6-Substituted 2,4-Diamino-5-methylpyrido[2,3-*d*]pyrimidines as Inhibitors of Dihydrofolate Reductases from *Pneumocystis carinii* and *Toxoplasma gondii* and as Antitumor Agents[1]

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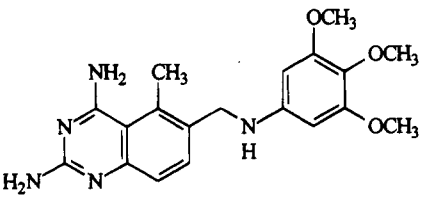
Received December 27, 1994®

The synthesis and biological activity of 15 6-substituted 2,4-diamino-5-methylpyrido[2,3-*d*]-pyrimidines are reported. These compounds were synthesized in improved yields by modifications of procedures previously reported by us. Specifically, dimethoxyphenyl-substituted compounds with H and CH_3 at the N-10 position and trimethoxyphenyl-substituted compounds with N-10 ethyl, isopropyl, and propargyl moieties were synthesized. These compounds were evaluated as inhibitors of dihydrofolate reductases (DHFR) from *Pneumocystis carinii*, *Toxoplasma gondii*, and rat liver, and selected analogues were evaluated as inhibitors of the growth of *T. gondii* and tumor cells in culture. All the compounds showed increased selectivity (vs rat liver DHFR) for *T. gondii* DHFR compared to trimetrexate. In general, for the trimethoxy-substituted analogues, increasing the size of the N-10 substituent from a methyl group to larger groups resulted in a decrease in selectivity and potency for both *P. carinii* and *T. gondii* DHFR. For the dimethoxy-substituted analogues, N-10 methylation in general decreased potency but increased selectivity for *T. gondii* DHFR. In an attempt to improve the cell penetration of these analogues, the N-10 naphthyl-substituted analogues were also synthesized. These analogues displayed excellent cell penetration and inhibition of *T. gondii* cells in culture. Further, these analogues were potent inhibitors of the growth of tumor cells in the preclinical *in-vitro* screening program of the National Cancer Institute with IC_{50}s in the nanomolar range.

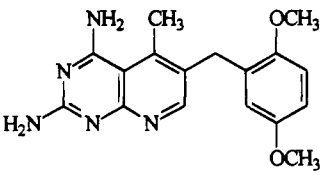
Infections with *Pneumocystis carinii* and *Toxoplasma gondii* remain the principal cause of death in patients afflicted with acquired immunodeficiency syndrome (AIDS).[2] Currently available treatments are beset with high cost, lack of selectivity, or host toxicity.[3] Trimethoprim (TMP) and pyrimethamine, both of which are weak inhibitors of *P. carinii* dihydrofolate reductase (pcDHFR) and *T. gondii* dihydrofolate reductase (tgDHFR),[4] are currently used in combination with sulfonamides in the treatment of *P. carinii* and *T. gondii* infections, respectively.

Lipophilic, nonclassical, bicyclic antifolates such as trimetrexate (TMQ), a quinazoline, and piritrexim (PTX), a pyrido[2,3-*d*]pyrimidine, have been reported as potent inhibitors of pcDHFR and tgDHRF.[5,6] TMQ (NeuTrexin) has recently been approved as an agent for the treatment of *P. carinii* infections.[7] These nonclassical antifolates enter cells *via* passive diffusion, circumventing the need for the folate transport system(s) necessary for the uptake of classical antifolates like methotrexate (MTX), and are able to penetrate *P. carinii* and *T. gondii* cells which lack the active transport system(s) for folates. However, TMQ and PTX lack selectivity for pcDHFR and tgDHFR compared to mammalian DHFR. Thus TMQ has been approved for clinical use against *P. carinii* infections only with leucovorin rescue.

We[8] have previously reported the synthesis and



TMQ



PTX

biological activities of a series of nonclassical pyrido-[2,3-*d*]pyrimidine antifolates as inhibitors of pcDHFR and tgDHFR. Several of these compounds were more potent and selective than TMQ and PTX. Compound **1b**, for example, was 5 times as potent and 8 times as selective for pcDHFR as TMQ.[8] Against tgDHFR, **1b** was 12 times as potent and 31 times as selective as TMQ. However, despite their potent DHFR inhibition and selectivity, compounds **1a,b** were poorly active against the growth of these organisms in culture.[8] In an attempt to extend our[9–11] efforts aimed at developing lipophilic, nonclassical antifolates with both high potency and selectivity for pcDHFR and/or tgDHFR, and to establish a structure–activity/selectivity relationship

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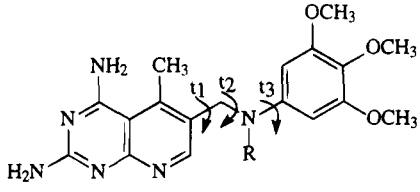
® Abstract published in *Advance ACS Abstracts*, April 15, 1995.

0022-2623/95/1838-1778$09.00/0

for 6-substituted 2,4-diamino-5-methylpyrido[2,3-*d*]-pyrimidines, we synthesized compounds **2a–e**, **3a–e**, **4a–c**, and **5a,b**. Compounds **2a–e** were designed to investigate the importance of the third methoxy group of **1a,b** toward selectivity and potency against pcDHFR and tgDHFR as well as to the inhibition of the growth of these organisms in culture. Sequential removal of one of the trimethoxy groups of TMP results in a significant decrease in inhibition of bacterial DHFR.[12] We were interested in determining if a similar structure–activity relationship exists for **1a,b** against pcDHFR and tgDHFR. The effects on biological activity of altering the substitution pattern of the methoxy groups on the phenyl ring were of interest and were also addressed by compounds **2a–e**. Since N-10 methylation in the trimethoxy series resulted in significant increase in the selectivity and potency of **1b** toward both pcDHFR and tgDHFR compared to the nonmethylated analogue **1a**,[8] compounds **3a–e** were synthesized with the idea that a similar increase in selectivity and potency of the dimethoxy-substituted analogues could be achieved *via* N-10 methylation. Compounds **4a–c** were designed to evaluate the inhibitory effects of different N-10 substitutions. These substituents serve to partially restrain the conformational flexibility around t_2 and t_3 and perhaps provide for additional interactions of the N-10 substituent with the enzyme–cofactor complex which could result in greater selectivity and/or potency as was observed for **1b** compared to **1a**.[8] In spite of the



1a. R = H
1b. R = CH_3

significant inhibitory activity of **1a,b** against tgDHFR, both compounds were considerably less potent against the growth of *T. gondii* cells in culture (Table 2), which could be attributed in part to their lack of sufficient lipid solubility. Compounds **5a,b** with N-10 naphthyl and N-10 4-methoxynaphthyl substitutions were synthesized to increase lipophilicity and the cell penetration of these analogues.

Chemistry

The syntheses of the target compounds are shown in Scheme 1 and required the intermediate 2,4-diamino-5-methylpyrido[2,3-*d*]pyrimidine-6-carbonitrile (**6**). One of the several methods of synthesizing the pyrido[2,3-*d*]pyrimidine ring system is by the reaction of *o*-amino nitriles with guanidine.[13] The synthesis of **6** was achieved according to the method of Elslager and Davoll,[14] later adapted by Piper *et al.*[15] for the synthesis of classical 5-substitued pyrido[2,3-*d*]pyrimidine antifolates. This method involves the dechlorination of 2-chloro-3,5-dicyano-4-methyl-6-aminopyridine to the amino nitrile followed by cyclization with guanidine hydrochloride. We carried out the dechlorination in dioxane, a lower boiling solvent than DMF,[15] which facilitated workup of the reaction. In addition, we found that the use of 5% Pd/C, which is less expensive than $Pd/BaCO_3$, resulted in a slight improvement in the yield of the dechlorinated product compared to the literature methods (90%; lit.[15] 86%). A significant improvement in the yield of the guanidine cyclization step to compound **6** was achieved by increasing the reaction time from 5 to 8 days and the amount of guanidine hydrochloride from 1 to 4 mol equiv (74%; lit.[15] 58%). Reductive condensation of **6** with the appropriately substituted dimethoxyanilines and aminonaphthylenes afforded target compounds **2a–e** and **5a,b**. The yields of these condensations were generally low (25–40%). In an attempt to improve the overall yield of the target compounds, the intermediate **6** was converted to the 6-carboxaldehyde **7** with 97% formic acid and either Raney nickel or a Ni–Al alloy for 2 h to yield the crude aldehyde **7** (75–80%). Reductive amination of 2,5-dimethoxyaniline with **7** in 5 N HCl/MeOH and $NaCNBH_3$ at pH 5 afforded target compound **2a**. The use of 4 Å molecular sieves resulted in slight improvement in yields. The low yield of this reaction is presumably due to the poor solubility of **7** in MeOH. The overall yield of **2a** from the nitrile *via* the aldehyde was inferior to that obtained *via* the direct reductive condensation of 2,5-dimethoxyaniline with the nitrile.

