Irreversible Enzyme Inhibitors. CXXIII.[1,2] Candidate Irreversible Inhibitors of Guanine Deaminase and Xanthine Oxidase Derived from 9-Phenylguanine Substituted with a Terminal Sulfonyl Fluoride

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9-[*p*-(*p*-Fluorosulfonylbenzamido)phenyl]guanine (**2**) at 0.7×10^{-6} *M* was an excellent active-site-directed irreversible inhibitor of xanthine oxidase, causing 85% inactivation in 1 min at 37° and 97% in 24 min; in contrast, **2** fails to inactivate guanine deaminase at 21×10^{-6} *M*. When the fluorosulfonylbenzamido moiety was moved to the *meta* position (**3**, **4**), better reversible inhibitors emerged, but **3** and **4** showed no irreversible inhibition of either xanthine oxidase or guanine deaminase.

9-Phenylguanine is a good inhibitor of both guanine deaminase[3,4] and xanthine oxidase.[5,6] The 9-phenyl group is hydrophobically bonded[7] to the enzyme, being 28 and 140 times more effective as an inhibitor, respectively, than 9-methylguanine. Insertion of the *p*-bromoacetamido group to give **1** resulted in an active-site-directed irreversible inhibitor[8] of xanthine oxidase, but **1** failed to inactivate guanine deaminase;[9] thus the bromomethyl group of **1** was properly juxtaposed to an appropriate nucleophilic group on the enzyme surface of xanthine oxidase that allowed covalent bond formation by neighboring-group reaction, but such was not the case with guanine deaminase. There are three major reasons why a candidate irreversible inhibitor such as **1** may have failed to inactivate guanine deaminase: (a) the leaving group of the inhibitor may lie in a hydrophobic bonding region of the enzyme; (b) the juxtaposed nucleophilic group on the enzyme may not have the chemical ability to interact with the leaving group on the inhibitor; and (c) the leaving group may not be in contact with the enzyme surface.

A study was performed on the dimensions of the hydrophobic bonding region of guanine deaminase; the ability of substituted 9-phenylguanines to bind to the enzyme was used for mapping purposes. The same compounds could be used for mapping the hydrophobic bonding region of xanthine oxidase.[3] The striking similarity of the hydrophobic bonding region of these two enzymes was remarkable and may have significance in the evolution and coding of these two enzymes. The fact that the bromomethyl group of **1** did not reside in a hydrophobic bonding region of either enzyme suggested that a different leaving group on the inhibitor be investigated. The recent successes with terminal sulfonyl fluorides for inactivation of dihydrofolic reductase,[10] chymotrypsin,[11] and trypsin[12] by the active-site-directed mechanism[8] encouraged the study of 9-phenylguanines bearing a terminal sulfonyl fluoride as candidate irreversible inhibitors of guanine deaminase and xanthine oxidase. The results with four such sulfonyl fluorides (**2**–**5**) are the subject of this paper.

Enzyme Results.—As a result of the earlier studies on the hydrophobic regions of the two enzymes,[3] **1**–**5** were probably not complexed to the enzyme in the conformations indicated in Figure 1. No evidence was available on whether the guanine complexed with the pyrimidine moiety to the left, as indicated, or the right by a "flipped over" purine; however, strong evidence was accumulated to show that the 9-phenyl group was coplanar to the guanine when complexed to the two enzymes. Further evidence was presented that the sulfonyl fluoride groups of **2**–**5** would not be in a hydrophobic bonding region when complexed to the enzyme and therefore were good candidates for active-site-directed irreversible inhibitors.

