Design, Synthesis and Biological Evaluation of 2,4-Diamino-6-methyl-5-substitutedpyrrolo[2,3-*d*]pyrimidines as Dihydrofolate Reductase Inhibitors

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Nine novel nonclassical 2,4-diamino-6-methyl-5-thioarylsubstituted-7*H*-pyrrolo[2,3-*d*]pyrimidines **2-10** were synthesized as potential inhibitors of dihydrofolate reductase and as antitumor agents. The analogues contain various electron donating and electron withdrawing substituents on the phenylsulfanyl ring of the side chains and were synthesized from the key intermediate 2,6-diamino-6-methyl-7*H*-pyrrolo[2,3-*d*]-pyrimidine, **14**. Compound **14**, was in turn obtained by chlorination of 4-position of 2-amino-6-methylpyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one, **16** followed by displacement with ammonia. Appropriately substituted phenyl thiols were appended to the 5-position of **14** *via* an oxidative addition reaction using iodine, ethanol and water. The compounds were evaluated against rat liver, rat-derived Pneumocystis, *Mycobacterium avium* and *Toxoplasma gondii* dihydrofolate reductase. The most potent and selective inhibitor, (**2**) has a 1-naphthyl side chain. In this series of compounds electron-withdrawing and bulky substituents in the side chain afford marginally active dihydrofolate reductase inhibitors. The single atom sulfur bridge in the side chain of these compounds is not conducive to potent dihydrofolate reductase inhibition.

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Introduction.

Combination therapy remains the primary treatment for *Pneumocystis jirovecii* [1a] [formerly known as *Pneumocystis carinii*] and *Toxoplasma gondii* which are often fatal opportunistic infections in Acquired Immune Deficiency Syndrome patients [1b,2]. *Mycobacterium avium* complex, a group of organisms that is responsible for disseminated infections in AIDS patients, additionally decreases the quality of life of patients with Acquired Immune Deficiency Syndrome. Methotrexate, a classical antifolate, binds very tightly to the dihydrofolate reductase from these organisms but it cannot be used clinically, since these organism lack the carrier system(s) responsible for effective, uptake and transport of methotrexate in mammalian cells [3] and methotrexate is not selective for the dihydrofolate reductase from these organisms. The drawback of classical antifolates has spawned the development of lipophilic nonclassical antifolates that do not require the carrier system(s) as inhibitors of dihydrofolate reductase from opportunistic pathogens. The combination of potency along with selectivity in inhibiting dihydrofolate reductase from these organisms has been an active area of research for a number of years [4,5]. However, inhibitors with these properties have thus far remained elusive. The combination of a weak dihydrofolate reductase inhibitor along with a potent dihydropteroate synthase inhibitor, to produce synergy, is currently used to treat infections caused by opportunistic pathogens. *Pneumocystis jirovecii* infection is treated with a combination of trimethoprim and sulfamethoxazole or with pentamidine [6-8]. A combination of pyrimethamine and sulfadiazine is used to treat *Toxoplasma gondii* infection. Although combination chemotherapy is effective in most patients with these infections, relapse rates are high and the side effects are severe enough that in 50% of the cases the treatment has to be discontinued [9,10].

Two other agents that are used to treat these pathogenic organisms include the nonclassical antifolate trimetrexate (TMQ) and piritrexim (PTX) [11,12]. These agents inhibit the dihydrofolate reductase from both human cells and the pathogenic organism, which results in host toxicity due to lack of selectivity. Hence, these agents are co-administered with leucovorin, the classical folate cofactor 5-formyltetrahydrofolate which selectively rescues the host cell. Leucovorin is transported into the host cell by the carrier-mediated transport system (only present on the host cell surface) and overcomes the effect of dihydrofolate reductase inhibition. The potential drawbacks of dihydrofolate reductase inhibitor/leucovorin combination therapy include the high cost of leucovorin and the unpredictable effect of leucovorin. Thus there is still a dire need for dihydrofolate reductase inhibitors that are both potent and selective.

