crystals: mp 224–227 °C (lit.[11] mp 224 °C); NMR δ 8.84 (br s, $^+NH_3$), 8.25 (d, J = 8.4 Hz, 3,5-H), 7.80 (d, J = 8.4 Hz, 2,6-H), 4.16 (s, CH_2).

p-Aminobenzylamine. A solution of p-aminobenzonitrile (10.0 g, 0.085 mol) in dry ether (100 mL) was added to a stirred suspension of $LiAlH_4$ (6.42 g, 0.169 mol) in dry ether (200 mL) at such a rate that the reaction mixture refluxed gently. The greenish suspension was heated at reflux for 1.5 h and, after cooling to room temperature, ethyl acetate (50 mL) was added slowly to decompose excess $LiAlH_4$. A solution of NaOH (7.0 g, 0.175 mol) in H_2O (30 mL) was added, and the copious precipitate which developed was filtered with suction and washed with ethyl acetate (3 × 50 mL). The washings were combined with the filtrate and the solvent removed in vacuo. The remaining yellow paste was mixed with ethyl acetate (25 mL), and insoluble material was removed by filtration. The filtered material was washed with a further 10-mL portion of solvent, and the combined filtrates were concentrated in vacuo leaving a viscous oil. Distillation yielded 4.2 g (37%) of light yellow oil: bp 116–120 °C (0.4 mm) [lit.[12] bp 142–143 °C (10 mm)]; NMR δ 6.97 (d, J = 8.1 Hz, 2,6-H), 6.49 (d, J = 8.1 Hz, 3,5-H), 4.81 (br s, Ar-NH_2), 3.52 (s, CH_2), 1.89 (br s, -CNH_2). (A significant amount of residue from the distillation remained; it distilled over the range 170–250 °C (0.4 mm) and appeared (NMR) to be composed of polymeric materials.) p-Aminobenzylamine dihydrochloride was crystallized from ethanol and had mp >300 °C.

p-Acetamidobenzylamine Hydrochloride. Acetic anhydride (1.1 g, 0.0107 mol) was added to a solution of p-aminobenzylamine dihydrochloride (2.1 g, 0.0107 mol) in H_2O (50 mL). Sodium acetate trihydrate (1.5 g, 0.0107 mol) was added, and the reaction mixture was stirred at room temperature for 15 min. Excess concentrated HCl was added, and the solution was concentrated to ~15 mL and chilled yielding 1.8 g (86%) of nearly colorless product, which, after crystallization from 50% ethanol, had mp 261–263 °C: NMR δ 9.99 (s, CONH), 8.5 (br s, NH_3^+), 7.61 (d, J = 8.4 Hz, 3,5-H), 7.35 (d, J = 8.4 Hz, 2,6-H), 3.94 (s, CH_2), 2.04 (s, CH_3).

The free base was obtained by neutralizing an aqueous solution of the hydrochloride with sodium bicarbonate, evaporating to dryness, and extracting the residue with hot benzene. The solid remaining after evaporation of the solvent was crystallized from benzene giving p-acetamidobenzylamine as colorless crystals: mp 124–126 °C; NMR δ 9.79 (s, CONH), 7.49 (d, J = 8.5 Hz, 2.6-H), 7.20 (d, J = 8.5 Hz, 3,5-H), 3.63 (s, CH_2), 2.02 (s, CH_3). Anal. ($C_9H_{12}N_2O$) C, H, N.

6-(p-Acetamidobenzylamino)uracil (12). A mixture of 6-chlorouracil (0.5 g, 0.0034 mol), p-acetamidobenzylamine hydrochloride (1.4 g, 0.0068 mol), and sodium acetate trihydrate (0.7 g, 0.0051 mol) was heated at reflux in glyme (15 mL) for 17 h. The reaction mixture was chilled, and 0.6 g of light brown precipitate was filtered with suction. Concentration of the filtrate and addition of 10 mL of H_2O to the residue produced a further 0.3 g of solid. The solids were combined and crystallized from 50% acetic acid yielding 0.56 g (62%) of **12** as a nearly colorless product: mp 310–312 °C; NMR δ 10.11 (br s, 3-H), 9.89 (br s, 1-H and CONH), 7.56 (d, J = 8.4 Hz, 3′,5′-H), 7.22 (d, J = 8.4 Hz, 2′,6′-H), 6.50 (t, J = 5.3 Hz, 6-NH), 4.40 (s, 5-H), 4.19 (d, J = 5.3 Hz, CH_2), 2.03 (s, CH_3). Anal. ($C_{13}H_{14}N_4O_3 \cdot H_2O$) C, H, N.

6-(p-Aminobenzylamino)uracil (13). A solution of **13** (0.417 g, 0.00152 mol) in 2.5 N NaOH (15 mL) was heated at reflux for 1 h. The solution was brought to pH 7 with concentrated HCl and chilled producing a yellowish precipitate. This was isolated by filtration and crystallized from H_2O giving 0.19 g (54%) of light yellow solid: mp >320 °C; NMR δ 10.09 (br s, 1.3-H), 6.98 (d, J = 8.4 Hz, 2′,6′-H), 6.53 (d, J = 8.4 Hz, 3′,5′-H), 6.38 (s, 6-NH), 5.85 (br s, NH_2), 4.42 (s, 5-H), 4.01 (s, CH_2). Anal. ($C_{11}H_{12}N_4O_2 \cdot 2H_2O$) C, H, N.

