Brominated Trimetrexate Analogues as Inhibitors of *Pneumocystis carinii* and *Toxoplasma gondii* Dihydrofolate Reductase

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Five previously undescribed trimetrexate analogues with bulky 2'-bromo substitution on the phenyl ring were synthesized in order to assess the effect of this structure modification on dihydrofolate reductase inhibition. Condensation of 2-[2-(2-bromo-3,4,5-trimethoxyphenyl)ethyl]-1,1-dicyanopropene with sulfur in the presence of *N,N*-diethylamine afforded 2-amino-5-(2'-bromo-3',4',5'-trimethoxybenzyl)-4-methylthiophene-3-carbonitrile (**15**) and 2-amino-4-[2-(2'-bromo-3',4',5'-trimethoxyphenyl)ethyl]thiophene-3-carbonitrile (**16**). Further reaction with chloroformamidine hydrochloride converted **15** and **16** into 2,4-diamino-5-(2'-bromo-3',4',5'-trimethoxybenzyl)-4-methylthieno[2,3-*d*]pyrimidine (**8a**) and 2,4-diamino-4-[2-(2'-bromo-3',4',5'-trimethoxyphenyl)ethylthieno[2,3-*d*]pyrimidine (**12**) respectively. Other analogues, obtained by reductive coupling of the appropriate 2,4-diaminoquinazoline-6(or 5)-carbonitriles with 2-bromo-3,4,5-trimethoxyaniline, were 2,4-diamino-6-(2'-bromo-3',4',5'-trimethoxyanilinomethyl)-5-chloroquinazoline (**9a**), 2,4-diamino-5-(2'-bromo-3',4',5'-trimethoxyanilinomethyl)quinazoline (**10**), and 2,4-diamino-6-(2'-bromo-3',4',5'-trimethoxyanilinomethyl)quinazoline (**11**). Enzyme inhibition assays revealed that space-filling 2'-bromo substitution in this limited series of dicyclic 2,4-diaminopyrimidines with a 3',4',5'-trimethoxyphenyl side chain and a CH_2, CH_2CH_2, or CH_2NH bridge failed to improve species selectivity against either *P. carinii* or *T. gondii* dihydrofolate reductase relative to rat liver dihydrofolate reductase.

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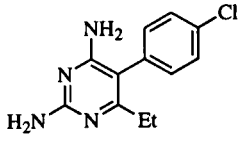
A hallmark of the clinical pathology of patients with AIDS is that their severely compromised immune system leaves them highly vulnerable to a multiplicity of life-threatening microbial infections, of which *Pneumocystis carinii* pneumonia and toxoplasmosis are but two examples [1,2]. While progress over the past few years in the prophylaxis and treatment of *Pneumocystis carinii* pneumonia has been considerable [3], toxoplasmosis in AIDS patients remains an intractable clinical problem [4]. The lipophilic dihydrofolate reductase inhibitors pyrimethamine (**1**) and trimethoprim (**2**), typically in combination with a sulfa drug, are currently the drugs used most often to treat AIDS patients with toxoplasmosis [5] and *Pneumocystis carinii* pneumonia [6], respectively. Epiroprim (**3**), a second-generation analog of trimethoprim with a pyrrole ring replacing the 4'-methoxy group, has recently shown promise when used against *Toxoplasma gondii* in laboratory models in combination with dapsone [7]. Trimetrexate (**4**), originally developed as an antiparasitic agent [8] and used more recently in a number of trials against cancer [9,10], has likewise been evaluated in AIDS patients with toxoplasmosis [11,12]. Piritrexim (**5**), a compound resembling trimetrexate, has been tested against *Toxoplasma gondii* in laboratory models [13,14], but thus far has not had a formal clinical trial against toxoplasmosis in patients with AIDS.

Trimethoprim is highly species-selective in its dihydrofolate reductase affinity, and thus has a high therapeutic index even though its potency is relatively low. In contrast, trimetrexate and piritrexim are much more tightly bound, but are also better inhibitors of mammalian dihydrofolate reductase than they are of *Pneumocystis carinii*



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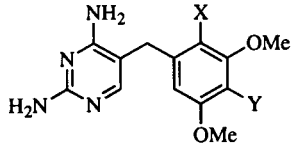
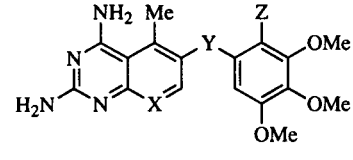


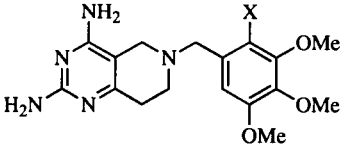
2: X = H, Y = OMe
3: X = H, Y = pyrrolo
7: X = Br, Y = OMe



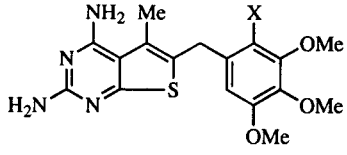
4: X = CH, Y = CH_2NH, Z = H
5: X = N, Y = CH_2, Z = H

or *Toxoplasma gondii* dihydrofolate reductase [14-18]; thus, these agents have to be given in combination with 5-formyl-5,6,7,8-tetrahydrofolate (leucovorin), which, because of its inability to be taken up by the parasites, can selectively protect the host [11]. Lipophilic antifolates combining the *high species selectivity of trimethoprim with the high potency of trimetrexate and piritrexim* at the level of the target enzyme have been the object of a vigorous synthetic effort in this laboratory [19-23] and others [18,24-28]. Several analyses of screening data on archival samples of inhibitors originally synthesized for other purposes have also appeared [16,17,29-31]. To date, the number of compounds with the desired combination of selectivity and potency, which would in principle allow them to be used without leucovorin, has been disappointingly low.

