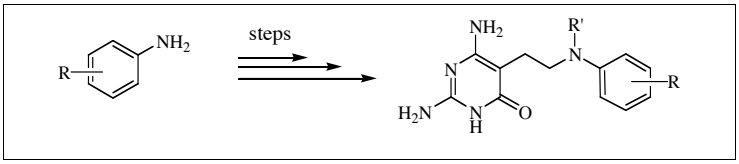
Synthesis of 2,6-Diamino-5-[(2-substituted phenylamino)ethyl]pyrimidin-4(3*H*)-one as Inhibitors of Folate Metabolizing Enzymes [1a,b]

Aleem Gangjee* [a], Ying Wang [a], Sherry F. Queener [b], and Roy L. Kisliuk [c]

[a] Division of Medicinal Chemistry, Graduate School of Pharmaceutical Sciences, Duquesne University, Pittsburgh, Pennsylvania 15282.
[b] Department of Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, Indiana, 46202.
[c] Department of Biochemistry, Tufts University School of Medicine, Boston, Massachusetts 02111

Received January 12, 2006

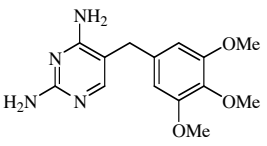


A series of eleven novel 2,6-diamino-5-[(2-substituted phenylamino)ethyl]pyrimidin-4(3*H*)-one derivatives were synthesized as potential inhibitors of dihydrofolate reductase (DHFR) and thymidylate synthase (TS). The synthesis of analogues **2a-f**, **3a** and **3e** was achieved *via* an improved method. Commercially available anilines **12a-f** were used as starting materials which on reaction with chloroacetaldehyde followed by cyanoacetate and cyclocondensation with guanidine afforded 2,6-diamino-5-[(2-substituted phenylamino)ethyl]pyrimidin-4(3*H*)-one **2a-f** in three steps. The N-methyl analogues **3a-3e** were prepared by reductive methylation. These compounds were evaluated against dihydrofolate reductase from *Escherichia coli*, *Toxoplasma gondii*, *Pneumocystis carinii*, human, and rat liver. Few compounds were marginally active against dihydrofolate reductase. The most potent inhibitor, (**2c**) which has a 1-naphthyl substituent on the side chain, has an IC_{50} = 150 μM and 9.1 μM against *Escherichia coli* and *Toxoplasma gondii* DHFR, respectively.

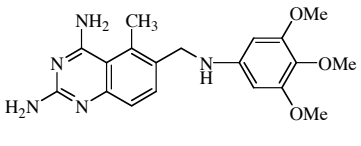
J. Heterocyclic Chem., **43**, 1523 (2006).

Introduction.

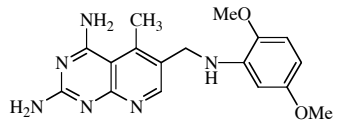
Inhibition of folate metabolism has long been an attractive biochemical target [2,3]. Opportunistic infections with *Pneumocystis carinii* and *Toxoplasma gondii* associated with loss of cell-mediated immunity remains among the principal causes of morbidity and mortality in patients with acquired immunodeficiency syndrome (AIDS) in the United States [4]. A therapeutic approach is to treat these infections *via* dihydrofolate reductase inhibitors.



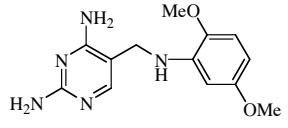
trimethoprim (TMP)



trimetrexate (TMQ)



piritrexim (PTX)



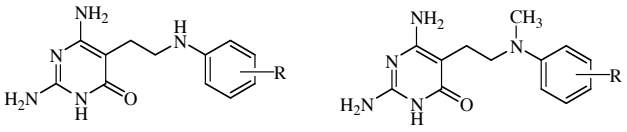
1

Figure 1

Several classical antifolates have been reported in the literature as inhibitors of dihydrofolate reductase and thymidylate synthase [5]. The disadvantage of classical compounds is that they require active transport to enter cells. Cells that lack these transport mechanisms, such as bacterial and protozoa cells are not susceptible to the action of classical antifolates. In an attempt to overcome these problems, nonclassical, lipophilic antifolates were developed [6]. The treatment of *Pneumocystis carinii* and *Toxoplasma gondii* infections with nonclassical antifolates takes advantages of the fact that these organisms are permeable to lipophilic, nonclassical antifolates and, unlike mammalian cells, lack a carrier-mediated active transport mechanism for the uptake of classical folates and antifolates with polar glutamate side chains [7]. Thus, host tissues can be selectively rescued from the toxic effects by a reduced folate, typically leucovorin, which is taken up only by host cells [7,8].

The clinically used lipophilic agents trimethoprim and pyrimethamine, used for *Pneumocystis carinii* and *Toxoplasma gondii* infections, respectively, are selective but weak inhibitors of dihydrofolate reductase from *Pneumocystis carinii* and *Toxoplasma gondii* and must be used with sulfonamides to provide synergistic effects [7,9]. Trimetrexate and piritrexim, which are 100-10,000 times more potent than trimethoprim or pyrimethamine against dihydrofolate reductase from *Pneumocystis carinii*

and *Toxoplasma gondii*, are also potent inhibitors of dihydrofolate reductase from mammalian sources [10,11].

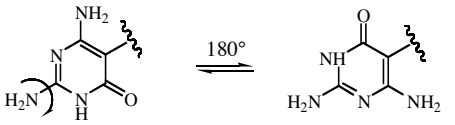


2a: R = H
2b: R = 4-Cl
2c: R = 2,3-C_4H_4
2d: R = 3,4-C_4H_4
2e: R = 3,4,5-triOMe
2f: R = 2,5-diOMe

3a: R = H
3b: R = 4-Cl
3c: R = 2,3-C_4H_4
3d: R = 3,4-C_4H_4
3e: R = 3,4,5-triOMe

Figure 2

Due to a complete lack of selectivity, these drugs have to be used along with leucovorin for host rescue. However, leucovorin is expensive and the side effects due to a lack of effective rescue sometimes require cessation of therapy. Thus the development of selective and potent nonclassical, lipophilic antifolates is a desirable goal.



2,4-diamino mode (binding to DHFR)

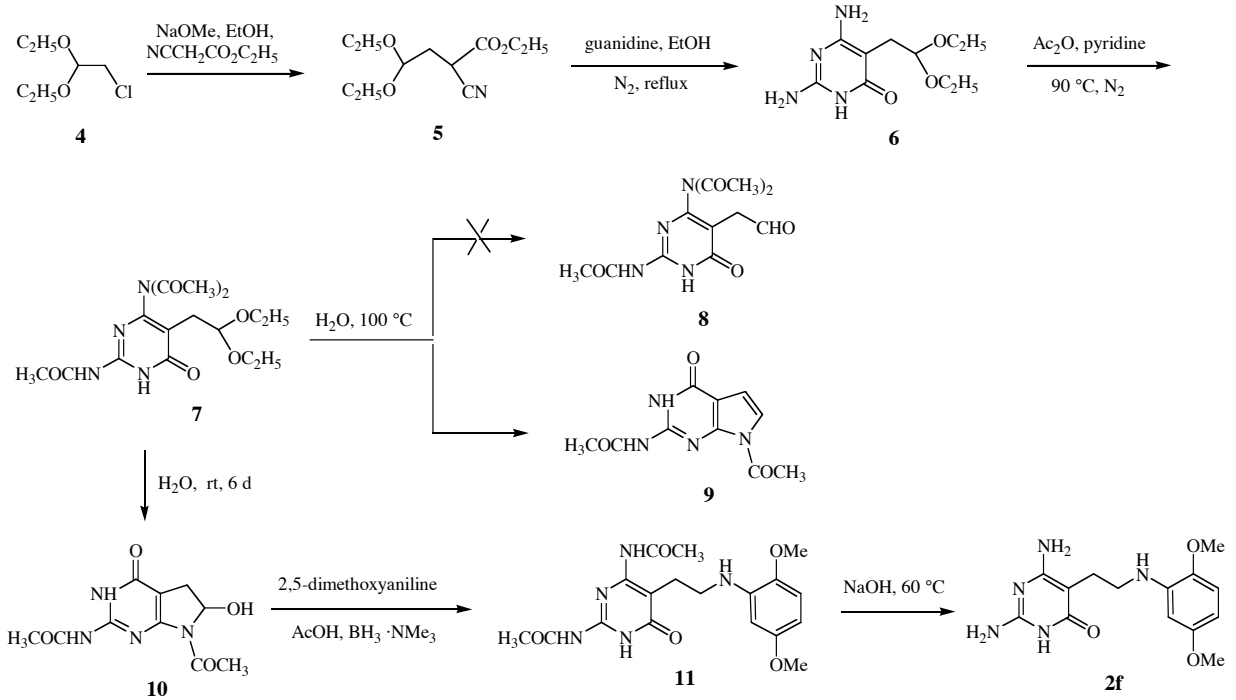
2-amino-4-oxo mode (binding to TS)

