6-Substituted 2,4-Diaminopyrido[3,2-*d*]pyrimidine Analogues of Piritrexim as Inhibitors of Dihydrofolate Reductase from Rat Liver, *Pneumocystis carinii*, and *Toxoplasma gondii* and as Antitumor Agents[1]

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The synthesis and biological activity are reported for 21 6-substituted 2,4-diaminopyrido[3,2-*d*]pyrimidine analogues (**4**−**24**) of piritrexim (PTX) as inhibitors of dihydrofolate reductase (DHFR) and as antitumor agents. Recombinant DHFR from *Pneumocystis carinii* (pc) and native DHFR from *Toxoplasma gondii* (tg) were the target enzymes tested; these organisms are responsible for fatal opportunistic infections in AIDS patients. Rat liver (rl) DHFR served as the mammalian reference enzyme to determine selectivity for the pathogenic DHFR. The synthesis of S9-bridged compounds **4**−**6** was achieved by aryl displacement of 2,4-diamino-6-chloropyrido[3,2-*d*]pyrimidine (**27**) with thiol nucleophiles. Oxidation of **4**−**6** with hydrogen peroxide in glacial acetic acid afforded the corresponding sulfone analogues **7**−**9**. The N9-bridged compounds **10**−**24** were synthesized from their precursor 3-amino-6-(arylamino)-2-pyridinecarbonitriles via a thermal cyclization with chloroformamidine hydrochloride. Unlike the S9-bridged compounds, the arylamino side chains of the N9-bridged analogues were introduced prior to the formation of the 2,4-diaminopyrido[3,2-*d*]pyrimidine nucleus. A reversed two-atom-bridged analogue (**25**) was also synthesized using a synthetic strategy similar to that utilized for compounds **10**−**24**. The IC$_{50}$ values of these compounds against pcDHFR ranged from 0.0023×10^{-6} M for 2,4-diamino-6-(*N*-methyl-3′,4′-dimethoxyanilino)pyrido[3,2-*d*]pyrimidine (**21**), which was the most potent, to 90.4×10^{-6} M for 2,4-diamino-6-(4′-methoxyanilino)-pyrido[3,2-*d*]pyrimidine (**12**), which was the least potent. The three S9-bridged compounds tested were more potent than the corresponding sulfone-bridged compounds for all three DHFRs. N9-Methylation increased the potency by as much as 17 000-fold (compounds **15** and **21**). None of the analogues were selective for pcDHFR. Against tgDHFR the most potent analogue was again **21** with an IC$_{50}$ value of 0.00088×10^{-6} M and the least potent was **12** with an IC$_{50}$ of 2.8×10^{-6} M. N9-Methylation afforded an increase in potency of up to 770-fold (compound **15** NH vs **21** N-CH$_3$) compared to the corresponding N9-H analogue. In contrast to pcDHFR, several analogues had a greater selectivity ratio for tgDHFR compared to trimetrexate (TMQ) or PTX, most notably 2,4-diamino-6-[(3′,4′- dimethoxyphenyl)thio]pyrido[3,2-*d*]pyrimidine (**4**), 2,4-diamino-6-[(2′-methoxyphenyl)sulfonyl]pyrido[3,2-*d*]pyrimidine (**7**), and 2,4-diamino-6-(2′,5′-dimethoxyanilino)pyrido[3,2-*d*]pyrimidine (**14**) which combined relatively high potency at 10^{-7}−10^{-8} M along with selectivity ratios of 3.97, 6.67, and 4.93, respectively. Several analogues synthesized had better selectivity ratios than TMQ or PTX for both pcDHFR and tgDHFR, and the potencies of the N9-methylated compounds were comparable to or greater than that of TMQ or PTX. Selected compounds were evaluated as inhibitors of the growth of a variety of tumor cells in culture. The N9-CH$_3$ analogues were, in general, highly potent with GI$_{50}$ values in the nanomolar range. The N9-H and S9 analogues were less potent with GI$_{50}$ values in the millimolar to micromolar range.

Introduction

Piritrexim (PTX) (Chart 1) is a lipophilic, nonclassical antifolate. It is a potent inhibitor of dihydrofolate reductase (DHFR) and is currently in phase II clinical trials as an anticancer agent against a variety of tumors.[2−5] PTX differs from other antitumor dihydrofolate reductase inhibitors such as methotrexate (MTX) and trimetrexate (TMQ) in that it contains a single-atom side chain bridge rather than the usual two-atom bridge. In sharp contrast to the large number of two-atom-bridged antifolates, both classical and nonclassical, which have been developed as antitumor agents,[6,7] there is a remarkable dearth of PTX analogues in the literature. It was therefore of interest to explore one-atom bridge analogues related to PTX as potential antitumor agents. We initially elected to do so with sulfur and nitrogen as the single-atom bridges.

Lipophilic antifolates[7,8] have also been explored as agents against the often fatal opportunistic infections caused by *Pneumocystis carinii* and *Toxoplasma gondii* in patients with AIDS and others with compromised immune systems such as organ transplant recipients and cancer patients undergoing chemotherapy and/or

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Chart 1
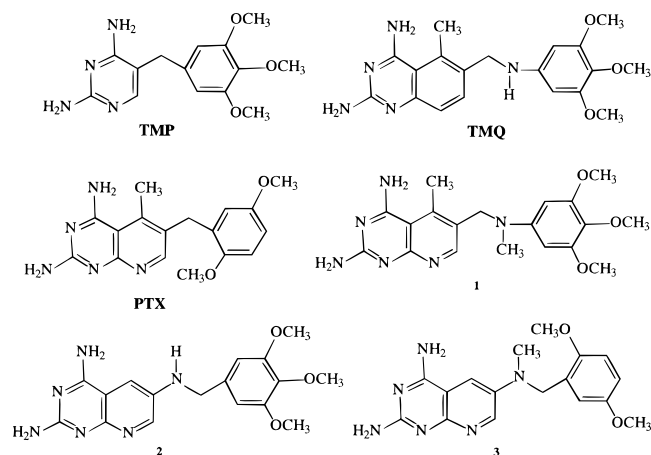
radiation treatment. Trimethoprim (TMP), a monocyclic 2,4-diaminopyrimidine antifolate, in combination with sulfamethoxazole is the first-line agent against *P. carinii* and is also used as a prophylactic agent.[9,10] In addition, pyrimethamine along with sulfa drug combinations is the preferred agent against cerebral toxoplasmosis.[11] TMQ is a potent, nonselective DHFR inhibitor and is approved for clinical use against *P. carinii* infections.[12] Since *P. carinii* and *T. gondii* lack folate transport systems,[13,14] the classical antifolates, which require transport into cells, are not viable agents against these infections. Thus, lipophilic nonclassical antifolates which are DHFR inhibitors such as TMP and TMQ[8,12] and can penetrate these organisms are approved agents against these opportunistic infections. TMQ must be administered with leucovorin, which is actively transported into host cells and converted to the folate cofactor 5,10-methylenetetrahydrofolate, thus selectively protecting host cells from fatal DHFR inhibition.[15] PTX, though not a clinically approved entity, has undergone clinical trials against *P. carinii* and *T. gondii*.[16,17] It was therefore also of interest to explore the 2,4-diamino 6-substituted pyrido[3,2-*d*]pyrimidines with sulfur and nitrogen as the single-atom bridge as inhibitors of *P. carinii* (pc) DHFR and *T. gondii* (tg) DHFR.

