6,8-Dimethyl-8-ergolene (4i, Agroclavine) from Chanoclavine I (5a) and Thionyl Chloride. Chanoclavine I (10 mg, 0.039 mmol) was dissolved in dioxane (10 ml), treated with thionyl chloride (20 mg, 0.168 mmol) in dioxane (2 ml), and stirred at room temperature for 0.5 hr under N_2 atmosphere. It was then neutralized with dilute $NaHCO_3$ solution and extracted with $CHCl_3$ (3 × 50 ml). The $CHCl_3$ was washed with H_2O, dried over Na_2SO_4, and evaporated to a syrup. The syrup was chromatographed on a 20 × 20 cm × 2.0 mm Brinkmann silplate, developing with $CHCl_3$–MeOH (8:2) to give 5 mg (54%) of **4i**. The ir and TLC [$CHCl_3$–MeOH (8:2), Me_2CO–EtOAc–DMF (5:5:1)] were identical with that of authentic agroclavine.

6,8-Dimethyl-8-ergolene (4i, Agroclavine) from 8-Chloromethyl-6-methyl-8-ergolene (4h). 8-Chloromethyl-6-methyl-8-ergolene (50 mg, 0.183 mmol) was added to a stirred slurry of $LiAlH_4$ (200 mg, 5.27 mmol) in THF (20 ml) and refluxed for 1.5 hr under N_2. The mixture was then combined with H_2O and $CHCl_3$ (150 ml), washed with H_2O, dried over Na_2SO_4, and chromatographed on a silica gel column (10 g). Elution with $CHCl_3$–MeOH (95:5) gave after trituration with hexane 17 mg (39%) of **4i**. The ir and TLC (see previous experiment) were identical with an authentic sample of agroclavine.

6-Methyl-8-anilinomethyl-8-ergolene (4m). 8-Chloromethyl-6-methyl-8-ergolene (**4h**, 100.0 mg, 0.368 mmol) was dissolved in 15 ml of DMSO under N_2. Aniline (0.6 ml, 0.62 g, 6.6 mmol) was added via syringe and allowed to react for 65 hr. The mixture was poured into 100 ml of water with 5 ml of saturated $NaHCO_3$ solution and extracted with 3 × 100 ml of EtOAc. The organic extracts were dried over Na_2SO_4 and concentrated in vacuo. The residual oil was chromatographed on silica gel using gradient elution from CH_2Cl_2 to 10% MeOH in CH_2Cl_2 to easily remove unreacted aniline from alkaloid products. Fractions containing the desired product were rechromatographed on a silica gel plate (10 × 20 cm × 2 mm) eluting with 9:1 CH_2Cl_2–MeOH to afford, after extraction with same solvent system, 107 mg (89%) of a solid foam, **4m**: ir (KBr) 2.93 (NH), 6.25, 6.67, 9.75, and 13.50 μ; MS (low resolution) *m/e* 329 (4), 328 (6), 237 (58), 236 (100), 235 (36), 167 (29), 154 (29), and 93 (28); NMR δ 2.4–4.0 (m, 12 H), 2.54 (NCH_3, s, 3 H), 6.35–7.4 (m, ~9 H); MS (high resolution) calcd for $C_{22}H_{22}N_3$ (M − 1), 328.181; found, 328.183. This sensitive compound was acetylated for further characterization.

6-Methyl-8-acetanilinomethyl-8-ergolene (4n). 8-Anilinomethyl-6-methyl-8-ergolene (**4m**, 100 mg, 0.304 mmol) was stirred in 3 ml of MeOH and 5 ml of acetic anhydride for 5 hr. After dilution with water and basification with Na_2CO_3 the mixture was extracted with 4 × 50 ml of CH_2Cl_2. The extract was dried over Na_2SO_4 and concentrated in vacuo. The residue was chromatographed on an alumina plate (10 × 20 cm × 1.5 mm) eluting with EtOAc–PhH to afford on extraction with EtOAc 69 mg (61%) of **4n**. Two recrystallizations from THF–petroleum ether gave an analytical sample: mp 185–186° dec; ir (KBr) 6.02 (C=O), 7.81 (CO), 13.51, and 14.50 μ; uv (MeOH) 222 (26,000), 274 (5310), 282 (5570), and 292 (4710); NMR (100 MHz) δ 1.88 ($NCOCH_3$, s, 3 H), 2.51 (NCH_3, s, 3 H), 2.4–3.8 (m, 6 H), 4.14 (–NCH_2–, d, J = 14, 1 H), and 8.13 (indole NH, s, 1 H); MS (low resolution) *m/e* 371 (M^+, 19), 237 (100), 167 (19), and 154 (21); MS (high resolution) calcd for $C_{24}H_{25}N_3O$, 371.200; found, 371.200. Anal. ($C_{24}H_{25}N_3O$) C, H, N.

