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Synthesis of New 2,4-Diaminopyrido[2,3-*d*]pyrimidine and 2,4-Diaminopyrrolo[2,3-*d*]pyrimidine Inhibitors of *Pneumocystis carinii*, *Toxoplasma gondii*, and *Mycobacterium avium* Dihydrofolate Reductase

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Abstract—A concise new route allowing easy access to five previously unreported 2,4-diamino-6-(substituted benzyl)pyrido[2,3-*d*]pyrimidines (**2a–e**) was developed, involving condensation of 2,4-dipivaloylamino-5-bromopyrido[2,3-*d*]pyrimidine (**6**) with an organozinc halide in the presence of a catalytic amount of [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II)·CH_2Cl_2, followed by removal of the pivaloyl groups with base. Also prepared via a scheme based on the Taylor ring expansion/ring annulation synthesis were three heretofore undescribed 2,4-diamino-5-(substituted benzyl)-7*H*-pyrrolo[2,3-*d*]pyrimidines (**3b–c**). Standard spectrophotometric assays were used to compare the ability of **2a–e** and **3b–c** to inhibit dihydrofolate reductase (DHFR) from *Pneumocystis carinii*, *Toxoplasma gondii*, and *Mycobacterium avium*, three examples of opportunistic pathogens to which AIDS patients are highly vulnerable because of their immunocompromised state. For comparison, 13 previously untested 2,4-diamino-6-(substituted benzyl)quinazolines (**17a–m**) were also evaluated as inhibitors of these enzymes, as well as the enzyme from rat liver. None of the quinazolines or pyridopyrimidines tested was more potent against the *P. carinii* enzyme than the structurally related reference compound piritrexim (**1**), and none showed selectivity for the *P. carinii* enzyme over the rat enzyme. One of the pyridopyrimidines (**2c**) showed 10-fold selectivity for *T. gondii* versus rat DHFR, and two of them (**2b**, **2c**) showed selectivity for the *M. avium* enzyme. However, this gain in species selectivity was achieved at the cost of decreased in potency, as has been noted with many other lipophilic DHFR inhibitors.

Introduction

2,4-Diaminopyrido[2,3-*d*]pyrimidines containing a substituted phenyl or other aromatic ring attached to C6 via by a short bridge comprise a family of lipophilic small-molecule antifolates with potential applications in the treatment and prophylaxis of AIDS-associated opportunistic microbial infections. Among the often fatal opportunistic pathogens frequently identified in immunocompromised AIDS patients are *Pneumocystis carinii, Toxoplasma gondii*, and the *Mycobacterium avium* complex (MAC).[1] Indeed, identification of one or more of these organisms is often the earliest sign that human immunodeficiency virus (HIV) is spreading rapidly through the immune system. The only 2,4-diaminopyrido[2,3-*d*]pyrimidine tested clinically against *P. carinii* in AIDS patients to date has been piritrexim (**1**).[2] Because this is an extremely potent inhibitor of dihydrofolate reductase (DHFR), and its affinity is higher for human dihydrofolate reductase (DHFR) than it is for the *P. carinii* or *T. gondii* enzyme, **1** is highly toxic to the hematopoietic system and has to be used in combination with leucovorin (LV) to selectively protect the bone marrow and other sensitive host tissues. LV is readily taken up by mammalian cells, whereupon it reconstitutes the tetrahydrofolate pool and thus acts as an antidote to the DHFR inhibitor. The selectivity of LV in this context is considered to reflect the fact that its uptake is mediated by a reduced folate carrier (RFC) protein that is present in the plasma membrane of mammalian cells but not parasitic eukaroytic species such as *P. carinii*.[3] Lipophilic antifolates like **1** do not

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depend on the RFC for uptake, and thus would be selectively toxic to *P. carinii* in the absence of LV only if their affinity for the DHFR of *P. carinii* were higher than their affinity for human enzyme. This would of course be true, as well, for other microbial organisms that lack the RFC system of mammalian cells.

A large variety 2,4-diaminopyrido[2,3-*d*]pyrimidines with a substituted aromatic ring joined to C6 by a short bridge have been studied in vitro as part of a broad search for compounds with a preferential binding affinity for *P. carinii* and *T. gondii* DHFR. These have included variously substituted 2,4-diamino-6-(*N*-arylamino)methyl,[4,5] 2,4-diamino-6-(2-arylethyl),[5,6] 2,4-diamino-6-(*N*-aralkyl)amino,[7] and 2,4-diamino-6-arylthio[7] derivatives. The 6-(*N*-aralkyl)-amino derivatives have also been tested against DHFR from *M. avium*.[8] However, to our knowledge, inhibition of *P. carinii*, *T. gondii*, and *M. avium* DHFR has not been studied with any 2,4-diamino-6-(substituted benzyl)pyrido[2,3-*d*]pyrimidines other than **1** itself, in which a 5-Me group is also present. In the present paper, we describe a synthesis of the previously unknown 2,4-diamino-6-(substituted benzyl)pyrido[2,3-*d*]pyrimidines **2a**–**e** via a convenient Pd(0) mediated carbon–carbon bond-forming reaction developed recently in our laboratory as a route to 2,4-diamino-6-(substituted benzyl)quinazolines from 2,4-diamino-6-iodoquinazoline.[9]

More distantly related to **1**, but nonetheless of potential interest in the context of the search for newer lipophilic antifolates for use in the treatment of AIDS-related opportunistic infections, are 2,4-diamino-5-(anilinomethyl) and 5-(arylthiomethyl-7*H*-pyrrolo[2,3-*d*]pyrimidines, of which several examples have been tested.[10] Apart from the presence of an electron-donating instead of electron-withdrawing nitrogen next to the pyrimidine ring, a notable feature of these five-membered ring analogues was that the side chain had a different spatial orientation with respect to the heterocyclic moiety than the side chain of pyrido[2,3-*d*]pyrimidine derivatives. Several analogous pyrrolo[2,3-*d*]-pyrimidines with a substituted phenyl group at C5 were subsequently reported by our own group.[11] In the present paper, in addition to **2a**–**e**, we report the synthesis and antifolate activity of the previously unknown 5-(substituted benzyl) derivatives **3a**–**c**. The structures of **1** (piritrexim), the pyrido[2,3-*d*]pyrimidines **2a**–**e**, and the pyrrolo[2,3-*d*]pyrimidines **3a**–**c** are shown in Figure 1.

