Synthesis of New 2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidines *via* the Taylor Ring Transformation/Ring Annulation Strategy

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Selected examples 2,4-diamino-7*H*-pyrrolo[2,3-*d*]pyrimidines with a phenyl or benzyl group at the 5-position were synthesized as inhibitors of dihydrofolate reductase (DHFR) from *Pneumocystis carinii* and *Toxoplasma gondii*, two potentially life-threatening opportunistic pathogens associated with AIDS and other disorders of the immune system. Aldol condensation of paraformaldehyde with substituted benzaldehydes or with phenylacetaldehyde afforded α-hydroxyketones with a phenyl or benzyl group at the 4-position. Further reaction of the hydroxyketones with malononitrile afforded 2-aminofuran-3-carbonitriles, which upon heating with guanidine underwent ring transformation/ring annulation to produce 2,4-diamino-7*H*-pyrrolo[2,3-*d*]pyrimidines rather than 2,4-diaminofuro[2,3-*d*]pyrimidines. One of the target compounds obtained in this manner, 2,4-diamino-5-(3,4,5-trimethoxyphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (**1d**), may be viewed as a conformationally restricted analogue of trimethoprim, an antimicrobial agent widely used in combination with a sulfa drug to treat *P. carinii* and *T. gondii* opportunistic infections in patients with AIDS. Compound **1d** inhibited *P. carinii* and *T. gondii* DHFR with IC_{50} values of 8.3 and 14 μ*M*, respectively. This potency was somewhat greater than that of trimethoprim. However, because this compound was also more potent than trimethoprim against mammalian (rat liver) DHFR rat liver it lacked species selectivity. The other 2,4-diamino-7*H*-pyrrolo[2,3-*d*]pyrimidines synthesized were neither potent nor selective.

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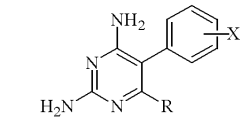
As part of in an ongoing program of design, synthesis, and biochemical and biological evaluation of various types of lipophilic condensed 2,4-diaminopyrimidine ring systems as inhibitors of dihydrofolate reductase (DHFR) and potential drugs against life-threatening opportunistic infections in AIDS patients with a dysfunctional immune system [1,2], we wish to report the synthesis of several previously unknown 2,4-diaminopyrrolo[2,3-*d*]-pyrimidines with a substituted phenyl and, in one example, an unsubstituted benzyl substituent at the 5-position. 2,4-Diaminopyrrolo[2,3-*d*]pyrimidines are but one of a number of condensed diaminopyrimidine ring systems known to bind tightly to dihydrofolate reductase (DHFR), the enzyme responsible for maintaining adequate levels of essential tetrahydrofolate cofactors in dividing cells. Mammalian cells take up reduced folates efficiently from their exogenous environment by making use of membrane-associated transporter proteins, and thus do not have to synthesize these folates *de novo*. However these transporter proteins are not present in *Pneumocystis carinii* and *Toxoplasma gondii*, two common opportunistic pathogens that often provide the earliest clue that someone has AIDS, and can themselves prove fatal if left untreated, especially if both organisms are present concurrently. The rationale for using lipophilic DHFR inhibitors as drugs against *P. carinii*, *T. gondii* and other opportunistic parasites lacking folate transporter proteins on their surface is that sensitive host tissues such as the bone marrow can be preferentially spared from the toxic effects of the antifolate by co-administration of an exogenous source of tetrahydrofolate, namely leucovorin (5-formyl-tetrahydrofolate). Experimental regimens using highly toxic DHFR inhibitors to treat severe *P. carinii* and *T. gondii* infections refractory to other classes of drugs are based on this rationale. An alternative approach that would obviate the need to co-administer leucovorin would be to use a single agent that binds preferentially to the DHFR from *P. carinii* or *T. gondii*, thereby decreasing the risk to the patient. A number of newer DHFR inhibitors that bind more tightly to *P. carinii* DHFR, and especially to *T. gondii* DHFR, have been discovered in the last few years, but thus far have not been tested clinically. A most vexing problem in designing such drugs has been that, in most of the examples studied thus far, selectivity of binding appears to be inversely correlated with potency. Thus, a major goal of our research and that of investigators working in this area has been to find a DHFR inhibitor that is both potent and selective.

