7-Methyl Trimethoprim Analogues as Inhibitors of the Folate Metabolizing Enzymes [1]

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Received January 17, 2003

A series of 5-(1-phenylethyl)pyrimidines **2-10** (Table I) were designed and synthesized as potent and selective inhibitors of *Pneumocystis carinii* (*P. carinii)*, *Toxoplasma gondii* (*T. gondii*) and *Mycobacterium avium* (*M. avium*) dihydrofolate reductases (DHFR). The structure of **2-10** incorporates a 7–methyl group to increase the potency of monocyclic trimethoprim (TMP). The target compounds were synthesized by an acid catalyzed condensation of ethyl cyanoacetate and appropriately substituted benzaldehydes followed by a Michael addition using methyl copper-lithium. The resulting adduct was cyclocondensed with guanidine to afford 2,6-diamino-4-hydroxy-5-(1-phenylethyl)pyrimidines **2-7**. Both amino moieties of **2-4** were protected with pivaloyl groups and their 4-hydroxy group chlorinated with phosphorus oxychloride. The resulting intermediates were subjected to hydrogenation and deprotection to afford **8-10**. Compound **7** was a good inhibitor of DHFR, however the other compounds were poor inhibitors of *P. carinii*, *T. gondii* and *M. avium* DHFR.

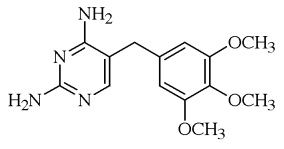
J. Heterocyclic Chem., **40**, 507 (2003).

Opportunistic infections, especially those caused by *Pneumocystis carinii* (*P. carinii*) and *Toxoplasma gondii* (*T. gondii*) are the leading cause of death in patients with acquired immunodeficiency syndrome (AIDS) [2,3]. In addition, *Mycobacterium avium* complex, a group of organisms that is responsible for disseminated infections in AIDS patients, decreases the quality of life of patients with AIDS. *P. carinii* infection is treated with antifolates like trimethoprim along with sulfamethoxazole or with pentamidine. Successful as they are in prolonging the period of survival of AIDS patients, current therapies are effective in only 50-75% of cases with considerable loss of efficacy in patients with two or more episodes of infection [4-6]. For the treatment of *T. gondii* infection, a combination of the antifolate pyrimethamine and sulfadiazine is used. In the current regimens for both infections, relapse rates are high and the side effects are severe enough that in 50% of the cases, the treatment has to be discontinued [7,8].

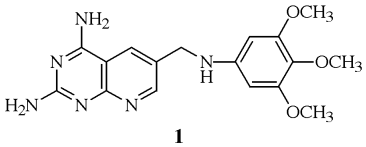
Trimethoprim was reported [9,10] as a weak but selective inhibitor of *P. carinii* dihydrofolate reductase (DHFR) and *T. gondii* DHFR, and has to be administered with sulfonamides to provide synergistic effects.

With potencies 100-10000 times that of trimethoprim against *P. carinni* and *T. gondii* DHFR, trimetrexate (TMQ) and the pyrido[2,3-*d*]pyrimidine piritrexim (PTX) [11,12] would appear to be better choices for treatment. However, due to a complete lack of selectivity resulting in unacceptable toxicities, these drugs have to be used along with leucovorin for host rescue. This provides the host but not *P. carinii* or *T. gondii* (because it can not be taken up efficiently by these organisms) with sufficient reduced folate to circumvent the dihydrofolate reductase inhibition. Up to 60 percent of patients being treated with TMQ suffer from relapse within 3 months [13].

Thus, the dihydrofolate reductase inhibitors currently used clinically are either selective but weak (*e.g*. TMP), or potent but non-selective (*e.g*. TMQ) against these pathogenic dihydrofolate reductases. Thus the discovery of dihydrofolate reductase inhibitors that are both potent and selective is a most desirable goal.



