidines[3] with a terminal fluorosulfonylbenzamido group on the benzene ring.

(9) B. R. Baker and R. B. Meyer, Jr., *J. Med. Chem.*, **11**, 489 (1968), paper CXIX 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	% inactvn
1	$(CH_2)_2CONHC_6H_4SO_2F$-*p*	W256[h]	0.020	0.003	0.050	95	<1, 10	90, 90[i]
		Rat liver[h]	0.0060	0.001	0.050	98	<2, 60	70, 70[i]
		L1210/FR8[h]	0.080	0.01	0.070	84	<2, 10	84, 84[i]
		Mouse liver			0.070		2, 60	38, 38[i]
					0.4		2, 60	59, 59[i]
2[h]	$CONHC_6H_4SO_2F$-*p*	W256	21	4	25	88	<1	100
		L1210/FR8	600	100	25	20	60	45
3	$(CH_2)_2CONHC_6H_4SO_2F$-*m*	W256	0.0080[j]	0.001	0.060	98	5	100[j]
					0.010	88	60	0
		Rat liver	0.012	0.002	0.060	97	2, 20	72, 72[i]
		L1210/FR8	0.044[j]	0.007	0.20	96	60	0[j]
6	$CH{=}CHCONHC_6H_4SO_2F$-*p*	W256	6.5	1.0	25	96	0	0
		L1210/FR8	2.8	0.5	14	97	60	0
7	$CH{=}CHCONHC_6H_4SO_2F$-*m*	W256	1.3	0.2	8	98	60	55
8	$CH_2NHCONHC_6H_4SO_2F$-*p*	W256	0.010	0.002	0.050	97	60	0
		L1210/FR8	0.075	0.01	0.38	97	60	0
9	$CH_2NHCONHC_6H_4SO_2F$-*m*	W256	0.0092	0.002	0.046	97	60	0
		L1210/FR8	0.028	0.005	0.14	97	60	0[k]
10	$(CH_2)_2NHSO_2C_6H_4SO_2F$-*p*	W256	0.039	0.006	0.20	97	30, 60	38, 38[i]
		L1210/FR8	0.092	0.02	0.46	97	60	0
11	$(CH_2)_2NHSO_2C_6H_4SO_2F$-*m*	W256	0.023	0.004	0.12	97	60	0
		L1210/FR8	0.15	0.02	0.75	97	60	0
12	$(CH_2)_2NHCOC_6H_4SO_2F$-*p*	W256	0.0078	0.001	0.039	97	60	27
					0.0078	87	5, 60	10, 10[i]
		L1210/FR8	0.036	0.006	0.18	97	60	0
13	$OCH_2CONHC_6H_4SO_2F$-*p*	W256	0.015	0.003	0.060	95	60	0
		L1210/FR8	0.048	0.008	0.24	97	60	0
14	$OCH_2CONHC_6H_4SO_2F$-*m*	W256	0.010	0.002	0.050	97	60	0
		L1210/FR8	0.069	0.01	0.22	93	60	0
15	$O(CH_2)_2OC_6H_4SO_2F$-*p*	W256	0.016	0.003	0.079	97	60	0
		L1210/FR8	0.052	0.01	0.26	97	60	0
16	$(CH_2)_2C_6H_4SO_2F$-*p*	W256	0.016	0.003	0.080	97	60	0
		L1210/FR8	0.039	0.006	0.19	97	60	0
17	C_2H_5	L1210/FR8	0.53	0.09				
18	OCH_3	L1210/FR8	0.68	0.1				
19	$(CH_2)_2CONH_2$	L1210/FR8	0.28	0.05				
20	H	L1210/FR8	0.21[l]	0.04				

[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.[6] [d] Incubated at 37° in pH 7.4 Tris buffer in the presence of 60 μM TPNH as previously described.[6] [e] I_{50} = concentration for 50% inhibition. [f] Estimated from $K_i = K_m[I]_{50}/[S]$ which is valid since $[S] = 6K_m = 6\ \mu M$ dihydrofolate; see ref 5, p 202. [g] Calcd from $[EI] = [E_t]/(1 + K_i/[I])$ where [EI] is the amount of total enzyme (E_t) reversibly complexed.[13] [h] Data from ref 6. [i] From time study plot; see ref 6. [j] Data from ref 7. [k] A low I_0 point indicated that there may have been some irreversible inhibitor occurring in the first few seconds. [l] Data from ref 16.

first two questions were answered by this study which is the subject of this paper. The third goal was not achieved by bridge modification, but was achieved with a different type of modification of **1**.[10]

Enzyme Results.—In an earlier paper it was reported[6] that the parent **1** could inactivate dihydrofolic reductase from Walker 256 about eightfold more rapidly than the enzyme from rat liver. Furthermore, at 0.05 μM of **1** the inactivation stopped at 90% with the rat tumor enzyme and 70% with the rat liver enzyme; it was noted that the different dihydrofolic reductase preparations could decompose the inhibitor, but since these were not pure enzyme preparations it could not be stated with any certainty that the enzyme *per se* was catalyzing the hydrolysis of the sulfonyl fluoride group. Since the same decomposition of appropriate sulfonyl fluorides was observed with pure α-chymotrypsin[11] and pure trypsin,[12] it was established that two reactions occurred within the enzyme–inhibitor complex; the first was covalent linkage to the enzyme with inactivation and the second was indeed enzyme-catalyzed hydrolysis of the sulfonyl fluoride. The relative rates of these two reactions could be dependent upon the relative positioning and an enzymic hydroxyl group to the sulfonyl fluoride; thus, if the complex juxtaposed

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

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

(12) B. R. Baker and E. H. Erickson, *ibid.*, **11**, 245 (1968), paper CXV of this series.