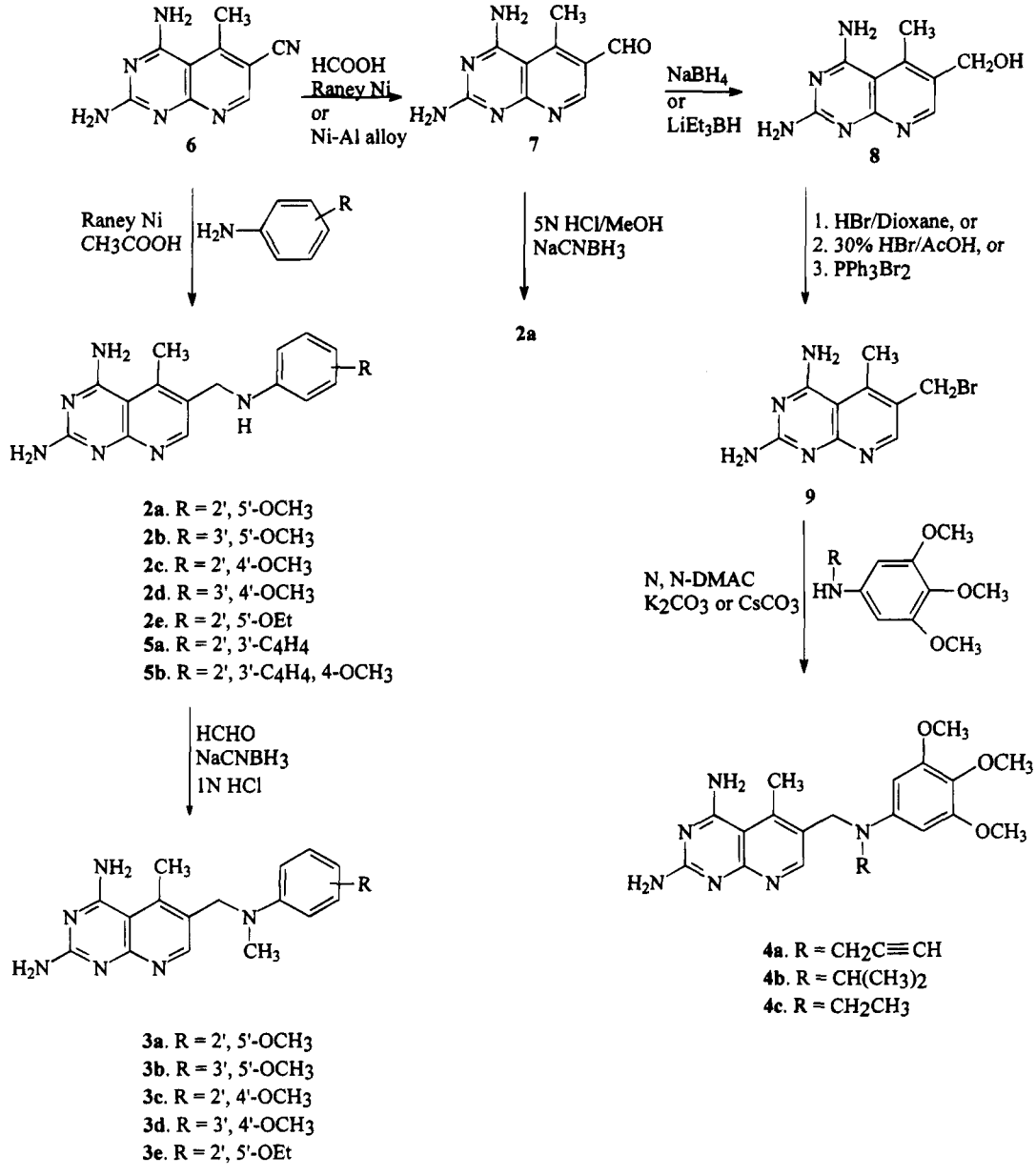
N-10 methylation of compounds **2a–e** to provide **3a–e** was carried out by a modification of an earlier procedure developed by us.[8] Reductive methylations in acetonitrile with $NaCNBH_3$ and HCHO was facilitated by the dropwise addition of sufficient 1 N HCl to effect solution (usually at a pH of 3–4). This methodology affords high yields (70–80%) of pure N-10-methylated product without the need for chromatographic purification of the products.

Compound **7** was reduced to the alcohol **8** with $NaBH_4$ in methanol. Long reaction times result in over-reduction of the aldehyde to the 6-methyl compound which has been shown by Taylor *et al.*[16] to be recalcitrant to any further transformations. The use of lithium triethylborohydride (Super Hydride) afforded **8** in yields comparable to that obtained with $NaBH_4$. The 6-hydroxymethyl compound **8** was then brominated using one of three methods: (a) triphenylphosphine dibromide in acetonitrile,[15] (b) 30% HBr in acetic acid,[17] (c) anhydrous HBr in dioxane.[18] In our hands, method b afforded the highest yield (91%) of the 6-bromomethyl compound **9**. This compound was very reactive as its hydrobromide salt and was used immediately in the next step. The *N*-ethyl- and *N*-propargyl-3,4,5-trimethoxyanilines were obtained by alkylating 3,4,5-trimethoxyaniline with the appropriate alkyl halide. The reaction was also carried out using a hindered base[19] to prevent dialkylation of the aniline. The best yield (63%) of the monoalkylated product was obtained with Hunigs base. *N*-Isopropyltrimethoxyaniline was obtained by the reductive amination of acetone with trimethoxyaniline.

Biological Results and Discussion

Compounds **2a–e**, **3a–e**, **4a–c**, and **5a,b** were evaluated as inhibitors of DHFRs from *P. carinii* (pc), *T. gondii* (tg), and rat liver (rl), and the results are listed in Table 1. Selectivity ratios were determined using rlDHFR as the mammalian source and are also listed in Table 1. Replacement of the three methoxy groups with two methoxy groups at various positions on the

Scheme 1



2a. R = 2', 5'-OCH_3
2b. R = 3', 5'-OCH_3
2c. R = 2', 4'-OCH_3
2d. R = 3', 4'-OCH_3
2e. R = 2', 5'-OEt
5a. R = 2', 3'-C_4H_4
5b. R = 2', 3'-C_4H_4, 4-OCH_3

3a. R = 2', 5'-OCH_3
3b. R = 3', 5'-OCH_3
3c. R = 2', 4'-OCH_3
3d. R = 3', 4'-OCH_3
3e. R = 2', 5'-OEt

