2,4-Diaminothieno[2,3-*d*]pyrimidine Lipophilic Antifolates as Inhibitors of *Pneumocystis carinii* and *Toxoplasma gondii* Dihydrofolate Reductase

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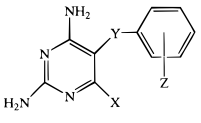
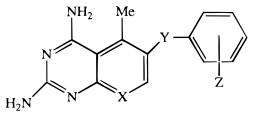
Ten previously unreported 2,4-diaminothieno[2,3-*d*]pyrimidine lipophilic dihydrofolate reductase inhibitors were synthesized as potential inhibitors of *Pneumocystis carinii* and *Toxoplasma gondii* dihydrofolate reductase. Pivaloylation of 2,4-diamino-5-methylthieno[2,3-*d*]pyrimidine followed by dibromination with *N*-bromosuccinimide in the presence of benzoyl peroxide gave 2,4-bis(pivaloylamino)-6-bromo-5-(bromomethyl)thieno[2,3-*d*]pyrimidine, which after condensation with substituted anilines or *N*-methylanilines and deprotection with base yielded 2,4-diamino-6-bromo-5-[(substituted anilino)methyl]thieno[2,3-*d*]pyrimidines. Removal of the 6-bromo substituent was accomplished with sodium borohydride and palladium chloride. The reaction yields were generally good to excellent. The products were tested as inhibitors of dihydrofolate reductase (DHFR) from *P. carinii*, *T. gondii*, and rat liver. Although the IC_{50} could not be reached for the 6-unsubstituted compounds because of their extremely poor solubility, three of the five 6-bromo derivatives were soluble enough to allow the IC_{50} to be determined against all three enzymes. 2,4-Diamino-5-[3,5-dichloro-4-(1-pyrrolo)anilino]methyl]-6-bromothieno[2,3-*d*]pyrimidine was the most active of the 6-bromo derivatives, with an IC_{50} of 7.5 μM against *P. carinii* DHFR, but showed no selectivity for either *P. carinii* or *T. gondii* DHFR relative to the enzyme from rat liver.

Pneumocystis carinii and *Toxoplasma gondii* are prevalent life-threatening opportunistic microbes in individuals with compromized immune systems. For this reason, AIDS patients, immunosuppressed organ transplant recipients, and patients receiving cancer chemotherapy are at high risk of contracting these infections.[1−3] Recent advances in the treatment of AIDS with two- and three-drug cocktails combining nucleosides and protease inhibitors are very promising, but these new regimens are not universally effective and the durability of their antiviral effect is not yet established. In addition, the high cost of these treatments and the fact that they have to be given frequently and over a long period with close medical supervision are impediments to their use in developing and underdeveloped countries, where new AIDS cases continue to be reported with alarming frequency and now greatly exceed the number of cases in the industrialized world. Thus, until a practicable approach to worldwide control of HIV-1 by means of antiviral vaccination or chemotherapy is achieved,[4] the development of new drugs for the management *P. carinii* and *T. gondii* opportunistic infections in AIDS patients remains an important goal.



1: X = H, Y = CH_2; Z = 3,4,5-$(OMe)_3$
2: X = Et, Y = none, Z = 4-Cl
3: X = H, Y = CH_2, Z = 3,5-$(OEt)_2$-4-(1-pyrrolo)
4: X = H, Y = CH_2, Z = 4-Br-3,5-$(OMe)_2$
5: X = H, Y = CH_2, Z = 3,5-Cl_2-4-(1-pyrrolo)



6: X = CH, Y = CH_2NH, Z = 3,4,5-$(OMe)_3$
7: X = N, Y = CH_2, Z = 2,5-$(OMe)_2$

Trimethoprim (TMP, **1**) and pyrimethamine (PM, **2**) are clinically approved lipophilic dihydrofolate reductase (DHFR) inhibitors for the treatment of infection by *P. carinii*, *T. gondii*, and other opportunistic parasites.[5−8] TMP is most often used against *P. carinii* pneumonia (PCP), whereas PM is most often prescribed for toxoplasmosis. While these drugs have a high degree of binding selectivity for *P. carinii* and *T. gondii* DHFR versus mammalian DHFR, they are not very potent or effective when used as single agents, and thus are generally used in combination with a sulfa drug such as sulfadiazine, sulfamethoxazole, or dapsone. Recently, epiroprim (EPM, **3**), a second generation analogue of TMP, has shown promise,[9−11] and another analogue, brodimoprim (**4**), has been advocated as an alternative to TMP because of its tighter DHFR binding and more favorable pharmacokinetics.[12,13] Analogues of epiroprim containing phenyl substituents other than ethoxy at the 3- and 5-position have also been described, such as the 3,5-dichloro analogue **5**, which had an IC_{50} of 23 μM against *P. carinii* DHFR and showed 13-fold selectivity for this enzyme relative to human DHFR.[14] Two other lipophilic antifolates, trimetrexate (TMQ, **6**) and piritrexim (PTX, **7**), which were originally developed as anticancer drugs, have been used to treat *P. carinii* and *T. gondii* infections in AIDS patients.[15−17] Unlike TMP and PM, these dicyclic molecules are very potent, but unfortunately bind better to mammalian DHFR species than they do to the *P. carinii* or *T. gondii* enzyme. For this reason, the rescue agent leucovorin (5-formyl-5,6,7,8-tetrahydrofolate) had to be used to prevent myelosuppression in the clinical trials with TMQ and PTX. Lipophilic DHFR inhibitors combining the potency of TMQ and PTX with the selectivity of TMP and PM would presumably have avoided the need for