Reaction of p-Aminobenzylamine with 3-Methyl-6-aminouracil. A mixture of p-aminobenzylamine (0.9 g, 0.007 mol), 3-methyl-6-aminouracil (0.5 g, 0.0035 mol), and acetic acid (0.4 g, 0.007 mol) was heated at reflux in glyme (15 mL) for 6 h. The solvent was removed in vacuo, H_2O (5 mL) was added to the residue, and, after chilling, a brownish precipitate developed. Crystallization from 50% ethanol gave 0.36 g (45%) of **20** as a nearly colorless solid: mp 258–260 °C; NMR δ 10.13 (br s, 1 H), 6.88 (d, 2 H, J = 8.4 Hz), 6.42 (d, 2 H, J = 8.4 Hz), 5.84 (br s, 2 H), 4.69 (br s, 2 H), 3.35 (s, 2 H), 3.06 (s, 3 H). Anal. Calcd for $C_{12}H_{14}N_4O_2 \cdot H_2O$: C, 54.53; H, 6.10; N, 21.20. Found: C, 54.72; H, 5.99; N, 21.33.

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References and Notes

(1) G. E. Wright and N. C. Brown, *J. Med. Chem.*, preceding paper in this issue.
(2) J. M. Mackenzie, M. M. Neville, G. E. Wright, and N. C. Brown, *Proc. Natl. Acad. Sci. U.S.A.*, **70**, 512 (1973).
(3) G. E. Wright and N. C. Brown, *J. Med. Chem.*, **17**, 1277 (1974).
(4) B. R. Baker and W. Rzeszotarski, *J. Med. Chem.*, **10**, 1109 (1967).
(5) B. R. Baker and W. Rzeszotarski, *J. Med. Chem.*, 11, 639 (1968).
(6) N. C. Brown and G. E. Wright in "Symposium on Drug Action at the Molecular Level", G. C. K. Roberts, Ed., Macmillan, New York, N.Y., in press.
(7) K. B. Gass and N. R. Cozzarelli, *J. Biol. Chem.*, **248**, 7688 (1973).
(8) G. E. Wright, *J. Heterocycl. Chem.*, **13**, 539 (1976).
(9) H. H. Fox, *J. Org. Chem.*, **13**, 438 (1948).
(10) H. Salkowski, *Ber.*, **22**, 2137 (1889).
(11) E. L. Holmes and C. K. Ingold, *J. Chem. Soc.*, 1800 (1925).
(12) N. Kornblum and D. C. Iffland, *J. Am. Chem. Soc.*, **71**, 2137 (1949).

2-Pyridylimidazoles as Inhibitors of Xanthine Oxidase

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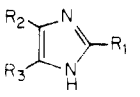
A series of 28 4-substituted and 4,5-disubstituted 2-pyridylimidazoles was synthesized and evaluated in vitro for inhibition of xanthine oxidase. Included within this group are examples of 2-pyridylimidazopyridines and halo-substituted 2-pyridylbenzimidazoles. Five compounds exhibited inhibitory activity in the same range as the standards, 4-hydroxypyrazolo[3,4-*d*]pyrimidine and 2-(4-pyridyl)-4-trifluoromethylimidazole (**22**). Two examples, 2-(4-pyridyl)-4,5-dicyanoimidazole (**16**) and 2-(4-pyridyl)-4-nitroimidazole (**3**), were at least an order of magnitude more active than the standards and therefore rank among the most potent known inhibitors of the enzyme.

Various 2-aryl-4(5)-trifluoromethylimidazoles have been reported to be in vitro inhibitors of the enzyme xanthine oxidase.[1] These compounds resulted from a search for specific inhibitors of the enzyme which were unrelated to