The *p*-fluorosulfonylbenzamido group (**2**) on the *para* position of 9-phenylguanine (**6**) led to little change in reversible binding to the two enzymes; that this lack of change was due to a repulsion of the carboxamide group with a compensating polar binding of the fluorosulfonylphenyl groups was previously discussed.[3] When **2** at its I_{50} concentration (0.7 μM) was incubated with xanthine oxidase at 37° and pH 7.4, rapid inactivation occurred; 85% inactivation occurred in 1 min and 97% in 24 min (Table I). That this inactivation proceeded through a reversible complex by the active-site-directed mechanism[8] was supported by the observation that *p*-acetamidobenzenesulfonyl fluoride at 70 μM failed to show any inactivation of xanthine oxidase in 60 min at 37°. In contrast to the results with xanthine oxidase, **2** at 21 μM failed to inactivate guanine deaminase. These results again show that two enzymes that are closely related in their ability to bind reversible inhibitors can be readily inhibited

(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 W. F. Wood, *J. Med. Chem.*, **11**, 644 (1968).

(3) B. R. Baker and D. V. Santi, *ibid.*, **10**, 62 (1967), paper LXXIV of this series.

(4) For the possible chemotherapeutic utility of tissue-selective irreversible inhibitors of guanine deaminase see B. R. Baker, *ibid.*, **10**, 59 (1967), paper LXXIII of this series.

(5) B. R. Baker, *J. Pharm. Sci.*, **56**, 959 (1967), paper XCIII of this series.

(6) For the possible chemotherapeutic utility of tissue-selective irreversible inhibitors of xanthine oxidase see B. R. Baker and J. L. Hendrickson, *ibid.*, **56**, 955 (1967), paper XCII of this series.

(7) B. R. Baker and W. F. Wood, *J. Med. Chem.*, **10**, 1101 (1967), paper CII of this series.

(8) 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.

(9) B. R. Baker and W. F. Wood, *J. Med. Chem.*, **10**, 1106 (1967), paper III of this series.

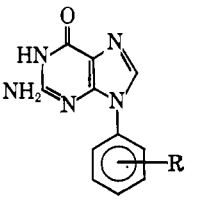
(10) (a) B. R. Baker and G. J. Lourens, *ibid.*, **10**, 1113 (1967), paper CV of this series; (b) B. R. Baker and G. J. Lourens, *ibid.*, **11**, 39 (1968), paper CXII of this series; (c) B. R. Baker and R. B. Meyer, Jr., *ibid.*, **11**, 489 (1968), paper CXIX of this series; (d) B. R. Baker and P. C. Hsiung, *ibid.*, **11**, 495 (1968), paper CXX of this series.

(11) (a) B. R. Baker and J. A. Duribon, *ibid.*, **11**, 233 (1968), paper CXIII of this series; (b) *ibid.*, **11**, 241 (1968), paper CXIV of this series.

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

TABLE I

INHIBITION[a] OF XANTHINE OXIDASE AND GUANINE DEAMINASE BY



		Xanthine oxidase					Guanine deaminase				
		Reversible[b]		Irreversible[c]			Reversible[d]		Irreversible[e]		
No.	R	I_{50},[f] μM	$([S]/[I])_{0.5}$[g]	Concn, μM	Time, min	% inactn	I_{50},[f] μM	$([S]/[I])_{0.5}$[g]	Concn, μM	Time, min	% inactn
1[h]	*p*-$NHCOCH_2Br$	1.9	4.20	5	40	50[i,j]	13	1.0	16	120	0
				5	120	95					
2	*p*-$NHCOC_6H_4SO_2F$-*p*	0.70	12	0.7	1,24	85, 97[j]	18	0.74	21	120	0
3	*m*-$NHCOC_6H_4SO_2F$-*p*	0.051	160	0.12	60	0	0.092	140	0.20	90	0
4	*m*-$NHCOC_6H_4SO_2F$-*m*	0.11	74	0.50	60	0	0.12	110	0.60	60	0
5	*m*-$NHCONHC_6H_4SO_2F$-*m*	1.8	4.5	9.0	60	0	0.10	120	0.50	60	0
6	H	0.41[h]	20				10[h]	1.3			