Although a number of 2,4-diaminopyrrolo[2,3-*d*]-pyrimidines with lipophilic aromatic substituents at the 5-position have been described [3,4], they all contain a CH_2NH, $CH_2N(Me)$, or CH_2S bridge between the pyrrole and aryl moiety. In the present work we were interested in preparing compounds in which the two halves of the molecule are joined *via* a shorter bridge in order to assess the effect of this particular structural modification on potency and selectivity. To this end, we took advantage of an elegant strategy first described by Taylor and coworkers [5,6], called "one-step ring-transformation/ring annulation". At the heart of this strategy is the intriguing

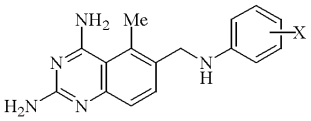
proclivity of 2-aminofuran-3-carbonitriles with a substituent at the 4-position to form 2,4-diaminopyrrolo[2,3-*d*]pyrimidines rather than 2,4-diaminofuro[2,3-*d*]pyrimidines upon being heated in the presence guanidine. Although two plausible pathways were proposed for this novel transformation, a choice between these pathways could not be made.

Among the simple model compounds reported by Taylor [5] was 2,4-diamino-5-phenylpyrrolo[2,3-*d*]pyrimidine (**1a**), which formed in 42% yield when 2-amino-4-phenylfuran-3-carbonitrile (**1b**) was heated with guanidine in refluxing methanol for 24 hours (Scheme 1). Several other 2,4-diaminopyrrolo[2,3-*d*]pyrimidines with methyl, ethyl, phenyl groups at the 5- and/or 6-position were obtained in similar fashion, as well as analogs in which the 5-substituent was a 2-phenylethyl or 3-phenylpropyl group with a CO_2Me group at the *para* position. The latter esters were used to prepare classical DHFR inhibitors with a glutamic acid side chain.

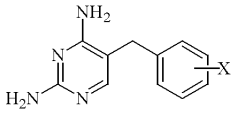
Because of the common presence of aromatic chloro and methoxy groups in therapeutically useful lipophilic DHFR inhibitors, a selected number of analogs of **1a** containing this pharmacophoric motif were prepared, the form of compounds **1b-e** (Scheme 1) In addition, because Taylor and coworkers had not used the ring transformation/ring annulation method to make 2,4-diamino-5-benzylpyrrolo[2,3-*d*]pyrimidine (**1e**, Scheme 1) their method was used to also prepare this compound as the simplest example of a 5-benzyl derivative. The 4-chloro- and 3,4-dichlorophenyl derivatives **1b** and **1c** may be viewed as analogs of pyrimethamine and metoprine, and the 3,4,5-trimethoxy derivative **1d** as an analog of trimethoprim. The structures of trimethoprim, pyrimethamine, metoprine, piritrexim, and trimetrexate are given in Figure 1.



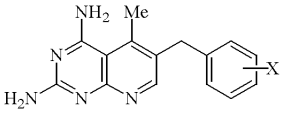
Pyrimethamine: R = Et, X = 4-Cl
Metoprine: R = Me, X = 3,4-Cl_2



