Early studies by Kato, Chiesara, and Vassanelli,[18] have suggested that while **1** produces a profound inhibitory effect acutely it may stimulate microsomal hydroxylation when given chronically. This possibility was further investigated in the present study. It was found that rats receiving **1** at a level of 22 mg/kg/day for 6 days demethylated butynamine about 2.5 times faster than normal (Table VIII). Microsomes from these rats also metabolized butynamine faster than control in the *in vitro* system. In a second experiment in which rats received 22 mg/kg/day for 12 days no increase in the *in vivo* demethylation of diphenamid[10] occurred although an increase in the rate of the *in vitro* demethylation of propoxyphene was observed. Phenobarbital, a widely used agent for stimulation of the microsomal system was very effective in increasing both the *in vivo* rate of demethylation of diphenamid and butynamide as well as the *in vitro* dealkylation of propoxyphene and butynamine. The results of these studies suggest that while **1** can stimulate microsomal oxygenases when given chronically its activity is only marginal.

(18) R. Kato, E. Chiesara, and P. Vassanelli, *Med. Exp.*, **6**, 254 (1962).

Irreversible Enzyme Inhibitors. CXLVI.[1,2] Active-Site-Directed Irreversible Inhibitors of Xanthine Oxidase[3] Derived from 9-(Acylamidophenyl)guanines Substituted with a Terminal Sulfonyl Fluoride

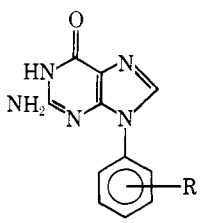
B. R. BAKER AND WILLIAM F. WOOD

Department of Chemistry, University of California at Santa Barbara, Santa Barbara, California 93106

Received October 21, 1968

Twenty candidate active-site-directed irreversible inhibitors of xanthine oxidase have been synthesized and evaluated. These were derived from *m*- or *p*-amino-9-phenylguanine attached to benzenesulfonyl fluoride by a sulfonamide, carboxamide, or urea bridge. Three excellent irreversible inhibitors emerged that at a concentration of 10^{-6} to 10^{-7} *M* gave total inactivation of xanthine oxidase with a half-life of 1 min or less; these were 9-phenylguanines substituted by a *p*-(*p*-fluorosulfonylbenzamido) (**2**), *p*-(*m*-fluorosulfonylbenzamido) (**7**), or *m*-(*p*-fluorosulfonylbenzenesulfonamido) (**21**) moiety.

9-Phenylguanine (**1**) is a good reversible inhibitor of



1, R = H
2, R = *p*-$NHCOC_6H_4SO_2F$-*p*
3, R = *m*-$NHCOC_6H_4SO_2F$-*p*
4, R = *m*-$NHCOC_6H_4SO_2F$-*m*
5, R = *m*-$NHCONHC_6H_4SO_2F$-*m*
6, R = *p*-$O(CH_2)_nNH{\sim}SO_2F$-*m*

xanthine oxidase[4,5] with the 9-phenyl group interacting with the enzyme by hydrophobic bonding.[6] A study of the nature and dimensions of this hydrophobic bonding region on the enzyme was performed to determine where the hydrophobic bonding region ended. Then a leaving group could be properly positioned on the inhibitor to form a covalent bond in a polar region of the enzyme surface.[7] As a result of this study, **2–5** were designed as irreversible inhibitors of xanthine oxidase; **2** was found to be a rapid irreversible inhibitor, but three related compounds (**3–5**) were not.[8] Studies of compounds related to **2–5** have now been extended and are the subject of this paper; sulfonyl fluorides bridged with an ether group (**6**) are the subject of the paper that follows.[9]

Enzyme Results.—An additional 20 analogs of **1** have been synthesized and evaluated as summarized in Table I. The three best of the 25 irreversible inhibitors were the parent **2** and its analogs **7** and **21**, all of which gave essentially total inactivation of xanthine oxidase when incubated at an I_{50} concentration of compound.[10] All three at an I_{50} concentration showed a half-life of inactivation of 1 min or less. Since **2**, **7**, and **21** differed less than threefold as reversible inhibitors, no one could be considered the best.