Gangjee et al.[18–22] and others[23–25] have recently reported several bicyclic lipophilic antifolates as potent and/or selective inhibitors of DHFR isolated from *P. carinii* and *T. gondii*. Some of these compounds have superior properties in both selectivity and potency compared to TMQ and PTX against pcDHFR and tgDHFR.[18–21,23] In particular, 2,4-diamino-5-methyl-6-[(3′,4′,5′-trimethoxy-*N*-methylanilino)methyl]pyrido[2,3-*d*]pyrimidine (**1**)[20] combines the structural features of both TMP and PTX. Compound **1** has both increased potency against pcDHFR and tgDHFR and increased selectivity ratios for both enzymes versus reference mammalian rat liver (rl) DHFR when compared with TMQ or PTX (10- and 100-fold for pcDHFR and tgDHFR, respectively, compared with PTX). Further structure–activity relationship (SAR) studies by Gangjee et al.[21] provided 2,4-diamino 6-substituted pyrido[2,3-*d*]pyrimidines **2** and **3** which are 5-desmethyl analogues of TMQ that exhibit excellent selectivity toward tgDHFR and pcDHFR using human (h) DHFR as the reference enzyme. The selectivity ratios of compounds **2** and
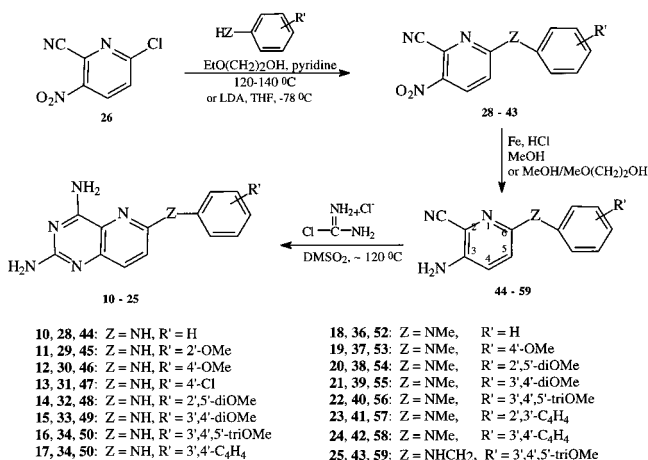
3 for tgDHFR were 200 and 300, respectively, and the potency of **3** against tgDHFR was significant at IC$_{50}$ = 6.3 nM. The importance of the 5-methyl group of 5-deaza folates for potent inhibition of DHFR from mammalian sources and in some instances bacterial sources is well-documented in the literature.[26–28] Thus, removal of the 5-methyl group from these agents increased their selectivity for *P. carinii* and *T. gondii*, and this increased selectivity was attributed to the decrease in potency against pcDHFR and tgDHFR which was less than the decrease against hDHFR.[21,29] A comparison of the structural features of PTX, which contains a 5-methyl group, with those of 5-desmethyl analogues **2** and **3** thus led to the hypothesis that 5-desmethyl analogues of PTX could increase the selectivity for pcDHFR and tgDHFR over mammalian DHFR while maintaining the potency of PTX. Therefore, it was of considerable interest to synthesize pyrido[3,2-*d*]pyrimidine analogues **4–17** of PTX as potentially potent and selective inhibitors of pcDHFR and tgDHFR.

One of the differences in the structure of pcDHFR as compared to hDHFR is that Val-115 in hDHFR is replaced with Ile-123 in pcDHFR.[30] On the basis of X-ray crystal structures of DHFR and molecular modeling studies using Sybyl 6.3,[31] Gangjee et al.[29] have attributed the selectivity of compound **3** (compared to TMQ or PTX), in part, to the preferential hydrophobic interaction of the N9-methyl moiety with Ile-123 in pcDHFR. The isobutyl side chain of Ile-123 in the active site of pcDHFR and the isopropyl side chain of Val-115 at the active site of hDHFR interact with the 5-methyl moiety of TMQ or PTX in a hydrophobic interaction. However, when the methyl group was transposed from the 5-position to the 9-position in compound **3**, molecular modeling suggests that only the longer isobutyl group of Ile-123 (pcDHFR) is able to make a hydrophobic contact with this N9-methyl group. The shorter isopropyl group of Val-115 of hDHFR does not make this interaction. This difference could, in part, account for the difference in potency of **3** against pcDHFR as compared to hDHFR and consequently the selectivity of **3** for pcDHFR. Thus, it was of interest to determine if N9-methylation of the nitrogen-bridged analogues of PTX in the pyrido[3,2-*d*]pyrimidines would also impart similar selectivity for pcDHFR. Thus the N9-methylated compounds **18–24** were also synthesized.

Gangjee et al.[18] have reported that the pyrido[3,2-*d*]-pyrimidine analogues of pteridine with a two-atom bridge possess potent inhibitory activity against the growth of a variety of tumor cell lines in culture, and some of these analogues are currently undergoing antitumor evaluation in animals under the auspices of the National Cancer Institute. As an extension of this previous work, a reversed two-atom-bridged 8-deaza analogue (**25**) was also synthesized and tested.

Chemistry

The target compounds **4–9** with sulfur or sulfone linkage at the 6-position were synthesized (Scheme 1) from 2,4-diamino-6-chloropyrido[3,2-*d*]pyrimidine (**27**), a known compound, which in turn was obtained from 2,6-dichloropyridine via a four-step synthetic procedure.[32,33] Thus, 2,6-dichloropyridine was nitrated with 90% nitric acid and sulfuric acid, followed by substitu-

Scheme 1. Synthetic Route for 6-Substituted 2,4-Diaminopyrido[3,2-*d*]pyrimidines **4**−**9**

7: R' = 2'-OMe
8: R' = 4'-OMe
9: R' = 3',4'-diOMe

4 R' = 2'-OMe
5 R' = 4'-OMe
6 R' = 3',4'-diOMe
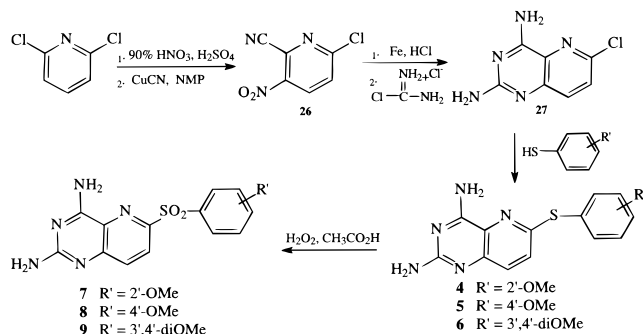
Scheme 2. Synthetic Route of 6-Substituted 2,4-Diaminopyrido[3,2-*d*]pyrimidines **10**−**25**

10, 28, 44: Z = NH, R' = H
11, 29, 45: Z = NH, R' = 2'-OMe
12, 30, 46: Z = NH, R' = 4'-OMe
13, 31, 47: Z = NH, R' = 4'-Cl
14, 32, 48: Z = NH, R' = 2',5'-diOMe
15, 33, 49: Z = NH, R' = 3',4'-diOMe
16, 34, 50: Z = NH, R' = 3',4',5'-triOMe
17, 34, 50: Z = NH, R' = 3',4'-C₄H₄

18, 36, 52: Z = NMe, R' = H
19, 37, 53: Z = NMe, R' = 4'-OMe
20, 38, 54: Z = NMe, R' = 2',5'-diOMe
21, 39, 55: Z = NMe, R' = 3',4'-diOMe
22, 40, 56: Z = NMe, R' = 3',4',5'-triOMe
23, 41, 57: Z = NMe, R' = 2',3'-C₄H₄
24, 42, 58: Z = NMe, R' = 3',4'-C₄H₄
25, 43, 59: Z = NHCH₂, R' = 3',4',5'-triOMe

tion of the 2-chloro moiety with cuprous cyanide at 180 °C to afford 2-cyano-3-nitro-6-chloropyridine (**26**) (Scheme 1). Following the reduction of **26** with iron powder in acidic methanol, the 2-cyano-3-amino function was set up to condense with chloroformamidine hydrochloride[32,33] in dimethyl sulfone to afford the crucial intermediate **27** (85% over two steps). Nucleophilic aromatic displacement of the 6-chloro of **27** with thiophenols afforded the target molecules **4**−**6** in yields of 65−79%. Oxidation of **4**−**6** with hydrogen peroxide in glacial acetic acid[32] provided the sulfone analogues **7**−**9**, respectively, in 50−72% yields.