4a. R = $CH_2C{\equiv}CH$
4b. R = $CH(CH_3)_2$
4c. R = CH_2CH_3

phenyl ring in an attempt to assess the contribution of the third methoxy substituent toward the selectivity observed for **1b**[8] afforded varied results. Substitution of a methoxy group at the *meta* position was important for potency toward both pcDHFR and tgDHFR. Thus, **2b**, which has both *meta* positions substituted, was the most potent in this series and was 4 and 2 times as potent as **1a** against pcDHFR and tgDHFR, respectively, and its selectivity for tgDHFR matches that of **1b**. Compounds **2a,d**, both of which have one *m*-methoxy substituent, had similar inhibitory activities against pcDHFR and tgDHFR. Compound **2a** was 140-fold more selective than **1a** and 4.5-fold as selective as **1b** as an inhibitor of pcDHFR. It was as selective as **1b** for tgDHFR. Compounds **2d**, however, was significantly more potent against rlDHFR and hence was not selective for pcDHFR or tgDHFR. Comparison of the biological activity of **2b,d** and **2a,c** defines the role of a *p*-methoxy group toward selectivity of the dimethoxy-substituted analogues. Compound **2b**, which differs from **2d** in the position of one methoxy group, was almost 12-fold more selective for pcDHFR and more than 10-fold more selective for tgDHFR than **2d**. Similarly, **2a** was more selective and potent than **2c**, which lacks a *meta* substituent and was the least potent analogue of the dimethoxy series. Clearly, the di-*m*-methoxy substitution in the dimethoxy series was conducive to both potency and selectivity toward pcDHFR and tgDHFR. In an attempt to increase the conformational restriction around t_3 and the size of the 2',5'-substituents, the 2',5'-diethoxy analogue **2e** was synthesized. Comparison of the activity of **2e** with **2a** indicated no significant difference in potency toward either pcDHFR or tgDHFR. However, there was a loss in selectivity of **2e** due to a 7-fold increase in the activity against rlDHFR.

The ^1H NMR data for analogues **2a**–**e** in deuterated dimethyl sulfoxide (Table 3) indicates, as anticipated, that the chemical shift of the N-10 H is strongly influenced by the position of the methoxy groups. It was interesting to note that the activity against pcDHFR and tgDHFR of analogues **2a**–**e** was inversely dependent on the mesomeric effect of the methoxy groups and hence the electron density on the N-10 nitrogen; ana-

Table 1. Inhibitory Concentrations (IC_{50}, nM) and Selectivity Ratios against pcDHFR, tgDHFR, rlDHFR, and lcDHFR

compd	pcDHFR	rlDHFR	rl/pc	tgDHFR	rl/tg	lcDHFR[24]
1a	86	2.1	0.02	7.4	0.28	22
1b	13.2	7.6	0.58	0.85	8.94	200
2a	46	128	2.8	16	8.0	39
2b	22.9	42.5	1.9	4.8	8.85	
2c	316	214	0.68	56.5	3.8	
2d	44	7.6	0.17	8.8	0.86	83
2e	76.7	17.4	0.23	17.0	1.0	
3a	216	407	1.9	30.1	13.5	
3b	130	170	1.3	58	2.9	
3c	510	330	0.6	38	8.7	
3d	320	44	0.14	29	1.5	
3e	3100	3000	1.0	100	30.0	
4a	53.5	11.8	0.22	7.7	1.5	
4b	13.4	17.5	1.3	6.7	2.6	210
4c	49.7	10.5	0.21	2.7	3.9	
5a	573	29.6	0.05	14.5	2.0	
5b	41	54	1.3	23	2.3	
TMQ	42	3.0	0.07	10	0.3	27
PTX	38	1.5	0.04	11	0.14	
TMP	12 000	133 000	11.1	2700	49	103

[a] This value was incorrectly reported as 0.58 nM in our previous publication.[8]

Table 2. Comparison of the Inhibition (IC_{50}, μM) of *T. gondii* Cells in Culture vs the Inhibition of tgDHFR

compd	tgDHFR	tg culture	culture/enzyme
1a	0.0074	23.0	3108
1b	0.00085	4.7	5529
2a	0.016	1.5	94
2b	0.0048	0.6	125
2e	0.017	0.32	19
3a	0.0301	1.05	35
3c	0.038	1.73	46
5a	0.0145	0.10	7
5b	0.02	0.04	2

Table 3. Correlation of Chemical Shifts (δ) of the N-10 H and the Inhibition (IC_{50}, nM) of pcDHFR and tgDHFR

compd	^{1}H NMR (N-10 H)	pcDHFR	tgDHFR
2b	5.91	22.9	4.8
2d	5.53	44.0	8.8
2a	5.18	46.0	16.0
2e	5.21	76.7	17.0
2c	4.61	316	56.5

logue **2b**, in which the N-10 H is not affected by the mesomeric effect of the methoxy groups, was the most potent and selective analogue against both pcDHFR and tgDHFR in the dimethoxyphenyl series. Similarly, the N-10 H of **2c** was the most upfield of those of the N-10 H analogues due to the mesomeric effects of the *o*- and *p*-methoxy groups, and **2c** was the least potent. We are currently investigating 4′-monosubstituted phenyl moieties with electron-withdrawing and -donating groups to further investigate this observation. This effect was not observed against rlDHFR, nor was it observed with the N-10-substituted analogues.

N-10 methylation, in general, resulted in a decrease in activity against both pcDHFR and tgDHFR compared to the N-10 H analogues **2a**–**e** (with the exception of **2c**). This is in sharp contrast to the 3,4,5-trimethoxy-substituted series[8] where N-10 methylation significantly increased activity. Further, the selectivity of **3a**–**d** toward pcDHFR was decreased compared to that of **2a**–**d**, again in contrast to the 3,4,5-trimethoxy analogues. This was surprising and suggests that extrapolation of results obtained in the 3,4,5-trimethoxy-substituted series to the dimethoxy-substituted series was inappropriate. One redeeming feature of N-10 methylation in the dimethoxy series was the increase in selectivity toward tgDHFR observed for all the compounds in this series (except **2b**). Compounds **3a**,**e** have greater selectivity for tgDHFR than **1b**, and compound **3e** in particular was 200-fold more selective for tgDHFR than TMQ and was the most selective inhibitor of the dimethoxy series.

In the 3,4,5-trimethoxy series, increasing the size of the N-10 substituent from a methyl group to larger alkyl groups in an attempt to increase the degree of conformational restriction around t_2 and t_3 and/or provide for additional interactions with the enzyme afforded varying results. Compound **4c**, the N-10 ethyl analogue, was less potent against all the three DHFRs compared to the N-10 methyl analogue **1b**. Similarly, introducing a propargyl group as in **4a** results in a decrease in activity toward all three DHFRs. Both **4a**,**c** were more potent and selective than **1a**, the N-10 H analogue, against pcDHFR. Compound **4a** was equipotent with **1a**, and both **4a**,**c** had greater selectivity than **1a** against tgDHFR, indicating that substitution on the N-10 nitrogen is conducive to selective inhibition of pcDHFR and tgDHFR. Compound **4b** was 4-fold more potent than **4a**,**c** against pcDHFR, and its potency parallels that of **1b**. Further **4b** was 65-fold more selective than **1a** and more than 2 times as selective as **1b** for pcDHFR. However its potency and selectivity toward tgDHFR were similar to those of **4a**,**c**. These results further substantiate the differences between DHFRs from *P. carinii*, *T. gondii*, and rat liver which should yield analogues selective for pcDHFR and/or tgDHFR.

Inhibition of *Lactobacillius casei* DHFR (lcDHFR) by compounds **2a**,**d** and **4b** is included in Table 1. Compound **2a** was more potent than TMP; however, it lacks the selectivity for lcDHFR vs rlDHFR seen with TMP. Removal of the 5-methoxy group of **1a** to give **2d** decreases lcDHFR inhibition by 4-fold and indicates that this modification is detrimental to lcDHFR inhibition. The inhibitory activity observed for **4b**, together with that of **1b**,[8] indicates that N-10 alkylation is also detrimental to the inhibition of lcDHFR in the 3,4,5-trimethoxy series.

