9-(3-Amino-4-methoxyphenyl)guanine hydrochloride (28) was prepared in 31% yield from 3-acetamido-4-methoxyaniline as described for 9-(*p*-aminophenyl)guanine;[6] mp >300°; λ_{max} (pH 1) 232, 260 (infl), (pH 13) 247, 268 mμ (infl). *Anal.* ($C_{12}H_{13}ClN_6O_2 \cdot H_2O$) C, H, N. For further transformations, the hydrochloride was converted first to the free base.[5]

O-(*p*-Nitrophenyl) N-(3-fluorosulfonyl-4-methylphenyl)carbamate (29) was prepared in 62% yield, mp 162–164°, by the previously described general method[12] from 2-methyl-5-aminobenzenesulfonyl fluoride[15] and *p*-nitrophenyl chloroformate. *Anal.* ($C_{14}H_{13}FN_2O_6S$) C, H, F.

Enzyme Assays.—The assay of the inactivation of xanthine oxidase by measurement of the rate of conversion of 8.1 μ*M* hypoxanthine or uric acid has been previously described.[10] Assay of the enzyme with 2,6-dichlorophenolindophenol (DCPI) was performed as follows.

Bovine milk xanthine oxidase (40 units/ml; 1 unit converts 1 μmole of xanthine or uric acid in 1 min) was purchased. Buffer was 0.05 *M* Tris (pH 7.4). Bulk enzyme was diluted 1:400 with buffer for assay. DCPI was dissolved in H_2O at 0.1 mg/ml. In a tube labeled I_{60} were placed 0.90 ml of diluted enzyme and 0.90 ml of buffer, then 0.45 ml was removed into each of three tubes labeled I_∞, C_∞, and C_{60}. The C_0 and I_0 tubes were placed in an ice bath until ready for assay. To the C_∞ tube was added 50 μl of DMSO and to the I_{60} tube was added 50 μl of DMSO containing inhibitor; these were incubated at 37° for 1 hr, then cooled in an ice bath until ready for assay.

In a 1-ml glass cuvette were placed 0.75 ml of buffer, 50 μl of 320 μ*M* hypoxanthine (cuvette concentration = 16 μ*M*), and 100 μl of DCPI. A base line was run at 600 mμ then 100 μl of C_{60} or I_{60} aliquot was added and the decrease in OD was observed on a Gilford recording spectrophotometer. For the C_0 tube, 50 μl of DMSO was added prior to assay; similarly, 50 μl of DMSO containing inhibitor was added to the I_0 tube just before assay. The C_0 tube under these conditions shows an OD change of 0.01–0.015 unit/min. The OD change is linear with enzyme concentration and therefore suitable for determining the extent of inactivation of the enzyme. For a time study, a larger volume of I_{60} solution is prepared and aliquots are withdrawn at appropriate intervals, then quenched in an ice bath until ready for assay.

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

Irreversible Enzyme Inhibitors. CXLVII.[1,2] Candidate Active-Site-Directed Irreversible Inhibitors of Xanthine Oxidase Derived from 9-(*p*-Acylamidoalkoxyphenyl)guanines Bearing a Terminal Sulfonyl Fluoride

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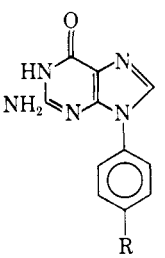
Twelve candidate irreversible inhibitors of xanthine oxidase were synthesized from 9-(*p*-aminoethoxyphenyl)guanine (**23a**) and 9-(*p*-aminopropoxyphenyl)guanine (**23b**) by connection to a benzenesulfonyl fluoride with an amide (**24**) or urea (**25**) bridge. Reversible inhibition results indicated the benzenesulfonyl fluoride moiety of the inhibitors was in contact with the enzyme surface within the enzyme–inhibitor complex; nevertheless, none of the twelve compounds was an irreversible inhibitor.