A similar strategy for the synthesis of target compounds **10**−**25** using the aromatic nucleophilic substitution reaction of **27** with appropriate anilines did not proceed as expected. The failure of the reaction was attributed to the relatively low nucleophilicity of the amino group compared to the thiol. Thus, the 6-arylamino and benzylamino side chains for the N9- and N9−C10-bridged analogues had to be introduced prior to the formation of the bicyclic pyrido[3,2-*d*]pyrimidine nucleus (Scheme 2). Thus **26** was substituted with the appropriate anilines, β-naphthylamine, and benzylamine in monoethyl glycol in the presence of pyridine at 120−140 °C to afford intermediates **28**−**35** and **43**. The synthesis of the intermediates **36**−**42** required *N*-methylarylamines. Those which were not commercially available were synthesized via the N-methylation of the corresponding arylamines with iodomethane and triethylamine as base. The *N*-methylarylamines were then separated chromatographically on silica gel from

the *N*,*N*-dimethylarylamines. Using similar reaction conditions mentioned above for **4**−**6**, the 3-nitro-6-(*N*-methylarylamino)pyridine nitrile intermediates **36**−**42** were obtained, except for **38** and **41**. For the synthesis of **38** and **41**, the starting materials appeared to undergo polymerization under the reaction conditions. To circumvent this problem, *N*-methyl-2,5-dimethoxyaniline and *N*-methyl-l-naphthylamine were first treated with LDA at −78 °C to afford a more reactive nitrogen anion, followed by displacement of the 6-chloro group of **26** to afford **38** and **41** in 70% and 51% yields, respectively. The nitro compounds **28**−**43** were then reduced with iron powder in an acidic solution of methanol or an acidic solution of methanol and 2-methoxyethanol to afford the corresponding 3-amino derivatives **44**−**59**. The advantage of the solvent mixture was not only to increase the solubility of the starting materials but also to facilitate the reaction by decreasing side product formation to trace amounts which significantly increased the yields of the reactions. The 2-cyano-3-amino functional groups in **44**−**59** were now poised for the cyclization with chloroformamidine hydrochloride to afford the desired target compounds **10**−**25**. The yields depended on the substitutions on the aryl side chains and varied from 17% for **25** to 100% for **24**.

Biological Results and Discussions

The compounds were evaluated as inhibitors of pcDHFR, tgDHFR, and rlDHFR. Selectivity ratios were determined versus rlDHFR as described previously.[34,35] The potency and selectivity data are listed in Table 1.

In compounds **4**−**6**, the 9-position in the bridge is a sulfur atom and the substitution (R′) on the phenyl ring is varied in position and/or the degree of substitution. For the 2′-OMe analogue **4** the potency against tgDHFR was comparable to that of PTX, while the potency against pcDHFR was decreased. However, the potency against rlDHFR was also decreased; thus the selectivity of **4** against tgDHFR increased 45-fold compared to that of PTX. Transposition of the methoxy substitution from the 2′- to the 4′-position (**5**) afforded improved activity against both pcDHFR and rlDHFR compared to **4**, while the activity against tgDHFR remained unchanged and the selectivity toward tgDHFR was decreased. This result indicates that the position of substitution can influence both activity and selectivity. The 3′,4′-diOMe analogue **6** showed increased activity against all three DHFRs, especially for pcDHFR (8-fold increase), but the selectivity for tgDHFR decreased as compared to the monomethoxy analogues **5** and **6**. Thus, an additional methoxy substituent was conducive to potency but detrimental to selectivity.

Oxidation of the S9 bridge of compounds **4**−**6** to the corresponding sulfones (**7**−**9**) generally decreased the potency against all three enzymes (compare **4** and **7**, **5** and **8**, **6** and **9**). However, the selectivity for tgDHFR of **8** and **9** was similar to that of **5** and **6**, respectively. Compound **7**, with a 2′-OMe substitution, provided the best selectivity against tgDHFR for the sulfur-containing analogues. Compared to PTX, the selectivity ratio of **7** for tgDHFR increased 75-fold. In general, the side chain sulfur-bridged analogues were more selective than PTX for both pcDHFR and tgDHFR, and oxidation of the sulfur to the sulfone had modest effects on selectivity.

Table 1. Inhibitory Concentrations (IC$_{50}$, μM) and Selectivity Ratios Against pcDHFR and tgDHFR versus rlDHFR[31,32]
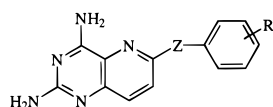
compd	Z	R'	pcDHFR	rlDHFR	rl/pc	tgDHFR	rl/tg
4	S	2'-OMe	2.2	0.23	0.10	0.058	3.97
5	S	4'-OMe	0.7	0.075	0.11	0.045	1.67
6	S	3',4'-diOMe	0.086	0.018	0.21	0.019	0.95
7	SO$_2$	2'-OMe	3.2	1.4	0.44	0.21	6.67
8	SO$_2$	4'-OMe	10.5	2.0	0.19	1.0	2.00
9	SO$_2$	3',4'-diOMe	2.7	0.88	0.33	0.94	0.94
11	NH	2'-OMe	8.7	0.26	0.03	0.46	0.57
12	NH	4'-OMe	90.4	3.8	0.04	2.8	1.36
15	NH	3',4'-diOMe	40.4	1.1	0.03	0.68	1.62
19	NCH$_3$	4'-OMe	0.22	0.0068	0.03	0.0086	0.79
21	NCH$_3$	3',4'-diOMe	0.0023	0.0004	0.17	0.00088	0.45
14	NH	2',5'-diOMe	16.1	3.6	0.22	0.73	4.93
20	NCH$_3$	2',5'-diOMe	0.034	0.0042	0.12	0.041	0.10
16	NH	3',4',5'-triOMe	25.9	3.2	0.12	2.4	1.33
22	NCH$_3$	3',4',5'-triOMe	0.021	0.0037	0.18	0.0076	0.49
17	NH	3',4'-C$_4$H$_4$	15	2.0	0.13	1.1	1.82
24	NCH$_3$	3',4'-C$_4$H$_4$	0.0317	0.0099	0.31	0.0145	0.68
10	NH	H	8.3	0.43	0.05	0.3	1.43
18	NCH$_3$	H	0.0995	0.0013	0.01	0.0022	0.59
13	NH	4'-Cl	14.6	0.82	0.06	0.83	0.99
23	NCH$_3$	2',3'-C$_4$H$_4$	5.1	3.3	0.65	2.1	1.57
25	NHCH$_2$	3',4',5'-triOMe	29.4	1.4	0.05	0.49	2.86
TMP[a]			12	133	11	2.7	49
TMQ[a]			0.042	0.003	0.07	0.01	0.30
PTX[a]			0.031	0.0015	0.048	0.017	0.088

[a] Data taken from ref 3.

For compounds **10**–**24**, the sulfur or sulfone bridge in the side chain of **4**–**9** was replaced by nitrogen, and their inhibitory concentrations along with their selectvity ratios are reported in Table 1. For the phenyl unsubstituted analogue **10**, the potency against all three DHFRs was decreased when compared with that of PTX, but the selectivity against tgDHFR increased 16-fold. *N*-Methylation of **10** (compound **18**) resulted in increased potency against pcDHFR, rlDHFR, and tgDH-FR (83-, 330-, and 136-fold, respectively). Substitution of the phenyl group of **10** with a 2'-OMe (**11**) did not afford any increase in potency as compared to **10**. For compound **12**, the 4'-OMe group decreased the inhibitory activity of **10** almost 10-fold against all three DHFRs, but the selectivity for pcDHFR and tgDHFR remained unchanged. N-Methylation of **12** to afford **19** resulted, as in the case of **10** and **18**, in a dramatic increase in inhibitory activities against all three DH-FRs. However, the selectivity of **19** for tgDHFR was only one-half that of its *N*-desmethyl analogue **12**. The electron-withdrawing chloro moiety at the 4'-position displayed a better activity profile than the electron-donating 4'-OMe (compare **13** and **12**); however, the selectivity for tgDHFR was slightly decreased. None of the monosubstituted analogues provided selectivity for pcDHFR.

