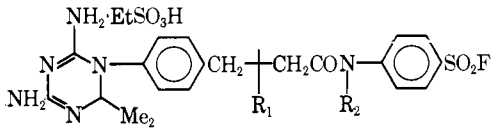
TABLE III

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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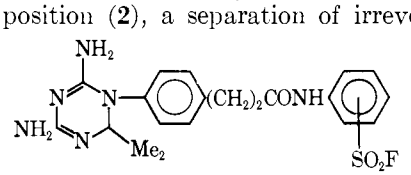
The title compound (1) is a fairly general irreversible inhibitor of dihydrofolic reductase, being able to rapidly inactivate this enzyme from Walker 256 tumor and liver from the rat and L1210/FR8 leukemia and liver from the mouse. Furthermore, the enzyme could catalyze the hydrolysis of the sulfonyl 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^{-3} *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).

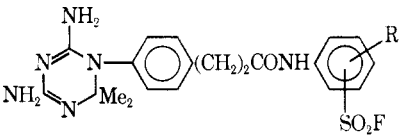
(3) G. J. L. wishes to thank the Council for Scientific and Industrial Research, Republic of South Africa, for a tuition fellowship.

(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.

Table I

Inhibition[a] of Dihydrofolic Reductase by



No.	SO_2F position	R	Enzyme source[b]	Reversible[c] I_{50},[e] μM	Reversible[c] K_i,[f] μM	Irreversible[d] Inhib. concn, μM	Irreversible[d] % EI[g]	Irreversible[d] Time, min	Irreversible[d] % inactn
1[h]	4	H	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	95	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]
						0.4		2, 60	59, 59[i]
2[h]	3	H	W256	0.0080	0.001	0.060	98	5	100[i]
						0.01	88	60	0
			Rat liver	0.012	0.002	0.060	97	2, 20	72, 72[i]
			L1210/FR8	0.044	0.007	0.20	96	60	0
3	4	2-Me	W256	0.0058	0.001	0.06	98	2, 8, 60	72, 90, 90[i]
						0.006	87	8, 60	10, 10[i]
			Rat liver			0.06		1, 3, 60	50, 89, 89[i]
			L1210/FR8	0.019	0.003	0.095	97	60	0
4	4	3-Me	W256	0.013	0.002	0.065	97	60	100
						0.013	87	60	0
			L1210/FR8	0.047	0.008	0.047	87	2, 8, 60	71, 78, 78[i]
						0.01	55	2, 60	10, 10[i]
			Mouse liver			0.047		4, 60	15, 15[i]
						0.24		<30, 60	44, 44[i]
5	4	3-OMe	W256	0.035	0.006	0.44	98	60	95
						0.035	87	60	0
			L1210/FR8	0.026	0.004	0.13	97	60	95
						0.026	87	60	0
6	3	6-OMe	W256	0.0084	0.001	0.042	97	60	20
			L1210/FR8	0.038	0.006	0.20	97	60	0
7	3	4-OMe	W256	0.0057	0.001	0.029	97	60	0
			L1210/FR8	0.038	0.006	0.19	97	60	0
8	3	4-Me	W256	0.0095	0.002	0.048	97	60	55
						0.0095	87	8, 60	10, 10[i]
			L1210/FR8	0.041	0.007	0.21	97	60	74
						0.041	87	5, 60	10, 10[i]

[a] The technical assistance of Jean Reeder, Diane Shea, and Sharon Laffer 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] Calcd from $[EI] = [E_t]/(1 + K_i/[I])$ where [EI] is the amount of total enzyme (E_t) reversibly complexed; see ref 4, Chapter VIII. [h] Data from ref 5, 7, 10. [i] From time study plot; see ref 5.

zyme from L1210/FR8 mouse leukemia was not.[5] Based on the bridge principle of specificity,[6] three approaches were initiated to try to separate the ability of a compound to inactivate both the tumor and liver enzymes: (a) modification of the propionamido bridge of **1** and **2**,[7] (b) substitution on the propionamido bridge,[2] and (c) substitution on one of the benzene rings of **1** and **2**; the results of this third approach are reported in this paper.

Enzyme Results.—For a discussion of relative inactivation rates one must bear in mind three factors:[8] (a) the rate of irreversible inhibition is dependent upon the amount of enzyme in reversible complex [EI]; (b) depending upon the position of the SO_2F group in the enzyme–inhibitor complex, the enzyme may become inactivated, the enzyme may catalyze hydrolysis of the SO_2F group, or both can occur;[9] (c) if less than 70% inactivation is seen at $5I_{50}$ of the inhibitor, which is sufficient to complex 97% of the enzyme, poor inactivation is usually seen at an I_{50} concentration.