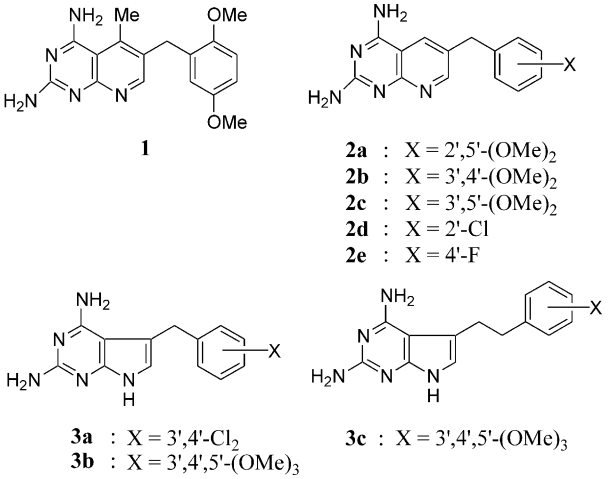
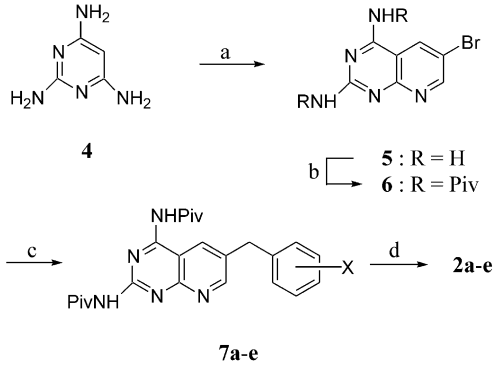


Figure 1. Structures of piritrexim (**1**), 2,4-diaminopyrido[2,3-*d*]pyrimidines **2a**–**e**, and 2,4-diamino-7*H*-pyrrolo[2,3-*d*]pyrimidines **3a**–**c**.

Chemistry

As shown in Scheme 1, condensation of 2,4,6-triaminopyrimidine (**4**) with freshly prepared bromomalonaldehyde according to Gangjee and coworkers[4c] afforded 2,4-diamino-6-bromopyrido[2,3-*d*]pyrimidine (**5**). Upon further reaction with pivalic anhydride in refluxing pyridine, **5** was converted to a product whose ^{1}H NMR spectrum showed it to consist mostly of the desired N^2,N^4-dipivaloyl derivative **6**, along with a smaller amount of material that appeared to be more extensively pivaloylated. Silica gel chromatography followed by recrystallization of pooled TLC-homogeneous fractions from acetone afforded pure **6** in 29% overall yield (two steps). In a typical coupling reaction, a catalytic amount (2.5 mol.%) of 1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II).CH_2Cl_2 was added to a commercially supplied solution of the substituted benzylzinc chloride in dry THF under an atmosphere of dry N_2. The solution was stirred at room temperature for 5 min to allow the black palladium–zinc complex to form, and a solution of **6** in dry THF was added via a cannula while maintaining the system continuously under N_2. When addition was complete, the mixture was refluxed for ca. 3 h, during which the progress of the reaction was monitored by TLC. After routine workup as described in Experimental, the pivaloylated coupling products (**7a**–**e**) were passed through a silica gel column and converted directly to **2a**–**e** by removal of the pivaloyl groups with NaOH in aqueous MeOH, followed by recrystallization from mixtures of aqueous DMF, mixtures of MeOH, EtOH, and H_2O, or mixtures of DMF, EtOH, and H_2O. Although the non-optimized combined yields for the coupling and deprotection reactions were <40%, the obvious merits of the synthesis are that it requires only four steps from **4**, and that it has the potential to easily generate a library of 2,4-



Scheme 1. Reagents: (a) bromomalonaldehyde/HCl/EtOH; (b) Piv_2O/C_5H_5N; (c) arymethylzinc chloride/$(DPPF)_2PdCl_2{\cdot}CH_2Cl_2$/THF; (d) NaOH/MeOH.

diamino - 6 - (arylmethyl)pyrido[2,3 - *d*]pyrimidines from commercially available arylmethylzinc halide reagents by parallel synthesis.

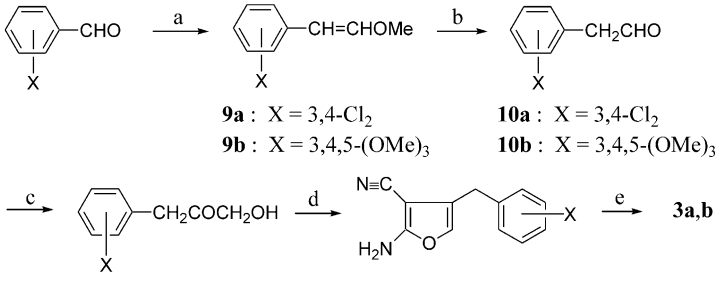
For the synthesis of **3a** and **3b**, the method we previously used for the synthesis of 2,4-diamino-5-benzyl-7*H*-pyrrolo[2,3-*d*]pyrimidine itself[11] was followed with minor changes. As shown in Scheme 2, the 2-amino-4-(substituted aralkyl)furan-3-carbonitriles **8a–c** were synthesized from 3,4,5-trimethoxybenzaldehyde, 3,4-dichlorobenzaldehyde, and 3,4,5-trimethoxyphenylacetaldehyde, respectively, via the ring transformation/ring annulation method of Taylor and coworkers.[12] Although the oily or semi-solid intermediates **9a**,**b** (enol ethers), **10a**,**b** (phenylacetaldehdyes), and **11a**,**b** (α-hydroketones) were not analyzed, they were carefully purified by column chromatography, and ^{1}H NMR spectra were obtained on the chromatographed products in order to confirm both their structure and purity. The enol ethers **9a**,**b** were each used as mixtures of *E* and *Z* isomers whose vinylic doublets could be readily assigned from their coupling constants of 7.0 and 13.2 Hz, respectively, in the case of **9a** and 7.2 and 12.8 Hz, respectively, in the case of **9b**. The benzylic and aldehyde protons in **10b** produced singlets at δ 3.87 and δ 9.66, whereas the corresponding singlets in **10a** were shifted downfield, as expected, to δ 5.05 and δ 9.97. In contrast, upfield rather than downfield shifts of the benzylic protons were observed in **11b** (δ 3.31) and **8b** (δ 3.55) relative to **11a** (δ 3.93) and **8a** (δ 3.65). As anticipated, the 2-furanyl proton in **8a** and **8b** (both at ca. δ 6.8) was unaffected by the electron-donating or electron-withdrawing character of the phenyl substituents. Critically important confirmation that the ring transformation/ring annulation reaction had proceeded in the desired manner to form the pyrrolopyrimidines **3a** and **3b**, and not the corresponding furopyrimidines, was provided by the ^{1}H NMR spectrum, which revealed the presence of the C2 and N7 protons as singlets at δ 6.40 and δ 10.42 in the case of **3a** and δ 6.4 and δ10.54 in the case of **3b**.

For the synthesis of the analogue **3c** with an extra CH_2 group in the bridge (Scheme 3), the commercially available starting material 3,4,5-trimethoxycinnemaldehyde (**12**) was reduced with $LiAlH_4$, the resulting alcohol (**13**) was re-oxidized to 3-(3,4,5-trimethoxypheny)propanal (**14**) with CrO_3/pyridine, and the Taylor approach was followed to convert **14** to **3c** via the α-hydroxyketone **15** and the furan aminonitrile **16**. As in the synthesis of **3a** and **3c**, the intermediates **13–16** were all oils or semi-solids whose identity and purity after chromatography on silica gel were established from their ^{1}H NMR spectra, which were essentially the same as those of the corresponding 3,4,5-trimethoxybenzyl intermediates **8b–11b** with the exception of an extra peak in the CH_2 region. Furthermore, as in the case of **3b**, the ^{1}H NMR spectrum of **3c** showed the peaks at δ 6.41 (pyrrole =CH) and δ 10.35 (pyrrole NH) expected from a pyrrolo[2,3-*d*]pyrimidine structure.

