Irreversible Enzyme Inhibitors. CXXV.[1,2] Active-Site-Directed Irreversible Inhibitors of Xanthine Oxidase Derived from Arylpurines and Pyrazolo[3,4-*d*]pyrimidines Bearing a Terminal Sulfonyl Fluoride

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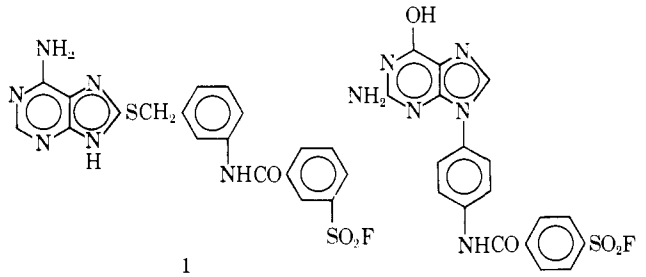
4-Hydroxy-6-phenylpyrazolo[3,4-*d*]pyrimidines with *m*-fluorosulfonylbenzamido (**14**) or *p*-fluorosulfonylbenzamido (**16**) groups on the *meta* position are active-site-directed irreversible inhibitors of xanthine oxidase; in contrast, the *m*-fluorosulfonylbenzamido substituent on the *para* position (**17**) does not give an irreversible inhibitor. Replacement of the 4-hydroxy group of **14** or **16** by amino (**11**, **12**) gives no loss of reversible inhibition, but irreversible inhibition is eradicated. Similarly, these fluorosulfonylbenzamido groups on the *meta* or *para* position of 8-phenyladenine (**8**–**10**) afford better reversible inhibitors, but irreversible inhibition is again lost. The seven types of active-site-directed irreversible inhibitors of xanthine oxidase found to date are compared in efficiency.

Two classes of active-site-directed irreversible inhibitors[3] of xanthine oxidase[4] that are derived from purines bearing a terminal sulfonyl fluoride[5] have been found; the first type was derived from 9-phenylguanine with a *p*-fluorosulfonylbenzamido substituent (**2**)[6] and the



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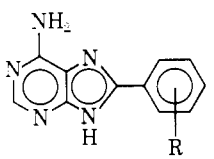

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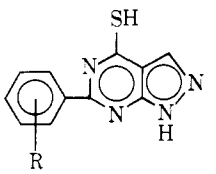

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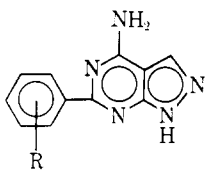

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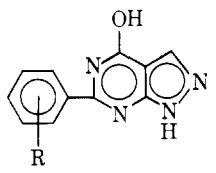

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second type[2] was derived from 8-benzylthioadenine (**1**) and 8-benzylthiohypoxanthine. Since 8-phenyladenines of type **3** were found to be excellent reversible inhibitors[7] of xanthine oxidase that complexed much better to the enzyme than the substrate, hypoxanthine, candidate irreversible inhibitors of the sulfonyl fluoride type derived from **3** were considered worthy of exploration. Furthermore, 4-mercapto-6-phenylpyrazolo[3,4-*d*]pyrimidines of type **4** were also good reversible inhibitors[8] of xanthine oxidase; therefore, candidate irreversible inhibitors derived from 6-phenylpyrazolo[3,4-*d*]pyrimidines and bearing a terminal sulfonyl fluoride group such as **5** and **6** were also considered. The synthesis and enzymic evaluation of these sulfonyl fluoride type irreversible inhibitors derived from **2**, **5**, and **6** are the subjects of this paper.

Enzymic Evaluation.—The sulfonyl fluoride (**1**) derived from 8-benzylthioadenine was previously found to be both a good reversible and irreversible inhibitor[2] of xanthine oxidase; **1** was complexed 19-fold better than the substrate, hypoxanthine, and at 0.4 μM showed rapid inactivation of xanthine oxidase with a half-life of 2 min (Table I). Removal of the sulfur atom from **1** to give a sulfonyl fluoride derived from 8-benzyladenine (**7**) led to an 83-fold loss in reversible inhibition; even though **7** could still inactivate xanthine oxidase at 70 μM, it was considerably slower than **1**.[9]

The derivatives of 8-phenyladenine (**8**–**10**) (Table I) were excellent reversible inhibitors of xanthine oxidase being complexed 24-, 400-, and 300-fold better, respectively, to the enzyme than the substrate. Unfortunately, at $2\text{-}5I_{50}$[9] none showed irreversible inhibition of xanthine oxidase. The fact that the *p*-fluorosulfonylbenzamido group on the *para* position of 9-phenylguanine (**2**)[6] showed irreversible inhibition of xanthine oxidase, but this same group on the *para* position of 8-phenyladenine (**10**) does not, shows that **2** and **10** are not complexed in a fashion that positions their sulfonyl fluoride in the same place within their enzyme–inhibitor complexes; this difference will be considered in more detail in the following paper.[7]

Three sulfonyl fluorides (**11**–**13**) (Table II) derived from 4-amino-6-phenylpyrazolo[3,4-*d*]pyrimidine bear-

(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 J. A. Kozma, *J. Med. Chem.*, **11**, 652 (1968).

(3) 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.