The significant selectivity and/or potency for tgDHFR of compounds **2a**,**b**,**e** and **3a**,**c** prompted their evaluation as inhibitors of the growth of *T. gondii* cells in culture. All five compounds showed greater inhibition of cell growth than **1a**,**b**, despite having lower or comparable IC_{50}s against isolated tgDHFR (Table 2). Clearly, the dimethoxyphenyl- and diethoxyphenyl-substituted compounds have a greater degree of cell penetration compared to the trimethoxyphenyl analogues. The ratios of IC_{50} values in isolated enzyme (tgDHFR) and cell culture are also shown in Table 2. The trimethoxy analogue **1b** had a ratio >5000, indicating poor cell penetration. The dimethoxy analogues had ratios which were 44–250 times better than the trimethoxy analogues and indicate significantly improved cell penetration. In an attempt to further increase the cellular penetration, we synthesized analogues with bicyclic N-10 side chains containing a naphthylene and a 4-methoxynaphthylene (**5a**,**b**, respectively) as an extension of our previously reported bicyclic side chain-containing analogues.[20,21] The goal of significantly improving cell penetration with the naphthyl analogues

Table 4. Cytotoxicity Evaluation (GI_{50}, M) against Selected Tumor Cell Lines[23]

	2c	3a	3b	5b
		Leukemia		
CCRF-CEM	4.29×10^{-6}	7.15×10^{-6}	5.36×10^{-7}	7.66×10^{-8}
K-562	3.05×10^{-7}	4.99×10^{-7}	2.09×10^{-7}	1.61×10^{-8}
MOLT-4	1.22×10^{-6}	3.16×10^{-6}	7.42×10^{-7}	9.11×10^{-8}
SR	3.53×10^{-6}	7.45×10^{-6}	5.41×10^{-7}	7.60×10^{-8}
		Non-small-cell Lung Cancer		
A549/ATCC	6.35×10^{-7}	7.32×10^{-7}	4.42×10^{-7}	3.84×10^{-8}
NCI-H322M	5.06×10^{-5}	5.56×10^{-6}	ND	8.50×10^{-8}
NCI-H460	4.81×10^{-7}	ND	5.44×10^{-7}	4.91×10^{-8}
		Colon Cancer		
HCT-116	1.73×10^{-7}	3.97×10^{-6}	4.10×10^{-7}	1.17×10^{-8}
HCT-15	2.20×10^{-6}	2.54×10^{-6}	2.12×10^{-6}	4.56×10^{-8}
HT29	2.38×10^{-6}	3.22×10^{-6}	5.84×10^{-6}	7.23×10^{-8}
SW-620	2.24	5.04×10^{-6}	5.50×10^{-6}	7.96×10^{-8}
		CNS Cancer		
SF-268	4.17×10^{-6}	5.91×10^{-6}	7.17×10^{-7}	6.04×10^{-8}
SF-295	1.61×10^{-6}	3.41×10^{-6}	5.15×10^{-6}	8.32×10^{-8}
U251	5.81×10^{-6}	4.21×10^{-6}	5.29×10^{-7}	7.29×10^{-8}
		Melanoma		
LOX IMVI	3.35×10^{-6}	5.82×10^{-6}	1.99×10^{-6}	6.94×10^{-8}
SK-MEL-5	1.15×10^{-6}	1.56×10^{-6}	5.96×10^{-7}	5.78×10^{-8}
UACC-257	1.00×10^{-4}	2.00×10^{-5}	6.20×10^{-7}	8.68×10^{-8}
UACC-62	1.26×10^{-4}	1.15×10^{-6}	6.28×10^{-7}	4.32×10^{-8}
		Ovarian Cancer		
IGROV1	6.27×10^{-6}	5.45×10^{-6}	9.56×10^{-7}	8.68×10^{-8}
		Renal Cancer		
786-0	5.88×10^{-7}	8.43×10^{-7}	6.30×10^{-7}	5.50×10^{-8}
ACHN	2.89×10^{-6}	3.75×10^{-6}	8.16×10^{-7}	4.79×10^{-8}
		Prostate Cancer		
PC-3	8.52×10^{-7}	7.83×10^{-7}	5.94×10^{-7}	7.98×10^{-8}
DU-145	2.83×10^{-6}	2.71×10^{-6}	4.36×10^{-6}	5.21×10^{-8}
		Breast Cancer		
MDA-MB-231/ATCC	1.91×10^{-6}	7.64×10^{-7}	9.22×10^{-6}	1.88×10^{-8}
MDA-MB-435	3.53×10^{-6}	4.18×10^{-6}	6.93×10^{-6}	6.21×10^{-8}
MDA-N	3.59×10^{-7}	6.62×10^{-6}	5.27×10^{-7}	4.25×10^{-8}

was realized as indicated in Table 2. Thus, the IC_{50} ratios for **5a,b** were 7 and 2, respectively. While this work was in progress, Piper *et al.*[22] reported excellent *T. gondii* cell penetration of compound **5a**.

Compounds **2c**, **3a,b**, and **5b** were selected for evaluation as antitumor agents by the National Cancer Institute in the preclinical *in-vitro* screening program[23] (Table 4). Compounds **2c** and **3a** displayed reasonable antitumor activity with IC_{50}s in the range of 10^{-4}–10^{-7} M against various cell lines. The cytotoxicity of **3b** was approximately 1 order of magnitude better than that of **1b** and **3a** in various cell lines including colon cancer, central nervous system (CNS) cancer, melanoma, ovarian cancer, and renal cancer. This corroborates the potent inhibition of mammalian DHFR (rlDHFR) of **3b** ($IC_{50} = 1.7 \times 10^{-7}$ M). However, its inhibition of rlDHFR was less than that of **1b**, suggesting possibly an enhanced cellular uptake compared to **1b**. The excellent cell penetration of **5b** was further demonstrated by its potent inhibition ($GI_{50} = (1-9) \times 10^{-8}$ M) of the growth of 26 cell lines.

On the basis of this report and our previous study,[8] it is apparent that N-10 side chain substituents play a pivotal role in the activity and selectivity of nonclassical pyrido[2,3-*d*]pyrimidine antifolates against DHFRs from *P. carinii* and *T. gondii* and that extrapolations of structure–activity/selectivity relationships from one series to another are inappropriate and need to be carried out separately for each series. In addition, cell penetration, which is an important attribute for a clinically viable agent, can be significantly improved by incorporating a naphthyl side chain. The challenge however remains to develop highly potent and selective DHFR inhibitors with good cell penetration ability as clinical agents against *P. carinii* and *T. gondii* infections.

Experimental Section

Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Infrared spectra (IR) were recorded with a Perkin Elmer Model 1430 spectrometer, in Nujol mulls. Nuclear magnetic resonance spectra for proton (^{1}H NMR) were recorded on a Brucker WH-300 (300 MHz) spectrometer. The data were accumulated by 16K size with 0.5 s delay time and 70° tip angle with internal standard TMS; s = singlet, br s = broad singlet, d = doublet, t = triplet, q = quartet, and m = multiplet. Low-resolution mass spectra were obtained on an LKB-9000 instrument. Thin layer chromatography was performed on silica gel plates with fluorescent indicator, and the plates were visualized with light at 254 and 366 nm. Column chromatography was performed with 230–400 mesh silica gel purchased from Aldrich Chemical Co., Milwaukee, WI. All anhydrous solvents were purchased from Aldrich Chemical Co. and used without further purification. Samples for microanalysis were dried *in vacuo* over phosphorus pentoxide at 70 or 110 °C. Microanalyses were performed by Atlantic Microlabs, Norcoss, GA.