TMP



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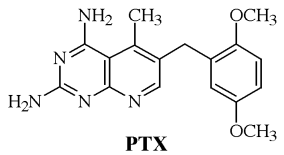
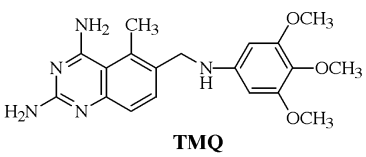


PTX

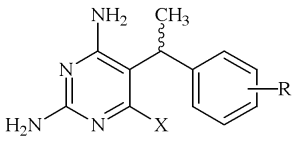


TMQ

Figure 1

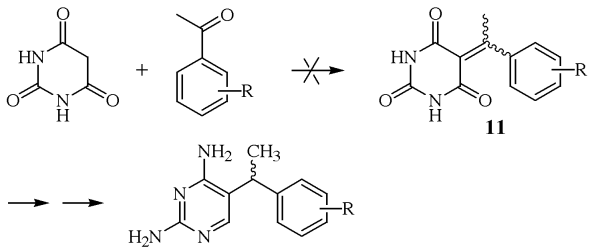
Both TMQ and PTX (Figure I) have a 6-6 ring system and possess a 5-methyl group. Gangjee *et al*. [14] designed and synthesized a 5-desmethylpyrido[2,3-*d*]pyrimidine analogue of TMQ, **1**, whose potency was decreased compared to the 5-methyl analogue. These results suggest that the 5-methyl group in TMQ and perhaps PTX is important for high potency. On the basis of X-ray crystal structure [15,16] and molecular modeling, the 5-methyl group is shown to interact with amino acid residues in dihydrofolate reductase and perhaps also affect the conformation of the 6-side chain. Thus we designed a series of 7-methyl TMP analogues and their derivatives **2-10** (Table I). As in the case of TMP, TMQ, and PTX, all but one of these analogues contains electron donating di- or tri-methoxy substitutions in the phenyl side chain. Compound **7** has electron withdrawing 2,4-dichloro substitutions and was included for comparison. The addition of a methyl group at the 7-position of TMP not only introduces a certain degree of conformational restriction to the side chain (thus decreasing the number of accessible low energy conformations), but should also increase the hydrophobic interaction with dihydrofolate reductase like the 5-methyl moiety of TMQ and PTX. We anticipated that this modification could afford increased potency with retention of selectivity in TMP analogues.

Table I



2	X = OH, R = 3,4-diOMe
3	X = OH, R = 2,4-diOMe
4	X = OH, R = 2,3,4-triOMe
5	X = OH, R = 2,4,5-triOMe
6	X = OH, R = 2,4,6-triOMe
7	X = OH, R = 2,4-diChloro
8	X = H, R = 3,4-diOMe
9	X = H, R = 2,4-diOMe
10	X = H, R = 2,4,5-triOMe

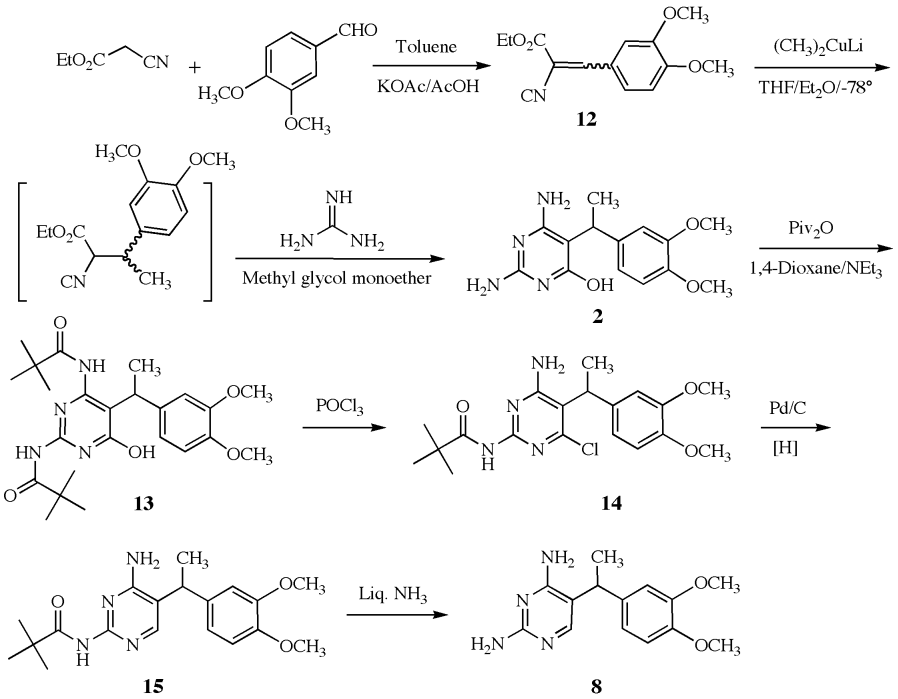
Scheme I



The initial synthetic strategy was to condense barbituric acid and substituted acetophenones to afford the desired heterocycle shown in Scheme I. The condensation product **11** could be converted to the desired target compounds using a literature procedure reported by Chan and Roth [17]. While the barbituric acid readily condensed with benzaldehydes, the acetophenones were extremely inert and did not condense.