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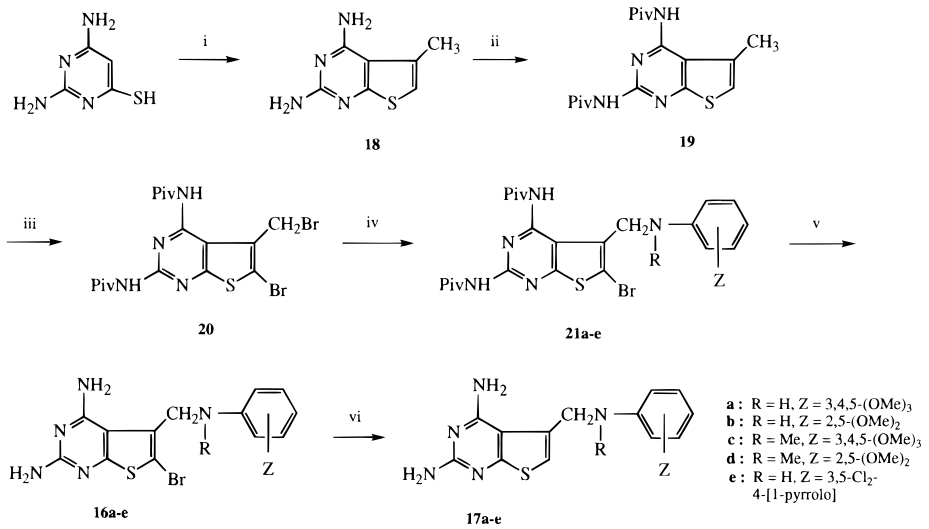
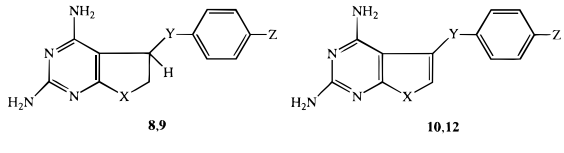
Scheme 1[a]

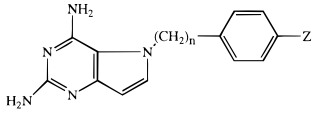


[a] (i) Chloroacetone, $KHCO_3$, DMF (77%); (ii) $(Me_3CCO)_2O$, pyridine (80%; (iii) NBS, Bz_2O_2, $CHCl_3$ (96%); (iv) arylamine (e.g., 3,4,5-trimethoxyaniline), $NaHCO_3$, DMF (56−88%); (v) NaOH, MeOH−H_2O (34−89%); (vi) $NaBH_4$, $PdCl_2$, THF−H_2O (52−98%).

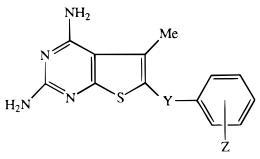
leucovorin, but unfortunately such compounds are yet to be developed.



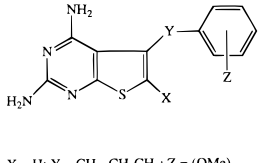
8: X = CH_2; Y = CH_2, CH_2CHR (R = Me, Et), CH_2NH, CH_2NMe; Z = COGlu
9: X = NH; Y = $(CH_2)_n$ (n = 2,3), CH_2NH, CH_2NMe; Z = COGlu
10: X = NH; Y = $(CH_2)_n$ (n = 2,3), CH_2NH, CH_2NMe; Z = COGlu
12: X = O; Y = CH_2CH_2, CH_2NHCH_2, CH_2NH, CH_2; Z = COGlu



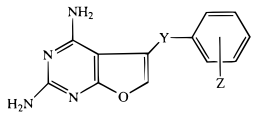
11: n = 2,3; Z = COGlu



13: Y = none, CH_2, CH_2CH_2; Z = 3,4,5-$(OMe)_3$, 2,5-$(OMe)_2$



14: X = H; Y = CH_2, CH_2CH_2; Z = $(OMe)_2$
16: X = Br; Y = CH_2NH, CH_2NMe; Z = 3,4,5-$(OMe)_3$ 2,5-$(OMe)_2$, 3,5-$(OMe)_2$-4-(1-pyrrolo)
17: X = H; Y = CH_2NH, CH_2NMe; Z - 3,4,5-$(OMe)_3$ 2,5-$(OMe)_2$, 3,5-$(OMe)_2$-4-(1-pyrrolo)



15: Y = CH_2NH; Z = 3,4,5-$(OMe)_3$, 2,5-$(OMe)_2$, 3,4-Cl_2, 3,4,5-Cl_3

Several potent DHFR inhibitors with 6/5-fused heterocyclic rings and a classical glutamate side chain have recently been reported to have good antitumor activity, including the cyclopenta[*c*]pyrimidines **8**,[18,19] pyrrolo[2,3-*d*]pyrimidines **9** and **10**,[20−24] pyrrolo[3,2-*d*]pyrimidines **11**,[25] and furo[2,3-*d*]pyrimidines **12**.[26,27] Despite their very tight binding to DHFR, these hydrophilic derivatives would not be appropriate to use against *P. carinii* or *T. gondii* because the plasma membrane of these cells lacks the reduced folate carrier protein whose natural function in mammalian cells is to take up exogenous reduced folates, as well as 2,4-diamino antifolates with a glutamate side chain.

Lipophilic 6/5-fused analogues whose uptake is not expected to require active transport by the reduced folate carrier protein have also been described, the first of which were the 2,4-diaminothieno[2,3-*d*]pyrimidines **13** and **14**, which lacked a nitrogen atom in the bridge.[28] A related group of nonclassical analogues with a lipophilic side chain (**15**) were also reported.[26] In the present paper we report the synthesis of 10 new 2,4-diaminothieno[2,3-*d*]pyrimidines (**16**, **17**) which, to our knowledge, are the first examples of this 6/5 ring system with a carbon−nitrogen bridge. Because of the closer bioisosteric relationship between a sulfur atom and two carbons, we speculated that thieno[2,3-*d*]pyrimidines would give a better approximation of the quinazoline ring system than is provided by furo[2,3-*d*]pyrimidines.