Insertion of a methyl group (**3**) *ortho* to the carboxamide function of **1** (Table I) gave about a threefold increment in binding to the dihydrofolic reductase from Walker 256 rat tumor. At a $10I_{50}$ concentration, **3** could still inactivate the Walker 256 enzyme; however, near I_{50} concentration there was only 10% inactivation. Hence, insertion of this methyl group has a detrimental effect on the ratio of the rate of inactivation of the enzyme to the rate of enzyme-catalyzed hydrolysis of the sulfonyl fluoride,[9] since **1** can still inactivate the enzyme at an I_{50} concentration.

(6) (a) See ref 4, pp 172–183; (b) B. R. Baker, *Biochem. Pharmacol.*, **11**, 1155 (1962).

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

(8) See ref 2 for more detail.

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

Furthermore, there was no apparent separation of inactivation of the Walker 256 and rat liver enzymes. With the L1210/FR8 mouse leukemia enzyme, the binding by **3** was also increased by about fourfold; however, even at a $5I_{50}$ concentration, **3** failed to inactivate the enzyme.

Insertion of a methyl group (**4**) *ortho* to the sulfonyl fluoride of **1** gave little change in reversible binding to the two tumor enzymes. Although the Walker 256 enzyme could be completely inactivated with $5I_{50}$ of **4**, at an I_{50} concentration no irreversible inhibition was seen. In contrast, the L1210/FR8 enzyme was still inactivated 78% by an I_{50} concentration of **4**; however, the rate was clearly slower than with an I_{50} concentration of **1**.

The same I_{50} concentration (5×10^{-8} *M*) of **4** that gave 80% inactivation of the L1210/FR8 mouse leukemia enzyme showed only 15% inactivation of the mouse liver enzyme. Thus the ratio of the rate of enzyme inactivation by **4** to the rate of enzyme-catalyzed hydrolysis[9] of **4** by the L1210/FR8 enzyme was little changed compared to **1**; in contrast this ratio was decreased with the mouse liver enzyme. Hence **4** shows greater selectivity of action against the L1210/FR8 *vs.* liver enzymes than does the parent **1**.

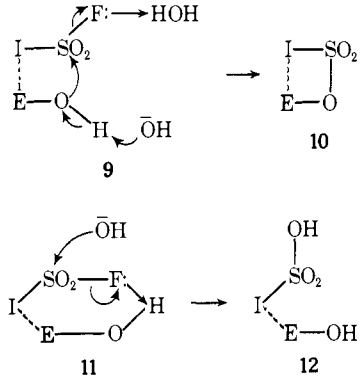
Insertion of a methoxyl group (**5**) *ortho* to the sulfonyl fluoride of **1** gave only a 2–3-fold change in reversible binding to the two tumor enzymes. The irreversible pattern with **5** on the Walker 256 enzyme was similar to that of **4**. In contrast, the methoxyl (**5**) and methyl (**4**) groups had opposite effects on the L1210/FR8 enzyme, that is, **5** could inactivate the enzyme at a concentration of $5I_{50}$ but not at I_{50}.

It was previously reported[10] that when the sulfonyl fluoride group of **1** was moved to the *meta* position (**2**), the Walker 256 enzyme could still be inactivated by $5I_{50}$ of **2**; the same concentration of **2** was just as effective on the rat liver enzyme. In contrast, the L1210/FR8 was not inactivated by a $5I_{50}$ concentration of **2** (Table I). Therefore the effect of *ortho* and *para* substituents on the activity of **2** was investigated. No change in reversible binding to the two tumor enzymes was seen when a methoxyl group (**6**) was inserted *para* to the sulfonyl fluoride group of **2**. This methoxyl group was severely detrimental to inactivation of the Walker 256 enzyme by **6** compared to **2**; no irreversible inhibition of the L1210/FR8 enzyme was seen with either **2** or **6**.

Insertion of the methoxyl group (**7**) *ortho* to the sulfonyl fluoride of **2** gave no change in reversible binding to the Walker 256 enzyme, but irreversible inhibition was lost. Insertion of an *o*-methyl group (**8**) in **2** gave slightly better reversible binding to the Walker 256 enzyme; however, **8** was not as effective an irreversible inhibitor as **2** since at $5I_{50}$, **8** only inactivated 55% and at I_{50} only 10%.