2,4-Diamino-5-methyl-6-[(2′,5′-dimethoxyanilino)methyl]pyrido[2,3-*d*]pyrimidine (2a). To a solution of the nitrile **6** (1.50 g, 7.50 mmol) in 200 mL of 85% acetic acid was added 5.00 g of Raney nickel followed immediately by 2,5-dimethoxyaniline (1.74 g, 11.34 mmol), and the mixture was hydrogenated at atmospheric pressure and room temperature for 5 h. The mixture was then filtered (Norit, Celite) and the filtrate concentrated *in vacuo* to yield a gummy residue. To a solution of this residue in warm ethanol was added a solution of 1 N

Na_2CO_3 dropwise, and the pH of the mixture was adjusted to 8–8.5. The precipitate obtained was filtered and the residue washed with copius amounts of acetone and ethyl acetate until the washings were colorless. To a solution of the residue in 200 mL of warm (45 °C) methanol, containing 1 mL of 1 N HCl, was added 1.0 g of silica gel, and the solvents were evaporated using a pump to yield a solid plug which was spread evenly on the surface of the silica gel in a 1.05 in. × 23 in. column. The column was eluted with $CHCl_3$:CH_3OH (6:1), and 10 mL fractions were collected. Fractions 12–36 were combined, and the solvent was evaporated to yield 0.80 g (30%) of **2a** as a yellow solid. Mp > 250 °C dec. IR (Nujol): 3210, 3000 (NH_2) cm^{-1}. ^1H NMR (Me_2SO-d_6): δ 2.66 (s, 3 H, 5-CH_3), 3.62 (s, 3 H, 2′-OCH_3), 3.72 (s, 3 H, 5′-OCH_3), 4.27 (d, 2 H, CH_2NH), 5.18 (t, 1 H, CH_2NH), 6.07 (s, 1H, 6′-H), 6.13 (d, 1 H, 4′-H), 6.20 (br s, 2 H, 4-NH_2), 6.70 (d, 1 H, 3′-H), 6.98 (br s, 2 H, 2-NH_2), 8.43 (s, 1 H, 7-H). Anal. ($C_{17}H_{20}N_6O_{20}$·0.5HCl·1.0H_2O) C, H, N, Cl.

2,4-Diamino-5-methyl-6-[(3′,5′-dimethoxyanilino)methyl]pyrido[2,3-*d*]pyrimidine (2b). To a solution of the nitrile **6** (1.50 g, 7.50 mmol) in 250 mL of 85% acetic acid was added 5.00 g of Raney nickel followed immediately by 3,5-dimethoxyaniline (1.74 g, 11.38 mmol). The mixture was hydrogenated at room temperature and atmospheric pressure for 5 h. The reaction mixture was then treated with Norit and filtered through Celite and the filtrate concentrated to yield a red gummy residue. This residue was taken up in 35 mL of warm ethanol and added with constant stirring to 50 mL of a 1 N solution of Na_2CO_3 to deposit crude **2b**. The precipitate was washed repeatedly with ethyl acetate and acetone to remove the unreacted aniline. The residue was then dissolved in 200 mL of methanol, 1.00 g of silica gel added, and the solution evaporated to yield a dry plug. Column chromatography as described previously for the purification of **2a** afforded 0.98 g (37% yield) of **2b** as a yellow solid in fractions 14–36. Mp > 250 °C dec. IR (Nujol): 3300, 3100 (NH_2) cm^{-1}. ^1H NMR (Me_2SO-d_6): δ 2.65 (s, 3 H, 5-CH_3), 3.64 (s, 6 H, 3′,5′-OCH_3), 4.16 (d, 2 H, CH_2NH), 5.74 (s, 1 H, 4′-H), 5.82 (s, 2 H, 2′,6′-H), 5.91 (t, 1 H, CH_2NH), 6.19 (br s, 2 H, 4-NH_2), 6.96 (br s, 2 H, 2-NH_2), 8.46 (s, 1 H, 7-H). Anal. ($C_{17}H_{20}N_6O_2$·0.5H_2O) C, H, N.

2,4-Diamino-5-methyl-6-[(2′,4′-dimethoxyanilino)methyl]pyrido[2,3-*d*]pyrimidine (2c). To a solution of the nitrile **6** (1.20 g, 6.00 mmol) in 200 mL of 80% acetic acid was added 2,4-dimethoxyaniline (1.37 g, 9.00 mmol) followed by 4.50 g of Raney nickel. The mixture was hydrogenated at atmospheric pressure and room temperature for 5 h, treated with Norit, and filtered through a Celite mat. The filtrate was concentrated to yield a red residue. This residue was dissolved in 100 mL of warm ethanol and neutralized with potassium carbonate. A dry plug for column chromatography was prepared as before and applied to the surface of a 1.05 in. × 23 in. column packed with silica gel and eluted with $CHCl_3$:CH_3OH (10:1) to yield 0.63 g (31%) of **2c** as a yellow solid. Mp = 230–235 °C dec. IR (Nujol): 3300, 3100 (NH_2) cm^{-1}. ^1H NMR (Me_2SO-d_6): δ 2.66 (s, 3 H, 5-CH_3), 3.64 (s, 3 H, 2′-OCH_3), 3.75 (s, 3 H, 4′-OCH_3), 4.21 (d, 2 H, CH_2NH), 4.61 (br s, 1 H, CH_2NH), 6.23 (br s, 2 H, 2-NH_2), 6.33 (m, 1 H, 6′-H), 6.48 (m, 2 H, 3′,5′-H), 6.8 (s, 2 H, 4-NH_2), 8.40 (s, 1 H, 7-H). Anal. ($C_{17}H_{20}N_6O_2$·0.5H_2O) C, H, N.

2,4-Diamino-5-methyl-6-[(3′,4′-dimethoxyanilino)methyl]pyrido[2,3-*d*]pyrimidine (2d). To a solution of **6** (1.50 g, 7.50 mmol) in 200 mL of 85% acetic acid was added 4.50 g of Raney nickel followed immediately by 3,4-dimethoxyaniline (1.74 g, 11.38 mmol), and the mixture was hydrogenated at room temperature and atmospheric pressure for 5 h. TLC analysis ($CHCl_3$:CH_3OH, 5:1) showed the absence of **6** and the presence of a new spot (R_f = 0.4). Workup and purification as described for **2a** afforded 0.69 g (34%) of **2d** as an HCl salt. Mp > 250 °C dec. IR (Nujol): 3290, 3830 (NH_2) cm^{-1}. ^1H NMR (Me_2SO-d_6): δ 2.67 (s, 3 H, 5-CH_3), 3.61 (s, 3 H, 4-OCH_3), 3.68 (s, 3 H, 3-OCH_3), 4.16 (d, 2 H, CH_2NH), 5.53 (br t, 1 H, CH_2NH), 6.10 (d, 1 H, 5′-H), 6.35 (s, 1 H, 2′-H), 6.42 (br s, 2 H, 4-NH_2), 6.71 (d, 1 H, 6′-H), 7.00 (br s, 2 H, 2-NH_2), 8.47 (s, 1 H, 7-H). Anal. ($C_{17}H_{20}N_6O_2$·0.5HCl·0.5H_2O) C, H, N, Cl.

2,4-Diamino-5-methyl-6-[(2′,5′-diethoxyanilino)methyl]pyrido[2,3-*d*]pyrimidine (2e). To a solution of **6** (1.10 g, 5.50 mmol) in 200 mL of 85% acetic acid was added 2,5-diethoxyaniline (1.49 g, 8.25 mmol) followed by 4.00 g of Raney nickel. The mixture was hydrogenated for 4.5 h, at the end of which TLC analysis indicated the absence of **6**. The reaction mixture was filtered (Norit, Celite) and the filtrate evaporated to yield crude **2e**. The red residue was dissolved in 30 mL of methanol and chromatographed on a 1.05 in. × 23 in. silica gel column eluted with $CHCl_3$:CH_3OH (10:1) to yield 0.60 g (32%) of **2e** as an acetate salt. Mp > 250 °C dec. IR (Nujol): 3300, 3130 (NH_2) cm^{-1}. ^1H NMR (Me_2SO-d_6): δ 1.21 (t, 3 H, OCH_2CH_3), 1.30 (t, 3 H, OCH_2CH_3), 2.67 (s, 3 H, 5-CH_3), 3.66 (q, 2 H, OCH_2CH_3), 3.91 (q, 2 H, OCH_2CH_3), 4.27 (d, 2 H, CH_2NH), 5.25 (t, 1 H, CH_2NH), 6.03 (d, 1 H, 4′-H), 6.07 (s, 1 H, 6′-H), 6.68 (d, 1 H, 6′-H), 6.89 (br s, 2 H, 4-NH_2), 7.6 (br s, 2 H, 2-NH_2), 8.45 (s, 1 H, 7-H). Anal. ($C_{19}H_{24}N_6O_2$·0.5CH_3COOH) C, H, N.