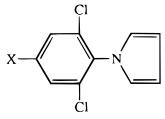
Chemistry

With commercially available 2,4-diamino-6-mercaptopyrimidine as the starting material (Scheme 1), the brominated intermediates **16a**−**e** can be synthesized in five steps, and the final target compounds **17a**−**e** can be obtained by an additional reductive debromination. Initial efforts to obtain compounds of type **17** via 2,4-diamino-5-(chloromethyl)thieno[2,3-*d*]pyrimidine proved unpromising, inasmuch as all attempts to generate this intermediate from 1,3-dichloroacetone and 2,4-diamino-6-mercaptopyrimidine in DMF in the presence of sodium bicarbonate unexpectedly yielded a complex mixture of products, of which none had the desired structure. The failure of this reaction was in contrast to the analogous reaction of 2,4-diamino-6-hydroxypyrimidine, which produces 2,4-diamino-5-chloromethylfuro[2,3-*d*]pyrimidine in good yield.[27,29] Fortunately, a more successful route to **17a**−**e** was found to be via the known intermediate 2,4-diamino-6-methylthieno[2,3-*d*]pyrimidine (**18**).[30] Thus the synthesis began with the reaction of 2,4-diamino-6-mercaptopyrimidine with chloroacetone in refluxing DMF in the presence of potassium bicarbonate, which gave a 77% yield of **18** after recrystallization from methanol. Protection of the amino groups

with pivalic anhydride in refluxing pyridine gave **19** in 80% yield after recrystallization from ethyl acetate. Attempted monobromination of **19** with 1 equiv of NBS in the presence of benzoyl peroxide occurred with predominant attack on the thiophene ring, as evidenced by the complete disappearance of the SCH═ singlet at δ 6.5 in the ^{1}H-NMR spectrum of the crude product. On the other hand, dibromination with 2.2 equiv of NBS in chloroform containing a catalytic amount of benzoyl peroxide afforded **20** in 96% yield after column chromatography on silica gel.

Reaction of **20** with substituted anilines and *N*-methylanilines in DMF containing excess sodium bicarbonate gave intermediates **21a**−**e** in yields of 56−88% depending on the aniline. The coupled products were isolated by silica column chromatography. Deprotection with sodium hydroxide in methanol and water gave **16a**−**e** in yields of 34−89%. Attempted dehalogenation of **16a** in 1:1 $CHCl_3$−MeOH solution with H_2 (50 lb/in.^{2}) in the presence of 10% Pd/C yielded a complex mixture of compounds from which the retrosynthetic 3,4,5-trimethoxyaniline was isolated in high yield. Treatment with 1.1 equiv of tributyltin hydride in refluxing THF for 3 days gave back 30% of unchanged **21a** along with a complex mixture of unidentified products. However hydrodebromination of **21a**−**d** was successfully accomplished with $NaBH_4$ and $PdCl_2$ in aqueous THF, which afforded **17a**−**d** in 52−98% yield.

The commercially unavailable *N*-methylanilines needed for the synthesis of **17c** and **17d** were prepared from 3,4,5-trimethoxy- and 2,5-dimethoxyaniline by acylation with 98% HCO_2H and reduction with $LiAlH_4$ in THF as previously reported.[31] The starting material for the synthesis of **17e**, the hitherto unknown pyrrole **22**, was prepared in excellent yield from commercially available 2,6-dichloro-4-nitroaniline in two steps. Treatment with 2,4-dimethoxytetrahydrofuran in refluxing AcOH afforded *N*-(2,6-dichloro-4-nitrophenyl)pyrrole (**22**, 82% yield), and the latter was reduced with $SnCl_2$ to obtain **23** (93% yield).



22 : X = NO_2
23 : X = NH_2

Enzyme Inhibition Assays

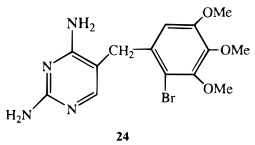
Compounds **16a**−**c** and **17a**−**e** were tested as inhibitors of DHFR from *P. carinii*, *T. gondii*, and rat liver as described previously.[32,33] Unfortunately, most of the thienopyrimidines were too insoluble to allow an IC_{50} to be determined. Thus it was not possible to compare the DHFR binding affinity of the two series. To our surprise, however, three of the brominated derivatives (**16a**,**c**,**e**) proved to be more soluble than their nonbrominated counterparts, allowing an IC_{50} to be reached. As shown in Table 1, the IC_{50} varied from 7.5 to 31 μM against the *P. carinii* enzyme, from 26 to 127 μM against the *T. gondii* enzyme, and from 10 to 33 μM against the rat liver enzyme. The most potent compound against each enzyme was the pyrrole derivative **16e**, whereas the least potent was the 3,4,5-trimethoxy analogue **16c**. A methyl group on N^{10} appeared to increase potency slightly depending on the enzyme, with

Table 1. Inhibition of *P. carinii, T. gondii,* and Rat Liver Dihydrofolate Reductase

	IC_{50} (μM)[b]			selectivity ratio[c]	
compd[a]	rat liver	*P. carinii*	*T. gondii*	*P. carinii*	*T. gondii*
16a	17	13	34	1.2	0.49
16b	33	>100	>100	ND	ND
16c	28	31	127	0.88	0.22
16d	>10	>10	>10	ND	ND
16e	10	7.5	26	1.4	0.39
TMP (**1**)	130	12	2.7	11	48
PM (**2**)	2.3	3.7	0.39	0.62	5.9
TMQ (**3**)	0.003	0.042	0.01	0.07	0.30
PTX (**4**)	0.015	0.031	0.017	0.048	0.088

[a] Compounds **17a**−**e** had IC_{50} values of >10 μM against all three enzymes with <30% inhibition at 10 μM. Higher concentrations could not be tested because of insufficient aqueous solubility. Data shown for TMP, PM, TMQ, and PTX for comparison purposes are from ref 35. [b] Enzyme activity was determined spectrophotometrically at 340 nm according to a standardized and highly reliable method which has been in continuous use in this program for a number of years.[26,27,31−35] As an illustration of the reproducibility of the assay, the IC_{50} value (mean ± standard error) obtained by S.F.Q. over a 5-year period using the pyrimethamine against rat liver and Pc DHFR has been 1.52 ± 0.32 and 2.39 ± 0.42 μM, respectively. [c] IC_{50} (rat liver)/IC_{50} (*P. carinii* or *T. gondii*).