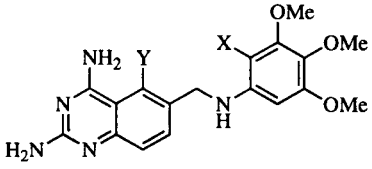
As part of our larger search for new inhibitors of *Pneumocystis carinii* and *Toxoplasma gondii* dihydrofolate reductase with improved potency and/or selectivity, we made the chance observation that 2,4-diamino-6-(2'-bromo-3',4',5'-trimethoxybenzyl)-5,6,7,8-tetrahydropyrido[4,3-*c*]pyrimidine (**6a**) was more active than the non-brominated parent compound **6b** [21]. Moreover, the difference in potency between **6a** and **6b** was greater for the mammalian enzyme than for the *P. carinii* enzyme, suggesting that a space-filling 2'-bromo substituent might favor species selectivity. In considering the possible scope of these findings, we noted that the 2'-bromo derivative **7** of trimethoprim had been reported to be 14-fold more potent against *P. carinii* dihydrofolate reductase than trimethoprim itself [30]. However, except for **7**, the effect on *P. carinii* or *T. gondii* dihydrofolate reductase binding by a space-filling atom or group at the *ortho* position of the 3',4',5'-trimethoxyphenyl ring was unknown. It was therefore of interest to synthesize some 2'-bromo-3',4',5'-trimethoxyphenyl analogs of the 6/6 and 6/5 fused-ring type with either one or two atoms in the bridge between the phenyl ring and heterocyclic moiety. The hypothesis to be tested was that a space-filling 2'-bromo substituent might have a greater effect when the bridge between the condensed diaminopyrimidine moiety and the phenyl ring is short, resulting in greater steric crowding. This paper reports five such compounds, of which two, **8a** and **9a**, could be compared directly with their previously described non-brominated counterparts **8b** and **9b** [19,20]. Three additional 2'-bromo derivatives **10-12** were also prepared. Compound **8a** was like **6a** in that it contained a one-atom bridge (CH_2), whereas **9a** and **10-12** all contained a two-atom bridge (CH_2NH or CH_2CH_2). Thus, if the hypothesis was correct, we expected the greatest effect of 2'-bromo substitution would be in **8a**.



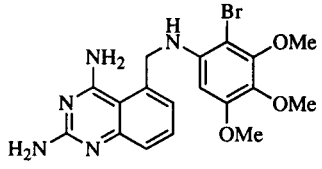
6a: X = Br
6b: X = H



8a: X = Br
8b: X = H



9a: X = Br, Y = Cl
9b: X = H, Y = Cl
11: X = Br, Y = H



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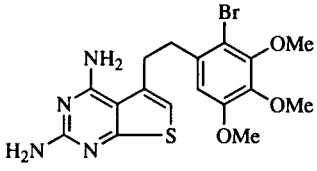

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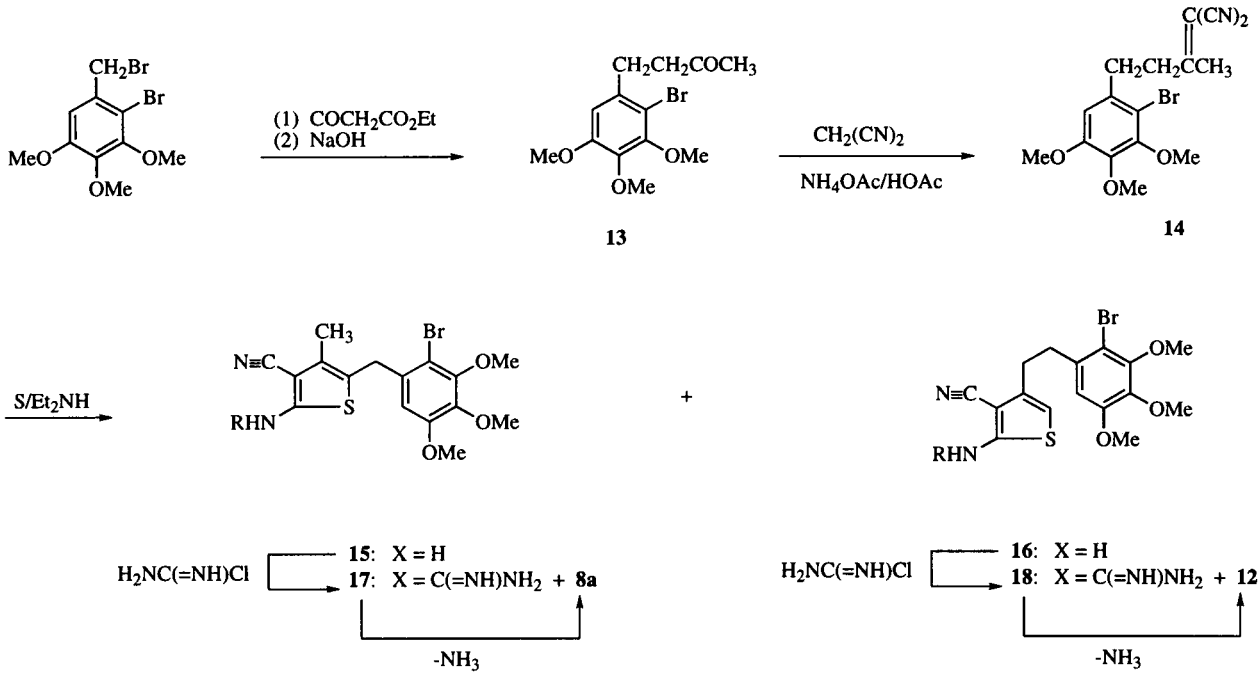
Chemistry.