It can be calculated that the xanthine oxidase concentration in the incubation is about 0.08 μM when assayed by the uric acid method[10] and 0.02 μM when assayed by the indophenol method; this calculation is based on an OD change of 0.01/min when the incubation mixture is diluted tenfold, on the molecular weight

(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 J. A. Hurlbut, *J. Med. Chem.*, **12**, 118 (1969).

(3) For the previous paper on this enzyme see B. R. Baker, W. F. Wood, and J. A Kozma, *ibid.*, **11**, 661 (1968), paper CXXVI of the series.

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

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

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

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

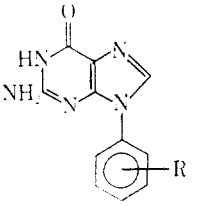
(8) B. R. Baker and W. F. Wood, *ibid.*, **11**, 650 (1968), paper CXXIII of this series.

(9) B. R. Baker and W. F. Wood, *ibid.*, **12**, 214 (1969), paper CXLVII of this series.

(10) B. R. Baker and J. A. Kozma, *ibid.*, **10**, 682 (1967), paper XCV of this series.

Table I

Inhibition[a] of Xanthine Oxidase by



No.	R	I_{50},[b] μM	Concn, μM	Time, min	% inactn	Assay method
2	*p*-NHCOC$_6$H$_4$SO$_2$F-*p*	0.70[d]	0.70	1, 24	85, 97[d,e]	Uric
			0.70	10	95	DCPI
7	*p*-NHCOC$_6$H$_4$SO$_2$F-*m*	0.28	0.28	60	100	Uric
			0.28	<1, 2	50, 93[e]	Uric
			0.14	60	100	DCPI
8	*m*-NHCOC$_6$H$_3$-4-Me-3-SO$_2$F	0.092	0.16	60	0	Uric, DCPI
9	*m*-NHCOC$_6$H$_3$-2-Cl-5-SO$_2$F	0.070	0.35	60	0	Uric, DCPI
10	*m*-NHCONHC$_6$H$_3$-4-Me-3-SO$_2$F	0.018	0.090	60	0	Uric, DCPI
11	*m*-NHCONHC$_6$H$_3$-2-OMe-5-SO$_2$F	0.016	0.080	60	0	Uric, DCPI
12	*m*-NHCONHC$_6$H$_4$SO$_2$F-*p*	0.024	0.12	60	0	Uric, DCPI
13	*m*-NHCONHC$_6$H$_3$-2-Cl-5-SO$_2$F	0.053	0.11	60	0	Uric, DCPI
14	*m*-NHCONHC$_6$H$_3$-3-Cl-4-SO$_2$F	0.033	0.17	60	0	Uric, DCPI
15	*m*-NHCOCH$_2$C$_6$H$_4$SO$_2$F-*p*	0.015	0.075	60	0	Uric, DCPI
16	*m*-NHCO(CH$_2$)$_2$C$_6$H$_4$SO$_2$F-*p*	0.026	0.13	60	0	Uric, DCPI
17	*m*-NHCO(CH$_2$)$_4$C$_6$H$_4$SO$_2$F-*p*	0.010	0.12	60	0	Uric, DCPI
18	*m*-NHCOCH$_2$OC$_6$H$_4$SO$_2$F-*p*	0.10	0.50	60	0	Uric
19	*p*-NHSO$_2$C$_6$H$_4$SO$_2$F-*p*	2.5	12	60	0	Uric
			33	60	0	DCPI
20	*p*-NHSO$_2$C$_6$H$_4$SO$_2$F-*m*	0.94	4.7	60	69	Uric
21	*m*-NHSO$_2$C$_6$H$_4$SO$_2$F-*p*	0.72	0.72	60	100	Uric, DCPI
			0.72	1, 4	50, 83[e]	Uric
			0.24	60	89	Uric
			0.24	60	100	DCPI
			0.080	60	36	Uric
			0.080	60	100	DCPI
			0.027	60	0	Uric
			0.0090	60	30	DCPI
22	*m*-NHSO$_2$C$_6$H$_4$SO$_2$F-*m*	1.3	2.6	60	89	Uric
			2.6	60	76	DCPI
23	*p*-OMe-*m*-NHCOC$_6$H$_3$SO$_2$F-*m*	4.9	25	60	0	Uric
24	*p*-OMe-*m*-NHCOC$_6$H$_4$SO$_2$F-*p*	4.1	12	60	0	Uric
25	*p*-OMe-*m*-NHCONHC$_6$H$_4$SO$_2$F-*m*	5.6	17	60	0	Uric
26	*p*-OMe-*m*-NHCONHC$_6$H$_4$SO$_2$F-*p*	4.5	13	60	0	Uric