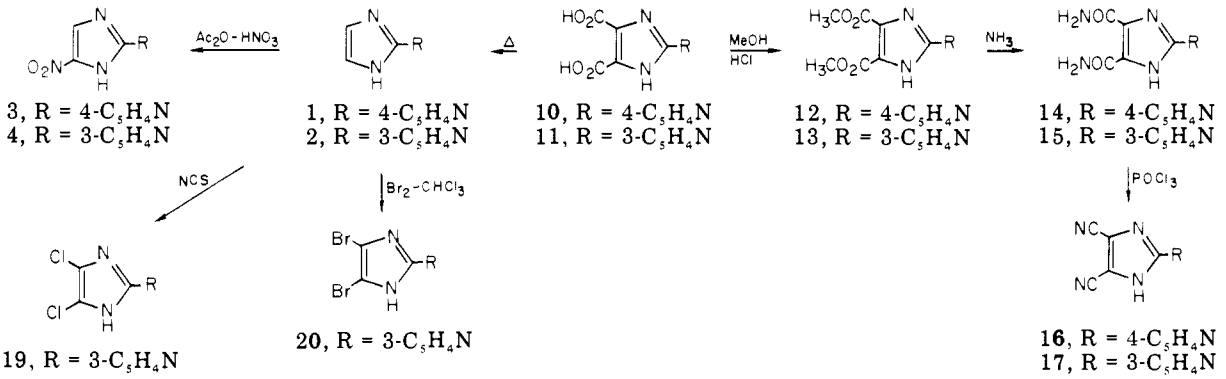
Table I. 2-Pyridylimidazoles



No.	R_1	R_2	R_3	Crystn solvent	Mp, °C	Yield, %	Formula	Analyses	I_{50}, X.O. inhibn[d]
1	4-Pyridyl	H	H		206-208	74.6	$C_8H_7N_3$	C, H, N	Inact.
2	3-Pyridyl	H	H		206-207	77	$C_8H_7N_3$	C, H, N	Inact.
3	4-Pyridyl	H	NO_2	MeOH-H_2O	>300	25	$C_8H_6N_4O_2$	C, H, N	3.5×10^{-7} M
4	3-Pyridyl	H	NO_2	MeOH-H_2O	280-282	11	$C_8H_6N_4O_2$	C, H, N	1.3×10^{-6} M
5	4-Pyridyl	H	CH_3	H_3CCN	154-156	12	$C_9H_9N_3$	C, H, N	Inact.
6	3-Pyridyl	H	CH_3	H_3CCN	166-167	15	$C_9H_9N_3$	C, H, N	Inact.
7[c]	4-Pyridyl	H	CO_2H						34% at 2×10^{-5} M[a]
8	4-Pyridyl	H	CO_2CH_3	H_2O	219-220	68.4	$C_{10}H_9N_3O_2$	C, H, N	10% at 2×10^{-5} M[a]
9	4-Pyridyl	H	CN	H_2O-H_3CCN	295-297	45	$C_9H_6N_4$	C, H, N	5.6×10^{-6} M
10	4-Pyridyl	CO_2H	CO_2H		308	34.8	$C_{10}H_7N_3O_4$	C, H, N	5.8×10^{-9} M
11	3-Pyridyl	CO_2H	CO_2H		302-303	40	$C_{10}H_7N_3O_4$	C, H, N	1×10^{-6} M
12	4-Pyridyl	CO_2CH_3	CO_2CH_3	H_3CCN	196	76.9	$C_{12}H_{11}N_3O_4$	C, H, N	Inact.
13	3-Pyridyl	CO_2CH_3	CO_2CH_3	H_2O	212-214	57	$C_{12}H_{11}N_3O_4$	C, H, N	Inact.
14	4-Pyridyl	$CONH_2$	$CONH_2$	DMF-H_2O	>300	70.6	$C_{10}H_9N_5O_2$	C, H, N	14% at 2×10^{-5} M[a]
15	3-Pyridyl	$CONH_2$	$CONH_2$	DMF-H_2O	>300	53	$C_{10}H_9N_5O_2$	C, H, N	Inact.
16	4-Pyridyl	CN	CN	H_2O	>300	24	$C_{10}H_5N_5$	C, H, N	2×10^{-8} M
17	3-Pyridyl	CN	CN	MeOH-H_2O	300-305	40	$C_{10}H_5N_5$	b	3.5×10^{-5} M
18	4-Pyridyl	CN	$CONH_2$	H_3CCN-H_2O	>300	12.9	$C_{10}H_7N_5O$	C, H, N	5×10^{-7} M
19	3-Pyridyl	Cl	Cl	H_3CCN	237-238	18.7	$C_8H_5Cl_2N_3$	C, H, N	29% at 2×10^{-5} M[a]
20	3-Pyridyl	Br	Br	H_3CCN	226-227	23.2	$C_8H_5Br_2N_3$	C, H, N	2×10^{-5} M
21[c]	4-Pyridyl	H	CF_3						2×10^{-6} M
22[c]	3-Pyridyl	H	CF_3						4×10^{-5} M

[a] Inhibition was determined at only 2×10^{-5} M. [b] Anal. Calcd: C, 61.53; H, 2.58; N, 35.88. Found: C, 60.86; H, 2.67; N, 35.66. [c] See ref 1. [d] X.O. = xanthine oxidase.

Scheme I



the purine type as exemplified by allopurinol, 4-hydroxypyrazolo[3,4-*d*]pyrimidine, since inhibitors of this latter type may be converted to nucleotides having antimetabolite activity.[2-8]

In order to further explore the structural requirements for enzymatic inhibitory activity among the 2-arylimidazoles, an investigation was initiated to evaluate the effect of substituents, other than trifluoromethyl, on biological activity. Since the preferred aryl substituent in this series was pyridyl,[1] the nuclei chosen as the bases for the study were 2-(3-pyridyl) and 2-(4-pyridyl)imidazole. Substituents differing in electronegativity were incorporated into either the 4 or the 4 and 5 positions of the imidazole ring. Selected fused ring analogues of the benzimidazole and imidazopyridine types were also prepared and evaluated as examples of the 4,5-disubstituted imidazole class.

Chemistry. The 4(5)-monosubstituted and 4,5-disubstituted derivatives of the 2-pyridylimidazoles, 1 and 2, prepared during this study, are presented in Table I.

As outlined in Scheme I, the imidazole-4,5-dicarboxylic acids, **10** and **11**, prepared by the Maquenne procedure,[9] served as convenient starting materials. Thermal decarboxylation of **10** and **11** yielded the 2-pyridylimidazoles, **1** and **2**. Nitration of **1** and **2** with nitric acid in acetic anhydride[10] yielded the mononitro derivatives, **3** and **4**.

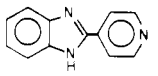
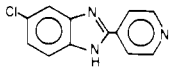
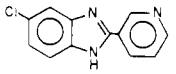
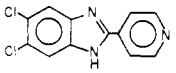
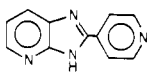
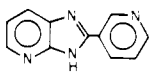
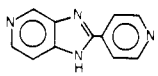
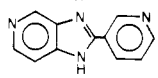
Bromination of **2** with bromine in chloroform gave **20** while chlorination with *N*-chlorosuccinimide (NCS) yielded **19**. Unlike **2**, 2-(4-pyridyl)imidazole (**1**) failed to either chlorinate or brominate satisfactorily under similar reaction conditions.