Bioassay

The ability of compounds **2a–e** and **3a–c** to inhibit reduction of dihydrofolate by *P. carinii, T. gondii*, *M. avium*, and rat DHFR in the presence of NADPH was assayed spectrophotometrically at 340 nm as previously described.[13] Also tested for the purpose of comparison were the 2,4-diamino-6-(substituted benzyl)quinazolines (**17a–m**, Table 1) whose chemistry we published recently without DHFR binding data.[9] The results for both groups of inhibitors are presented in Table 1. The IC_{50} values of **2a–e** against *P. carinii* DHFR were only in the 0.2–0.5 μM range, and thus were approximately one order of magnitude higher than that of **1** (IC_{50} 0.03 μM). Compound **2a**, with the identical 2,5-dimethoxy substitution as **1**, was a 10-fold poorer inhibitor, pointing to an important role for the 5-methyl group in binding to the enzyme. The IC_{50} values of **17a–m** were in the 0.05–0.3 μM range, and in four of the five examples for which a side-by-side comparison could be made the quinazolines tended to be slightly more potent than the pyrido[2,3-*d*]pyrimidines. With regard to selectivity, it can be seen that both types of diaminoheterocycles were more potent against the rat enzyme than against the *P. carinii* enzyme, resulting in SI values of <1.

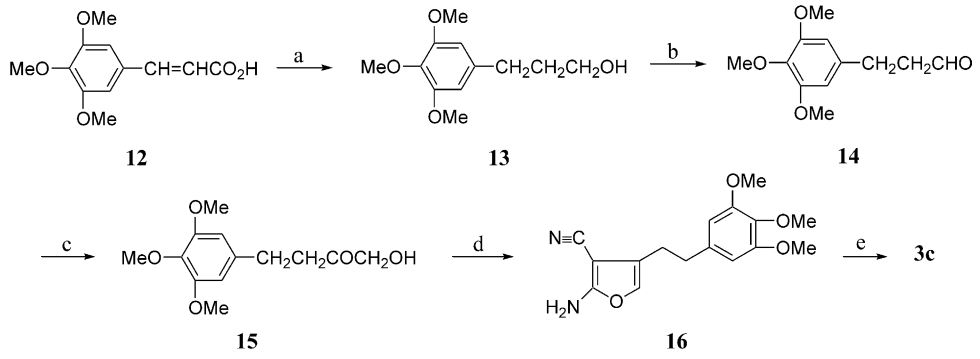
The IC_{50} values of **2a–e** against *T. gondii* DHFR were in the 0.01–0.07 μM range, and thus were consistently lower than their IC_{50} values against the *P. carinii* enzyme. However, the potency of these compounds against *T. gondii* DHFR was lower than that of **1**. On the other hand, the 2-chlorobenzyl analogue **2d** dis-



Scheme 2. Reagents: (a) $MeOCH{=}PPh_3$; (b) aq $HClO_4/Et_2O$; (c) $(CH_2O)_n$/*N*-benzothiazolium bromide/Et_3N; (d) $CH_2(CN)_2/Et_3N$; (e) $H_2NC(=NH)NH_2$.

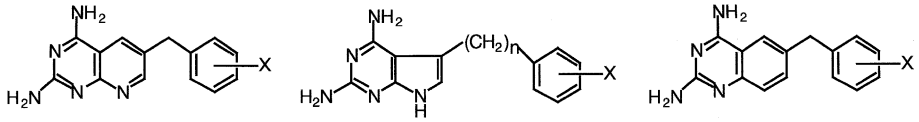
played a modest SI of 4.3 in comparison with the highly unfavorable value of 0.077 observed with **1**. An even better SI of 10.3 was found with the 3,5-dimethoxybenzyl analogue **2c**, although this was unfortunately accompanied by a small decrease in potency. As in the case of the *P. carinii* enzyme, the quinazoline inhibitors tended to be somewhat more potent than the corresponding pyrido[2,3-*d*]pyrimidines. However the increased potency of the quinazolines was, once again, accompanied by decrease in selectivity; cf. for example the SI values of **17f** versus **2c** and **17h** versus **2d**. Interestingly, the compound with the best combination of potency and selectivity against *T. gondii*, relative to **1**, was the 3,5-dimethoxy analogue **2c** rather than the 2,5-dimethoxy analogue **2a**. This difference relative to **1** may be due to the absence of a methyl group at C5.

In general, the IC_{50} values of **2a**–**e** and **17a**–**m** against *M. avium* DHFR were similar to those against *T. gondii*



Scheme 3. Reagents: (a) $LiAlH_4$/THF; (b) CrO_3/pyridine; (c) $(CH_2O)_n$/*N*-ethylbenzothiazolium bromide/Et_3N; (d) $CH_2(CN)_2/Et_3N$; (e) $H_2NC(=NH)NH_2$.

Table 1. Inhibition of *P. carinii*, *T. gondii*, *M. avium*, and rat liver dihydrofolate reductase by 2,4-diamino-6-(substituted benzyl)-pyrido[2,3-*d*]pyrimidines, 2,4-diamino-5-(substituted benzyl)-7*H*-pyrido[2,3-*d*]pyrimidines, and 2,4-diamino-6-(substituted benzyl)quinazolines



2a-e **3a-c** **17a-m**

Compd	R	IC_{50} (μM)[a]				Selectivity index (SI)[b]		
		P. carinii	*T. gondii*	*M. avium*	Rat liver	*P. carinii*	*T. gondii*	*M. avium*
1[c]		0.013	0.0043	0.0006	0.00033	0.026	0.077	0.55
2a	2,5-$(OMe)_2$	0.28	0.034	0.059	0.054	0.19	1.6	0.92
2b	3,4-$(OMe)_2$	0.24	0.064	0.028	0.21	0.88	3.3	7.5
2c	3,5-$(OMe)_2$	0.21	0.036	0.041	0.37	1.8	10.3	9.0
2d	2-Cl	0.51	0.017	0.039	0.073	0.14	4.3	1.9
2e	4-F	0.52	0.040	0.057	0.13	0.25	3.3	2.3
3a	3,4-Cl_2, $n=1$	29	3.3	14	9.6	0.30	2.9	0.69
3b	3,4,5-$(OMe)_3$, $n=1$	72	14	67	52	0.72	3.7	0.78
3c	3,4,5-$(OMe)_3$, $n=2$	0.77	0.037	0.077	0.20	0.26	5.4	2.6
17a	2-OMe	0.31	0.011	0.016	0.054	0.17	4.9	3.4
17b	3-OMe	0.20	0.010	0.021	0.038	0.19	3.8	1.8
17c	4-OMe	0.23	0.018	0.013	0.10	0.43	5.6	7.7
17d	2,5-$(OMe)_2$	0.11	0.010	0.011	0.0094	0.085	0.94	0.85
17e	3,4-$(OMe)_2$	0.092	0.013	0.009	0.028	0.30	2.2	3.1
17f	3,5-$(OMe)_2$	0.11	0.0099	0.017	0.030	0.27	3.0	1.8
17g	3,4,5-$(OMe)_3$	0.15	0.017	0.007	0.042	0.28	2.5	6.0
17h	2-Cl	0.11	0.0064	0.009	0.011	0.10	1.2	0.52
17i	3-Cl	0.17	0.016	0.021	0.040	0.24	2.5	1.9
17j	4-Cl	0.15	0.022	0.025	0.040	0.27	1.8	1.6
17k	3,4-Cl_2	0.050	0.056	0.021	0.028	0.56	0.50	1.3
17l	4-F	0.67	0.026	0.030	0.068	0.10	2.6	2.3
17m	[d]	0.087	0.022	0.020	0.015	0.17	0.68	0.75

