Synthesis and Biological Evaluation of Nonclassical 2,4-Diamino-5-methylpyrido[2,3-*d*]pyrimidines with Novel Side Chain Substituents as Potential Inhibitors of Dihydrofolate Reductases[1]

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Nine novel 2,4-diamino-5-methyl-6-substituted-pyrido[2,3-*d*]pyrimidines, **2**−**10**, were synthesized as potential inhibitors of *Pneumocystis carinii* dihydrofolate reductase (pcDHFR) and *Toxoplasma gondii* dihydrofolate reductase (tgDHFR). Compounds **2**−**5** were designed as conformationally restricted analogues of trimetrexate (TMQ), in which rotation around τ_3 was constrained by incorporation of the side chain nitrogen as part of an indoline or an indole ring. Analogue **6**, which has an extra atom between the side chain nitrogen and the phenyl ring, has its nitrogen as part of a tetrahydroisoquinoline ring. Analogues **7**−**9** are epiroprim (Ro 11-8958) analogues and contain a pyrrole ring as part of the side chain substitution on the phenyl ring similar to epiroprim. These analogues were designed to investigate the role of the pyrrole substitution on the phenyl ring of 2,4-diamino-5-methyl-6-(anilinomethyl)pyrido[2,3-*d*]pyrimidines. Molecular modeling indicated that a pyrrole substituent in the ortho position of the side chain phenyl ring was most likely to interact with pcDHFR in a manner similar to the pyrrole moiety of epiroprim. Analogue **10**, in which a phenyl ring replaced a methoxy group, was synthesized to determine the contribution of a phenyl ring on selectivity, lipophilicity, and cell penetration. The synthesis of analogues **2**−**4** was achieved *via* reductive amination of 2,4-diamino-5-methyl 6-carboxaldehyde with the appropriately substituted indolines. The indolines were obtained from the corresponding indoles *via* $NaCNBH_3$ reductions. Analogues **5**−**10** were synthesized by nucleophilic displacement of 2,4-diamino-5-methyl-6-(bromomethyl)-pyrido[2,3-*d*]pyrimidine with the 5-methoxyindolyl anion, 6,7-dimethoxytetrahydroisoquinoline, the appropriately substituted pyrroloaniline or 2-methoxy-5-phenylaniline. The pyrroloanilines were synthesized in two steps by treating the substituted nitroanilines with 2,5-dimethoxy-tetrahydrofuran to afford the nitropyrrole intermediates, followed by reduction of the nitro group with Raney Ni. The analogues were more potent than trimethoprim and epiroprim and more selective than TMQ and piritrexim against pcDHFR and tgDHFR. Compounds **5** and **10** had IC_{50} values of 1 and 0.64 μM, respectively, for the inhibition of the growth of *T. gondii* cells in culture, and showed excellent culture IC_{50}/enzyme IC_{50} ratios, which were correlated with their calculated log *P* values, indicating a direct relationship between calculated lipophilicity and cell penetration.

The principal causes of morbidity and mortality in patients with AIDS are opportunistic infections with *Pneumocystis carinii* and *Toxoplasma gondii*.[2] One of the ways of tackling *P. carinii* infections is by prophylaxis including the dihydrofolate reductase (DHFR) inhibitor, trimethoprim (TMP). TMP is a weak inhibitor of *P. carinii* (pc) DHFR and *T. gondii* (tg) DHFR and hence is used along with sulfamethoxazole (SMX) to augment its inhibition. Other drugs such as atovaquone[3] and pentamidine[4] have been evaluated for the treatment of these infections, but TMP/SMX is considered the most effective first-line treatment for *P. carinii* pneumonia (PCP). The combination of pyrimethamine and sulfadiazine is currently approved for the treatment of *T. gondii* infections. Both TMP/SMX and pyrimethamine/sulfadiazine suffer from disadvantages which often force discontinuation of therapy.[5] Drugs suggested as substitutes include trimetrexate (TMQ) and piritrexim (PTX), two potent lipophilic, nonclassical inhibitors of pcDHFR and tgDHFR.[6−8] These drugs are able to penetrate *P. carinii* and *T. gondii* cells by passive diffusion, thus circumventing the requirement for the carrier-mediated active transport system(s) needed for the uptake of classical antifolates such as methotrexate (MTX), but both TMQ and PTX lack selectivity for either pcDHFR or tgDHFR, and their use as a single agent is associated with considerable host toxicity. As a result, TMQ has to be coadministered with leucovorin (N5 formyl tetrahydrofolate) which is selectively taken up by host cells, thus providing a rescue of host cells and decreasing toxicity.

With the recent report of the crystal structure of pcDHFR,[9] the search for potent and selective inhibitors of pcDHFR has gained momentum. Gangjee *et al.*[10−12] recently reported the synthesis and biological activities of series of 2,4-diamino-6-substituted-pyrido[2,3-*d*]pyrimidines with a variety of side chain substituents as semirigid analogues of TMQ, in an attempt to increase the selectivity and/or potency of TMQ. In general, these analogues were more selective than TMQ against pcDHFR and tgDHFR. Some analogues displayed 1000−1400-fold greater selectivity than TMQ against tgDHFR and pcDHFR, respectively. It was observed