Although insertion of an *o*-methyl group (**8**) in **2** decreased the effectiveness of irreversible inhibition on the Walker 256 enzyme, this substitution increased the effectiveness of irreversible inhibition of the L1210/FR8 enzyme. Note that the parent **2** showed no irreversible inhibition of L1210/FR8 enzyme at $5I_{50}$, but **8** at $5I_{50}$ gave 74% inactivation of this enzyme; **8** was almost ineffective as an irreversible inhibitor of the L1210/FR8 enzyme at I_{50}.

The diverse effects on irreversible inhibition of the two tumor enzymes by substitution on the benzene ring containing the sulfonyl fluoride can be rationalized by consideration of the transition state involved in inactivation of the enzyme or involved in the enzyme-catalyzed hydrolysis of the sulfonyl fluoride. The following mechanisms had been previously proposed to



account for either enzyme inactivation or enzyme-catalyzed hydrolyis.[9] In order for the S–F bond to be broken by nucleophilic attack, assistance by a hydrogen bond from the F is required;[11] this assistance can occur with water, thus allowing an enzymic hydroxyl group to act as the nucleophile (**9**) that results in covalent bond formation (**10**). In contrast, the enzymic hydroxyl group can form a hydrogen bond with the F, then hydroxide ion from water can act as the nucleophile (**11**), thus resulting in enzyme-catalyzed hydrolysis of the SO_2F group (**12**). Which reaction occurs will depend upon the positioning of the SO_2F group within the enzyme–inhibitor complex, the difference between the position in **9** and **11** being about 3 Å. Thus, substitution on the benzene ring bearing the sulfonyl fluoride could move the positioning of sulfonyl fluoride within the complex to account for these results which are summarized as follows.

(1) Substitution caused loss of irreversible inhibition; this was seen with **1** *vs.* **3** on the L1210/FR8 enzyme and **2** *vs.* **7** on the Walker 256 enzyme. These results can be due to either the sulfonyl fluoride being moved sufficiently in complex **9** to give only complex **11** or the sulfonyl fluoride moving sufficiently within the complex that no reaction occurs; these two mechanisms cannot be distinguished kinetically by the methods used,[5] but can be distinguished by product isolation.[9] Since either mechanism is a chemotherapeutic dead end, no further work was considered worthwhile with compounds showing these results.

(2) The ratio of the rate of inactivation of the enzyme to the rate of enzyme-catalyzed hydrolysis was less favorable, but irreversible inhibition was still detectable; this was seen with **1** *vs.* **3**, **4**, or **5**, and **2** *vs.* **6** or **8** on the Walker 256 enzyme, as well as with **1** *vs.* **5** on the L1210/FR8 enzyme. These results can be explained on the basis that the substitution on the benzene ring shifted the position of the sulfonyl fluoride in the complex so that it was less favorable for conversion of **9** to **10** and more favorable for conversion of **11** to **12**.

(10) B. R. Baker and G. J. Lourens, *J. Med. Chem.* **11**, 39 (1968), paper CXII of this series.

(11) C. A. Bunton and J. H. Fendler, *J. Org. Chem.*, **31**, 2307 (1966).

(3) The opposite of 1 was seen in the case of **2** *vs.* **8** on the L1210/FR8 enzyme, that is, the parent **2** showed no inactivation of the enzyme, but substitution to give **8** was favorable; either **2** was completely in the form of the complex **11** leading to hydrolysis or the sulfonyl fluoride of **2** was not juxtaposed to an appropriate enzymic nucleophilic group within the enzyme-inhibitor complex (**9**).

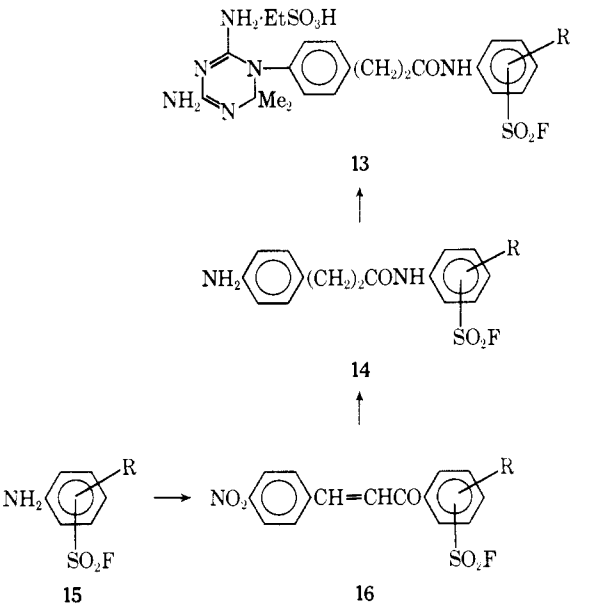
(4) The ratio of the rate of inactivation of the enzyme to the rate of enzyme-catalyzed hydrolysis was decreased more on the liver enzyme than the tumor enzyme from the same animal; this was seen with **1** *vs.* **4** on the L1210/FR8 and liver enzymes from the mouse. It is this change in ratio of rates to give less inactivation of the liver enzyme than the tumor enzyme that was sought at the outset of the problem as described in this and the previous two papers.[2,7] That the opposite effect can occur, that is, more rapid inactivation of the liver enzyme than the tumor enzyme, was seen with **1** in the rat system when an α-phenyl group was introduced on the bridge.[2]