9-(*p*-Ethoxyphenyl)guanine (**1**) is an excellent reversible inhibitor of xanthine oxidase, being complexed 16-fold better than the substrate, hypoxanthine; the phenyl group interacts with the enzyme by hydrophobic bonding.[3] As a result of a study on the nature and dimensions of the hydrophobic bonding region,[4] it was shown that the phenoxypropyl derivative (**2**) was as good an inhibitor as the ethoxy derivative (**1**), indicating a bulk tolerance for large groups on the ethoxy moiety. Furthermore, the active-site-directed irreversible inhibitor[5a] of xanthine oxidase[5b] (**4**)[2,6] was believed to complex to the enzyme in a different manner than **1** and **2**.[4] Therefore, a series of candidate irreversible inhibitors of type **3** was synthesized for evaluation on xanthine oxidase. The results are the subject of this paper.



1, R = OC_2H_5
2, R = $O(CH_2)_3C_6H_5$
3, R = $O(CH_2)_nNHCORSO_2F$
4, R = $NHCOC_6H_4SO_2F$-*p*

Enzyme Results.—The results with the twelve candidate irreversible inhibitors are collated in Table I. Since the I_{50}'s varied between 0.067 and 1 μ*M* but **1** had I_{50} = 0.11 μ*M*,[4] it is clear that the acylamido moiety on the *para* side chain was in contact with the enzyme. Nevertheless, none of the compounds was an irreversible inhibitor of xanthine oxidase, most likely because the SO_2F moiety was not juxtaposed to an appropriate nucleophile on the enzyme surface such as a serine hydroxyl.

Chemistry.—The irreversible inhibitors in Table I can be divided into two types. The carboxamides (**24**) were made by acylation of **23** with the appropriate

(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.*, **12**, 211 (1969).

(3) B. R. Baker and W. F. Wood, *ibid.*, **10**, 1101 (1967), paper CII of this series.

(4) B. R. Baker and W. F. Wood, *ibid.*, **11**, 644 (1968), paper CXXII of this series.

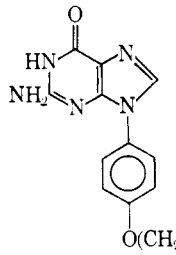
(5) (a) B. R. Baker, "Design of Active-Site-Directed Irreversible Enzyme Inhibitors. The Organic Chemistry of the Enzymic Active-Site," John Wiley and Sons, Inc., New York, N. Y., 1967; (b) for the chemotherapeutic utility of such inhibitors see B. R. Baker and J. L. Hendrickson, *J. Pharm. Sci.*, **56**, 955 (1967), paper XCII of this series.

(6) B. R. Baker and W. F. Wood, *J. Med. Chem.*, **11**, 650 (1968), paper CXXIII of this series.

acid chloride[7] in DMF. The phenylureas (**25**) were made by condensation of **23** with the appropriate O-phenyl N-(fluorosulfonylphenyl)carbamate,[8] O-(*p*-nitrophenyl) carbamate,[9] or *m*-fluorosulfonylphenyl isocyanate in DMSO or DMF. The requisite amines (**23**) were made from **17** (Scheme I). Reaction of **17** with hexamethylenetetramine followed by acid hydrolysis by the Delepine synthesis gave **18** which was converted to **19** with acetic anhydride. Catalytic reduction to **20** followed by condensation with 2-amino-6-chloro-5-phenylazo-4-pyrimidinol[3,10] afforded