The thieno[2,3-*d*]pyrimidines **8a** and **12** were prepared *via* a route similar to the one used earlier to obtain other 5- and 6-substituted 2,4-diaminothieno[2,3-*d*]pyrimidines [19]. As shown in Scheme 1, the unknown starting ketone for the synthesis, 4-(2'-bromo-3',4',5'-trimethoxyphenyl)-2-butanone (**13**), was produced in good yield from 2-bromo-3,4,5-trimethoxybenzyl bromide [32] by reaction with ethyl acetoacetate, followed by hydrolysis and decarboxylation. Cope reaction of malononitrile with **13** in the presence of acetic acid and ammonium acetate in refluxing benzene afforded the ylidenemalononitrile **14** (58%). Further reaction with sulfur in the presence of *N,N*-diethylamine at room temperature for 18 hours as described by Gewald [33] yielded a roughly equal mixture of two isomeric amino nitriles **15** and **16**, whose separation by flash chromatography on silica gel proved very difficult. Only the less polar isomer, which eluted first from the column, could be isolated in pure state. It was unambiguously identified as **15** from its ^{1}H nmr spectrum, which contained the expected singlets at δ 2.16 (4-CH_3) and δ 3.94 (bridge CH_2). Fusion of **15** with chloroformamidine hydrochloride at 120° (internal temperature) for 20 minutes under an argon atmosphere yielded a mixture of the desired product **8a** and a small amount of the non-cyclized intermediate **17**, which was detectable by tlc as a less mobile spot. Ring closure to **8a** (23%) was readily completed by heating the mixture at 65° under reduced pressure (0.1 Torr) for 16 hours [19]. The ^{1}H nmr spectrum of **8a**, taken at 500 MHz in d_6-dimethylsulfoxide solution, contained the expected singlets at δ 2.39 (5-CH_3), δ 4.04 (bridge CH_2), and δ 6.76 (6'-H) in addition to broad peaks at δ 5.89 and δ 6.34 for the amino groups and three sharply-resolved singlets in the δ 3.72-3.75 region for the aromatic methoxy groups.

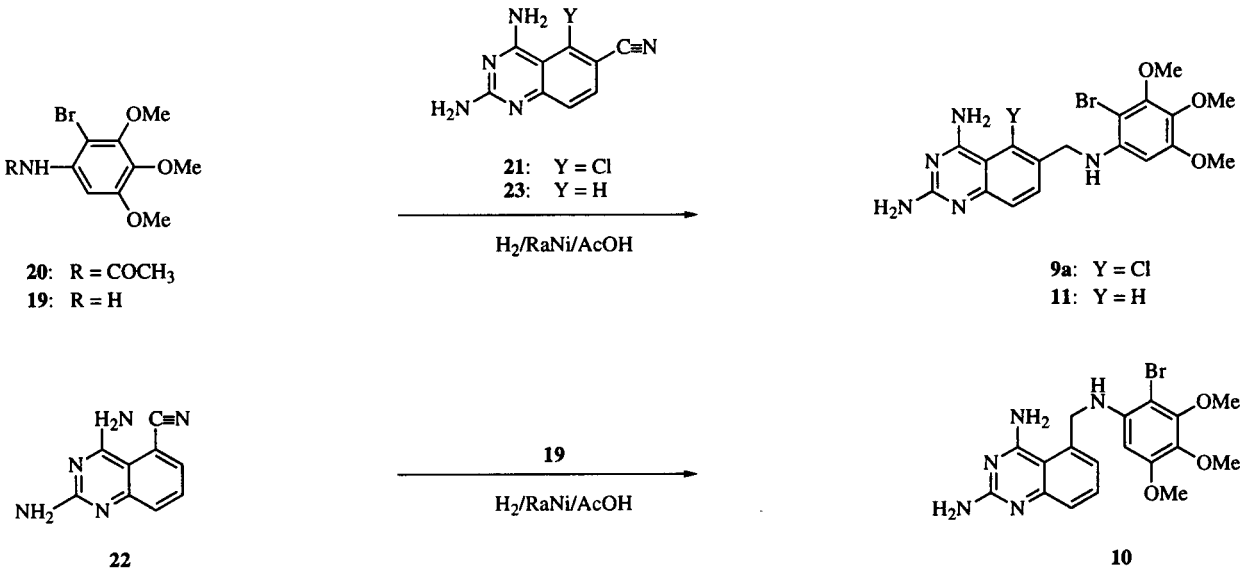
Although a completely purified sample of the second product **16** from the Gewald reaction could not be obtained by flash chromatography, we were gratified to find that, when the chloroformadine hydrochloride fusion reaction was done with the 1:1 mixture of **15** and **16**, the annulation products **8a** and **12** could be separated with relative ease. Not surprisingly, the fusion reaction yielded, in this case, an initial product whose tlc was consistent with a mixture of the **8a** (R_f 0.48) and **12** (R_f 0.56) and the more polar non-cyclized intermediates **17** (R_f 0.35) and **18** (R_f 0.32). When this four-component mixture was heated under reduced pressure (see above) the two slowest-moving spots disappeared. Careful flash chromatography of the remaining material allowed **8a** and **12** to be separated cleanly in yields of 35% and 31% respectively, based on a 1:1 mixture of amino nitrites. The structure of **12**, and hence of **16**, was confirmed by ^{1}H nmr, which showed the bridge CH_2 groups as a pair of multiplets at δ 3.3 and δ 3.0 and the thiophene 6-H as a singlet at δ 6.5.