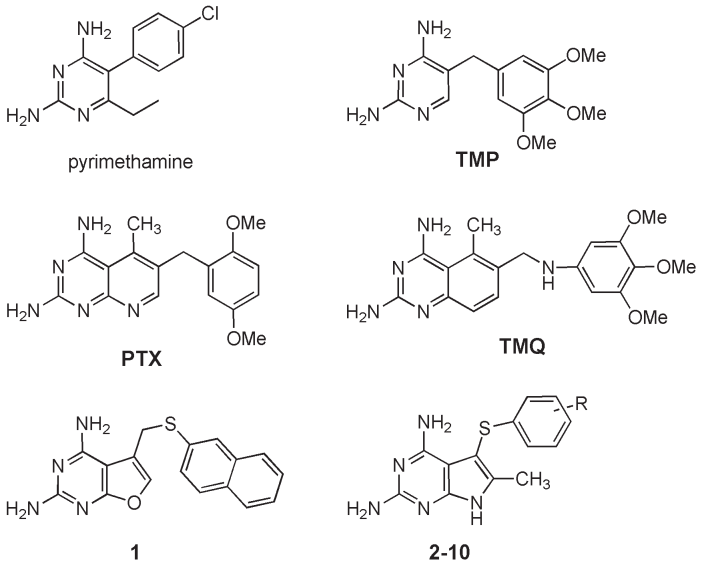
The X-ray crystal structures [11] of dihydrofolate reductase from both human and pathogenic organisms have shown that most of the catalytic site residues are conserved. There are however subtle differences in the amino acid sequences that can be exploited to design dihydrofolate reductase inhibitors that could be selective for these pathogens. One such difference that has recently been exploited by Gangjee *et al.* [18] is the hydrophilic asparagine 64 in human dihydrofolate reductase; a

hydrophobic phenylalanine appears at the corresponding position 69 in the enzyme from rat-derived Pneumocystis [18], or position 91 in *Toxoplasma gondii* [12] whereas a valine appears at position 58 in *Mycobacterium avium* dihydrofolate reductase [13]. Based on the observed differences it should be possible to design selective dihydrofolate reductase inhibitors that interact specifically with these hydrophobic residues in the pathogenic dihydrofolate reductase thus providing selectivity.

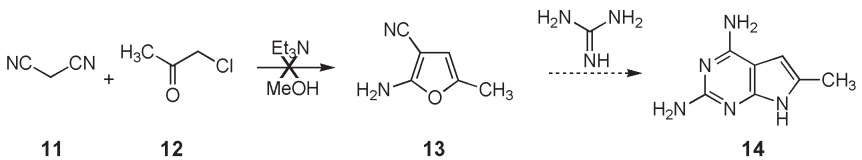
Gangjee *et al*. [14] have previously reported compound **1** (Figure 1) as a selective inhibitor of rat-derived Pneumocystis *and Toxoplasma gondii* dihydrofolate reductase compared to rat liver dihydrofolate reductase. The crystal structure of **1** with rat-derived Pneumocystis dihydrofolate reductase revealed a close hydrophobic interaction between the 2-naphthyl side chain and phenylalanine 69 of rat-derived Pneumocystis dihydrofolate reductase that is suggested to be responsible for its selectivity [14]. Compound **1** has a 6-5 fused furo[2,3-*d*]pyrimidine ring system. Various other [15-18] 6-5 fused ring systems have recently been shown to afford good potency and selectivity against these pathogenic organisms. As part of a program aimed at designing potent and selective inhibitor of pathogenic dihydrofolate reductase we decided to explore the 2,4-diamino-6-methyl-5-thioarylsubstituted-7*H*-pyrrolo-[2,3-*d*]pyrimidines **2-10** as potential dihydrofolate reductase inhibitors.

The key intermediate in the synthesis of compounds **2-10** was **14**. Compound **14** was purportedly obtained in the literature by Jong-Gab [19]. However no method of preparation or supporting physical data for the caracterization of **14** was reported [19]. The reaction of malononitrile (**11**) (Scheme I) with chloroacetone (**12**) using triethylamine as the base was alleged to yield 2-amino-3-cyano-5-methylfuran (**13**), which on further reaction with guanidine in refluxing methanol purportedly afforded, **14**. In our hands, however, a number attempts to obtain **14** *via* this route were unsuccessful. Adding the reactants in different order, variation of temperature (0-50 °C) and bases (DBU, sodium methoxide) also failed to afford the desired furan **13**. The progress of the