Figure 3

Gangjee *et al.* [12] simplified the structure of trimetrexate and designed a series of monocyclic nonclassical trimethoprim analogues bearing a C-C bridge in the side chain, as potent and selective pathogen dihydrofolate reductase inhibitors (Figure 1). Among these, compound **1** was the most potent and had an activity profile (potency and selectivity) against *Pneumocystis carinii* dihydrofolate reductase similar to trimethoprim. Molecular modeling using SYBYL 6.8 [13] and superimposition of **1** onto trimetrexate indicated that the distance between the pyrimidine ring and side chain substituted phenyl ring in **1** was shorter than that in trimetrexate. Increasing the length of the bridge by one atom could afford optimal chain length for enzyme interaction similar to **1**. In this report we synthesized compounds **2a-f** and **3a-e** with a C-C-N side chain in an attempt to improve the potency and selectivity of **1** against *Pneumocystis carinii* dihydrofolate reductase and *Toxoplasma gondii* dihydrofolate reductase (Figure 2).

In addition, it was also of interest to combine both dihydrofolate reductase and thymidylate synthase inhibitory activity in one nonclassical antifolate molecule. Gangjee *et al.* [14] recently reported the successful design, synthesis and evaluation of dual DHFR-TS inhibitors. Such dual inhibitors could act at two different sites, *i.e.*, dihydrofolate reductase and thymidylate synthase, and could possess "combination chemotherapy" potential in a single molecule without the pharmacokinetic disadvantages of two separate entities. Since the 2,4-diamino portion of the pyrimidine

Scheme 1



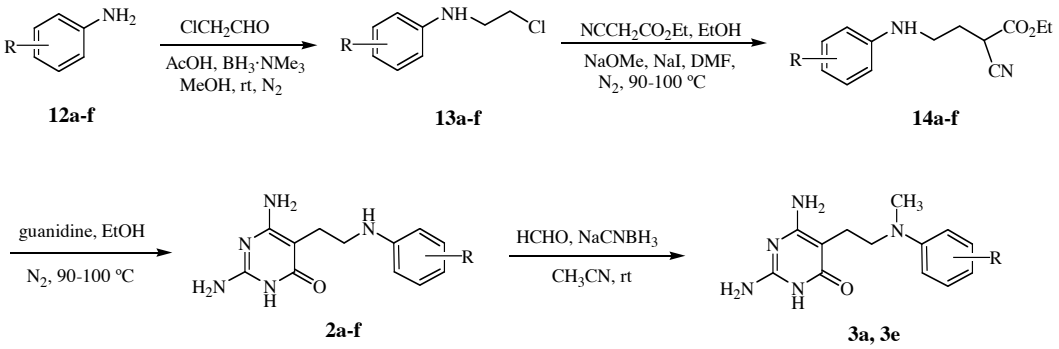
ring is the preferred binding mode for dihydrofolate reductase (*e.g.* trimethoprim) [15] and 2-amino-4-oxo portion of the pyrimidine ring is the preferred binding mode for TS [*e.g.* 2-amino-4-oxo-*N*10-propargyl-5,8-dideazafolate (PDDF)] [16], compounds **2a-f** and **3a-e**, as potential nonclassical dual inhibitors of dihydrofolate reductase and thymidylate synthase could adopt both "2,4-diamino" mode as well as "2-amino-4-oxo" mode *via* rotation of the C_2-NH_2 bond by 180 °C, using the 6-oxo to mimic the 4-oxo group of PDDF (Figure 3).

Results and Discussion.

The initial synthetic strategy for **2a-f** is shown in Scheme 1. The synthesis commenced from the commercially available 2-chloroacetaldehyde diethyl acetal **4** and ethyl cyanoacetate using sodium methoxide as base and a crystal of sodium iodide as catalyst to afford ethyl 2-cyano-4,4-diethoxy-butyl-anoate **5** using a modification of a literature procedure [17]. Compound **5** condensed with guanidine in ethanol to provide 2,6-diamino-5-(3,3-diethoxyethyl)pyrim-idin-4(3*H*)-one **6** in 16% yield. The amino groups in **6** were protected by acetylation with acetic anhydride and pyridine to give the triacetyl aminopyrimidine **7**. To deprotect the acetal, **7** was heated in water at 100 ºC. Interestingly, cyclized compound **9** with a stable pyrrole ring was formed in this process instead of the desired aldehyde **8**. ^{1}H nmr of **9** indicated the absence of the 5-formylmethyl protons as well as the presence of two vinyl protons (doublet, J = 3.6 Hz) between 6.55-7.49 ppm, confirmed that cyclization and elim-ination had occurred simultaneously to form the pyrrolo[2,3-*d*]pyrimidine. However, treatment of **7** in water at ambient temperature afforded hydrolysis of the acetal, deacetylation, and cyclization to give **10** in 32% yield. Reaction of **10** with 2,5-dimethoxyaniline in the presence of acetic acid and borane-trimethylamine gave the desired diacetylated analogue **11** in 9% yield. Deprotection of **12** was readily accomplished with hot 1 *N* sodium hydroxide to give **2f** in 36% yield.

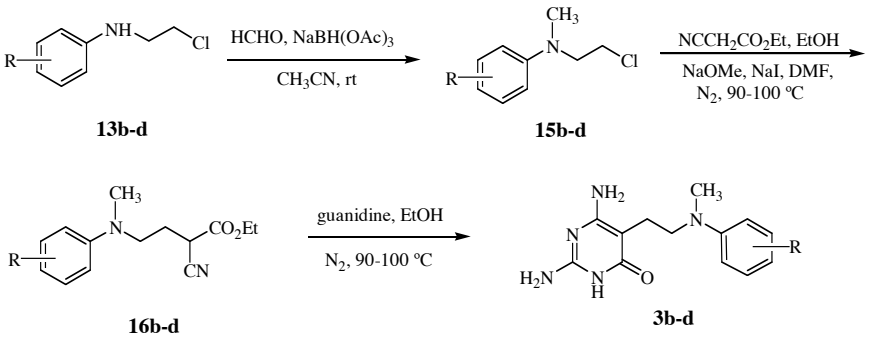
Since the overall yield was low in the synthetic sequence for **2f**, a more efficient synthetic route was developed for the target compounds (Scheme 2). Anilines **12a-f** were condensed with 2-chloroacetaldehyde to afford **13a-f** in 72-98% yields in the presence of borane-trimethylamine and acetic acid at room temperature. Intermediates **13a-f** were then reacted with ethyl cyanoacetate to afford **14a-f** in 30-45% yields. Finally **14a-f** were cyclocondensed with guanidine to give the target compounds **2a-f** in moderate yields. The N-methyl analogues **3a** and **3e** were prepared from **2a** and **2e** *via* reductive methylation using formaldehyde and sodium

Scheme 2



12a-f **13a-f** **14a-f** **2a-f** **3a, 3e**

Scheme 3



13b-d **15b-d** **16b-d** **3b-d**

cyanoborohydride in 44% and 47% yield, respectively. Analogues **3b-d** were synthesized *via* a slightly modified sequence (Scheme 3). Methylation of **13b-d** under classical method provided **15a-f**, which were converted to the desired analogues **3b-d** in two steps. The structures of all target compounds **2a-f** and **3a-e** were confirmed by ^{1}H NMR and elemental analysis or high resolution mass spectrum.