The starting amine **19** for the synthesis of **9a**, **10**, and **11** was obtained from 3,4,5-trimethoxyaniline by successive *N*-acetylation, treatment with *N*-bromosuccinimide, and hydrolysis of the anilide **20** with sodium hydroxide. The amine was obtained as a purplish oil which could not be induced to form crystals, tended to darken rapidly at

Scheme 1



Scheme 2



room temperature, and was therefore used without purification [34]. Reductive coupling with 2,4-diamino-5-chloroquinazoline-6-carbonitrile (**21**) [20,36], 2,4-diaminoquinazoline-5-carbonitrile (**22**) [22,37], and 2,4-diaminoquinazoline-6-carbonitrile (**23**) [36] in glacial acetic acid in the presence of Raney nickel afforded the desired products (Scheme 2). As in condensations of **21** and **22** we have carried out using other anilines substituted with methoxy groups [20,22], the yields in these reactions were low.

Enzyme Inhibition.

Compounds **8a**, **9a**, and **10-12** were tested as inhibitors of rat liver, *Pneumocystis carinii, Toxoplasma gondii* and rat liver dihydrofolate reductase as described previously [16,17]. The results are listed in the Experimental. The brominated 2,4-diaminothieno[2,3-*d*]pyrimidine analog **8a** was more active against *T. gondii* dihydrofolate reductase than against rat liver dihydrofolate reductase. In contrast, the activity of **8a** against the *P. carinii* enzyme was very low. Thus, while this compound showed modest species selectivity against the *T. gondii* enzyme, its selectivity against the *P. carinii* enzyme was unfavorable. The IC_{50} values of the 2,4-diamino-5-chloroquinazoline analog **9a** against rat liver, *Pneumocystis carinii,* and *Toxoplasma gondii* dihydrofolate reductase were much lower than those of **6a** or **8a**, in agreement with our previous findings with other highly potent 2,4-diamino-5-chloroquinazolines related to trimetrexate and piritrexim [20]. However, the activity of **9a** against each enzyme did not differ substantially from that of its non-brominated counterpart **9b**, showing that bromination did not have a favorable effect on either potency or selectivity when the bridge was CH_2NH. Thus, an effect on species-specific binding to dihydrofolate reductase by a space-filling 2'-bromo substituent was not observed in a 6/6 compound when the bridge was longer than one atom in length. As expected from our other recent work on 5- versus 6-substituted-2,4-diaminoquinazoline antifolates [22], compounds **10** and **11** were less active than **9a** against all the enzymes, though **11** did have a modest degree of species selectivity (8.5-fold) against the *T. gondii* enzyme. Compound **12** was consistently less active than **8a**, in agreement with previous structure-activity correlations in the 2,4-diaminothieno-[2,3-*d*]pyrimidine series [19]. Taken together, the enzyme assay results failed to validate the hypothesis of this work, which had been that a space-filling 2'-bromo substituent on the phenyl ring might favorably increase the potency and/or selectivity of trimethoprim analogues with a short one-carbon bridge between the heterocyclic moiety and the phenyl ring. We had speculated that, in such compounds, steric constraints in the region between the heterocylic moiety and the phenyl ring might be less easily relieved by torsional change than in compounds with a longer bridge, but unfortunately the enzyme from rat liver proved more sensitive to this type of steric effect than the enzyme from *T. gondii or P. carinii.*

The antiparasitic effect of an antifolate against intact *P. carinii* and *T. gondii* organisms and its effects on sensitive tissues of the host obviously depend on a number of factors other than enzyme inhibition (*e.g.* cellular uptake). However, since the 2'-bromo compound reported in this paper did not display either a significantly greater potency

or a significantly greater selectivity than their non-brominated counterparts against cell-free dihydrofolate reductase, they were not studied further.

EXPERIMENTAL

The ir spectra were obtained on a Perkin-Elmer Model 781 double-beam recording spectrophotometer; only peaks with wave numbers greater than 1200 cm^{-1} are reported. Quantitative uv absorbance spectra were measured on a Varian Model 210 instrument. The ^1H nmr spectra were recorded on a Varian Model EM360 instrument using tetramethylsilane as the reference, or in some instances on a Varian Model VXR500 instrument. Tlc separations were done on Baker Si250F silica gel plates, with spots being visualized under 254-nm illumination. Column chromatography was on Baker 7024 flash silica gel (40 mm particle size). Other chemicals, as well as dry solvents in Sure-Seal bottles, were purchased from Aldrich (Milwaukee WI). Melting points were determined in Pyrex capillary tubes using a Mel-Temp apparatus (Laboratory Devices Inc., Cambridge MA) and are not corrected. Microanalyses were done by QTI Laboratories, Whitehouse, NJ.

4-(2'-Bromo-3',4',5'-trimethoxyphenyl)-2-butanone (**13**).