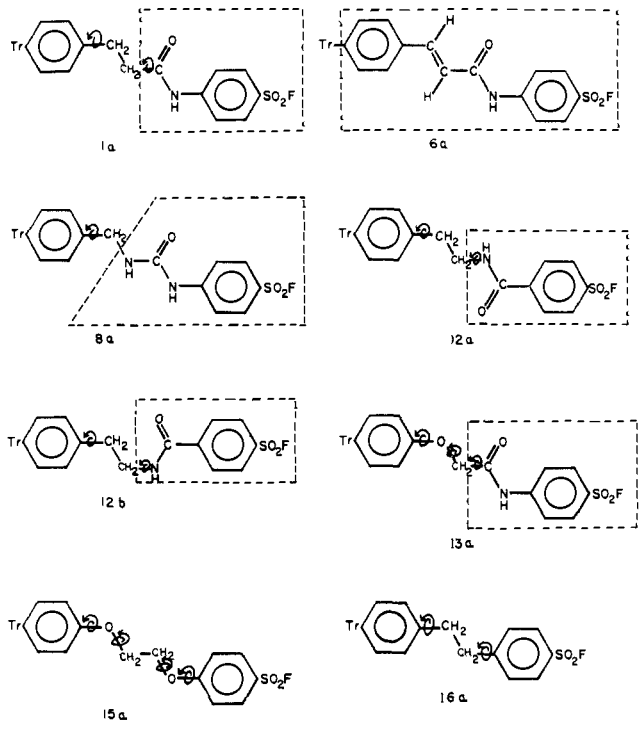
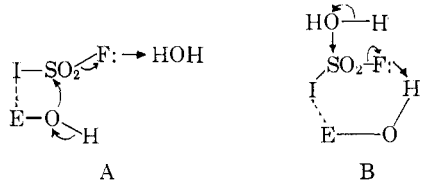


Figure 1.—Conformations of candidate irreversible inhibitors of dihydrofolic reductase. In addition to the preferred staggered conformation of the ethane moiety, the dotted areas indicate a preferred conformation in the ground state where any rotation requires energy. The arrows indicate which bonds have some freedom of rotation. Tr = 4,6-diamino-1,2-dihydro-2,2-dimethyl-*s*-triazin-1-yl.

the sulfonyl fluoride as in A, covalent bond formation occurred, but if juxtaposed as in B, hydrolysis occurred.[11] The two extremes in positioning differed by only about 3 Å.



A similar observation has now been made with **1** on inactivation of the dihydrofolic reductases from L1210/FR8 and mouse liver (Table I). The leukemia enzyme was 84% inactivated in <2 min by 0.07 μM of **1**, but a similar concentration of **1** gave only 38% inactivation of the mouse liver enzyme; when **1** was increased to 0.4 μM, the total inactivation of the mouse liver enzyme was raised to 59%.

Since a structural change in an irreversible inhibitor can change either the reversible binding or the rate of inactivation or both, two compounds should not be compared at equal I concentrations but at near equal concentrations of reversible complex, EI; such a comparison considers only the ability to inactivate within the EI complex.[13] When the propionamide bridge of **1** was changed to the acrylamido bridge of **6**, not only was reversible binding greatly decreased, but **6** failed to inactivate either of the tumor enzymes. In contrast, when the propionamido bridge of **1** was changed to the methyleneureido bridge of **8**, reversible binding did not change, but irreversible inhibition was still lost. These results can be rationalized by consideration of the allowable ground-state conformations of **1**, **6**, and **8** as shown in Figure 1.

The conjugated cinnamamide system of **6a** has no rotation about any of its bonds in its ground-state conformation that does not require energy; the benzenesulfonyl moiety is in a plane 38° from the plane of the carboxamide[14] and little energy-free rotation can occur. Thus, the loss in reversible binding by **6** is due to the presence of a coplanar substituent on the *para* position of the 1-phenyl-*s*-triazine, a situation known not to be well tolerated by the surface of vertebrate dihydrofolic reductase.[15,16] In contrast, **1a** has two degrees of free rotation not requiring energy as indicated in Figure 1; thus **1a** does not take conformation **6a** for either reversible or irreversible inhibition of the dihydrofolic reductases. The urea (**8a**) is intermediate between **6a** and **1a** in that **8a** has only one degree of free rotation; this one degree of free rotation apparently allows a conformation suitable for good reversible binding, but does not allow suitable juxtaposition of the sulfonyl fluoride for covalent bond formation. It follows that **1a** assumes one of its possible ground-state conformations where rotation about both degrees of freedom have occurred (compared to **6** and **8**) in order to properly juxtapose the sulfonyl fluoride group to an enzymic nucleophilic group, presumably a hydroxyl of serine or threonine.