Compounds **2a-f** and **3a-e** were evaluated as inhibitors of isolated dihydrofolate reductase against *Escherichia coli*, *Toxoplasma gondii*, *Pneumocystis carinii*, human, and rat liver along with trimetrexate (TMQ) and trimethoprim (TMP). None of the target compounds inhibited these enzymes at 24 μM except **2c** for which the IC_{50} values were 150 μM and 9.1 μM against *Escherichia coli* and *Toxoplasma gondii*, respectively. This clearly indicated that both C-C and C-C-N bridged 5-substituted nonclassical monocyclic pyrimidine systems are not conductive to dihydrofolate reductase inhibitory activity. The inhibitory activity of these analogues against thymidylate synthase will be reported in due course.

EXPERIMENTAL

All evaporations were carried out *in vacuo* with a rotary evaporator. Analytical samples were dried *in vacuo* (0.2 mmHg) in an Abderhalden drying apparatus over P_2O_5 and ethanol at reflux. Thin layer chromatography (tlc) was performed on silica gel plates with fluorescent indicator. Spots were visualized by UV light (254 and 365 nm). 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 (200-400 mesh). The amount (weight) of silica gel for column chromatography was in the range of 50-100 times the amount (weight) of the crude compounds being separated. Columns were dry packed unless specified otherwise. Solvent systems are reported as volume percent mixture. Melting points were determined on a Mel-Temp II melting point apparatus with a digital thermometer 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, t = triplet, q = quartet, m = multiplet, bs = broad singlet. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA. Elemental compositions were within ± 0.4% of the calculated values. Fractional moles of water or organic solvents frequently found in some analytical samples of antifolates could not be removed despite 24 h of drying in vacuum and were confirmed, where possible, by their presence in the ^{1}H nmr spectrum. All solvents and chemicals were purchased from Aldrich Chemical Co. and Fisher Scientific and were used as received.

2-Cyano-4,4-diethoxybutyric Acid Ethyl Ester (**5**).

To a solution of sodium methoxide (1.51 g, 26.5 mmol) in anhydrous ethanol (10 ml) was added ethyl cyanoacetate (3.0 g, 26.5 mmol), and the reaction mixture was stirred at room temperature for 20 minutes. The solvent was removed under reduced pressure at 40 °C, and the residue was dissolved in anhydrous DMF (10 ml). To this solution were added 2-chloroacetaldehyde diethyl acetal (**4**, 4.10 g, 26.5 mmol) and a crystal of NaI, and the mixture was protected with a drying tube and stirred for 4 hours at 95 °C. The solution was cooled, poured into ice water (15 ml), and extracted with ethyl ether (6 × 20 ml). The organic phase was washed with water (2 × 10 ml), brine (10 ml), dried with Na_2SO_4, and filtered. The filtrate was concentrated *in vacuo* to give 3.11 g (51%) of **5** as a pale brown liquid and was used for next step without further purification.

2,6-Diamino-5-(3,3-diethoxyethyl)pyrimidin-4(3*H*)-one (**6**).

To a solution of sodium methoxide (1.54 g, 27.16 mmol) in anhydrous ethanol (20 ml) were added guanidine hydrochloride (1.30 g, 13.58 mmol) and crude **5** (3.11 g, 13.58 mmol). The mixture that resulted was refluxed at 95 °C for 2.5 hours, and then further stirred at room temperature for 12 hours. To this solution were added methanol (20 ml) and silica gel (10.0 g), and the solvent evaporated. The silica gel plug obtained was loaded on a silica gel column and eluted with $CHCl_3$:MeOH (28-30 % ammonia in methanol) (100:5). Fractions containing the product (tlc) were pooled and the solvent evaporated to afford 0.55 g (17%) of **6** as a white solid, mp 185-187.5 °C (lit., [18] 187-189 °C); tlc R_f 0.44 ($CHCl_3$:MeOH, 5:1); ^{1}H nmr (DMSO-d_6) δ 1.07 (t, 6H, J = 7.1 Hz, CH_3), 2.42 (d, 2H, J = 5.5 Hz, CH_2), 3.39 (m, 2H, OCH_2), 3.59 (m, 2H, OCH_2), 4.42 (t, 1H, J = 5.3 Hz, CH), 5.58 (s, 2H, NH_2), 5.98 (s, 2H, NH_2), 9.85 (s, 1H, NH).

N-[4-Diacetylamino-5-(2,2-diethoxyethyl)-6-oxo-1,6-dihydropyrimidin-2-yl)acetamide (**7**).

To a solution of **6** (0.30 g, 1.24 mmol) in anhydrous pyridine (10 ml) was added acetic anhydride (1.32 ml, 13.97 mmol). The reaction mixture was stirred at 90 °C under a nitrogen atmosphere for 5 hours and then further stirred at room temperature for 12 hours. The solution was evaporated *in vacuo* to give an oil. To this residue were added methanol (20 ml) and silica gel (5.0 g), and the solvent evaporated. The silica gel plug obtained was loaded on a silica gel column and eluted with $CHCl_3$:MeOH (20:2). Fractions containing the product (tlc) were pooled and the solvent evaporated to afford 0.27 g (59%) of **7** as a pale yellow solid; tlc R_f 0.79 ($CHCl_3$:MeOH, 5:1); ^{1}H nmr (DMSO-d_6) δ 1.07 (t, 6H, OCH_2CH_3), 2.14 (s, 3H, $COCH_3$), 2.30 (s, 6H, $COCH_3$), 2.64 (d, 2H, CH_2), 3.43 (m, 2H, OCH_2), 3.55 (m, 2H, OCH_2), 4.77 (t, 1H, CH), 9.50 (bs, 1H, NHCO), 11.91 (bs, 1H, NH).

N-(7-Acetyl-4-oxo-4,5,6,7-tetrahydro-3*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl)acetamide (**9**).

The triacetylated pyrimidinone **7** (0.18 g, 0.50 mmol) was heated with water (5 ml) on a steam bath for 4 h. Water was evaporated at 50 °C *in vacuo* to afford a residual oil. To this residue were added methanol (8 ml) and silica gel (5.0 g), and the solvent evaporated. The silica gel plug obtained was loaded on a silica gel column and eluted with $CHCl_3$:MeOH (a gradient elution, 100:0.5 to 100:5). Fractions containing the product (tlc) were pooled and the solvent evaporated to afford 0.057 g of **9** (23%) as a colorless oil; tlc R_f 0.72 ($CHCl_3$:MeOH, 5:1); ^{1}H nmr (DMSO-d_6) δ 2.09 (s, 3H, $NCOCH_3$), 2.26 (s, 3H, $NCOCH_3$),

6.55 (d, 1H, J = 3.6 Hz, CH), 7.49 (d, 1H, J = 3.6 Hz, CH), 11.80 (bs, 2H, NH and 2-NHCO).

(±)-*N*-(7-Acetyl-6-hydroxy-4-oxo-4,5,6,7-tetrahydro-3*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl]acetamide (**10**).

A solution of the triacetylated pyrimidinone **7** (0.27 g, 0.73 mmol) in water (10 ml) was stirred at room temperature for 6 days. TLC showed that the starting material was still present and there were two new spots. Water was evaporated at 50 °C *in vacuo* to afford a residual solid. To this residue were added methanol (20 ml) and silica gel (10.0 g), and the solvent evaporated. The silica gel plug obtained was loaded on a silica gel column and eluted with $CHCl_3$:MeOH (a gradient elution, 100:0.5 to 100:5). Fractions containing the product (tlc) were pooled and the solvent evaporated to afford 0.07 g of **10** (32%) as a white solid; tlc R_f 0.52 ($CHCl_3$:MeOH, 5:1); ^{1}H nmr (DMSO-d_6) δ 2.15 (s, 3H, $NCOCH_3$), 2.49 (s, 3H, $NCOCH_3$), 2.89-2.97 (q, 2H, CH_2), 5.87 (t, 1H, CHOH), 6.55 (d, 1H, J = 5.9 Hz, OH), 11.61-11.71 (bs, 2H, NH and 2-NHCO).