[a]Assays of DHFR inhibition were performed and described in refs 13a and b (*P. carinii*, *T. gondii*, rat liver) and 13c (*M. avium*). The standard deviation of these values, based on recent data obtained with **1** against rat liver DHFR is estimated to be ±10%.
[b]SI = IC_{50} (rat liver)/IC_{50} (*P. avium*, *T. gondii*, or *M. avium*).
[c]IC_{50} values of **1** against *P. carinii*, *T. gondii*, and rat liver DHFR differ somewhat from those reported recently in ref 15, which had been obtained with an older reference sample acquired from the NCI drug repository through the courtesy of Dr. Mohamed Nasr. The data reported here were obtained on a different sample kindly provided by Dr. Gary Smith, Glaxo-Wellcome, Research Triangle Park, NC, USA.
[d]2-Naphthylmethyl.

DHFR, but lower than those against *P. carinii* DHFR. Moreover the quinazolines tended once again to be more potent than the corresponding pyrido[2,3-*d*]pyrimidines; cf. for example, **17d–f** versus **2a–c**. The most potent member of the series in the case of the *M. avium* enzyme was **17g**, with an IC_{50} of 0.007 μM. Unlike **1**, **17g** displayed a modest level of selectivity (SI = 6.0).

The pyrrolopyrimidine analogues **3a** and **3b** displayed a dramatic decrease in potency relative to the corresponding quinazolines (**3a** vs **17k**, **3b** vs **17g**), indicating that a bulky benzyl group at the 5-position is very poorly tolerated when the six-membered aromatic B-ring is replaced by a pyrrole. However, potency was substantially restored when an extra CH_2 group was added to the bridge (**3c** vs **3b**). This suggested that a benzyl group at the 5-position, as in **3a** or **3b**, can interfere with the proper positioning of the 4-amino group in the active site, and that this steric constraint is relieved in **3c** because the phenethyl group is able to rotate out of the way.

Although the selectivity of **2a–e** and **17a–m** against the three species of microbial DHFR was less than had been hoped, we felt that, in light of the well-known ability of lipophilic DHFR inhibitors to overcome transport-based resistance to classical antifolates like methotrexate, it might be of interest to test these compounds as inhibitors of the growth of tumor cells in culture. Accordingly, a standard 72-h growth inhibition assay was performed with these compounds against CCRF-CEM human leukemic lymphoblasts. As shown in Table 2, the IC_{50} values of pyridopyrimidines **2a–e** ranged from 0.20 μM (**2b**) to 1.9 μM (**2c**), and those of quinazolines **17a–m** ranged from 0.089 μM (**17h**) to 0.38 μM (**17g**). Where pairs of structures with identical aryl substituents could be compared, the quinazolines were more potent than the pyridopyrimidines in nearly every case (e.g., **17d** > **2a**, **17h** > **2d**, and **17l** > **2e**), in agreement with their greater ability to bind to DHFR. However, in one instance (**17e** vs **2b**) no difference in activity between the two heterocyclic systems was observed.

Table 2. Growth inhibition of CCRF-CEM human leukemic cells by 2,4-diamino-6-(substituted benzyl)pyrido[2,3-*d*][pyrimidines and 2,4-diamino-6-(substituted benzyl)quinazolines

Compd	IC_{50} (μM)	Compd	IC_{50} (μM)	Compd	IC_{50} (μM)
2a	0.31	**17a**	0.21	**17h**	0.089
2b	0.20	**17b**	0.30	**17i**	0.32
2c	1.89	**17c**	0.33	**17j**	0.28
2d	0.45	**17d**	0.13	**17k**	0.32
2e	1.4	**17e**	0.20	**17l**	0.21
		17g	0.38	**17m**	0.27

Cells were grown in 96-well plates for 72 h under a 5% CO_2 humidified atmosphere in RPMI-1640 medium containing 10% fetal bovine serum, 2 mM L-glutamine, and antibiotics. IC_{50} values for each compound were determined by generating a composite growth curve using combined numbers from either two or three separate experiments on different days. IC_{50} values determined from individual experiments with a given compound did not differ from the composite value for that compound by more than ±20%.

Although there have been almost no systematic studies focusing on the ability of compounds such as those described here to inhibit the growth of human tumor cells in culture, the results in Table 2 generally met our expectation that these compounds would probably have IC_{50} values in the 0.1–1 μM range. As a point of reference, the IC_{50} of **1** against CCRF-CEM cells after 48 h of drug exposure can be estimated from published growth curves to be roughly in the 0.01–0.1 μM range.[14] Thus, our somewhat higher IC_{50} for **2a** as compared to **1** was consistent with a view of the 5-Me substituent as being a positive contributor to the biological activity of **1** by virtue of its ability to either promote cellular uptake or interact with nonpolar residues in the active site of DHFR. Conversely, the finding that **2a** and several of its congeners were selective for the *T. gondii* and *M. avium* DHFR, whereas **1** was not, lends support to the view that the smaller active site of the DHFR from these species may not be able to accommodate the 5-Me group as readily as the more 'open' mammalian enzyme. As a corollary, it is conceivable that 5-unsusbtituted analogues would be less likely than **1** to require co-administration of leucovorin when given to AIDS patients with *P. carinii* pneumonia or other opportunistic infections.

Experimental

IR spectra were obtained on a Perkin–Elmer model 781 double-beam spectrophotometer. Only peaks with wavenumbers greater than 1400 cm^{-1} are reported. ^1H NMR spectra were recorded at 200 MHz on a Varian VX200 or, where specified, on a Varian EM360 instrument. Mass spectra in the fast atom bombardment (FAB) mode were obtained by staff of the Dana-Farber Cancer Institute Molecular Biology Core Facility. TLC analyses were performed on Whatman MK6F silica gel plates with UV illumination at 254 nm. Column chromatography was performed on Baker 7025 flash silica gel (40 μm particle size). Melting points were measured in Pyrex capillary tubes in a Mel-Temp 'Electrothermal' apparatus (Fisher Scientific), and are not corrected. [1,1′-Bis(diphenylphosphino)ferrocene] dichloropalladium(III)·CH_2Cl_2 [$(DPPF)_2PdCl_2CH_2Cl_2$] was purchased from Aldrich, Milwaukee, WI, USA. Other chemicals, including the Rieke organozinc reagents as 0.5 M solutions in THF in 'Sure-Seal' bottles, were also from Aldrich. The organozinc chloride solutions were transferred to the reaction flask with the aid of a cannula and dry N_2, and glassware was thoroughly dried and purged with dry N_2 before use. The THF in the Pd(0) catalyzed cross coupling reactions was freshly distilled from Na benzophenone ketyl under a dry N_2, atmosphere. Elemental analyses were performed by Robertson Laboratories, Madison, NJ, USA, and were within ±0.4% of theoretical values unless otherwise specified. The 2,4-diamino-6-benzylquinazolines **17a–m** were prepared as previously reported.[9] 2,4-Diamino-6-bromopyrido[2,3-*d*]pyrimidine (**5**) and 2,4-dipivaloyl-6-bromopyrido[2,3-*d*]pyrimidine (**6**) were synthesized by a slight modification of the method of Gangjee and coworkers[4c] as described below.