(5) Substitution of a methoxyl group *ortho* to the sulfonyl fluoride group was more detrimental to the ratio of the rate of inactivation to the rate of enzyme-catalyzed hydrolysis than was an *o*-methyl group as seen with **4** *vs.* **5** and **7** *vs.* **8** on both tumor enzymes; this detrimental effect has also been seen with an appropriate sulfonyl fluoride type of irreversible inhibitors of trypsin substituted *ortho* to the sulfonyl fluoride by a methoxyl or ethoxyl group.[12] One possible explanation is that the polar ether oxygen has sufficient affinity for water that the water is held in complex for hydrolysis (**11**), thus being less favorable for the complex for inactivation (**9**) compared to the nonpolar *o*-methyl group).

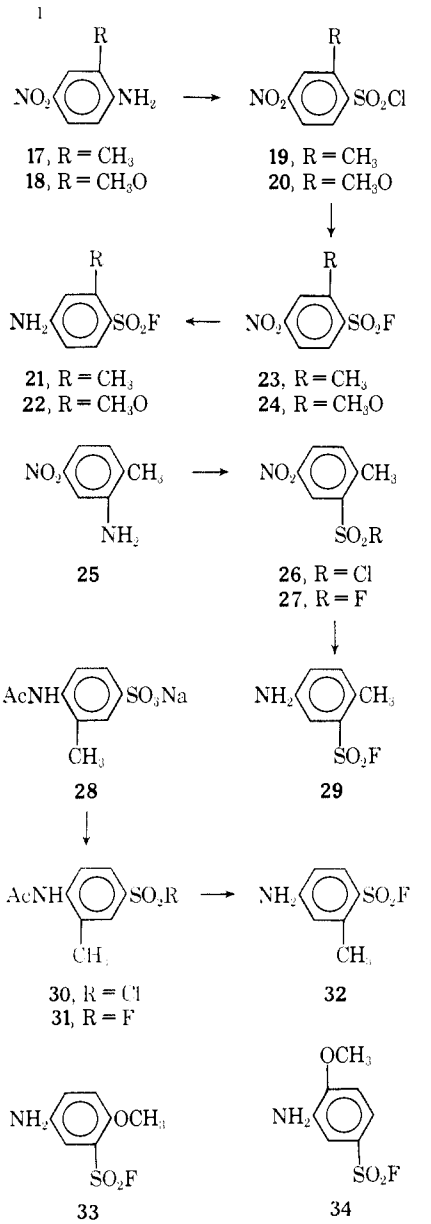
In summary, with an irreversible inhibitor, substitution on the benzene ring bearing a sulfonyl fluoride may have one of a gamut of effects; these vary from being completely unfavorable (**1** *vs.* **3**) for chemotherapy to being more favorable (**1** *vs.* **4**). Thus each type of inhibitor for each enzyme will have to be studied individually, except perhaps for the *o*-alkoxyl substitution where so far only detrimental results have been observed. Furthermore, appropriate substitution on an otherwise nonirreversible inhibitor may allow an irreversible inhibitor to emerge by changing the complex more akin to **9**, as seen with **2** *vs.* **8** in the L1210/FR8 system; other examples have been seen in this laboratory and will be published in future papers.