Analogues with 2',5'-diOMe and 3',4'-diOMe (**14** and **15**) afforded no increase in inhibitory potency as compared to **10**. However, compound **14** with a 2',5'-diOMe substitution is 4 times more selective for tgDHFR than **10**. It is the second most selective compound for tgDHFR in this series. N-Methylation of **14** and **15** (compounds **20** and **21**) provided improved potencies against all three DHFRs, particularly compound **21**. Compound **21** is the most potent inhibitor against all

three DHFRs in this series and is more potent than PTX. Further, its selectivity for pcDHFR and tgDHFR was increased 4- and 5-fold, respectively, compared to that of PTX.

Trisubstituted analogue 3',4',5'-triOMe (**16**), with a N9-H, displayed potency which was decreased compared to that of the unsubstituted compound **10**, while the selectivity for tgDHFR remained unchanged. N-Methylation of **16** to afford **22** resulted in a similar, significant increase in potencies against all three DH-FRs as compared to the mono- and di-substituted analogues; selectivity was unchanged for pcDHFR and slightly decreased for tgDHFR.

Compound **17**, with a β-naphthyl side chain, was prepared to determine the effect of size on potency and selectivity against pcDHFR and tgDHFR. It has been reported that a naphthyl substituent in place of a phenyl in methotrexate allows for retention of DHFR inhibition.[36] Compound **17** was less potent against all three enzymes as compared to **10**. Compound **24**, the N-methylated analogue of **17**, had IC$_{50}$ values in the nanomolar and subnanomolar range and was significantly more potent than its desmethyl analogue **17** which has IC$_{50}$ values in the micromolar range. Contrary to all other *N*-methyl analogues, which generally have much higher potencies against all three DHFRs compared to the corresponding N9-H analogues, compound **23** with a *N*-methyl-α-naphthylamino side chain was a relatively weak inhibitor with IC$_{50}$ values in the micromolar range.

In compound **25**, the single-atom N9 bridge was extended to two atoms (N9–Cl0) to afford a longer, more flexible side chain. Comparison of **25** with **16**, which has identical substitution on the phenyl ring, showed that the potencies against rlDHFR and tgDHFR were

Table 2. Inhibition of the Growth (GI$_{50}$, μM) of Selected Tumor Cells in Culture by Selected Compounds

	10	18	19	20	21	22	24
			Leukemia				
MOLT-4	2.18	0.06	0.02	<0.01 (54)^a	<0.01 (57)^a	0.04	0.02
RPMI-8226	0.67	<0.01 (59)^a	<0.01 (59)^a	<0.01 (68)	<0.01 (69)	<0.01 (56)^a	<0.01 (66)^a
SR	2.91	0.05	<0.01 (53)	<0.01 (68)	<0.01 (69)	0.03	<0.01 (57)
			Non-Small-Cell Lung Cancer				
A549/ATCC	0.64	<0.01 (65)	<0.01 (67)	<0.01 (72)	<0.01 (70)	<0.01 (67)	<0.01 (65)
NCI-H23	3.67	0.03	<0.01 (54)	<0.01 (78)	<0.01 (82)	<0.01 (51)	<0.01 (63)
NCI-H322M	8.02	0.07	0.02	<0.01 (58)	<0.01 (53)	11.0	<0.01 (56)
NCI-H460	0.43	<0.01 (80)	<0.01 (84)	<0.01(82)	<0.01 (83)	<0.01 (79)	<0.01 (79)
NCI-H522	4.26	0.06	<0.01 (53)	<0.01 (67)	<0.01 (59)	0.03	<0.01 (60)
			Colon Cancer				
HCT-116	0.51	<0.01 (80)	<0.01 (83)	<0.01 (83)	<0.01 (83)	<0.01 (76)	<0.01 (85)
HCT-15	0.85	<0.01 (66)	<0.01 (67)	<0.01 (70)	<0.01 (75)	<0.01 (57)	<0.01 (69)
KM-12	5.94	0.19	0.02	<0.01 (55)	<0.01 (56)	0.04	0.01
			CNS Cancer				
SF-268	6.05	0.01	<0.01 (68)	<0.01 (57)	<0.01 (52)	<0.01 (61)	<0.01 (52)
SF-295	1.43	<0.01 (59)	<0.01 (62)	<0.01 (69)	<0.01 (68)	<0.01 (53)	<0.01 (63)
SF-539	2.95	<0.01 (52)	0.03	<0.01 (65)	<0.01 (67)	0.02	<0.01 (66)
SNB-19	1.57	0.04	<0.01 (51)	<0.01 (73)	<0.01 (90)	<0.01 (53)	<0.01 (62)
			Melanoma				
M14	1.07	0.01	<0.01 (55)	<0.01 (61)	<0.01 (59)	0.02	<0.01 (54)
SK-MEL-5	0.83	<0.01 (59)	<0.01 (65)	<0.01 (76)	<0.01 (83)	<0.01 (60)	<0.01 (77)
			Ovarian Cancer				
IGROVI	3.27	<0.01 (53)	<0.01 (62)	<0.01 (66)	<0.01 (65)	<0.01 (52)	<0.01 (65)
OVCAR-8	0.99	<0.01 (91)	<0.01 (90)	<0.01 (75)	<0.01 (76)	<0.01 (57)	<0.01 (95)
			Renal Cancer				
786-0	0.89	0.22	0.59	<0.01 (55)	<0.01 (53)	0.02	<0.01 (54)
CAKI-1	3.58	0.01	<0.01 (59)	<0.01 (70)	<0.01 (81)	0.01	<0.01 (74)
UO-31	<0.01 (57)	0.03	<0.01 (74)	<0.01 (100)	<0.01 (100)	<0.01 (65)	<0.01 (54)
			Prostate Cancer				
DU-145	4.99	0.12	<0.01 (51)	<0.01 (57)	<0.01 (57)	0.22	<0.01 (51)
			Breast Cancer				
MCF7	6.45	0.14	0.06	<0.01 (58)	<0.01 (60)	0.04	0.03
MCF7/ADR-RES	2.69	<0.01 (83)	<0.01 (100)	<0.01 (82)	<0.01 (84)	0.01	<0.01 (100)
MDA-MB-435	0.57	<0.01 (73)	<0.01 (68)	<0.01 (70)	<0.01 (72)	<0.01 (56)	<0.01 (66)
MDA-N	0.68	<0.01 (68)	<0.01 (66)	<0.01 (59)	<0.01 (63)	<0.01 (58)	<0.01 (75)

^{a} Numbers in parentheses are percent inhibition at 0.01 μM.

increased slightly, as was the selectivity for tgDHFR. However, the potency against pcDHFR remained unchanged.

Several of the analogues were selected by the National Cancer Institute for evaluation in their preclinical in vitro antitumor screening program against a panel of 56 different tumor cell lines.[37] On the basis of their activities against rlDHFR, the N9-Me analogues were the most inhibitory in the series. The results (GI$_{50}$) of the inhibition of the growth of tumor cells in culture for compounds **18**−**22** and **24** along with the N9-H analogue **10** are listed in Table 2. The remarkable increase observed in rlDHFR inhibitory activity on N9-methylation (Table 1) translated into nanomolar, and perhaps in some instances subnanomolar, growth inhibitory potencies against several of the tumor cells in culture. The cytotoxicity of these analogues, in part, can be attributed to their high DHFR inhibitory potency and to their ability to penetrate tumor cells in culture. The sulfur and sulfone analogues were not potent inhibitors as compared with the N9-Me analogues and had GI$_{50}$ values in the 10^{-4}−10^{-6} M range. The GI$_{50}$ values of the N9-H analogues are typified by **10** in Table 2.