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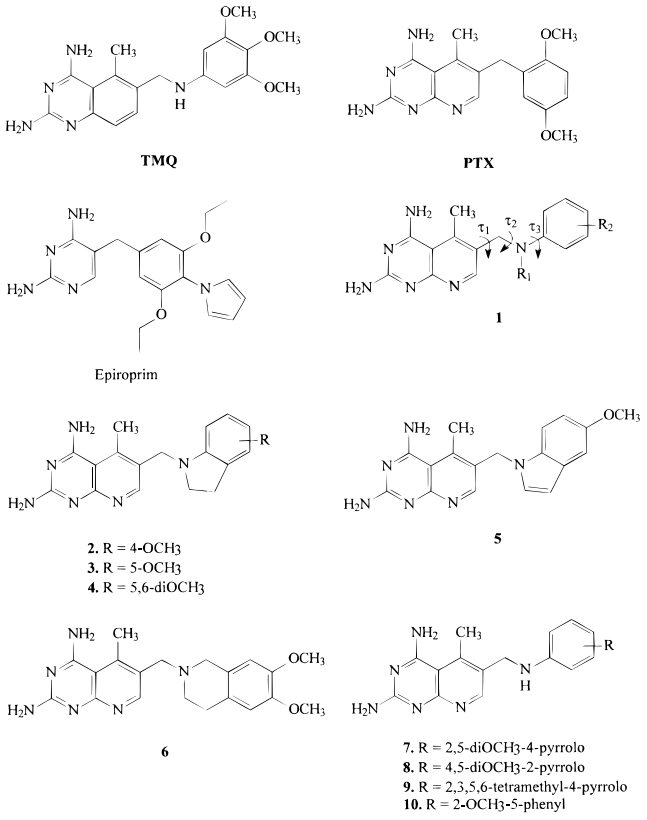
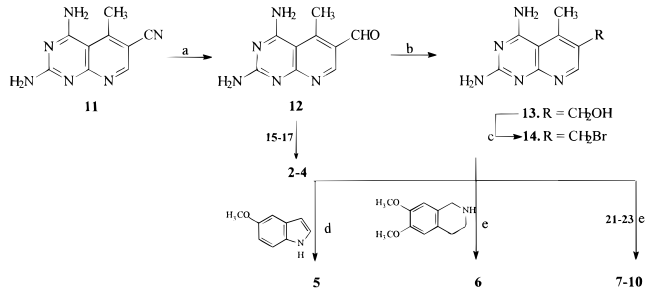
that the presence of a methyl moiety on the side chain bridge nitrogen resulted in increased potency and, in some instances, improved selectivity against pcDHFR and tgDHFR. This suggested that conformational restriction about τ_2 and τ_3 induced by the N-methyl moiety may be conducive to potency and selectivity, in addition to the hydrophobic interactions of the methyl group with the enzyme. The ability of these analogues to penetrate *T. gondii* cells in culture, an important attribute of a clinically viable agent, was also studied. Gangjee *et al.*[11] have reported that incorporation of bulky naphthalene or 4-methoxynaphthalene rings in the side chain resulted in excellent cell penetration. Further, dimethoxyphenyl-substituted analogues displayed greater cell penetration compared to trimethoxyphenyl-substituted analogues. As part of our continuing effort to synthesize potent and selective inhibitors of pcDHFR and tgDHFR, we synthesized compounds **2**−**10**. Analogues **2**−**5** were designed as conformationally restricted analogues of TMQ in which torsion angle τ_3 was restricted by incorporating the side chain nitrogen within an indoline or indole ring. In our previous report,[10] we found that 2,4-diamino-5-methyl-6-[(3′,4′,5′-trimethoxy-*N*-methylanilino)methyl]pyrido[2,3-*d*]pyrimidine (**1,** $R_1 = CH_3$; $R_2 = 3,4,5\text{-}OCH_3$) was both a selective (IC_{50} rlDHFR/IC_{50} tgDHFR = 8.94) and potent (IC_{50} = 0.85 nM) inhibitor of tgDHFR. Further, the N10 formyl analogue was as selective against tgDHFR as the N10 methyl analogue. Molecular modeling using Sybyl 6.0[13] indicated that restriction about torsion angles τ_2 and τ_3, imposed by the N10 substituent, could be responsible for the selective inhibition of tgDHFR, in addition to a possible selective interaction of the N10 substituent with the enzyme. Compounds **2**−**5** are conformationally restricted analogues of the N10 methyl analogue (**1**, $R_1 = CH_3$; $R_2 = 3,4,5\text{-}OCH_3$), which could serve to further increase the potency and/or selectivity of these analogues by orienting the side chain in a more favorable conformation for interaction with tgDHFR. In addition, in a series of nonclassical tetrahydroquinazoline analogues recently reported by Gangjee *et al.*,[14] an indoline substitution resulted in a selectivity ratio of 6 for tgDHFR (*vs* rlDHFR). Thus, it was of interest to evaluate compounds **2**−**5** as inhibitors of pcDHFR and tgDHFR. Comparison of the pcDHFR and tgDHFR inhibitory activities as well as the *in vitro P. carinii* and *T. gondii* cell culture inhibition results of an indoline and indole analogue would provide an insight into the role of a flat, heteroaromatic system (such as an indole) *vs* a bulky substitution (such as an indoline) on the side chain for inhibition of pcDHFR and tgDHFR as well as for cell penetration. This aspect was of special interest, since the hydrophobic interactions of the indole and the indoline moieties with the enzyme as well as their contribution to the overall lipophilicity (as reflected in their calculated log *P* values[15]) of the compound, and hence cell penetration was anticipated to be different. In their study of trimethoprim analogues, Rauckmann *et al.*,[16] had reported significant differences in DHFR inhibition and selectivities for *E. coli* (ec) DHFR with the quinoline and tetrahydroquinoline moieties, lending further credence to the idea that the indoline and indole analogues were worth investigating as inhibitors of pcDHFR and tgDHFR as well as inhibitors of *P. carinii* and *T. gondii* cells in culture.



Analogue **6** was designed to study the effect of extending the bridge length between the pyrido[2,3-*d*]-pyrimidine moiety and the side chain phenyl ring by an extra atom, thus probing any additional contacts that the extended side chain phenyl ring might provide with either pcDHFR and/or tgDHFR. Such an extension of the bridge between the 2,4-diamino-substituted heterocyclic moiety and the side chain phenyl ring from two atoms to three atoms has been recently reported by Gangjee *et al.*[17]

Then *et al.*[18] reported a series of trimethoprim analogues, one of which, epiroprim (Ro 11-8958), had a reported 200-fold selectivity for pcDHFR *vs* human (h) DHFR. This is the most selective inhibitor of pcDHFR known to date. In addition, epiroprim was also an extremely selective (3×10^5-fold) and potent inhibitor of ecDHFR. The ability of epiroprim to inhibit PCP in combination with other antifolates has been studied, and some synergy has been observed with SMX and with dapsone.[19] The analogues of epiroprim which displayed appreciable selectivity (>20-fold) for pcDHFR (*vs* hDHFR) had a pyrrole ring as part of the side chain, suggesting a selective interaction of the pyrrole ring with pcDHFR which was absent with hDHFR. Recently, epiroprim has been evaluated against tgDHFR[20] and was found to be 650-fold more selective for tgDHFR (*vs* human DHFR) and was 86-fold more selective compared to pyrimethamine. When administered intraperitoneally in combination with sulfadiazine at the relatively high doses of 300 and 375 mg/kg, respectively, 100% survival was seen in mice acutely infected with the recombinant human strain of *T. gondii*. Either drug alone was ineffective at the same doses, establishing effective synergy in this model. The potency of epiroprim, however, is in the micromolar range. In an attempt to

Scheme 1[a]



[a] Reagents: (a) Raney Ni or Ni Al; (b) $NaBH_4$ or $LiEtBH_3$; (c) PPh_3Br_2 or anhd. HBr/dioxane or 30% HBr/AcOH; (d) NaH, DMAC; (e) K_2CO_3 or $CsCO_3$.