[a] The technical assistance of Pepper Caseria and Maureen Baker with these assays is acknowledged. [b] Commercial xanthine oxidase from bovine milk was assayed with 8.1 μM hypoxanthine in Tris buffer (pH 7.4) containing 10% DMSO as previously described.[6] [c] Inactivation of xanthine oxidase was performed in pH 7.4 Tris buffer containing 5% DMSO at 37° as previously described by B. R. Baker and J. Kozma, *J. Med. Chem.*, **10**, 682 (1967). [d] Commercial guanine deaminase from rabbit liver was assayed with 13.3 μM guanine in pH 7.4 Tris buffer containing 3% DMSO as previously described by B. R. Baker, *ibid.*, **10**, 59 (1967). [e] Inactivation of guanine deaminase in pH 7.4 Tris buffer containing 5% DMSO at 37° was performed as previously described.[9] [f] Concentration necessary for 50% inhibition. [g] Ratio of concentration of substrate to inhibitor required for 50% reversible inhibition. [h] Data from ref 9. [i] Half-time of reaction. [j] From time study plot.

differentially by appropriate irreversible inhibitors.[8]

The sulfonyl fluorides (**3**, **4**) derived by *meta* substitution on 9-phenylguanine (**6**) were much better reversible inhibitors than **1**, **2**, and **6** primarily due to donor–acceptor binding of the carboxamide oxygen.[2] For reasons previously discussed,[2] **5** was a poorer reversible inhibitor of xanthine oxidase than **6**. However, **3**–**5** failed to show irreversible inhibitors of either enzyme. These negative results are not too surprising in view of the fact that the ability of a sulfonyl fluoride for inactivating other enzymes such as dihydrofolic reductase[10] is extremely position sensitive; for example, moving of a sulfonyl fluoride group from *meta* to *para* on a benzene ring was sufficient to give an all or nothing effect on irreversible inhibition.[10b] As a result of these studies and the earlier studies[2] on the mapping of the hydrophobic bonding region of guanine deaminase,[2] there are numerous other possibilities for candidate irreversible inhibitors of this enzyme; such studies are being vigorously pursued to further differentiate guanine deaminase and xanthine oxidase as well as to differentially inhibit each of these enzymes from different sources such as tumor and liver of the same animal.

Chemistry.—Two routes were investigated for the synthesis of 9-phenylguanine bearing a terminal sulfonyl fluoride group. Although the sulfonyl fluoride group is reasonably stable to boiling water and alcohol,[13] the sulfonyl fluoride group did not withstand the rigors of a total synthesis from 2-amino-6-chloro-5-phenylazo-4-pyrimidinol and the appropriate arylamine;[7,14] such a sequence involves a zinc–formic acid reduction and a formamide–formic acid ring closure at the boiling point.[7]

The candidate irreversible inhibitors of type **9** were synthesized from the appropriate 9-(aminophenyl)-guanine (**7**) by acylation with *m*- or *p*-fluorosulfonylbenzoyl chloride in DMF in the presence of Et_3N as an acid acceptor. The urea-linked sulfonyl fluoride (**5**) was synthesized from **7** by reaction with *m*-fluorosulfonylphenyl isocyanate in DMF.

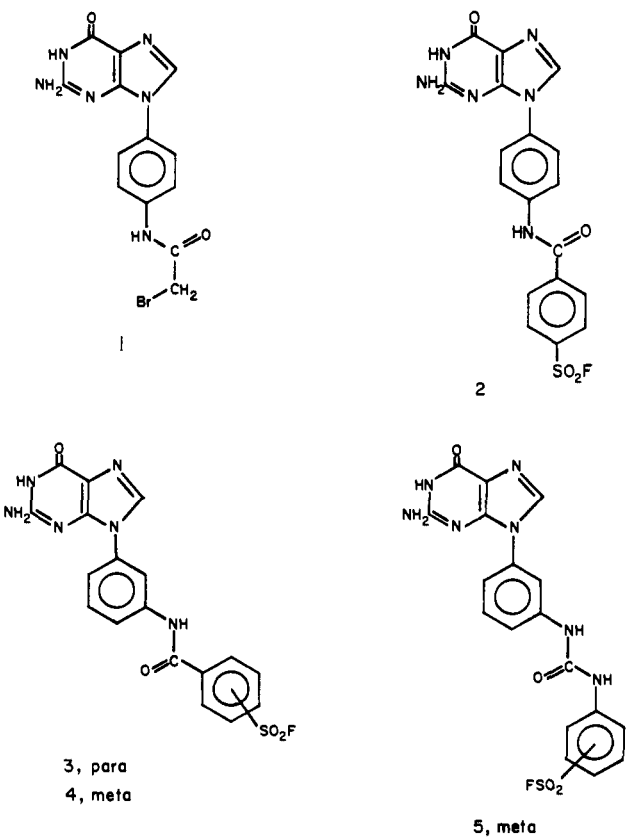
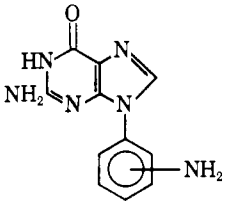