The failure of the previous methodology prompted a total synthetic strategy shown in Scheme II. The synthesis

Scheme II



of **2** started with the condensation reaction of 3,4-dimethoxybenzaldehyde with ethyl cyanoacetate, the reaction was carried out in toluene and catalyzed by ammonium acetate and acetic acid. Compound **12** was washed with water and extracted with ethyl acetate. This condensation product was then subjected to a Michael addition with one equivalent of methyl copper lithium which was in turn generated *in situ* by adding two equivalents of 1.5 *M* methyl lithium solution in ethyl ether to one equivalent of copper (I) iodide. The reaction proceeded in dry THF at –78 °C for two hours and was quenched with saturated aqueous ammonium chloride, and the product extracted with ethyl ether which was purified with column chromatography (hexane/ethyl acetate: 6/1, silica gel). Without further purification, the resulting compound was subjected to a condensation reaction with guanidine free base that was in turn obtained from equal amounts of guanidine hydrochloride and sodium methoxide. The reaction occurred in ethoxyethanol and required 3 to 6 hours to reach completion. The resulting reaction mixture was then chromatographed with chloroform/methanol (20/1) on a silica gel column to afford the 4-hydroxy analogue **2**.

The 2,6-diamino groups of **2** were protected with pivaloyl chloride. The reaction was carried out in 1,4-dioxane with triethylamine as the base to afford, after chromatographic purification, a 60% yield of the pivaloyl protected analogue **13**. The protected compound **13** was reacted with phosphorus oxychloride, which afforded the 4-chloro analogue **14**. Catalytic hydrogenation with 5% palladium-charcoal and 50 psi of hydrogen afforded compound **15**. Final depivaloylation was carried out in closed vessel at 150 °C with liquid ammonia to afford compound **8**.

The target compounds **3-7** and **9-10**, were synthesized using the same procedure as in Scheme I without isolation of the intermediates as shown in Scheme III. Compounds **3-7** were obtained in 10-20% overall yield. Compounds **9** and **10** were synthesized from **3** and **5** in 9% and 6% yield, respectively.

Analogues **2-10** were evaluated as inhibitors of *P. carinii*, *T. gondii*, *Mycobacterium avium* and rat liver dihydrofolate reductase and the results are listed in Table II. Compound **7**, the only analogue that had electron withdrawing chloro groups on the phenyl ring was the most active and selective dihydrofolate reductase inhibitor of the series. It inhibited *T. gondii* dihydrofolate reductase with an IC_{50} of 0.58 μM and had a selectivity ratio, versus rat liver dihydrofolate reductase, of 15.3. This analogue was 5-fold more potent and 3-fold less selective as compared to TMP. Compound **7** also inhibited *M. avium* dihydrofolate reductase with an IC_{50} of 0.29 μM and a selectivity ratio of 30.5. The 4-H compounds **8–10** were in general more potent than their 4-hydroxy counterparts (**2**, **3** and **5**) indicating that the 4-hydroxy moiety was detrimental to dihydrofolate reductase inhibition. These results suggest that perhaps the

Scheme III

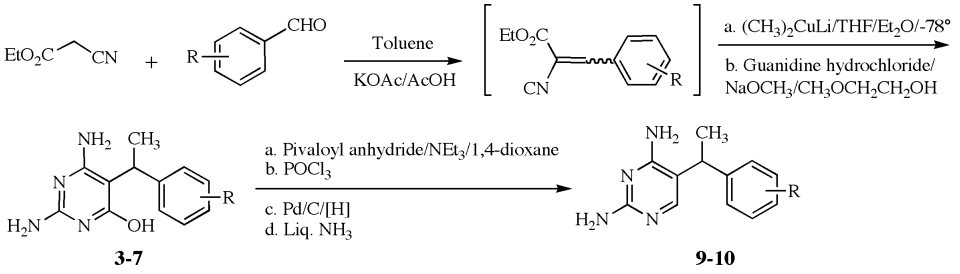


Table II

Inhibitory Concentrations (IC_{50}) in μM and Selectivity Ratios of Analogues **2-10** against *P. carinii* DHFR, *T. gondii* DHFR, *M. avium* DHFR versus Rat Liver DHFR