(4) For the chemotherapeutic utility of a tissue-specific blockade of this enzyme see B. R. Baker and J. L. Hendrickson, *J. Pharm. Sci.*, **56**, 955 (1967), paper XCII of this series.

(5) (a) The use of the terminal sulfonyl fluoride group for active-site-directed irreversible inhibitors of the exo type was first described for dihydrofolic reductase by B. R. Baker and G. J. Lourens, *J. Med. Chem.*, **10**, 1113 (1967), paper CV of this series; (b) phenylmethanesulfonyl fluoride, an endo-type irreversible inhibitor of chymotrypsin, was described earlier by D. E. Fahrney and A. M. Gold, *J. Am. Chem. Soc.*, **85**, 997 (1963).

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

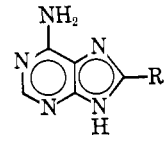
(7) B. R. Baker, W. F. Wood, and J. A. Kozma, *ibid.*, **11**, 661 (1968), paper CXXVI of this series.

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

(9) (a) It should be noted in comparing two active-site-directed irreversible inhibitors that the rate of inactivation is dependent upon the amount of (EI) complex and not directly on [I]; in this paper, the compounds are sometimes compared as a function of I_{50}, the concentration for 50% inhibition. (b) See ref 3, Chapter VIII, for the kinetics of irreversible inhibition.

TABLE I

INHIBITION[a] OF XANTHINE OXIDASE BY

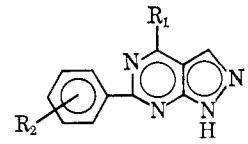


		Reversible[b]		Irreversible[c]		
No.	R	I_{50},[d] μM	$([S]/[I])_{0.5}$[e]	Inhib concn, μM	Time, min	% inactn
1[f]	$SCH_2C_6H_4(NHCOC_6H_4SO_2F\text{-}m)\text{-}m$	0.42	19	0.42	2, 15, 35	50, 88, 88[g]
7	$CH_2C_6H_4(NHCOC_6H_4SO_2F\text{-}p)\text{-}m$	35	0.23	70	12, 60	50, 79[g]
8	$C_6H_4(NHCOC_6H_4SO_2F\text{-}p)\text{-}m$	0.33	24	0.55	60	0
9	$C_6H_4(NHCOC_6H_4SO_2F\text{-}m)\text{-}p$	0.020	400	0.10	60	0
10	$C_6H_4(NHCOC_6H_4SO_2F\text{-}p)\text{-}p$	0.027	300	0.17	60	0

[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.[4] [c] Inactivation of xanthine oxidase was performed at 37° in pH 7.4 Tris buffer containing 5% DMSO as previously described,[16] except the zero point was determined by removal of an aliquot prior to addition of the inhibitor.[5a] [d] Concentration necessary for 50% inhibition. [e] Ratio of concentration of substrate to inhibitor for 50% inhibition. [f] Data from ref 2. [g] From time study; see ref 16 and 5a.

TABLE II

INHIBITION[a] OF XANTHINE OXIDASE BY



			Reversible[b]		Irreversible[c]		
No.	R_1	R_2	I_{50},[d] μM	$([S]/[I])_{0.5}$[e]	Inhib concn, μM	Time, min	% inactn
11	NH_2	$m\text{-}NHCOC_6H_4SO_2F\text{-}m$	7.0	1.1	14	60	0
12	NH_2	$m\text{-}NHCOC_6H_4SO_2F\text{-}p$	2.3	3.5	5.0	60	~0
13	NH_2	$m\text{-}NHCONHC_6H_4SO_2F\text{-}m$	0.75	11	3.8	60	0
14	OH	$m\text{-}NHCOC_6H_4SO_2F\text{-}m$	13	0.62	13	4, 30, 60	50, 97, 100[f]
					4.0	22, 60	50, 60[f]
15	OH	$m\text{-}NHCONHC_6H_4SO_2F\text{-}m$	0.68	12	3.4	16, 60	50, 57[f]
16	OH	$m\text{-}NHCOC_6H_4SO_2F\text{-}p$	15	0.54	20	8, 60	50, 90[f]
					10	30, 60	50, 50[f]
17	OH	$p\text{-}NHCOC_6H_4SO_2F\text{-}m$	0.018	450	0.060	60	0
18	OH	$m\text{-}NHCOC_6H_3\text{-}CH_3\text{-}4\text{-}SO_2F\text{-}3$	12	0.67	10	18, 30, 60	50, 63, 76[f,g]
					4.0	30, 60	63, 73[f,g]

[a–e] See corresponding footnotes in Table I. [f] From time study; see ref 16 and 5a. [g] Inactivation still taking place.

ing a fluorosulfonyl substituent on the *meta* position were synthesized and evaluated. Although **10–13** were reasonably good reversible inhibitors of xanthine oxidase that were complexed **1.1**-, 3.5-, and **11**-fold better than the substrate, at 2–5I_{50}[9] none of the three showed irreversible inhibition of xanthine oxidase; fortunately, better results were obtained with candidate irreversible inhibitors derived from 4-hydroxy-6-phenylpyrazolo[3,4-*d*]pyrimidine.