When the carboxamide group of **1** was reversed to give **12**, little change in reversible binding to the two enzymes occurred; however, **12** showed no irreversible inhibition of the L1210/FR8 enzyme and only a small amount of irreversible inhibition of the Walker 256 enzyme before the inhibitor was hydrolyzed by enzyme catalysis. Thus, the positioning of the sulfonyl fluoride of **12** is slightly different in the enzyme–inhibitor complex than with **1**, but is probably changed less than 3 Å, or else the Walker 256 enzyme could not catalyze hydrolysis of the sulfonyl fluoride of **12**. Two explanations were considered for this difference. If the carboxamide oxygen of **1** is complexed to the enzyme, then **12** would have to assume conformation **12b** to give the same carbonyl interaction with the enzyme; such a change from **12a** would move the SO_2F group about 2.7 Å. That the amount of binding by the carboxamide moiety of **1** was only about twofold was shown by the comparison of the binding of the *p*-ethyl group of **17** with the *p*-propionamide group of **19**; thus, conformation **12b** would not be a likely explanation for the difference between **12** and **1**, since conformation **12a** could be assumed with little loss of binding energy. The second explanation involved the difference in angles between the phenyl plane and the carboxamide plane of **12** and **1**. In the case of **12a** the amide and benzene moieties are coplanar, but in the case of **1**, the two planes are at an angle of 38° as previously noted.[14] This 38° angle could have one of two effects; it could change the position of the sulfonyl fluoride group by 0.8 Å between **12** and **1** or it could change the angle of the sulfonyl fluoride to the attacking group, perhaps negating a transition state for reaction.

(13) For a discussion of the kinetics of active-site-directed irreversible enzyme inhibition see ref 5, Chapter VIII.

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

(15) (a) B. R. Baker and B.-T. Ho, *J. Pharm. Sci.*, **55**, 470 (1966); (b) B. R. Baker, *J. Med. Chem.*, **10**, 912 (1967), paper XCVII of this series.

(16) B. R. Baker, *ibid.*, **11**, 483 (1968), paper CXVII of this series.

Changing the propionamido group of **1** to sulfonamidoethyl (**10**, **11**) gave similar effects that can be accounted for by the bond angles; reversible inhibition was the same, but irreversible inhibition varied between poor and none.

Considerably more difficult to rationalize is the change of the propionamido bridge of **1** to oxyacetamido bridge (**13**). Reversible inhibition was similar, but irreversible inhibition was lost. The bridge of **13a** now has three degrees of free rotation compared to two degrees for **1a** (Figure 1). Thus **13a** can assume any conformation that **1a** can and would therefore be expected to position favorably for irreversible inhibition; the –O– and $-CH_2-$ bond angles are almost identical and could not account for this difference. Since it was experimentally observed that **13** and **1** do not position the sulfonyl fluoride in the same way within the enzyme–inhibitor complex, one possible explanation is that the third degree of rotation of **13** allows a conformation more favorable for reversible binding, but does not juxtapose the sulfonyl fluoride moiety properly; however, such a suggestion was not strongly supported by comparing the binding increments between **18** and **13** (14-fold) and **17** and **1** (sevenfold).

A similar loss of irreversible inhibition was observed in the *m*-sulfonyl fluoride series (**3** *vs.* **13**).

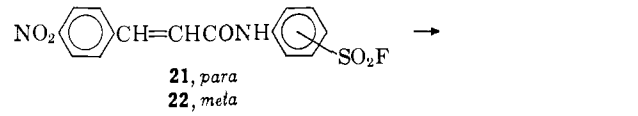
Replacement of the propionamido bridge of **1** with ethylenedioxy to give **15** resulted in little change in reversible binding, but again irreversible inhibition was lost. The bridge of **15a** now has four degrees of freedom of rotation, each –O–C bond being able to rotate (Figure 1). Again the only apparent rationalization is that the four degrees of rotation allow **15** to take a conformation most favorable for reversible binding, which does not properly juxtapose the sulfonyl fluoride for covalent bond formation with the enzyme.

That a more favorable conformation for reversible binding can lead to loss of juxtaposition of the sulfonyl fluoride moiety for covalent bond formation was more clearly shown with the comparison of the carboxamide-bridged (**2**) with the ethane-bridged (**16**) sulfonyl fluoride. Since **2** has a large linear side chain at the *para* position, it is a poor reversible inhibitor as expected;[15,16] that it could irreversibly inhibit both tumor enzymes was previously reported.[6] The ethane-bridge (**16**) could then take a noncoplanar conformation leading to a good reversible inhibitor,[15] but apparently this better conformation for reversible binding no longer allows juxtaposition of the sulfonyl fluoride with the enzymic nucleophilic group within the reversible complex.