Compd	P. carinii	Rat liver	Rat liver/*P. carinii*	T. gondii	Rat liver/*T. gondii*	*M. avium*	Rat liver/*M. avium*
2	>95 (10)*	> 95(11)*	ND	89.8	ND	>95(9)*	ND
3	>14 (4)*	>14(12)*	ND	>54 (7)*	ND	>54(8)*	ND
4	>58 (19)*	>58(2)*	ND	>58 (14)*	ND	>58 (14)*	ND
5	135	>131(0)*	ND	268	ND	>131(8)*	ND
6	>23 (1)*	>23(8)*	ND	>23 (18)*	ND	>23(5)*	ND
7	23.2	8.85	0.4	0.58	15.3	0.29	30.5
8	>147 (17)*	>147 (12)*	ND	50.5	ND	19.3	ND
9	>33 (18)*	65.6	ND	17.5	3.7	61.1	1.07
10	>16 (8)*	>16 (4)*	ND	>16 (13)*	ND	5.4	ND
TMQ	0.042	0.003	0.07	0.01	0.3	ND	ND
TMP	12	133	11.1	2.7	49	ND	ND

* Numbers in parentheses are percent inhibition at the highest concentration.

methyl group on the bridge prevents these molecules from assuming the biologically active conformation of TMP and thus renders these compounds (except **7**) poorly active against *P. carinii* dihydrofolate reductase. However, **3**, with electron donating 2,4-dimethoxy substitutions is much less potent and selective than the corresponding electron withdrawing substituents containing analog **7**. This indicates that the nature of the substituent on the phenyl ring does play a major role in the inhibition of these pathogenic DHFR for this series of compounds.

In conclusion, based on the fact that the 5-methyl group is conducive to dihydrofolate reductase inhibitory activities in the case of some dihydrofolate reductase inhibitors with 6,6-fused ring systems, we synthesized 7-methyl trimethoprim analogues **2-10** as novel non-classical dihydrofolate reductase inhibitors using a total synthetic strategy. With the exception of analogue **7**, all the analogues in the series were poor inhibitors of *P. carinii*, *T. gondii* and *M. avium* dihydrofolate reductase. Compound **7** with 2,4-dichloro substituents in the phenyl ring was more potent (compared to TMP) against *T. gondii* and *M. avium* dihydrofolate reductase with some selectivity against *T. gondii* and *M. avium* dihydrofolate reductase.

EXPERIMENTAL

Melting points were determined on a Mel-Temp apparatus and are uncorrected. Nuclear Magnetic Resonance Spectra (^{1}H nmr) were recorded on a Brucker WH-300 (300 MHz). Internal standard trimethysilane (TMS); s = singlet, br s = broad singlet, d = doublet, t = triplet, q = quartet, m = multiplet. High resolution mass spectra (HRMS) were obtained on a VG7070E-HF instrument. Thin layer chromatography was performed on Aldrich silica gel plates with a fluorescent indicator and were visualized with UV-lamp at 254 nm and 366 nm. Column chromatography was performed with (60 Å, 230-400 mesh) silica gel from Aldrich Chemical Company WI, employing gravity or flash columns (2.4 x 15 cm) unless otherwise stated. Elutions were performed using a gradient (specifically stated in the experimental) and 10 ml fractions were collected. Solvents for column chromatography were purchased from Fisher Scientific, PA. Samples for microanalysis were dried under vacuum over phosphorous pentoxide at 75 °C for 24-48 hours utilizing the Chem-Dry apparatus. Analyses were performed by Atlantic Microlabs, Georgia and are within ± 0.4% of the calculated value. Fractional amounts of solvents could not be removed from some of the compounds despite drying under vacuum and were confirmed where possible by their presence in the ^{1}H NMR.

Ethyl-3-(3,4-dimethoxyphenyl)-2-cyanoprop-2-enoate (*E*/*Z* mixture) (**12**).

In a 250 ml round bottom flask was charged 10 g (60 mmol) of 3,4-dimethoxybenzaldehyde, which was followed by the addition of 30 ml of toluene. To the resulting clear solution was added 4.7 g (60 mmol) of NH_4OAc, 11 g (54 mmol) of AcOH and 6.6 g (58 mmol) of ethyl cyanoacetate. The mixture was kept refluxing under a Dean-Stark apparatus for 21 hours. The reaction was then cooled to room temperature. The yellow precipitate was collected and washed with water repeatedly to afford 12.7 g (83%) of a yellow powder. mp 153.1-155.0 °C, tlc R_f=0.29 (hexane/ethyl: acetate 4/1, silica gel); ^{1}H nmr (DMSO-d_6): δ 1.29 (t, 3H, $COCH_2CH_3$), 3.83 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 4.30 (q, 2H, $COCH_2CH_3$), 7.17 (d, 2H, Ar-H), 7.71-7.76 (m, 2H, Ar-H), 8.29 (s, 1H, vinyl proton); HRMS (EI): *m/e* calcd for (M+) $C_{14}H_{15}NO_4$ 261.1001; found 261.0991.