2,4-Diamino-6-(2′,5′-dimethoxybenzyl)pyrido[2,3-*d*]pyrimidine (2a). Step 1. $(DPPF)_2PdCl_2 \cdot CH_2Cl_2$ (42 mg, 0.05 mmol) was added to a solution of 2,5-dimethoxybenzylzinc chloride in THF (0.5 M, 4 mL, calculated to contain 2 mmol), and the mixture stirred at room temperature for 5 min under dry N_2. A solution containing **6** (203 mg, 0.5 mmol) in dry THF (2 mL) was added via a cannula. The reaction mixture was stirred under reflux and monitored periodically by TLC. After 3 h, the reaction was quenched with saturated aqueous NH_4Cl (10 mL) followed by saturated aqueous Na_2EDTA (10 mL). After being stirred at room temperature for 30 min, the brown mixture was extracted with CH_2Cl_2, and the extracts were washed with brine, dried (Na_2SO_4) and concentrated under reduced pressure. Chromatography of the residue on silica gel (3% MeOH in CH_2Cl_2) yielded **7a** as a pale-yellow solid that was used directly in the next step (100 mg, 48%); ^{1}H NMR (DMSO-d_6) δ 1.21 (s, 9H, Me_3C), 1.23 (s, 9H, Me_3C), 3.66 (s, 3H, 2′- or 5′-OMe). 3.73 (s, 3H, 2′- or 5′-OMe), 4.00 (s, 2H, CH_2), 6.73–6.90 (m, 3H, 3′-, 4′-, and 6′-H), 8.51 (d, J = 2.4 Hz, 1H, H-5), 8.82 (d, J = 2.4 Hz, 1H, H-7), 11.31 (br, s, CONH).

Step 2. A solution of **7a** (90 mg, 0.214 mmol) in MeOH (10 mL) was treated with 1 N aqueous NaOH (10 mL) and stirred at room temperature under N_2 for 5 days. The solid precipitate was filtered, washed to neutrality with distilled H_2O, and recrystallized from DMF/H_2O to obtain **2a** as a white solid (21 mg, 31%); mp > 300 °C; MS m/z: found 312, calcd 312 $(M+1)^+$; ^{1}H NMR (DMSO-d_6) δ 3.67 (s, 3H, 2′- or 5′-OMe), 3.73 (s, 3H, 2′- or 5′-OMe), 3.85 (s, 2H, CH_2), 6.21 (br s, 2H, NH_2), 6.73 (d, J = 2.8 Hz, 1H, 6′-H), 6.75 (dd, J = 9.6 Hz, 2.8 Hz, 1H, 4′-H), 6.88 (d, J = 9.6 Hz, 1H, 3′-H), 7.41 (br s, 2H, NH_2), 8.20 (d, J = 2.4 Hz, 1H, H-5), 8.49 (d, J = 2.4 Hz, 1H, H-7). IR (KBr) ν 3410, 3340, 3180, 2940, 2840, 1640, 1620, 1580, 1555, 1500 cm^{-1}. Anal. calcd for $C_{16}H_{17}N_5O_2 \cdot 0.35H_2O$: C, 60.50; H, 5.62; N, 22.05. Found: C, 60.56; H, 5.46; N, 21.74.

2,4-Diamino-6-(3′,4′-dimethoxybenzyl)pyrido[2,3-*d*]pyrimidine (2b). Step 1. Addition of **6** (203 mg, 0.5 mmol) in dry THF (2 mL) to 3,4-dimethoxybenzylzinc chloride in dry THF (0.5 M, 4 mL, calculated to contain 2.0 mmol), followed by a workup similar to the one for **7a**, yielded **7b** as a pale-yellow solid (121 mg, 58%); ^{1}H NMR (DMSO-d_6) δ 1.21 (s, 9H, CMe_3), 1.25 (s, 9H, CMe_3), 3.64 (s, 3H, 3′- or 4′-OMe), 3.68 (s, 3H, 3′- or 4′-OMe), 4.02 (s, 2H, CH_2), 6.78 (dd, J = 8.6 Hz, 3.0 Hz, 1H, 6′-H), 6.91 (d, J = 3.0 Hz, 1H, 2′-H), 6.92 (d, J = 8.6 Hz, 5′-H), 8.51 (d, J = 2.4 Hz, 1H, H-5), 8.88 (d, J = 2.4 Hz, 1H, H-7), 11.31 (br, s, CONH).

Step 2. Starting from **7b** (84 mg, 0.20 mmol), **2b** was obtained by the same method as **2a** except that recrystallization was from EtOH/MeOH/H_2O; white solid (20 mg, 31%); mp > 300 °C; MS m/z found 312, found 312 $(M+1)^+$; ^{1}H NMR (DMSO-d_6) δ 3.68 (s, 3H, 3′- or 4′-OMe), 3.71 (s, 3H, 3′- or 4′-OMe), 3.86 (s, 2H, CH_2), 6.26 (br s, 2H, NH_2), 6.71 (d, J = 8.8 Hz, 1H, 6′-H), 6.85 (m, 2H, 2′ and 5′-H), 7.46 (br s, 2H, NH_2), 8.23 (s, 1H, H-5), 8.53 (s, 1H, H-7); IR (KBr) ν 3405, 3340, 3130, 2840, 1650, 1620, 1580, 1555, 1500, 1460 cm^{-1}. Anal. calcd for $C_{16}H_{17}N_5O_2 \cdot 0.3H_2O \cdot 0.15$ MeOH: C, 60.33; H, 5.70; N, 21.78. Found: C, 60.56; H, 5.56; N, 21.43.

2,4-Diamino-6-(3′,5′-dimethoxybenzyl)pyrido[2,3-*d*]pyrimidine (2c). Step 1. Addition of **6** (203 mg, 0.5 mmol) in dry THF (2 mL) to 3,5-dimethoxybenzylzinc chloride in THF (0.5 M, 4 mL, calculated to contain 2 mmol), followed by a workup similar to the one for **7a**, yielded **7c** as a pale yellow solid (117 mg, 56%); ^{1}H NMR (DMSO-d_6) δ 1.23 (s, 9H, Me_3C), 1.25 (s, 9H, Me_3C), 3.70 (s, 6H, 3′- and 5′-OMe), 4.04 (s, 2H, CH_2), 6.35 (t, J = 2.4 Hz, 1H, 4′-H), 6.48 (d, J = 2.4 Hz, 2H, 2′-H and 6′-H), 8.54 (d, J = 2.4 Hz, 1H, H-5), 8.85 (d, J = 2.4 Hz,1H, H-7), 11.31 (br, s, CONH).