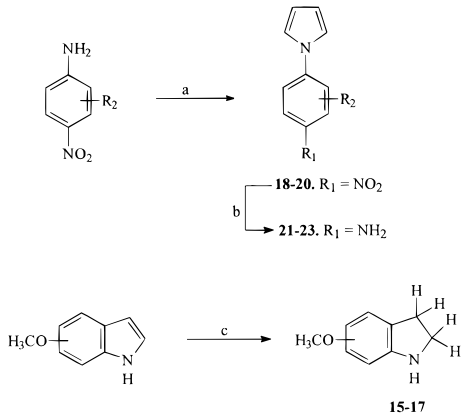
incorporate the selectivity of epiroprim into potent pyrido[2,3-*d*]pyrimidines, we synthesized the pyrrole-substituted analogues **7**−**9**. The 2′,5′-dimethoxy-substituted analogue (**1,** R_1 = H; R_2 = 2,5-OCH_3) displayed a 3-fold selectivity for pcDHFR and an 8-fold selectivity for tgDHFR (*vs* rlDHFR).[11] Comparison of the biological activity of this analogue with analogue **7** would permit an assessment of the contribution to the activity and selectivity of a pyrrole moiety in the pyrido[2,3-*d*]-pyrimidine series. Molecular modeling using Sybyl 6.0 indicated that a pyrrole substituent in the ortho position of the side chain phenyl ring was most likely to interact with pcDHFR in the same manner as the pyrrole moiety of epiroprim. Further, the substitution of a pyrrole moiety at the ortho position as in **8** might also serve to increase the conformational restriction about τ_3, a feature which could provide increased selectivity and/or potency against pcDHFR and/or tgDHFR. Compound **9** was synthesized to significantly increase the lipid solubility of **7** by replacing the 2′,5′-dimethoxy moieties with methyl moieties and further adding two methyl groups at the 3′ and 6′ positions. Analogue **10**, in which a methoxy group has been replaced with a phenyl ring, would provide an assessment of the contribution of a hydrophobic phenyl moiety in place of a methoxy group, in particular with respect to *P. carinii* and *T. gondii* cell penetration, since this analogue is anticipated to possess improved lipid solubility compared to **1** (R_1 = H; R_2 = 2,5-diOCH_3).

Chemistry

The synthetic route adopted for these analogues was a modification of that reported earlier.[11] This involved the synthesis of 2,4-diamino-5-methylpyrido[2,3-*d*]pyrimidine-6-carbonitrile, **11**, in improved yields[11] compared to the literature procedure[21] (74%; lit. 54%). This nitrile was then reduced to the 6-carboxaldehyde **12** (Scheme 1) using either Raney Ni or a Ni−Al alloy in formic acid. Due to the instability of the aldehyde, it was not purified but used directly for further transformations.

We have previously reported[14] the reductive amination of 2,4-(bisacetylamino)-5,6,7,8-tetrahydroquinazoline-6-carboxaldehyde with various secondary amines including indoline. We adopted a similar strategy for the synthesis of compounds **2**−**4** (Scheme 1). The indoline precursors were obtained in quantitative yield *via* the reduction of the corresponding methoxy-substituted indole analogues using $NaCNBH_3$ in acetic acid (Scheme 2).[22] The presence of two triplets in the ^{1}H NMR spectra in deuterated chloroform, corresponding

Scheme 2[a]



[a] Reagents: (a) 2,5-dimethoxytetrahydrofuran, CH_3COOH, reflux; (b) Raney Ni, EtOH, 35 psi; (c) $NaCNBH_3$, CH_3COOH.

to the two pairs of methylene protons, confirmed the product. Reductive amination of the 6-carboxaldehyde with these indolines was attempted in methanol.[14] Despite variation in reaction time and temperature, the yield of the desired analogues, as evidenced from TLC analysis, was poor (<5%). One of the reasons for the poor yield was the insolubility of the aldehyde in methanol. To improve the solubility of the aldehyde, it was converted to its acetate salt. The reductive amination was then attempted using the more soluble acetate salt of the aldehyde and the appropriate indoline using $NaCNBH_3$. HCl (6 N) in methanol was added to the reaction mixture to maintain the pH at 6. The use of freshly activated molecular sieves[23] afforded analogues **2**−**4** in 30−35% yields.

For the synthesis of the indole analogue **5**, the 6-aldehyde **12** was reduced to the 6-alcohol **13** using $NaBH_4$ in methanol.[11] The alcohol **13** was brominated using one of three methods reported in the literature: (a) triphenylphosphine dibromide,[21] (b) anhydrous HBr in dioxane,[24] and (c) 30% HBr in acetic acid.[25] The bromination was most efficient using 30% HBr/AcOH and afforded the 6-bromomethyl analogue **14** as a hydrobromide salt in 90% yield. Initial attempts at alkylating the sodium salt of the indole nitrogen with the bromomethyl compound did not afford the desired analogue due to rapid quenching of the anion with the hydrogen bromide associated with the bromomethyl compound. Hence, the bromomethyl compound **14** was first treated with triethylamine under an atmosphere of argon, and then the sodium salt of the indole was added dropwise at room temperature to afford the desired analogue in 30% yield. The regioselectivity of the alkylation (N1 *vs* C3) was confirmed from the ^{1}H NMR which indicated the presence of the two doublets for C2-H and C3-H of the indole moiety and the absence of the N1-H of the indole.

The synthesis of the target compounds **7**−**9** required the synthesis of the appropriate pyrrole-substituted anilines. These were synthesized in two steps, starting from the appropriately substituted nitroanilines (Scheme 2). Refluxing these anilines with 2,5-dimethoxytetrahydrofuran in acetic acid afforded the pyrrolonitrobenzenes in quantitative yield. For the tetramethylnitroaniline, it was necessary to reflux the reaction for 10 h to allow for complete formation of the pyrrole (TLC), presumably due to steric hindrance of the amino group. Selective reduction of the nitro group using Raney nickel

Table 1. Inhibitory Concentrations (IC_{50}, μM) and Selectivity Ratios against *P. carinii* (pc), *T. gondii* (tg), and Rat Liver (rl) DHFR[a]

	pcDHFR	rlDHFR	rl/pc	tgDHFR	rl/tg
1 (R_1 = H; R_2 = 2,5OCH_3)	0.046[b]	0.13[b]	2.8[b]	0.016[b]	8.0[b]
2	0.29	0.15	0.52	0.048	3.1
3	0.25	0.17	0.68	0.057	3.0
4	0.41	0.23	0.56	0.049	4.7
5	0.57	0.47	0.83	0.077	6.1
6	0.85	0.13	0.2	0.11	1.2
7	0.35	0.23	0.7	0.033	7.0
8	1.8	3.5	1.9	0.6	5.8
9	0.62	0.17	0.3	0.075	2.3
10	0.64	0.44	0.7	0.068	6.5
TMQ	0.042	0.003	0.07	0.010	0.3
PTX	0.038	0.0015	0.04	0.011	0.14
TMP	12	133	11.1	2.7	49
epiroprim	2.6	33.2	12.8	0.48	70.6

[a] These assays were carried out at 37 °C, under conditions of substrate (90 μM dihydrofolic acid) and cofactor (119 μM NaDPH) in the presence of 150 mM KCl and 2-mercaptoethanol (8.9 mM), in a sodium phosphate buffer (pH 7.4).[26,27] [b] Data from ref 11.