TABLE I

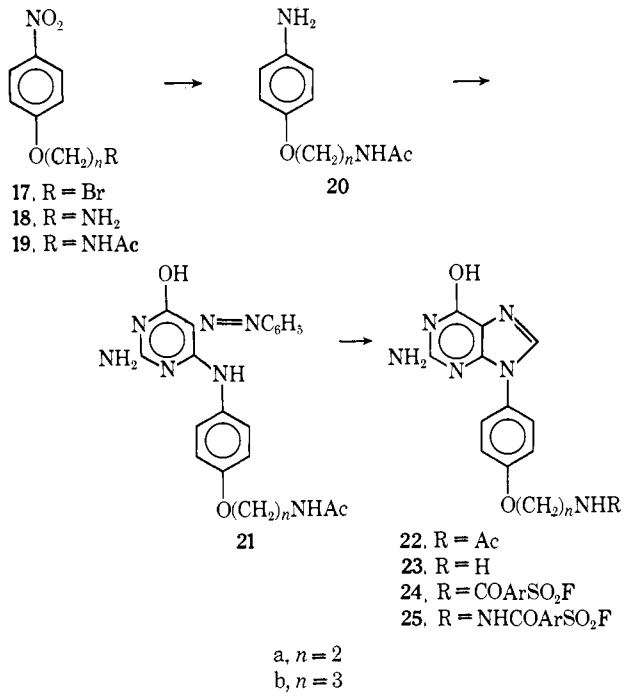
INHIBITION[a] OF XANTHINE OXIDASE[b] BY



No.	*n*	R	I_{50},[c] μM
5	2	$C_6H_4SO_2F$-*m*	0.53
6	2	$C_6H_4SO_2F$-*p*	0.69
7	2	C_6H_3-4-Me-3-SO_2F	0.49
8	2	$NHC_6H_4SO_2F$-*m*	0.18
9	2	$NHC_6H_4SO_2F$-*p*	0.33
10	2	NHC_6H_3-2-Cl-5-SO_2F	0.092
11	2	NHC_6H_3-4-Me-3-SO_2F	0.12
12	2	NHC_6H_3-2-MeO-5-SO_2F	0.067
13	3	$C_6H_4SO_2F$-*m*	0.63
14	3	$C_6H_4SO_2F$-*p*	1.0
15	3	$NHC_6H_4SO_2F$-*m*	0.40
16	3	$NHC_6H_4SO_2F$-*p*	0.67

[a] The technical assistance of Julie Leseman and Maureen Baker with these assays is acknowledged. [b] Xanthine oxidase was a commercial preparation from bovine milk that was assayed with 8.1 μM hypoxanthine and O_2 in 0.05 M Tris buffer (pH 7.4) containing 10% DMSO as previously described.[6b] Assays for irreversible inhibition were performed by either the uric acid assay or the 2,6-dichlorophenolindophenol assay or both[2]; the indophenol assay was utilized when I_{50} was <0.1 μM.[2] [c] I_{50} = concentration for 50% inhibition.

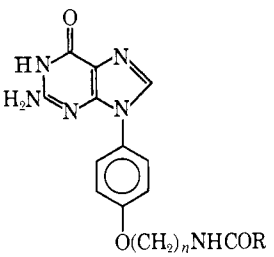
SCHEME I



a, $n=2$
b, $n=3$

TABLE II

PHYSICAL PROPERTIES OF



No.	*n*	R	Method[a]	% yield[b]	Mp, °C[c]	Formula[d]
5	2	$C_6H_4SO_2F$-*m*	A	62	260	$C_{20}H_{17}FN_6O_5S\cdot 0.5H_2O$
6	2	$C_6H_4SO_2F$-*p*	A	51	250	$C_{20}H_{17}FN_6O_5S\cdot 0.5H_2O$
7	2	C_6H_3-4-Me-3-SO_2F	A	27	250	$C_{21}H_{19}FN_6O_5S$
8	2	$NHC_6H_4SO_2F$-*m*	C	45	250	$C_{20}H_{18}FN_7O_5S\cdot H_2O$
9	2	$NHC_6H_4SO_2F$-*p*	B	53	220	$C_{20}H_{18}FN_7O_5S\cdot H_2O$
10	2	NHC_6H_3-2-Cl-5-SO_2F	D	35	250	$C_{20}H_{17}ClFN_7O_5S\cdot 0.5CH_3OCH_2CH_2OH$[e]
11	2	NHC_6H_3-4-Me-3-SO_2F	D	46	235	$C_{21}H_{20}FN_7O_5S$
12	2	NHC_6H_3-2-MeO-5-SO_2F	D	52	240	$C_{21}H_{20}FN_7O_6S$
13	3	$C_6H_4SO_2F$-*m*	A	35	245	$C_{21}H_{19}FN_6O_5S$
14	3	$C_6H_4SO_2F$-*p*	A	37	270	$C_{21}H_{19}FN_6O_5S$
15	3	$NHC_6H_4SO_2F$-*m*	D	20	215	$C_{21}H_{20}FN_7O_5S$
16	3	$NHC_6H_4SO_2F$-*p*	B	12	190	$C_{21}H_{20}FN_7O_5S$