N-{4-Acetylamino-5-[2-(2,5-dimethoxyphenylamino)ethyl]-6-oxo-1,6-dihydro-pyrimidin-2-yl}acetamide (**11**).

To a solution of **10** (69 mg, 0.27 mmol) in methanol (20 ml) were added 2,5-dimethoxyaniline (45 mg, 0.30 mmol), borane trimethylamine (41 mg, 0.54 mmol), and acetic acid (0.02 ml, 0.27 mmol). The resulting mixture was stirred at room temperature for 12 hours. The pH of the solution was adjusted to 8 with conc. ammonium hydroxide in an ice bath. To this solution was added silica gel (5.0 g) and the solvent evaporated. The silica gel plug obtained was loaded on a silica gel column and eluted with $CHCl_3$:MeOH (a gradient elution, 100:0.5 to 100:3). Fractions containing the product (tlc) were pooled and the solvent evaporated to afford 10 mg of **11** (9%) as a gray solid, mp 215-217 °C; tlc R_f 0.79 ($CHCl_3$:MeOH, 5:1); ^{1}H nmr (DMSO-d_6) δ 2.05 (s, 3H, $NCOCH_3$), 2.14 (s, 3H, $NCOCH_3$), 2.57 (t, 2H, 7-CH_2), 3.13 (t,, 2H, NCH_2), 3.66 (s, 3H, OCH_3), 3.69 (s, 3H, OCH_3), 5.04 (t, 1H, 9-NH), 6.03 (dd, 1H, C_6H_3), 6.24 (d, 1H, J = 2.6 Hz, C_6H_3), 6.65 (d, 1H, J = 8.5 Hz, C_6H_3), 9.70 (s, 1H, NH), 11.54 (bs, 1H, NHCO), 11.85 (bs, 1H, NHCO).

Anal. Calcd for $C_{18}H_{23}N_5O_5$: C, 55.52; H, 5.95; N, 17.98. Found: C, 55.31; H, 5.78; N, 18.23.

2,6-Diamino-5-[2-(2,5-dimethoxyphenylamino)ethyl]pyrimidin-4(3*H*)-one (**2f**).

A stirred solution of **11** (39 mg, 0.10 mmol) and 1 *N* aq. NaOH (2 ml) was heated at 60 ºC for 10 hours and then stirred at room temperature for 12 hours. The pH of the solution was adjusted to 8 with 1 *N* HCl in an ice bath. To this solution was added silica gel (2.0 g) and the solvent evaporated. The silica gel plug obtained was loaded on a silica gel column and eluted with $CHCl_3$:MeOH:NH_4OH (10:1:0.1). Fractions containing the product (tlc) were pooled and the solvent evaporated to afford 11 mg of **2f** (36%) as a white solid, mp 246-249 °C; tlc R_f 0.47 ($CHCl_3$:MeOH, 5:1); ^{1}H nmr (DMSO-d_6) δ 2.48 (m, 2H, CH_2), 2.93 (m, 2H, NCH_2), 3.64 (s, 3H, OMe), 3.67 (s, 3H, OMe), 5.02 (t, 1H, J = 5.1 Hz, NH), 5.84 (s, 2H, NH_2), 5.97-6.02 (m, 3H, C_6H_3 and NH_2), 6.11 (d, 1H, J = 2.6 Hz, C_6H_3), 6.63 (d, 1H, J = 8.6 Hz, C_6H_3), 9.90 (s, 1H, NH).

Anal. Calcd for $C_{14}H_{19}N_5O_3 \cdot 0.4\ H_2O$: C, 53.80, H, 6.39; N, 22.40. Found: C, 54.08; H, 6.14; N, 22.09.

General Procedure for the Synthesis of Compounds **13a-f**.

To a solution of the appropriate aniline (**12a-f**) in methanol were added chloroacetaldehyde, acetic acid, and borane trimethylamine. The mixture was stirred at room temperature under nitrogen for 10-18 hours. If the reaction was not complete (tlc) after 6 hours, an additional amount of acetic acid (0.5 eq), chloroacetaldehyde (1.0 eq) and borane trimethylamine (1.0 eq) were added, and the mixture was further stirred for 10-12 hours. The pH of the solution was adjusted to 8 with conc. ammonium hydroxide. To this solution was added silica gel and the solvent evaporated. The silica gel plug obtained was loaded on a silica gel column and eluted with ethyl acetate:hexanes (1:20). Fractions containing the product (tlc) were pooled and evaporated to afford the desired product.

(2-Chloroethyl)phenylamine (**13a**).

Compound **13a** was obtained from aniline **12a** (3.0 g, 32.25 mmol), chloroacetaldehyde (4.89 ml, 38.50 mmol), borane trimethylamine (4.71 g, 62.57 mmol) and acetic acid (0.27 ml, 4.70 mmol) by using the method described above to afford 4.91 g (98%) of **13a** as a yellow oil; tlc R_f 0.76 (ethyl acetate:hexanes, 1:2); ^{1}H nmr (DMSO-d_6) δ 3.48 (t, 2H, NCH_2), 3.80 (t, 2H, CH_2Cl), 6.16 (t, 1H, NH), 6.52 (m, 3H, C_6H_5), 7.07 (m, 2H, C_6H_5).

(2-Chloroethyl)-(4-chlorophenyl)amine (**13b**).

Compound **13b** was obtained from 4-chloroaniline **12b** (4.0 g, 31.35 mmol), chloroacetaldehyde (4.75 ml, 36.62 mmol), borane trimethylamine (6.87 g, 94.07 mmol) and acetic acid (0.30 ml, 5.22 mmol) by using the method described above to afford 5.06 g (85%) of **13b** as a gray oil; tlc R_f 0.81 (ethyl acetate:hexanes, 1:2); ^{1}H nmr (DMSO-d_6): δ 3.37 (t, 2H, NCH_2), 3.68 (t, 2H, CH_2Cl), 6.05 (bs, 1H, NH), 6.60 (dd, 2H, C_6H_4), 7.09 (dd, 2H, C_6H_4).

(2-Chloroethyl)naphthalen-1-yl-amine (**13c**).

Compound **13c** was obtained from 1-aminonaphthalene **12c** (3.0 g, 20.98 mmol), chloroacetaldehyde (3.18 ml, 25.04 mmol), borane trimethylamine (3.06 g, 40.66 mmol) and acetic acid (0.36 ml, 6.27 mmol) by using the method described above to afford 3.28 g (76%) of **13c** as a red oil; tlc R_f 0.43 (ethyl acetate:hexanes, 1:7); ^{1}H nmr (DMSO-d_6) δ 3.61 (q, 2H, NCH_2), 3.85 (t, 2H, J = 6.4 Hz, CH_2Cl), 6.40 (t, 1H, J = 5.4 Hz, NH), 6.57 (d, 1H, J = 7.6 Hz, $C_{10}H_7$), 7.14 (d, 1H, J = 8.0 Hz, $C_{10}H_7$), 7.29 (t, 1H, $C_{10}H_7$), 7.43 (m, 2H, $C_{10}H_7$), 7.76 (d, 1H, $C_{10}H_7$), 8.13 (d, 1H, $C_{10}H_7$).

(2-Chloroethyl)naphthalen-2-yl-amine (**13d**).

Compound **13d** was obtained from 2-aminonaphthalene **12d** (3.00 g, 20.97 mmol), chloroacetaldehyde (3.18 ml, 25.04 mmol), borane trimethylamine (3.06 g, 40.66 mmol) and acetic acid (0.24 ml, 4.18 mmol) by using the method described above to afford 3.89 g (90%) of **13d** as a deep red oil; tlc R_f 0.63 (ethyl acetate:hexanes, 1:2); ^{1}H nmr (DMSO-d_6) δ 3.51 (q, 2H, NCH_2), 3.80 (t, 2H, J = 6.4 Hz, CH_2Cl), 6.19 (t, 1H, J = 5.7 Hz, NH), 6.79 (s, 1H, $C_{10}H_7$), 6.99 (t, 1H, $C_{10}H_7$), 7.11 (q, 1H, $C_{10}H_7$), 7.31 (t, 1H, $C_{10}H_7$), 7.61 (m, 3H, $C_{10}H_7$).