Although rationalization of the lack of irreversible inhibition by some of the bridge changes in **1**–**16** is difficult, the fact remains that the proper positioning of the sulfonyl fluoride to an enzymic nucleophilic group within the enzyme–inhibitor complex is extremely sensitive to small conformational changes in the inhibitor. Such sensitivity is desirable for tissue-specific active-site-directed irreversible enzyme inhibitors as has been shown with three classes[3,9,10] of candidate irreversible inhibitors of dihydrofolic reductase, but the bridge changes in **1** (Table I) are too drastic. More subtle changes in **1** that allow retention of irreversible inhibition of the L1210/FR8 enzyme, with a lesser effect on the mouse liver enzyme, is described in an accompanying paper.[10]

Chemistry.—All of the dihydro-*s*-triazines in this study were made by the three-component method of Modest,[17] that is, the condensation of the appropriate arylamine salt with cyanoguanidine and acetone; the appropriate arylamines were made as follows.

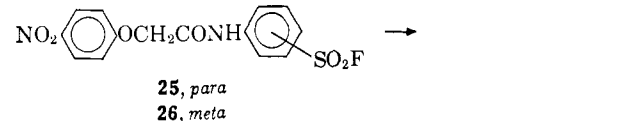
p-Nitrocinnamoylsulfanilyl fluoride (**21**)[6] was selectively reduced to the amine (**23**) with Fe powder in a



21, *para*
22, *meta*

23, *para*
24, *meta*

mixture of MeCN and HOAc;[18] the *meta* isomer (**24**) was made similarly from **22**.[7] Acylation of sulfanilyl fluoride with *p*-nitrophenoxyacetyl chloride in boiling toluene afforded **25** in 82% yield. Catalytic reduction of the nitro group with a Raney Ni catalyst afforded the desired amine (**27**); **28** was prepared similarly from metanilyl fluoride.

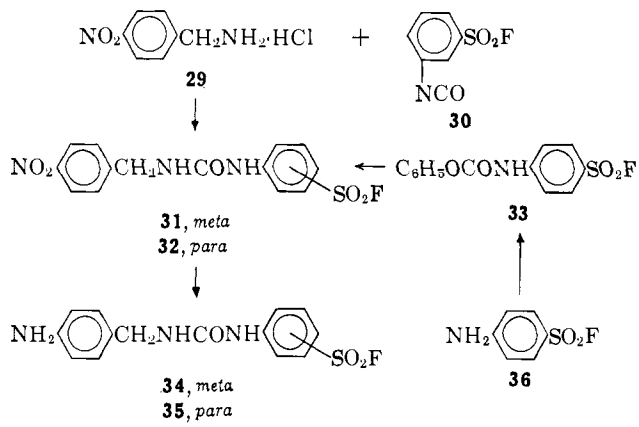


25, *para*
26, *meta*

27, *para*
28, *meta*

Condensation of *p*-nitrobenzylamine hydrochloride (**29**)[19] with commercial *m*-fluorosulfonylphenyl isocyanate (**30**) proceeded smoothly in $CHCl_3$ at 0° when neutralized with triethylamine; the mixed urea (**31**) was obtained analytically pure in 49% yield (Scheme I). The nitro group was then catalytically reduced to

SCHEME I



29
30
31, *meta*
32, *para*
33
34, *meta*
35, *para*
36

34 with Raney Ni catalyst. The *para* isomer (**32**) could not be made the same way from the *para* isomer of **30** since sulfanilyl fluoride failed to react smoothly with phosgene presumably due to the deactivation of

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

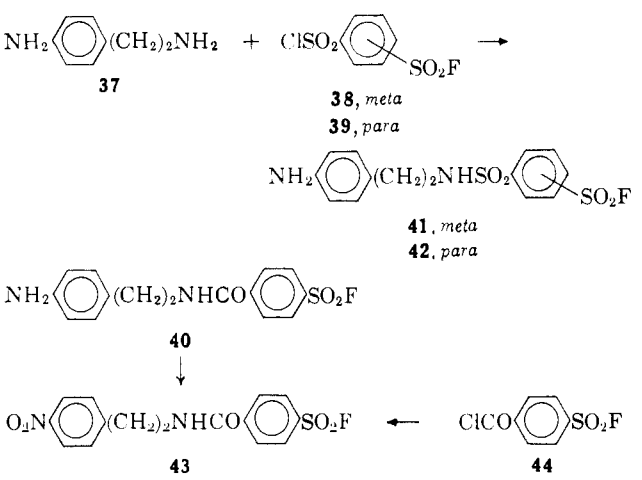
(18) J. Jaeken and R. L. Jansseune, British Patent 857,028 (1960); *Chem. Abstr.*, **62**, P682*a* (1965).