2,4-Diamino-5-methyl-6-[(1′-naphthylamino)methyl]pyrido[2,3-*d*]pyrimidine (5a). To a solution of the nitrile **6** (1.20 g, 6.00 mmol) in 80% acetic acid was added 1-aminonaphthalene (1.29 g, 9.00 mmol) followed by Raney Ni (5.00 g). The reaction mixture was stirred at room temperature and atmospheric pressure in a hydrogenator for 7 h and filtered, the solvent evaporated, and the residue dissolved in 10 mL of warm methanol. The solution was then chromatographed on silica gel in a 1.05 in. × 23 in. column and eluted with $CHCl_3$:MeOH (7:1). Fractions 15–39 were pooled and evaporated to yield 0.63 g (32%) of **5a** as a bown solid. Mp > 220 °C dec. IR (Nujol): 3300, 3150 (NH_2) cm^{-1}. ^1H NMR (Me_2SO-d_6): δ 2.73 (s, 3 H, 5-CH_3), 4.45 (d, 2 H, CH_2NH), 6.45 (br s, 1 H, CH_2NH), 6.51 (m, 3 H, 2-NH_2, 2′-H), 7.12 (d, 1 H, 4′-H), 7.27 (m, 3 H, 4-NH_2, 3′-H), 7.42 (m, 2 H, 6′,7′-H), 7.76 (d, 1 H, 8′-H), 8.21 (d, 1 H, 5′-H), 8.50 (s, 1 H, 7-H). Anal. ($C_{19}H_{18}N_6$·0.5CH_3-COOH·1.0H_2O) C, H, N.

2,4-Diamino-5-methyl-6-[[(4′-methoxy-1′-naphthyl)amino]methyl]pyrido[2,3-*d*]pyrimidine (5b). 4-Methoxy-1-nitronaphthalene (1.22 g, 6.00 mmol) was dissolved in 10 mL of absolute ethanol, Raney Ni (3.00 g) added, and the mixture hydrogenated at 35 psi for 3 h. To this was added the nitrile **6** (0.80 g, 4.00 mmol) dissolved in 125 mL of 80% acetic acid followed by another 6.00 g of Raney Ni, and the reaction mixture was hydrogenated at room temperature and atmospheric pressure for 6 h and filtered and the solvents evaporated. The residue was dissolved in 50 mL of ethanol and cooled and 1 N Na_2CO_3 added with stirring to precipitate crude **5b**. The solid was collected, washed repeatedly with acetone and ether until the washings were colorless, and then dissolved in 1 L of warm methanol, 1.00 g of silica gel was added, and the methanol was evaporated to yield a dry plug. This plug was placed on a silica gel column, eluted with $CHCl_3$:MeOH (6:1), and fractions 21–38 were combined, evaporated, and dried to yield 0.36 g (25%) of pure **5b** as a yellow solid. Mp > 230 °C. IR (Nujol): 3320, 3180 (NH_2) cm^{-1}. ^1H NMR (Me_2SO-d_6): δ 2.72 (s, 3 H, 5-CH_3), 3.85 (s, 3 H, 4′-OCH_3), 4.37 (d, 2 H, CH_2NH), 6.01 (t, 1 H, CH_2NH), 6.19 (br s, 2 H, 2-NH_2), 6.45 (d, 1 H, 1′-H), 6.78 (d, 1 H, 3′-H), 6.99 (br s, 2 H, 4-NH_2), 7.46 (m, 2 H, 6′,7′-H), 8.08 (d, 1 H, 5′-H), 8.18 (d, 1 H, 8′-H), 8.49 (s, 1 H, 7-H). Anal. ($C_{20}H_{20}N_6O$·0.5H_2O) C, H, N.

2,4-Diamino-5-methylpyrido[2,3-*d*]pyrimidine-6-carboxaldehyde (7). To a solution of **6** (5.00 g) in 125 mL of warm formic acid was added Raney nickel or a Ni–Al alloy (20.00 g), and the mixture was heated at 90 °C under nitrogen for 1.5 h. TLC analysis indicated the absence of **6** and the appearance of a major spot corresponding to the aldehyde (positive (2,4-dinitrophenyl)hydrazine) and a minor spot. The reaction mixture was filtered hot (Norit, Celit) and the filtrate evaporated to dryness to afford an orange residue. This residue was dissolved in 100 mL of boiling water, treated with Norit, and filtered and the filtrate neutralized with concentrated NH_4OH at 0–5 °C to yield 4.20 g of crude **7**. Due to the instability of this compound it was used directly in the next step without purification. ^1H NMR (Me_2SO-d_6): δ 2.96 (s, 3 H, 5-CH_3), 8.85 (s, 1 H, 7-H), 10.16 (s, 1 H, CHO).

2,4-Diamino-5-methyl-6-(hydroxymethyl)pyrido[2,3-*d*]pyrimidine (8). The crude aldehyde **7** (2.00 g) was stirred overnight in anhydrous methanol (1 L) under nitrogen. To this suspension was added $NaBH_4$ (0.50 g) in four separate

portions at 25 °C (exothermic). After the final addition, the reaction mixture was stirred for a further 6–7 h. (It was found that allowing the reaction mixture to stir longer resulted in over-reduction of the alcohol to the 6-methyl compound.) At the end of 6–7 h, the reaction mixture was filtered, water (30 mL) was added to the filtrate, which was concentrated to 20 mL and refrigerated, and the yellow solid obtained was collected by filtration to yield 1.10 g (54%) of **8**. ^{1}H NMR (Me_2SO-d_6): δ 2.66 (s, 3 H, 5-CH_3), 4.51 (s, 2 H, CH_2OH), 8.45 (s, 1 H, 7-H). Anal. ($C_9H_{11}N_5O\cdot1.0H_2O$) C, H, N.

2,4-Diamino-5-methyl-6-(bromomethyl)pyrido[2,3-*d*]pyrimidine Hydrobromide (9). Method A. To freshly prepared triphenylphosphine dibromide (1.39 g, 3.29 mmol) in 20 mL of *N*,*N*-dimethylacetamide was added **8** (225 mg, 1.09 mmol) with stirring. After 2 h, 20 mL of toluene was added which precipitated a brown solid. The clear liquid was decanted, the remaining solid was washed with toluene and ethyl ether, and then sufficient 48% HBr was added to dissolve the solid. This solution was added to cold acetonitrile (ice bath) to deposit a brown solid which was chilled in the refrigerator, filtered, washed with ethyl ether, and dried at 40 °C to yield 0.23 g (77%) of **9**.

Method B. To 0.15 g (0.72 mmol) of the alcohol **8** in round-bottomed flask was added 20 mL of glacial acetic acid, and the reaction mixture was heated to 80 °C to dissolve **7**. The solution was cooled to room temperature, 25 mL of 30% HBr in acetic acid added, and the mixture then stirred at room temperature for 48 h. TLC analysis indicated that most of the starting material had been consumed. The acetic acid was evaporated, and ethyl ether was added to precipitate 0.95 g (91%) of **9**.

Method C. The alcohol **8** (0.25 g, 1.21 mmol) was stirred in anhydrous dioxane and the suspension saturated with dry HBr gas. The reaction mixture was then stirred at room temperature in a tightly sealed flask for 24 h. Some undissolved solid that remained was filtered and the filtrate concentrated to a small volume (approximately 10 mL). To this was added anhydrous ether to precipitate 0.26 g (80%) of **9**. MS: *m/e* 268 and 270 $(M + 1)^+$. ^{1}H NMR (Me_2SO-d_6): δ 2.77 (s, 3 H, 5-CH_3), 4.93 (s, 2 H, CH_2Br), 8.25 (br s, 2 H, 4-NH_2), 8.86 (s, 1 H, 7-H), 9.40 (br s, 2 H, 2-NH_2).