In summary, for S9-bridged 2,4-diaminopyrido[3,2-*d*]pyrimidine analogues of piritrexim, the 4′-OMe provides higher potencies against all three DHFRs as compared to the 2′-OMe. Selectivity for pcDHFR and tgDHFR in the 4′-OMe analogues was decreased. 3′,4′-Dimethoxy substitution increased the activity compared to the monomethoxy analogue but decreased selectivity. Compounds with a sulfone bridge were less potent than the corresponding S-bridged compounds; while the selectivity against pcDHFR was decreased slightly, the selectivity against tgDHFR was unchanged or increased. Thus, a strong electron-withdrawing group which possesses steric bulk such as the sulfone at the 6-position was detrimental to potency against DHFR. For the N9-bridged pyrido[3,2-*d*]pyrimidine analogues of piritrexim, the N9-desmethyl analogues generally had low activities against pcDHFR, rlDHFR, and tgDHFR, whereas the N9-methylated compounds were much more potent and had inhibitory activities similar to or better than that of PTX. Thus it is apparent that the loss of the 5-CH$_3$ moiety of PTX in the pyrido[3,2-*d*]pyrimidines in this study resulted in a decrease in inhibition of DHFR and that the potency is restored when the N9 was methylated.

Substitutions on the phenyl ring of the side chain of the N9-desmethyl analogues generally decreased potencies against all three DHFRs, irrespective of the electronic nature of the substitution(s). In contrast, substitution on the phenyl ring of the N9-methylated analogues either increased potency (**21** vs **18**) or decreased potency (**19** vs **18**). Compound **21**, which was the most potent compound in this series, is currently

Table 3. Experimental for the Synthesis of Compounds **10**−**25** Except **15** (which is described in the general procedure)
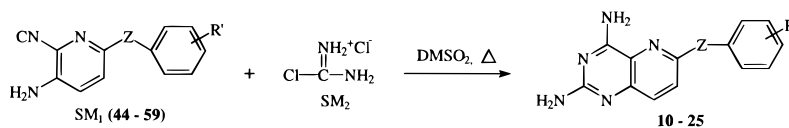
compd	SM$_1$ [no., g (mmol)]	SM$_2$ [g (mmol)]	DMSO$_2$ (g)	T (°C)	time (min)	R_f (MeOH/CHCl$_3$, v/v)	solv for column (MeOH/CHCl$_3$, v/v)	yield [g (%)]	mp (°C)
10	**44**, 0.30 (1.8)	0.43 (3.7)	3.0	175	12	0.24 (1:9)	15:85	0.27 (58)	211−213
11	**45**, 0.60 (2.5)	0.58 (5.0)	2.5	150	20	0.26 (1:9)	*a*	0.59 (84)	335−337
12	**46**, 0.62 (2.7)	0.62 (5.4)	2.8	150	25	0.24 (1:9)	15:85	0.51 (67)	244−246
13	**47**, 0.70 (2.9)	0.66 (5.7)	2.8	175	35	0.20 (1:9)	20:80	0.65 (79)	249−251
14	**48**, 0.35 (1.3)	0.30 (2.6)	2.0	127−137	15	0.29 (2:8)	15:85^b	0.23 (57)	198−200
16	**50**, 0.30 (1.0)	0.23 (2.0)	1.0	127−137	19	0.24 (2:8)	25:75	0.23 (67)	218−219
17	**51**, 0.45 (1.7)	0.40 (3.4)	1.8	127−137	15	0.24 (2:8)	20:80	0.25 (50)	227−229
18	**52**, 0.40 (1.8)	0.41 (3.6)	2.0	128	20	0.34 (2:8)	10:90	0.41 (86)	237−239
19	**53**, 0.72 (2.8)	0.65 (5.6)	3.0	125−135	20	0.37 (2:8)	15:85	0.51 (61)	217−219
20	**54**, 0.60 (2.1)	0.48 (4.2)	2.5	130	20	0.40 (2:8)	*c*	0.55 (80)	242−244
21	**55**, 0.80 (2.8)	0.65 (5.6)	3.0	125−135	20	0.46 (2:8)	*d*	0.57 (62)	207−209
22	**56**, 0.58 (1.9)	0.40 (3.5)	2.0	130	20	0.37 (2:8)	15:85	0.41 (61)	209−211
23	**57**, 0.30 (1.1)	0.25 (2.2)	1.2	120−130	20	0.34 (2:8)	15:85	0.08 (23)	176 dec
24	**58**, 0.30 (1.1)	0.25 (2.2)	1.2	115	20	0.35 (2:8)	15:85	0.35 (100)	221−222.5
25	**59**, 0.83 (2.6)	0.65 (5.6)	3.0	123−125	20	0.24 (2:8)	25:75	0.16 (17)	187 dec

^{a} The procedure to purify **11** is as follows: After a workup procedure described for **15**, the precipitate formed upon basification was filtered, triturated in methanol−0.1 N HCl (9:1) to remove impurities, washed with 20% methanol in chloroform, and dried in vacuum. ^{b} An analytically pure sample was obtained by carefully washing the product obtained from column chromatography with a small amount of 0.01 N HCl. ^{c} Purified via recrystallization of the precipitate in 95% methanol. ^{d} Purified via recrystallization of the precipitate in 40% acetone in methanol.

undergoing further investigation as an antitumor agent. Extending the bridge from one to two atoms did not increase inhibitory activity against the DHFR evaluated. The selectivity against tgDHFR was increased, compared to PTX, for all of the analogues tested in this study by as much as 75-fold (compound **14**); however, the compounds were not as selective as the pyrido[2,3-*d*]pyrimidine series reported earlier.[20,21] This indicates that for this series of analogues the absence of the 5-methyl moiety and/or the presence of a N9-methyl group does not afford compounds which are highly selective for pcDHFR or tgDHFR. The N9-CH$_3$ analogues were significantly inhibitory against the growth of a variety of tumor cells in culture with IG$_{50}$ values < 1.00×10^{-8} M. The N9-H and S9 compounds were much less inhibitory with IG$_{50}$ values in the 10^{-4}−10^{-6} M range. In addition to **21**, the other N9-CH$_3$ analogues are also currently undergoing further evaluation as antitumor agents.

Experimental Section

All evaporations were carried out in vacuo with a rotary evaporator or by short-path distillation. Analytical samples were dried in vacuo (0.2 mmHg) in an Abderhalden drying apparatus over P$_2$O$_5$ and refluxing ethanol or toluene. Thin-layer chromatography (TLC) was performed on Eastman Kodak silica gel chromatogram plates with fluorescent indicator. Spots were visualized by UV light (254 and 350 nm) or using vanillin as an indicator. All analytical samples were homogeneous on TLC in at least two different solvent systems. Purification by column and flash chromatography was carried out using Merck silica gel 60 (230−400 mesh). The amount (weight) of silica gel for column chromatography was in the range of 20−30 times the amount (weight) of the crude compounds being separated. Columns were wet packed unless specified otherwise. Melting points were determined by capillary method on a Fisher-Johns or Thomas-Hoover melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker WH-300 (300 MHz) NMR spectrometer. The chemical shift (δ) values are reported as parts per million

(ppm) relative to tetramethylsilane as internal standard; s = singlet, d = doublet, dd = doublet of doublet, t = triplet, q = quartet, m = multiplet, br = broad peak, br s = broad singlet, exch = protons exchangeable by addition of D$_2$O. Elemental analyses were performed by Atlantic Microlab, Inc., Atlanta, GA. Elemental compositions were within ±0.4% of the calculated values. Fractional moles of water or organic solvents frequently found in some analytic samples of antifolates could not be removed despite 24 h of drying in vacuo and were confirmed, where possible, by their presence in the ^{1}H NMR spectra. All solvents and chemicals were purchased from Aldrich Chemical Co. and Fisher Scientific and were used as received except anhydrous solvents which were freshly dried in the laboratory.