Trimetrexate: X = 3,4,5-$(OMe)_3$



Trimethoprim: X = 3,4,5-$(OMe)_3$

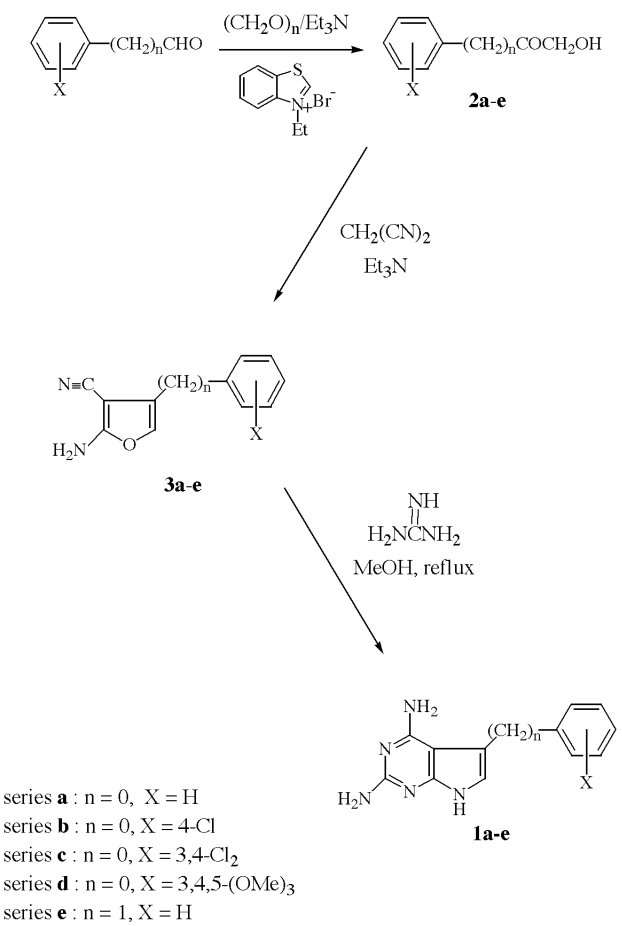


Piritrexim: X = 2,5-$(OMe)_2$

Figure 1. Structures of clinically used lipophilic DHFR inhibitors.

As shown in Scheme 1, aldol condensation of appropriately substituted benzaldehydes with paraformaldehyde in the presence of *N*-ethylbenzothiazolium bromide and triethylamine [5-7] afforded the desired α-hydroxyketones **2a-d**. Further reaction of **2a-d** with malononitrile and triethylamine in refluxing methanol yielded the furans **3a-d**, which upon heating with 1.5 molar equivalents of guanidine in refluxing ethanol, typically for 30 hours, were converted to the pyrrolopyrimidines **1a-d**. In the case of **1e**, phenylacetaldehyde was used in place of benzaldehyde, giving rise to the α-hydroxyketone **2e** and the furan **3e**.

Scheme 1



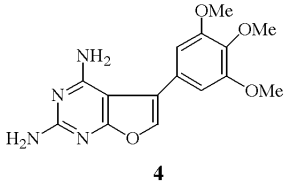
Compounds **1a-e** were all obtained in analytically pure form by column chromatography on silica gel, and were characterized spectroscopically and by microchemical analysis. A characteristic feature of each product was the presence in the ^{1}H-nmr spectrum of a singlet at ca. δ 6.8, which could be assigned to the C6 proton of a

pyrrolo[2,3-*d*]pyrimidine and was in agreement with the reported value for **1a** [5]. The benzylic CH_2 group in the furan **3e** gave a singlet at δ 3.61, and thus was less deshielded than the same proton in the pyrrole **1e** (δ 4.00). Interestingly, the vinylic C6 proton in **3e** (δ 6.76) was less deshielded than the same proton in **3a** (δ 6.99) and the C6 proton in **1e** (δ 6.32) was less deshielded than the same proton in **1a** (δ 6.78). Wahid and coworkers [8] recently reported that the vinylic C6 proton in 2,4-diamino-5-(3,4,5-trimethoxyphenyl)furo[2,3-*d*]pyrimidine (**4**) gives rise to a singlet at δ 7.53, a chemical shift considerably higher than the one we observed for **1d**. Final proof that **1a-e** were pyrrolo[2,3-*d*]pyrimidines rather than furo-[2,3-*d*]pyrimidines, as would have been the case if the guanidine reaction had occurred without rearrangement, came from the observation that all these compounds showed an exchangeable singlet at ca. δ 11.0 corresponding to the pyrrole NH.