The hydroxy analog (**14**) of **11** changed only twofold in reversible inhibition, but was dramatically different in irreversible inhibition. At an I_{50} concentration **14** completely inactivated xanthine oxidase with a half-life of 4 min. However, when the concentration of **14** was reduced to 0.3I_{50}, inactivation was incomplete; such kinetics have been previously shown to be due to the competition of two reactions within the enzyme–inhibitor complex, namely, (a) covalent bond formation with resultant inactivation,[3] and (b) enzyme-catalyzed hydrolysis of the sulfonyl fluoride group.[10]

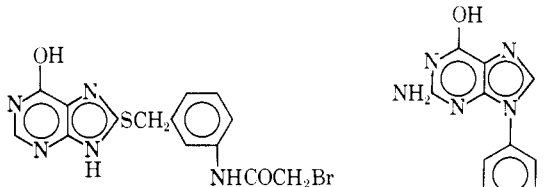
When the sulfonyl fluoride group of **14** was changed to the *para* position (**16**), reversible inhibition did not change; however, the rate of irreversible inhibition of **16** was only about one-half that of **14**. Replacement of the carboxamide bridge of **14** by urea (**15**) led to a 20-fold better reversible inhibitor; however, **15** was not as good an irreversible inhibitor since at 5I_{50}, **15** showed only 57% inactivation of xanthine oxidase before the enzyme had destroyed the inhibitor by catalytic hydrolysis.[10] When the *m*-fluorosulfonylbenzamido group of **14** was moved to the *para* position (**17**), a much better reversible inhibitor resulted; **17** was reversibly complexed to xanthine oxidase 450-fold better than hypoxanthine and 730-fold better than **14**. Unfortunately, **17** at 3I_{50} failed to show any irreversible inhibition of the enzyme.

Substitution of an *o*-methyl group on **14** to give **18** resulted in no change in reversible inhibition; however, two effects were seen on irreversible inhibition. At near I_{50} concentration, **14** inactivated xanthine oxidase about five times faster than **18**; however, at 0.3I_{50}, **18** gave more inactivation than **1** **4**, indicating that the ratio of the rate of inactivation to enzyme

(10) (a) B. R. Baker and J. A. Hurlbut, *J. Med. Chem.*, **11**, 233 (1968), paper CXIII of this series; (b) B. R. Baker and E. H. Erickson, *ibid.*, **11**, 245 (1968), paper CXV of this series.

catalyzed hydrolysis[10] was more favorable with **18** than **14**.[11]

To date seven different types of active-site-directed irreversible inhibitors[3] of xanthine oxidase have been found. In addition to type **6** in Table II and types **1**[12] and **2**,[13] other types found were bromoacetamides derived from 8-benzylthiohypoxanthine (**19**)[16] and 9-phenylguanine (**20**)[17] and two types of sulfonyl fluorides derived from 8-benzylthiohypoxanthine (**21**) and 2-benzylthiohypoxanthine (**22**). In order to compare the efficiency of active-site-directed irreversible



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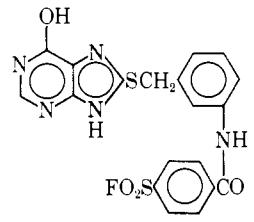

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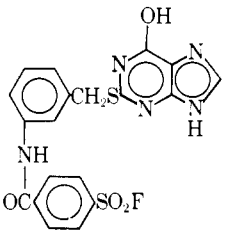

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inhibitors or in order to state whether such an inhibitor is sufficiently effective to be considered for chemotherapy in animals, the following parameters should be considered.[5a]

(a) The enzyme should be inactivated by 10^{-7}–10^{-9} M inhibitor since the lower the effective concentration the more selective the inhibitor is apt to be with respect to other enzymes; secondly, this concentration range would be suitable for *in vivo* dosage. The concentration of inhibitor required for inactivation is in turn dependent upon the reversible dissociation constant, K_i, of the enzyme–inhibitor complex and the concentration of the inhibitor.[9]

(b) The inhibitor should preferably inactivate the enzyme with a half-life of less than **10** min; inhibitors might still be effective *in vivo* at longer half-life, but metabolism and/or excretion of the drug then become more important factors.

(c) The inhibitor should give no significant amount of irreversible attack of serum proteins by a random bimolecular reaction;[9b] such random attack would place a haptenic determinate on the protein(s) that would lead to antibody formation and subsequent allergic reaction.[18] The sulfonyl fluorides appear to be considerably superior to bromoacetamides in their low random attack of proteins.[5a,10a]

(d) The inhibitor should be able to penetrate cell membranes. The types of compounds under discussion should be able to penetrate by passive diffusion since they are relatively nonpolar.[19]

(e) The inhibitor should inactivate the target enzyme in the target cell with minimal attack of the target enzyme in host tissues.[20]

(f) The inhibitor should be able to inactivate the target enzyme at a concentration $\leq K_i$; if a greater concentration is required, then selectivity may be lost due to high reversible inhibition of the target enzyme in all tissues. Since hypoxanthine was used at 8.1 μM and its K_i = 8.5 μM,[21] as a first approximation $I_{50} \simeq K_i$; therefore, in the case of xanthine oxidase, near complete irreversible inhibition at the I_{50} concentration of inhibitor would be necessary.