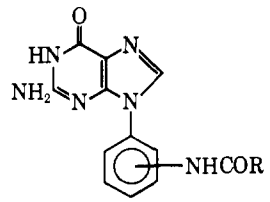
Figure 1.—Binding conformations of candidate irreversible inhibitors when complexed to guanine deaminase and xanthine oxidase.

(13) For a discussion of the chemistry of sulfonyl fluorides see ref 10a.

(14) (a) H. C. Koppel, D. E. O'Brien, and R. K. Robins, *J. Am. Chem. Soc.*, **81**, 3046 (1959); (b) C. W. Noell and R. K. Robins *J. Med. Pharm. Chem* **5**, 558 (1962).



7, *meta*
8, *para*



5, R = *m*-$NHC_6H_4SO_2F$-*m*
9, R = $C_6H_5SO_2F$

Experimental Section[15]

9-[*m*-(*m*-Fluorosulfonylphenylureido)phenyl]guanine (5).—To a solution of 100 mg (0.41 mmole) of **7**[7] in 5 ml of DMF was added 92 mg (0.45 mmole) of *m*-fluorosulfonylphenyl isocyanate. After being stirred 1 hr at ambient temperature, the mixture was added to 50 ml of 0.2 *N* HCl. The product was collected on a filter and washed with H_2O. Two recrystallizations from MeOEtOH–H_2O gave nearly white crystals with a negative Bratton-Marshall test for aromatic amine;[16] yield 50 mg (28%); λ_{max} (mμ) pH 1, 261; pH 13, 261. *Anal.* ($C_{20}H_{14}FN_7O_5S$) C, H, F.

9-[*p*-(*p*-Fluorosulfonylbenzamido)phenyl]guanine (2).—To a solution of **8**·HCl (0.36 mmole) in 5 ml of DMF containing 73 mg (0.72 mmole) of Et_3N was added 115 mg (0.54 mmole) of *p*-fluorosulfonylbenzoyl chloride. After being stirred 1 hr at ambient temperature, the solution gave a negative Bratton-Marshall test for aromatic amine.[16] The mixture was added to 50 ml of H_2O containing 61 mg (0.72 mmole) of $NaHCO_3$. The product was collected and washed with water, then dissolved in 5 ml of warm DMF. Addition of H_2O gave 70 mg (45%) of product as a nearly white powder: λ_{max} (mμ) pH 1, 228, 282; pH 13, 272. *Anal.* ($C_{18}H_{13}FN_6O_4S$) C, H, N.

9-[*m*-(*p*-Fluorosulfonylbenzamido)phenyl]guanine (3) was prepared in 44% yield as described for **2**: λ_{max} (mμ) pH 1, 230, 280; pH 13, 271. *Anal.* ($C_{18}H_{13}FN_6O_4S$) C, H, N.

9-[*p*-(*m*-Fluorosulfonylbenzamido)phenyl]guanine (4) was prepared in 29% yield as described for **2** except the product was recrystallized from MeOEtOH–H_2O; λ_{max} (mμ) pH 1, 267; pH 13, 270. *Anal.* ($C_{18}H_{13}FN_6O_4S\cdot 0.5H_2O$) C, H, F.

(15) All analytical samples gave combustion values within 0.3% of theoretical; each moved as a single spot on tlc with EtOH–$CHCl_3$ (3:5) on Brinkmann silica gel GF when detected under uv light and each had ir and uv spectra compatible with their assigned structures. None showed a melting point below 300°.