Chemistry.—The new candidate irreversible inhibitors (**3**–**8**) of dihydrofolic reductase in Table I can be generalized by structure **13**; these were synthesized by the general method developed earlier for **1**[5] from the appropriate aminobenzenesulfonyl fluoride (**15**) (Scheme I). The latter compounds were condensed with *p*-nitrocinnamoyl chloride in boiling toluene to give **16**. Reduction of the nitro group and double bond to **14** was accomplished with H_2 and Raney Ni; the resultant amines (**14**) were condensed with cyanoguanidine and acetone in the presence of ethanesulfonic acid to the candidate irreversible inhibitors (**13**), the so-called three-component method of Modest.[13]

Scheme I



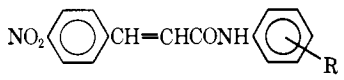
Scheme II



(12) B. R. Baker and E. Erickson, manuscript in preparation.
(13) E. J. Modest, *J. Org. Chem.*, **21**, 1 (1956).

TABLE II

PHYSICAL CONSTANTS OF



No.[a]	R	% yield	Mp, °C dec	Formula[b]
16a	2-Me-4-SO_2F	61	269–271	$C_{16}H_{13}FN_2O_5S$
16b	3-Me-4-SO_2F	68	>255	$C_{16}H_{13}FN_2O_5S$
16c	3-MeO-4-SO_2F	49[c]	255–257	$C_{16}H_{13}FN_2O_6S$
16d	2-MeO-5-SO_2F	54	291–293	$C_{16}H_{13}FN_2O_6S$
16e	4-MeO-3-SO_2F	40	260–262	$C_{16}H_{13}FN_2O_6S$
16f	4-Me-3-SO_2F	43[c]	261–263	$C_{16}H_{13}FN_2O_5S$

[a] Prepared by method A[2] and recrystallized from MeOEtOH. [b] All compounds showed a correct analysis for C, H, N. [c] The intermediate amine was not isolated; over-all yield from NO_2 compound.

The required sulfonyl fluorides (**15**) were prepared by one of two methods. The first method is represented by the synthesis of **21** (Scheme II). Diazotization of 2-methyl-4-nitroaniline followed by treatment with SO_2 in HOAc[14] gave the corresponding sulfonyl chloride (**19**) in 50% yield; although the melting point of **19** did not correspond to that in the literature for **19** prepared by an alternate method,[15] treatment with NH_4OH gave the same amide, mp 155–156°. Reaction of compound **19** with KF in H_2O[16] afforded the sulfonyl fluoride (**23**) in 46% yield of analytically pure material. Catalytic reduction of **23** with a Raney Ni catalyst afforded the desired 2-methylsulfanilyl fluoride (**21**) in 62% yield. Similarly, **18** was converted to **22** *via* **20** and **24**; 6-methylmetanilyl fluoride (**29**) was prepared from **25** *via* **26** and **27** in the same manner.

N-Acetyl-3-methylsulfanilyl chloride (**30**)[17] was prepared *via* the sodium sulfonate (**28**)[17] with $ClSO_3H$. Reaction of **30** with KF in dioxane–H_2O suspension[16] afforded **31** in 53% yield. Hydrolysis of **31** with 1:1 EtOH–12 *N* HCl gave the desired 3-methylsulfanilyl fluoride (**32**) in 73% yield.

Of the remaining two sulfonyl fluorides needed for this study, **34** was commerically available and **33** has been previously described.[16]

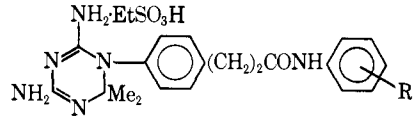
Experimental Section[18]

Methods A and B were the same as previously described;[2] compounds prepared by these methods are listed in Tables II and III.

2-Methyl-4-nitrobenzenesulfonyl Chloride (19) (Method C).—To a stirred mixture of 38 g (0.25 mole) of **17** and 100 ml of 12 *N* HCl cooled in an ice–salt bath was added dropwise a solution of 19 g (0.28 mole) of $NaNO_2$ in 30 ml of H_2O at such a rate that the temperature was −5 to 0°. This mixture was added slowly to 200 ml of HOAc saturated with SO_2 that contained 10 g of H_2O and 10 g of $CuCl_2 \cdot 2H_2O$; the temperature was maintained at about 25° and N_2 was evolved. After being stirred an additional 10 min, the mixture was diluted with 500 ml of ice water, then filtered. The product was washed [H_2O, then petroleum ether (bp 30–60°)]. Recrystallization from petroleum ether (bp 60–110°) gave 28 g (51%) of light yellow crystals: mp 69–70°; ν_{max} 1395, 1180 (SO_2), 1340 cm^{-1} (NO_2); lit.[15] mp 106°. Reaction with NH_4OH gave the sulfonamide, mp 155–156°, lit.[15] mp 157°. For other sulfonyl chloride prepared by this method see Table IV.