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4-Trifluoromethylimidazoles and 5-(4-Pyridyl)-1,2,4-triazoles, New Classes of Xanthine Oxidase Inhibitors

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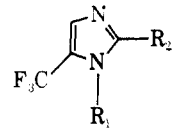
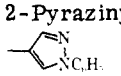
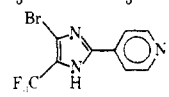
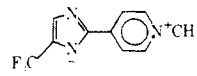
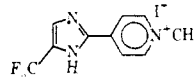
The syntheses of a number of 2-substituted 4-trifluoromethylimidazoles and 3-substituted 5-(4-pyridyl)-1,2,4-triazoles are described. The trifluoromethylimidazoles were prepared from 3,3-dibromo-1,1,1-trifluoroacetone after hydrolysis with aqueous sodium acetate solution and condensation with an aldehyde in the presence of ammonia. Basic hydrolysis of the trifluoromethyl group was found to provide a facile method for the synthesis of imidazole-4-carboxylic acids. In the imidazole series a 2-aryl substituent and a free imino group were required for xanthine oxidase inhibitory activity. The triazoles were obtained through the reaction of an aroylhydrazine and an imino ether followed by thermal ring closure of the intermediate acylamidrazone. As in the imidazole series, a free imino group is an absolute requirement for in vitro activity. Additional structure–activity relationships of these compounds are presented.

Various purine analogs have been shown by many investigators to inhibit the enzyme xanthine oxidase. Among these, allopurinol, 4-hydroxypyrazolo[3,4-*d*]pyrimidine, a potent xanthine oxidase inhibitor,[1,2] has found use therapeutically in the treatment of hyperuricemia associated with gout.[3,4] However, the reported conversion of such purine analogs into nucleotides[5–13] with resulting antimetabolite activity led to a search for specific xanthine oxidase in-

Table I. 4-Trifluoromethylimidazoles



No.	R_1	R_2	Crystn solvent	Mp. °C	Yield, %	Formula	Analyses	XO inhibn, I_{50}
5	H	H	H_2O	148.5–149.5	51.2	$C_4H_3F_3N_2$	C, H, N, F	Inact
6	H	H_3C	H_2O	161–165	8	$C_5H_5F_3N_2$	C, H, N, F	Inact
7	H	$-CH(CH_3)_2$	EtOH–H_2O	201–202	40	$C_7H_9F_3N_2$	C, H, N	Inact
8	H	C_6H_5	H_3CCN–H_2O	210–211	53.6	$C_{10}H_7F_3N_2$	C, H, N	2×10^{-5}
9	H	p-ClC_6H_4	$H_3CC_6H_5$	228–230	47.2	$C_{10}H_7ClF_3N_2$	C, H, N	1×10^{-5}
10	H	p-$H_3COC_6H_4$	H_3CCN	204–206	16.9	$C_{11}H_9F_3N_2O$	C, H, N	1×10^{-5}
11	H	p-$H_3CCONHC_6H_4$	H_3CCN	273–274	30.5	$C_{12}H_{10}F_3N_3O$	C, H, N	4×10^{-6}
12	H	p-$O_2NC_6H_4$	C_6H_6	195–196.5	30	$C_{10}H_6F_3N_3O_2$	C, H, N	2×10^{-5}
13	H	p-$(CH_3)_2NC_6H_4$	H_3CCN	264–265	7.1	$C_{12}H_{12}F_3N_3$	C, H, N	5×10^{-5}
14	H	p-NCC_6H_4	C_6H_6	207–208	14.3	$C_{11}H_6F_3N_3$	C, H, N	Inact
15	H	p-FC_6H_4	C_6H_6	206.5–207.5	48.4	$C_{10}H_6F_4N_2$	C, H, N	3.5×10^{-5}
16	H	p-$HO_2CC_6H_4$	H_3CNO_2	287 dec	16	$C_{11}H_7F_3N_2O_2$	C, H, N	4×10^{-6}
17	H	m-ClC_6H_4	C_6H_6	186.5–187.5	36.9	$C_{10}H_6ClF_3N_2$	C, H, N	3×10^{-6}
18	H	o-ClC_6H_4	C_6H_6–C_6H_{12}	165–167	37	$C_{10}H_6ClF_3N_2$	C, H, N	Inact
19	H	3,4-$Cl_2C_6H_3$	C_6H_6	212.5–213.5	19.4	$C_{10}H_5Cl_2F_3N_2$	C, H, N	1.5×10^{-6}
20	H	2,4-$Cl_2C_6H_3$	H_3CCN	191–193	37.2	$C_{10}H_5Cl_2F_3N_2$	C, H, N	Inact
21	H	4-Pyridyl	H_2O	211–212	32	$C_9H_6F_3N_3$	C, H, N	2×10^{-6}
22	H	3-Pyridyl	H_3CCN	228–228.5	26.7	$C_9H_6F_3N_3$	C, H, N	4×10^{-5}
23	H	2-Pyridyl	H_3CCN–H_2O	156–157.5	42.7	$C_9H_6F_3N_3$	C, H, N	Inact
24	H	2-Quinolyl	$H_3CC_6H_{11}$	156–158	19	$C_{13}H_8F_3N_3$	C, H, N	2×10^{-5}
25	H	6-Quinolyl	H_3CCN	253.5–255	22.3	$C_{13}H_8F_3N_3$	C, H, N	7×10^{-7}
26	H	4-Quinolyl	H_3CCN	206–207	25.9	$C_{13}H_8F_3N_3$	C, H, N	Inact
27	H	7-Quinolyl	H_3CCN	268–270	30.29	$C_{13}H_8F_3N_3$	C, H, N	6×10^{-6}
28	H	2-Pyrazinyl	H_3CCN	237–238	21	$C_8H_5F_3N_4$	C, H, N	2×10^{-7}
29	H		H_3CCN	205–206	21.6	$C_{13}H_9F_3N_4$	C, H, N	2×10^{-7}
30	CH_3	4-CF_3	H_2O	122–124	8	$C_{10}H_8F_3N_3$	C, H, N	Inact
31	CH_3	5-CF_3	Hexane	50–52	5.7	$C_{10}H_8F_3N_3$	C, H, N	Inact
36			H_3CCN	216–217	42.8	$C_9H_{15}BrF_3N_3$	C, H, N	3×10^{-7}
37			C_6H_6–H_3CCN	250–255	56.2	$C_{10}H_8F_3N_3$	C, H, N	Inact
38			$(CH_3)_2CHOH$	230–232	67.6	$C_{10}H_9F_3IN_3$	C, H, N	4×10^{-5}