2,6-Diamino-5-[(3',4'-dimethoxyphenyl)ethyl]pyrimidin-4-ol (**2**).

In a 250 ml flask was placed 6.4 g (33.6 mmol) of copper(I) iodide in 30 ml of anhydrous diethyl ether, followed by the addition of 45 ml of 1.5 *M* solution of methyl lithium in diethyl ether (67.2 mmol). The mixture was stirred under nitrogen at –78 °C for 30 min, 8.0 g (30.6 mmol) of **12** dissolved in 30 ml of THF was then added to the mixture. The reaction was allowed to proceed at -78 °C for 2 hours. The reaction mixture was diluted with 40 ml of brine, extracted with ethyl ether (3 x 40 ml), and the extract was dried over anhydrous sodium sulfate and chromatographed (Hexane/ethyl acetate: 6/1, 800 ml) to afford a colorless oil (4.0 g, 48%). This product (1.5 g) was treated with 17.5 mmol of guanidine free base (from 1.7 g (17.5 mmol) of guanidine hydrochloride and 0.95 g (17.5 mmol) of sodium methoxide) in 100 ml of glycol ethyl monoether. The reaction mixture was kept at reflux for 6 hours. The reaction mixture was filtered and the clear filtrate was stripped of solvent *in vacuo*. The residue was then chromatographed (chloroform:methanol, 10/1). The fractions containing the desired spot were pooled and the solvent evaporated *in vacuo*. The residue was recrystallized from ethyl acetate to afford 0.4 g (18%) of the product as a white powder; mp 269.8-274.4 °C, tlc R_f=0.57 (chloroform/methanol: 5/1), ^{1}H nmr (DMSO-d_6): δ 1.47 (d, 3H, $CHCH_3$), 3.69 (s, 6H, 3,4-OMe), 4.07 (q, 1H, $CHCH_3$), 5.32 (s, 2H, NH_2), 5.95 (s, 2H, NH_2), 6.79-6.88 (m, 2H, Ar-H), 6.95 (s, 1H, Ar-H), 9.77 (s, 1H, 4-OH).

Anal. Calcd. for $C_{14}H_{18}N_4O_3 \bullet 0.4H_2O$: C, 56.52; H, 6.37; N, 18.83. Found: C, 56.56; H, 6.15; N, 19.12.

2,4-Dipivaloylamino-5-[(3',4'-dimethoxyphenyl)ethyl]-6-hydroxypyrimidine (**13**).

In a 250 ml round-bottom flask was placed 300 mg (1.0 mmol) of 2, which was dissolved in 30 ml of 1,4-dioxane. Triethylamine (0.52 g, 0.7 ml, 5.0 mmol) was then added, followed by the addition of 0.62 g of pivaloyl chloride (0.64 ml, 5.0 mmol). The reaction was allowed to proceed under reflux for 2 hours. The solvent was then evaporated under reduced pressure. The residue was then chromatographed (hexane/ethyl acetate: 1/1 and then ethyl acetate) to afford 0.25 g (52%) of product; tlc R_f=0.85 (chloroform/methanol: 10/1), ^{1}H nmr (DMSO-d_6): δ 1.20 (m, 18H, 2 $C(CH_3)_3$), 1.63 (d, 3H, $CHCH_3$), 3.74 (s, 6H, 3,4-OCH_3), 3.95 (q, 1H, $CHCH_3$), 6.90 (m, 3H, Ar-H), 9.43 (s, 1H, NH), 11.10 (s, 1H, NH), 11.98 (s, 1H, OH). The product was used without further purification.

4-Amino-6-chloro-2-pivaloylamino-5-[(3',4'-dimethoxyphenyl)-ethyl]pyrimidine (**14**).