(2-Chloroethyl)-(3,4,5-trimethoxyphenyl)amine (**13e**).

Compound **13e** was obtained from 3,4,5-trimethoxyaniline **12e** (4.11 g, 21.78 mmol), chloroacetaldehyde (3.30 ml, 25.98 mmol), borane trimethylamine (3.18 g, 42.24 mmol) and acetic acid (0.24 ml, 4.18 mmol) by using the method described above to afford 4.47 g (81%) of **13e** as an oil; tlc R_f 0.42 (ethyl acetate:hexanes, 1:2); ^{1}H nmr (DMSO-d_6): δ 3.36 (m, 2H, N-CH_2), 3.45 (s, 2H, CH_2Cl), 3.51 (s, 3H, 4-OMe), 3.69 (s, 6H, 3,5-diOMe), 5.6 (bs, 1H, NH), 5.89 (s, 2H, C_6H_2).

(2-Chloroethyl)-(2,5-dimethoxyphenyl)amine (**13f**).

Compound **13f** was obtained from 2,5-dimethoxyaniline **12f** (2.75 g, 18.0 mmol), chloroacetaldehyde (3.43 ml, 27.0 mmol), borane trimethylamine (2.71 g, 36.0 mmol) and acetic acid (0.24 ml, 4.18 mmol) by using method described above to afford 2.82 g (72%) of **13f** as an oil; tlc R_f 0.68 (ethyl acetate:hexanes, 1:2); ^{1}H nmr (DMSO-d_6) δ 3.44 (m, 2H, NCH_2), 3.65 (s, 3H, OCH_3), 3.79 (m, 5H, OCH_3 and CH_2Cl), 5.14 (t, 1H, J = 6.0 Hz, NH), 6.11 (d, 1H, C_6H_3), 6.23 (s, 1H, C_6H_3), 6.74 (dd, 1H, C_6H_3).

General Procedure for Synthesis of Compounds **14a-f**.

To a stirred solution of sodium methoxide in absolute ethanol was added ethyl cyanoacetate. The resulting mixture was stirred at room temperature for 5 minutes. The solvent was evaporated *in vacuo* at 40 °C, and the residual white solid was dissolved in dry DMF. To this solution were added the appropriate intermediate **13a-f** and a crystal of NaI, and the solution was heated at 90 °C under nitrogen for 4 hours. The reaction mixture was poured into water and extracted with methylene chloride. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography and eluted with ethyl acetate:hexanes (1:10 to 1:5). Fractions containing the pure product (tlc) were pooled, evaporated and immediately used for the next step.

2-Cyano-3-phenylaminopropionic Acid Ethyl Ester (**14a**).

Compound **14a** was obtained from intermediate **13a** (1.0 g, 6.43 mmol), sodium methoxide (0.35 g, 6.43 mmol), ethyl cyanoacetate (1.03 ml, 9.64 mmol) and a crystal of NaI to afford 0.44 g (30%) of **14a** as a yellow oil; tlc R_f 0.57 (ethyl acetate:hexanes, 1:2); ^{1}H nmr (DMSO-d_6) δ 1.21 (t, 3H, CH_3), 2.09 (m, 2H, CH_2), 3.17 (q, 2H, N-CH_2), 4.18 (m, 3H, CHCN and $COOCH_2$), 5.71 (t, 1H, NH), 6.57 (m, 3H, C_6H_5), 7.08 (t, 2H, C_6H_5).

3-(4-Chlorophenylamino)-2-cyanopropionic Acid Ethyl Ester (**14b**).

Compound **14b** was obtained from intermediate **13b** (1.0 g, 5.26 mmol), sodium methoxide (0.31 g, 5.78 mmol), ethyl cyanoacetate (0.84 ml, 7.89 mmol) and a crystal of NaI to afford 0.42 g (30%) of **14b** as a pale brown oil; tlc R_f 0.45 (ethyl acetate:hexanes, 1:2); ^{1}H nmr (DMSO-d_6) δ 1.15 (t, 3H, CH_3), 2.09 (m, 2H, CH_2), 3.15 (m, 2H, NCH_2), 4.12-4.29 (m, 3H, CHCN and $COOCH_2$), 5.95 (t, 1H, NH), 6.56 (d, 2H, C_6H_4), 7.10 (d, 2H, C_6H_4).

2-Cyano-3-(naphthalen-1-ylamino)propionic Acid Ethyl Ester (**14c**).

Compound **14c** was obtained from intermediate **13c** (1.84 g, 8.94 mmol), sodium methoxide (0.73 g, 13.41 mmol), ethyl cyanoacetate (1.76 ml, 16.09 mmol) and a crystal of NaI to afford 0.89 g (35%) of **14c** as a gray oil; tlc R_f 0.58 (ethyl acetate:hexanes, 1:2); ^{1}H nmr (DMSO-d_6) δ 1.16 (t, 3H, CH_3), 2.27 (m, 2H, CH_2), 3.16 (m, 2H, NCH_2), 4.15 (m, 3H, CHCN and $COOCH_2$), 6.27 (t, 1H, J = 5.3 Hz, NH), 6.54 (d, 1H, J = 7.6 Hz, $C_{10}H_7$), 7.14 (d, 1H, J = 8.0 Hz, $C_{10}H_7$), 7.29 (t, 1H, $C_{10}H_7$), 7.43 (m, 2H, $C_{10}H_7$), 7.76 (d, 1H, J = 7.3 Hz, $C_{10}H_7$), 8.13 (d, 1H, J = 8.0 Hz, $C_{10}H_7$). HRMS (EI): Calculated for $C_{17}H_{18}N_2O_2$: *m/z* = 282.3387. Found: *m/z* = 282.3374.

2-Cyano-3-(naphthalen-2-yl-amino)propionic Acid Ethyl Ester (**14d**).

Compound **14d** was obtained from intermediate **13d** (0.80 g, 3.89 mmol), sodium methoxide (0.23 g, 4.28 mmol), ethyl cyanoacetate (0.62 ml, 5.84 mmol) and a crystal of NaI to afford 0.35 g (32%) of **14d** as a brown oil; tlc R_f 0.32 (ethyl acetate:hexanes, 1:2); ^{1}H nmr (DMSO-d_6): δ 1,23 (m, 3H, CH_3), 2.22 (m, 2H, CH_2), 3.28 (m, 2H, NCH_2), 4.13-4.35 (m, 3H, CHCN and $COOCH_2$), 6.08 (t, 1H, NH), 6.75 (s, 1H, $C_{10}H_7$), 6.95 (dd, 1H, $C_{10}H_7$), 7.12 (t, 1H, $C_{10}H_7$), 7.31 (t, 1H, $C_{10}H_7$), 7.61 (q, 3H, $C_{10}H_7$).

2-Cyano-3-(3,4,5-trimethoxyphenylamino)propionic Acid Ethyl Ester (**14e**).

Compound **14e** was obtained from intermediate **13e** (2.0 g, 8.15 mmol), sodium methoxide (0.48 g, 8.96 mmol), ethyl cyanoacetate (1.13 ml, 10.60 mmol) and a crystal of NaI to afford 2.13 g (45%) of **14e** as a pale yellow oil; tlc R_f 0.64 (ethyl acetate:hexanes, 2:1); ^{1}H nmr (DMSO-d_6) δ 1.19 (t, 3H, CH_3), 2.07 (m, 2H, CH_2), 3.14 (m, 2H, N-CH_2), 3.51 (s, 3H, 4'-OCH_3), 3.70 (s, 6H, 3',5'-diOCH_3), 4.02 (m, 2H, $COOCH_2$), 4.20 (m, 1H, CHCN), 5.58 (t, 1H, NH), 5.85 (s, 2H, C_6H_2).

2-Cyano-3-(2,5-dimethoxyphenylamino)propionic Acid Ethyl Ester (**14f**).