TABLE III

PHYSICAL CONSTANTS OF

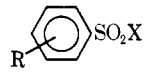


No.[a]	R	% yield[b]	Mp, °C dec	Formula[c]
3	2-Me-4-SO_2F	28[d]	208–210	$C_{21}H_{25}FN_6O_8S \cdot C_2H_6SO_3H$
4	3-Me-4-SO_2F	47[d]	219–220	$C_{21}H_{25}FN_6O_8S \cdot C_2H_6SO_3H$[e]
5	3-MeO-4-SO_2F	53[f]	199–200	$C_{21}H_{25}FN_6O_4S \cdot C_2H_6SO_3H$
6	2-MeO-5-SO_2F	33[f]	210–211	$C_{21}H_{25}FN_6O_4S \cdot C_2H_6SO_3H$
7	4-MeO-3-SO_2F	41[f]	235–237	$C_{21}H_{25}FN_6O_4S \cdot C_2H_6SO_3H$
8	4-Me-3-SO_2F	60[d]	221–223	$C_{21}H_{25}FN_6O_8S \cdot C_2H_6SO_3H$

[a] Prepared by method B;[2] each had an ir band at 1395–1405 cm^{-1} characteristic of SO_2F. [b] Analytically pure product. [c] Each compound showed a correct analysis for C, H, F unless otherwise indicated. [d] Recrystallized from *i*-PrOH–H_2O. [e] Showed a correct analysis for C, H, N. [f] Recrystallized from EtOH–H_2O.

TABLE IV

PHYSICAL CONSTANTS OF



No.	X	R	Method	% yield	Mp, °C	Formula[a]
19	Cl	2-Me-4-NO_2	C	51[b]	69–70[c]	$C_7H_6ClNO_4S$
20	Cl	2-MeO-4-NO_2	C	64[d]	97–98	$C_7H_6ClNO_5S$
21	F	2-Me-4-NH_2	G	62[e]	81–82	$C_7H_8FNO_2S$
22	F	2-MeO-4-NH_2	G		[f]	$C_7H_8FNO_3S$
23	F	2-Me-4-NO_2	D	46[g]	54–56	$C_7H_5FNO_4S$
24	F	2-MeO-4-NO_2	E	38[h]	108–109	$C_7H_6FNO_5S$
26	Cl	2-Me-5-NO_2	C	57[d]	43–44[i]	$C_7H_6ClNO_4S$
27	F	2-Me-5-NO_2	E	48[g]	48–49[j]	$C_7H_5FNO_4S$
29	F	2-Me-5-NH_2	G		[f]	$C_7H_8FNO_2S$
31	F	3-Me-4-AcNH	F	53[e]	186–187	$C_9H_{10}FNO_3S$
32	F	3-Me-4-NH_2	H	73[e]	108–109	$C_7H_8FNO_2S$

[a] All compounds showed a correct analysis for C, H, N. [b] Recrystallized from petroleum ether (bp 60–110°). [c] Lit.[15] mp 106°, prepared by different route; see Experimental Section. [d] Recrystallized from C_6H_6–petroleum ether (bp 60–110°). [e] Recrystallized from EtOH–H_2O. [f] Converted to **16** (Table II) without purification. [g] Recrystallized from MeOH–H_2O. [h] Recrystallized from EtOH. [i] Mp 43–45°, prepared by an alternate method by E. F. Elslager, M. Maienthal, and D. R. Smith, *J. Org. Chem.*, **21**, 1528 (1956). [j] Mp 57–58°, prepared by an alternate method by W. Steinkopf, *J. Prakt. Chem.*, **117**, 1 (1929).

2-Methyl-4-nitrobenzenesulfonyl Fluoride (23) (Method D).—To a stirred solution of 8.7 g (0.15 mole) of KF in 9 ml of H_2O in a bath at 125–130° was added 23.6 g (0.1 mole) of **19** in portions over a period of 10 min. After being stirred an additional 50 min in the bath, 100 ml of hot H_2O was added. The water was decanted from the thick oil which was extracted twice with two 100-ml portions of hot H_2O. Trituration of the oil with cold H_2O gave a solid which was collected on a filter. Two recrystallizations from MeOH–H_2O gave 10 g (46%) of light yellow crystals, mp 54–56°. See Table IV for additional compounds prepared by this method.

