Inhibitors of *Bacillus subtilis* DNA Polymerase III. 6-(Arylalkylamino)uracils and 6-Anilinouracils

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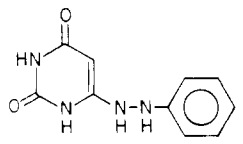
6-(Benzylamino)uracils and substituted 6-anilinouracils have been found to be potent inhibitors of *Bacillus subtilis* DNA polymerase III by a mechanism identical with that of 6-(phenylhydrazino)uracils. Higher phenylalkylamino homologues are progressively weaker inhibitors of the enzyme. Examination of the effects of substituents on the activity of 6-(benzylamino)uracils against wild-type and mutant enzymes and preliminary results for 6-anilinouracils have permitted further dissection of the mechanism of inhibition. The experimental results indicate that (1) the polymerase inhibitor binding site is compact, accommodating only small alterations in the distance between the uracil and phenyl rings, (2) the phenyl ring, which provides the major contribution to inhibitor–enzyme binding, adopts a specific active conformation, and (3) an enzyme site which interacts with substituents in the phenyl ring forms a part of the active site of DNA polymerase III.

6-(Phenylhydrazino)uracils, e.g., **1**, inhibit DNA polymerase III (pol III) of *Bacillus subtilis* by promoting the formation of a catalytically inactive, ternary complex with DNA and enzyme. The mechanism of complex formation specifically involves (1) hydrogen bonding of the uracil and its 6-NH moiety with cytosine in the DNA template and (2) binding of the phenyl group with a hydrophobic site on the enzyme.[1] We have continued attempts to dissect further the specific mechanism of drug-induced inhibition of pol III, placing particular emphasis on the design of active drug molecules which do not require reduction to maintain the inhibitory forms and which may form the basic for the synthesis of useful, irreversible enzyme inhibitors. This paper describes alterations in the hydrazino group of inhibitors via (1) replacement of the 1′-NH moiety with CH_2 groups to yield a series of 6-(benzylamino)uracils and several higher 6-(phenylalkylamino)uracils and (2) elimination of the 1′-NH moiety to give 6-anilinouracils. The results presented below agree with and clarify several aspects of the model proposed for the inhibitor binding site of pol III.



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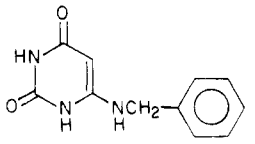

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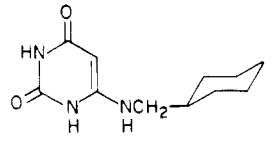

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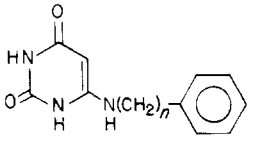
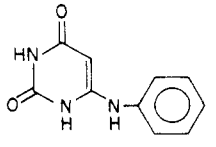


4, n = 2
5, n = 3
6, n = 4



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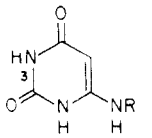
Results

6-(Phenylalkylamino)uracils. Our previous studies indicated[2] that the hydrazino NH group adjacent to the uracil ring of **1** is intimately involved in binding of pol III inhibitors to cytosine residues of the DNA template; these studies did not indicate unequivocally whether the distal 1′-NH also participated in inhibitor binding, particularly to an enzyme group with which it could form a hydrogen bond. We tested this possibility by replacing 1′-NH by CH_2; the resulting compound, 6-(benzylamino)uracil (**2**), is a very active pol III inhibitor (see Table I) with K_i = 2.3 μM, a value close to that of **1** (1.4 μM). Replacement of the 1′-NH group by CH_2 also did not alter the mechanism of inhibition or the ability of the compound to mimic a specific purine substrate. Additional experiments (results not shown) indicated that the inhibitory effect of **2** was specifically and completely reversed by dGTP.