A 100 ml round-bottom flask was charged with 250 mg (0.53 mmol) of **13**, which was then dissolved in 30 ml of phosphorus oxychloride. The mixture was kept at reflux for 1.5 hours; phosphorus oxychloride was then evaporated under reduced pressure. The residue was neutralized with concentrated ammonium hydroxide and extracted with chloroform (3x40 ml). The extract

was dried over sodium sulfate and chromatographed (chloroform:methanol, 20/1). 164 mg (75%) of the product was obtained as a light brown powder; mp 152.6-159.3 °C, tlc R_f=0.79 (chloroform:methanol, 10/1), ^{1}H nmr (DMSO-d_6): δ 1.09-1.24 (m, 9H, C(CH_3)$_3$), 1.57 (d, 3H, CHCH_3), 3.71 (s, 3H, OMe), 3.72 (s, 3H, OMe), 4.55 (q, 1H, CHCH$_3$), 6.53 (br s, 2H, NH$_2$), 6.70-6.81 (m, 2H, Ar-H), 6.90 (d, 1H, Ar-H), 9.59 (s, 1H, NH); HRMS (EI): *m/e* calcd for (M+) $C_{19}H_{25}ClN_4O_3$ 392.1615; found 392.1618.

4-Amino-2-pivaloylamino-5-[(3',4'-dimethoxyphenyl)ethyl]-pyrimidine (**15**).

Compound **14**, 150 mg (0.51 mmol), was hydrogenated in 40 ml of ethanol over 100 mg of 5% palladium charcoal under 40 psi pressure for 12 hours. The catalyst was then removed by filtration and the solvent removed *in vacuo*, the residue was chromatographed to afford 119 mg (87%) of the product as light brown powder; ^{1}H nmr (DMSO-d_6): δ 1.16 (s, 9H, C(CH_3)$_3$), 1.48 (d, J=7.1 Hz, 3H, CHCH_3), 3.70 (s, 3H, OCH$_3$), 3.72 (s, 3H, OCH$_3$) 4.01 (q, 1H, CHCH$_3$), 6.54 (br s, 2H, NH$_2$), 6.84-6.90 (m, 1H, Ar-H), 7.91 (d, 1H, Ar-H), 9.29 (s, 1H, NH). The product was used without further purification.

2,4-Diamino-5-[(3',4'-dimethoxyphenyl)ethyl]pyrimidine (**8**).

Compound **15**, 116 mg (0.48 mmol), was placed in a closed vessel, followed by the addition of 30 ml of liquid ammonia. The container was kept in an oil bath with external temperature at 160 °C overnight. The liquid ammonia was allowed to evaporate at room temperature, the residue was first neutralized with acetic acid and then purified with chromatography (chloroform:methanol, 10/1) to afford 27 mg (31%) of **8** as a white powder; mp 201.6-205.5 °C; tlc *Rf*=0.51 (chloroform:methanol, 5/1); ^{1}H nmr (DMSO-d_6): δ 1.40 (d, 3H, CHCH_3), 3.70 (s, 3H, OCH$_3$), 3.72(s, 3H, OCH$_3$), 3.92 (q, 1H, CHCH$_3$), 6.41 (s, 2H, NH$_2$), 6.71 (s, 2H, NH$_2$), 6.77 (s, 1H, Ar-H), 6.87 (d, 2H, Ar-H), 7.60 (s, 1H, 6-H); HRMS (EI): *m/e* calcd for (M+) $C_{14}H_{18}N_4O_2$ 274.1430; found 274.1434.

General Procedure for the Synthesis of Analogues **3-7**.

A round bottom flask was charged with 1 equivalent of the appropriately substituted benzaldehyde, followed by the addition of 30 ml of toluene. To the resulting clear solution was added 1 equivalent of NH_4OAc, 3 equivalents of AcOH and 1 equivalent of ethyl cyanoacetate. The mixture was kept at reflux under a Dean-Stark apparatus for 21 hours. The reaction was then cooled to room temperature. The precipitate that formed was collected and washed with water repeatedly and added to a ethyl ether solution of 1 equivalent of methyl copper lithium (from 1 equivalent of copper(I) iodide and 2 equivalents of methyl lithium) without purification. The reaction was allowed to proceed at –78 °C for 2 hours. The reaction mixture was diluted with 40 ml of brine, extracted with ethyl ether (3 x 40 ml), and the extract was dried over anhydrous sodium sulfate and chromatographed (Hexane/ethyl acetate: 6/1) to afford a colorless oil, which was treated with 3 equivalents of guanidine free base (from 3 equivalents of guanidine hydrochloride and 3 equivalents of sodium methoxide) in 100 ml of glycol ethyl monoether. The reaction mixture was kept at reflux for 6 hours. The solid obtained on cooling was filtered and the clear filtrate was stripped of solvent *in vacuo*. The residue was then chromatographed (chloroform: methanol, 10/1) on a silica gel column. The fractions containing the desired spot were pooled and the solvent evaporated *in vacuo*. The residue was recrystallized from ethyl acetate to afford the product as a white powder.