Esterification of the dicarboxylic acids, **10** and **11**, under standard acid-catalyzed conditions gave the diesters, **12** and **13**. Ammonolysis of the esters yielded the diamides, **14** and **15**, which were converted to the dinitriles, **16** and **17**, on treatment with phosphorus oxychloride. With **14**, under milder conditions, the nitrile amide, **18**, was obtained as the principal product. The corresponding nitrile amide, derivable from **15**, was a minor contaminant in the dinitrile **17**.

The 4-methylimidazoles, **5** and **6**, were prepared by the Weidenhagen modification of the Radziszewski procedure[11] involving the reaction of the pyridinealdehydes with ammonia and acetoxyacetone in the presence of cupric acetate.

The 4-trifluoromethylimidazole, **21**, served as the

Table II. Pyridylbenzimidazoles and Imidazopyridines

No.	Compd	Crystn solvent	Mp, °C	Yield, %	Formula	Analyses	I_{50}, X.O. inhibn
23[b]							Inact.
24		EtOH-H_2O	306-307	49.1	$C_{12}H_8ClN_3$	C, H, N	11% at 2 × 10^{-5} M[a]
25		H_3CCN-EtOH	244-246	52.4	$C_{12}H_8ClN_3$	C, H, N	Inact.
26		EtOH-H_2O	329-330	13.3	$C_{12}H_7Cl_2N_3$	C, H, N	1.5 × 10^{-5} M
27		H_3CCN-H_2O	297	11.2	$C_{11}H_8N_4$	C, H, N	Inact.
28		MeOH-H_2O	284	15.3	$C_{11}H_8N_4$	C, H, N	Inact.
29		H_3CCN-H_2O	285-286	15.3	$C_{11}H_8N_4$	C, H, N	1.3 × 10^{-5} M
30		H_3CCN-H_2O	247-249	20.4	$C_{11}H_8N_4$	C, H, N	23% at 2 × 10^{-5} M[a]
	Allopurinol						3 × 10^{-6} M

[a] Inhibition was determined at only 2 × 10^{-5} M. [b] See ref 12.

starting point for the synthesis of the cyano derivative, **9**. The intermediate imidazole-4(5)-carboxylic acid, **7**, was prepared by the previously reported basic hydrolysis of **21**.[1] Similar treatment of the 3-pyridyl analogue, **22**, resulted in extensive decomposition and failed to yield the corresponding 2-(3-pyridyl)imidazole-4(5)-carboxylic acid. Esterification of the acid, **7**, gave the methyl ester, **8**; ammonolysis of **8** yielded the amide which was converted without purification to the nitrile, **9**.

The fused ring analogues, presented in Table II, were obtained through the reaction of *o*-diamines with nicotinic and isonicotinic acids. The benzimidazole, **23**, was prepared according to the procedure of Bastic[12] involving the reaction of *o*-phenylenediamine with isonicotinic acid at elevated temperature. Similarly, treatment of either 2,3-diaminopyridine or 3,4-diaminopyridine with nicotinic or isonicotinic acids at elevated temperatures gave the imidazopyridines, **27–30**. Attempts to prepare halogenated derivatives of **23** under similar conditions failed; however, in the presence of polyphosphoric acid,[13] benzimidazole formation occurred and compounds **24–26** were obtained.

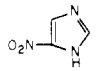
Product yields, as listed in Tables I and II, are based on analytically pure material and no attempts were made to optimize reaction conditions.

Representative apparent pK_a values of selected examples are recorded in Table III. These values were obtained by potentiometric titration in 30% EtOH-H_2O; all examples studied were weak acids being half-ionized in the pH range of 9–11.

Discussion

It has been previously determined that 4-trifluoromethylimidazoles require a 2-aryl or heteroaryl substituent and an imino hydrogen for maximum xanthine oxidase inhibitory activity.[1] In an extension of this work the effect of substituents in the 4 or 4 and 5 positions of the imidazole ring on intrinsic activity has been examined. As indicated by the I_{50} values listed in Tables I and II, the nature of these substituents can exert a significant influence on intrinsic activity. The introduction of a nitro group as in **3** or two cyano substituents as in **16** yields

Table III. Apparent pK_a

Compd	Apparent pK_a[a]		I_{50}, X.O. inhibn
12	3.98[b]	7.15[c]	Inact.
21	3.76[b]	9.73[c]	2 × 10^{-6} M
22	3.50[b]	10.21[c]	4 × 10^{-5} M
23	3.85[b]	10.95[c]	Inact.
24	3.50[b]	10.00[c]	11% at 2 × 10^{-5} M
26	3.00[b]	9.33[c]	1.5 × 10^{-5} M
		9[c]	Inact.

[a] Apparent pK_a values were determined by potentiometric titration in 30% EtOH-H_2O. [b] Proton gained. [c] Proton lost.

compounds having inhibitory activity one to two orders of magnitude greater than that of either allopurinol or the trifluoromethylimidazole, **21**. Substitution of the trifluoromethyl moiety in **21** with a cyano group yields a compound, **9**, of equivalent potency, whereas replacement with hydrogen, methyl, or carbomethoxy significantly reduces inhibitory activity.