Table 2. *T. gondii* Cell Culture Inhibition (IC_{50}, μM) of Selected Analogues[a]

	IC_{50}			
	tgDHFR	culture	culture/enz	log *P*
4	0.049	25.10	512	2.3343
5	0.077	1.0	13	3.0668
10	0.068	0.64	10	4.7690

[a] *T. gondii* cell culture inhibition was assessed by measuring the incorporation of [^{3}H]uracil by *T. gondii* cells.[26]

in ethanol at 35 psi afforded the substituted pyrroloanilines. Alkylation (Scheme 1) of 6,7-dimethoxytetrahydroisoquinoline, the pyrrole-substituted anilines and 2-methoxy-5-phenylaniline, with the 6-bromomethyl compound **14** using either potassium carbonate or cesium carbonate afforded analogues **6**−**10** in 65−70% yield after purification *via* column chromatography.

Biological Evaluation and Discussion

All the analogues were evaluated against *P. carinii* (pcDHFR), *T. gondii* (tgDHFR), and rat liver DHFR (rlDHFR), and the results are indicated in Table 1. Selectivity ratios are expressed as ratios of rlDHFR IC_{50}/pcDHFR IC_{50} and rlDHFR IC_{50}/tgDHFR IC_{50}, and are also indicated in Table 1. Selected analogues were evaluated for the inhibition of the growth of *T. gondii* cells in culture, and the results are reported in Table 2. All of the analogues were more selective than TMQ and PTX against both pcDHFR and tgDHFR. Comparison of the biological activities of analogues **2**−**4** indicates the effect of conformational restriction around τ_3 in the form of an indoline ring. The results show that analogues **2**−**4** have similar inhibitory effects against pcDHFR, tgDHFR, and rlDHFR, suggesting that conformational restriction *via* an indoline ring orients the side chain of these three analogues in similar positions, enabling these analogues to interact with all three DHFRs to the same extent, and that moving the position of the methoxy group in the phenyl ring does not afford any differential inhibition. However, analogues **2**−**4** were more selective than TMQ and PTX against both pcDHFR and tgDHFR. Analogue **4,** in particular was 16-fold more selective than TMQ and 33-fold more selective than PTX against tgDHFR. Comparison of the DHFR inhibitory concentrations of **3** and **5** indicate that the effect of a substituted indoline and an indole on all three DHFRs is quite similar, although a 2-fold increase in selectivity with the indole analogue **5,** for tgDHFR, was obtained.

Incorporation of the side chain nitrogen in a tetrahydroisoquinoline ring as in analogue **6** resulted in submicromolar inhibitory activity against all three DHFRs. The weak activity of this analogue against pcDHFR and tgDHFR could be attributed to the lack of sufficient space in the active site of the enzyme to accommodate the bulky isoquinoline moiety, or to the presence of a basic, protonated nitrogen in the side chain, or both. The active site of DHFR has been shown by Piper *et al.*[25] to be sufficiently large to accommodate bulky substituents on the side chain. Gangjee *et al.*[17] have speculated on the role of a basic, protonated nitrogen in the side chain as being one of the reasons for the lack of significant DHFR inhibitory activity of classical furo[2,3-*d*]pyrimidine analogues. Results obtained with analogue **6** corroborate that initial[17] assumption. However, further studies are required to assess the exact role of a basic nitrogen in the side chain of DHFR inhibitors toward inhibition of the enzyme.

Analogues **7**−**9** (which were designed to investigate the importance and/or interaction of the pyrrole ring) were more active than epiroprim against pcDHFR and rlDHFR; **7** and **9** were more active than epiroprim against tgDHFR, while **8** was equipotent with epiroprim. Substitution of a pyrrole ring at the *para* position of the phenyl ring as in analogue **7** resulted in a decrease in activity against pcDHFR and tgDHFR as well as rlDHFR compared to the pyrrole-unsubstituted analogue (**1,** R_1 = H; R_2 = 2,5-OCH_3). Compound **7** retained the selectivity for tgDHFR displayed by compound **1**. Further, it was 15-fold more potent than epiroprim against tgDHFR. Analogue **8**, with an *ortho* pyrrolo-4,5-dimethoxy substituent, displayed IC_{50} values for all three DHFRs in the micromolar range. This result indicates that substitution of a pyrrole ring in the *ortho* position is detrimental to potency against pcDHFR and tgDHFR; nevertheless, this analogue displayed a selectivity ratio of 1.9 for pcDHFR (*vs* rlDHFR) and was the most pcDHFR selective analogue of this series. The analogue also had a 6-fold selectivity for tgDHFR. Analogue **10**, in which one of the methoxy groups was replaced with a phenyl ring, provided interesting results. Comparison of its activity with that of the 2′,5′-dimethoxyphenyl-substituted analogue (**1,** R_1 = H; R_2 = 2,5-OCH_3)[11] indicates the importance of the substitution at the 5′-position for inhibition of pcDHFR. Replacement of the 5′-methoxy moiety with a phenyl ring results in a decrease in potency against all of these DHFR, but pcDHFR is most strongly affected with a 14-fold decrease in potency compared to the parent analogue (**1**, R_1 = H, R_2 = 2,5-OCH_3).[11] These results once again underscore the point that the active site of these DHFR are quite different and that a separate structure−activity/selectivity relationship needs to be established for each enzyme.

Three of the analogues (**4**, **5**, and **10**) were evaluated as inhibitors of the growth of *T. gondii* cells in culture,[26] and the results are shown in Table 2. The corresponding calculated log *P* values of these analogues are also listed. Thus, while the indoline and indole analogues (**4** and **5,** respectively) displayed similar inhibition of

isolated tgDHFR, in *T. gondii* cell culture studies, the indole analogue was 25-fold superior. One of the reasons for this enhanced inhibition of the indole analogue could be a differential transport across the cell membrane, as is reflected in the higher calculated log *P* value of **5** as compared to that of **4**. This assumption is corroborated by the lower IC_{50} ratio of culture/enzyme of **10**, which has a higher calculated log *P* value than that of **5**. It should be noted that an increase in calculated log *P* value beyond a certain limit will result in decreased cell penetration. This is substantiated in Table 2, where there is a leveling off of the IC_{50} ratio of culture/enzyme with an increase in log *P*, which attests to the fact that a suitable balance between lipophilicity and hydrophilicity is needed for superior cell penetration.