2-Methoxy-4-nitrobenzenesulfonyl Fluoride (24) (Method E).—A mixture of 25.1 g (0.10 mole) of **20**, 14.5 g (0.25 mole) of KF, and 50 ml of DMF was stirred at 100° for 45 min, then poured into 900 ml of cold H_2O. The product was collected on a filter, then washed with H_2O and recrystallized from EtOH; yield 7.0 g (31%), mp 108–109°. See Table IV for additional data.

(14) (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), paper XC of this series.

(15) An alternate synthesis of **19**, mp 106°, has been reported by P. Pfeiffer and H. Jäger, *Ber.*, **75B**, 1885 (1942); treatment with ammonia was reported to give an amide, mp 157°.

(16) A. H. deCat and R. K. vanPoucke, *J. Org. Chem.*, **28**, 3426 (1963), and references therein.

(17) This compound (**30**) was previously prepared from **28** and PCl_5; see L. Szabo, *Bull. Soc. Chim. France*, 771 (1953).

(18) Melting points were taken in capillary tubes on a Mel-Temp block and are uncorrected. Each analytical sample had uv and ir spectra compatible with its assigned structure and gave combustion values for C, H, and N or F within 0.4% of theory.

N-Acetyl-3-methylsulfanilyl Fluoride (31) (Method F).—To a stirred mixture of 24.7 g (0.1 mole) of **30** (prepared from **28** with $ClSO_3H$[17]) and 30 ml of dioxane in a bath at 120–125° was added a solution of 8.7 g (1.50 moles) of KF in 9 ml of H_2O over a period of about 2 min. The mixture was stirred under reflux for 30 min, then diluted with 100 ml of H_2O. The product was collected on a filter, washed with H_2O, and recrystallized from EtOH–H_2O; yield 12.2 g (53%), mp 186–187°. See Table IV for additional data.

2-Methylsulfanilyl Fluoride (21) (Method G).—A mixture of 2.19 g (10 moles) of **23**, 100 ml of EtOH, and 5 ml of Raney Ni was shaken with H_2 at 2–3 atm for 30 min when reduction was complete. The filtered solution was evaporated *in vacuo* and the residue was recrystallized from EtOH–H_2O; yield 1.17 g (62%) of white crystals, mp 81–82°. See Table IV for additional data.

3-Methylsulfanilyl Fluoride (32) (Method H). To a stirred mixture of 10.4 g (45 mmoles) of **31** and 40 ml of EtOH heated under reflux was added 40 ml of 12 *N* HCl. After 45 min the cooled mixture was carefully poured into a mixture of 41 g of $NaHCO_3$ and 400 ml of ice–H_2O. The product was collected on a filter, washed with H_2O, and recrystallized from EtOH–H_2O; yield 6.18 g (73%), mp 108–109°. See Table IV for additional data.

Irreversible Enzyme Inhibitors. CXXX.[1,2] Cytosine Nucleoside Deaminase. 1. Hydrophobic Bonding with Monosubstituted Uracils

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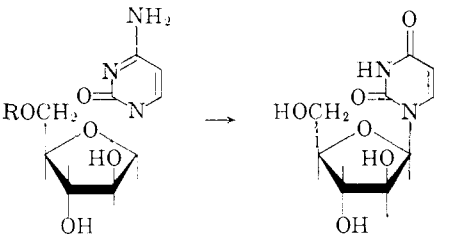
Thirty-eight derivatives of uracil and five derivatives of thymidine have been investigated as inhibitors of the cytosine nucleoside deaminase from *E. coli* B. Hydrophobic bonding to the enzyme could be detected with 1-substituted uracils, 6-substituted uracils, and 5′-substituted thymidines. Thymidine itself is a weak inhibitor of this enzyme and was employed as the base-line standard compound. The best inhibitors of the 1-substituted uracil class were 1-phenylbutyl- (**15**), 1-phenylamyl- (**16**), and 1-phenoxypropyluracil (**18**) which were complexed to the enzyme about as effectively as thymidine. The best inhibitors of the 6-substituted uracil class were 6-benzyl- (**25**) and 6-phenylpropyluracil (**26**) which were also complexed as well as thymidine. In the thymidine series, the best inhibitors were the 5′-O-carbophenoxy (**37**) and 5-O-(N-phenylpropylcarbamoyl) (**38**) derivatives which were 3–4-fold more effective than thymidine; in contrast, 5′-O-carbamoylthymidine (**35**) was less effective than thymidine. The possible biological role of this hydrophobic bonding region adjacent to the active site of cytosine nucleoside deaminase is discussed.