Syntheses of compounds **10**−**25** and their physical properties are summarized in Table 3 except **15** which is described in the general procedure below. Their ^{1}H NMR data are summarized in Table 6 (Supporting Information). Syntheses of compounds **28**−**37**, **39**, **40**, **42**, and **43** are summarized in Table 4 except **33** which is described in the general procedure for the synthesis of these compounds. The ^{1}H NMR data are summarized in Table 7 (Supporting Information). The synthesis of compound **44** is described in the general procedure for the synthesis of compounds **45**−**59** which are summarized in Table 5, and their ^{1}H NMR data are in Table 8 (Supporting Information).

General Procedure for the Synthesis of Compounds 4−**6 Exemplified by the Synthesis of 2,4-Diamino-6-[(2′-methoxyphenyl)thio]pyrido[3,2-*d*]pyrimidine (4).** A mixture of 2,4-diamino-6-chloropyrido[3,2-*d*]pyrimidine (**27**) (0.50 g, 2.6 mmol), 2-methoxyphenylthiol (0.50 g, 2.6 mmol), and dimethyl sulfone (2.50 g) was heated at 190 °C (oil bath) for 20 min. The hot mixture was poured into water with stirring to afford a sticky syrup. The aqueous mixture was then made basic with ammonium hydroxide to pH 10. Acetone was added dropwise with stirring, and the syrup was transformed into a solid precipitate which was separated, washed with water, and ether, respectively, suspended in a small amount of 8:2 acetone−methanol, and sonicated for 1 h. Filtration, washing with ether, and drying under vacuum afforded 0.59 g of **4** (77%) as an off-white solid: TLC R_f 0.19 (MeOH/CHCl$_3$, 1:9); mp 201−202 °C dec; ^{1}H NMR (DMSO-d_6) δ 3.72 (s, 3 H, 2′OCH$_3$), 6.16 (br s, 2 H, 2-NH$_2$, exch), 6.94−7.17 (m, 2 H, C$_6$H$_4$ and

Table 4. Experimental for the Synthesis of Compounds **28–37**, **39**, **40**, **42**, and **43** Except **33** (which is described in the general procedure)
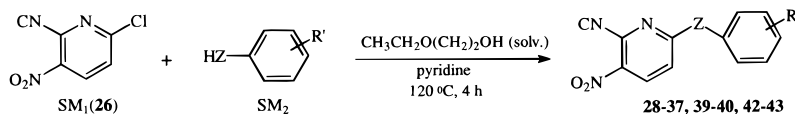
product	SM$_1$ (**26**) [g, (mmol)]	R′	Z	SM$_2$ (amount) [g mmol]	solv^e (mL)	pyridine [g (mmol)]	yield [g (%)]
28	0.75 (4.1)	H	NH	0.46 (4.9)	20	0.39 (4.9)	0.86 (88)
29	1.50 (8.2)	2-OMe	NH	1.11 (9.0)	40	0.78 (9.8)	1.35 (61)
30	0.75 (4.1)	4-OMe	NH	0.60 (4.9)	20	0.39 (4.9)	1.06 (96)
31^a	0.50 (2.7)	4-Cl	NH	0.44 (3.5)	10	0.26 (3.3)	0.62 (55)
32	0.75 (4.1)	2,5-diOMe	NH	0.80 (5.2)	20	0.39 (4.9)	1.02 (83)
34	4.0 (22.0)	3,4,5-triOMe	NH	4.83 (26.4)	40	2.08 (26.4)	7.18 (83)
35	1.0 (5.5)	3,4-C$_4$H$_4$	NH	0.86 (6.0)	30	0.52 (6.6)	0.76 (63)
36	1.0 (5.5)	H	NCH$_3$	0.60 (6.0)	30	0.52 (6.6)	1.00 (72)
37^b	1.0 (5.5)	4-OMe	NCH$_3$	0.83 (6.0)	20	0.52 (6.6)	1.30 (87)
39^d	1.25 (6.8)	3,4-diOMe	NCH$_3$	1.25 (7.5)	20	0.62 (8.1)	1.60 (75)
40^d	0.95 (5.2)	3,4,5-triOMe	NCH$_3$	1.13 (5.7)	20	0.49 (6.2)	1.02 (57)
42^c	0.88 (4.8)	3,4-C$_4$H$_4$	NCH$_3$	0.83 (5.3)	20	0.39 (4.9)	0.50 (34)
43^d	1.50 (8.2)	3,4,5-triOMe	NCH$_3$	1.94 (9.8)	40	0.78 (9.8)	2.02 (72)

^{a} Purified by recrystallization from ethanol. ^{b} The reaction was carried out at 135 °C for 3 h. ^{c} The reaction was carried out at 120–130 °C for 2 h, and the compound was purified through column chromatography using 70:30 chloroform–hexane (v/v) as eluent. ^{d} The reaction was carried out at 120–130 °C for 3 h. ^{e} The solvent used was 2-ethoxyethanol.

Table 5. Experimental for the Synthesis of Compounds **44–59** Except **44** (which is described in the general procedure)
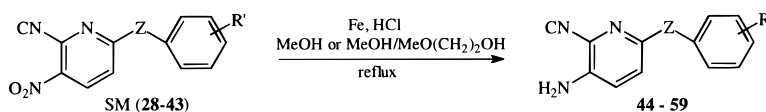
product	SM [no., g (mmol)]	Fe [g (mmol)]	MeOH (mL)	MeO(CH$_2$)$_2$OH (mL)	concd HCl (mL)	time (h)	solv for column (v/v)	yield [g (%)]
45	**29**, 1.30 (4.8)	1.34 (23.9)	30	10	6.0	2.5	50:50:1 AcOEt–CHCl$_3$–MeOH	0.68 (59)
46	**30**, 1.00 (3.7)	1.10 (19.6)	30	0	4.0	1.1	50:50 AcOEt–CHCl$_3$	0.69 (78)
47	**31**, 1.20 (4.4)	1.20 (21.4)	30	5.0	4.0	1.5	50:50 hexane–acetone	0.75 (70)
48	**32**, 1.50 (5.0)	0.94 (16.8)	20	0	4.2	2.0	60:40:1 AcOEt–CHCl$_3$–MeOH	0.47 (35)
49	**33**, 0.70 (2.4)	0.47 (8.3)	13	0	3.0	1.5	60:40:1 AcOEt–CHCl$_3$–MeOH	0.41 (65)
50	**34**, 7.0 (21.2)	5.78 (100)	110	0	20	1.3	60:40:1 AcOEt–CHCl$_3$–MeOH	4.30 (67)
51	**36**, 0.70 (2.4)	0.74 (13.2)	14	7.0	3.0	1.5	5:95 MeOH–CHCl$_3$	0.51 (81)
52	**36**, 1.00 (5.5)	1.05 (18.7)	20	10	4.5	1.5	20:80 AcOEt–CHCl$_3$	0.47 (56)
53	**37**, 1.20 (4.2)	1.25 (22.3)	30	8.0	5.0	1.5	20:80 AcOEt–CHCl$_3$	0.84 (78)
54	**38**, 1.00 (3.3)	1.05 (18.7)	20	10	4.5	0.92	15:85 AcOEt–CHCl$_3$	0.71 (79)
55	**39**, 1.50 (4.8)	0.91 (16.2)	20	5.0	4.0	2.0	20:80 AcOEt–CHCl$_3$	0.93 (69)
56	**40**, 0.90 (2.6)	0.80 (14.3)	14	7.0	3.0	3.0	20:80 AcOEt–CHCl$_3$	0.65 (78)
57	**41**, 0.58 (1.9)	0.54 (9.5)	15	0	2.5	4.0	CHCl$_3$	0.34 (65)
58	**42**, 0.50 (1.6)	0.50 (9.0)	20	0	2.0	1.0	15:85 AcOEt–CHCl$_3$	0.30 (67)
59	**43**, 1.90 (5.5)	1.50 (26.8)	30	0	6.0	1.0	33:67 AcOEt–CHCl$_3$	1.29 (74)

7-H/8-H), 7.14 (d, 1 H, C$_6$H$_4$), 7.37–7.44 (m, 3 H, C$_6$H$_4$ and 7-H/8-H), 7.18–7.52 (br, 2 H, 4-NH$_2$, exch). Anal. (C$_{14}$H$_{13}$N$_5$-OS·0.30NH$_4$Cl) C, H, N, S.