2,6-Diamino-5-[(2',4'-dimethoxyphenyl)ethyl]pyrimidin-4-ol (**3**).

This compound was synthesized using general procedure from 2,4-dimethoxybenzaldehyde in 16% overall yield; mp 202.0-204.0 °C; tlc R_f=0.60 (chloroform/methanol: 5/1), ^{1}H nmr (DMSO-d_6): δ 1.45 (d, 3H, CHCH_3), 3.71 (s, 3H, OCH$_3$), 3.77 (s, 3H, OCH$_3$), 4.10 (q, 1H, CHCH$_3$), 5.31 (s, 2H, NH$_2$), 5.93 (s, 2H, NH$_2$), 6.43 (t, 2H, Ar-H), 7.39 (d, 1H, Ar-H), 9.71 (s, 1H, 4-OH).

Anal. Calcd. for $C_{14}H_{18}N_4O_3$•0.5CH$_3$OH: C, 56.85; H, 6.58; N, 18.29. Found: C, 56.66; H, 6.32; N, 18.65.

2,6-Diamino-5-[(2',3',4'-trimethoxyphenyl)ethyl]pyrimidin-4-ol (**4**).

This compound was synthesized using general procedure from 2,3,4-trimethoxybenzaldehyde in 14% overall yield; mp 197.6-199.4 °C, tlc R_f=0.60 (chloroform/methanol: 5/1); ^{1}H nmr (DMSO-d_6): δ 1.47 (d, 3H, CHCH_3), 3.68 (s, 3H, OCH$_3$), 3.71 (s, 3H, OCH$_3$), 3.74 (s, 3H, OCH$_3$), 4.06 (q, 1H, CHCH$_3$), 5.35 (s, 2H, NH$_2$), 5.92 (s, 2H, NH$_2$), 6.72 (d, 1H, Ar-H), 7.24 (d, 1H, Ar-H), 9.70 (s, 1H, 4-OH).

Anal. Calcd. for $C_{15}H_{20}N_4O_4$•0.2CH$_3$COOC$_2$H$_5$: C, 56.15; H, 6.44; N, 16.58. Found: C, 56.00; H, 6.35; N, 16.61.

2,6-Diamino-5-[(2',4',5'-trimethoxyphenyl)ethyl]pyrimidin-4-ol (**5**).

This compound was synthesized using general procedure from 2,4,5-trimethoxybenzaldehyde in 20% overall yield; mp 227.2-232.2 °C, tlc R_f=0.60 (chloroform/methanol: 5/1), ^{1}H nmr (DMSO-d_6): δ 1.50 (d, 3H, CHCH_3), 3.64 (s, 3H, OCH$_3$), 3.70 (s, 3H, OCH$_3$), 3.74 (s, 3H, OCH$_3$), 4.05 (q, 1H, CHCH$_3$), 5.39 (s, 2H, NH$_2$), 5.96 (s, 2H, NH$_2$), 6.59 (s, 1H, Ar-H), 7.24 (s, 1H, Ar-H), 9.73 (s, 1H, 4-OH).

Anal. Calcd. for $C_{15}H_{20}N_4O_4$: C, 56.23; H, 6.29; N, 17.49. Found: C, 56.08; H, 6.36; N, 17.44.

2,6-Diamino-5-[(2',4',6'-trimethoxyphenyl)ethyl]pyrimidin-4-ol (**6**).

This compound was synthesized using general procedure from 2,4,6-trimethoxybenzaldehyde in 11% overall yield; mp 208.9-212.6 °C; tlc R_f=0.56 (chloroform/methanol: 5/1); ^{1}H nmr (DMSO-d_6): δ 1.37 (d, 3H, CHCH_3), 3.70 (s, 6H, 2, 6-OCH$_3$), 3.73 (s, 3H, 4-OCH$_3$), 4.56 (q, 1H, CHCH$_3$), 5.32 (s, 2H, NH$_2$), 5.82 (s, 2H, NH$_2$), 6.19 (s, 2H, Ar-H), 9.59 (s, 1H, 4-OH).

Anal. Calcd. for $C_{15}H_{20}N_4O_4$•0.1CH$_3$COOC$_2$H$_5$: C, 56.19; H, 6.37; N, 17.02. Found: C, 56.23; H, 6.28; N, 16.88.