Of the mono- and disubstituted 2-pyridylimidazoles described, those compounds having a 2-(4-pyridyl) substituent generally exhibited greater inhibitory activity in the in vitro system than the corresponding 2-(3-pyridyl) analogues. This may result from the difference in the apparent pK_a of the imino hydrogen. As seen in Table III, the apparent pK_a values of **21** and **22** differ by half a pK_a unit, the 4-pyridyl derivative being the more acidic.

Among the monosubstituted examples, **1–9**, intrinsic activity increased as the electronegativity of the substituent increased. The unsubstituted and 4-methyl derivatives, examples **1**, **2**, **5**, and **6**, were devoid of significant activity while those compounds bearing highly electronegative substituents such as cyano and nitro groups were the most potent inhibitors of the enzyme. Since the ester **8** was less active than might be expected, the molecular radius of the substituent may also play a critical role as a determinant of activity. This steric effect may be more clearly seen in

the case of 4,5-disubstituted examples.

Such disubstitution was not only allowed with groups having small molecular radii, such as carboxy and cyano, but the intrinsic activity was increased from 1 to 3 log units. When two bulky groups were present, such as the diesters and diamides, **12–15**, the compounds were virtually inactive at a concentration of 2×10^{-5} M. As previously mentioned, steric restraints may be imposed upon the 4-substituent. Increasing bulk may result in decreasing activity; this effect is either intensified by the introduction of a second bulky group or alleviated by the introduction of a small electron-withdrawing moiety as seen in example **18**.

The presence of an acidic imino hydrogen having an apparent pK_a below 10 appears to be a requirement for inhibitory activity. The top of this apparent pK_a range was bracketed by the benzimidazole derivatives, **23**, **24**, and **26**. The unsubstituted pyridylbenzimidazole **23** with an apparent pK_a above 10 was inactive while both **24** and **26** with apparent pK_a values at and below 10 possessed measurable inhibitory activity.

An aryl substituent in the 2 position is also critical. This is most clearly illustrated with 4-nitroimidazole; this compound has an apparent pK_a of 9, yet is inactive at a concentration of 2×10^{-5} M. The introduction of a pyridyl substituent into the 2 position yields compounds **3** and **4** which are highly potent inhibitors. This enhancing effect of the aryl substituent may indicate the presence of a hydrophobic binding region on the enzymatic surface. Such a hydrophobic site near the active center of xanthine oxidase has been suggested by Baker and Wood[14,15] based on studies with 9-arylpurines.

With inhibitors of the 2-arylimidazole type, it is possible that binding with the enzyme occurs at two points, at a hydrophobic site and at a center capable of hydrogen bonding with the weakly acidic imino hydrogen. Although it is not as yet established that inhibitors of the imidazole class interact with the active site, the similarity between the structural requirements for activity and those found by Baker and Wood together with the acidic nature of the natural substrate, xanthine,[16] suggest that binding at or near the active site is a likely possibility.

In summary, the intrinsic xanthine oxidase inhibitory activity of 2-pyridylimidazoles is profoundly influenced by the substituents present in the 4 or 4 and 5 positions of the imidazole ring. The introduction of small, electronegative groups in these positions yields compounds such as **3**, **17**, and **18** which are up to two orders of magnitude more active than allopurinol and rank among the most potent known inhibitors of the enzyme.

Experimental Section

IR spectra were obtained on a Perkin-Elmer Model 137; NMR spectra were obtained on a Varian A-60 or Varian T-60. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Where analyses are indicated only by symbols of the elements, analytical results obtained are within 0.4% of the theoretical values.

Preparation of 2-Pyridylimidazole-4,5-dicarboxylic Acids. The preparation of 2-(4-pyridyl)imidazole-4,5-dicarboxylic acid (**10**) is presented as an example; the 2-(3-pyridyl) derivative, **11**, was obtained by essentially the same procedure and had IR (Nujol) 3150 and 1550 cm^{-1}.

H_2SO_4 (200 mL) was added dropwise to a solution of *d*-tartaric acid (50 g, 0.33 mol) in HNO_3 (108 mL). HNO_3 (90%, 108 mL) was then added while maintaining the mixture at 38 °C. The reaction was stirred 2 h at 0 °C and the resulting tartaric acid dinitrate filtered and added with stirring to crushed ice (400 g). Concentrated NH_4OH (300 mL) was added at −5 to −10 °C. Pyridine-4-carboxaldehyde (40 g, 0.37 mol) was added and the mixture allowed to warm to 27 °C over 16 h. The resulting solid was filtered and dissolved in H_2O and the solution acidified with HCl to yield 30 g of **10**: IR (Nujol) 3400, 3100, and 1550 cm^{-1}.

Preparation of 2-Pyridylimidazoles. The preparation of 2-(3-pyridyl)imidazole (**2**) is presented as an example; the corresponding 4-pyridyl analogue, **1**,[17] was obtained by essentially the same procedure and had IR (Nujol) 3350, 1610, and 1575 cm^{-1}; NMR (Me_2SO-d_6) δ 8.63 (d, 2 H), 8.00 (d, 2 H), and 7.37 (s, 2 H).

The diacid **11** (23 g, 0.098 mol) was heated at 290–310 °C under reduced pressure (30 mm). After the evolution of CO_2 had ceased, the residue was sublimed at 190–210 °C at 0.5 mm and resublimed at the same temperature and pressure to yield 11 g of **2** (lit.[18] 196–198 °C): NMR (Me_2SO-d_6) δ 9.18 (d, 1 H), 8.55 (dd, 1 H), 8.28 (m, 1 H), 7.45 (dd, 1 H), and 7.2 (s, 2 H).