In summary, conformational restriction in the form of an indoline or an indole ring, while providing selective analogues, did not provide analogues which were better in potency or selectivity than the open-chain analogues synthesized previously.[11] Further, incorporation of a pyrrole moiety in the side chain of these molecules did not provide any beneficial selectivity as compared to epiroprim, suggesting that whatever interactions the side chain of epiroprim undergoes with pcDHFR could not be exploited with analogues **7**−**9**. Despite the fact that selective inhibitors of pcDHFR and tgDHFR were attained in this study, along with other analogues with excellent cell penetration properties, the challenge remains to design analogues which combine both of these attributes in a single molecule for the treatment of *P. carinii* and *T. gondii* infections.

Experimental Section

All evaporations were carried out *in vacuo* with a rotary evaporator. Analytical samples were dried *in vacuo* (0.2 mmHg) in an Abderhalden drying apparatus over phosphorous pentoxide and refluxing ethanol or toluene. Thin layer chromatography was performed on Sigma Aldrich silica gel chromatogram plates with fluorescent indicator. Spots were visualized by UV light (254 and 350 nm). Proportions of solvents used are by volume. All analytical samples were homogeneous in at least three different solvent systems. Melting points were determined on a Fisher Johns melting point apparatus or a Meltemp and are uncorrected. Purification by gravity column chromatography and flash chromatography were carried out using Merck silica gel 60 (230−400 mesh). Infrared (IR) spectra were determined neat or in Nujol mulls on a Perkin-Elmer 1430 ratio recording infrared spectrophotometer and are reported in reciprocal centimeters. The ^{1}H NMR spectra were recorded on a Bruker WH-300 (300 MHz) spectrometer. The chemical shift (δ) values are expressed in ppm (parts per million) relative to tetramethylsilane as internal standard: s = singlet, d = doublet, t = triplet, m = multiplet, exch = protons exchangeable on addition of deuterium oxide. Elemental analyses were performed at Atlantic Microlabs and are within +0.4 of the calculated values. All solvents and chemicals were purchased from Aldrich Chemical Co. and Fisher Scientific and were used as received.

General Procedure for the Synthesis of the Methoxyindolines. To a solution of the methoxyindole (1 equiv) in acetic acid was added $NaCNBH_3$ (3 equiv) in portions (0.5 equiv each time) over a 10 min period (**caution**: exothermic reaction). The reaction was stirred at room temperature for one-half hour after the last addition. TLC analysis ($CHCl_3$:Et_2O, 5:1) indicated the presence of a new spot corresponding to the product. A small amount of water (~2−3 mL) was added to the reaction, the acetic acid evaporated under reduced pressure (0.1 mm, 45 °C bath tempeature) and the residue cooled (0 to −5 °C) to afford the desired indoline.

4-Methoxyindoline (15). This was synthesized as a colorless oil from 4-methoxyindole (0.30 g, 1.84 mmol), and $NaCNBH_3$ (0.35 g, 5.52 mmol) in 20 mL of acetic acid (0.30 g, 98%): TLC (hexane:EtOAc, 1:1) R_f = 0.45; ^{1}H NMR ($CDCl_3$) δ 3.04 (m, 2 H, NH$CH_2$$CH_2$), 3.65 (t, 2 H, $CH_2$$CH_2$), 3.81 (s, 3 H, 4-$OCH_3$), 6.45 (d, 1 H, 7-H), 6.53 (d, 1 H, 5-H), 7.06 (t, 1 H, 6-H).

Anal. ($C_9H_{11}NO$·0.2CH_3COOH) C, H, N.

5-Methoxyindoline (16). This was synthesized as an oil from 5-methoxyindole (0.40 g, 2.45 mmol), and $NaCNBH_3$ (0.46 g, 7.36 mmol) in 25 mL of acetic acid (0.38 g, 94%): TLC (hexane:EtOAc, 1:1) R_f = 0.46; ^{1}H NMR ($CDCl_3$) δ 2.92 (t, 2 H, NH$CH_2$$CH_2$), 3.34 (t, 2 H, $CH_2$$CH_2$), 3.73 (s, 3 H, 5-$OCH_3$), 6.41 (d, 1H, 7-H), 6.64 (m, 1 H, 6-H), 6.72 (s, 1 H, 4-H).

Anal. ($C_9H_{11}NO$·0.6CH_3COOH) C, H, N.

5,6-Dimethoxyindoline (17). This was synthesized as a colorless oil from 5,6-dimethoxyindole (0.35 g, 1.81 mmol), and $NaCNBH_3$ (0.34 g, 5.44 mmol) in 20 mL of acetic acid (0.32 g, 92%): TLC (hexane:EtOAc, 1:1) R_f = 0.54; ^{1}H NMR ($CDCl_3$) δ 3.41 (m, 2 H, NH$CH_2$$CH_2$), 3.67 (t, 2 H, $CH_2$$CH_2$), 3.75 (s, 3 H, 5-$OCH_3$), 3.79 (s, 3 H, 6-$OCH_3$), 6.68 (s, 1 H, 7-H), 6.75 (s, 1 H, 4-H).

Anal. ($C_{10}H_{13}NO_2$·0.5CH_3COOH) C, H, N.

2,4-Diamino-5-methyl-6-[(4′-methoxyindolinyl)methyl]-pyrido[2,3-*d*]pyrimidine (2). To a solution of the acetate salt of 2,4-diamino-5-methylpyrido[2,3-*d*]pyrimidine-6-carboxaldehyde (**12**, 0.25 g, 1.23 mmol) in 25 mL of absolute methanol was added 4 Å molecular sieves (previously activated by heating at 100 °C), followed by 4-methoxyindoline (0.27 g, 1.85 mmol). The reaction mixture was stirred under nitrogen for 6 h, after which $NaCNBH_3$ (0.19 g, 3.05 mmol) was added. HCl (6 N) in methanol was then added dropwise periodically, to maintain the pH at 6. After 44 h, 2 mL of water was added to the reaction mixture which was then filtered, 1.0 g of silica gel was added to the filtrate, and the solvent was evaporated under vacuum. The resulting dry plug was chromatographed on a silica gel column (1.05 in. × 23 in.) using $CHCl_3$:CH_3OH (10:1) as the eluant. Fractions containing the desired product (R_f = 0.61) were pooled and evaporated to afford compound **2** as a brown solid (0.27 g, 66%): mp 277 °C; IR (Nujol) 3320, 3140 (NH_2) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.79 (s, 3 H, 5-CH_3), 3.10 (t, 2 H, $CH_2$$CH_2$), 3.61 (t, 2 H, $CH_2$$CH_2$), 3.71 (s, 3 H, OCH_3), 4.34 (s, 2 H, CH_2), 6.87 (d, 1 H, 7′-H), 7.02−7.07 (m, 2 H, 5′-H and 6′-H), 8.6 (s, 1 H, H-7).