Sodium metal (344 mg, 15 mmoles) was added to anhydrous ethanol (40 ml) in a round-bottomed flask under an argon atmosphere. When all the sodium dissolved, ethyl acetoacetate (1.38 ml, 15 mmoles) was added dropwise at room temperature and the mixture was stirred for another 15 minutes. A solution of 2-bromo-3,4,5-trimethoxybenzyl bromide [32] (5.0 g, 14.6 mmoles) in ethanol (40 ml) was then added dropwise, and stirring at room temperature was continued for 2 hours. The reaction mixture was concentrated to a volume of *ca.* 1 ml by rotary evaporation, and the product was partitioned between water (50 ml) and ether (50 ml). The layers were separated and the aqueous phase was re-extracted with ether (3 x 50 ml). The combined organic layers were washed with water (50 ml), dried over magnesium sulfate, and evaporated to dryness. Chromatography of the oily residue on silica gel with 4:1 hexanes-ethyl acetate as the eluent yielded a faster-moving band with R_f 0.32 (silica gel, 4:1 hexanes-ethyl acetate) and a slower moving band with R_f 0.12. Evaporation of the faster-moving band afforded an oil (3.8 g, 68%); ir (thin film): ν 2970, 2930, 2830, 1735 (C=O), 1715 (C=O), 1580, 1560, 1480-1450, 1420, 1390, 1340 br, 1250 br, 1200 br cm^{-1}; ^1H nmr (500 MHz, deuteriochloroform): δ 1.21 (t, 3H, CH_3CH_2), 2.23 (s, 3H, MeCO), 3.18-3.4 (m, 2H, CH_2CH), 3.81 (s, 3H, 5-OMe), 3.84 (s, 3H, 4-OMe), 3.87 (s, 3H, 3-OMe), 3.88-3.94 (m, 1H, CH_2CHCO), 4.14 (q, 2H, CH_2CH_3), 6.63 (s, 1H, 6-H). A portion of this material (3.6 g, 9.2 mmoles) was stirred at room temperature in 1*N* sodium hydroxide (20 ml) until complete dissolution occurred (*ca.* 3 hours). The reaction mixture was diluted with water (20 ml) and extracted with ether (3 x 30 ml). The aqueous layer was acidified to *p*H 2 with 6*N* hydrochloric acid, heated at 60° for 1 hour, and extracted with ether (3 x 50 ml). The combined extracts were dried over magnesium sulfate and evaporated to dryness. Chromatography of the residue on silica gel with 3:1 hexanes-ethyl acetate as the eluent yielded the title compound as a colorless oil (1.88 g, 64%); ir (thin film): ν 3000-2800, 1710 (C=O), 1580, 1560, 1480, 1460, 1440, 1420, 1390, 1350-1330, 1270, 1235, 1200 cm^{-1}; ^1H nmr (deuteriochloroform): δ 2.15 (s, 3H, MeCO), 2.75-2.77 (m, 2H, CH_2CH_2CO), 2.94-2.97 (m, 2H, CH_2CH_2CO), 3.89 (s, 3H, 3-OMe), 3.85 (s, 3H, 4-OMe), 3.83 (s, 3H, 5-OMe), 6.62 (s, 1H, 6-H).

Anal. Calcd. for $C_{13}H_{17}BrO_4$: C, 49.23; H, 5.41; Br, 25.19. Found: C, 49.04; H, 5.42; Br, 24.99.

2-[2-(2-Bromo-3,4,5-trimethoxyphenyl)ethyl]-1,1-dicyanopropene (**14**).

Glacial acetic acid (70 µl) and ammonium acetate (47 mg) were added to a stirred solution of the foregoing ketone (704 mg, 2.2 mmoles) and malononitrile (1.38 mg, 2.1 mmoles) in benzene (15 ml), and the mixture was refluxed for 3.5 hours, then cooled and poured into water (30 ml). Extraction with ethyl acetate (3 x 30 ml) followed by washing of the combined extracts with water (25 ml), drying over magnesium sulfate, and rotary evaporation left an oil, which was crystallized from ether-hexanes to obtain white prisms (470 mg, 58%); R_f 0.19 (silica gel, 3:1 hexanes- ethyl acetate); mp 91-93°; ir (thin film): ν 3420 br, 3000-2800, 2225 (C≡N), 1600, 1580, 1560, 1480, 1465, 1450, 1425, 1375, 1340, 1320, 1300, 1260, 1240, 1200 cm^{-1}; ^1H nmr (500 MHz, deuteriochloroform): δ 2.35 (s, 3H, MeC=), 2.85-2.88 (m, 2H, CH_2CH_2C=), 2.95-2.98 (m, 2H, CH_2CH_2C=), 3.89 (s, 3H, 3-OMe), 3.87 (s, 3H, 4-OMe), 3.86 (s, 3H, 5-OMe), 6.57 (s, 1H, 6-H).

Anal. Calcd. for $C_{16}H_{17}BrN_2O_3$: C, 52.62; H, 4.69; Br, 21.88; N, 7.67. Found: C, 52.54; H, 4.69; Br, 22.04; N, 7.54.

2-Amino-5-(2'-bromo-3',4',5'-trimethoxybenzyl)-4-methylthiophene-3-carbonitrile (**15**).

N,N-Diisopropylamine (0.15 ml, 1.09 mmoles) was added dropwise to a stirred mixture of the ylidenemalononitrile **14** (400 mg, 1.1 mmoles) and powdered sulfur (38 mg, 1.2 mmoles) in 95%, ethanol (4 ml), and the mixture was left to stir at room temperature for 18 hours. The volume of the solution was then reduced to *ca.* 0.5 ml by rotary evaporation (bath temperature 35°), 0.2 *N* hydrochloric acid (20 ml) was added, and the product was extracted into ethyl acetate (3 x 40 ml). The combined extracts were washed with water (30 ml), dried over magnesium sulfate, and evaporated to a brown oil under reduced pressure. Column chromatography (flash silica gel, 35 g, 210 x 21 mm) with 3:1 hexanes-ethyl acetate as the eluent afforded two rough fractions, A followed by B. Although both fractions had essentially the same R_f on tlc plates (R_f 0.35, 0.36; silica gel, 3:1 hexanes-ethyl acetate), the ^1H nmr spectrum of fraction A showed it to contain **15** as the major component, whereas the spectrum of fraction B indicated it to be an unresolved mixture of **15** and 2-amino-4-[2-(2'-bromo-3',4',5'-trimethoxyphenyl)ethyl]thiophene-3-carbonitrile (**16**). Recrystallization of fraction A from ether afforded pure **15** as orange prisms (121 mg, 28%), mp 124-126°; ir (potassium bromide): ν 3385, 3300, 3000, 2925, 2830, 2195, 1620, 1570, 1515, 1460, 1440, 1430, 1420, 1390, 1270, 1240, 1200 cm^{-1}; ^1H nmr (500 MHz, d_6-dimethyl sulfoxide): δ 2.16 (s, 3H, 4-Me), 3.79 (s, 3H, 5'-OMe), 3.86 (s, 3H, 4'-OMe), 3.89 (s, 3H, 3'-OMe), 3.94 (s, 2H, CH_2), 4.45 (br s, 2H, NH_2), 6.47 (s, 1H, 6'-H). Fraction A was fused with chloroformamidine hydrochloride to obtain **8a**; fraction **B** was used to obtain a mixture of **8a** and **12** (see below).