2,4-Diamino-6-[(4′-methoxyphenyl)thio]pyrido[3,2-*d*]-pyrimidine (5). Compound **27** (0.50 g, 2.6 mmol) was reacted with 4-methoxyphenylthiol (0.42 g, 3.0 mmol) in dimethyl sulfone (2.50 g) under the same conditions described for **4** to afford 0.52 g (68%) of **5**: TLC R_f 0.21 (MeOH/CHCl$_3$, 1:9); mp 220–222 °C; ^{1}H NMR (DMSO-d_6) δ 3.79 (s, 3 H, 4′OCH$_3$), 7.03 [m, 5 H, 2-NH$_2$ (exch), 7-H and two phenyl Hs], 7.53 (m, 3 H, 8-H and two phenyl Hs), 7.71 (br, 1 H, 4-NH$_2$), 8.11 (br, 1 H, 4-NH$_2$). Anal. (C$_{14}$H$_{13}$N$_5$OS·0.20H$_2$O) C, H, N, S.

2,4-Diamino-6-[(3′,4′-dimethoxyphenyl)thio]pyrido[3,2-*d*]pyrimidine (6). Nucleophilic displacement of the 6-chloro group of **27** (0.50 g, 2.6 mmol) with 3,4-dimethoxyphenylthiol (0.50 g, 3.0 mmol) in dimethyl sulfone (2.50 g) at 190 °C for 1.5 h and a similar workup procedure as described above for **4** (except that the pH was adjusted to 5–6 with ammonium hydroxide rather than to pH 10) afforded 0.66 g (79%) of **6**: TLC R_f 0.22 (MeOH/CHCl$_3$, 1:9); mp 218–219.5 °C; ^{1}H NMR (DMSO-d_6) δ 3.75, 3.70 (2 s, 6 H, 3′-OCH$_3$ and 4′-OCH$_3$), 6.15

(br s, 2 H, 2-NH$_2$, exch), 6.96–7.30 (br m, 6 H, 4-NH$_2$, C$_6$H$_4$, and 7-H/8-H), 7.41 (d, 1 H, 7-H/8-H). Anal. (C$_{15}$H$_{15}$N$_5$O$_2$S·0.50HCl) C, H, N, S.

General Procedure for the Synthesis of Compounds 7–9 Exemplified by the Synthesis of 2,4-Diamino-6-[(2′-methoxyphenyl)sulfonyl]pyrido[3,2-*d*]pyrimidine (7). A suspension of **4** (0.30 g, 1.0 mmol), 7.0 mL of glacial acetic acid, and 30% hydrogen peroxide (1.8 mL, 18 mmol) was stirred at room temperature for 2 days. The acetic acid was then evaporated with a rotary evaporator, and to the residue was added water (10 mL) with stirring. The resulting solution was carefully brought to pH 9–10 with ammonium hydroxide, and the precipitate formed was filtered, washed with water and ether, and redissolved in 50:50 methanol–acetone (v/v). Silica gel (3.0 g) was added to the solution followed by removal of the solvent under reduced pressure. The silica gel plug thus formed was loaded onto a triethylamine-pretreated silica gel column and eluted with 15% methanol in chloroform. The fractions containing the product (monitored on TLC) were pooled and evaporated to afford 0.24 g (72%) of **7**: TLC R_f 0.17 (MeOH/CHCl$_3$, 1:9); mp 205–207 °C; ^{1}H NMR (DMSO-d_6) δ 3.68 (s, 3 H, 2′-OCH$_3$), 6.52 (br s, 2 H, 2-NH$_2$, exch), 7.07 (d, 1

H, 7-H/8-H), 7.19 (t, 2 H, C$_6$H$_4$), 7.52 (d, 2 H, C$_6$H$_4$), 7.60 (br s, 2 H, 4-NH$_2$, exch), 7.72 (d, 1 H, 7-H/8-H). Anal. (C$_{14}$H$_{13}$N$_5$O$_3$S) C, H, N, S.

2,4-Diamino-6-[(4′-methoxyphenyl)sulfonyl]pyrido[3,2-*d*]pyrimidine (8). A mixture of **5** (0.20 g, 0.7 mmol) and 30% hydrogen peroxide (1.8 mL, 18 mmol) in glacial acetic acid (9.0 mL) was stirred at room temperature for 2 days. Following the workup procedure described above for compound **7** and column chromatographic purification using 15% methanol in chloroform as eluent afforded 0.12 g (54%) of **8**: TLC *R$_f$* 0.16 (MeOH/CHCl$_3$, 1:9); mp 216−218 °C; ^{1}H NMR (DMSO-*d$_6$*) δ 3.73 (s, 3 H, 4′-OCH$_3$), 6.47 (br s, 2 H, 2-NH$_2$, exch), 7.03 (d, 2 H, C$_6$H$_4$), 7.53 (br, 2 H, 4-NH$_2$, exch), 7.67 (d, 1 H, 7-H/8-H), 7.72 (d, 2 H, C$_6$H$_4$), 7.88 (d, 1 H, 7-H/8-H). Anal. (C$_{14}$H$_{13}$N$_5$O$_3$S· 0.15H$_2$O) C, H, N, S.

2,4-Diamino-6-[(3′,4′-dimethoxyphenyl)sulfonyl]pyrido-[3,2-*d*]pyrimidine (9). Using the same procedure as described above for **7**, compound **6** (0.18 g, 0.5 mmol) was oxidized with 30% hydrogen peroxide (1.5 mL, 15 mmol) in glacial acetic acid (8.0 mL) to afford 0.09 g (40%) of **9**: TLC *R$_f$* 0.18 (MeOH/CHCl$_3$, 1:9); mp 231−233 °C; ^{1}H NMR (DMSO-*d$_6$*) δ 3.75, 3.72 (2 s, 6 H, 3′-OCH$_3$ and 4′-OCH$_3$), 6.46 (br s, 2 H, 2-NH$_2$, exch), 7.05 (d, 1 H, aryl H), 7.32−7.36 (m, 2 H, aryl Hs), 7.57 (br d, 2 H, 4-NH$_2$, exch), 7.66 (d, 1 H, aryl H), 7.89 (d, 1 H, aryl H). Anal. (C$_{15}$H$_{15}$N$_5$O$_4$S·0.05C$_6$H$_{14}$) C, H, N, S.