At this stage only parameters a–c and f need be considered since parameter e has not yet been determined for this enzyme and the assumption in parameter d is most efficiently checked by tissue culture studies after parameter e has been established. It is apparent that none of the seven types yet meet all the parameters of a–c and f.

The bromoacetamides (**19**, **20**) require 1.5 and 5 μM for complexing 50% of the enzyme and thus do not meet parameter a; furthermore, these two compounds do not meet parameter c, as discussed previously, nor do they meet parameter b. Therefore, the best chance of success would probably be with sulfonyl fluorides, which rapidly inactivate when they are irreversible inhibitors and which give less, if any, random attack on serum proteins.

The sulfonyl fluorides derived from 8-benzylthioadenine (**1**) and 8-benzylthiohypoxanthine (**21**) come close to meeting the four parameters under discussion. Both show 80–90% inactivation in less than 10 min at an $I_{50} \simeq K_i$ concentration; however, the K_i's are just outside the range of 10^{-7} M by a factor of 4. To obtain a 4–10-fold increment in binding by simple substituents on one or the other of the benzene rings has not been too difficult to achieve in other enzyme systems. A similar shortcoming exists with **2** which might be overcome in the same manner.

In contrast to **21**, the 2-benzylthiohypoxanthine derivative (**22**) is a good reversible inhibitor with $K_i \simeq 10^{-7}$ M but does not give sufficient inactivation at a K_i concentration. Since **22** can inactivate >80% at $6K_i$, it is probable that the ratio of rate of inactivation by **22** to the rate of enzyme-catalyzed hydrolysis of **22** is unfavorable;[10] it is not unlikely that this ratio can be changed favorably by substitution on one of the benzene rings as noted with **14** *vs.* **18**.[11]

The derivatives of 6-phenylpyrazolo[3,4-*d*]pyrimidine (**14**, **18**) suffer from being insufficiently good enough reversible inhibitors by a factor of about 120-fold;

(11) That substituents on one of the two benzene rings of an inhibitor such as **18** can influence the ratio of the rates of these two reactions within the reversible complex was observed previously in this laboratory with a number of other enzyme systems, such as dihydrofolic reductase,[12,13] chymotrypsin,[14] and trypsin.[15]

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

(13) B. R. Baker and N. M. J. Vermeulen, unpublished.

(14) B. R. Baker and J. A. Hurlbut, unpublished.

(15) B. R. Baker and E. H. Erickson, unpublished.

(16) B. R. Baker and J. Kozma, *J. Med. Chem.*, **10**, 682 (1967), paper XCV of this series.

(17) B. R. Baker and W. F. Wood, *ibid.*, **10**, 1106 (1967), paper CIII of this series.

(18) See ref 3, pp 153–155.

(19) See ref 3, pp 263–266.

(20) Inactivation of the dihydrofolic reductase from a tumor with minimal effect on the same enzyme from the liver of the same animal has been observed with appropriate irreversible inhibitors.[5a,22]

(21) B. R. Baker and R. B. Meyer, Jr., *J. Med. Chem.*, **11**, 189 (1968), paper CXIX of this series.

(22) B. R. Baker and P. C. Hanig, *ibid.*, **11**, 495 (1968), paper CXX of this series.

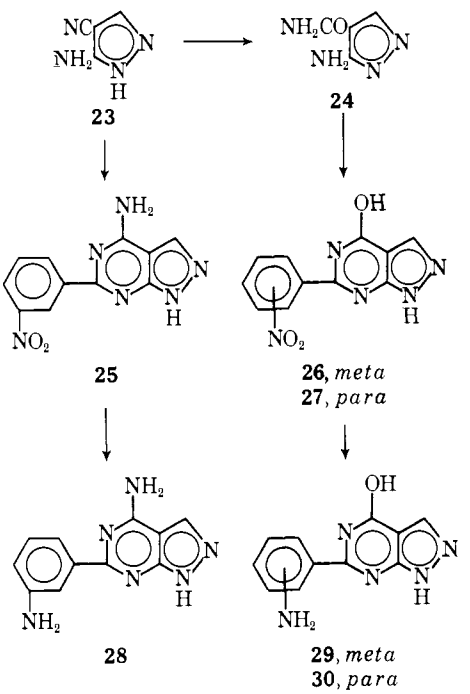
(23) J. B. Wyngaarden, *J. Biol. Chem.*, **224**, 153 (1957).

whether or not a 120-fold increment in binding by appropriate substitution on this type of compound can be achieved is highly questionable. Since **17** is the best reversible inhibitor in Table II, but shows no irreversible inhibition, further studies should be made in variation of the *para* substituent on the benzene ring. Therefore, future work for parameter e, selective irreversible inhibition[4] of a tumor enzyme compared to a liver enzyme, would be best studied with compounds related in structure to **1**, **2**, **21**, and **22**, and perhaps **17**.

Chemistry.—A number of methods have been described for conversion of aminopyrazoles such as **23** and **24** to pyrazolo[3,4-*d*]pyrimidines with 6 substituents. Acylation of **23** with aliphatic anhydrides followed by alkaline ring closure afforded 6-alkylpyrazolo-[3,4-*d*]pyrimidines;[24] this method proceeded poorly in this laboratory with aromatic acid derivatives. Since other methods have operational difficulties,[25] a new method for synthesis of 6-phenylpyrazolo[3,4-*d*]-pyrimidines was devised that was based on the following observations.