(19) Prepared by alkylation of potassium phthalimide with *p*-nitrobenzyl bromide in DMF by the method of H. R. Ing and R. Robinson, *J. Chem. Soc.*, 1663 (1926), followed by cleavage with $N_2H_4 \cdot H_2O$ according to H. R. Ing and R. H. F. Manske, *ibid.*, 2350 (1936).

the *p*-amino group by the electron-withdrawing *p*-sulfonyl fluoride group. However, the Crosby method[20] for mixed urea formation was successful. Reaction of **36** with phenyl chloroformate in boiling C_6H_6 afforded crystalline **33** in 52% yield; the phenoxy group of **33** was readily displaced by *p*-nitrobenzylamine (**29**) in DMF at room temperature. The nitro group was again reduced catalytically with a Raney Ni catalyst to give **35**.

Since the aliphatic amino group of the commercially available *p*-aminophenethylamine (**37**) should be considerably more reactive than the aromatic amino group and the sulfonyl chloride group is considerably more reactive than a sulfonyl fluoride group,[21] the selective acylation of **37** with **38** was investigated; when the reaction was performed in THF at 0° with Et_3N as an acid acceptor, the desired aminophenylsulfonyl fluoride (**41**) was obtained in 54% yield of analytically pure material (Scheme II); that acylation had proceeded on

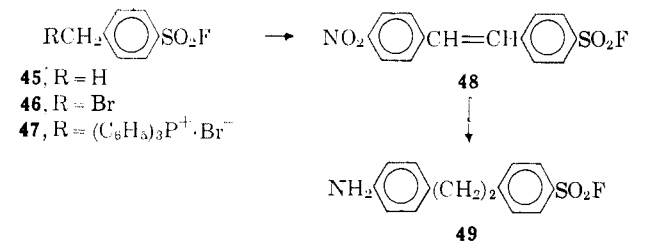
Scheme II



the aliphatic amino group was shown by the positive Bratton–Marshall test for aromatic amine[22] and the presence of SO_2F by an ir band at 1400 cm^{-1}. Similarly, the *para* isomer (**42**) was prepared in 37% yield from **39**. Reaction of the corresponding benzoyl chloride (**44**) with **37** was not sufficiently selective even at relatively high dilution at −70°; considerable reaction also occurred on the aromatic amine as indicted by isolation of a high-melting product with a negative Bratton–Marshall test.[22] Therefore *p*-nitrophenethylamine was acylated with **44** to give **43**, which was catalytically reduced to the desired **40**.

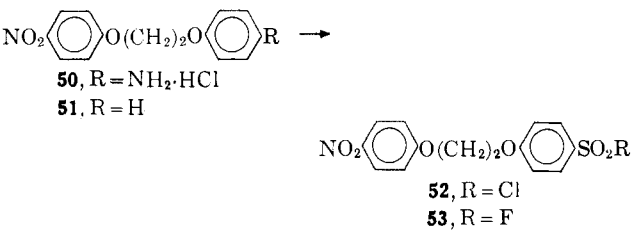
Reaction of *p*-tolylsulfonyl fluoride (**45**) with NBS in CCl_4 in the presence of benzoyl peroxide and uv light afforded the bromomethyl derivative (**46**). This compound (**46**) should be handled with care since it is a severe skin irritant; it is best treated directly with triphenylphosphine to give the Wittig reagent **47**. Wittig condensation of **47** with *p*-nitrobenzaldehyde performed in MeOH at 0° in the presence of Et_3N, conditions which caused the product to separate from solution before it could overreact, gave **48** in 55% yield

Scheme III



(Scheme III); the more standard conditions of KOBu-*t* in DMF gave lower yields of less pure product.

Two routes were investigated for synthesis of **53**. The first, diazotization of the known **50**[23] followed by treatment with SO_2 by the Meerwein procedure,[24] was unsuccessful due to insolubility. Direct chlorosulfonation of **51**[25] proceeded to the sulfonyl chloride **52** which was not purified but was converted to the more stable sulfonyl fluoride with KF in DMF.[26] The re-



quired aromatic amine was prepared from **53** by Raney Ni catalyzed hydrogenation, then coverted to the dihydro-*s*-triazine (**15**).

Experimental Section[27]

***p*-Aminocinnamoylsulfanilyl Fluoride (23).**—To a stirred suspension of 1.75 g (5 mmoles) of **21**[6] in 50 ml of MeCN, 20 ml of HOAc, and 5 ml of H_2O was added 2 g of Fe powder in portions over 20 min. After being stirred an additional 20 min at ambient temperature, the mixture was refluxed for 1 hr. The mixture was filtered and the cake was washed with 50 ml of Me_2CO. The combined filtrate and washings were poured in 500 ml of stirred H_2O containing 15 g of $NaHCO_3$. The product was collected on a filter, washed with H_2O, and recrystallized from *i*-PrOH; yield, 0.72 g (45%) of yellow solid which gradually decomposed over 195° and which moved as a single spot on tlc with 2:1 EtOAc–petroleum ether (bp 30–60°). *Anal.* ($C_{15}H_{13}FN_2O_3S$) C, H, N.