Table I. Activity of Inhibitors on *B. subtilis* DNA Polymerase III



Compd	R	K_i, μM[a]
2	$CH_2C_6H_5$	2.3
3	CH_2-c-C_6H_{11}	Inactive[b]
4	$(CH_2)_2C_6H_5$	31.7
5	$(CH_2)_3C_6H_5$	2000
6	$(CH_2)_4C_6H_5$	Inactive
7	C_6H_5	Inactive
8	$CH_2C_6H_5$(3-CH_3)	5.1
9	$(CH_2)_2C_6H_5$(3-CH_3)	20
10	$CH(CH_3)C_6H_5$	Inactive
11	$CH_2C_6H_4$-*p*-NO_2	5.2
12	$CH_2C_6H_4$-*p*-$NHCOCH_3$	2300
13	$CH_2C_6H_4$-*p*-NH_2	17
14	C_6H_4-*m*-CH_3	27.5
15	C_6H_4-*p*-CH_3	20.4
16	C_6H_3-*m*,*p*-$(CH_3)_2$	1.0
17	C_6H_3-*m*,*p*-$(CH_2)_3$	0.4

[a] K_i is the concentration of inhibitor giving 50% inhibition of enzyme activity in truncated DNA synthesis (−dGTP); see ref 1 for details of assay procedure. [b] Compounds which produce <10% inhibition at 1 mM are considered inactive.

The identification of **2** and several substituted derivatives (see below) as active compounds clearly indicates three structural characteristics relating to inhibitor function. First, the 1′-NH group of hydrazino derivatives does not interact significantly with the enzyme. Second, the CH_2 group of **2** does not interfere with inhibitor–cytosine pairing. Third, both 1′-NH and CH_2 groups permit essentially identical active conformations of the phenyl ring.

The lack of activity of the saturated analogue of **2**, 6-(cyclohexylmethylamino)uracil (**3**), demonstrates the requirement of a planar, i.e., aromatic, group on the inhibitors for binding to the enzyme. Inactivity of **3** and of

6-amino- and 6-hydrazinouracils confirms the suggestions[1] that the phenyl ring is the site of inhibitor–enzyme binding and that the enzyme binding region is probably planar.

Several homologues of **2** (Table I) were prepared in an attempt to determine the distance between the pyrimidine and phenyl rings which would optimize inhibitory activity. A higher homologue of **2**, 6-(β-phenethylamino)uracil (**4**) had considerable activity, binding only about 15-fold more weakly than **2** to the enzyme. However, further separation of the pyrimidine and phenyl rings, viz. compounds **5** and **6**, led to rapidly decreasing activity; indeed, these compounds have lost practically all activity. These results are in accordance with the compactness of the pol III inhibitor binding site.

6-Anilinouracil (**7**), a lower "homologue", is inactive as an inhibitor of the enzyme. Apparently, the phenyl ring, being closer to the uracil ring, is no longer in intimate contact with the inhibitor binding site. However, several substituted 6-anilinouracils were found to be quite potent pol III inhibitors (see below).

Substituted 6-(Benzylamino)uracils. Methylation of the uracil 3 position of phenylalkylaminouracils had no deleterious effect on inhibitor activity; indeed these compounds, e.g., **8** and **9** (Table I), were approximately equipotent with their nonmethylated analogues **2** and **3**. However, the presence of a methyl group on the α-carbon of **2** yielded a compound, **10**, which was devoid of inhibitory activity. The effect of the α-methyl group is even more pronounced than the negative effect of a 1′-NMe group on the activity of **1**, which decreased inhibitor potency by several hundred-fold.[1,3] This observation reinforces our view that such substituents interfere with rotation of the phenyl ring into an active conformation.