Preparation of 4(5)-Methyl-2-pyridylimidazoles. The preparation of 4(5)-methyl-2-(4-pyridyl)imidazole (**5**) is presented as an example; the corresponding 3-pyridyl analogue, **6**, was obtained by essentially the same procedure and had NMR ($CDCl_3$) δ 9.12 (d, 1 H), 8.45 (dd, 1 H), 8.20 (m, 1 H), 7.23 (dd, 1 H), 6.87 (s, 1 H), and 2.23 (s, 3 H).

A solution of α-acetoxyacetone (3.5 g, 0.03 mol), $Cu(OAc)_2 \cdot H_2O$ (12 g, 0.06 mol), and pyridine-4-carboxaldehyde (3.2 g, 0.03 mol) in concentrated NH_4OH (75 mL) and MeOH (75 mL) was heated 3 h at reflux. After cooling to room temperature, the precipitated solid was removed by filtration and suspended in H_2O at 80 °C. H_2S was bubbled into the suspension for 1 h. After filtration, the filtrate was saturated with Na_2CO_3 and extracted with $CHCl_3$. The $CHCl_3$ extract was dried over Na_2SO_4 and concentrated to dryness under reduced pressure. The resulting residue was chromatographed on alumina, activity grade II, and eluted with $CHCl_3$. After recrystallization from H_3CCN, 0.55 g of **5** was obtained: NMR ($CDCl_3$) δ 8.50 (m, 2 H), 7.78 (d, 2 H), 6.9 (s, 1 H), and 2.25 (s, 3 H).

Preparation of 2-(4-Pyridyl)-4(5)-nitroimidazole. The preparation of 2-(4-pyridyl)-4(5)-nitroimidazole (**3**) is presented as an example; the 2-(3-pyridyl) derivative, **4**, was obtained by essentially the same procedure and had IR (KBr) 3450, 3130, 1520, and 1320 cm^{-1}; NMR (Me_2SO-d_6) δ 9.16 (br s, 1 H), 8.65 (dd, 1 H), 8.50 (s, 1 H), 8.33 (m, 1 H), and 7.50 (dd, 1 H).

A suspension of **1** (3.0 g, 0.02 mol) in $(CH_3CO)_2O$ (45 mL) was warmed until solution was obtained and then cooled to 30 °C. A solution of fuming HNO_3 (90%, 1.8 mL) in $(CH_3CO)_2O$ (6 mL) was added dropwise with stirring over 10 min. After stirring 5 min at room temperature, the reaction mixture was heated to 80 °C at which point the reaction became exothermic and the temperature rose to 110 °C. The reaction mixture was cooled to room temperature, poured onto ice, and neutralized with 40% NaOH solution. A solid was obtained and chromatographed on silica gel using 9:1 $CHCl_3$–MeOH as eluent. After recrystallization from MeOH–H_2O, 1 g of **3** was obtained: IR (KBr) 3450, 3155, 1520, and 1360 cm^{-1}; NMR (Me_2SO-d_6) δ 8.77 (m, 2 H), 8.62 (s, 1 H), and 8.00 (d, 2 H).

Preparation of Dimethyl 2-Pyridylimidazole-4,5-dicarboxylate. The preparation of dimethyl 2-(4-pyridyl)-imidazole-4,5-dicarboxylate (**12**) is presented as an example; the 2-(3-pyridyl) derivative, **13**, was obtained by essentially the same procedure and had IR (Nujol) 3400, 1740, and 1700 cm^{-1}; NMR (Me_2SO-d_6) δ 9.37 (br d, 1 H), 8.73 (dd, 1 H), 8.55 (m, 1 H), 7.60 (dd, 1 H), and 4.0 (s, 6 H).

HCl gas was bubbled into a suspension of **10** (30 g, 0.13 mol) in MeOH (2.2 L) at reflux for 1 h. After an additional hour of reflux, the reaction mixture was concentrated to a solid and dissolved in H_2O (500 mL) and the resulting diester precipitated by the addition of saturated $NaHCO_3$ solution. After recrystallization from H_3CCN, 27 g of **12** was obtained: NMR (Me_2SO-d_6) δ 8.55 (d, 2 H), 8.03 (d, 2 H), and 3.90 (s, 6 H).

Preparation of 2-Pyridylimidazole-4,5-dicarboxamide. The preparation of 2-(4-pyridyl)-4,5-dicarboxamide (**14**) is presented as an example; the 2-(3-pyridyl) derivative, **15**, was obtained by essentially the same procedure and had IR (Nujol) 3175, 1680, 1630, and 1610 cm^{-1}; NMR (Me_2SO-d_6) δ 9.00 (br d, 1 H), 8.17 (m, 2 H), and 7.17 (dd, 1 H).

A mixture of **12** (10 g, 0.038 mol) and NH_3 (15 g) in MeOH (200 mL) was heated 18 h at 150 °C. The resulting solid was filtered and recrystallized from DMF–H_2O to yield 6.2 g of **14**; IR (Nujol) 3400, 3200, 3050, 1650, and 1600 cm^{-1}; NMR (Me_2SO-d_6) δ 8.66 (br s, 2 H) and 8.10 (d, 2 H).