hibitors of novel structural type unrelated to the purine nucleus. During the investigation, a series of 2-aryl-4-nitroimidazoles was found to exhibit a significant degree of inhibition in vitro (Duggan and Novello, unpublished results).

Based on the assumption that the nitro substituent conferred biological activity through enhancing the acidity of the imino hydrogen, effort was directed toward both the replacement of the nitro moiety by other electronegative groups and the utilization of related nitrogen heterocycles having an imino hydrogen with a pK_a in the same range as the nitroimidazoles. This report will concentrate on the use of the trifluoromethyl moiety as the replacement for nitro and the use of 1,2,4-triazole as the basis for the pK_a equivalency study.

Chemistry. Although 2-trifluoromethylimidazoles have been previously described,[12] the corresponding 4-isomer had not been reported in the literature. After the completion of this work, six 2,5-disubstituted examples of the 2-aryl-4-phenyl-5-trifluoromethylimidazole type were described.[13] Scheme I depicts the general synthetic sequence used in the synthesis of the 4-trifluoromethylimidazoles.

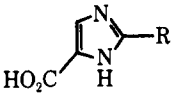
Table II. Trifluoromethylimidazoles. Apparent pK_a

Compd	pK_a[a]	Compd	pK_a[a]
Imidazole	6.67[b]	9	10.34[c]
5	2.90[b]	19	9.96[c]
6	10.15[c]	21	3.76,[b] 9.73[c]
7	10.70[c]	22	3.50.[b] 10.21[c]
8	11.07[c]	23	3.23.[b] 10.88[c]

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

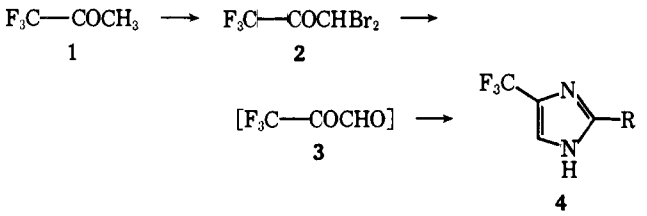
1,1,1-Trifluoroacetone was brominated according to the method of McBee and Burton[14] to yield **2** which was converted to **3** on treatment with aqueous sodium acetate. The glyoxal **3** was not isolated but allowed to react in situ with an aldehyde and ammonia to yield the imidazole **4**. The 2-

Table III. Imidazole-4-carboxylic Acids



No.	R	Crystn solvent	Mp, °C	Yield, %	Formula	Analyses	XO inhibn, I_{50}
33	3,4-$Cl_2C_6H_3$	H_3CCN–H_2O	249–250 dec	41.7	$C_{10}H_6Cl_2N_2O_2$	C, H, N	4×10^{-7}
34	4-Pyridyl	Me_2SO–H_2O	>300	41	$C_9H_7N_3O_2$	C, H, N	3×10^{-5}
35	*p*-ClC_6H_4	H_3CCN–H_2O	261 dec	86.1	$C_{10}H_7ClN_2O_2$	C, H, N	1×10^{-5}

Scheme I

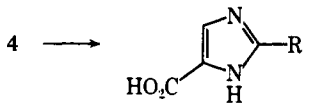


substituted derivatives prepared in this manner are presented in Table I.

The half-neutralization points or pK_a's of representative examples were determined by potentiometric titration in 30% EtOH–H_2O. A decrease in basicity was observed upon the introduction of a trifluoromethyl substituent into the 4 position of imidazole. The pK_a of protonation decreased from 6.67 for imidazole to 2.90 for the trifluoromethyl derivative **5**. In contrast to this, the 2-aryl-4-trifluoromethylimidazoles were weak acids being half ionized in the pH range of 9.7–11. Typical pK_a values are recorded in Table II.