Spectrophotometric assays of the ability of **1a-e** to inhibit partly purified DHFR from *P. carinii*, *T. gondii*, and rat liver were carried out according to a standardized protocol one of us (R.F.Q.) developed and extensively used in the past to test compounds synthesized in this program [2]. With the exception of the 3,4,5-trimethoxy derivative **1d**, whose IC_{50} values were 8.3 μM against the *P. carinii* enzyme, 14 μM against the *T. gondii* enzyme, and 27 μM against the rat liver enzyme, all the compounds were weak inhibitors, and appeared to lack species selectivity.

Gangjee and coworkers [3] have presented DHFR binding data for 2,4-diamino-5-(3,4,5-trimethoxy-anilino)methyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (**5**), an analog of **1d** in which the two halves of the molecule are joined via a CH_2NH bridge. The IC_{50} values of **5** were >23 μM against *P. carinii* DHFR, 8.1 μM against *T. gondii* DHFR, and 56 μM against rat liver DHFR. Thus our data suggest that removal of the CH_2NH bridge from **5** increased potency against the *P. carinii* and rat enzyme while decreasing it against the *T. gondii* enzyme. Also of interest is that 2,4-diamino-(3,4,5-trimethoxyphenyl)furo[2,3-*d*]pyrimidine (**4**), the oxygen analog of **1e**, is reported to have an IC_{50} >300 μM against both *P. carinii* and human DHFR [8]. From our results it would appear that the acidic proton at N7 must play a significant role in the binding of **1e** to DHFR.

An interesting analog of trimethoprim and **1d**, synthesized and studied in detail in 1996 by Kuyper and coworkers [9], is 2-amino-4-methyl-5-(3,4,5-trimethoxyphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (**6**), in which the normal role of the 4-amino group in DHFR binding is now played by the pyrrole nitrogen. The IC_{50} values of **6** against rat DHFR was found to be 29 μM as compared with 350 μM for trimethoprim, a difference of 12-fold. The corresponding values against *Escherichia coli* DHFR, whose active site is more compact than that of mammalian enzymes, were 0.44 and 0.007 μM respectively, a difference of 63-fold. Crystallographic analysis of the binary complex of **6** with *Escherichia coli* DHFR suggested that the 2-amino and 3,4,5-trimethoxyphenyl groups occupy approximately the same locations in the enzyme active site as the 2-amino and 3,4,5-trimethoxybenzyl groups of trimethoprim, with a slight displacement to accommodate the sterically hindered biphenyl-like structure of **6**, whose two halves are forced to lie in a nearly perpendicular orientation to each another. Although structural analysis of the ternary enzyme complex containing NADPH as well as the ligand in the active was not carried out, the authors speculated that the stronger binding of **6** to both the bacterial and mammalian enzyme might be due to its higher pK_a, which results in 6% protonation at neutral pH as compared with 56% in the case of trimethoprim. Because **1d** is a 2,4-diamino derivative its pK_a is likely to be closer to trimethoprim than to **6**. Thus our finding that **1d** and **6** have almost the same IC_{50} against rat DHFR suggests that other factors than just basicity may have to be considered in order to explain the improved binding of these conformationally restricted analogs relative to trimethoprim.



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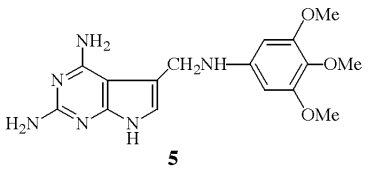

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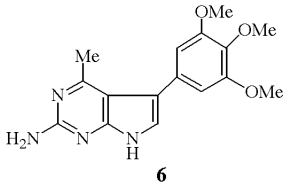

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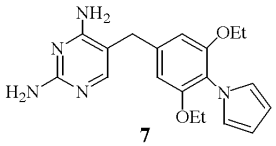

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Table 1

Dihydrofolate Reductase Inhibition by Pyrrolo[2,3-*d*]pyrimidines **1a-e**

Compound	IC_{50} (μ*M*) [a]			Selectivity Index [b]	
	P. carinii	*T. gondii*	Rat Liver	*P. carinii*	*T. gondii*
1a	30% @ 186 μ*M*	12	9.1	[c]	0.76
1b	27% @ 161 μ*M*	113	62	[c]	0.55
1c	33	16	23	0.68	1.5
1d	8.3	14	27	3.2	1.9
1e	30% @ 189 μ*M*	44% @ 130	270	[c]	[c]
Trimethoprim [d]	12	2.7	130	11	48