P. carinii and *T. gondii* DHFR being a little more sensitive to this substitution than the mammalian enzyme. Compound **16b** inhibited rat liver DHFR with an IC_{50} of 33 μM, whereas the corresponding value for **16a** was 17 μM, suggesting that 2,5-dimethoxy substitution was slightly less favorable than 3,4,5-trimethoxy substitution, as had also been the case with other thienopyrimidines studied earlier.[28] The presence of a space-filling Br atom at the 6-position was notable in view of previous reports showing that three other compounds with a bromine atom adjacent to the bridge, namely 2,4-diamino-5-(2-bromo-3,4,5-trimethoxybenzyl)-pyrimidine (**24**),[12] 2,4-diamino-6-(2-bromo-3,4,5-trimethoxybenzyl)-5,6,7,8-tetrahydropyrido[4,3-*c*]pyrimidine (**25**),[34] and 2,4-diamino-6-(2-bromo-3,4,5-trimethoxybenzyl)-5-methylthieno[2,3-*d*]pyrimidine (**26**),[35] were better inhibitors of DHFR than their nonbrominated counterparts. In the present work, in contrast to compounds **24**−**26**, a space-filling Br atom on the heterocyclic moiety adjacent to the CH_2NH or CH_2NMe bridge did not seem to have a markedly favorable effect on either potency or selectivity.



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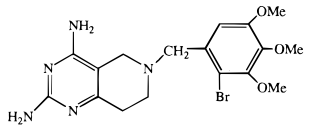

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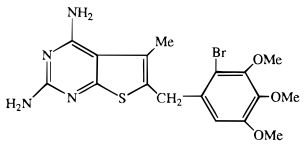

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Experimental Section

IR spectra were obtained on a Perkin-Elmer Model 781 double-beam recording spectrophotometer. ^{1}H NMR spectra were recorded at 60 MHz on a Varian Model EM360 instrument using Me_4Si as the reference or at 500 MHz on a Varian VX500 instrument. TLC analyses were done on Whatman

MK6F silica gel plates, using 254-nm illumination to visualize the spots. Column chromatography was on Baker 7024 flash silica gel (40 mm particle size). Solvents for moisture-sensitve reactions were purchased from Aldrich. Melting points were determined in Pyrex capillary tubes using a Mel-Temp apparatus (Laboratory Devices, Inc., Cambridge, MA) and are not corrected. Elemental analyses were performed by QTI Laboratories, Whitehouse, NJ, or Robertson Laboratories, Madison, NJ, and were within ±0.4% of theoretical values unless otherwise indicated.

2,4-Diamino-5-methylthieno[2,3-*d*]pyrimidine (18). Chloroacetone (16.4 g, 177 mmol) was added to a stirred mixture of 2,4-diamino-6-mercaptopyrimidine hemisulfate (33.8 g, 177 mmol) and $KHCO_3$ (18.6 g, 186 mmol) in dry DMF (250 mL). The mixture was heated at reflux overnight under N_2 and then allowed to cool to room temperature. The mixture was concentrated to dryness by rotary evaporation, EtOAc was added to the residue, and the insoluble portion was filtered off. Evaporation of the filtrate and recrystallization of the residue from MeOH afforded a white solid (25.9 g, 77%): mp 213−215 °C (lit.[30] mp 210−212 °C); ^{1}H NMR ($CDCl_3$) δ 2.4 (s, 3H, CH_3), 5.9 (s, 2H, NH_2), 6.4 (s, 2H, NH_2), 6.5 (s, 1H, SCH=).

2,4-Bis(pivaloylamino)-5-(bromomethyl)-6-bromothieno[2,3-*d*]pyrimidine (20). A mixture of pivalic anhydride (32.8 g, 16.2 mmol) and **18** (1.34 g, 7.44 mmol) in dry pyridine (16 mL) was heated at reflux under N_2 overnight, cooled to room temperature, and evaporated to dryness under reduced pressure. The residue was dissolved in Et_2O (500 mL), the solution was washed with 5% aqueous $NaHCO_3$ (2 × 100 mL), and the organic layer was dried ($MgSO_4$) and evaporated. Recrystallization from Et_2O yielded the dipivaloyl drivative **19** as a white solid pure enough to use directly in the next reaction: yield 2.06 g (80%); mp 179.5 °C; ^{1}H NMR (CD_3OD) δ 1.3 (s, 18H, Me_3C), 2.5 (s, 3H, Me), 7.2 (s, 1H, SCH=); IR (KBr) ν 3420, 3210, 2960, 2870, 1685, 1600, 1550, 1470, 1420, 1295, 1160, 925, 750, 730 cm^{-1}.