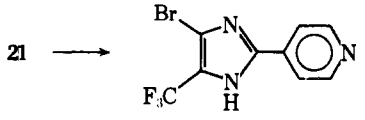
These findings are similar to those reported by Lombardino[12] in which the introduction of a 2-trifluoromethyl group into a 4,5-bis(*p*-methoxyphenyl)imidazole increased acidity, lowering the pK_a from the 12.5–14.5 range expected for simple arylimidazoles to 10.7.

As part of the investigation into the chemistry of this system the stability of the trifluoromethyl group was evaluated and found to be sensitive to aqueous base, thus providing a facile entry into the imidazole-4-carboxylic acid system. When a 2-aryl-4-trifluoromethylimidazole was subjected to treatment with 1 *N* sodium hydroxide solution while warming, solution was rapidly achieved and subsequent neutralization afforded the corresponding acid **32.**

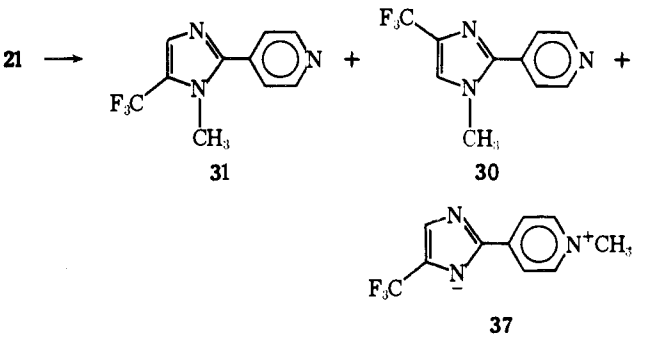


The monocarboxylic acids prepared to establish the utility of this conversion are summarized in Table III.

Bromination and alkylation of the pharmacologically most interesting compound, 2-(4-pyridyl)-4-trifluoromethylimidazole (**21**), were investigated. This compound underwent facile bromination with bromine in chloroform to yield 2-(4-pyridyl)-4(5)-bromo-5(4)-trifluoromethylimidazole (**36**).

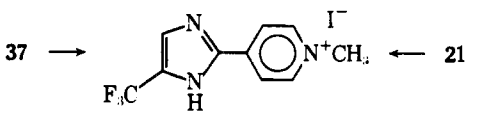


Treatment of **21** with diazomethane in ether–ethanol gave particularly interesting results yielding both the expected 1-methyl-4-trifluoromethyl isomer **30** and the 1-methyl-5-trifluoromethyl isomer **31.** However, the major reaction product was the betaine **37.** The assigned structures were consistent with spectral data and were further confirmed by chemical means. Structural assignments between the two 1-methyl isomers were based on NMR spectra; the isomer having the downfield imidazole proton at δ 8.0 was tentatively assigned structure **30.**



The *N*-methyl derivatives **30** and **31** were separated and isomeric purity was ascertained by GLC analysis. Methylation of **21** in toluene–dimethyl sulfoxide using sodium hydride as base with methyl iodide as the alkylating agent gave a single *N*-methyl derivative which was identical in all respects with the isomer obtained from the diazomethane alkylation to which structure **30** had been assigned.

Treatment of the betaine **37** with hydriodic acid gave a quaternary methiodide which was identical in all respects with the product obtained on the reaction of **21** with methyl iodide in methanol.



Scheme II depicts the general synthetic sequence used in the synthesis of the various 3-aryl-5-pyridyl-1,2,4-triazoles and is essentially that described by Browne and Polya.[15]

Condensation of an imino ether **40** and an acylhydrazine

Scheme II

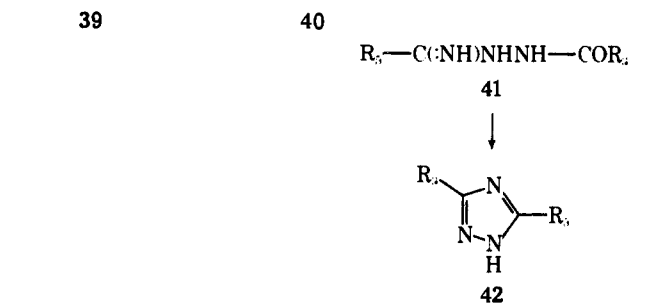
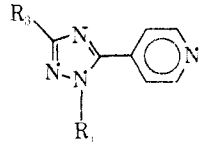
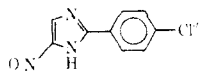
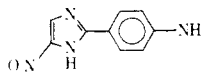