(a) Condensation of guanidine with **23** afforded 4,6-diaminopyrazolo[3,4-*d*]pyrimidine,[26] and (b) fusion

SCHEME I

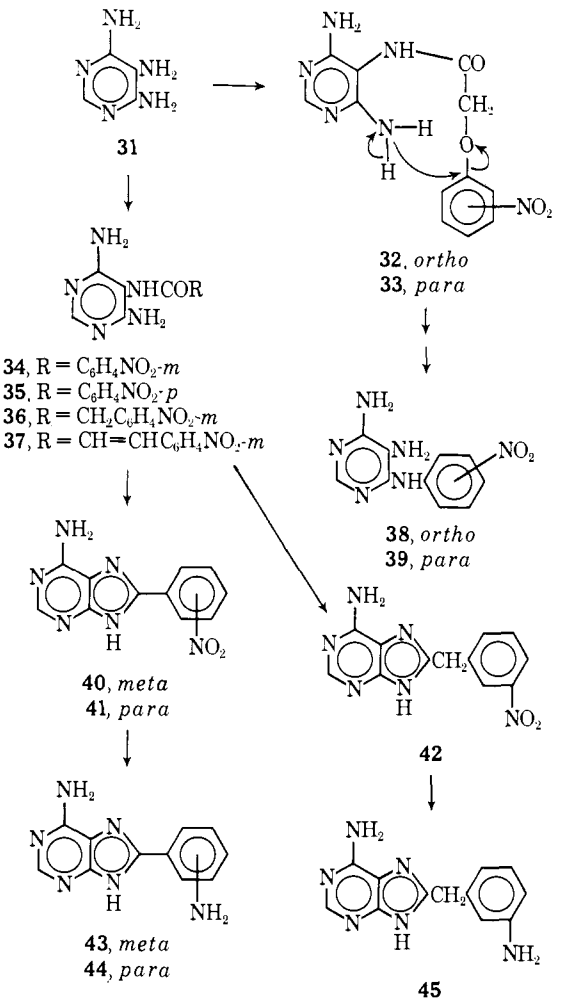


of 4,5-diaminopyrimidines with amidines afforded 8-substituted purines.[27] Therefore fusion of **23** or **24** with substituted benzamidines to form 6-phenylpyrazolo[3,4-*d*]pyrimidines of types **25–27** was investigated (Scheme I). This reaction proceeded smoothly at 200° and the products were readily purified. The method appeared to be quite general since benzamidines substituted with either the electron-withdrawing nitro group or the electron-donating hydroxyl group could be used.

The nitro group of **25–27** was catalytically reduced to give the amines (**28–30**) using a Pd catalyst. The various candidate irreversible inhibitors in Table II were then prepared from **28–30** by reaction with *m*-fluorosulfonylphenyl isocyanate or the appropriate fluorosulfonylbenzoyl chloride in DMF–Et_3N by the previously described method.[2]

Acylation of 4,5,6-triaminopyrimidine (**31**) with the appropriate acid chloride in aqueous NaOH[28,29] afforded 5-acylamidopyrimidines (**32–37**) in 15–60% yield of analytically pure material (Scheme II). Cyclization

SCHEME II



of **34–36** to **40–42** proceeded smoothly with polyphosphoric acid,[28,30] but this reagent decomposed **32**, **33**, and **37**; attempted cyclization of **37** with aqueous NaOH[28] was also unsuccessful. Attempted cyclization of **32** or **33** to the corresponding purines with aqueous NaOH led to rearrangement followed by cleavage; the products had an empirical formula in agreement with **38** and **39** but a rigorous structure proof was not attempted. The formation of **38** and **39** is envisioned as

(24) (a) C. C. Cheng and R. K. Robins, *J. Org. Chem.*, **23**, 191 (1958); (b) S. Irone, H. Nagaro, E. A. Nodiff, and A. J. Saggimo, *J. Med. Chem.*, **7**, 816 (1964).

(25) (a) P. S. Schmidt, K. Eichenberger, and M. Wilhelm, *Angew. Chem.*, **73**, 15 (1961); (b) P. S. Schmidt, K. Eichenberger, and M. Wilhelm, *Helv. Chim. Acta*, **45**, 1620 (1962); (c) E. C. Taylor and A. L. Borror, *J. Org. Chem.*, **26**, 4967 (1961); (d) E. C. Taylor and J. A. Zoltewicz, *J. Am. Chem. Soc.*, **83**, 248 (1961).

(26) J. Danal and K. A. Kerridge, *J. Chem. Soc.*, 2589 (1961).

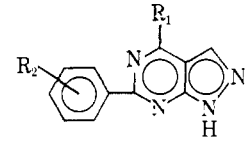
(27) (a) F. Bergmann, M. Rashi, M. Kleiner, and R. Knafo, *ibid.*, 1254 (1967); (b) F. Bergmann and M. Tamari, *ibid.*, 4468 (1961).

(28) B. R. Baker and D. V. Santi, *J. Heterocycl. Chem.*, **4**, 216 (1967), paper XCIV of this series.

(29) G. B. Elion, E. Burgi, and G. H. Hitchings, *J. Am. Chem. Soc.*, **73**, 5235 (1951).