Compound **14f** was obtained from intermediate **13f** (1.07 g, 4.96 mmol), sodium methoxide (0.27 g, 4.96 mmol), ethyl cyanoacetate (0.80 ml, 7.44 mmol), and a crystal of NaI to afford 0.58 g (40%) of **14f** as a gray oil; tlc R_f 0.57 (ethyl acetate:hexanes, 1:2); ^{1}H nmr (DMSO-d_6) δ 1.20 (t, 3H, CH_3), 2.10 (m, 2H, CH_2), 3.18 (m, 2H, NCH_2), 3.65 (s, 3H, OMe), 3.68 (s, 3 H, OMe), 4.17 (m, 3H, $COOCH_2$ and CHCN), 5.14 (t, 1H, J = 6.1 Hz, NH), 6.06-6.12 (m, 2H, C_6H_3), 6.68 (d, 1H, J = 8.4 Hz, C_6H_3).

General Procedure for the Synthesis of Compounds **2a-f**.

To a solution of sodium methoxide in absolute ethanol was added guanidine hydrochloride. The reaction mixture was stirred at room temperature for 30 minutes, the appropriate intermediate (**14a-f**) was added. The reaction mixture was stirred under nitrogen at 95 °C for 2-4 hours and quenched by adding water. To this solution was added silica gel and the solvent evaporated. The silica gel plug obtained was loaded on a silica gel column and eluted with $CHCl_3$:MeOH:NH_4OH (10:1:0.1). Fractions containing the pure product (tlc) were pooled and evaporated to afford the product.

2,6-Diamino-5-(2-phenylaminoethyl)pyrimidin-4(3*H*)-one (**2a**).

Compound **2a** was synthesized from intermediate **14a** (0.44 g, 1.90 mmol), sodium methoxide (0.64 g, 11.76 mmol) and guanidine hydrochloride (1.09 g, 11.40 mmol) using the general

procedure described above to afford 0.20 g (45%) of **2a** as a white solid, mp > 240 °C dec; tlc R_f 0.33 ($CHCl_3$:MeOH, 5:1); ^{1}H nmr (DMSO-d_6) δ 2.47 (t, 2H, CH_2), 2.92 (m, 2H, NCH_2), 5.47 (t, 1H, NH), 5.81 (s, 2H, NH_2), 5.99 (s, 2H, NH_2), 6.50 (m, 3H, C_6H_5), 7.04 (t, 2H, C_6H_5) 9.88 (s, 1H, NH).

Anal. Calcd for $C_{12}H_{15}N_5O$: C, 58.76; H, 6.16; N, 28.30. Found: C, 58.48; H, 6.06; N, 28.20.

2,6-Diamino-5-[2-(4-chlorophenylamino)ethyl]pyrimidin-4(3*H*)-one (**2b**).

Compound **2b** was synthesized from intermediate **14b** (0.21 g, 0.77 mmol), sodium methoxide (0.27 g, 4.98 mmol) and guanidine hydrochloride (0.52 g, 4.62 mmol) using the general procedure described above to afford 0.10 g (48%) of **2b** as a white solid, mp > 250 °C dec; tlc R_f 0.35 ($CHCl_3$:MeOH, 5:1); ^{1}H nmr (DMSO-d_6) δ 2.45 (t, 2H, CH_2), 2.89 (t, 2H, NCH_2), 5.74 (s, 1H, NH), 5.82 (s, 2H, NH_2), 5.99 (s, 2H, NH_2), 6.52 (d, 2H, C_6H_5), 7.04 (d, 2H, C_6H_5), 9.84 (s, 1H, NH).

Anal. Calcd for $C_{12}H_{14}N_5OCl$•0.1 H_2O: C, 51.20; H, 5.08; N, 24.88; Cl, 12.59. Found: C, 51.06; H, 5.00; N, 24.70; Cl, 12.61.

2,6-Diamino-5-[2-(naphthalen-1-yl-amino)ethyl]pyrimidin-4(3*H*)-one (**2c**).

Compound **2c** was synthesized from intermediate **14c** (0.54 g, 1.92 mmol), sodium methoxide (0.15 g, 2.76 mmol) and guanidine hydrochloride (0.22 g, 2.30 mmol) using the general procedure described above to afford 0.35 g (10%) of **2c** as a gray solid, mp > 260 °C dec; tlc R_f 0.47 ($CHCl_3$:MeOH, 5:1); ^{1}H nmr (DMSO-d_6) δ 2.67 (t, 2H, CH_2), 3.11 (t, 2H, CH_2-N), 6.00 (bs, 4H, 2-NH_2 and 4-NH_2), 6.35 (d, 1H, J = 7.5 Hz, $C_{10}H_7$), 6.61 (s, 1H, NH), 7.01 (d, 1H, J = 8.0 Hz, $C_{10}H_7$), 7.23 (t, 1H, $C_{10}H_7$), 7.36 (m, 2H, $C_{10}H_7$), 7.70 (d, 1H, J = 7.9 Hz, $C_{10}H_7$), 8.01 (d, 1H, J = 8.0 Hz, $C_{10}H_7$), 10.16 (s, 1H, NH).

Anal. Calcd for $C_{16}H_{17}N_5O$•0.6 $CH_3COOC_2H_5$: C, 63.47; H, 6.31; N, 20.11. Found: C, 63.57; H, 6.26; N, 19.96.

2,6-Diamino-5-[2-(naphthalen-2-yl-amino)ethyl]pyrimidin-4(3*H*)-one (**2d**).

Compound **2d** was synthesized from intermediate **14d** (0.45 g, 1.77 mmol), sodium methoxide (0.50 g, 9.22 mmol) and guanidine hydrochloride (0.85 g, 8.85 mmol) using the general procedure described above to afford 0.13 g (28%) of **2d** as a light brown solid, mp > 243 °C dec; tlc R_f 0.47 ($CHCl_3$:MeOH, 5:1); ^{1}H nmr (DMSO-d_6) δ 2.55 (t, 2H, CH_2) , 3.02 (m, 2H, CH_2), 5.86 (bs, 3H, 9-NH and NH_2), 6.01 (s, 2H, NH_2), 6.68 (s, 1H, $C_{10}H_7$), 6.90 (d, 1H, J = 8.3 Hz, $C_{10}H_7$), 7.06 (t, 1H, $C_{10}H_7$), 7.27 (t, 1H, $C_{10}H_7$), 7.52-7.62 (m, 3H, $C_{10}H_7$), 9.88 (s, 1H, NH).

Anal. Calcd for $C_{16}H_{17}N_5O$•0.5 H_2O: C, 63.14; H, 5.96; N, 23.01. Found: C, 63.19; H, 5.72; N, 22.84.

2,6-Diamino-5-[2-(3,4,5-trimethoxyphenylamino)ethyl]pyrimidin-4(3*H*)-one (**2e**).

Compound **2e** was synthesized from intermediate **14e** (0.58 g, 1.95 mmol), sodium methoxide (1.14 g, 21.12 mmol) and guanidine hydrochloride (1.89 g, 19.80 mmol) using the general procedure described above to afford 0.27 g (45%) of **2e** as a light pink solid, mp 181-183 °C; tlc R_f 0.36 ($CHCl_3$:MeOH, 5:1); ^{1}H nmr (DMSO-d_6) δ 2.45 (t, 2H, CH_2) , 2.90 (m, 2H, N-CH_2), 3.51 (s, 3H, 4'-OMe), 3.70 (s, 6H, 3',5'-diOMe), 5.31 (t, 1H, 9-NH), 5.81 (s, 2H, NH_2), 5.86 (s, 2H, C_6H_2), 5.98 (s, 2H, NH_2), 9.88 (s, 1H, NH).

Anal. Calcd for $C_{15}H_{21}N_5O_4$: C, 53.72; H, 6.31; N, 20.88. Found: C, 53.84; H, 6.48; N, 20.67.

2,6-Diamino-5-[2-(2,5-dimethoxyphenylamino)ethyl]pyrimidin-4(3*H*)-one (**2f**).

Compound **2f** was synthesized from intermediate **14f** (0.57 g, 1.95 mmol), sodium methoxide (1.14 g, 21.12 mmol) and guanidine hydrochloride (1.89 g, 19.80 mmol) using the general procedure described above to afford 0.16 g (28%) of **2f** as a white solid. The compound obtained was identical to that synthesized using the alternative synthesis reported above.

General Procedure for Synthesis of Compounds **3a** and **3e**.