***N*-Isopropyl-3,4,5-trimethoxyaniline (10).** To a solution of 3,4,5-trimethoxyaniline (0.50 g, 2.73 mmol) in 25 mL of acetonitrile was added acetone (0.24 g, 4.10 mmol) followed by $NaCNBH_3$ (0.52 g, 8.19 mmol). Acetic acid (0.50 mL) was added to the reaction mixture at 0.5 h intervals over a 2 h period. The solution was allowed to stir at room temperature for a further 24 h, a small amount of water was added to the reaction mixture, and the solvents were evaporated. The residue was dissolved in 5 mL of $CHCl_3$ and chromatographed on a silica gel column using $CHCl_3$:diethyl ether (1:1) as the eluant. Fractions 18–29 were pooled together and evaporated to dryness to yield 0.33 g (55%) of *N*-isopropyltrimethoxyaniline as a brown solid. ^{1}H NMR ($CDCl_3$): δ 1.21 (d, 6 H, $CH(CH_3)_2$), 2.91 (br s, 1 H, *NH*), 3.56 (m, 1 H, $CH(CH_3)_2$), 3.76 (s, 3 H, 4-OCH_3), 3.82 (s, 6 H, 3-,5-OCH_3), 5.84 (s, 2 H, 2,6-H). Anal. ($C_{12}H_{19}NO_3$) C, H, N.

***N*-Ethyl-3,4,5-trimethoxyaniline (11).** To a solution of 3,4,5-trimethoxyaniline (2.50 g, 13.60 mmol) in 50 mL of warm toluene (60 °C) was added iodoethane (2.13 g, 13.60 mmol) followed by *N*,*N*-diisopropylethylamine (1.76 g, 13.60 mmol) and the mixture refluxed for 24 h. At the end of this period, the solvent was evaporated and the residue dissolved in ~5 mL of chloroform and chromatographed on a 1.5 in. × 23 in. column using $CHCl_3$:diethyl ether (5:1) as the eluant. Fractions containing pure monoalkylated product were pooled and evaporated to yield an oil which solidified on refrigerating (1.53 g, 53%). ^{1}H NMR ($CDCl_3$): δ 1.23 (t, 3 H, CH_3), 2.80 (br s, 1 H, NH), 3.15 (m, 2 H, CH_2), 3.76 (s, 3 H, 4-OCH_3), 3.78 (s, 6 H, 3,5-OCH_3), 5.86 (s, 2 H, 2,6-H). Anal. ($C_{11}H_{17}NO_3$) C, H, N.

***N*-Propargyl-3,4,5-trimethoxyaniline (12).** A solution of 3,4,5-trimethoxyaniline (3.00 g, 16.00 mmol), propargyl bromide (1.94 g, 16.00 mmol), and *N*,*N*-diisopropylethylamine (2.10 g, 16.00 mmol) in toluene was heated at 60 °C (bath temperature) for 24 h. Workup and purification were carried out in a manner similar to that mentioned previously for the *N*-ethyl analogue to yield 1.87 g (53%) of pure monoalkylated product. ^{1}H NMR ($CDCl_3$): δ 2.65 (t, 1 H, NH), 4.17 (s, 3 H, 4-OCH_3), 4.23 (s, 6 H, 3,5-OCH_3), 4.31 (d, 2 H, CH_2), 3.21 (br s, 1 H, CH), 6.34 (s, 2 H, 2,6-H). Anal. ($C_{12}H_{15}NO_3$) C, H, N.

2,4-Diamino-5-methyl-6-[(2′,5′-dimethoxy-*N*-methylanilino)methyl]pyrido[2,3-*d*]pyrimidine (3a). To a suspension of **2a** (0.12 g, 0.35 mmol) in 15 mL of acetonitrile was added formaldehyde (0.12 mL) followed by $NaCNBH_3$ (0.07 g, 1.06 mmol). The mixture was stirred for 5 min, and 1 N HCl was added dropwise to the suspension until the reaction became a clear solution (usually at a pH of 3–4). TLC analysis at that time indicated that the N-methylated product had already begun to form. Within 10–15 min, a yellow solid began to fall out of solution. The reaction mixture was stirred for a total of 4 h at the end of which the solvent was removed, a small amount of water added to the residue, and the suspension filtered to yield 0.10 g of analytically pure **3a** (79%). Mp > 250 °C dec. IR (Nujol): 3350, 3190 (NH_2) cm^{-1}. ^{1}H NMR (Me_2SO-d_6): δ 2.54 (s, 3 H, 5-CH_3), 2.68 (s, 3 H, N-10-CH_3), 3.68 (s, 3 H, 2′-OCH_3), 3.74 (s, 3 H, 5′-OCH_3), 4.17 (s, 2 H, CH_2N), 6.45 (br s, 2 H, 4-NH_2), 6.50 (d, 1 H, 4′-H), 6.53 (s, 1 H, 6′-H), 6.87 (d, 1 H, 3′-H), 7.19 (br s, 2 H, 2-NH_2), 8.46 (s, 1 H, 7-H). Anal. ($C_{18}H_{22}N_6O_2\cdot0.5HCl\cdot0.5H_2O$) C, H, N, Cl.

2,4-Diamino-5-methyl-6-[(3′,5′-dimethoxy-*N*-methylanilino)methyl]pyrido[2,3-*d*]pyrimidine (3b). To a suspension of **2b** (0.13 g, 0.37 mmol) in CH_3CN was added formaldehyde (0.13 mL) followed by $NaCNBH_3$ (0.07 g, 1.10 mmol), and the suspension was allowed to stir for 5–10 min after which 1 N HCl was added dropwise until a clear solution was obtained. The reaction mixture was allowed to stir for an additional 3–4 h after which a small amount of water was added to the reaction mixture and the acetonitrile evaporated. The suspension was refrigerated overnight (0–5 °C), filtered, and dried to yield 0.10 g (76%) of analytically pure **3b** as an orange solid. Mp > 250 °C dec. IR (Nujol): 3290, 3120 (NH_2) cm^{-1}. ^{1}H NMR (Me_2SO-d_6): δ 2.62 (s, 3 H, 5-CH_3), 2.84 (s, 3 H, N-10-CH_3), 3.58 (s, 6 H, 3′,5′-OCH_3), 4.49 (s, 2 H, CH_2-N), 5.86 (s, 3 H, 2′,4′,6′-H), 6.33 (br s, 2 H, NH_2), 7.07 (br s, 2 H, NH_2), 8.27 (s, 1 H, 7-H). Anal. ($C_{18}H_{22}N_6O_2\cdot0.5HCl$) C, H, N, Cl.

2,4-Diamino-5-methyl-6-[(2′,4′-dimethoxy-*N*-methylanilino)methyl]pyrido[2,3-*d*]pyrimidine (3c). To a suspension of **2c** (0.15 g, 0.43 mmol) in acetonitrile was added 37% formaldehyde (0.15 mL) followed by $NaCNBH_3$ (0.08 g, 1.28 mmol). The reaction mixture was stirred for 10 min, and 1 N HCl was added dropwise to adjust the pH to 4. The resulting clear solution was stirred at room temperature for 3 h, and a small amount of water was added to the reaction mixture, concentrated to a small volume (aspirator) and refrigerated, to deposit 0.1 g (65%) of **3c** as a yellow solid. Mp > 270 °C dec. IR (Nujol): 3300, 3120 (NH_2) cm^{-1}. ^{1}H NMR (Me_2SO-d_6): δ 2.68 (s, 3 H, 5-CH_3), 2.71 (s, 3 H, N-10-CH_3), 3.65 (s, 3 H, 2′-OCH_3), 3.70 (s, 3 H, 4′-OCH_3), 4.23 (s, 2 H, CH_2N), 6.42–6.56 (m, 5 H, 3′,5′,6′-H, 4-NH_2), 7.24 (br s, 2 H, 2-NH_2), 8.44 (s, 1 H, 7-H). Anal. ($C_{18}H_{22}N_6O_2\cdot0.5HCl\cdot1.0H_2O$) C, H, N, Cl.