(30) S.-C. J. Fu, E. Chinoporos, and H. Terzian, *J. Org. Chem.*, **30**, 1916 (1965).

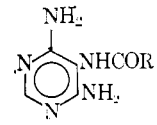
Table III
Physical Properties of



No.	R_1	R_2	Method	% yield	Mp, °C	Formula	Analyses	λ_{max}, mμ[a] pH 1	pH 13
11	NH_2	*m*-$NHCOC_6H_4SO_2F$-*m*	F[b]	76[c]	293–295 dec	$C_{18}H_{13}FN_6O_3S$	C, H, F	247, 271, 296[d]	277, 302[d]
12	NH_2	*m*-$NHCOC_6H_4SO_2F$-*p*	F[b]	71[c]	297–300 dec	$C_{18}H_{13}FN_6O_3S$	C, H, F	228, 278	252, 280,[d] 310[d]
13	NH_2	*m*-$NHCONHC_6H_4SO_2F$-*m*	G[e]	87[c]	Indef	$C_{18}H_{14}FN_7O_3S$	C, H, F	232, 260, 292	255, 310[d]
14	OH	*m*-$NHCOC_6H_4SO_2F$-*m*	F[b]	55[c]	311–313 dec	$C_{18}H_{12}FN_5O_4S$	C, H, F	229, 279	244,[d] 273[d]
15	OH	*m*-$NHCONHC_6H_4SO_2F$-*m*	G[e]	85[c]	>350	$C_{18}H_{13}FN_6O_4S$	C, H, F	221, 261, 301	254, 310[d]
16	OH	*m*-$NHCOC_6H_4SO_2F$-*p*	F[b]	34[c]	>350	$C_{18}H_{12}FN_5O_4S$	C, H, F	229, 278	243, 278[d]
17	OH	*p*-$NHCOC_6H_4SO_2F$-*m*	F[b]	37[c]	298–300 dec	$C_{18}H_{12}FN_5O_4S$	C, H, F	229, 302	285
18	OH	*m*-$NHCOC_6H_3$-4-CH_3-3-SO_2F	F[b,c]	77	283–285 dec	$C_{19}H_{14}FN_5O_4S \cdot 0.5H_2O$	C, H, F	234, 277, 282	271
25	NH_2	*m*-NO_2	A	39[b]	314–315	$C_{11}H_8N_6O_2 \cdot 0.25H_2O$	C, H, N	230, 265	243, 273, 318[f]
26	OH	*m*-NO_2	B	65[c]	>360	$C_{11}H_7N_5O_4$	C, H, N	265	242, 270
27	OH	*p*-NO_2	B	27[c]	>350	$C_{11}H_7N_5O_3$	C, H, N	249, 310	283, 350[d]
28	NH_2	*m*-NH_2	C	72[h]	275–277	$C_{11}H_{10}N_6$	C, H, N	238, 275	278,[d] 310
29	OH	*m*-NH_2	C	83[c]	339–341	$C_{11}H_9N_5O$	C, H, N	231, 280	249,[d] 308[d]
30	OH	*p*-NH_2	C	72[c]	>360	$C_{11}H_9N_5O$	C, H, N	233, 284	286
46	NH_2	H	A	42[h]	277–278[i]	$C_{11}H_9N_5$	C, H, N	242, 277	245, 278, 315[d]
47	OH	H	B	62[c]	338–339	$C_{11}H_8N_4O$	C, G, N	235, 277	236, 276,[d] 308[d]
48	OH	*p*-OH	B	36[c]	>360	$C_{11}H_8N_4O_2$	C, H, N	260, 297	317
49	NH_2	*p*-OH	A	48[b]	>360	$C_{11}H_9N_5O$	C, H, N	264,[d] 301	290, 320

[a] Spectra taken in 10% EtOH. [b] See method A, ref 2. [c] Recrystallized from DMF–H_2O. [d] Inflection. [e] See method B, ref 2. [f] The required fluorosulfonylbenzoyl chloride was synthesized in this laboratory by R. B. Meyer, Jr., unpublished. [g] Reprecipitated from dilute NaOH with HOAc. [h] Recrystallized from MeOH. [i] Lit.[25c] mp 275–277°.

Table IV
Physical Properties of



No.	R'	% yield	Mp, °C dec	Formula	λ_{max}, mμ[a] pH 1	pH 13
32	$CH_2OC_6H_4NO_2$-*o*	15	242–244	$C_{12}H_{12}N_6O_4$	262, 332	273, 336
33	$CH_2OC_6H_4NO_2$-*p*	49	266–268	$C_{12}H_{12}N_6O_4$	264, 303	276, 314
34	$C_6H_4NO_2$-*m*	25	>350	$C_{11}H_{10}N_6O_4$	266	276
35	$C_6H_4NO_2$-*p*	35	>350	$C_{11}H_{10}N_6O_4$	265	276
36	$CH_2C_6H_4NO_2$-*m*	60	281–283	$C_{12}H_{12}N_6O_4$	265	272
37	$CH{=}CHC_6H_4NO_2$-*m*	23	309–311	$C_{13}H_{12}N_6O_4$	265	270

[a] Compounds prepared by method D, recrystallized from DMF–H_2O, and analyzed for C, H, N. [b] In 10% EtOH.

a concerted neighboring group reaction where the 6-amino group displaces the nitro-activated alkoxy group of **32** or **33** followed by base cleavage of the resultant labile glycolic amide.