Table IV. 3-Substituted 5-(4-Pyridyl)-1,2,4-triazoles



No.	R_1	R_3	Crystn solvent	Mp, °C	Yield, %	Formula	Analyses	XO inhibn. I_{50}
43	H	4-Pyridyl	EtOH	286–287	23.8	$C_{12}H_9N_5$	C. H. N	6×10^{-8}
44	H	3-Pyridyl	EtOH	235–237	44.8	$C_{12}H_9N_5$	C. H. N	2×10^{-8}
45	H	2-Pyridyl	H_3CCN-H_2O	261–262	34	$C_{12}H_9N_5$	C. H. N	2×10^{-5}
46	H	C_6H_5	H_3CCN-H_2O	241–242	31.5	$C_{13}H_{10}N_4$	C. H. N	1×10^{-6}
47	H	p-ClC_6H_4	$EtOH-H_2O$	265.5–266.5	34	$C_{13}H_9ClN_4$	C. H. N	6×10^{-7}
48	H	m-ClC_6H_4	EtOH	269–271	37	$C_{13}H_9ClN_4$	C. H. N	2×10^{-8}
49	H	p-$H_2NSO_2C_6H_4$	$EtOH-H_2O$	301–303	35	$C_{13}H_{11}N_5O_2S$	C. H, N	3×10^{-6}
50	H	3,5-$Cl_2C_6H_3$	$EtOH-H_2O$	298–299.5	38	$C_{13}H_8Cl_2N_4$	C. H, N	2×10^{-8}
51	H	3,4-$Cl_2C_6H_3$	$EtOH-H_2O$	345–346.5	20	$C_{13}H_8Cl_2N_4$	C. H. N	1×10^{-7}
52	H	3,5-$(H_3CO)_2C_6H_3$	EtOH	252–253.5	15	$C_{15}H_{14}N_4O_2$	C, H, N	2×10^{-7}
53	H	6-Quinolyl	MeOH	313–314.5	12	$C_{16}H_{11}N_5$	C. H. N	2.5×10^{-8}
54	H	2-Furyl	H_3CCN	216–217	38	$C_{11}H_8N_4O$	C. H, N	1.5×10^{-5}
55	H	4-Pyridazinyl	H_3CCN-H_2O	276–278	31.8	$C_{11}H_8N_6$	C. H. N	8×10^{-8}
56	H	2-Thienyl	H_3CCN	240–241.5	31.6	$C_{11}H_8N_4S$	C. H, N. S	1×10^{-4}
57	H	2-Pyrimidinyl	H_2O	274–276	31.2	$C_{11}H_8N_6$	C. H, N	4×10^{-5}
58	H	4-Pyrimidinyl	EtOH	285–286.5	42.7	$C_{11}H_8N_6$	C. H. N	4×10^{-8}
59	H	4-Pyrazinyl	H_3CCN-H_2O	251–252.5	31.9	$C_{11}H_8N_6$	C. H. N	5×10^{-7}
60	CH_3	4-Pyridyl	EtOH	170–171.5	36.9	$C_{13}H_{11}N_5$	C. H, N	Inact

Table V. 3-Substituted 5-(4-Pyridyl)-1,2,4-triazoles. Apparent pK_a

Compd	pK_a[a]	XO inhibn. I_{50}
	9.00[c]	
	7.99[c]	1×10^{-7}
	8.80[c]	1×10^{-5}
43	3.10,[b] 3.93,[b] 7.10[c]	
44	2.95,[b] 3.68,[b] 7.60[c]	
45	2.90,[b] 3.50,[b] 8.10[c]	
46	3.75,[b] 8.00[c]	
47	3.65,[b] 7.72[c]	
50	3.33,[b] 8.10[c]	
54	3.90,[b] 7.85[c]	
59	3.56,[b] 7.05[c]	

[a] pK_a values were determined by potentiometric titration in 30% EtOH-H_2O. [b] Proton gained. [c] Proton lost. [d] See ref 16.

39 generated the acylamidrazone 41, which was thermally cyclized to the triazole 42. The choice of 4-cyanopyridine or isonicotinoylhydrazine as the reagent depended upon the availability or ease of synthesis of the coreactant. The various 3-substituted 5-(4-pyridyl)-1,2,4-triazoles prepared during the course of this study are summarized in Table IV.

Representative pK_a values of several examples in comparison with the 4-nitroimidazole lead are recorded in Table V. These values were obtained by potentiometric titration in 30% EtOH-H_2O.

Discussion

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 a 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".

In the 4-trifluoromethylimidazole series, the requirements for significant in vitro xanthine oxidase inhibitory activity were the presence of an aryl or heteroaryl group in the 2 position and the presence of an unsubstituted imino group. Both the replacement of the 2-aryl substituent by hydrogen or an alkyl group and the methylation of the imidazole ring resulted in loss of intrinsic activity.

Substitution in the 2-aryl moiety had little effect on inhibitory activity, the exceptions being the cyano derivative 14 and a negative effect by o-chlorine as seen in 18. Among the heterocyclic substituents, the intrinsic activity was strongly influenced by the position of the heteroatom; this can be clearly seen by an examination of the relative activities among the pyridyl and quinolyl derivatives 21–27.

The 2-(4-pyridyl) derivative 21 was selected as being the most interesting member of the series and was subjected to a detailed pharmacological evaluation.