[a] The technical assistance of Maureen Baker, Julie Leseman, and Jean Reeder is acknowledged. [b] I_{50} = concentration for 50% inhibition when 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.[5] [c] Inactivation of xanthine oxidase was performed in pH 7.4 Tris buffer containing 5% DMSO at 37° then assayed by uric acid formation as previously described,[10] or by the dichlorophenolindophenol (DCPI) assay in the Experimental Section. [d] Data from ref 4. [e] From time study plot.

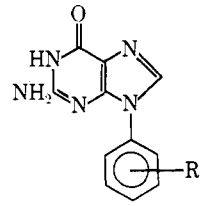
of 290,000, and on the turnover number of 0.33 unit/mg per protein (1 unit = 1 mole of product/min). In order to check the sensitivity of the incubation assay, the inhibitor concentration of **21** was lowered by factors of three. With the uric acid assay using about 0.08 μM enzyme, 0.24, 0.08, and 0.027 μM inhibitor gave 89, 36, and 0% inactivation, respectively; thus this assay should detect an irreversible inhibitor down to about 0.1 μM. With the more sensitive indophenol assay,[11] the inhibitor concentration in the incubation could be reduced to about 0.01 μM; thus 0.08 and 0.009 μM of **21** gave 100 and 30% inactivation, respectively. The limitation on incubation concentration is the I_{50}; that is, $5I_{50}$ can be used in the incubation since it is diluted tenfold for assay, but higher concentrations cannot be used since the amount of reversible inhibition of the incubation aliquot becomes too high. Those compounds (**8**–**17**) with potent I_{50}'s in the range of 0.01–0.07 μM were reassayed by the indophenol method, but none were irreversible inhibitors.

The following conclusions regarding irreversible inhibition of xanthine oxidase by 9-phenylguanines bridged with an amide to a sulfonyl fluoride are pertinent. (a) None of the *m*-carboxamide-bridged compounds (**9**–**18**) were irreversible inhibitors. (b) In contrast, the *m*-sulfonamide-bridged compound (**21**) was an excellent irreversible inhibitor. This difference between **21** and the corresponding *m*-carboxamide (**3**) is probably due to the difference in the bond angle between CO–NH and SO_2–NH and the probability that the carboxamide oxygen is complexed to the enzyme,[7] but the sulfonamide oxygen is not. (c) The *p*-carboxamide-bridged compounds (**1**, **7**) are good

(11) P. Feigelson, *J. Biol. Chem.*, **197**, 843 (1952).