Similarly, ***p*-aminocinnamoylmetanilyl fluoride (24)** was prepared from **22**, in 31% yield, dec pt >190°. *Anal.* ($C_{15}H_{13}FN_2O_3S$) C, H, N.

***p*-Nitrophenoxyacetylsulfanilyl Fluoride (25).**—A mixture of 2.96 g (15 mmoles) of *p*-nitrophenoxyacetic acid, 5 ml of $SOCl_2$, and 15 ml of C_6H_6 was refluxed with magnetic stirring for 2 hr, then the solution was spin evaporated *in vacuo*. To the residual acid chloride were added 85 ml of $C_6H_5CH_3$ and 2.62 g (15 mmoles) of sulfanilyl fluoride. After being refluxed with stirring for 5.5 hr, the mixture was cooled to room temperature. The product was collected on a filter and washed with C_6H_6. Recrystallization from MeOEtOH–H_2O gave 4.38 g (82%) of white crystals, mp 186–188°. *Anal.* ($C_{14}H_{11}FN_2O_6S$) C, H, N.

(20) (a) D. G. Crosby and C. Niemann, *J. Am. Chem. Soc.*, **76**, 4458 (1954); (b) B. R. Baker and R. P. Ratel, *J. Pharm. Sci.*, **52**, 927 (1963).

(21) For a review of the chemical reactivity of the sulfonyl fluoride group see ref 6.

(22) B. R. Baker, D. V. Santi, J. K. Coward, H. S. Shapiro, and J. H. Jordaan, *J. Heterocycl. Chem.*, **3**, 425 (1966).

(23) B. R. Baker and G. J. Lourens, *J. Med. Chem.*, **11**, 26 (1968).

(24) (a) H. Meerwein, G. Dittmar, R. Göllner, K. Hafner, F. Mensch, and O. Steinfort, *Chem. Ber.*, **90**, 841 (1957); (b) B. R. Baker and J. K. Coward, *J. Heterocycl. Chem.*, **4**, 195 (1967).

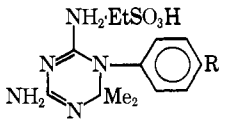
(25) H. Ryan and T. Kenny, *Sci. Proc. Roy. Dublin Soc.*, **17**, 305 (1924).

(26) D. E. Fahrney and A. M. Gold, *J. Am. Chem. Soc.*, **85**, 997 (1963).

(27) 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 taken in capillary tubes on a Mel-Temp block and are uncorrected.

TABLE II

PHYSICAL PROPERTIES OF



No.	R	% yield	Mp, °C dec	Formula	Analyses
6	CH=CHCONHC$_6$H$_4$SO$_2$F-*p*	61[a]	220–222	C$_{20}$H$_{21}$FN$_6$O$_3$S·C$_2$H$_5$SO$_3$H	C, H, N
7	CH=CHCONHC$_6$H$_4$SO$_2$F-*m*	56[a]	225–227	C$_{20}$H$_{21}$FN$_6$O$_3$S·C$_2$H$_5$SO$_3$H	C, H, F
8	CH$_2$NHCONHC$_6$H$_4$SO$_2$F-*p*	45[a]	215–218	C$_{19}$H$_{22}$FN$_7$O$_3$S·C$_2$H$_5$SO$_3$H	C, H, N
9	CH$_2$NHCONHC$_6$H$_4$SO$_2$F-*m*	39[a,b]	>130[c]	C$_{19}$H$_{22}$FN$_7$O$_3$S·C$_2$H$_5$SO$_3$H	C, H, N
10	(CH$_2$)$_2$NHSO$_2$C$_6$H$_4$SO$_2$F-*p*	52[a]	214–216	C$_{19}$H$_2$ FN$_6$O$_4$S·C$_2$H$_5$SO$_3$H	C, H, N
11	(CH$_2$)$_2$NHSO$_2$C$_6$H$_4$SO$_2$F-*m*	49[a]	189–191	C$_{19}$H$_{23}$FN$_6$O$_4$S·C$_2$H$_5$SO$_3$H	C, H, N
12	(CH$_2$)$_2$NHCOC$_6$H$_4$SO$_2$F-*p*	33[a,b]	196–197	C$_{19}$H$_{23}$FN$_6$O$_4$S·C$_2$H$_5$SO$_3$H·0.5C$_3$H$_8$O	C, H, F
13	OCH$_2$CONHC$_6$H$_4$SO$_2$F-*p*	60[a]	219–220	C$_{19}$H$_{21}$FN$_6$O$_4$S·C$_2$H$_5$SO$_3$H	C, H, N
14	OCH$_2$CONHC$_6$H$_4$SO$_2$F-*m*	49[a,b]	214–215	C$_{19}$H$_{21}$FN$_6$O$_4$S·C$_2$H$_5$SO$_3$H	C, H, N
15	O(CH$_2$)$_2$OC$_6$H$_4$SO$_2$F-*p*	45[a,b]	221–222	C$_{19}$H$_{22}$FN$_5$O$_4$S·C$_2$H$_5$SO$_3$H	C, H, F
16	(CH$_2$)$_2$C$_6$H$_4$SO$_2$F-*p*	52[a,b]	211–213	C$_{19}$H$_{22}$FN$_5$O$_2$S·C$_2$H$_5$SO$_3$H	C, H, F
17	C$_2$H$_5$	53[a,d]	210–212	C$_{13}$H$_{19}$N$_5$·HCl	C, H, N
18	OCH$_3$	66[d,e]	206–209[f]		
19	(CH$_2$)$_2$CONH$_2$	16[b,e,g]	216–217	C$_{14}$H$_{20}$N$_6$O·C$_2$H$_5$SO$_3$H	C, H, N