To 600 mL of stirred $CHCl_3$, cooled to 0 °C in an ice bath, were added **19** (2.61 g, 7.48 mmol), NBS (1.62 g, 9.08 mmol), and Bz_2O_2 (0.209 g, 0.825 mmol). The ice bath was removed, and the resulting solution was allowed to warm to room temperature. After overnight stirring, more NBS (9.10 g, 51 mmol) and Bz_2O_2 (1.16 g, 4.6 mmol) were added, and stirring was continued for a total of 6 days. A yellow precipitate was filtered off, and the filtrate was washed with H_2O (2 × 50 mL), dried (Na_2SO_4), and evaporated. Silica gel column chromatography using EtOAc−heptanes (1:1) gave a light-yellow solid (3.64 g, 96%): mp 230−332 °C dec >200 °C; ^{1}H NMR ($CDCl_3$) δ 1.35 (s, 18H, Me_3C), 5.15 (s, 2H, CH_2), 8.25 (s, 2H, NH); IR (KBr) ν 3250, 2850, 2880, 1690, 1660, 1600, 1550, 1440, 1365, 1290, 1165, 940, 780 cm^{-1}. Anal. ($C_{17}H_{22}Br_2N_4O_2S$) C, N; H: calcd, 4.38; found, 3.92.

***N*-(2,6-Dichloro-4-nitrophenyl)pyrrole (22).** A solution of 2,5-dimethoxytetrahydrofuran (3.52 g, 0.0266 mol) and 2,6-dichloro-4-nitroaniline (5.0 g, 0.0242 mol) in glacial AcOH (150 mL) was heated at reflux overnight under N_2 and then cooled to room temperature. The AcOH was removed by rotary evaporation, and the crude residue was taken up in Et_2O (100 mL). The Et_2O solution was washed with concentrated aqueous $NaHCO_3$ (30 mL), rinsed with H_2O (2 × 20 mL), dried (Na_2SO_4), and evaporated. Silica gel column chromatography using 1:4 EtOAc−heptanes, followed by recrystallization from the same solvent mixture, gave a yellow-orange solid (5.09 g, 82%); mp 92.5−93.5 °C; ^{1}H NMR ($CDCl_3$) δ 6.4 (m, 2H, pyrrole 3-H), 6.75 (m, 2H, pyrrole 2-H), 8.35 (s, 2H, phenyl protons); IR (KBr) ν 3140, 3080, 1530, 1490, 1345, 1155, 1080, 1010, 905, 890, 810, 760, 730 cm^{-1}. Anal. ($C_{10}H_6Cl_2N_2O_2$) C, H, N.

***N*-(2,6-Dichloro-4-aminophenyl)pyrrole (23).** $SnCl_2 \cdot H_2O$ (18.6 g, 0.098 mol) was added to a stirred solution of **22** (18.6 g, 0.0198 mol) in EtOAc (70 mL), and the mixture was heated at reflux under N_2 for 3.5 h and then cooled to room temperature. The reaction mixture was diluted with EtOAc (100 mL) and treated with 5% aqueous $NaHCO_3$ (500 mL). A white precipitate was filtered off, the two layers in the filtrate were separated, and the organic layer was dried (Na_2SO_4) and evaporated to a yellow solid (4.2 g, 93%): mp 172−174 °C; ^{1}H NMR ($CDCl_3$) δ 3.9 (s, 2H, NH_2), 6.3 (m, 2H, pyrrole 3-H), 6.7 (m, 2H, pyrrole 2-H), 6.75 (s, 2H, phenyl protons); IR (KBr) ν 3440, 3350, 3125, 3100, 1625, 1500, 1430, 1280, 1200, 1065, 1010, 805 cm^{-1}. Anal. ($C_{10}H_8Cl_2N_2$) C, H, N.

2,4-Bis(pivaloylamino)-5-[(3,4,5-trimethoxyanilino)methyl]-6-bromothieno[2,3-*d*]pyrimidine (21a). A mixture of **20** (500 mg, 1.05 mmol), 3,4,5-trimethoxyaniline (125 mg, 0.682 mmol), and $NaHCO_3$ (888 mg, 10.6 mmol) in dry DMF (4 mL) was stirred at 55 °C with stirring for 1 day. The DMF was removed by rotary evaporation, and the residue was triturated with EtOAc (50 mL). The undissolved solid was filtered off, the filtrate was evaporated, and the residue was purified by silica gel column chromatography using 1:1 EtOAc−heptanes: yield 232 mg (56% based on the aniline); mp 211−214 °C dec; ^{1}H NMR ($CDCl_3$) δ 1.1−1.4 (m, 18H, Me_3C), 3.8 (m, 9H, OMe), 4.2−4.4 (m, 3H, NH, CH_2), 5.85 (s, 1H, phenyl proton), 6.2 (s, 1H, phenyl proton), 8.2 (s, 1H, NH), 8.8 (s, 1H, NH); IR (KBr) ν 3400, 3215, 2985, 2915, 1715, 1600, 1430, 1235, 1160, 1130, 1005, 780 cm^{-1}. Anal. ($C_{26}H_{34}BrN_5O_5S \cdot H_2O$) C, H, N.

2,4-Bis(pivaloylamino)-5-[(2,5-dimethoxyanilino)methyl]-6-bromothieno[2,3-*d*]pyrimidine (21b). A mixture of **20** (0.130 g, 0.273 mol), 2,5-dimethoxyaniline (0.027 g, 0.177 mmol), and $NaHCO_3$ (0.231 g, 2.75 mmol) in dry DMF (2 mL) was stirred at 60 °C overnight and worked up as in the synthesis of **21a**: yield 74 mg (72% based on the aniline); mp 225−226 °C dec; ^{1}H NMR ($CDCl_3$) δ 1.35 (s, 18H, Me_3C), 3.78 (s, 3H, OMe), 3.8 (s, 3H, OMe), 4.35 (s, 2H, CH_2), 6.4 (d, 1H, phenyl proton), 6.45 (s, 1H, phenyl proton), 6.75 (d, 1H, phenyl proton), 8.9 (s, 2H, CONH); IR (KBr) ν 3440, 3320, 2950, 1690, 1595, 1550, 1500, 1455, 1285, 1160 cm^{-1}. Anal. ($C_{25}H_{32}BrN_5O_4S$) C, H, N.