2,6-Diamino-5-[(2',4'-dichlorophenyl)ethyl]pyrimidin-4-ol (**7**).

This compound was synthesized using general procedure from 2,4-dichlorobenzaldehyde in 12% overall yield; mp 208.9-212.6 °C; tlc R_f=0.58 (chloroform/methanol: 5/1); ^{1}H nmr (DMSO-d_6): δ 1.45 (d, 3H, CHCH_3), 4.38 (q, 1H, CHCH$_3$), 5.65 (s, 2H, NH$_2$), 5.95 (s, 2H, NH$_2$), 7.29-7.32 (m, 1H, Ar-H), 7.40 (s, 1H, Ar-H), 7.62 (d, 1H, Ar-H), 9.61 (s, 1H, 4-OH).

Anal. Calcd. for $C_{12}H_{12}Cl_2N_4O$: C, 48.18; H, 4.04; N, 18.73, Cl, 23.70. Found: C, 48.42; H, 4.09; N, 18.51; Cl, 23.99.

General Procedure for the Synthesis of Analogues **10** and **11**.

To a 250 ml flask was added 1 equivalent of **3** or **5** dissolved in 30 ml of 1,4-dioxane. Triethylamine, 5 equivalents, was then added, followed by the addition of 5 equivalents of pivaloyl chloride. The reaction was allowed to proceed under reflux for 2 hours.

The solvent was evaporated under reduced pressure. The residue was chromatographed (hexane/ethyl acetate: 1/1 and then ethyl acetate) to afford an oil, which was dissolved directly in 30 ml of phosphorus oxychloride. The mixture was refluxed for 1.5 hours; phosphorus oxychloride was then evaporated under reduced pressure. The residue was neutralized with concentrated ammonium hydroxide and extracted with chloroform (3x40 ml). The extract was dried over sodium sulfate and chromatographed (chloroform:methanol, 20/1), the fractions with the desired spot were pooled and the solvent evaporated, the oily residue was hydrogenated in 40 ml of ethanol over 100 mg of palladium charcoal under 40 psi pressure for 12 hours. The catalyst was then removed by filtration and the solvent removed *in vacuo*. The residue was chromatographed to afford a brown oil, which was transferred to a closed vessel, followed by the addition of 30 ml of liquid ammonia. The container was heated in an oil bath with external temperature at 160 °C overnight. The liquid ammonia was allowed to evaporated at room temperature, the residue was first neutralized with acetic acid and then chromatographically purified using silica gel and chloroform:methanol, 10/1.

2,4-Diamino-5-[(2',4'-dimethoxyphenyl)ethyl]pyrimidine (**9**).

This compound was synthesized using the general procedure from **3** in 9% overall yield; mp 182.4-184.5 °C; tlc R_f=0.56 (chloroform/methanol: 5/1); ^{1}H nmr (DMSO-d_6): δ 1.35 (d, J=6.8Hz, 3H, CHCH_3), 3.70 (s, 3H, OCH_3), 3.75 (s, 3H, OCH_3), 4.07 (q, 1H, C$H$$CH_3$), 5.71 (s, 2H, NH_2), 5.87 (s, 2H, NH_2), 6.47-6.53 (m, 2H, Ar-H), 7.02 (d, 1H, Ar-H), 7.47 (s, 1H, 6-H).

Anal. Calcd. for $C_{14}H_{18}N_4O_2$•0.5CH_3COOH: C, 59.20; H, 6.62; N, 18.41. Found: C, 58.84; H, 6.37; N, 18.52.

2,4-Diamino-5-[(2',4',5'-trimethoxyphenyl)ethyl]pyrimidine (**10**).

This compound was synthesized using general procedure from **5** in 6% overall yield; mp 267.0-271.9 °C; tlc R_f=0.35 (chloroform/methanol: 5/1); ^{1}H nmr (DMSO-d_6): δ 1.40 (d, 3H, CHCH_3), 3.70 (s, 3H, OCH_3), 3.75 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 4.15 (q, 1H, C$H$$CH_3$), 6.70 (s, 1H, Ar-H), 6.87 (d, 1H, Ar-H), 7.33 (s, 1H, 6-H), 7.37 (s, 2H, NH_2), 7.61 (br s, 2H, NH_2); HRMS (EI): *m/e* calcd for (M+) $C_{15}H_{20}N_4O_3$ 304.1535; found 304.1548.

Acknowledgement.

This work was supported in part by NIH grants AI41743 (A. G.) and AI44661 (A. G.) from the National Institute of Allergy and Infectious Diseases.

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