[a] Methods: A, from the appropriate acid chloride[7] plus amine in DMF containing Et_3N[3]; B, reaction of amine with O-phenyl N-(*m*-fluorosulfonylphenyl)carbamate[8] in DMSO; C, amine plus *m*-fluorosulfonylphenyl isocyanate in DMF;[6] D, amine plus appropriate O-(*p*-nitrophenyl) carbamate[2,9] in DMF. [b] Recrystallized from MeOEtOH–H_2O. [c] Wide decomposition range starting at temperature indicated. [d] Analyzed for C, H, F. [e] Contained 1050-cm^{-1} C–O–C band not present in other compounds in Table II.

(7) B. R. Baker and R. B. Meyer, Jr., *J. Med. Chem.*, **12**, 104 (1969), paper CXLII of this series.

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

(9) B. R. Baker and N. M. J. Vermeulen, *ibid.*, **12**, 74 (1969), paper CXXXIV of this series; **12**, 79 (1969), paper CXXXV of this series.

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

21. Reductive formylation of the azo linkage followed by ring closure in formamide–formic acid[3] gave the substituted 9-phenylguanine (**22**). Acid hydrolysis removed the acetyl blocking group with generation of **23**.

Experimental Section

Melting points were taken in capillary tubes on a Mel-Temp block and are uncorrected. Each analytical sample showed proper uv and ir spectra, moved as a single spot on tlc on Brinkmann silica gel GF, and gave combustion analysis for C, H, and N or F within 0.4% of theory. The physical properties of **5**–**16** are listed in Table II.

Acetamidoethyl *p*-Nitrophenyl Ether (19a). **A.**—To a solution of 4.92 g (20 mmoles) of **17a**[11] in 40 ml of $CHCl_3$ was added 3.1 g (22 mmoles) of hexamethylenetetramine. The mixture was refluxed with stirring for 100 hr, during when the intermediate heximonium bromide separated. The latter was collected on a filter, washed ($CHCl_3$), dried, and added to a mixture of 8 ml of H_2O, 40 ml of EtOH, and 10 ml of 12 *N* HCl. After 24 hr the mixture was filtered from the separated NH_4Cl, then evaporated *in vacuo*. The residual, crude **18a**·HCl was dissolved in 50 ml of $CHCl_3$ and 8.1 g of Et_3N. To the hot solution was added 4.1 g of Ac_2O over a period of about 10 min. The cooled solution was washed with three 100-ml portions of H_2O, then dried ($MgSO_4$), and evaporated *in vacuo*. Recrystallization from C_6H_6 gave 2.0 g (45%) of crystals, mp 121–122°. *Anal.* ($C_{16}H_{12}N_2O_4$) C, H, N.

B.—To a suspension of 10 g (33 mmoles) of *p*-nitrophenyl phthalimidoethyl ether[12] in 100 ml of refluxing EtOH was added 5.77 g (0.1 mole) of 85% hydrazine hydrate. Solution was complete in 2 min and a precipitate separated in 15 min. After a total of 20 min, the hot mixture was treated with 200 ml of 3 *N* HCl. After 10 min, the mixture was cooled and the phthalhydrazide was removed by filtration. The filtrate was evaporated *in vacuo* and the residual **18a**·HCl + N_2H_4·HCl was acetylated as above. Recrystallization from C_6H_6 gave 4.0 g (53%) of crystals, mp 120–121°, that were identical with preparation A.

Acetamidopropyl *p*-nitrophenyl ether (19b) was prepared from **17b**[11] and hexamethylenetetramine as described for **17a**; yield 46%, mp 117–118°. *Anal.* ($C_{11}H_{14}N_2O_4$) C, H, N.