Step 2. Starting from **7c** (103 mg, 0.245 mmol), **2c** was prepared by the same method as **2a** except that it was recrystallized from MeOH/H_2O: white solid (29 mg, 38%); mp > 300 °C; MS m/z found 312, calcd 312 $(M+1)^+$; ^{1}H NMR (DMSO-d_6) δ 3.69 (s, 6H, 3′- and 5′-OMe), 3.85 (s, 2H, CH_2), 6.30 (br s, 2H, NH_2), 6.32 (t, J = 2.2 Hz, 1H, H-6′), 6.40 (d, J = 2.2 Hz, 2H, 2′-H and 6′-H), 7.50 (br s, 2H, NH_2), 8.25 (d, J = 2.4 Hz, 1H, H-5), 8.55 (d, J = 2.4 Hz, 1H, H-7), IR (KBr) ν 3410, 3340, 3130, 2960, 2840, 1610, 1585, 1555, 1500, 1460 cm^{-1}. Anal. calcd for $C_{16}H_{17}N_5O_2 \cdot 1.1H_2O$: C, 58.03; H, 5.84; N, 21.15. Found: C, 58.00; H, 5.81; N, 20.87.

2,4-Diamino-6-(2′-chlorobenzyl)pyrido[2,3-*d*]pyrimidine (2d). Step 1. Addition of **6** (203 mg, 0.5 mmol) in dry THF (2 mL) to 2-chlorobenzylzinc chloride in THF (0.5 M, 4 mL, calculated to contain 2 mmol), followed by a workup similar to the one for **7a**, yielded **7d** as a pale-yellow solid (107 mg, 54%); ^{1}H NMR (DMSO-d_6) δ 1.20 (s, 9H, Me_3C), 1.24 (s, 9H, Me_3C), 4.24 (s, 2H, CH_2), 7.28–7.47 (m, 4H, 3′-, 4′-, 5′-, and 6′-H), 8.50 (s,1H, H-5), 8.87 (s, 1H, H-7), 11.31 (br s, CONH).

Step 2. Starting from **7d** (95 mg, 0.24 mmol), **2d** was prepared by the same method as **2a** except that recrystallization was from DMF/EtOH/H_2O, white solid (24 mg, 34%); mp > 300 °C; MS m/z found 286, calcd 286 $(M+1)^+$; ^{1}H NMR (DMSO-d_6) δ 4.05 (s, 2H, CH_2), 6.26 (br s, 2H, NH_2), 7.25–7.42 (m, 3H, 4′-, 5′-, and 6′-H), 7.43 (d, J = 8.8 Hz, 1H, 3′-H), 7.47 (br s, 2H, NH_2), 8.22 (d, J = 2.4 Hz, 1H, H-5), 8.51 (d, J = 2.4 Hz, 1H, H-7); IR (KBr) ν 3410, 3340, 3140, 1650, 1620, 1580, 1560, 1460, 1375 cm^{-1}. Anal. calcd for $C_{14}H_{12}N_5Cl \cdot 0.2H_2O$: C, 58.12; H, 4.32; N, 24.20; Cl, 12.25. Found: C, 57.89; H, 4.06; N, 23.80; Cl, 12.73.

2,4-Diamino-6-(4′-fluorobenzyl)pyrido[2,3-*d*]pyrimidine (2e). Step 1. Addition of **6** (203 mg, 0.5 mmol) in dry THF (2 mL) to 4-fluorobenzylzinc chloride in THF (0.5 M, 4 mL, calculated to contain 2 mmol), followed by a workup similar to the one for **7a**, afforded **7e** as a pale-yellow solid (144 mg, 76%); ^{1}H NMR (DMSO-d_6) δ 1.20 (s, 9H, Me_3C), 1.23 (s, 9H, Me_3C), 4.12 (s, 2H, CH_2), 7.09–7.18 (m, 2H, 2′- and 6′-H), 7.30–7.37 (m, 2H, 3′- and 5′-H), 8.46 (s, 1H, H-5), 8.84 (s, 1H, H-7), 11.32 (br s, CONH).

Step 2. Starting from **7e** (140 mg, 0.37 mmol), **2e** was prepared by the same method as **2a** except that recrystallization was from DMF/EtOH/H_2O; white solid (40 mg, 40%); mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 3.91 (s, 2H, CH_2), 6.20 (br s, 2H, NH_2), 7.05–7.08 (m, 2H, 2′- and 6′-H), 7.25–7.29 (m, 2H, 3′- and 5′-H), 7.40 (br s, 2H, NH_2), 8.22 (d, J = 2.4 Hz, 1H, H-5), 8.51 (d, J = 2.4 Hz, 1H, H-7); IR (KBr) ν 3410, 3340, 3140, 1650, 1620, 1580, 1560, 1460, 1375 cm^{-1}. Anal. calcd for $C_{14}H_{12}N_5F{\cdot}0.2H_2O$), C, 61.62; H, 4.58; N, 25.66; F, 6.95. Found: C, 61.82; H, 4.47; N, 25.96; F, 6.84.

2,4-Diamino-5-(3′,4′-dichlorobenzyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (3a). Step 1. Solid (methoxymethyl)-triphenylphosphonium chloride (17.7 g, 50 mmol) was added to a heat-dried 1000-mL three-necked flask containing dry THF (300 mL) which had been freshly distilled from Na benzophenone ketyl under an argon atmosphere. The reaction mixture was then stirred and cooled in a dry ice/acetone bath while *n*-butyllithium (2.5 M in hexane, 20 mL, 3.2 g, 55 mmol) was added dropwise over 30 min. To the flask was then added dropwise with vigorous stirring over 45 min a solution of 3,4-dichlorobenzaldehyde (9.2 g, 50 mmol) in dry THF (100 mL). The solution changed slowly in color from red to yellow and became turbid. After being left to stir at room temperature overnight, the reaction mixture was filtered to remove the triphenylphosphine oxide. The solvent was evaporated under reduced pressure, and the resulting yellow oil was stirred with EtOAc (50 mL) until a second crop of triphenylphosphine oxide crystallized out. The latter was filtered off, the filtrate was evaporated, and the residue was subjected to flash chromatography (silica gel, 95:5 EtOAc–hexane). A nonpolar component identified as 1-(3,4-dichlorophenyl)butene was discarded, and the slower-moving band, consisting of a mixture of the *Z* and *E* isomer of **9a** (total yield 7.7 g, 76%), was used directly in the next step; for the *Z* isomer: ^{1}H NMR (DMSO-d_6) δ 3.63 (s, 3H, OMe), 5.23 (d, J = 7.0 Hz, 1H, CH=C*H*OMe), 6.40 (d, J = 7.0 Hz, 1H, C*H*=CHOMe), 7.22 (d, J = 8.8 Hz, 1H, 6-H), 7.27 (d, J = 8.8 Hz, 1H, 5-H), 7.34 (s, 1H, 2-H); for the *E* isomer: ^{1}H NMR (DMSO-d_6) δ 3.78 (s, 3H, OMe), 5.80 (d, J = 13.2 Hz, 1H, CH=C*H*OMe), 7.37 (d, J = 13.2 Hz, 1H, C*H*=CHOMe), 7.40 (d, J = 8.8 Hz, 1H, 6-H), 7.44 (s, 1H, 5-H), 7.53 (d, J = 8.8 Hz, 1H, 2-H).