Anal. ($C_{18}H_{20}N_6O$·1.0H_2O) C, H, N.

2,4-Diamino-5-methyl-6-[(5′-methoxyindolinyl)methyl]-pyrido[2,3-*d*]pyrimidine (3). To a solution of the acetate salt of **12** (0.30 g, 1.48 mmol) in 30 mL of absolute methanol (charged with freshly activated 4 Å molecular sieves) was added 5-methoxyindoline (0.33 g, 2.20 mmol) and the reaction mixture was stirred under an atmosphere of nitrogen. After 5 h, $NaCNBH_3$ (0.23 g, 3.7 mmol) was added, followed by the periodic addition of 6 N HCl in absolute methanol to maintain the pH at 6. After 48 h, 2 mL of water was added, the reaction mixture filtered, and the filtrate purified as described for **2** to afford pure **3** as a brown solid (0.31 g, 62%): mp 266 °C; IR (Nujol) 3330, 3140 (NH_2) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.72 (s, 3 H, 5-CH_3), 2.98 (t, 2 H, $CH_2$$CH_2$), 3.47 (t, 2 H, $CH_2$$CH_2$), 3.78 (s, 3 H, OCH_3), 4.49 (s, 2 H, CH_2), 6.47 (d, 1 H, 7′-H), 6.63 (d, 1 H, 6′-H), 6.78 (br s, 2 H, 2-NH_2), 7.10 (d, 1 H, 4′-H), 7.63 (s, 2 H, 4-NH_2), 8.41 (s, 1 H, H-7).

Anal. ($C_{18}H_{20}N_6O$·0.6H_2O) C, H, N.

2,4-Diamino-5-methyl-6-[(5′,6′-dimethoxyindolinyl)-methyl]pyrido[2,3-*d*]pyrimidine (4). To a solution of the acetate salt of **12** (0.23 g, 1.13 mmol) in 25 mL of absolute methanol was added 5,6-dimethoxyindoline (0.29 g, 1.65 mmol), followed by 4 Å molecular sieves. The reaction mixture was stirred under an atmosphere of nitrogen for 6 h, following which $NaCNBH_3$ (0.26 g, 4.13 mmol) was added followed by 6 N HCl in methanol to maintain the pH at 6. The reaction mixture was then stirred for 22 h and filtered, 1.0 g of silica gel was added to the filtrate, and the filtrate was evaporated to dryness to afford a dry plug. This plug was loaded on the surface of a silica gel column (1.05 in. × 23 in.) and eluted using $CHCl_3$:CH_3OH (10:1). Fractions containing the desired product were pooled and evaporated to afford a yellow solid

(0.28 g, 67%): mp 267 °C; IR (Nujol) 3330, 3140 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.52 (s, 3 H, CH_3), 3.01 (t, 2 H, CH_2), 3.62 (t, 2 H, CH_2), 3.76 (s, 6 H, OCH_3), 4.35 (s, 2 H, CH_2), 6.45 (s, 1 H, 7′-H), 6.72 (s, 2 H, 2-NH_2), 6.91 (s, 1 H, 4′-H), 7.62 (s, 2 H, 4-NH_2) 8.48 (s, 1 H, H-7).

Anal. ($C_{19}H_{22}N_6O_2 \cdot 1.0H_2O$) C, H, N.

2,4-Diamino-5-methyl-6-[(5′-methoxyindolyl)methyl]-pyrido[2,3-*d*]pyrimidine (5). To a solution of 2,4-diamino-5-methyl-6-(bromomethyl)pyrido[2,3-*d*]pyrimidine (**14,** (0.20 g, 0.75 mmol), in 20 mL of anhydrous *N*,*N*-dimethylacetamide (DMAC) was added triethylamine dropwise until a pH of 8. The mixture was rapidly filtered and the filtrate stirred under nitrogen for 10 min. In another flask, 5-methoxyindole (0.11 g, 0.75 mmol) was dissolved in 15 mL of DMAC and NaH (0.03 g, 1.13 mmol), as a 60% dispersion in mineral oil was added. The reaction mixture was stirred for 15 min and then was added dropwise *via* a double-tipped canula to the solution of the 6-bromomethyl compound under an atmosphere of nitrogen. The reaction mixture was stirred for 24 h while being protected from light, at the end of which 5 mL of water was added to the reaction mixture and the solvent evaporated under reduced pressure. The residue was then dissolved in 100 mL of methanol, 1.0 g of silica gel was added, and the methanol was evaporated to afford a dry plug. This plug was eluted with $CHCl_3$:CH_3OH (15:1), and fractions containing pure product were pooled together and evaporated to afford pure **5** as a brown solid (0.16 g, 63%): mp 300 °C dec; TLC ($CHCl_3$:CH_3OH, 5:1 R_f = 0.64) IR (Nujol) 3290, 3130 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.71 (s, 3 H, 5-CH_3), 3.81 (s, 3 H, OCH_3), 4.49 (s, 2 H, CH_2), 6.51 (s, 2 H, 2-NH_2), 6.87 (d, 1 H, 7′-H), 7.02 (m, 2 H, 4′-H and 6′-H), 7.09 (d, 1 H, 3′-H), 7.16 (d, 1 H, 2′-H), 7.62 (s, 2 H, 4-NH_2), 8.6 (s, 1 H, H-7).

Anal. ($C_{18}H_{18}N_6O \cdot 1.0H_2O$) C, H, N.