[a] Recrystallized from *i*-PrOH–H$_2$O. [b] Over-all yield from the nitro compound since the amine was not isolated. [c] Gradually decomposes higher than this temperature. [d] HCl salt. [e] Recrystallized from EtOH–H$_2$O. [f] M. Furukawa, Y. Seto, and S. Toyoshima, *Chem. Pharm. Bull.* (Tokyo), **9**, 914 (1961), have recorded mp 204–205°. [g] For starting *p*-nitrocinnamamide see G. Carrara, R. Ettore, F. Fava, R. Rolland, E. Testa, and A. Vecchi, *J. Am. Chem. Soc.*, **76**, 4391 (1954).

***p*-Nitrophenoxyacetylmetanilyl fluoride (26)** was prepared as described for **25** in 77% yield, mp 172–173°. *Anal.* (C$_{14}$H$_{11}$FN$_2$O$_5$S) C, H, N.

N-Carbophenoxysulfanilyl Fluoride (33).—A mixture of 1.56 g (10 mmoles) of phenyl chloroformate, 1.31 g (7.5 mmoles) of sulfanilyl fluoride (**36**), and 20 ml of C$_6$H$_6$ was refluxed with magnetic stirring for 2 hr during which time the product began to separate. The cooled mixture was filtered and the product was washed with C$_6$H$_6$. Recrystallization from C$_6$H$_6$ gave 1.15 g (52%) of white crystals, mp 166–167°. *Anal.* (C$_{13}$H$_{10}$FNO$_4$S) C, H, N.

N-(*m*-Fluorosulfonylphenyl)-N′-(*p*-nitrobenzyl)urea (31).—To a stirred mixture of 1.41 g (7.5 mmoles) of **29**[19] and 0.77 g (7.5 mmoles) of Et$_3$N in 10 ml of CHCl$_3$ cooled in an ice bath was added a solution of 1.61 g (8 mmoles) of **30** in 5 ml of CHCl$_3$. After being stirred in the ice bath for 30 min, the mixture was filtered and the product was washed with CHCl$_3$. Recrystallization from EtOH gave 1.31 g (49%) of white crystals, mp 214–215°. *Anal.* (C$_{14}$H$_{12}$FN$_3$O$_5$S) C, H, N.

N-(*p*-Fluorosulfonylphenyl)-N′-(*p*-nitrobenzyl)urea (32).—A mixture of 1.89 g (10 mmoles) of **29**,[19] 2.95 g (10 mmoles) of **33**, 8 ml of DMF, and 1.02 g (10 mmoles) of Et$_3$N was stirred at ambient temperature for 7 hr. Addition of 5 ml of C$_6$H$_6$ and 30 ml of H$_2$O gave a gummy solid which was triturated with 15 ml of 2:1 C$_6$H$_6$–petroleum ether (bp 30–60°). The product was collected on a filter and washed with C$_6$H$_6$. Recrystallization from EtOH with the aid of charcoal gave 2.75 g (80%) of white crystals, mp 200–201°. *Anal.* (C$_{14}$H$_{12}$FN$_3$O$_5$S) C, H, N.

N-(*p*-Aminophenethyl)-*p*-fluorosulfonylbenzenesulfonamide (42).—To a stirred solution of 1.36 g (10 mmoles) of **37** and 1.11 g (11 mmoles) of Et$_3$N in 7.5 ml of THF cooled in an ice bath was added dropwise a solution of 2.58 g (10 mmoles) of **39** in 7.5 ml of THF over a period of 8 min. The mixture was stirred in the ice bath for an additional 1 hr, then filtered, and the Et$_3$N·HCl was washed with 20 ml of THF. The combined filtrate and washings were evaporated *in vacuo*. Trituration of the oily residue with a mixture of 5 ml of C$_6$H$_6$ and 25 ml of H$_2$O gave a solid which was collected on a filter and washed with 20 ml of 1:1 C$_6$H$_6$–petroleum ether (bp 30–60°). Two recrystallizations from EtOH–H$_2$O gave 1.34 g (37%) of light yellow crystals, mp 134–136°, that showed a positive Bratton–Marshall test for aromatic amine.[21] *Anal.* (C$_{14}$H$_{15}$FN$_2$O$_4$S$_2$) C, H, N.