2,4-Diamino-5-methyl-6-[(3′,4′-dimethoxy-*N*-methylanilino)methyl]pyrido[2,3-*d*]pyrimidine (3d). To a suspension of **2d** (0.12 g, 0.36 mmol) in CH_3CN was added formaldehyde (0.12 mL) followed by $NaCNBH_3$ (0.07 g, 1.08 mmol), and HCl was added dropwise to adjust the pH to 4. After 4 h, a small amount of water was added to the reaction mixture, the acetonitrile evaporated, and the suspension refrigerated to afford 0.09 g (75%) of **3d** as a white solid. Mp > 270 °C dec. IR (Nujol): 3310, 3190 (NH_2) cm^{-1}. ^{1}H NMR (Me_2SO-d_6): δ 2.67 (s, 3 H, 5-CH_3), 2.81 (s, 3 H, N-10-CH_3), 3.66 (s, 3 H, 4′-OCH_3), 3.72 (s, 3 H, 3′-OCH_3), 4.41 (d, 2 H, CH_2N), 6.36 (br s, 2 H, 2′,6′-H), 6.78 (d, 1 H, 5′-H), 7.12 (br s, 4 H, 2,4-NH_2), 8.24 (s, 1 H, 7-H). Anal. ($C_{18}H_{22}N_6O_2\cdot1.0HCl$) C, H, N, Cl.

2,4-Diamino-5-methyl-6-[(2′,5′-diethoxy-*N*-methylanilino)methyl]pyrido[2,3-*d*]pyrimidine (3e). To a suspension of **2e** (0.11 g, 0.3 mmol) in CH_3CN was added HCHO (0.11 mL) followed by $NaCNBH_3$ (0.06 g, 0.9 mmol), and the pH of the reaction mixture was adjusted to 4 by the dropwise

addition of 1 N HCl. The reaction mixture was stirred at 25 °C for 4 h. At the end of that time a small amount of water was added to the reaction mixture, and the acetonitrile was removed *via* an aspirator. The suspension was refrigerated overnight to afford 0.08 g (72%) of analytically pure **3e** as a white solid. IR (Nujol): 3320, 3160 (NH_2) cm^{-1}. ^1H NMR (Me_2SO-d_6): δ 1.27 (t, 6 H, OCH_2CH_3), 2.51 (s, 3 H, 5-CH_3), 2.68 (s, 3 H, N-10-CH_3), 3.95 (m, 4 H, OCH_2CH_3), 4.17 (s, 2 H, CH_2N), 6.21 (br s, 2 H, 4-NH_2), 6.43–6.50 (m, 4 H, 4′,6′-H, 2-NH_2), 6.82 (d, 1 H, 3′-H), 8.47 (s, 1 H, 7-H). Anal. ($C_{20}H_{26}N_6O_2 \cdot 1.0HCl \cdot 0.5H_2O$) C, H, N, Cl.

2,4-Diamino-5-methyl-6-[(3′,4′,5′-trimethoxy-*N*-propargylanilino)methyl]pyrido[2,3-*d*]pyrimidine (4a). To a solution of compound **9** (0.21 g, 0.78 mmol) in 15 mL of anhydrous *N*,*N*-dimethylacetamide was added *N*-proparyltrimethoxyaniline (0.19 g, 0.86 mmol) followed by potassium carbonate (0.11 g, 0.78 mmol). The mixture was stirred under an atmosphere of nitrogen for 48 h. TLC analysis in $CHCl_3$:CH_3OH (5:1) indicated the presence of a major spot (corresponding to the product, R_f = 0.5), excess aniline, and some impurity at the base. The reaction mixture was filtered and the solvent evaporated *in vacuo* at 60 °C. The residue was dissolved in 30 mL of methanol, 0.25 g of silica gel added, and the methanol evaporated to yield a dry plug. This plug was applied to the surface of a 1.05 in. × 23 in. column packed with silica gel and eluted with $CHCl_3$:CH_3OH (5:1). Fractions containing pure product were pooled and evaporated to yield 0.22 g (67%) of **4a** as a tan solid. Mp > 250 °C dec. IR (Nujol): 3500 (CH stretch), 3250, 3100 (NH_2) cm^{-1}. ^1H NMR (Me_2SO-d_6): δ 2.57 (s, 3 H, 5-CH_3), 3.17 (s, 1 H, C≡CH), 3.58 (s, 3 H, 4′-OCH_3), 3.73 (s, 6 H, 3″,5′-OCH_3), 3.97 (s, 2 H, CH_2-C≡CH), 4.42 (s, 2 H, CH_2N), 6.24 (s, 4 H, 2′,6′-H, 4-NH_2), 6.99 (br s, 2 H, 2-NH_2), 8.40 (s, 1 H, 7-H). Anal. ($C_{21}H_{24}N_6O_3 \cdot 0.5H_2O$), C, H. N.

2,4-Diamino-5-methyl-6-[(3′,4′,5′-trimethoxy-*N*-isopropylanilino)methyl]pyrido[2,3-*d*]pyrimidine (4b). To a solution of compound **9** (0.25 g, 0.93 mmol) in 15 mL of anhydrous *N*,*N*-dimethylacetamide was added *N*-isopropyltrimethoxyaniline (0.23 g, 1.02 mmol) followed by cesium carbonate (0.20 g, 1.02 mmol). The brownish solution was stirred at room temperature under nitrogen for 60 h. At the end of this period, TLC analysis in $CHCl_3$:CH_3OH (5:1) showed the presence of a major spot (corresponding to the product, R_f = 0.48). The reaction mixture was filtered and the solvent removed *in vacuo* using a pump (bath temperature ~60 °C). A small amount of acetic acid (~2 mL) was added to the residue followed by 30 mL of methanol and 0.50 g of silica gel. Column chromatography as described above for the purification of **4a** yielded 0.24 g (61%) of **4b** as a tan solid. Mp > 270 °C dec. IR (Nujol): 3300, 3060 (NH_2) cm^{-1}. ^1H NMR (Me_2SO-d_6): δ 1.16 (d, 6 H, $CH(CH_3)_2$), 2.69 (s, 3 H, 5-CH_3), 3.52 (s, 3 H, 4′-OCH_3), 3.62 (s, 6 H, 3′,5′-OCH_3), 4.14 (m, 1 H, $CH(CH_3)_2$), 4.31 (s, 2 H, CH_2N), 5.96 (s, 2 H, 2′,6′-H), 6.15 (br s, 2 H, 4-NH_2), 6.95 (br s, 2 H, 2-NH_2), 8.26 (s, 1 H, 7-H). Anal. ($C_{21}H_{32}N_6O_3 \cdot 1.0H_2O$), C, H, N.

2,4-Diamino-5-methyl-6-[(3′,4′,5′-trimethoxy-*N*-ethylanilino)methyl]pyrido[2,3-*d*]pyrimidine (4c). To a solution of **9** (0.18 g, 0.66 mmol) in 15 mL of anhydrous *N*,*N*-dimethylacetamide was added *N*-ethyltrimethoxyaniline (0.15 g, 0.73 mmol) followed immediately by cesium carbonate (0.14 g, 0.73 mmol). The reaction mixture was stirred under nitrogen for 48 h. TLC analysis indicated the presence of a major spot at R_f = 0.46. Workup and purification as described above for **4a** yielded 0.19 g (71%) of pure **4c**. Mp > 280 °C dec. IR (Nujol): 3300, 3140 (NH_2) cm^{-1}. ^1H NMR (Me_2SO-d_6): δ 1.06 (t, 3 H, CH_2CH_3), 2.64 (s, 3 H, 5-CH_3), 3.36 (q, 2 H, CH_2CH_3), 3.54 (s, 3 H, 4′-OCH_3), 3.68 (s, 6 H, 3′,5′-OCH_3), 4.45 (s, 2 H, CH_2N), 5.99 (s, 2 H, 2′,6′-H), 6.61 (br s, 2 H, 4-NH_2), 7.37 (br s, 2 H, 2-NH_2), 8.30 (s, 1 H, 7-H). Anal. ($C_{20}H_{26}N_6O_3 \cdot 1.0H_2O$) C, H, N.

Acknowledgment. This work was supported in part by NIH Grants GM40998 (A.G.) and CA10914 (R.L.K.) and NIH Contracts NO1-AI-87240 (S.F.Q.) and NO1-AI-35171 (S.F.Q.) Division of AIDS.

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JM9408631