2,4-Bis(pivaloylamino)-5-[(3,4,5-trimethoxy-*N*-methylanilino)methyl]-6-bromothieno[2,3-*d*]pyrimidine (21c). A mixture of **20** (0.250 g, 0.525 mmol), 3,4,5-trimethoxy-*N*-methylaniline (0.067 g, 0.340 mmol), and $NaHCO_3$ (0.444 g, 5.28 mmol) was stirred at 55 °C overnight and worked up as in the synthesis of **21a**: yield 163 mg (77% based on the aniline); mp softening 112−115 °C; ^{1}H NMR ($CDCl_3$) δ 1.2 (s, 9H, Me_3C), 1.3 (s, 9H, Me_3C), 2.8 (s, 3H, NMe), 3.8 (s, 9H, OMe), 4.35 (s, 2H, CH_2), 6.3 (s, 2H, phenyl protons), 8.7 (s, 2H, CONH); IR (KBr) ν 3550, 3230, 2960, 2870, 1700, 1600, 1550 1420, 1395, 1235, 1125, 1000, 785 cm^{-1}. Anal. ($C_{27}H_{36}BrN_5O_5S$) C, H, N.

2,4-Diamino-5-[(3,4,5-trimethoxyanilino)methyl]-6-bromothieno[2,3-*d*]pyrimidine (16a). A solution of **21a** (472 mg, 0.758 mmol) in MeOH (100 mL) was treated with 1 M aqueous NaOH (50 mL) and stirred at 35−40 °C under N_2 for 1 day. The white solid that precipitated was filtered, washed with distilled H_2O (3 × 10 mL), and dried in air: yield 210 mg (63%); mp 214.5−215.5 °C; ^{1}H NMR ($CDCl_3$) δ 3.8 (s, 3H, 4-OMe), 3.85 (s, 6H, 3- and 5-OMe), 4.35 (d, 2H, CH_2), 4.8 (s, 2H, NH_2), 5.0−6.0 (broad s, 2H, NH_2), 6.1 (s, 2H, aryl protons), 6.2 (broad s, 1H, NH); IR (KBr) ν 3440, 3320, 3180, 2910, 2830, 1610, 1550, 1500, 1445, 1230, 1125, 1000, 900, 780 cm^{-1}. Anal. ($C_{16}H_{18}BrN_5O_3S \cdot 0.25H_2O$) C, H, N.

2,4-Diamino-5-[(2,5-dimethoxyanilino)methyl]-6-bromothieno[2,3-*d*]pyrimidine (16b). Treatment of **21b** (605 mg, 1.05 mmol) with 1 M NaOH (50 mL) in MeOH (100 mL) at 35−40 °C under N_2 for 36 h was followed by a workup similar to that of **16a**: yield 235 mg (55%); mp 225 °C; ^{1}H NMR ($CDCl_3$) δ 3.79 (s, 6H, OMe), 4.33 (s, 2H, CH_2), 4.76 (s, 2H, NH_2), 6.18 (broad s, 2H, NH_2), 6.37 (d, 1H, phenyl proton), 6.5 (s, 1H, phenyl proton), 6.75 (d, 1H, phenyl proton); IR (KBr) ν 3480, 3410, 3340, 3120, 2960, 2830, 1605, 1560, 1510, 1220, 1135, 1015, 910, 850 cm^{-1}. Anal. ($C_{15}H_{16}BrN_5O_2S$) C, H, N.

2,4-Diamino-5-[(3,4,5-trimethoxy-*N*-methylanilino)methyl]-6-bromothieno[2,3-*d*]pyrimidine (16c). A solution of **21c** (472 mg, 0.758 mmol) in MeOH (100 mL) was treated with 1 M aqueous NaOH (50 mL) and stirred at 35−40 °C under N_2 for 1 day. The white precipitate was collected, washed with distilled H_2O (3 × 10 mL), and dried in air, yield 210 mg (61%). The filtrate was extracted with $CHCl_3$ (3 × 100 mL), and the combined extracts were dried (Na_2SO_4), evaporated, and chromatographed on silica gel using MeOH−$CHCl_3$ (1:9) to recover an additional 91 mg (26%, total yield

87%): mp 205.5−207.5 °C; ^{1}H NMR ($CDCl_3$) δ 2.75 (s, 3H, NMe), 3.8 (s, 3H, 4-OMe), 3.85 (s, 6H, 3- and 5-OMe), 4.25 (s, 2H, CH_2), 4.78 (s, 2H, NH_2), 5.0−6.0 (broad s, 2H, NH_2), 6.3 (s, 2H, phenyl protons); IR (KBr) ν 3550, 3470, 3260, 3120, 2940, 2840, 1640, 1600, 1510, 1230, 1130, 990, 800 cm^{-1}. Anal. ($C_{17}H_{20}N_5O_3 \cdot 0.4H_2O$) C, H, N.

2,4-Diamino-5-[(2,5-dimethoxy-*N*-methylanilino)-methyl]-6-bromothieno[2,3-*d*]pyrimidine (16d). A mixture of **20** (0.910 g, 1.91 mmol), 2,5-dimethoxy-*N*-methylaniline (0.209 g, 1.25 mmol), and $NaHCO_3$ (1.05 g, 12.5 mmol) in dry DMF (5 mL) was stirred at 50−55 °C overnight and worked up as in the synthesis of **21a**. The product (**21d**) was pure enough to use directly in the next step: yield 654 mg (86% based on the aniline); mp 149−151.5 °C; ^{1}H NMR ($CDCl_3$) δ 1.3 (s, 18H, Me_3C), 2.8 (s, 3H, NMe), 3.6 (s, 3H, OMe), 3.8 (s, 3H, OMe), 4.3 (s, 2H, CH_2), 6.6 (m, 2H, aryl protons), 6.8 (m, 1H, aryl proton), 8.6 (s, 2H, CONH); IR (KBr) ν 3470, 3200, 2950, 2870, 1700, 1690, 1600, 1550, 1415, 1325, 1270, 1220, 1150, 1050, 1025, 940, 795, 785 cm^{-1}.