Anal. Calcd. for $C_{16}H_{17}BrN_2O_3S$: C, 48.37; H, 4.31; Br, 20.11; N, 7.05; S, 8.07. Found: C, 48.44; H, 4.19; Br, 20.40; N, 6.88; S, 8.02.

2,4-Diamino-5-(2'-bromo-3',4',5'-trimethoxybenzyl)-4-methylthieno[2,3-*d*]pyrimidine (**8a**).

A finely ground mixture of pure **15** (120 mg, 0.3 mmole) and chloroformamidine hydrochloride (137 mg, 1.21 mmoles) was immersed in an oil bath and kept at an internal temperature of 120° for 20 minutes under a gentle stream of argon. The reaction mixture was left to cool, the solid dissolved in methanol (1 ml), and the solution diluted with dichloromethane (15 ml). The resulting precipitate was filtered and the filtrate was concentrated by rotary evaporation to obtain the non-cyclized intermediate **17**; R_f 0.35 (silica gel, 100:10:1 chloroform-methanol-28% ammonium hydroxide). Chromatography on silica gel (100:10:1 dichloromethane-methanol-28% ammonium hydroxide) followed by heating of the purified product under reduced pressure (0.1 Torr) at 75° for 16 hours gave a semi-solid containing mainly cyclized product (R_f 0.48) along with a remnant of noncyclized intermediate (R_f 0.35). Recrystallization from hot methanol afforded pure **8a** as a beige powder (31 mg, 23%), mp 224-225°; ir (potassium bromide): ν 3480, 3405, 3305, 3180, 2930, 1625, 1560, 1520, 1475, 1445, 1395, 1330, 1290, 1245, 1200 cm^{-1}; uv (ethanol): λ max 236 nm (ε 27,064), 282 (15,137); ^1H nmr (d_6-dimethyl sulfoxide): δ 2.39 (s, 3H, 4-Me), 3.72 (s, 3H, 5'-OMe), 3.73 (s, 3H, 4'-OMe), 3.75 (s, 3H, 3'-OMe), 4.04 (s, 2H, CH_2), 5.89 (br s, 2H, NH_2), 6.34 (br s, 2H, NH_2), 6.76 (s, 1H, 6'-H).

Anal. Calcd. for $C_{17}H_{19}BrN_4O_3S \cdot 0.3H_2O$): C, 45.87; H, 4.44; N, 12.59; S, 7.21. Found: C, 45.92; H, 4.33; N, 12.33; S, 7.04.

2,4-Diamino-4-[2-(2'-bromo-3',4',5'-trimethoxyphenyl)ethyl] thieno[2,3-*d*]pyrimidine (**12**).

A fnely ground mixture of chloroformamidine hydrochloride (120 mg, 1.05 mmoles) and a 1:1 mixture of thiophenes **15** and **16** (fraction B from the synthesis of **15**; total 105 mg, 0.264 mmole) was heated and worked up as in the synthesis of **8a**. Tlc analysis (silica gel, 100:10:1 chloroform-methanol-28% ammonium hydroxide) showed the product to be a mixture of two slow-moving non-cyclized intermediates **17** and **18** (R_f 0.32, 0.35) and two fast-moving cyclized products **8a** and **12** (R_f 0.46, 0.56). The four-component mixture was heated (80°/0.1 Torr) for 16 hours, at which time only the fast-moving tlc spots remained. The mixture of cyclized products was filtered through a short plug of silica gel with 100:10:1 dichloromethane-methanol-28% ammonium hydroxide as the eluent, and the filtrate was evaporated under reduced pressure. The residue was rechromatographed on silica gel with 100:4:0.4 dichloromethane-methanol-28% ammonium hydroxide as the eluent, small individual fractions of eluent were carefully monitored by tlc (silica gel 100:10:1 chloroform-methanol-28% ammonium hydroxide), and appropriate fractions were combined and recrystallized from methanol to obtain each product as a beige powder. A mixed fraction (8 mg, 7%) was also recovered. The slower-moving product (41 mg, 35%; R_f 0.48) was indistinguishable from the authentic sample of **8a** obtained in the preceding experiment. The faster-moving product (R_f 0.56) was **12**, yield 36 mg (31%), mp 136-138°; ir (potassium bromide): ν 3405, 3180, 2925, 1600, 1550, 1500, 1480, 1460, 1450, 1425, 1395, 1340, 1320, 1285, 1240, 1200 cm^{-1}; uv (ethanol) λ_{max} 227 nm (ε 32,881), 276 (10,847); ^1H nmr (d_6-dimethyl sulfoxide): δ 2.97-3.03 (m, 2H, bridge CH_2), 3.27-3.30 (m, 2H, bridge CH_2), 3.72 (s, 3H, 5'-OMe), 3.73 (s, 3H, 4'-OMe), 3.74 (s, 3H, 3'-OMe), 5.98 (br s, 2H, NH_2), 6.38 (br s, 2H, NH_2), 6.52 (s, 1H, 6-H), 6.78 (s, 1H, 6'-H).