[a] Enzyme activity was determined according to a standardized method used this program reliably for a number of years [12,13]. Each drug concentration was tested in triplicate. As an example of the reproducibility of the assay, the IC_{50} values (mean ± standard error) in Dr. Queener's laboratory over a five-year period using pyrimethamine and partially purified dihydrofolate reductase from *P. carinii*, *T. gondii*, and rat liver have been 2.39 ± 0.42, 0.50 ± 0.23, and 1.52 ± 0.32 μ*M*, respectively; [b] Selectivity Index = IC_{50} (rat liver)/IC_{50} (*P. carinii* or *T. gondii*); [c] Not determined because the solubility of the drug was exceeded in one or both assays; [d] Data cited in reference 2.

The finding that **1d** is more potent than trimethoprim against *P. carinii* and rat liver DHFR while being less potent against the *T. gondii* enzyme is also of interest. While the distance between the phenyl ring and the diaminopyrimidine moiety is similar in **1d** and trimethoprim, the conformational flexibility of the 3,4,5-trimethoxybenzyl side chain of trimethoprim is clearly absent in **1d**. Molecular models indicate that the only way unfavorable steric interaction can be avoided between the 4-amino group and the *ortho* hydrogen of the phenyl ring is for the latter to adopt a severely twisted conformation, as has been found in the case of **6** [9] A consequence of this nearly orthogonal configuration is that the methoxy groups of **1d** must lie in a different spatial location in the DHFR active site than those of trimethoprim. Moreover, a consequence of the sterically constrained biphenyl-like structure of 2,4-diamino-5-aryl-7*H*-pyrrolo[2,3-*d*]pyrimidines is that these compounds can in principle exist as pairs of non-superimposable rotamers with different energies of binding to dihydrofolate reductase. Our results suggest that the orthogonal orientation of the rings in **1d** may be tolerated better in the active site of the *P. carinii* and rat enzyme than in the active site of the *T. gondii* enzyme.

Given the dissimilarity in their three-dimensional structures, the finding that **1d** and trimethoprim so closely resembles each other in their ability to inhibit DHFR is a novel and possibly exploitable lead. Substitution of a pyrrole ring for one of the methoxy groups in trimethoprim, as well replacement of one or more of the methoxy groups by other alkoxy groups, has been reported to selectively enhance binding to *P. carinii* versus mammalian DHFR [10], and was the basis for the recent development of 2,4-diamino-5-(3,5-diethoxy-4-pyrrolobenzyl)pyrimidine (**7**, epiroprim) as a promising new drug against various resistant microbial organisms including *P. carinii* [10]. Inasmuch as **1d** appears to represent a sparsely studied class of DHFR inhibitors, this compound may be viewed as a potential candidate for lead optimization studies aimed at improving potency and selectivity.

EXPERIMENTAL

Infrared spectra were obtained on a Perkin-Elmer Model 781 double-beam recording spectrophotometer. ^{1}H-nmr spectra were recorded at 60 MHz (Dana-Farber Cancer Institute) or at 200 or 500 MHz (Harvard Medical School). Low-resolution mass spectra were obtained by the Molecular Biology Core Facility, Dana-Farber Cancer Institute, using either the electron impact (EI) or fast atom bombardment (FAB) mode. Analytical thin-layer chromatography (tlc) was on Whatman MK6F silica gel slides (60 μm layer, 1.5 x 4.5 cm, with fluorescent indicator), using 254-nm illumination to visualize the spots. Column chromatography was on Baker 7024 flash silica gel (40 μm particle size). Melting points were determined in Pyrex capillary tubes using a Mel-Temp apparatus (Laboratory Devices, Inc., Cambridge, MA) and are not corrected. Solvents for moisture-sensitive reactions were of 'Sure-Seal' grade (Aldrich, Milwaukee, WI). Other chemicals obtained from commercial sources were of the best available purity. Elemental analyses were performed by QTI Laboratories, Madison, NJ, and were within ±0.4% of theoretical values unless otherwise indicated.