Step 2. A solution of **9a** (10 g, 49 mmol) in Et_2O (20 mL) was added dropwise to a stirred pre-cooled (ice bath) mixture of 70% $HClO_4$ (8.7 mL, 98 mmol), H_2O (8.7 mL), and Et_2O (20 mL). After the addition was complete, the yellow reaction mixture was stirred vigorously at room temperature for 1 h. Additional H_2O (30 mL) and Et_2O (30 mL) were added, the layers were separated, and the aqueous layer was extracted with Et_2O (2×20 mL). The combined organic layers were washed sequentially with H_2O (2×20 mL), 5% $NaHCO_3$ (2×20 mL), and brine (2×20 mL), and finally were dried ($MgSO_4$) and evaporated. Column chromatography (silica gel, 7:3 hexane–EtOAc) afforded **10a** as an amber-colored oil (6.5 g, 70%) that was pure enough to be used directly in the next step; ^{1}H NMR (DMSO-d_6) δ 5.05 (s, 1H, CH_2), 7.27 (d, J = 8.4 Hz, 1H, 6-H), 7.45 (d, J = 8.4 Hz, 1H, 5-H), 9.97 (s, 1H, CHO).

Step 3. Paraformaldehyde (1.0 g, 34 mmol), *N*-ethylbenzothiazolium bromide (1.5 g, 6.2 mmol), and Et_3N (0.63 g, 6.2 mmol) were added to a solution of **10a** (6.5 g, 34 mmol) in absolute EtOH (30 mL), and the reaction mixture was stirred under reflux for 24 h. After removal of the solvent under reduced pressure, the residue was shaken with EtOAc (50 mL) until a white solid formed, which was collected, washed with EtOAc (3×20 mL), and discarded. The pooled filtrates were evaporated and the residue was chromatographed (silica gel, 7:3 hexane–EtOAc) to obtain α-hydroxyketone **11a** as a brown semi-solid (4.4 g, 57%) that was used directly in the next step; ^{1}H NMR (DMSO-d_6) δ 3.93 (s, 2H, CH_2), 4.04 (s, 2H, CH_2OH), 4.12 (t, 1H, CH_2O*H*), 7.22 (s, J = 8.5 Hz, 1H, 6′-H), 7.38 (s, 1H, 2′-H), 7.41 (d, J = 8.5 Hz, 1H, 5′-H).

Step 4. A solution of malononitrile (0.6 g, 10 mmol) and Et_3N (0.38 g, 3.8 mmol) in anhydrous MeOH (5 mL) was added dropwise to a solution of **11a** (2.2 g, 10 mmol) in anhydrous MeOH (30 mL) while keeping the reaction mixture under N_2. The solution was stirred at room temperature overnight, and the solvent was evaporated under reduced pressure. The residue was chromatographed (silica gel, 7:3 EtOAc–hexane) and appropriately pooled column fractions were recrystallized from CH_2Cl_2 to obtain furan amino nitrile **8a** as a brown solid (1.2 g, 45%) that was used directly in the next step; mp 187–188 °C; ^{1}H NMR (DMSO-d_6) δ 3.65 (s, 2H, CH_2), 6.80 (s, 1H, furan =CH). 7.20 (dd, J_{ortho} = 8.4 Hz, J_{meta} = 2.2 Hz, 1H, 6′-H), 7.29 (broad peak, NH_2), 7.46 (d, J = 2.2 Hz, 1H, 2′-H), 7.55 (d, J = 8.4 Hz, 1H, 5′-H).

Step 5. Aminonitrile **8a** (400 mg, 1.5 mmol) was added to a solution prepared from guanidine hydrochloride (215 mg, 2.3 mmol) and NaOMe (124 mg, 2.3 mmol) in anhydrous MeOH (25 mL). The reaction was stirred under reflux for 24 h, the solvent was evaporated, and the residue was chromatographed on silica gel (4:1 $CHCl_3$–MeOH). Evaporation of appropriately pooled fractions and recrystallization from MeOH afforded **3a** as a brown solid (350 mg, 50%); mp 235–236 °C; MS *m*/*e* found 308, calcd 308 $(M+1)^+$; IR (KBr) ν 3485, 3442, 3205, 1625, 1595, 1570, 1490, 1445 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 4.03 (s, 2H, CH_2), 5.44 (br s, 2H, NH_2), 5.89 (br s, 2H, NH_2), 6.44 (s, 1H, pyrrole =CH), 7.19 (dd, J_{ortho} = 8.2 Hz, J_{meta} = 2.0 Hz, 1H, 6′-H), 7.46 (d, 1H, 2′-H), 7.51 (d, J = 8.2 Hz, 1H, 5′-H), 10.54 (br s, 1H, pyrrole NH). Anal. calcd for $C_{13}H_{10}N_5Cl_2$: C, 50.83; H, 3.28; N, 22.80; Cl, 23.08. Found, C, 50.49; H, 3.44; N, 22.53; Cl, 23.06.

2,4-Diamino-5-(3′,4′,5′-trimethoxybenzyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (3b). Step 1. Starting from (methoxymethyl)triphenylphosphonium chloride (17.7 g, 50 mmol), n-butyllithium (2.5 M in hexane, 20 mL, calculated to contain 3.2 g, 55 mmol), and 3,4,5-trimethoxybenzaldehyde (10 g, 50 mmol), the same method as was used to prepare **9a** afforded enol ether **9b** (estimated to contain 65% of the *E* and 35% of the *Z* isomer by ^{1}H NMR) as a yellow oil (9.0 g, 80%, used directly in the

next step). For the *E* isomer: ^{1}H NMR (DMSO-d_6) δ 3.61 (s, 3H, =CHO*Me*), 3.73 (s, 3H, 4-OMe), 3.75 (s, 6H, 3- and 5-OMe), 5.77 (d, J = 7.2 Hz, 1H, CH=C*H*OMe), 6,58 (s, 2H, aromatic), 7.25 (d, J = 7.2 Hz, 1H, C*H*=CHOMe); for the *Z* isomer: ^{1}H NMR (DMSO-d_6) δ 3.63 (s, 3H, =CHO*Me*), 3.74 (s, 3H, 4-OMe), 3.76 (s, 6H, 3- and 5-OMe), 5.14 (d, J = 12.8 Hz, 1H, CH=C*H*OMe), 6,22 (d, J = 12.8 Hz, 1H, C*H* = CHOMe), 6.84 (s, 2H, 2- and 6-H).

Step 2. Starting from **9b** (9 g, 40 mmol) and 70% $HClO_4$ (7 mL, 80 mmol), aldehyde **10b** was obtained by the same method as **10a**, except that the column eluent was 4:1 hexane–EtOAc; yellow oil (6.0 g, 71%, used directly in the next step); ^{1}H NMR (DMSO-d_6) δ 3.75 (s, 2H, 3- and 5-OMe), 3.77 (s, 3H, 4-OMe), 3.87 (s, 2H, CH_2), 7.25 (s, 2H, 2- and 6-H), 9.66 (s, 1H, CH=O).