TABLE II

PHYSICAL PROPERTIES OF



No.	R	Method[a]	% yield	Mp, °C dec[b]	Formula[c]
7	*p*-NHCOC$_6$H$_4$SO$_2$F-*m*	A	45	300	C$_{18}$H$_{13}$FN$_6$O$_4$S[d]
8	*m*-NHCOC$_6$H$_3$-4-Me-3-SO$_2$F	A	45	300	C$_{19}$H$_{15}$FN$_6$O$_4$S
9	*m*-NHCOC$_6$H$_3$-2-Cl-5-SO$_2$F[e]	A	49	300	C$_{18}$H$_{12}$ClFN$_6$O$_4$S·0.5H$_2$O
10	*m*-NHCONHC$_6$H$_3$-4-Me-3-SO$_2$F[f]	C	48	300	C$_{19}$H$_{16}$FN$_7$O$_4$S
11	*m*-NHCONHC$_6$H$_3$-2-OMe-5-SO$_2$F[f]	C	20	290	C$_{19}$H$_{16}$FN$_7$O$_5$S·0.5CH$_3$OCH$_2$CH$_2$OH[g]
12	*m*-NHCONHC$_6$H$_4$SO$_2$F-*p*	C	67	300	C$_{18}$H$_{14}$FN$_7$O$_4$S·CH$_3$OCH$_2$CH$_2$OH[g]
13	*m*-NHCONHC$_6$H$_3$-2-Cl-5-SO$_2$F[f]	C	35	250	C$_{18}$H$_{13}$ClFN$_7$O$_4$S·2CH$_3$OCH$_2$CH$_2$OH[g]
14	*m*-NHCONHC$_6$H$_3$-3-Cl-4-SO$_2$F[f]	C	38	235	C$_{18}$H$_{13}$ClFN$_9$O$_4$S·CH$_3$OCH$_2$CH$_2$OH[g]
15	*m*-NHCOCH$_2$C$_6$H$_4$SO$_2$F-*p*[h]	A	17	300	C$_{19}$H$_{15}$FN$_6$O$_4$S
16	*m*-NHCO(CH$_2$)$_2$C$_6$H$_4$SO$_2$F-*p*	A[i]	33	300	C$_{20}$H$_{17}$FN$_6$O$_4$S·H$_2$O
17	*m*-NHCO(CH$_2$)$_4$C$_6$H$_4$SO$_2$F-*p*[h]	A[i]	10	265–267[j]	C$_{22}$H$_{21}$FN$_6$O$_4$S
18	*m*-NHCOCH$_2$OC$_6$H$_4$SO$_2$F-*p*[k]	A	20	195	C$_{19}$H$_{15}$FN$_6$O$_5$S·HCl
19	*p*-NHSO$_2$C$_6$H$_4$SO$_2$F-*p*	D	15	300	C$_{17}$H$_{13}$FN$_6$O$_5$S$_2$
20	*p*-NHSO$_2$C$_6$H$_4$SO$_2$F-*m*	D	47	300	C$_{17}$H$_{13}$FN$_6$O$_5$S$_2$
21	*m*-NHSO$_2$C$_6$H$_4$SO$_2$F-*p*	D	12	300	C$_{17}$H$_{13}$FN$_6$O$_5$S$_2$
22	*m*-NHSO$_2$C$_6$H$_4$SO$_2$F-*m*	D	18	285	C$_{17}$H$_{13}$FN$_6$O$_5$S$_2$
23	*p*-OMe-*m*-NHCOC$_6$H$_4$SO$_2$F-*m*	A	5	300	C$_{19}$H$_{15}$FN$_6$O$_5$S
24	*p*-OMe-*m*-NHCOC$_6$H$_4$SO$_2$F-*p*	A	16	300	C$_{19}$H$_{15}$FN$_6$O$_5$S·H$_2$O
25	*p*-OMe-*m*-NHCONHC$_6$H$_4$SO$_2$F-*m*[f]	C	53	290	C$_{19}$H$_{16}$FN$_7$O$_5$S
26	*p*-OMe-*m*-NHCONHC$_6$H$_4$SO$_2$F-*p*[f]	C	15	287	C$_{19}$H$_{16}$FN$_7$O$_5$S·H$_2$O

[a] For method A see the preparation of **2**;[8] for method B see the preparation of **5**.[8] Method D was the same as A with Et$_3$N replaced by pyridine dried with molecular sieves. [b] Temperature at which decomposition starts. [c] Analyzed for C, H, and F unless otherwise indicated. [d] Analyzed for C, H, N. [e] For intermediate acid see ref 13c. [f] For the intermediate O-nitrophenylurethan see ref 12. [g] Solvate confirmed by 1050 cm^{-1} C–O–C band in the ir absent in other compounds. [h] For intermediate acid see ref 13a. [i] Carboxyl activated with EtOCOCl in DMF–Et$_3$N. [j] Actual melting point. [k] See ref 13b for preparation of intermediate acid; nmr showed the SO$_2$F was *para*, as would be expected.

irreversible inhibitors; in contrast, the *p*-sulfonamido-bridged inhibitors show poor (**20**) to no (**19**) irreversible inhibition. (d) Attempts to change the binding conformation of the *m*-carboxamide-bridged inhibitors by introduction of an adjacent OMe group (**22–26**) led to large losses in reversible inhibition and no emergence of irreversible inhibition.