In this context, we questioned earlier[3] whether the weak activity of 6-(*p*-nitrophenylhydrazino)uracil resulted from repulsion of the NO_2 group by the enzyme or from a resonance interaction between this strongly electron-withdrawing group and the 1′-NH group, yielding an increased energy barrier to rotation of the phenyl ring. We resolved this question by examining the inhibitory activity of 6-(*p*-nitrobenzylamino)uracil (**11**), which does *not* have a nonbonded electron pair para to the NO_2 group; this compound (Table I) has fivefold greater potency than the analogous phenylhydrazino derivative (K_i = 5.2 vs. 26.3 μM). This decrease in influence of the *p*-NO_2 group of **11** must be a direct result of its lack of effect, via resonance, on phenyl conformation.

We hoped that a haloacetamido group in the phenyl ring of **2** might produce a compound capable of irreversibly binding to pol III. However, the prototype *p*-acetamido derivative, **12**, was too weak an inhibitor (K_i = ~2300 μM) to assure highly selective reaction of a haloacetamido analogue with the enzyme; the acetamido group and, particularly, a haloacetamido group would appear to be too bulky to be accommodated readily by the inhibitor binding site.

Effects of 6-(Phenylalkylamino)uracils on the Mutant Enzyme Pol III/azp-12. On the basis of studies with 6-(phenylhydrazino)uracils[1] we postulated that the *p*-OH group of 6-(*p*-hydroxyphenylhydrazino)uracil binds to a site on the enzyme which in pol III/azp-12 is mutated, yielding an enzyme specifically resistant to inhibitors bearing polar, hydrogen-bonding groups, i.e., OH, NH_2, in the phenyl ring. The results of studies comparing the effects of several phenylalkylamino inhibitors on wild-type pol III and pol III/azp-12 are presented in Table II. The data show that 6-(*p*-aminobenzylamino)uracil (**13**) is much less potent as an inhibitor of pol III/azp-12 than of the wild-type enzyme, whereas the unsubtituted inhibitors, **2** and **4**, are, as in the case of 6-(phenylhydrazino)uracil, equipotent inhibitors of both polymerases. Thus, the pattern of resistance/sensitivity of pol III/azp-12 to 6-(phenylalkylamino)uracils is identical with that reported[1] for 6-(phenylhydrazino)uracils.

Table II. K_i of 6-(Arylalkylamino)uracils against Wild-Type Pol III and Pol III/azp-12

		K_i, μM	
Compd	6-Substituent	Wild-type pol III	Pol III/azp-12
2	$NHCH_2C_6H_5$	2.3	2.5
4	$NHCH_2CH_2C_6H_5$	31.7	30
13	$NHCH_2C_6H_4$-*p*-NH_2	17	156

6-Anilinouracils. 6-Anilinouracil itself (**7**) is inactive against pol III. However, the data of Table I show that introduction of alkyl groups into the meta and/or para positions of the phenyl ring produces compounds (**14–17**) with markedly increased affinity for the enzyme; indeed, **17** is one of the most potent inhibitors of pol III yet examined. These compounds operate by the same mechanism as phenylhydrazino and benzylamino inhibitors because their inhibitory effects are reversed by dGTP, and they are equipotent with respect to inhibition of both wild-type and azp-12 polymerases (results not shown). It would appear that projection of alkyl groups into the drug-binding region of the enzyme serves to regain much of the binding energy lost in moving the phenyl ring closer to the uracil ring. An important implication of the ability of 6-anilinouracils to bind strongly with pol III is that the conformation of the phenyl ring of these compounds is fixed, in contrast to that of phenylhydrazino and benzylamino inhibitors.

Discussion

The similarities between the structure–activity relationships of 6-(phenylhydrazino)uracils and those of 6-(benzylamino)uracils affirm that both classes of pol III inhibitors operate by a common mechanism. The inhibitory effects of both classes of compounds are reversible by dGTP, display comparable substituent effects, and yield an identical pattern of resistance/sensitivity toward the azp-12 mutant enzyme.

Results obtained from the examination of a series of phenylalkylamino compounds, in which the phenyl ring is displaced relative to the pyrimidine ring, clearly show that the inhibitor binding site does not have a high degree of bulk tolerance.