A solution of **21d** (605 mg, 0.904 mmol) in MeOH (100 mL) was treated with 1 M NaOH (50 mL) and stirred at 35 °C under N_2 for 1 day. The precipitate was filtered, washed with distilled H_2O (2 × 10 mL), and dried in air, yield 290 mg. The filtrate was cooled at 5 °C overnight to obtain a second crop: total yield 342 mg (89%); mp 218.5−220 °C; ^{1}HNMR ($CDCl_3$) δ 2.63 (s, 3H, NMe), 3.78 (s, 3H, OMe), 3.81 (s, 3H, OMe), 4.22 (s, 2H, CH_2), 4.78 (s, 2H, NH_2), 6.1−6.3 (broad s, 2H, NH_2), 6.62 (d, 1H, phenyl proton), 6.8 (m, 2H, phenyl protons); IR (KBr) ν 3460, 3280, 3170, 3020, 2910, 2800, 2770, 1620, 1530, 1480, 1425, 1270, 1210, 1160, 1140, 1100, 1035, 1010, 895, 770 cm^{-1}. Anal. ($C_{16}H_{18}BrN_5O_2S$) C, H, N.

2,4-Diamino-5-[[3,5-dichloro-4-(1-pyrrolo)anilino]-methyl]-6-bromothieno[2,3-*d*]pyrimidine (16e). A mixture of **20** (0.209 g, 0.440 mmol), **23** (0.100 g, 0.440 mmol), and $NaHCO_3$ (0.370 g, 4.40 mmol) in dry DMF (3 mL) was stirred at 55−60 °C overnight and worked up as in the synthesis of **21a**. The product (**21e**) was pure enough to use in the next step: yield 253 mg (88% based on the aniline); mp 226−228 °C; ^{1}H NMR ($CDCl_3$) δ 1.4 (s, 18H, Me_3C), 4.1 (d 1H, NH), 4.7 (d, 2H, CH_2), 6.35 (m, 2H, pyrrole 3-H), 6.7 (m, 2H, pyrrole 2-H), 6.75 (m, 2H, aryl protons), 8.3 (s, 2H, CONH); IR (KBr) ν 3200, 2950, 2850, 1680, 1590, 1535, 1400, 1280, 1150, 1000, 700 cm^{-1}.

A solution of **21e** (95 mg, 0.758 mmol) in MeOH (20 mL) was treated with 1 M NaOH (20 mL) and worked up as in the synthesis of **16a**: yield 20 mg (34%); mp 260−261 °C; ^{1}H NMR ($CDCl_3$) δ 4.03 (m, 1H, NH), 4.4 (d, 2H, CH_2), 4.8 (s, 2H, NH_2), 5.8 (s, 2H, NH_2), 6.4 (m, 2H, pyrrole 3-H), 6.7 (m, 2H, pyrrole 2-H), 6.9 (s, 2H, phenyl protons); IR (KBr) ν 3480, 3380, 3230, 3100, 2825, 1600, 1500, 1390, 1290, 1080, 1010, 910, 810, 730 cm^{-1}. Anal. ($C_{17}H_{13}BrCl_2N_6S$) C, H, N.

2,4-Diamino-5-[(3,4,5-trimethoxyanilino)methyl]thieno-[2,3-*d*]pyrimidine (17a). To a stirred solution of **16a** (20 mg, 0.045 mmol) in 1:1 THF−H_2O (4 mL) cooled to 0 °C in an ice bath were added $PdCl_2$ (37 mg, 0.091 mmol) and $NaBH_4$ (17 mg, 0.45 mmol). After 5 min at 0 °C, the bath was removed, stirring was continued for 7 h, and the THF was removed by rotary evaporation. The mixture was diluted with H_2O (10 mL) and the product extracted with $CHCl_3$ (30 mL). The organic layer was washed with H_2O (2 × 10 mL), dried (Na_2SO_4), and concentrated to dryness. The residue was purified by preparative TLC on silica gel plates using 92:8 $CHCl_3$−MeOH: yield 16 mg (98%); mp 223−224.5 °C; ^{1}H NMR ($CDCl_3$) δ 3.8 (s, 3H, 4-OMe), 3.85 (s, 6H, 3- and 5-OMe), 4.3 (s, 2H, CH_2), 4.8 (s, 2H, NH_2), 5.0−6.0 (broad s, 1H, NH), 6.05 (s, 2H, phenyl protons), 6.2−6.4 (broad s, 2H, NH_2), 6.81 (s, 1H, SCH═); IR (KBr) ν 3420, 3360, 3200, 2960, 2920, 2860, 1600, 1550, 1500, 1290, 1235, 1130, 1000, 955, 920 cm^{-1}. Anal. ($C_{16}H_{19}N_5O_3S \cdot 0.25H_2O$) C, H, N.