2,4-Diamino-5-phenyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (**1a**).

Step 1 (Method A): Paraformaldehyde (0.63 g, 20 mmole), (*N*-ethylbenzothiazolium bromide (0.90 g, 3.6 mmole), and triethylamine (0.37 g, 3.6 mmole) were added with magnetic stirring to a solution of benzaldehyde (2.14 g, 20 mmole). The mixture was stirred under reflux overnight, the solvent was evaporated under reduced pressure, and the residue was taken up in ethyl acetate (30 mL). The white solid that formed gradually on standing was collected and washed with ethyl acetate (10 mL). The filtrate was evaporated to form a brown oil which slowly solidified in the refrigerator. Recrystallization from a 7:3 hexanes-ethyl acetate mixture afforded **2a** as colorless crystals (1.2 g, 44%, used directly in the next step), mp 63-64°; ^{1}H-nmr (60 MHz, deuteriochloroform) δ 3.51 (s, 1H, CH_2OH), 4.85 (s, 2H, CH_2OH), 7.41-7.98 (m, 5H, aromatic protons).

Step 2 (Method B) A solution of malononitrile (0.60 g, 9.1 mmole) and triethylamine (0.92 g, 9.1 mmole) in anhydrous methanol (10 m) was added dropwise under a nitrogen atmosphere to a solution of **2a** (1.1 g, 8.3 mmole) in anhydrous methanol (30 mL). The mixture was stirred magnetically at room

temperature for 15 hours. After removal of the solvent by rotary evaporation, the residue was chromatographed on silica gel with chloroform as the eluent to obtain **3a** as a yellow oil, which became partially solid on standing (0.58 g, 39%, used directly in the next step); ^{1}H-nmr (60 MHz, d_6-dimethylsulfoxide) δ 5.45 (br s, 2H, NH_2), 6.99 (s, 1H, C=CH), 7.21-7.85 (m, 5H, aromatic protons).

Step 3 (Method C). Amino nitrile **3a** (184 mg, 1 mmole) was added to a solution prepared from guanidine hydrochloride (140 mg, 1.5 mmole) and sodium methoxide (81 mg, 1.5 mmole) in anhydrous methanol (25 mL). The reaction mixture was stirred under reflux for 48 hours, the solvent was removed by rotary evaporation, and the residue was chromatographed on silica gel with 95:5 chloroform-methanol as the eluent. Fractions containing the desired product were combined and evaporated under reduced pressure to obtain **1a** as a slightly purplish white solid (128 mg, 57%), mp 215-216°; ms: theory for $C_{12}H_{11}N_5$ m/z 225; found 225; ir (potassium bromide) ν 3472, 3310, 3161, 1600, 1565, 1540, 1474, 1418, 1304, 1070, 960 cm^{-1}; ^{1}H-nmr (500 MHz, d_6-dimethylsulfoxide): δ 5.40-5.70 (br s, 2- and 4-NH_2), 6.78 (d, 1H, C=CH), 7.30-7.43 (m, 5H, aromatic protons), 10.95 (broad s, 1H, pyrrole NH).

Anal. Calcd. For $C_{12}H_{11}N_5$•0.1H_2O: C, 63.48; H, 4.97; N, 30.84. Found: 63.26; H, 4.79; N, 30.64.

2,4-Diamino-5-(4-chlorophenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (**1b**).