2,4-Diamino-5-methyl-6-[(6′,7′-dimethoxytetrahydroisoquinolinyl)methyl]pyrido[2,3-*d*]pyrimidine (6). To a solution of 2,4-diamino-5-methyl-6-(bromomethyl)pyrido[2,3-*d*]-pyrimidine (**14**) (0.23 g, 0.86 mmol) in 25 mL of anhydrous DMAC was added K_2CO_3 (0.24 g, 1.72 mmol) followed by 6,7-dimethoxytetrahydroisoquinoline (0.17 g, 0.86 mmol), and the reaction mixture was stirred under an atmosphere of nitrogen for 24 h. TLC analyses ($CHCl_3$:CH_3OH, 5:1) indicated the presence of the desired analogue (R_f = 0.6) along with some baseline contamination. The reaction mixture was filtered, 1.0 g of silica gel was added, and the filtrate was evaporated to dryness. This plug was eluted on a silica gel column (1.05 in. × 23 in.) with $CHCl_3$:CH_3OH (100:1 to 100:20), and fractions containing pure product were pooled and evaporated to afford **6** (0.22 g, 68%): mp 276 °C; ^1H NMR (DMSO-d_6) δ 2.61 (s, 3 H, CH_3), 2.79 (t, 2 H, 3′-CH_2), 3.18 (t, 2 H, 4′-CH_2), 4.01 (s, 2 H, CH_2), 4.40 (s, 2 H, CH_2), 6.48 (br s, 2 H, 2-NH_2), 6.66 (overlapping s, 2 H, 5′-H and 8′-H), 7.46 (s, 2 H, 4-NH_2), 8.48 (s, 1 H, H-7).

Anal. ($C_{20}H_{24}N_6O_2 \cdot 0.6H_2O$) C, H, N.

General Procedure for the Synthesis of the Pyrrolonitrobenzenes from the Corresponding Nitroanilines. To a solution of the commercially available nitroaniline (1.0 equiv) in 30 mL of glacial acetic acid, at reflux, was added 2,5-dimethoxytetrahydrofuran (1.0 equiv), and the mixture was heated for 10 min. (Note: For the tetramethylnitroaniline, the reaction mixture had to be refluxed for 1 h). TLC analyses (hexanes:EtOAc, 1:1) indicated the presence of a new spot, corresponding to the product. The acetic acid was evaporated and the resulting residue sonicated in diethyl ether for 30 min to afford the desired pyrrole as a solid.

2,5-Dimethoxy-4-pyrrolo-1-nitrobenzene (18). This was obtained as a brown solid from 2,5-dimethoxy-4-amino-1-nitrobenzene (0.44 g, 2.21 mmol) and 2,5-dimethoxytetrahydrofuran (0.29 g, 2.21 mmol) in 20 mL of acetic acid (0.50 g, 91%): TLC (hexane:EtOAc, 1:1) R_f = 0.54; ^1H NMR ($CDCl_3$) δ 3.86 (s, 3 H, 5-OCH_3), 3.96 (s, 3 H, 2-OCH_3), 6.37 (t, 2 H, 3′,4′-H), 7.02 (s, 1 H, 3-H), 7.09 (d, 2 H, 2′,5′-H), 7.65 (s, 1 H, 6-H).

Anal. ($C_{12}H_{12}N_2O_4 \cdot 0.6CH_3COOH$) C, H, N.

4,5-Dimethoxy-2-pyrrolo-1-nitrobenzene (19). This was obtained as a brown solid from 4,5-dimethoxy-2-amino-1-nitrobenzene (0.53 g, 2.66 mmol) and 2,5-dimethoxytetrahydrofuran (0.35 g, 2.66 mmol) in 25 mL of acetic acid (0.56 g, 85%): TLC (hexane:EtOAc, 1:1) R_f = 0.59; ^1H NMR ($CDCl_3$) δ 3.94 (s, 3 H, 5-OCH_3), 3.96 (s, 3 H, 4-OCH_3), 6.34 (t, 2 H, 3′,4′-H), 6.75 (d, 2 H, 2′,5′-H), 6.84 (s, 1H, 3-H), 7.50 (s, 1 H, 6-H).

Anal. ($C_{12}H_{12}N_2O_4 \cdot 0.3CH_3COOH$) C, H, N.

2,3,5,6-Tetramethyl-4-pyrrolo-1-nitrobenzene (20). This was obtained as a black solid from 2,3,5,6-tetramethyl-4-amino-1-nitrobenzene (0.50 g, 2.58 mmol) and 2,5-dimethoxytetrahydrofuran (0.34 g, 2.58 mmol) in 25 mL of acetic acid (0.42 g, 66%): TLC (hexane:EtOAc, 1:1) R_f = 0.76; ^1H NMR ($CDCl_3$) δ 1.88 (s, 6 H, 2- and 6-CH_3), 2.19 (s, 6 H, 3- and 5-CH_3), 6.33 (t, 2 H, 3′,4′-H), 6.54 (d, 2 H, 2′,5′-H).

Anal. ($C_{14}H_{16}N_2O_2 \cdot 0.7CH_3COOH$) C, H, N.

2,5-Dimethoxy-4-pyrroloaniline (21). This was obtained as a brown solid by hydrogenation, at 35 psi, of 2,5-dimethoxy-4-pyrrolo-1-nitrobenzene (**18**) (0.20 g, 0.81 mmol) and Raney Ni (1.0 g) in 10 mL of absolute ethanol for 2 h. The solvent was evaporated to afford pure **21** (0.12 g, 85%): TLC (hexane:EtOAc, 1:1) R_f = 0.63; ^1H NMR (DMSO-d_6) δ 3.69 (s, 3 H, 2-OCH_3), 3.82 (s, 2 H, 5-OCH_3), 6.16 (m, 3 H, NH_2 and 6-H), 6.99 (m, 5 H, 3-H and 2′,3′,5′,6′-H).

Anal. ($C_{12}H_{14}N_2O_2 \cdot 0.2CH_3COOH$) C, H, N.

4,5-Dimethoxy-2-pyrroloaniline (22). This was obtained as a brown solid from 4,5-dimethoxy-2-pyrrolo-1-nitrobenzene **19** (0.31 g, 1.25 mmol) and Raney Ni (1.5 g) (0.18 g, 81%): TLC (hexane:EtOAc, 1:1) R_f = 0.69; ^1H NMR ($CDCl_3$) δ 3.85 (s, 3 H, 5-OCH_3), 3.94 (s, 3 H, 4-OCH_3), 6.21 (m, 3 H, NH_2 and 6-H), 6.52 (s, 1 H, 3-H), 6.98 (m, 4 H, 2′,3′,4′,5′-H).

Anal. ($C_{12}H_{14}N_2O_2 \cdot 0.8CH_3COOH$) C, H, N.