The candidate irreversible inhibitors (**7–10**) were then prepared by catalytic reduction to the amines (**43–45**) followed by reaction with the appropriate fluorosulfonylbenzoyl chloride in DMF-Et_3N.[2]

Experimental Section[31]

4-Amino-6-phenylpyrazolo[3,4-*d*]pyrimidine (46) (Method A).—A mixture of 1.00 g (9.2 mmoles) of **23**, 2.02 g (13 mmoles) of benzamidine hydrochloride, and 1.47 g (18 mmoles) of NaOAc were well mixed, then heated in a bath at 200° until gas evolution (NH_3) essentially ceased. The cooled melt was dissolved in hot 3 *N* HCl. The solution was clarified with carbon, then adjusted to about pH 4 with 3 *N* NaOH. The product was collected on a filter and washed with H_2O. Recrystallization from MeOH gave white crystals, mp 277–278°, lit.[25c] mp 275–277°. See Table III for additional data and other compounds prepared by this method.

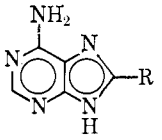
4-Hydroxy-6-phenylpyrazolo[3,4-*d*]pyrimidine (47) (Method B).—A mixture of 1.60 g (9.2 mmoles) of **24**, 2.02 g (13 mmoles) of benzamidine hydrochloride, and 2.40 g (31 mmoles) of NaOAc was well mixed then heated in a bath at 200° until gas evolution (NH_3) was essentially complete. The melt was dissolved in hot 2 *N* NaOH. The solution was clarified with carbon, then acidified with HOAc. The product was collected on a filter and washed with H_2O. Recrystallization from DMF–H_2O gave white crystals, mp 338–339°. See Table III for additional data and other compounds prepared by this method.

4-Amino-6-(*m*-aminophenyl)pyrazolo[3,4-*d*]pyrimidine (28) (Method C).—A mixture of 490 mg (1.87 mmoles) of **25**, 100 ml of MeOH, and 100 mg of 10% Pd–C was shaken with H_2 at 2–3 atm until reduction was complete. The filtered solution was evaporated *in vacuo*. Recrystallization from MeOH gave nearly white crystals, mp 275–277°. See Table III for additional data and Tables III and V for additional compounds prepared by this method.

(31) All analytical samples moved as a single spot on tlc with Brinkmann silica gel GF in EtOAc–MeOH; each had ir and uv spectra in agreement with their assigned structures and gave combustion values within 0.4% of theoretical unless otherwise indicated. Melting points were taken in capillary tubes on a Mel-Temp block and those below 230° are corrected; the uv spectral data are given since they are more reliable than melting points for these compounds.

TABLE V

PHYSICAL PROPERTIES OF



No.	R	Method	% yield	Mp, °C dec	Formula	Analyses	λ_{max}, mμ[a] pH 1	λ_{max}, mμ[a] pH 13
7	$CH_2C_6H_4(NHCOC_6H_4SO_2F\text{-}p)\text{-}m$	F[b]	62[c]	250–252	$C_{19}H_{15}FN_6O_3S\cdot H_2O$	C, H, N	270	272
8	$C_6H_4(NHCOC_6H_4SO_2F\text{-}p)\text{-}m$	F[b]	58[c]	>340	$C_{18}H_{13}FN_6O_3S\cdot 0.5H_2O$	C, H, N	226, 294	237,[d] 305
9	$C_6H_4(NHCOC_6H_4SO_2F\text{-}m)\text{-}p$	F[b]	59[c]	>340	$C_{18}H_{13}FN_6O_3S\cdot 0.5H_2O$	C, H, N	320	252,[d] 323
10	$C_6H_4(NHCOC_6H_4SO_2F\text{-}p)\text{-}p$	F[b]	65[e]	>340	$C_{18}H_{13}FN_6O_3S\cdot H_2O$	C, H; F[f]	322	251,[d] 325
40	$C_6H_4NO_2\text{-}m$	E	78[c]	>350	$C_{11}H_8N_6O_2$	C, H, N	224, 288	237, 304
41	$C_6H_4NO_2\text{-}p$	E	47[c]	>350	$C_{11}H_8N_6O_2$	C. H, N	247, 265, 326	259, 369
42	$CH_2C_6H_4NO_2\text{-}m$	E	70[c]	307–309	$C_{12}H_{10}N_6O_2$	C, H, N	266	272
43	$C_6H_4NH_2\text{-}m$	C	85[g]	>340	$C_{11}H_{10}N_6\cdot 0.5H_2O$	C, H, N	226, 294	239,[d] 306
44	$C_6H_4NH_2\text{-}p$	C	76[c]	>340	$C_{11}H_{10}N_6\cdot 0.25H_2O$	C, H, N	226, 295	254, 318
45	$CH_2C_6H_4NH_2\text{-}m$	C	53[g]	242–245	$C_{12}H_{12}N_6\cdot H_2O$	C, H	267	276

[a] In 10% EtOH. [b] See method A, ref 2. [c] Recrystallized from MeOEtOH–H_2O. [d] Inflection. [e] Recrystallized from EtOH–H_2O. [f] F: calcd, 4.42; found, 3.86. [g] Recrystallized from DMSO–H_2O.