4-Chloro-α-hydroxyacetophenone (**2b**) was obtained from 4-chlorobenzaldehyde according to Method A, and was used directly for the next step; yellow crystals (48%), mp 111-112° (from 7:3 hexanes-ethyl acetate); ^{1}H-nmr (60 MHz, deuteriochloroform): δ 3.85 (s, 1H, CH_2OH), 4.88 (s, 2H, CH_2OH), 7.45 (d, 2H, aromatic protons), 7.88 (d, 2H, aromatic protons). 2-Amino-4-(4-chlorophenyl)furan-3-carbonitrile (**3b**) was obtained from **2b** and malononitrile by Method B except that the product was chromatographed with ethyl acetate, then recrystallized from dichloromethane. The greenish-white product (53%, mp 155-156°) was used directly in the next step; ^{1}H-nmr (60 MHz, d_6-dimethylsulfoxide) δ 4.94 (s, 2H, NH_2), 6.97 (s,1H, C=CH), 7.25-7.71 (m, 4H, aromatic protons). Condensation of **2c** with guanidine was carried out by Method C to obtain **3c** as an off-white powder (51%), mp 226° dec; ms: theory for $C_{12}H_{10}N_5Cl$ m/z 260, 261; found 260, 261; ir (KBr) ν 3470, 3390, 3320, 3185, 1610, 1590, 1570, 1540, 1420, 1090, 960 cm^{-1}; ^{1}H-nmr (500 MHz, d_6-dimethylsulfoxide): δ 5.56 (br s, 2H, 2-NH_2), 5.60 (br s, 2H, 4-NH_2), 6.83 (s, 1H, C=CH), 7.33 (d, 4H, aromatic protons), 11.00 (br s, 1H pyrrole NH).

Anal. Calcd. for $C_{12}H_{10}N_5Cl$: C, 55.50; H, 3.88; N, 26.97; Cl, 13.66. Found: C, 55.53; H, 4.09; N, 26.68; Cl, 13.74.

2,4-Diamino-5-(3,4-chlorophenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (**1c**).

3,4-Dichloro-α-hydroxyacetophenone (**2c**) was obtained from 3,4-dichlorobenzaldehyde by Method A, and was used directly in the next step; tan crystals (34%), mp 115-116° (from 9:1 hexanes-ethyl acetate); ^{1}H-nmr (200 MHz, deuteriochloroform): δ 4.77 (s, 2H, CH_2OH), 5.22 (broad s, 1H, CH_2OH), 7.87 (s, 1H, aromatic proton), 8.12 (m, 3H, aromatic protons). 2-Amino-4-(3,4-dichlorophenyl)furan-3-carbonitrile (**3c**) was obtained from **2c** by Method B except that the product was chromatographed with ethyl acetate. The resultant yellow powder (41%, mp 92-93°) was used directly in the next step; ^{1}H-nmr (200 MHz, d_6-dimethylsulfoxide): δ 4.78 (s, 2H, NH_2), 7.17 (s, 1H, C=CH), 7.50-7.85 (m, 3H, aromatic protons). Condensation of **3c** with guanidine was carried out by Method C except that the product (**1c**) was chromatographed with 4:1 chloroform-methanol; off-white powder (49%), mp 264° dec; ms: theory for $C_{12}H_9N_5Cl_2$ m/z 294, 295, 296; found 294, 295, 296; ir (KBr) ν 3470, 3370, 3330, 3140, 1605, 1570, 1530, 1465, 1130, 1025, 980, 790 cm^{-1}; ^{1}H-nmr (200 MHz, d_6-dimethylsulfoxide) δ 5.59 (br s, 2H, 2-NH_2), 5.71 (br s, 2H, 2H, 4-NH_2), 6.95 (s, 1H, C=CH), 7.38 (dd, 1H, J_{ab} = 8 Hz, J_{ac} = 1.8 Hz, 5'-H), 7.61 (d, 1H, J_{ac} = 1.8 Hz, 2'-H), 7.63 (d, 1H, J_{ab} = 8.0 Hz, 4'-H), 11.08 (s, 1H, pyrrole NH).

Anal. Calcd. for $C_{12}H_9N_5Cl_2$•0.55CH_3OH: C, 48.35; H, 3.62; N, 22.46; Cl, 22.74. Found: C, 48.48; H, 3.22, N, 22.14; Cl, 22.60.

2,4-Diamino-5-(3,4,5-trimethoxyphenyl)-7*H*-pyrrolo[2,3-*d*]-pyrimidine (**1d**).