***p*-Aminophenyl Acetamidoethyl Ether (20a).**—A solution of 1.12 g (5 mmoles) of **19a** in 100 ml of EtOH was shaken with H_2 at 2–3 atm in the presence of 50 mg of PtO_2 until reduction of the NO_2 group was complete. The filtered solution was evaporated *in vacuo*. Recrystallization of the residue from toluene gave 0.85 g (87%) of white crystals, mp 93–94°. *Anal.* ($C_{10}H_{14}N_2O_2$) C, H, N.

Similarly, **20b** was prepared in 79% yield, mp 79–80°. *Anal.* ($C_{11}H_{16}N_2O_2$) C, H, N.

9-(*p*-Aminoethoxyphenyl)guanine (23a) was prepared from **20a** *via* **21a** as described for 9-(*p*-aminophenyl)guanine,[3] except the free base was released at pH 10 with NH_4OH; yield 22%; mp >300°; λ_{max} (pH 11) 270 (infl), (pH 13) 270 mμ. *Anal.* ($C_{13}H_{14}N_6O_2$) C, H, N.

Similarly, **23b** was prepared in 40% yield as white crystals that were uniform on tlc in 3:5 EtOH–$CHCl_3$, but gave erratic combustion values.

(11) B. R. Baker and C. J. Lourens, *J. Med. Chem.*, **11**, 26 (1968), paper CIX of this series.

(12) J. N. Ashley, R. F. Collins, M. Davis, and N. E. Sirett, *J. Chem. Soc.*, 3880 (1959).

Irreversible Enzyme Inhibitors. CXLVIII.[1,2] Active-Site-Directed Irreversible Inhibitors of Guanine Deaminase[3] Derived from 9-Phenylguanine Bearing a Terminal Sulfonyl Fluoride

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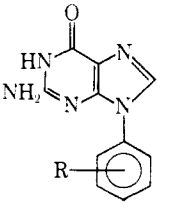
Thirty-eight candidate irreversible inhibitors of guanine deaminase derived from 9-phenylguanine with a terminal sulfonyl fluoride bridged to the *meta* or *para* position of the phenyl moiety by an amide or ether linkage were evaluated with the enzyme from Walker 256 rat tumor. Three of the compounds (**6**, **7**, **9**) were excellent irreversible inhibitors of this enzyme, but also showed no isozyme specificity since these could also inactivate the rat liver enzyme. Of the 13 compounds showing moderate irreversible inhibition of the Walker 256 enzyme, four (**5**, **28**, **30**, **31**) showed isozyme specificity with no inactivation of the rat liver enzyme.

The design, synthesis, and evaluation of active-site-directed irreversible inhibitors[4] of guanine deaminase[5] has been a project in this laboratory. 9-Phenylguanine (**1**) was found to be a good reversible inhibitor of the enzyme, being complexed slightly better than the substrate.[6] It was then established that the 28-fold increment in binding by the phenyl group of **1** was due to a hydrocarbon interaction with the enzyme.[7] From a study of the nature and dimensions of this hydrophobic bonding region,[8] compounds of types **2** and **3** were considered likely candidates as irreversible inhibitors of guanine deaminase that operate by the



1, R = H
2, R = *m*-NHCORSO$_2$F
3, R = *p*-O(CH$_2$)$_n$NHCORSO$_2$F

(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 and W. F. Wood, *J. Med. Chem.*, **12**, 214 (1969).

(3) For the previous paper on this enzyme see B. R. Baker and W. F. Wood, *ibid.*, **11**, 650 (1968), paper CXXIII of this series.

(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) For the chemotherapeutic utility of a tumor-specific inhibitor of guanine deaminase see B. R. Baker, *J. Med. Chem.*, **10**, 59 (1967), paper LXXIII of this series.

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

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

(8) B. R. Baker and W. F. Wood, *ibid.*, **11**, 641 (1968), paper CXXII of this series.