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

Irreversible Enzyme Inhibitors. CXXIV.[1,2] Active-Site-Directed Irreversible Inhibitors of Xanthine Oxidase Derived from 2- (and 8-) Benzylthiopurines Bearing a Terminal Sulfonyl Fluoride

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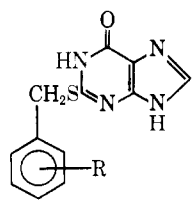
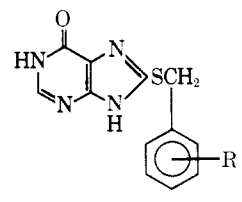
Twelve candidate irreversible inhibitors of xanthine oxidase have been synthesized and evaluated that bear a terminal sulfonyl fluoride for covalent bond formation within the inhibitor–enzyme complex. Four candidates were derived from 2-benzylthiohypoxanthine, five from 8-benzylthiohypoxanthine, and three from 8-benzylthioadenine where the sulfonyl fluoride was bridged to the phenyl moiety with a benzamido or phenylureido moiety. Of these twelve candidate irreversible inhibitors seven showed varying degrees of irreversible inhibition of xanthine oxidase and five showed none. The two best irreversible inhibitors were 8-[*m*-(*p*-fluorosulfonylbenzamido)benzylthio]hypoxanthine (**14**) and 8-[*m*-(*m*-fluorosulfonylbenzamido)benzylthio]adenine (**16**). Both **14** and **16** were reversibly complexed to xanthine oxidase about 20-fold better than the substrate. At 4×10^{-7} *M*, **16** inactivated 88% of the enzyme in 15 min with a half-life of 2 min; at the same concentration, **14** was fourfold slower since it inactivated xanthine oxidase with a half-life of 7 min.

In a previous paper of this series, 2-benzylthiohypoxanthine (**1**) and its 8 isomer (**2**) were shown to be good reversible inhibitors of xanthine oxidase, being complexed tenfold and eightfold better, respectively, than the substrate, hypoxanthine.[3] Of the four candidate active-site-directed irreversible inhibitors[4] (**3**, **7**, **9**, **13**), bearing a bromoacetamido group that were prepared in a subsequent study,[5] only **13** showed irreversible inhibition of xanthine oxidase; at a concentration of 1.5 μM ($2I_{50}$), **13** inactivated xanthine oxidase with a half-life of 50 min. With a related problem in this laboratory, it was discovered that the terminal sulfonyl fluoride group was much superior to the bromoacetamido group for effective active-site-directed irreversible inhibitors operating by the exo mechanism.[6] For example, 4,6-diamino-1,2-dihydro-1-phenyl-*s*-triazines bridged from the phenyl to a terminal sulfonyl fluoride





1, R = H
3, R = *o*-$NHCOCH_2Br$
7, R = *m*-$NHCOCH_2Br$
8, R = *m*-$NHCOC_6H_4SO_2F$-*p*

2, R = H
9, R = *o*-$NHCOCH_2Br$
13, R = *m*-$NHCOCH_2Br$
14, R = *m*-$NHCOC_6H_4SO_2F$-*p*

(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 W. F. Wood, *J. Med. Chem.*, **11**, 650 (1968).

(3) B. R. Baker and J. L. Hendrickson, *J. Pharm. Sci.*, **56**, 955 (1967), paper XCII of this series. In this paper is also discussed the chemotherapeutic utility for tissue-specific inhibitors of xanthine oxidase.

(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 J. Kozma, *J. Med. Chem.*, **10**, 682 (1967), paper XCV of this series.

(6) The exo mechanism is defined as formation of a covalent bond within an inhibitor–enzyme complex that occurs outside the active site; in contrast, the endo mechanism is defined as covalent bond formation within the active site.[7] The active site, in turn, is defined as containing those amino acid residues responsible for complex formation with the substrate and those amino acid residues responsible for the catalytic conversion of substrate to product.[8]

(7) See ref 5, Chapter I.

(8) See ref 5, p 188.