N-(*p*-Aminophenethyl)-*m*-fluorosulfonylbenzenesulfonamide (41) was prepared from **37** and **38** as described for **42**. Recrystallization from EtOH–C$_6$H$_6$ gave 1.92 g (54%) of light yellow crystals, mp 128–130°. *Anal.* (C$_{14}$H$_{15}$FN$_2$O$_4$S$_2$) C, H, N.

***p*-Fluorosulfonyl-N-(*p*-nitrophenethyl)benzamide (43).**—To a stirred mixture of 1.51 g (7.5 mmoles) of *p*-nitrophenethylamine hydrochloride,[28] 2 ml of DMF, and 2.27 g (22.5 mmoles) of Et$_3$N cooled in ice bath was added dropwise a solution of 1.99 g (8.5 mmoles) of *p*-fluorosulfonylbenzoyl chloride (**44**) in 3 ml of DMF. After 40 min at 0° and 1 hr at ambient temperature, the mixture was diluted with 10 ml of petroleum ether (bp 60–110°) and 50 ml of H$_2$O. The product was collected on a filter, washed with 50% EtOH–H$_2$O, then twice recrystallized from EtOH with the aid of charcoal in the first recrystallization; yield 1.20 g (47%), mp 108–109°. *Anal.* (C$_{15}$H$_{13}$FN$_2$O$_5$S) C, H.

***α*-Bromo-*p*-tolylsulfonyl Fluoride (46).**—A mixture of 0.87 g (5 mmoles) of **45**, 0.89 g (5 mmoles) of NBS, 10 mg of benzoyl peroxide, and 5 ml of CCl$_4$ was refluxed with stirring and irradiation by a uv lamp for 2 hr. The mixture was cooled in an ice bath, then filtered. The combined filtrate and washings were evaporated *in vacuo*. Recrystallization of the residue from petroleum ether (bp 30–60°) gave 0.56 g (44%) of white crystals, mp 73–74°, that gave a positive 4-(*p*-nitrobenzyl)pyridine test for activated halogen.[22] *Anal.* (C$_7$H$_6$BrFO$_2$S) C, H.

This compound is a severe skin irritant and should be handled with care; it is best converted to **47** without isolation as follows.

***p*-Fluorosulfonylbenzyl Triphenylphosphonium Bromide (47).**—A mixture of 17.4 g (0.1 mole) of **45**, 17.8 g (0.1 mole) of NBS, 250 mg of benzoyl peroxide, and 100 ml of CCl$_4$ was refluxed with stirring and uv radiation for 2 hr. The cooled mixture was filtered, the succinimide was washed with CCl$_4$, then the combined filtrate and washings were evaporated *in vacuo*. The residual crude **46** was dissolved in 200 ml of C$_6$H$_6$ and treated with 26.2 g (0.1 mole) of triphenylphosphine. A gummy precipitate that separated was removed by filtration. The solution was then stirred at ambient temperature for 48 hr. The product was collected on a filter and washed with C$_6$H$_6$. Recrystallization from MeOH–Et$_2$O gave 17.7 g (34%) of white crystals, mp 251–252° dec. *Anal.* (C$_{25}$H$_{21}$BrFO$_2$PS) C, H.

4-Fluorosulfonyl-4′-nitrostilbene (48).—To a mixture of 1.59 g (10 mmoles) of *p*-nitrobenzaldehyde, 5.16 g (10 mmoles) of **47**, and 5 ml of MeOH stirred in an ice bath and protected from moisture was added dropwise 1.22 g (10 mmoles) of Et$_3$N over a period of 5 min. After being stirred 1 hr at 0° and 3.5 hr at ambient temperature during which time the product separated, the mixture was kept overnight at −15°. The product was collected on a filter and washed with cold EtOH. The solid was stirred with 10 ml of warm 50% EtOH, then collected on a filter and washed successively with 50% EtOH and H$_2$O; yield 1.68 g (55%) of a yellow solid, mp 110–160°, that moved as a single spot on tlc in C$_6$H$_6$ and was used for further transformation. For analysis, a portion of this presumed mixture of *cis* and *trans*

(28) F. R. Goss, W. Hanhart, and C. K. Ingold, *J. Chem. Soc.*, 250 (1927).

isomers was recrystallized from EtOH to give yellow crystals, mp 216–218°. *Anal.* ($C_{14}H_{10}FNO_5S$) 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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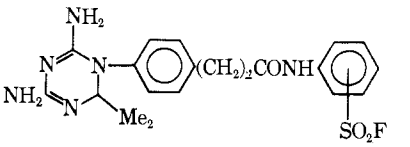
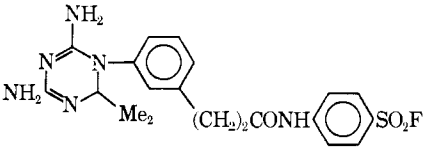
Received March 9, 1968

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 μM 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*



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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.