The lack of activity of a saturated analogue (compound **3**) further supports the contention that this binding site requires a planar inhibitor group, i.e., an aromatic ring.

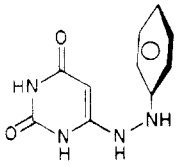
A considerable portion of the experimental results supports the hypothesis that inhibitory activity requires assumption of an active conformation of the phenyl ring relative to the uracil ring as shown in structure **18**. The α-methyl group of **10**, which might be expected to raise the energy barrier to rotation of the phenyl ring, destroys activity. The *p*-NO_2 group of **11** decreases activity considerably less than it does in the phenylhydrazino series, in which resonance interaction between the NO_2 group and the 1′-NH of the hydrazino group could raise the rotational barrier. Finally, alkyl-substituted 6-anilinouracils are potent inhibitors of DNA polymerase III; the phenyl ring of these compounds (see structure **19**) is constrained to lie in a plane very similar to that proposed for the active conformation of phenylhydrazino and benzylamino inhibitors (structure **18**). Whereas the unsubstituted

Table III. 6-(Arylalkylamino)uracils and 6-Anilinouracils

Compd	Rxn time,[a] h	Yield, %	Crystn solvent	Mp, °C	Formula[b]
3	6	75	Glyme	>320	$C_{11}H_{17}N_3O_2$
5	16	88	50% EtOH	286-289	$C_{13}H_{15}N_3O_2 \cdot 0.5H_2O$
6	16	78	50% EtOH	261-265	$C_{14}H_{17}N_3O_2 \cdot 0.5H_2O$
8	6	59	EtOH	289-293	$C_{12}H_{13}N_3O_2 \cdot 0.5H_2O$
9	8	59	EtOH	246-249	$C_{13}H_{15}N_3O_2$[c]
11	18	30	HAc	294-296	$C_{11}H_{10}N_4O_4 \cdot H_2O$
16	17	87	80% HAc	310-313	$C_{12}H_{13}N_3O_2$
17	16	96	80% HAc	312-315	$C_{13}H_{13}N_3O_2 \cdot 0.5H_2O$

[a] In refluxing glyme. [b] C, H, and N analyses. [c] N: calcd, 17.13; found, 17.59.

compound 7 does not significantly inhibit pol III, alkyl-substituted 6-anilinouracils, e.g., 14–17, are surprisingly potent inhibitors, suggesting that substituents in the plane of the phenyl ring project favorably into the binding site of the enzyme.



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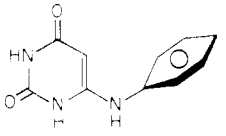

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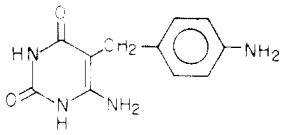
Many 6-substituted uracils, including arylalkylamino- and anilinouracils, had been found by Baker to be potent inhibitors of *E. coli* thymidine phosphorylase.[4,5] Our comparison of structure–activity relationships in the thymidine phosphorylase system with those in the pol III system has demonstrated two significant differences. First, thymidine phosphorylase inhibitors do not require a 6-NH group nor do they require a free 1-H on the uracil ring; these inhibitors do not bind to cytosine residues as part of their mechanism. Second, large phenyl substituents and polycyclic aromatic rings at the 6 position can be accommodated by the inhibitor-binding site of thymidine phosphorylase; indeed binding is enhanced by the presence of large, planar moieties.[4] Previous results[1,6] and the results reported in this paper have shown that the pol III inhibitor-binding site is compact, although it also accommodates planar groups.