Chemistry.—The candidate irreversible inhibitors with an amide linkage (**7–9, 15–24**) were prepared by acylation of the appropriate 9-(aminophenyl)guanine[6] with the appropriate acid chloride[8] or mixed anhydride. The urea-linked candidate irreversible inhibitors (**10–14, 25, 26**) were prepared from the same guanines by reaction with the appropriate O-(*p*-nitrophenyl)-N-phenylcarbamate.[12] The requisite acids[13] and O-(*p*-nitrophenyl)urethans[12] were prepared by literature methods[13] as indicated in Table II. Since fluorosulfonation of hydrocinnamic acid resulted in ring closure to 1-indanone,[13a] the corresponding methyl ester was fluorosulfonated, then hydrolyzed with aqueous acid to the desired *p*-fluorosulfonylhydrocinnamic acid (**27**).

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

(13) (a) W. Baker, G. E. Coate, and F. Glocking, *J. Chem. Soc.*, 1736 (1951); (b) Gevaent Photo-Producten N.V., Belgian Patent 586,694 (July 19, 1960); *Chem. Abstr.*, **57**, 15301*d* (1962); (c) B. R. Baker and R. B. Meyer, Jr., *J. Med. Chem.*, **12**, 104 (1969), paper CXLII of this series.

Experimental Section

Melting points were taken in capillary tubes on a Mel-Temp block and are uncorrected. All analytical samples moved as a single spot on tlc with Brinkmann silica gel GF, gave appropriate ir spectra, and gave combustion analyses for C, H, and N or F within 0.4% of theory.

9-[*m*-(*p*-Fluorosulfonylphenylureido)phenyl]guanine (12) (Method C).—To a solution of 242 mg (1 mmole) of 9-(*m*-aminophenyl)guanine[6] in 10 ml of DMF was added 374 mg (1.1 mmoles) of O-(*p*-nitrophenyl) N-(*p*-fluorosulfonylphenyl)carbamate.[12] After 1 hr at ambient temperature, the mixture was diluted with 50 ml of H$_2$O. The product was collected on a filter, washed (H$_2$O), then twice recrystallized from MeO(CH$_2$)$_2$OH–H$_2$O; yield, 350 mg (67%) of nearly white crystals that gradually decomposed over 300° and gave a negative Bratton-Marshall test for aromatic amine.[14] See Table II for additional data.

***p*-Fluorosulfonylhydrocinnamic Acid (27).**—To 40 ml of FSO$_3$H in a polyethylene container was added portionwise 16.4 g (0.1 mole) of methyl hydrocinnamate with cooling at such a rate that the temperature was 20–25°. After 3 hr at ambient temperature, the solution was poured into 300 g of ice and extracted with three 50-ml portions of CHCl$_3$. The combined extracts were washed with two 100-ml portions of 5% NaHCO$_3$, then H$_2$O. Dried with MgSO$_4$, the CHCl$_3$ solution was evaporated *in vacuo*. The residual oil was refluxed with a mixture of 50 ml of HOAc and 25 ml of 6 *N* HCl for 15 min, then diluted with 400 ml of H$_2$O. The product was collected on a filter and washed (H$_2$O). Two recrystallizations from H$_2$O gave 5.5 g (24%) of white needles, mp 155–156°, that were uniform on tlc in 5:3 EtOH–CHCl$_3$; the presence of a *p*-SO$_2$F group was confirmed by ir and its position by nmr. *Anal.* (C$_9$H$_9$FO$_4$S) C, H, F.

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

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.* ($C_{12}H_{14}ClN_5O_2 \cdot H_2O$) C, H, N. For further transformations, the hydrochloride was converted first to the free base.[6]

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_0, C_0, and C_{60}. The C_0 and I_0 tubes were placed in an ice bath until ready for assay. To the C_{60} 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**, 877 (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

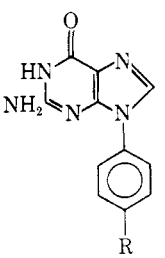
B. R. BAKER AND WILLIAM F. WOOD

Department of Chemistry, University of California at Santa Barbara, Santa Barbara, California 93106

Received October 21, 1968

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.