Anal. Calcd. for $C_{17}H_{19}BrN_4O_3S \cdot H_2O$: C, 44.64; H, 4.62; N, 12.24. Found: C, 44.49; H, 4.71; N, 11.90.

2-Bromo-3,4,5-trimethoxyaniline (**19**).

Acetic anhydride (10 ml) was added to a solution of 3,4,5-trimethoxyaniline (10.0 g, 54.6 mmoles) in dry pyridine (10 ml) under an argon atmosphere, and the reaction was left stirring at room temperature overnight. The mixture was poured into ice (100 g), and after the ice melted, the precipitated solid was filtered and washed copiously with water and dried (65°/0.05 Torr) for 14 hours to obtain 3,4,5-trimethoxyacetanilide as a white powder (12.3 g, 99%); mp 110-115°; ir (potassium bromide): ν 3980, 3350, 3040, 2990, 1980, 1650, 1590, 1550, 1505, 1410, 1370, 1300, 1280, 1235, 1220 cm^{-1}; ^1H nmr (deuteriochloroform): δ 2.1 (s, 3H, MeCO), 3.8 (m, 9H, 3-, 4-, and 5-OMe), 4.5 (br s, 1H, CONH), 6.8 (s, 2H, 2- and 6-H) A portion of this material (6.0 g, 16.6 mmoles) was added directly to chloroform (250 ml) at 0°, and *N*-bromosuccinimide (5.69 g, 31.9 mmoles) was added in small portions with stirring. After *ca.* 40 minutes, when the amide dissolved completely, the reaction mixture was washed with water (2 x 30 ml) and the organic layer was concentrated to dryness by rotary evaporation. Crystallization of the brown residue from warm ether afforded 2-bromo-3,4,5-trimethoxyacetanilide (**20**) as white needles (5.03 g, 62%) which were used without further purification, mp 92-93°; ir (thin film): ν 3410, 3275, 1660 (amide C=O), 1580, 1530, 1480, 1460, 1440, 1430, 1390, 1350, 1290, 1230 cm^{-1}; ^1H nmr (500 MHz, deuteriochloroform): δ 2.1 (s, 3H, MeCO), 3.8-3.9 (three overlapping singlets, 9H, 3-, 4-, and 5-OMe), 7.6 (br s, 1H, CONH), 7.9 (s, 1H, 6-H). A stirred suspension of the foregoing product (3.41 g, 11.2 mmoles) in 2*N* sodium hydroxide (35 ml) was heated under reflux for 3 hours, cooled, and extracted with ether (3 x 50 ml). The combined extracts were washed with water (2 x 20 ml), dried over magnesium sulfate, and concentrated to dryness by rotary evaporation. Chromatography of the residue on silica gel with 4:1 hexanes-ethyl acetate as the eluent yielded **16** as a light-sensitive colorless oil which did not crystallize even in the freezer; yield 2.97 g, *ca.* 100%) [34]; ir (potassium bromide): ν 3450, 3350, 3200, 2800-3000, 1610, 1565, 1480, 1450, 1415, 1405, 1370, 1280, 1230, 1210 cm^{-1}; ^1H nmr (500 MHz, deuteriochloroform): δ 3.78 (s, 3H, 5-OMe), 3.79 (s, 3H, 4-OMe), 3.89 (s, 3H, 3-OMe), 6.16 (s, 1H, 6-H). The broad, low-intensity signal at δ 3.7-3.9 due to the aromatic amino group could not be clearly visualized because it lay under the three OMe singlets.

Anal. Calcd. for $C_9H_{12}BrNO_3$: C, 41.24; H, 4.61; N, 5.34. Found: C, 41.16; H, 4.55; N, 5.25.

2,4-Diamino-6-(2'-bromo-3',4',5'-trimethoxyanilino)methyl-5-chloroquinazoline (**9a**).

Raney nickel (100 mg, 50% slurry in water) was added to a solution of **21** [20,36] (230 mg, 1.04 mmoles) and **17** (289 mg, 1.35 mmoles) in glacial acetic acid (100 ml), and the mixture was hydrogenated for 18 hours in a Parr apparatus at an initial pressure 32 lb/in^2. The reaction mixture was filtered through Celite, and the pad was washed with glacial acetic acid. The combined filtrates were reduced to *ca.* 1 ml by rotary evaporation, then diluted with water (20 ml), and basified with concentrated ammonia. The solution was extracted with chloroform (3 x 30 ml), and the organic layers were evaporated to obtain a yel-

low powder. After trituration with 1:1 ether-hexanes to remove excess starting amine, chromatography on silica gel with 100:10:1 chloroform-methanol-28% ammonium hydroxide as the eluent yielded two rough fractions, A followed by B. Evaporation of Fraction A yielded a semi-solid, which was recrystallized from hot 95% ethanol to obtain **9a** as beige prisms (45 mg, 9%); R_f 0.44 (silica gel, 100:10:1 chloroform-methanol-28% ammonium hydroxide), mp 162-163°; ir (potassium bromide): ν 3480, 3390, 2950, 2130, 1600, 1550, 1440-1460, 1400, 1375, 1360, 1345, 1310, 1240, 1210 cm^{-1}; ^1H nmr (500 MHz, d_6-dimethyl sulfoxide): δ 3.59 (s, 3H, 5'-OMe), 3.61 (s, 3H, 4'-OMe), 3.73 (s, 3H, 3'-OMe), 4.43 (d, 2H, 9-CH_2), 5.72 (m, 1H, 10-NH), 5.97 (s, 1H, 6'-H), 6.10 (br s, 2H, NH_2), 7.12 (d, 1H, 7-H), 7.36 (br s, 2H, NH_2), 7.42 (d, 1H, 8-H).