To a suspension of **2a** or **2e** in acetonitrile was added formaldehyde, followed by sodium cyanoborohydride. The pH of the resulting mixture was adjusted to 2-3 with conc. HCl. Within 5 minutes, tlc ($CHCl_3$:MeOH, 5:1, with a drop of NH_4OH) indicated the presence of a new spot and the starting material was consumed in 30 minutes. This suspension, cooled in an ice bath, was neutralized to pH 7-8 with conc. ammonium hydroxide. Methanol was added to dissolve the precipitate followed by silica gel and the solvent evaporated to afford a silica gel plug. The silica gel plug obtained was loaded on a silica gel column and eluted with $CHCl_3$:MeOH:NH_4OH (100:10:0.1). Fractions containing the product (tlc) were pooled and evaporated to afford the product.

2,6-Diamino-5-[2-(methylphenylamino)ethyl]pyrimidin-4(3*H*)-one (**3a**).

Compound **3a** was obtained from intermediate **2a** (0.10 g, 0.43 mmol), formaldehyde (0.12 ml, 4.30 mmol) and sodium cyanoborohydride (89 mg, 1.29 mmol) using the general procedure described above to afford 0.047 g (44%) of **3a** as a pale yellow solid, mp 218-219 °C; tlc R_f 0.39 ($CHCl_3$:MeOH, 5:1); ^{1}H nmr (DMSO-d_6) δ 2.37 (t, 2H, CH_2), 2.89 (s, 3 H, CH_3), 3.16 (t, 2 H, NCH_2), 5.75 (s, 2 H, NH_2), 5.95 (s, 2 H, NH_2), 6.53 (t, 1H, C_6H_5), 6.80 (d, 2H, C_6H_5), 7.10 (t, 2H, C_6H_5), 9.75 (s, 1H, NH).

Anal. Calcd for $C_{13}H_{17}N_5O$: C, 60.21; H, 6.61; N, 27.01. Found: C, 60.01; H, 6.60; N, 26.84.

2,6-Diamino-5-{2-[methyl-(3,4,5-trimethoxyphenyl)amino]ethyl}-pyrimidin-4(3*H*)-one (**3e**).

Compound **3e** was obtained from intermediate **2e** (100 mg, 0.31 mmol), formaldehyde (0.09 ml, 3.10 mmol), and sodium cyanoborohydride (64 mg, 0.93 mmol) using the general procedure described above to afford 0.05 g (47%) of **3e** as a pale orange solid, mp 190-192 °C; tlc R_f 0.41 ($CHCl_3$:MeOH, 5:1); ^{1}H nmr (DMSO-d_6): δ 2.40 (t, 2H, CH_2) , 2.92 (s, 1H, CH_3), 3.15 (m, 2H, N-CH_2), 3.53 (s, 3H, 4'-OMe), 3.77 (s, 6H, 3',5'-diOMe), 5.80 (s, 2H, NH_2), 5.93 (s, 2H, NH_2), 6.13 (s, 2H, C_6H_2), 9.91 (s, 1H, NH).

Anal. Calcd for $C_{16}H_{23}N_5O_4$• 0.3 H_2O: C, 54.17; H, 6.70; N, 19.74. Found: C, 53.81; H, 6.88; N, 20.10.

General Procedure for the Synthesis of Compounds **15b-d**.

To a mixture of **13b-d** and formaldehyde in 1,2-dichloro-ethane were added sodium triacetoxyborohydride and acetic acid. The resulting reaction mixture was stirred at room temperature for 24 hours and then poured into water and extracted with CH_2Cl_2. The organic layer was washed with

brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography and eluted with ethyl acetate:hexanes (1:30) to afford the product.

(2-Chloroethyl)-(4-chlorophenyl)methylamine (**15b**).

Compound **15b** was obtained from intermediate **13b** (0.76 g, 3.99 mmol) and formaldehyde (0.57 ml, 19.95 mmol), sodium triacetoxyborohydride (1.27 g, 7.98 mmol), and acetic acid (0.15 ml, 2.61 mmol) using the general procedure described above to afford 0.28 g (34%) of **15b** as a pale yellow oil; tlc R_f 0.47 (ethyl acetate:hexanes, 1:6); ^{1}H nmr (DMSO-d_6) δ 2.93 (s, 3H, NCH_3), 3.65-3.72 (m, 4H, NCH_2 and CH_2Cl), 6.72 (d, 2H, C_6H_4), 7.19 (d, 2H, C_6H_4). HRMS (EI): Calculated for $C_9H_{11}NCl_2$, *m/z* = 204.0968). Found: *m/z* = 204.0969.

(2-Chloroethyl)methylnaphthalen-1-yl-amine (**15c**).

Compound **15c** was obtained from intermediate **13c** (1.98 g, 9.61 mmol), formaldehyde (1.37 ml, 48.05 mmol), sodium triacetoxyborohydride (3.06 g, 19.23 mmol), and acetic acid (0.15 ml, 2.61 mmol) using the general procedure described above to afford 1.41 g (67%) of **15c** as a red oil; tlc R_f 0.47 (ethyl acetate:hexanes, 1:6); ^{1}H nmr (DMSO-d_6) δ 2.84 (s, 3H, NCH_3), 3.34 (m, 2H, CH_2N), 3.82 (t, 2H, J = 6.3 Hz, CH_2Cl), 7.21 (d, 1H, J = 7.4 Hz, $C_{10}H_7$), 7.40-7.53 (m, 3H, $C_{10}H_7$), 7.60 (d, 1H, J = 8.0 Hz, $C_{10}H_7$), 7.87 (t, 1H, $C_{10}H_7$), 8.23 (d, 1H, $C_{10}H_7$).

(2-Chloroethyl)methylnaphthalen-2-yl-amine (**15d**).

Compound **15d** was obtained from intermediate **13d** (0.20 g, 0.98 mmol), formaldehyde (3.60 ml, 4.89 mmol, 37% in water), sodium triacetoxyborohydride (0.62 g, 2.93 mmol), and acetic acid (0.15 ml, 2.61 mmol) using the general procedure described above to afford 0.75 g (35%) of **15d** as a yellow oil; tlc R_f 0.81 (ethyl acetate:hexanes, 1:2); ^{1}H nmr (DMSO-d_6) δ 3.05 (s, 3H, CH_3), 3.35 (m, 2H, CH_2N), 3.79 (t, 2H, CH_2Cl), 6.96 (s, 1H, $C_{10}H_7$), 7.14-7.37 (m, 3H, $C_{10}H_7$), 7.65-7.82 (m, 3H, $C_{10}H_7$).

General Procedure for the Synthesis of Compounds **3b-d**.

To a stirred solution of sodium methoxide in absolute ethanol was added ethyl cyanoacetate. The solution was stirred for 5 minutes at room temperature and then evaporated *in vacuo* at 40 °C, and the residue was dissolved in dry DMF. To the solution were added **15b-d** and a crystal of NaI, and the solution was stirred at 95-100 °C under nitrogen for 2 hours. The mixture was poured into water and extracted with CH_2Cl_2 (6 × 15 ml). The organic layer was washed with brine (2 × 30 ml), dried with Na_2SO_4, filtered and purified by flash column chromatography on silica gel eluted with ethyl acetate:hexanes (1:30) to afford **16b-d**. The freshly prepared **16b-d** was immediately added to a stirred mixture of sodium methoxide and guanidine hydrochloride that had been stirred for 30 minutes at room temperature. This mixture was stirred under nitrogen at 90-100 °C for 1 hour and quenched by adding water (1.0 ml). Silica gel was added to this mixture and the solvent evaporated to afford a dry plug. This plug was chromatographed on a silica gel column using a gradient of $CHCl_3$:MeOH (28-30 % NH_3 in MeOH) 20:1 and then 10:1 as the eluent. Fractions containing the pure product (tlc) were pooled and evaporated to afford the product.

2,6-Diamino-5-{2-[(4-chlorophenyl)methylamino]ethyl}-pyrimidin-4(3*H*)-one (**3b**).