Step 3. Starting from paraformaldehyde (0.84 g, 29 mmol), *N*-ethylbenzothiazolium bromide (1.3 g, 5.2 mmol), Et_3N (0.53 g, 5.2 mmol), and **10b** (6.0 g, 29 mmol), α-hydroxyketone **11b** was obtained by the same method as **11a**; yellow oil (3.1 g, 45%, used directly in the next step); ^{1}H NMR (DMSO-d_6) δ 3.31 (s, 2H, CH_2), 3.70 (s, 3H, 4-OMe), 3.88 (s, 6H, 3- and 5-OMe), 3.92 (s, 2H, C*H*$_2$OH), 3.98 (br s, 1H, CH$_2$O*H*), 7.21 (s, 2H, 2- and 6-H).

Step 4. Starting from malononitrile (0.25 g, 3.8 mmol), Et_3N (0.38 g, 3.8 mmol), and **11b** (0.90 g, 3.8 mmol), furan amino nitrile **8b** was obtained by the same method as **8a** except that the column eluent was $CHCl_3$; brown solid (0.66 g, 60%, used directly in the next step); mp 127–128 °C; ^{1}H NMR (DMSO-d_6) δ 3.55 (s, 2H, CH_2), 3.62 (s, 3H, 4′-OMe), 3.73 (s, 6H, 3′- and 5′-OMe), 6.55 (2′- and 6′-H), 6.77 (s, 1H, furan =CH).

Step 5. Starting from **8b** (200 mg, 0.7 mmol), guanidine hydrochloride (100 mg, 1.05 mmol) and NaOMe (57 mg, 1.05 mmol), **3b** was obtained by the same method as **3a** except that the column eluent was 85:15 $CHCl_3$–MeOH; yellowish-white solid (150 mg, 65%); mp 222–223 °C; MS *m*/*e* found 330, calcd 330 $(M+1)^+$; IR (KBr) ν 3440, 3365, 3210, 1610, 1555, 1510, 1465, 1430 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 3.60 (s, 3H, 4′-OMe), 3.68 (s, 6H, 3′- and 5′-OMe), 3.92 (s, 2H, CH_2), 5.35 (br s, 2H, NH_2), 5.56 (br s, 2H, NH_2), 6.40 (s, 1H, pyrrole =CH), 6.56 (s, 2H, 2′- and 6′-H), 10.42 (br s, 1H, pyrrole NH). Anal. calcd for $C_{16}H_{19}N_5O_3$: C, 58.35; H, 5.81; N, 21.26; Found, C, 57.97; H, 5.88; N, 20.97.

2,4-Diamino-5-[2-(3′,4′,5′-trimethoxyphenyl)ethyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (3c). Step 1. To a stirred suspension of $LiAlH_4$ (1.28 g, 32 mmol) in dry THF (50 mL, freshly distilled from CaH_2) at 0 °C under argon was added dropwise a solution of **12** (5 g, 20 mmol) in dry THF (50 mL). The mixture was stirred at 0 °C for 2 h and then at room temperature overnight. After being cooled again to 0 °C, the excess hydride was quenched by dropwise addition of 20% H_2O in THF (50 mL). The color of the suspension changed from gray to yellow. The mixture was filtered through Celite, and the filter cake was washed with Et_2O until the washings were colorless. Evaporation of the pooled filtrates afforded **13** as a pale-yellow oil (4.2 g, 93%, used directly in the next step); ^{1}H NMR (DMSO-d_6) δ 1.67 (m, 2H, CH_2O), 2.48 (t, 2H, benzylic CH_2), 3.36 (m, 2H, CH_2), 3.5 (s, 2H, 4-OMe), 3.69 (s, 6H, 3- and 5-OMe), 4.41 (t, 1H, OH), 6.43 (s, 2H, 2- and 6-H).

Step 2. CrO_3 (1.2 g, 12.2 mmol) was added in small portions to a stirred mixture of pyridine (2 mL) and anhydrous CH_2Cl_2 (15 mL) at room temperature. After 15 min, the brown suspension was treated with a solution of **13** (0.4 g, 2.0 mmol) in CH_2Cl_2 (2 mL), and stirring was continued for 1 h. The liquid was decanted and the residue triturated with Et_2O (3×50 mL). The pooled Et_2O extracts were washed successively with H_2O (4×20 mL), 5% NaOH (2×10 mL), 5% HCl (2×20 mL), and finally 50% saturated $NaHCO_3$ (2×20 mL) The organic layer was dried ($MgSO_4$) and evaporated under reduced pressure, and the residue was chromatographed on silica gel (9:1 $CHCl_3$–MeOH) to obtain aldehyde **14** as a yellow oil (244 mg, 53%, used directly in the next step); ^{1}H NMR (DMSO-d_6) δ 2.77 (m, 4H, CH_2CH_2), 3.60 (s, 3H, 4-OMe), 3.74 (s, 6H, 3- and 5-OMe), 6.52 (s, 2H, 2-H and 6-H).

Step 3. Starting from **14** (7 g, 31 mmol), paraformaldehyde (0.96 g, 32 mmol), *N*-ethylbenzothiazolium bromide (1.4 g, 5.6 mmol), and Et_3N (0.58 g, 5.6 mmol), α-hydroxyketone **15** was obtained by the same method as **11a**; brown oil (5.0 g, 63%, used directly in the next step); ^{1}H NMR ($CDCl_3$) δ 2.72 (m, 4H, CH_2CH_2), 3.60 (s, 3H, 4-OMe), 3.73 (s, 6H, 3- and 5-OMe), 4.80 (d, 2H, CH$_2$O*H*), 5.10 (s, 2H, 2- and 6-H).

Step 4. Starting from **15** (5 g, 20 mmol), malononitrile (1.4 g, 22 mmol) and Et_3N (2.2 g, 22 mmol), furan amino nitrile **16** was obtained by the same method as **8a** except that the column eluent was 95:5 $CHCl_3$–MeOH; yellow solid (5 g, 84%, used directly in the next step); mp 151–152 °C; ^{1}H NMR (DMSO-d_6) δ 2.56 (m, 2H, CH_2), 2.74 (m, 2H, benzylic CH_2), 3.61 (s, 3H, 4-OMe), 3.74 (s, 6H, 3- and 5-OMe), 6.50 (s, 2H, 2- and 6-H), 6.74 (s, 2H, furan =CH), 7.22 (br s, 2H, NH_2).

Step 5. Starting from **15** (200 mg, 0.66 mmol), guanidine hydrochloride (95 mg, 1.0 mmol) and NaOMe (54 mg, 1.0 mmol), **3c** was obtained by the same method as **3a**; brown powder (100 mg, 44%); mp 210–211 °C; MS *m*/*z* found 344, calcd 344 $(M+1)^+$; IR (KBr) ν 3440, 3260, 3000, 1620, 1575, 1530, 1480, 1445 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.77 (m, 2H, CH_2), 2.93 (m, 2H, benzylic CH_2), 3.60 (s, 3H, 4′-OMe), 3.73 (s, 6H, 3′- and 5′-OMe), 5.33 (br s, 2H, NH_2), 5.93 (br s, 2H, NH_2), 6.41 (s, 1H, pyrrole =CH), 6.54 (s, 2H, 2′- and 6′-H), 10.35 (br s, 1H, pyrrole NH). Anal. calcd for $C_{17}H_{21}N_5O_3$: C, 59.46; H, 6.16; N, 20.40. Found: C, 59.22; H, 6.22; N, 20.35.

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