One important similarity between thymidine phosphorylase and pol III inhibitors is that the active conformation of inhibitor molecules appears to be identical, i.e., the aromatic substituents have been postulated to adopt the conformations of structures 18 and 19 in both cases. It is likely, therefore, that inhibitors of pol III project the phenyl ring into a region of enzyme space near that which is normally occupied by the deoxyribose ring of nucleotide substrates, i.e., into the active site region of the polymerase.[1] It follows that mutations of pol III to azp-12 (see above) and similar[7] mutant enzymes affect amino acid(s) within or near the enzyme active site.

The discovery that 6-anilinouracils are inhibitors of *B. subtilis* DNA polymerase III has two implications for our future work with these compounds. First, because the phenyl ring of 6-anilinouracils is fixed in the active conformation, substituent effects in this series should reflect direct effects on inhibitor–enzyme binding and not be complicated by conformational uncertainties. Second, because the phenyl ring is closer to the uracil ring than in phenylhydrazino and benzylamino inhibitors, we may be able to introduce reactive groups into the phenyl ring to give effective, irreversible pol III inhibitors.

Chemistry. Several of the compounds employed in this work had been prepared[4,5] by reactions between 6-chlorouracil and the appropriate amines in aqueous solution. We have chosen to prepare these and new compounds (see Table III for details) using glyme as the solvent; in this solvent, reaction times can be reduced considerably to give comparable yields of products. 6-Aminouracil can also be employed as substrate, but with lower yields or increased reaction times. 3-Methyl analogues (8 and 9) were prepared from 3-methyl-6-aminouracil.[8]

It was not possible to prepare 13 directly from reactions between 6-chloro- or 6-aminouracil and *p*-aminobenzylamine. Reaction of 6-chlorouracil with *p*-aminobenzylamine gave unidentifiable products, whereas 6-amino- and 3-methyl-6-aminouracils gave products whose NMR spectra were suggestive of 5-substituted uracils. For example, the compound derived from 3-methyl-6-aminouracil and *p*-aminobenzylamine appears to be 20 (see Experimental Section). Definitive structural proof of 20 and analogous compounds and investigations of the mechanism of this reaction are in progress.



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Inability to prepare 13 directly led to the synthesis of *p*-acetamidobenzylamine, a previously unknown compound. An aqueous solution of *p*-aminobenzylamine dihydrochloride was treated with 1 mol of acetic anhydride and of sodium acetate to give this monoacetyl derivative, isolated both as the hydrochloride and as the free base. *p*-Acetamidobenzylamine reacted with 6-chlorouracil to give the *p*-acetamido inhibitor, 12; alkaline hydrolysis of 12 gave 13.

Experimental Section

Melting points were determined on a Fisher-Johns apparatus and are uncorrected. Elemental analyses were done by HetChem Co., Harrisonville, Mo.; all agreed to ±0.4% of calculated values. Nuclear magnetic resonance spectra were obtained with a Perkin-Elmer R-12B/TT7 Fourier transform instrument (60 MHz); proton spectra were obtained in Me_2SO-d_6 (internal Me_4Si) and were consistent with the proposed structures. Procedures for the preparation of inhibitors are exemplified by the description of the synthesis of 12; reaction conditions and properties of new compounds are listed in Table III. This general method was used to make compounds prepared previously by Baker: 2, 4, and 7[4] and 10, 14, and 15.[5] Details of inhibitor assays are to be found in ref 1.

***p*-Nitrobenzylamine Hydrochloride.** A solution of *N*-(*p*-nitrobenzyl)phthalimide (1.0 g, 0.0035 mol; obtained in 88% yield from potassium phthalimide and *p*-nitrobenzyl bromide[10]) and hydrazine hydrate (0.17 g, 0.0035 mol) in dimethylformamide (15 mL) was stirred for 3 h at room temperature. Five milliliters of 1 N HCl were added and the reaction mixture was concentrated in vacuo. Water (20 mL) was added to the residue and the brownish precipitate was removed by filtration. The filtrate was evaporated to dryness in vacuo, and the remaining solid was crystallized from ethanol yielding 0.55 g (83%) of pale yellow

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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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