Compound **3b** was obtained from intermediate **15b** (1.97 g, 9.64 mmol), sodium methoxide [(0.57 g, 10.61 mmol, first step) and (1.02 g, 18.86 mmol, second step)], ethyl cyanoacetate (1.62 ml, 14.46 mmol), and guanidine hydrochloride (1.74 g, 18.24 mmol) using the general procedure described above to afford 0.34 g (12% over two steps) of **3b** as a white solid, mp 252-253 °C; tlc R_f 0.35 ($CHCl_3$:MeOH, 5:1); ^{1}H nmr (DMSO-d_6) δ 2.37 (t, 2H, CH_2), 2.90 (s, 3H, NCH_3), 3.17 (t, 2H, NCH_2), 5.81 (s, 2H, NH_2), 5.99 (s, 2H, NH_2), 6.81 (d, 2H, C_6H_4), 7.13 (d, 2H, C_6H_4), 9.85 (s, 1H, NH).

Anal. Calcd for $C_{13}H_{16}N_5OCl$: C, 53.15; H, 5.49; N, 23.84; Cl, 12.10. Found: C, 52.86; H, 5.37; N, 23.63; Cl, 12.37.

2,6-Diamino-5-[2-(methylnaphthalen-1-yl-amino)ethyl]pyrimidin-4(3*H*)-one (**3c**).

Compound **3c** was obtained from intermediate **15c** (1.41 g, 6.42 mmol), sodium methoxide [(0.38 g, 7.07 mmol, first step) and (0.68 g, 12.57 mmol, second step)], ethyl cyanoacetate (1.08 ml, 9.64 mmol), and guanidine hydrochloride (1.16 g, 12.16 mmol) using the general procedure described above to afford 0.10 g (5% over two steps) of **3c** as a pale yellow solid, mp 113-115 °C; tlc R_f 0.33 ($CHCl_3$:MeOH, 5:1); ^{1}H nmr (DMSO-d_6) δ 2.57 (t, 2H, CH_2), 2.87 (s, 3H, CH_3), 2.95 (t, 2H, CH_2N), 5.70 (s, 2H, NH_2), 5.95 (s, 2H, NH_2), 7.13 (d, 1H, J = 7.2 Hz, $C_{10}H_7$), 7.35-7.52 (m, 4H, $C_{10}H_7$), 7.83 (d, 1H, J = 7.2 Hz, $C_{10}H_7$), 8.20 (d, 1H, J = 7.7 Hz, $C_{10}H_7$), 9.77 (s, 1H, NH).

Anal. Calcd for $C_{17}H_{19}N_5O$• 0.4 CH_3OH: C, 64.87; H, 6.44; N, 21.74. Found: C, 65.22; H, 6.34; N, 21.45.

2,6-Diamino-5-[2-(methylnaphthalen-2-yl-amino)ethyl]pyrimidin-4(3*H*)-one (**3d**).

Compound **3d** was obtained from intermediate **15d** (1.97 g, 9.64 mmol), sodium methoxide [(0.38 g, 7.07 mmol, first step) and (0.35 g, 6.49 mmol, second step)], ethyl cyanoacetate (1.08 ml, 9.64 mmol), and guanidine hydrochloride (0.60 g, 6.28 mmol) using the general procedure described above to afford 0.18 g (9% over two steps) of **3d** as a gray solid, mp 198-200 °C; tlc R_f 0.41 ($CHCl_3$:MeOH, 5:1); ^{1}H nmr (DMSO-d_6) δ 2.50 (s, 2H, CH_2) , 3.03 (s, 3H, CH_3), 3.34 (s, 2H, CH_2N), 5.81 (s, 1H, NH_2), 6.00 (s, 2H, NH_2), 6.98 (s, 1H, $C_{10}H_7$), 7.10 (t, 1H, $C_{10}H_7$), 7.29 (t, 1H, $C_{10}H_7$), 7.37 (d, 1H, $C_{10}H_7$), 7.57 (d, 1H, $C_{10}H_7$), 7.66 (d, 2H, $C_{10}H_7$), 9.81 (s, 1H, NH). HRMS (EI): Calculated for $C_{17}H_{19}N_5O$: *m/z* = 309.1590. Found: *m*/z = 309.1601.

Acknowledgement.

This work was supported in part by Grants AI 41743 (A.G.) and AI 44661 (A.G.), NIH Contracts N01-AI-87240 (S.F.Q.) and N01-AI-3171 (S.F.Q.) from the National Institutes of Allergy and Infectious Diseases, and CA10914 (R.L.K.) from the National Cancer Institute.

REFERENCES AND NOTES

* Author to whom correspondence should be addressed at Duquesne University: Tel: 412-396-6070. Fax: 412-396-5593.

[1a] Taken in part from the thesis submitted by Y. W. to the Graduate School of Pharmaceutical Sciences, Duquesne University, in partial fulfillment of the requirement for the degree of Master of Science, June 2004; [b] Presented in part at the American Chemical Society National Meeting, Anaheim, CA, March 21-25, 1999; MEDI 047.

[2] E. M. Berman and L. M. Werbel, *J. Med. Chem.*, **34**, 479 (1991).

[3] R. C. Jackson, Antifolate Drugs: Past and Future Perspectives. in *Antifolate Drugs in Cancer Therapy*, A. L. Jackman, Ed., Humana Press, Totowa, NJ, 1999, pp 1-12.

[4] J. Mills and H. Masur, *Sci. Am.* August, 50-57 (1991).

[5] A. Rosowsky, in *Progress in Medicinal Chemistry*, G. P. Ellis, G. B. West, Eds., Elsevier Science Publishers, Amsterdam, 1989, pp 1-252.

[6] L. M. Werbel, in *Folate Antagonists as Therapeutic Agents*. Vol **1**, Sirotnak, FL, pp 261 (1984).

[7] C. J. Allegra, J. A. Kovacs, J. C. Drake, L. C. Swan, B. A. Chabner and H. Masur, *J. Exp. Med.*, **165**, 926 (1987).

[8] H. Masur, M. A. Polis, C. U. Tuazon, D. Ogata-Aralali, J. A. Kovacs, D. Katz, D. Hilt, T. Simmons, I. Feurestein and B. Lundgren, *J. Infect. Dis.*, **167**, 1422 (1993).

[9] C. J. Allegra, J. A. Kovacs, J. C. Drake, L. C. Swan, B. A. Chabner and H. Masur, *J. Clin. Invest.*, **79**, 478 (1987).

[10] J. A. Kovas, C. J. Allegra, J. C. Swan, J. C. Drake, J. E. Parrillo, B. A. Chabner and H. Masur, *Antimicrob. Agents Chemother.*, **32**, 430 (1988).

[11] S. F. Queener, M. S. Bartlett, M. A. Jay, M. M. Durkin and J. W. Smith, *Antimicrob. Agents Chemother.*, **31**, 1323 (1987).

[12] A. Gangjee, M. Kothare, A. Vasudevan and S. F. Queener. Synthesis and Biological Activities of Homologs of Trimethoprim as Inhibitors of Dihydrofolate Reductase From *Pneumocystis carinii* and *Toxoplasma gondii*, MEDI 247, 213th ACS National Meeting, San Francisco, April 13-17 (1997).

[13] Tripos Inc., 1699 South Hanley Road, St. Louis, MO 63144.

[14] A. Gangjee, J. Yu, J. J. McGuire, V. Cody, N. Galitsky, R. L. Kisliuk and S. F. Queener, *J. Med. Chem.*, **43**, 3837 (2000).

[15] V. Cody, A. Wojtczak, T. I. Kalman, J. H. Friesheim and R. L. Blakley, in *Advances in Experimental Medicine and Biology, Chemistry, and Biology of Pteridines and Folates*; J. E. Ayling, M. G. Nair and C. M. Baugh, Eds., Plenum Press, New York, **338**, 481-486 (1993).

[16] A. C. Anderson, K. M. Perry, D. M. Freymann and R. M. Stroud, *J. Mol. Biol.*, **297**, 645 (2000).

[17] J. L. Kelley, E. W. McLean, N. K. Cohn, M. P. Edelstein, D. S. Duch, G. K. Smith, M. H. Hanlon and R. Ferone, *J. Med. Chem.*, **33**, 561 (1990).

[18] F. Seela and U. Leupke, *Chem. Ber.*, **110**, 1462 (1977).