Anal. Calcd. for $C_{18}H_{19}BrClN_5O_3 \cdot 2.6H_2O$: C, 41.92; H, 4.74; N, 13.59. Found: C, 41.73; H, 4.83; N, 14.04.

Evaporation of Fraction B (see above) yielded another 31 mg (6%) containing **9a** (R_f 0.44, 100:10:1 chloroform-methanol-28% ammonium hydroxide; developed twice for better resolution) and a slightly more polar product (R_f 0.41) assumed to be a *cis/trans* mixture of incompletely reduced imine adducts. Longer hydrogenation may be used to convert these imines to **9a**.

2,4-Diamino-5-(2'-bromo-3',4',5'-trimethoxyanilino)methylquinazoline (**10**).

A mixture of **22** [22,37] (400 mg, 2.16 mmoles) and **19** (500 mg, 2.34 mmoles) in glacial acetic acid (45 ml) was warmed until all the solids dissolved. Raney nickel (200 mg, 50% slurry in water) was then added, and the mixture was hydrogenated in a Parr apparatus (initial pressure 32 lb/in^2) for 18 hours. The catalyst was removed by filtration through Celite, and the pad was washed with glacial acetic acid. The filtrates were combined, concentrated to *ca.* 5 ml by rotary evaporation, and diluted with ice-water (20 ml). The precipitate was filtered, washed with cold water, and dried to obtain a yellow solid (200 mg) whose ^1H nmr spectrum indicated that reduction of the intermediate imine was incomplete. The solid was therefore redissolved in glacial acetic acid, another portion of Raney nickel (40 mg, 50% slurry in water) was added, and hydrogenation was resumed for an additional 18 hours. The filtrate from the second hydrogenation was concentrated and basified with concentrated ammonia, and the precipitated solid was filtered and chromatographed on silica gel with 100:8:1 chloroform-methanol-28% ammonium hydroxide as the eluent. Fractions containing a single tlc spot with R_f 0.34 (same eluent) were pooled and recrystallized from boiling ethanol to obtain pale-yellow needles (79 mg, 8%), mp 211-213°; ir (potassium hromide): ν 3460, 3395, 3360. 3270, 3170, 2960, 1630-1600, 1570, 1490-1450, 1420, 1390, 1340, 1250, 1240, 1210 cm^{-1}; ^1H nmr (500 MHz, d_6-dimethyl sulfoxide): δ 3.64 (s, 3H, 5'-OMe), 3.76 (s, 3H, 4'-OMe), 3.75 (s, 3H, 3'-OMe), 4.55 (d, 2H, 9-CH_2), 5.15 (m, 1H, 10-NH), 5.99 (br s, 2H, NH_2), 6.39 (s, 1H, 6'-H), 7.04 (m, 1H, 6-H), 7.05 (br s, 2H, NH_2), 7.16 (m, 1H, 7-H), 7.40 (t, 1H, 8-H).

Anal. Calcd. for $C_{18}H_{20}BrN_5O_3 \cdot 0.75H_2O$: C, 48.28; H, 4.84; N, 15.64. Found: C, 48.25; H, 4.74; N, 15.51.

2,4-Diamino-6-(2'-bromo-3',4',5'-trimethoxyanilino)methylquinazoline (**11**).

A mixture of **23** [36] (250 mg, 1.35 mmoles) and **18** (289 mg, 1.35 mmoles) in glacial acetic acid (15 ml) was warmed until all the solid dissolved. Raney nickel (45 mg, 50% slurry in water) was added, and the mixture was hydrogenated at 1 atmosphere for 18 hours using a balloon. The reaction mixture was filtered through a pad of Celite, and the pad was washed with acetic acid. The combined filtrate and wash solution were concentrated to *ca.* 5 ml by rotary evaporation and then diluted with ice-water (20 ml). The solution was basified with ammonia and chilled for 1 hour, and the precipitated solid was filtered, dried, and chromatographed on silica gel with 100:15:1 chloroform-methanol-28% ammonium hydroxide as the eluent. Appropriate fractions were pooled and recrystallized from hot ethanol to obtain **11** as beige needles (99 mg, 15%), mp 213-214°; ir (potassium bromide): ν 3600, 3410, 3380, 3090, 2930, 1645, 1620, 1600, 1565, 1505, 1450, 1350, 1280, 1240, 1200 cm^{-1}; ^1H nmr (500 MHz, d_6-dimethyl sulfoxide): δ 3.57 (s, 3H, 5'-OMe), 3.62 (s, 3H, 4'-OMe), 3.71 (s, 3H, 3'-OMe), 4.31 (d, 2H, 9-CH_2), 5.55 (m, 1H, 10-NH), 5.89 (br s, NH_2), 6.10 (1H, 6'-H), 7.13 (d, 1H, 7-H).

Anal. Calcd. for$C_{18}H_{20}BrN_5O_3 \cdot 2H_2O$: C, 45.97; H, 5.12; N, 14.89. Found: C, 46.17; H, 4.90; N, 15.04.

Enzyme Inhibition.

Compounds **8a**, **9a**, and **10-12** were assayed as dihydrofolate reductase inhibitors as described earlier [16,17]. Their IC_{50} values (50% inhibitory concentrations) against the *P. carinii, T. gondii*, and rat liver enzymes, respectively, were found to be: **8a**, >12, 0.21, 0.93 μ*M*; 9a, 0.028, 0.0078, 0.0098 μ*M*; **10**, 200, 25, 43 μ*M*; **11**, 0.55, 0.039, 0.33 μ*M*; **12**, 49, 2.5, 2.8 μ*M*. The corresponding IC_{50} values for the non-brominated compounds **8b** and **9b** were reported previously; **8b**, >8.0, 0.63, 51 μ*M* [19]; **9b**, 0.033, 0.0052, 0.0059 μ*M* [20].

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