3,4,5-Trimethoxy-α-hydroxyacetophenone (**2d**) was obtained from 3,4,5-trimethoxybenzaldehyde by Method A, and was used directly in the next step; colorless crystals (31%), mp 73-74° (90:10 hexanes-ethyl acetate); ^{1}H-nmr (200 MHz, d_6-dimethylsulfoxide): δ 3.72 (s, 3H, 4-OMe), 3.83 (s, 6H, 3- and 5-OMe), 4.79 (d, 2H, J = 5.8 Hz, CH_2OH), 5.02 (t, 1H, J = 5.8 Hz, CH_2OH), 7.21 (s, 2H, aromatic protons). 2-Amino-4-(3,4,5-trimethoxyphenyl)furan-3-carbonitrile (**3d**) was obtained from **2d** by Method B except that the product was chromatographed with 3:2 hexane-ethyl acetate; dark-brown powder (30%), mp 117-118°; ^{1}H-nmr (200 MHz, d_6-dimethylsulfoxide): δ 3.66 (3H, 4-OMe), 3.79 (s, 6H, 3- and 5-OMe), 4.82, br s, 2H, NH_2) 6.86 (s, 2H, 2'- and 6'-H), 7.41 (s, 1H, C=CH). Condensation of **3d** with guanidine was carried out by Method C except that the product (**1d**) was chromatographed with 4:1 chloroform-methanol; tan powder (55%), mp 265-266°; ir (potassium bromide) ν 3480, 3380, 3170, 1605, 1565, 1410, 1360, 1120, 995 cm^{-1}; ms: theory for $C_{15}H_{17}N_5O_3$ m/z 316, found 316; ^{1}H-nmr (200 MHz, d_6-dimethylsulfoxide): δ 3.67 (s, 3H, 4-OMe), 3,80 (s, 6H, 3- and 5-OMe), 5.52 (br s, 2H, 2-NH_2), 5.66 (br s, 2H, 4-NH_2), 6.68 (s, 2H, 2- and 6-H), 6.81 (s, 1H, C=CH), 10.90 (s, 1H, pyrrole NH).

Anal. Calcd for $C_{15}H_{17}N_5O_3$•0.1H_2O: C, 56.81; H, 5.47; N, 22.08. Found: C, 56.79; H, 5.28; N, 22.02.

2,4-Diamino-5-benzyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (**1e**).

1-Hydroxy-4-phenylpropan-2-one (**2e**) was obtained by Method A except that the product was purified by silica gel chromatography using 7:3 hexanes-ethyl acetate as the eluent; yellow oil (73%); ^{1}H-nmr (60 MHz, deuteriochloroform): δ 3.65 (s, 2H, benzylic CH_2), 4.80 (br m, 1H, CH_2OH), 4.22 (s, 2H, CH_2OH), 7.11-7.54 (m, 5H, aromatic protons). The ketone was condensed directly with malononitrile by Method B to obtain **3e** as a brown solid (43%), mp 105-106°; ^{1}H-nmr (200 MHz, d_6-dimethylsulfoxide) δ 3.31 (s, 2H, 4-NH_2), 3.61 (s, 2H, benzylic CH_2), 6.76 (s, 1H, C=CH), 7.21-7.32 (m, 5H, aromatic protons). Condensation of **3e** with guanidine was carried out by Method C; colorless crystals (37%) from methanol, mp 266-267°; ms: theory for $C_{13}H_{13}N_5$ m/z 239 (M^+), 240 (MH^+), found 239, 240; ir (potassium bromide) ν 3490, 3450, 3390, 3305, 3130, 1610, 1575, 1545, 1480, 1420, 1400, 1320, 1100, 700 cm^{-1}; ^{1}H-nmr

(200 MHz, d_6-dimethylsulfoxide): δ 4.00 (s, 2H, benzylic CH_2), 5.34 (br s, 2H, 2-NH_2), 5.70 (br s, 2H, 4-NH_2), 6.32 (s, 1H, C=CH), 7.12-7.31 (m, 5H, aromatic protons), 10.43 (s, 1H, pyrrole NH).

Anal. Calcd. for $C_{13}H_{13}N_5$: C, 65.26; H, 5.48; N, 29.27. Found: C, 65.00; H, 5.49; N, 29.49.

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