4,6-Diamino-5-(*m*-nitrobenzamido)pyrimidine (34) (Method D).—To a stirred mixture of 4.82 g (20 mmoles) of **31** sulfate on 40 ml of 1 *N* NaOH cooled in an ice bath was added dropwise a solution of 3.70 g (20 mmoles) of *m*-nitrobenzoyl chloride in 5 ml of dioxane over a period of 1 hr. The pH was maintained at 10–11 by addition of 1 *N* NaOH as needed. After being stirred for an additional 4 hr, the mixture was filtered and the product was washed with H_2O. Recrystallization from DMF–H_2O gave 1.75 g (32%) of pure product, mp >350°. See Table IV for additional data and other compounds prepared by this method.

8-(*m*-Nitrophenyl)adenine (40) (Method E).—To a mixture of 1.20 g of **34** and 12.5 g of P_2O_5 cooled in an ice bath was added 9 ml of 85% H_3PO_4. The mixture was heated in a bath at 165–170° for 1.5 hr, then cooled and poured into 20 ml of iced H_2O with stirring. The solution was adjusted to pH 8–9 with 4 *N* NaOH. The product was collected on a filter and washed with H_2O, then MeOH. Recrystallization from DMF–H_2O gave 0.60 g (55%) of pure product, mp >350°. See Table V for additional data and other compounds prepared by this method.

4-5-Diamino-6-(*o*-nitroanilino)pyrimidine (38).—A mixture of 500 mg (1.65 mmoles) of **32**, and 15 ml of 4 *N* NaOH was refluxed for 10 hr. The cooled suspension was filtered and the product was washed with H_2O. The solid was dissolved in 3 *N* H_2SO_4, then spin evaporated to a syrup *in vacuo*. The sulfate salt was collected on a filter and washed with Et_2O; yield 410 mg, mp 225–226°, that moved as a single spot on tlc. The salt was dissolved in H_2O and the free base precipitated by addition of 2 *N* NaOH. The product was collected on a filter and thoroughly washed with H_2O, then MeOH, and finally Et_2O; yield 260 mg (67%); mp 249–252° dec; λ_{max} (mμ) pH 1, 267, 401; pH 13, 280 (infl), 409. *Anal.* ($C_{10}H_{10}N_6O_2$) C, H, N.

The *para* isomer (**39**) was prepared similarly except that the sulfate salt was insoluble in cold 3 *N* H_2SO_4. The sulfate salt was collected by filtration and recrystallized from DMF–Et_2O; yield 270 mg (93%), mp 303–305° dec. *Anal.* ($C_{10}H_{10}N_6O_2\cdot 0.5H_2SO_4$) C, H. The free base was recrystallized from MeOEtOH–H_2O; yield 156 mg (64%); mp 328–331° dec; λ_{max} (mμ) pH 1, 261, 368; pH 13, 256, 382. *Anal.* ($C_{10}H_{10}N_6O_2$) C, H; N: calcd, 34.1; found, 33.5.

Irreversible Enzyme Inhibitors. CXXVI.[1,2] Hydrocarbon Interaction with Xanthine Oxidase by Phenyl Substituents on Purines and Pyrazolo[3,4-*d*]pyrimidines

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A hydrophobic bonding region exists on xanthine oxidase just adjacent to the active site that can complex aryl groups attached to purines and pyrazolo[3,4-*d*]pyrimidines. Inhibition by 57 purines and pyrazolo[3,4-*d*]-pyrimidines bearing polar groups or both polar and hydrophobic bonding groups was measured; no unifying theory emerged on the mode of binding of these heterocycles to xanthine oxidase, although it was established by several parameters that the heterocycles could bind in one of a number of rotomeric configurations depending upon the positions of polar and phenyl groups on the heterocycle. The three best reversible inhibitors of xanthine oxidase found in this study were 8-phenylhypoxanthine (**8**), 8-(*m*-nitrophenyl)adenine (**15**), and 6-(*m*-nitrophenyl)pyrazolo[3,4-*d*]pyrimidine (**42**), which were complexed 100–500-fold better than the substrate hypoxanthine (**5**) and 12–54-fold better than 4-hydroxypyrazolo[3,4-*d*]pyrimidine.

The Bergmann school has made extensive studies on the influence of substituents on the xanthine oxidase catalyzed oxidation of purines in order to elucidate the mode of binding of purines and the mechanism of action of the enzyme.[3] From their studies it was apparent that there were multiple modes of binding of purines to the enzyme depending upon the purine substituents. For example, hypoxanthine and 8-hydroxypurine were oxidized at the 2 position but 2-hydroxypurine was oxidized at the 8 position; in contrast, adenine was oxidized at the 8 position, but 2-amino- and 8-amino-

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(2) For the previous paper of this series see B. R. Baker and J. A. Kozma, *J. Med. Chem.*, **11**, 656 (1968).

(3) F. Bergmann, G. Levin, H. Kwietny-Gorvin, and H. Ungar, *Biochim. Biophys. Acta*, **47**, 1 (1961), and references therein.