2,3,5,6-Tetramethyl-4-pyrroloaniline (23). This was obtained as a black solid from 2,3,5,6-tetramethyl-4-pyrrolo-1-nitrobenzene (**20**, 0.50 g, 2.05 mmol) and Raney Ni (2.0 g) (0.29 g, 66%): TLC (hexane:EtOAc, 1:1) R_f = 0.83; ^1H NMR ($CDCl_3$) δ 1.81 (s, 6 H, CH_3), 2.21 (s, 6 H, CH_3), 6.14−6.21 (m, 4 H, 2′,3′,4′,5′-H), 6.35 (s, 2 H, NH_2). Anal. ($C_{14}H_{18}N_2 \cdot 0.3CH_3COOH$) C, H, N.

2,4-Diamino-5-methyl-6-[(2′,5′-dimethoxy-4′-pyrroloanilino)methyl]pyrido[2,3-*d*]pyrimidine (7). To a solution of **14** (0.20 g, 0.75 mmol) in 20 mL of DMAC was added 2,5-dimethoxy-4-pyrroloaniline (0.21 g, 1.13 mmol) followed by potassium carbonate (0.16 g, 1.13 mmol). The reaction mixture was stirred at room temperature under an atmosphere of nitrogen for 48 h. TLC analyses ($CHCl_3$:CH_3OH, 5:1) at the end of this period indicated the presence of a new spot corresponding to the product (R_f = 0.6), along with some unreacted starting material. The reaction mixture was filtered, 1.0 g of silica gel added to the filtrate, and the mixture evaporated to dryness under reduced pressure to afford a dry plug. This plug was loaded on the surface of a 1.05 in. × 23 in. silica gel column and eluted with $CHCl_3$:CH_3OH using gradient elution (99:1 to 80:20). Fractions containing the desired product were pooled and evaporated to afford pure **7** as a yellow solid (0.20 g, 69%): mp 277 °C; ^1H NMR (DMSO-d_6) δ 2.61 (s, 3 H, 5-CH_3), 3.67 (3, 3 H, 2′-OCH_3), 3.77 (s, 3 H, 5′-OCH_3), 4.42 (d, 2 H, CH_2), 6.20 (m, 2 H, NH and 6′-H), 6.64 (s, 2 H, 2-NH_2), 6.98 (m, 5 H, 3′-H and 2″-, 3″-, 5″-, and 6″-H), 7.47 (s, 2 H, 4-NH_2), 8.49 (s, 1 H, H-7).

Anal. ($C_{20}H_{23}N_7O_2 \cdot 0.9H_2O$) C, H, N.

2,4-Diamino-5-methyl-6-[(4′,5′-dimethoxy-2′-pyrroloanilino)methyl]pyrido[2,3-*d*]pyrimidine (8). To a solution of the 6-bromomethyl compound **14** (0.20 g, 0.75 mmol) in 30 mL of DMAC was added 4,5-dimethoxy-2-pyrroloaniline (0.21 g, 1.13 mmol), followed by potassium carbonate (0.16 g, 1.13 mmol), and the mixture was stirred for 48 h under an atmosphere of nitrogen. At the end of this period, the mixture was filtered and the solvent evaporated under reduced pressure. The residue was dissolved in methanol and 1.0 g of silica gel added to the solution and the methanol evaporated to afford a dry plug. This plug was eluted on a column using the same procedure as described for **7**. Fractions containing the desired product were pooled and evaporated to afford pure **8** as a brown solid (0.19 g, 66%): TLC ($CHCl_3$:CH_3OH, 6:1) R_f = 0.65; mp 270 °C; IR (Nujol) 3330, 3140 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.63 (s, 3 H, 5-CH_3), 3.69 (s, 3 H, 4′-OCH_3), 3.77 (s, 3 H, 5′-OCH_3), 4.47 (d, 2 H, CH_2), 6.21 (m, 2 H, NH and 6′-H), 6.71 (s, 2 H, 2-NH_2), 6.99 (s, 1 H, 3′-H), 7.01 (m, 4 H, 2″,3″,4″,5″-H), 7.81 (s, 2 H, 4-NH_2), 8.51 (s, 1 H, H-7).

Anal. ($C_{20}H_{23}N_7O_2 \cdot 0.5H_2O$) C, H, N.

2,4-Diamino-5-methyl-6-[(2′,3′,5′,6′-tetramethyl-4′-pyrroloanilino)methyl]pyrido[2,3-*d*]pyrimidine (9). To a solution of the 6-bromomethyl compound **14** (0.24 g, 0.89 mmol) in 25 mL of anhydrous DMAC was added 2,3,5,6-tetramethyl-4-pyrroloaniline (0.29 g, 1.34 mmol) followed by $CsCO_3$ (0.50 g), and the mixture was stirred under an atmosphere of nitrogen for 72 h. The reaction mixture was then filtered, and purification was carried out in the same manner as described previously for **7** to afford pure **9** as a brown solid (0.25 g, 69%): TLC ($CHCl_3$:CH_3OH, 5:1) $R_f = 0.78$; mp 299 °C dec; IR (Nujol) 3290, 3080 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.81 (s, 6 H, 2′,6′-CH_3), 2.20 (s, 6 H, 3′,5′-CH_3), 2.68 (s, 3 H, CH_3), 4.31 (d, 2 H, CH_2), 5.31 (t, 1 H, NH), 6.14−6.22 (m, 6-H, 2″,3″,4″,5″-H and 2-NH_2), 7.01 (s, 2 H, 4-NH_2), 8.48 (s, 1 H, H-7).

Anal. ($C_{23}H_{27}N_7 \cdot 1.0\ H_2O$) C, H, N.

2,4-Diamino-5-methyl-6-[(2′-methoxy-5′-phenylanilino)-methyl]pyrido[2,3-*d*]pyrimidine (10). To a solution of **14** (0.29 g, 1.09 mmol) in 25 mL of anhydrous DMAC was added 2-methoxy-5-phenylaniline (0.33 g, 1.63 mmol) followed by K_2CO_3 (0.22 g, 1.63 mmol). The reaction mixture was stirred under nitrogen for 48 h, filtered, and purified as described previously for **7** to afford pure **10** as a yellow solid (0.29 g, 71%): TLC ($CHCl_3$:CH_3OH, 5:1) $R_f = 0.84$; mp 265 °C dec; IR (Nujol) 3290, 3080 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.67 (s, 3 H, CH_3), 3.71 (s, 3 H, 2′-OCH_3), 4.41 (d, 2 H, CH_2), 5.22 (t, 1 H, NH), 6.12 (m, 2 H, 3′-H and 6′-H), 6.22 (s, 2 H, 2-NH_2), 6.29 (d, 1 H, 4′-H), 7.12 (s, 2 H, 4-NH_2), 7.36 (m, 5 H), 8.47 (s, 1 H, H-7).

Anal. ($C_{22}H_{22}N_6O \cdot 0.5H_2O$) C, H, N.

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