Several of the pyridyltriazole derivatives exhibited an intrinsic activity 2–3 log units higher than that observed in the trifluoromethylimidazole series, having I_{50}'s in the range of 2×10^{-7}–2×10^{-8} M.

With these highly potent compounds, variation of the 3-substituent was found to have a significant impact on inhibitory activity. The presence of the furyl or thienyl moiety, as in examples 53 and 55, resulted in only moderately active compounds, while the presence of a substituted aryl substituent resulted in compounds having high levels of in-

trinsic activity. Again the effect of the relative position of the heteroatom could be seen among the 3-heteroaryl derivatives and is most clearly illustrated by the pyridyl- and pyrimidinyl-substituted derivatives **43–45, 57,** and **48.** As in the case of the trifluoromethylimidazoles, a free imino group was required for in vitro activity as seen by the loss of intrinsic potency upon the introduction of a 1-methyl substituent, as in **60.**

The biological properties of three of the most active members of this series, **43, 55,** and **58,** are described in the accompanying paper.[21]

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.

General Preparation of 4-Trifluoromethylimidazoles. The preparation of 2-(4-pyridyl)-4-trifluoromethylimidazole is presented as an example; all others were obtained by essentially the same procedure utilizing the appropriate aldehyde in place of isonicotinaldehyde. The following intermediate aldehydes were prepared by literature procedures (all others were obtained from commercial sources): quinoline-4-carboxaldehyde,[17] quinoline-6-carboxaldehyde,[18] quinoline-7-carboxaldehyde,[18] pyrazinecarboxaldehyde,[19] and 1-phenyl-4-formylpyrazole.[20]

To a solution of $NaOAc \cdot 3H_2O$ (11.97 g, 0.088 mol) in H_2O (40 ml) was added 1,1,1-trifluoro-3,3-dibromoacetone (11.87 g, 0.044 mol). The stirred mixture was heated 30 min at steam bath temperature. After cooling, the solution was added to isonicotinaldehyde (4.28 g, 0.04 mol) in MeOH (200 ml) and concentrated NH_4OH (50 ml). After standing 5 hr at room temperature, the solution was concentrated under reduced pressure to 50 ml. H_2O (50 ml) was added; the resulting solid was filtered and recrystallized from H_2O to give **21** (2.7 g, 30%), mp 213–214°. Anal. ($C_9H_6F_3N_3$) C, H, N.

General Preparation of 2-Arylimidazole-4-carboxylic Acids. The preparation of 2-(4-chlorophenyl)imidazole-4-carboxylic acid is presented as an example; the 2-(4-pyridyl) and 2-(3,4-dichlorophenyl) derivatives, **33** and **34,** are obtained by essentially the same procedure.

A stirred suspension of **9** (17 g) in 1 *N* NaOH solution (1 l.) was heated at 90–95° until solution was obtained (1.5 hr). The solution was filtered and acidified with concentrated HCl; **35** precipitated. After filtration and recrystallization from 9:1 H_3CCN–H_2O, 15 g of **35** having a melting point of 261° was obtained. Anal. ($C_{10}H_7ClN_2O_2$) C, H, N.

Reaction of 2-(4-Pyridyl)-4-trifluoromethylimidazole with Diazomethane. To a solution of **21** (10 g, 0.047 mol) in ether (200 ml) and MeOH (25 ml) was added dropwise with stirring at 0–5° an ethereal solution of CH_2N_2 (4.5 g, 0.1 mol). After the addition was complete the solution was allowed to warm to room temperature. After standing 20 hr, the betaine **37** had separated and was filtered to yield, after recrystallization from C_6H_6–H_3CCN, 6 g, mp 250–254° dec. The ethereal mother liquor was concentrated under reduced pressure and the residue dissolved in refluxing hexane. On standing, **30** separated and was filtered and recrystallized from H_2O to yield 930 mg, mp 122–i24°. The hexane filtrate was concentrated under reduced pressure and the solid residue recrystallized from hexane to yield 610 mg of **31,** mp 50–52°.

GLC analysis was done on an F & M Model 810 flame ionization detection gas chromatograph equipped with glass columns 4 mm i.d. × 6 ft packed with a stationary phase of Chromosorb G which had been acid washed, dimethylchlorosilane treated, and coated with 5% QF-1 fluorinated alkylsilicone. Column temperature was 200° and the carrier gas was He. The materials were found to be isomerically homogeneous having retention times of 648 sec for **30** and 240 sec for **31.**

In the NMR (obtained in a solution of Me_2SO-*d* at 37° on a 60-MHz Varian A-60A spectrophotometer) the absorption of the imidazole proton in the two isomers was distinct, coming at δ 8.0 in **30** and δ 7.7 in **31.** Anal. For **30** ($C_{10}H_8F_3N_3$) C, H, N. Anal. For **31** ($C_{10}H_8F_3N_3$) C, H, N. Anal. For **37** ($C_{10}H_8F_3N_3$) C, H, N.