That β-D-arabinofuranosyl analogs of nucleosides could show cytotoxic action was first demonstrated by Pizer and Cohen[3] with **1**-β-D-arabinofuranosyluracil (*ara*-U) (**3**). Other arabinosyl nucleosides, such as 9-β-D-arabinofuranosyladenine[4] and **1**-β-D-arabinofuranosylcytidine (*ara*-C) (**1**),[5] with cytotoxic activity soon followed. Since *ara*-C (**1**) showed sufficient selective cytoxicity to be effective against certain animal tumors,[5a] a great deal of research at both the preclinical[6,7] and clinical levels[8] was published. The cytotoxicity of *ara*-C (**1**) is due to intracellular conversion to the nucleotide (**2**); the latter is most probably cytotoxic due to inhibition of the conversion of cytidylate to 2′-deoxycytidylate[6,9] at the triphosphate level, and hence the inhibition of DNA synthesis.

ara-C (**1**) is rapidly deaminated *in vivo* by cytosine nucleoside deaminase to *ara*-U (**3**),[8d,10] the latter being relatively nontoxic to mammalian cells.[6,9] Thus, one possible mechanism for selective action of *ara*-C (**1**) could be due to the poor ability of a susceptible cell line to deaminate *ara*-C (**1**) to *ara*-U (**2**) compared to normal tissues. Cytosine nucleoside deaminase normally uses cytidine or 2′-deoxycytidine as a substrate, the order of velocity with the *E. coli* B enzyme[3] being 2′-deoxycytidine > cytidine > *ara*-C; similarly, cytidine and 2′-deoxycytidine were more rapidly deaminated by the enzyme from human liver than *ara*-C.[11] The enzyme can be inhibited by classical-type antimetabolites,[12] that is, those having essentially isosteric changes in structure compared to the substrate. The most effective compound found was N-hydroxy-5-methyl-2′-deoxycytidine[12] which was complexed to the enzyme about tenfold better than *ara*-C; the enzyme can also be inhibited by thymidine, 2′-deoxyuridine, and uridine, but these are complexed to the enzyme less



1, $R = H$
2, $R = PO(OH)_2$
3

(1) This work was supported in part by Grant No. CA-08695 from the National Cancer Institute, U. S. Public Health Service.

(2) For the previous paper in this series see B. R. Baker and G. J. Loevers, *J. Med. Chem.*, **11**, 677 (1968).

(3) L. I. Pizer and S. S. Cohen, *J. Biol. Chem.*, **235**, 2387 (1960).

(4) W. W. Lee, A. Benitez, L. Goodman, and B. R. Baker, *J. Am. Chem. Soc.*, **82**, 2648 (1960).

(5) (a) J. S. Evans, E. A. Musser, G. D. Mengel, K. R. Forsblad, and J. H. Hunter, *Proc. Soc. Exptl. Biol. Med.*, **106**, 350 (1961); (b) J. H. Hunter, U. S. Patent 3,183,226 (1963).

(6) (a) R. L. Dixon and R. H. Adamson, *Cancer Chemotherapy Rept.*, **48**, 11 (1965); (b) I. Wodinsky and C. J. Kensler, *ibid.*, **47**, 65 (1965); (c) I. Kline, J. M. Venditti, D. D. Tyrer, and A. Goldin, *Proc. Am. Assoc. Cancer Res.*, **6**, 36 (1965).

(7) For a review see S. S. Cohen, *Progr. Nucleic Acid Res.*, **5**, 1 (1965).

(8) (a) R. W. Talley and V. K. Vaitkevicius, *Blood*, **21**, 352 (1963); (b) J. B. Block, W. Bell, J. Whang, and P. P. Carbone, *Proc. Am. Assoc. Cancer Res.*, **6**, 6 (1965); (c) R. W. Carey and R. R. Ellison, *Clin. Res.*, **13**, 337 (1965); (d) R. W. Talley, R. M. O'Bryan, W. G. Tucker, and R. V. Loo, *Cancer*, **20**, 809 (1967).

(9) M. Y. Chu and G. A. Fischer, *Biochem. Pharmacol.*, **11**, 423 (1962).

(10) G. W. Camiener and C. G. Smith, *ibid.*, **14**, 1405 (1965).

(11) G. W. Camiener, *ibid.*, **16**, 1691 (1967).

(12) (a) G. W. Camiener, *Proc. Am. Assoc. Cancer Res.*, **8**, 9 (1967); (b) E. A. Falco and J. J. Fox, *J. Med. Chem.*, **11**, 148 (1968), and references therein.