Preparation of 4,5-Dicyano-2-pyridylimidazoles. The preparation of 4,5-dicyano-2-(4-pyridyl)imidazole (**16**) is presented as an example; the 2-(3-pyridyl) derivative, **17**, was obtained by essentially the same procedure and had IR (Nujol) 3300 and 2230 cm^{-1}; NMR (Me_2SO-d_6) δ 9.23 (br d, 1 H), 8.65 (m, 2 H), and 7.77 (dd, 1 H).

A suspension of **14** (15 g, 0.065 mol) in $POCl_3$ (200 mL) was refluxed for 8 h. The excess $POCl_3$ was removed under reduced pressure; the resulting residue was dissolved in H_2O and neutralized with NH_4OH to yield 12.5 g of **16**. The solid was chromatographed on silica gel and eluted with 9:1 $CHCl_3$–MeOH and recrystallized from H_2O to yield 3 g of **16**: IR (Nujol) 3200, 2230, 1640, and 1600 cm^{-1}; NMR (Me_2SO-d_6) δ 8.66 (br s, 2 H) and 8.10 (d, 2 H).

4(5)-Cyano-2-(4-pyridyl)-5(4)-imidazolecarboxamide (18). A suspension of **14** (5 g, 0.02 mol) in $POCl_3$ (70 mL) was heated 5 h at reflux. The resulting solution was concentrated under reduced pressure to afford a residue which was dissolved in H_2O and neutralized with NH_4OH. The resulting solid was chromatographed on silica gel and eluted with 9:1 $CHCl_3$–MeOH to yield after recrystallization from H_3CCN–H_2O 550 mg of **18**: IR (Nujol) 3450, 3300, 3200, 2230, 1680, and 1600 cm^{-1}.

Methyl 2-(4-Pyridyl)imidazole-4(5)-carboxylate (8). HCl gas was introduced into a solution of **7** (3.5 g, 0.018 mol) in MeOH (150 mL) at reflux for 1 h. After cooling to room temperature, the reaction mixture was concentrated under reduced pressure to a solid. The residue was recrystallized from H_2O to yield 2.5 g of **8**: IR (Nujol) 3150, 1690, and 1610 cm^{-1}; NMR (Me_2SO-d_6) δ 8.77 (d, 2 H), 8.10 (s, 1 H), 7.95 (d, 2 H), and 3.57 (s, 3 H).

2-(4-Pyridyl)-4(5)-cyanoimidazole (9). **8** (20 g, 0.1 mol) and NH_3 (105 g) in MeOH (500 mL) were heated for 24 h at 120 °C. The reaction mixture was concentrated under reduced pressure to a solid which after recrystallization from H_3CCN–H_2O yielded 13.2 g of the amide, 2-(4-pyridyl)-4(5)-imidazolecarboxamide (70.2%), mp 256–260 °C.

A suspension of the amide (8.1 g, 0.043 mol) in $POCl_3$ (80 mL) was heated 5 h at reflux. The excess $POCl_3$ was removed under reduced pressure and aqueous $NaHCO_3$ solution was added to the residue; the resulting solid was filtered and recrystallized from H_2O–H_3CCN to yield 3.3 g of **9**: IR (Nujol) 3150, 2230, and 1620 cm^{-1}; NMR (Me_2SO-d_6) δ 8.75 (d, 2 H), 8.40 (s, 1 H), and 7.87 (d, 2 H).

2-(3-Pyridyl)-4,5-dichloroimidazole (19). To a suspension of **2** (1.5 g, 0.01 mol) in $CHCl_3$ (150 mL) was added *N*-chlorosuccinimide (2.6 g, 0.02 mol) portionwise with stirring at reflux over 1 h. After 3 h at reflux, the $CHCl_3$ was removed under reduced pressure; the residue was triturated with H_2O and filtered. After recrystallization from H_3CCN, 400 mg of **19** was obtained: IR (Nujol) 1620, 1560, and 1500 cm^{-1}; NMR (Me_2SO-d_6) δ 9.07 (br d, 1 H), 8.60 (dd, 1 H), 8.23 (m, 1 H), and 7.50 (dd, 1 H).

2-(3-Pyridyl)-4,5-dibromoimidazole (20). To a suspension of **2** (1.5 g, 0.01 mol) in $CHCl_3$ (150 mL) was added Br_2 (3.2 g, 0.02 mol) in $CHCl_3$ (5 mL) dropwise with stirring at room temperature. After 2 h, the $CHCl_3$ was removed by decantation and the residual material was triturated with H_2O (25 mL) containing $NaHSO_3$ (1 g). The resulting solid was filtered and recrystallized from H_3CCN to yield 0.7 g of **20**: IR (Nujol) 1600, 1540, and 1480 cm^{-1}; NMR (Me_2SO-d_6) δ 9.13 (br d, 1 H), 8.67 (dd, 1 H), 8.30 (m, 1 H), and 7.60 (dd, 1 H).

Preparation of 5-Chloro- and 5,6-Dichloro-2-pyridylbenzimidazoles. The preparation of **24** is presented as an example; the 5-chloro derivative **25**[19] and the 5,6-dichloro analogue **26** were prepared by essentially the same process. The 5-chloro-2-(3-pyridyl)benzimidazole (**25**) had IR (Nujol) 1580 and 1530 cm^{-1} and NMR (Me_2SO-d_6) δ 9.33 (d, 1 H), 8.67 (dd, 1 H), 8.47 (m, 3 H), 7.60 (m, 2 H), and 7.23 (dd, 1 H); and 5,6-dichloro-2-(4-pyridyl)benzimidazole (**26**) had IR (Nujol) 3200 and 1620 cm^{-1} and NMR (Me_2SO-d_6) δ 8.97 (d, 2 H), 8.00 (d, 2 H), and 7.87 (s, 2 H).