***N*-Methyl-4-(4-trifluoromethyl-2-imidazolyl)pyridinium Iodide (38). Method A.** MeI (7 g, 0.05 mol) was added with stirring at room temperature to a solution of **21** (2.13 g, 0.01 mol) in MeOH (50 ml). After standing 24 hr at room temperature, the solution was concentrated to a solid which, after recrystallization from isopropyl alcohol, gave **38** (2.4 g) having mp 230–232°. Anal. ($C_{10}H_9F_3IN_3$) C, H, N.

Method B. The betaine **37** (200 mg) was added with stirring to HI (2 ml). After 10 min the reaction was concentrated under reduced pressure to yield a solid which after recrystallization from isopropyl alcohol gave **38** (210 mg) melting at 230–232°. Anal. ($C_{10}H_9F_3IN_3$) C, H, N.

1-Methyl-2-(4-pyridyl)-4-trifluoromethylimidazole (30). To a solution of **21** (2.1 g, 0.01 mol) in 4:1 toluene–Me_2SO (15 ml) was added at room temperature with stirring NaH (57% in mineral oil, 0.42 g, 0.01 mol). After stirring 15 min, the reaction mixture was cooled to 0–5° and MeI (1.5 g, 0.01 mol) in 4:1 toluene–Me_2SO (3 ml) was added dropwise with stirring. The mixture was then allowed to stir 2 hr at room temperature and concentrated under reduced pressure to an oil. H_2O (15 ml) was added to the residue which solidified and was filtered. After recrystallization from H_2O, **30** (0.75 g, mp 123–126°) was obtained and found by mixture melting point, ir, and NMR to be identical with the higher melting isomer obtained from CH_2N_2 treatment of **21.** Anal. ($C_{10}H_8F_3N_3$) C, H, N.

2-(4-Pyridyl)-4(5)-bromo-5(4)-trifluoromethylimidazole (36). To a suspension of **21** (2.1 g, 0.01 mol) in $CHCl_3$ (100 ml) was added dropwise with stirring at room temperature Br_2 (0.6 g, 0.01 mol) in $CHCl_3$ (5 ml). The resulting solution was stirred 4 hr at room temperature and concentrated under reduced pressure. H_2O (25 ml) was added to the solid residue and the resulting solution neutralized with saturated aqueous $NaHCO_3$ solution. The precipitated solid was filtered and recrystallized from H_3CCN to yield **36** (1.25 g), mp 216–217°. Anal. ($C_9H_5BrF_3N_3$) C, H, N.

General Preparation of 3-Aryl-5-(4-pyridyl)-1,2,4-triazoles. Method A. The preparation of 3-(4-pyridazinyl)-5-(4-pyridyl)-1,2,4-triazole is presented as an example of the synthetic method employed utilizing 4-cyanopyridine and an aroylhydrazine. The intermediate aroylhydrazines used in the syntheses were prepared by standard literature methods.

To a solution of 4-cyanopyridine (1.4 g, 0.014 mol) in MeOH (50 ml) was added Na (100 mg). The solution was allowed to stand 45 min at room temperature and was then added to a solution of 4-pyridazinoylhydrazine (1.9 g, 0.014 mol) in MeOH (15 ml). The resulting solution was heated 3 hr at reflux and allowed to stand 16 hr at room temperature. The precipitated solid was filtered and washed with isopropyl alcohol yielding the intermediate acylamidrazone (2 g) which was then heated from 180 and 285° over 2 hr. After cooling the residue was recrystallized from 9:1 H_3CCN–H_2O to yield **57** (1 g) melting at 277–278.5°. Anal. ($C_{11}H_8N_6$) C, H, N.

Method B. The preparation of 3-(3,5-dimethoxyphenyl)-5-(4-pyridyl)-1,2,4-triazole is presented as an example of the method used employing isonicotinoylhydrazine and an aroylnitrile.

HCl gas was introduced over 35 min into a solution of 3,5-dimethoxybenzonitrile (14.7 g, 0.01 mol) and EtOH (4.6 g, 0.1 mol) in dry C_6H_6 (100 ml) at 0°. The solution was stirred 3 days at 0–10°; Et_2O (100 ml) was added and the resulting solid removed by filtration. The imino ether hydrochloride was converted to the free base (16.6 g) using cold, saturated K_2CO_3 solution. A solution of the imino ether (14.6 g, 0.07 mol) and isonicotinoylhydrazine (9.6 g, 0.07 mol) in MeOH (100 ml) was heated 1 hr at reflux. The solution was then concentrated under reduced pressure to 50 ml; the acylamidrazone separated and was filtered (15.6 g). The crude acylamidrazone (10 g) was then heated from 200 to 270° over 30 min and maintained at 270° for an additional 30 min. After cooling, the residue was recrystallized from EtOH to yield **52** (3 g), mp 252–253.5°. Anal. ($C_{15}H_{14}N_4O_2$) C, H, N.