2,4-Diamino-5-[(2,5-dimethoxyanilino)methyl]thieno-[2,3-*d*]pyrimidine (17b). To a stirred solution of **16b** (50 mg, 0.122 mmol) in 1:1 THF−H_2O (10 mL) cooled to 0 °C in an ice bath were added $PdCl_2$ (44 mg, 0.244 mmol) and $NaBH_4$ (46 mg, 1.22 mmol). After 20 min at 0 °C the bath was removed, stirring was contined for 4 h, and the reaction mixture was filtered through Celite. The Celite pad was rinsed with a 1:1 MeOH−H_2O, the MeOH and THF were removed by rotary evaporation, and the remaining aqueous phase was extracted with $CHCl_3$ (2 × 100 mL). The combined extracts were washed with H_2O (2 × 20 mL), dried (Na_2SO_4), and evaporated. The residue was purified by preparative TLC on silica gel plates using 95:5 $CHCl_3$−MeOH to obtain a light-yellow solid: yield 21 mg (52%); mp 196.5−198.5 °C; ^{1}H NMR ($CDCl_3$) δ 3.8 (s, 6H, OMe), 4.28 (s, 2H, CH_2), 4.42 (m, 1H, NH), 4.76 (s, 2H, NH_2), 6.18 (broad s, 2H, NH_2), 6.37 (d, 1H, phenyl proton), 6.5 (s, 1H, phenyl proton), 6.74 (d, 1H, phenyl proton), 6.8 (s, 1H, SCH═). Anal. ($C_{15}H_{17}N_5SO_2 \cdot {}^1/_2H_2O \cdot {}^1/_6CH_3OH$) C, H, N.

2,4-Diamino-5-[(3,4,5-trimethoxy-*N*-methylanilino)-methyl]thieno[2,3-*d*]pyrimidine (17c). To a solution of **16c** (100 mg, 0.220 mmol) in THF (25 mL) were added $PdCl_2$ (77 mg, 0.44 mmol) and H_2O (25 mL). The mixture was cooled to 0−5 °C, $NaBH_4$ (83 mg, 2.2 mmol) was added, and stirring was continued for 15 min in the ice bath and for 3.5 h at room temperature. The rest of the workup was similar to the preceding experiment except that 8.5:1 CH_2Cl_2−MeOH (4 × 100 mL) was used to extract the aqueous phase after removal of the THF and MeOH. The combined extracts were washed with H_2O (30 mL), dried (Na_2SO_4), and evaporated: yield 67 mg (81%); mp 185.5−187 °C (crystallized from MeOH); ^{1}H NMR ($CDCl_3$) δ 2.7 (s, 3H, NMe), 3.85 (s, 3 H, OMe), 3.9 (s, 6 H, OMe), 4.3 (s, 2H, CH_2N), 4.9 (s, 2H, NH_2), 6.4 (s, 2H, phenyl proton), 6.6 (s, 2H, NH_2), 6.75 (s, 1H, SCH═); IR (KBr) ν 3420, 3310, 3190, 2990, 2915, 2815, 1650, 1600, 1550, 1440, 1230, 1125, 1000, 960, 790, 770 cm^{-1}. Anal. ($C_{17}H_{21}N_5SO_3 \cdot 0.5H_2O$) C, H, N.

2,4-Diamino-5-[(2,5-dimethoxy-*N*-methylanilino)methyl]thieno[2,3-*d*]pyrimidine (17d). A solution of **16c** (100 mg, 0.236 mmol) in 1:1 THF−H_2O (50 mL) was cooled in an ice bath to 0−5 °C and treated with $PdCl_2$ (84 mg, 0.472 mmol) followed by $NaBH_4$ (89 mg, 2.4 mmol). The rest of the workup was similar to that of **17b** except that the combined $CHCl_3$ extracts were washed first with H_2O (20 mL) and then with brine (20 mL); yield 59 mg (72%). The analytical sample was purified by preparative tlc on silica gel with 8.5:1 $CHCl_3$−MeOH: mp 191.5−193.5 °C; ^{1}H NMR ($CDCl_3$) δ 2.59 (s, 3H, NMe), 3.78 (s, 3H, OMe), 3.83 (s, 3H, OMe), 4.15 (s, 2H, CH_2), 4.78 (s, 2H, NH_2), 5.4−6.0 (broad s, 2H, NH_2), 6.62 (d, 1H, phenyl proton), 6.71 (s, 1H, SCH═), 6.76 (d, 1H, phenyl proton), 6.82 (d, 1H, phenyl proton); IR (KBr) ν 3460, 3420, 3290, 3170, 2930, 2820, 1630, 1560, 1510, 1445,1220, 1025, 850 cm^{-1}. Anal. ($C_{16}H_{19}N_5SO_2 \cdot {}^1/_8H_2O$) C, H, N.

2,4-Diamino-5-[[3,5-dichloro-4-(1-pyrrolo)anilino]-methyl]thieno[2,3-*d*]pyrimidine (17e). To a mixture of 1:1 THF−H_2O (30 mL) cooled to 0 °C in an ice bath were added sequentially $PdCl_2$ (160 mg, 0.90 mmol), $NaBH_4$ (173 mg, 4.57 mmol), and **16e** (220 mg, 0.454 mmol). The cooling bath was removed, and the mixture was stirred overnight and then filtered through Celite. The Celite pad was rinsed with a 1:1 MeOH−H_2O and then MeOH alone. The solid which precipitated in the filtrate was collected, washed with H_2O (2 × 10 mL), and then dried in air, yield 77 mg. A second crop weighing 63 mg was also obtained: total yield 140 mg (76%); mp 231−232 °C; ^{1}H NMR ($CDCl_3$) δ 4.4 (m, 3H, CH_2 and NH), 5.2 (s, 2H, NH_2), 6.2 (s, 2H, NH_2), 6.35 (m, 2H, pyrrole 2-H), 6.65 (m, 2H, pyrrole 3-H), 6.85 (s, 1H, SCH═), 6.9 (s, 2H, phenyl protons); IR (KBr) ν 3405, 3305, 3195, 2950, 2850, 1625, 1600, 1550, 1395, 1290, 1180 1160, 1020, 1000, 920, 805, 725 cm^{-1}. Anal. ($C_{17}H_{14}Cl_2N_6S$) C, H, N.

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