To a mixture of isonicotinic acid (4.9 g, 0.04 mol) and 4-chloro-o-phenylenediamine (5.8 g, 0.041 mol) was added polyphosphoric acid (20 mL). The mixture was heated to 200 °C and maintained at this temperature for 45 min. After cooling, the reaction mixture was poured onto ice and the solution made basic with concentrated NH_4OH. The resulting yellow solid was removed by filtration, dissolved in 2-propanol, and filtered and the filtrate was concentrated to a solid. After recrystallization from EtOH–H_2O, 4.5 g of **24** was obtained: IR (Nujol) 1700 and 1610 cm^{-1}; NMR (Me_2SO-d_6) δ 8.83 (d, 2 H), 8.13 (d, 2 H), 7.70 (m, 2 H), and 7.30 (m, 1 H).

Preparation of 2-Pyridylimidazopyridines. The preparation of 2-(3-pyridyl)-1*H*-imidazo[4,5-*b*]pyridine (**28**) is presented as an example; compounds **27**, **29**, and **30** were prepared by essentially the same procedure. Compound **27** had IR (Nujol) 1620, 1590, 1570, 1550, 1320, and 1280 cm^{-1}; NMR (Me_2SO-d_6) δ 8.77 (d, 2 H), 8.38 (dd, 1 H), 8.10 (m, 3 H), and 7.30 (dd, 1 H). Compound **29** had IR (Nujol) 1640, 1620, 1600, 1560, 1540, 1290, and 1210 cm^{-1}. Compound **30** had IR (Nujol) 3400, 1640, 1600, 1290, and 1225 cm^{-1}.

A mixture of 2,3-diaminopyridine (5.4 g, 0.05 mol) and nicotinic acid (6.1 g, 0.05 mol) was heated at 240 °C for 30 min. The resulting dark melt was allowed to cool and then recrystallized four times from MeOH–H_2O to yield 1.5 g of **28**: IR (Nujol) 1640, 1610, 1580, 1545, 1320, and 1280 cm^{-1}.

Enzyme Assay. For enzyme inhibition measurements, reactions were started by addition of enzyme (typically 1.0 mL of 1:300 dilution of Worthington XOP grade bovine xanthine oxidase to produce ΔOD_{292} of 0.09–0.12 min^{-1}) to 2.0 mL of 0.05 M phosphate buffer, pH 7.5, containing hypoxanthine at 3×10^{-5} M and inhibitor. I_{50} values were calculated from log concentration vs. inhibition lines for a minimum of three inhibitor concentrations. When inhibition at 2×10^{-5} M was less than 10%, inhibitors are designated as "inactive".

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References and Notes

(1) J. J. Baldwin, P. A. Kasinger, F. C. Novello, J. M. Sprague, and D. E. Duggan, *J. Med. Chem.*, **18**, 895 (1975).
(2) H. G. Mandel, *Pharmacol. Rev.*, 11, 743 (1959).
(3) A. Hampton and A. R. P. Peterson, *Biochim. Biophys. Acta*, 114, 185 (1966).
(4) R. J. McCollister, W. R. Gilbert, D. M. Ashton, and J. B. Wyngaarden, *J. Biol. Chem.*, **239**, 1560 (1964).
(5) T. D. Beardmore and W. N. Relley, *Adv. Exp. Med. Biol.*, **41B**, 609 (1974).
(6) F. Delbarre, C. Auscher, A. Degery, and J. L. Olivier, *Presse Med.*, **76**, 2329 (1968).
(7) T. A. Krenitsky, G. B. Elion, R. A. Strelitz, and G. H. Hitchings, *J. Biol. Chem.*, **242**, 2675 (1967).
(8) R. W. Rundles, J. B. Wyngaarden, G. H. Hitchings, and G. B. Elion, *Annu. Rev. Pharmacol.*, **9**, 345 (1969).
(9) M. Maquenne, *Ann. Chim. Phys.*, **24**, 522 (1891).
(10) G. Powell and F. R. Johnson, "Organic Syntheses", Collect. Vol. II, Wiley, New York, N.Y., 1948, p 449.
(11) R. Weidenhagen and R. Herrmann, *Ber.*, **68**, 1953 (1935).
(12) B. L. Bastic, *Glas. Hem. Drus., Beograd.*, **16**, 141 (1951).
(13) D. W. Hein, R. G. Alheim, and J. J. Lewvitt, *J. Am. Chem. Soc.*, **79**, 427 (1957).
(14) (a) B. R. Baker and W. F. Wood, *J. Med. Chem.*, **10**, 1101 (1967); (b) *ibid.*, 11, 644 (1968).
(15) B. R. Baker, W. F. Wood, and J. D. Kuzma, *J. Med. Chem.*, 11, 661 (1968).
(16) G. Kortum, W. Vogel, and K. Andrussow, "Dissociation Constants of Organic Acids in Aqueous Solution", Butterworths, London, 1961.
(17) V. J. Bauer, G. E. Wiegand, W. J. Fanshawe, and S. R. Safir, *J. Med. Chem.*, **12**, 944 (1969).
(18) K. Fromherz and H. Spiegelberg, *Helv. Physiol. Pharmacol. Acta*, **6**, 42 (1948).
(19) M. Ridi, L. Lazzi, and P. Corti, *Boll. Chim. Farm.*, **107**, 667 (1968).