1-Methyl-3,5-bis(4-pyridyl)-1,2,4-triazole (60). Method A. To a solution of 4-cyanopyridine (4.16 g, 0.04 mol) in MeOH (60 ml) was added Na (0.2 g). The solution was allowed to stand 30 min at room temperature and was then added to 1-methyl-2-isonicotinoylhydrazine (6 g, 0.04 mol) in MeOH (80 ml). After heating 4 hr at reflux, the reaction mixture was concentrated under reduced pressure to an oil which solidified. After recrystallization from EtOH 3.5 g of **60** melting at 170–171.5° was obtained. Anal. ($C_{13}H_{11}N_5$) C, H, N.

Method B. To **43** (2.1 g, 0.01 mol) in dry THF (125 ml) was added NaH (57% in mineral oil, 0.42 g, 0.01 mol). The mixture was heated 30 min at reflux and MeI (1.4 g, 0.01 mol) was added. After heating at reflux for 3 hr, the mixture was filtered and concentrated under reduced pressure to a solid. After chromatography on silica gel using 19:1 $CHCl_3$–MeOH as eluent and recrystallization

from EtOH, 1 g of **60** was obtained, mp 170–171°. The product obtained by method B was identical with that obtained by method A as shown by mixture melting point, TLC, and ir.

Determination of Apparent pK_a. The half-neutralization points or pK_a's were determined by potentiometric titration. The sample (0.05 mmol) was dissolved in 30% EtOH–H_2O (30 ml) containing NaOH (0.15 mmol) and back titrated with 0.5 *N* HCl using a glass calomel electrode system on an expanded pH scale. The apparent pK_a's were taken from the neutralization curve as the pH at the one-half neutralization point.

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3,5-Disubstituted 1,2,4-Triazoles, a New Class of Xanthine Oxidase Inhibitor

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3,5-Bis(4-pyridyl)-1,2,4-triazole (PPT), 3-(4-pyrimidinyl)-5-(4-pyridyl)-1,2,4-triazole (PMPT), and 3-(4-pyridazinyl)-5-(4-pyridyl)-1,2,4-triazole (PZPT) are among the most active competitive inhibitors of xanthine oxidase among a series of 3,5-disubstituted triazoles synthesized for this purpose, inhibition constants being less than 1×10^{-7} *M* for each. ED_{50} values in squirrel monkeys derived from first-order rate constants for the first and rate-limiting step of the sequence, xanthine → uric acid → allantoin + CO_2, range from 0.04 to 0.08 mg kg^{-1} orally, with unusually long durations of action attributable to asymmetric distribution of inhibitor within liver and gut as a consequence of enterohepatic recirculation. Sensitivity of rats, dogs, and anthropoid species to these, as to other xanthine oxidase inhibitors, is markedly less than that of the squirrel monkey, but the triazoles are at least an order of magnitude more active than the representative purine analogs tested.

Inhibition of uric acid biosynthesis as an alternative to uricosuria in the therapy of gout has become firmly established with the introduction of the xanthine oxidase inhibitor 4-hydroxypyrazolo[3,4-*d*]pyrimidine or allopurinol into clinical use.[1]

Virtually all xanthine oxidase inhibitors which are structural analogs of xanthine or hypoxanthine are substrates for phosphoribosyl transferases, forming ribonucleotides which possess various spectra of antimetabolite activities, e.g., 2,6-diaminopurine, 6-thioguanine, 8-azaguanine, 6-mercaptopurine,[2] 6-chloropurine,[3] 4-hydroxypyrazolopyrimidine,[4] and 4-mercaptopyrazolopyrimidine.[5] Thus, in addition to possibly augmenting normal autoregulatory mechanisms involving soluble nucleotide effects upon de novo purine biosynthesis,[6] a variety of inhibitions of interconversions and utilization of polynucleotide precursors occurs.

Because of this common inability of purine isosteres to inhibit xanthine oxidase in a highly selective manner, testing for xanthine oxidase inhibitory activity among novel structural types, unrelated to the purine nucleus, was undertaken in these laboratories.[7] The biological properties of three of the more active members of one such series are the subject of the present report.

Experimental Section

Materials and Methods. The syntheses of 3,5-bis(4-pyridyl)-1,2,4-triazole (PPT), 3-(4-pyrimidinyl)-5-(4-pyridyl)-1,2,4-triazole (PMPT), and 3-(4-pyridazinyl)-5-(4-pyridyl)-1,2,4-triazole (PZPT) are described elsewhere.[7] Bovine milk xanthine oxidase, uricase, and xanthine were purchased from Nutritional Biochemical Corp. and uric acid-2-^{14}C from Amersham Searle. Xanthine-6-^{14}C was prepared from guanine-6-^{14}C as previously described.[8]

Enzyme Inhibition in Vitro. For aerobic oxidation of xanthine, reactions were started by addition of enzyme (typically 1.0 ml of 1:300 dilution of Worthington Grade XOP enzyme to produce a ΔOD_{290} of 0.09–0.12 min^{-1}) to 2.0 ml of 0.05 *M* phosphate buffer, pH 7.5, containing xanthine and inhibitor. Initial reaction rates were determined directly from continuous recordings of OD_{290} vs.