General Procedure for the Synthesis of Compounds 10−25 Exemplified by the Synthesis of 2,4-Diamino-6-(3′,4′-dimethoxyanilino)pyrido[3,2-*d*]pyrimidine (15). A mixture of 2-cyano-3-amino-6-(3′,4′-dimethoxyanilino)pyridine (**49**) (0.56 g, 2.1 mmol), chloroformamidine hydrochloride (0.48 g, 4.2 mmol), and dimethyl sulfone (2.0 g) was heated in an oil bath at 140 °C under nitrogen for 15 min. The oil bath was removed, and water (10 mL) was added slowly to the hot reaction mixture. The aqueous solution was cooled to room temperature and extracted with chloroform (3 × 5 mL) to remove dimethyl sulfone. The aqueous phase was made basic to pH 10 with ammonium hydroxide, followed by removal of water under reduced pressure. The residue was dissolved in a mixture of 50:50 methanol−acetone (v/v), and silica gel was added (3.0 g). After the removal of solvent with a rotary evaporator, the silica gel plug was loaded onto a column and eluted with 3:10:9 MeOH−CHCl$_3$−(CH$_3$)$_2$CO (v/v/v) to afford 0.41 g (63%) of **15**: ^{1}H NMR (DMSO-*d$_6$*) δ 3.72, 3.78 (2 s, 6 H, 3-OCH$_3$ and 4-OCH$_3$), 5.99 (br s, 2 H, 2-NH$_2$, exch), 6.30−7.27 (collapsed br, 2 H, 4-NH$_2$, exch), 6.90 (d, 1 H, 5′-H), 7.14 (d, 1 H, 7-H/8-H), 7.34 (dd, 1 H, 6′-H), 7.41 (d, 1 H, 2′-H), 7.47 (d, 1 H, 7-H/8-H), 9.14 (s, 1 H, N9-H, exch). Anal. (C$_{15}$H$_{16}$N$_6$O$_2$·0.5CH$_3$-OH) C, H, N.

General Procedure for the Synthesis of Compounds 28−37, 39, 40, 42, and 43 Exemplified by the Synthesis of 3-Nitro-6-(3′,4′-dimethoxyanilino)pyridine-2-carbonitrile (33). A solution of 6-chloro-3-nitro-2-pyridinecarbonitrile (**26**) (0.50 g, 2.7 mmol), 4-aminoveratrole (0.53 g, 3.5 mmol), and pyridine (0.26 g, 3.3 mmol) in 2-ethoxyethanol (10 mL) was heated at 120 °C under nitrogen. After 4 h, the solution was concentrated under reduced pressure and the concentrated solution poured into water and stirred at room temperature for 30 min. The precipitate obtained was filtered, washed twice with water and twice with ether successively, transferred into a 100-mL round-bottom flask, and redissolved in ethanol (20 mL). To this solution was added silica gel (3.5 g), and the solvent removed by evaporation. The silica gel plug obtained was loaded onto a silica gel column and eluted with 1:1 ethyl acetate−methylene chloride (v/v). The fractions containing the desired product (monitored on TLC) were pooled, and the solvent was evaporated to afford 0.71 g (87%) of **33**: ^{1}H NMR (DMSO-*d$_6$*) δ 3.73, 3.74 (2 s, 6 H, 3′-OCH$_3$ and 4′-OCH$_3$), 6.96 (d, *J* = 8.8 Hz, 1 H, 5′-H), 7.01 (d, *J* = 9.5 Hz, 1 H, 5-H), 7.13 (d, *J* = 8.8 Hz, 1 H, 6′-H), 7.25 (br s, 1 H, 2′-H), 8.30 (d, *J* = 9.5 Hz, 1 H, 4-H), 10.34 (s, 1 H, NH, exch).

General Procedure for the Synthesis of Compounds 38 and 41 Exemplified by the Synthesis of 3-Nitro-6-(*N*-methyl-2′,5′-dimethoxyanilino)pyridine-2-carbonitrile (38). To a stirred solution of *N*-methyl-2,5-dimethoxyaniline

(1.0 g, 6.0 mmol) in anhydrous THF (10 mL) at −78 °C was added 2 M LDA solution in hexane (3.0 mL, 6.0 mmol). After 20 min, the solution was allowed to warm to room temperature and then added dropwise to a 50-mL round-bottom flask containing a solution of 3-nitro-6-chloro-2-pyridinecarbonitrile (**26**) (1.0 g, 5.4 mmol) in anhydrous THF (15.0 mL) at −78 °C over a period of 10 min. The reaction mixture was stirred at −78 °C for an additional 30 min and was allowed to warm to room temperature. The reaction was continued for 2 h at room temperature and quenched with saturated NH$_4$Cl solution (1.0 mL), and the solvent THF was removed with a rotary evaporator. Chloroform (30.0 mL) was then added to the flask, and the resulting solution was transferred into a separatory funnel, washed with water and saturated sodium chloride solution successively, and dried over anhydrous Na$_2$SO$_4$ for 40 min. After removal of chloroform under reduced pressure, the residue was loaded onto a silica gel column and eluted gradually with CHCl$_3$ and CHCl$_3$−AcOEt (95:5) to afford 1.21 g (70%) of **38**: ^{1}H NMR (CDC$_3$) δ 3.51 (s, 3 H, NCH$_3$), 3.75, 3.77 (d, 6 H, 2′-OCH$_3$ and 5′-OCH$_3$), 6.33 (d, 1 H, 5-H), 6.72 (d, 1 H, 6′-H), 6.97 (m, 2 H, 3′-H and 4′-H), 8.06 (d, 1 H, 4-H).

3-Nitro-6-(*N*-methyl-1′-naphthylamino)pyridine-2-carbonitrile (41). Using the procedure described for the synthesis of **38**, compound **26** (0.87 g, 4.7 mmol) was reacted with *N*-methyl-2-naphthylamine (0.82 g, 5.2 mmol) to afford 0.58 g (40%) of **41**: ^{1}H NMR (DMSO-*d$_6$*) δ 3.41 (s, 3 H, NCH$_3$), 7.04 (d, 1 H, 5-H), 7.21 (d, 1 H, C$_{10}$H$_7$), 7.34 (t, 1 H, C$_{10}$H$_7$), 7.57 (m, 2 H, C$_{10}$H$_7$), 7.82 (d, 1 H, C$_{10}$H$_7$), 7.96 (dd, 1 H, C$_{10}$H$_7$), 8.17 (d, 1 H, 4-H).

General Procedure for the Synthesis of Compounds 44−59 Exemplified by the Synthesis of 3-Amino-6-anilinopyridine-2-carbonitrile (44). To a suspension of 3-nitro-6-anilino-2-pyridocarbonitrile (**28**) (0.83 g, 3.5 mmol) in methanol (20 mL) and concentrated hydrochloric acid (36%, 4.0 mL) was added iron powder (0.65 g, 11.6 mmol). The mixture was refluxed until all the starting material had reacted (monitored on TLC). The hot reaction mixture was poured into water (20 mL) and stirred for 5 min. The unreacted iron was removed using a stirring bar retriever, and the aqueous solution was neutralized with ammonium hydroxide to pH 4. The solution was then extracted with chloroform (3 × 20 mL), and the combined organic layer was washed with water (2 × 5.0 mL), sodium bicarbonate solution (5.0 mL), and brine (5.0 mL) and dried over anhydrous sodium sulfate. Chromatographic purification on a silica gel column using AcOEt−CHCl$_3$−MeOH (12:8:1) as eluent afforded 0.52 g (71%) of 3-amino-6-anilino-2-pyridinocarbonitrile (**44**): ^{1}H NMR (DMSO-*d$_6$*) δ 5.66 (br s, 2 H, 3-NH$_2$, exch), 6.80 (t, 1 H, 4′-H), 6.93 (d, 1 H, 4-H/5-H), 7.16 (d, 1 H, 4-H/5-H), 7.20 (t, 2 H, 3′-H and 5′-H), 7.52 (d, 2 H, 2′-H and 6′-H), 8.56 (br s, 1 H, NH, exch).

Supporting Information Available: ^{1}H NMR data for compounds **10−25**, **28−32**, **34−47**, and **49−59** (Tables 6−8) (6 pages). Ordering information is given on any current masthead page.

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