Figure 1

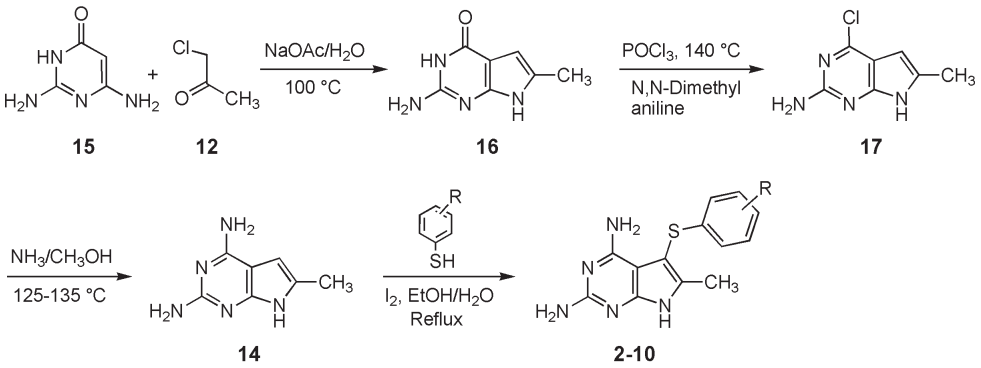


Scheme I



reaction on tlc indicated that no desired spot was formed even after 24 hours. Failure of the above method led us to explore a different strategy (Scheme II). It is well known in the literature [20] that 2-aminopyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one is readily converted to 2,4-diamino-7*H*-pyrrolo[2,3-*d*]pyrimidine by chlorination followed by nucleophilic displacement with ammonia of the resultant 4-chloro intermediate. Thus reaction between 2,6-diamino-4-pyrimidone (**15**) and chloroacetone in sodium acetate/water at 100 °C afforded 2-amino-6-methyl-pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one (**16**) [21]. Chlorination [22] with phosphorous oxychloride and *N*,*N*-dimethylaniline at 140 °C gave the desired 2-amino-4-chloro-6-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (**17**) in 32% yield. In the ^{1}H nmr, the disappearance of the lactam NH proton at 10.78 ppm along with deshielding of the remaining protons indicated that chlorination had indeed taken place. Nucleophilic displacement of the chloro group at the 4-position in **17** with ammonia in methanol at 125-135 °C in a sealed vessel gave the desired intermediate **14** in 76% yield [20]. The appearance of an additional amino peak at 5.22 ppm which exchanged on addition of deuterium oxide indicated that amination had taken place. Analogues **2-10** were obtained in a one-step oxidative addition of the appropriately substituted aryl thiols to the 5-position of **14** [21]. Thus, heating a mixture of **14** and the substituted aryl thiols in a mixture of ethanol/water (2:1) with two equivalents of iodine at reflux for a period of 3-4 hours afforded the desired target compounds **2-10**. Addition of sodium thiosulfate to remove the excess iodine followed by evaporation of the solvent under reduced pressure gave residues which were further purified by column chromatography to afford the target compounds **2-10** in 20-43% yields. The absence of the 5-aromatic proton and the presence of the appropriate protons of the phenyl side chain for **2-10** confirmed that substitution had occurred.

Scheme II



Analogues **2-10** were evaluated as inhibitors of rat-derived Pneumocystis, *Toxoplasma gondii*, *Mycobacterium avium* and rat liver dihydrofolate reductase and the results are listed in Table I. Compounds with electron-withdrawing chloro group(s) in the side chain (**3**, **5**, **6**) or bulk (**2**, **4**) in

Table I

Inhibitory Concentrations (IC_{50}, μM) against Dihydrofolate Reductase and Selectivity Ratios [a]

Compound	*P. carinii*	rat liver	rl/pc [a]	*T. gondii*	rl/tg [a]	*M. avium*	rl/ma [a]
1	0.65	12.3	18.9	11.6	1.1		
2	22.8	40	1.75	18.8	2.13	43.2	0.93
3	28.7	37.4	1.30	31.8	1.18	145	0.26
4	42.8	31	0.72	31	1.00	70	0.44
5	70.6	64	0.91	76.8	0.83	100	0.64
6	61	34.6	0.57	28.2	1.23	73.9	0.47
7	12%@48	9%@48	ND	23%@48	ND	10%@48	ND
8	11%@46	16%@46	ND	87	ND	11%@46	ND
9	12%@81	19%@81	ND	35%@81	ND	6%@81	ND
10	10%@33	16%@33	ND	81	ND	10%@33	ND
trimetrexate	0.042	0.003	0.07	0.010	0.3	0.0015	2.0
trimethoprim	12	180	14	2.8	65	0.3	610

[a] Selectivity ratios, rl/pc = IC_{50} rat liver dihydrofolate reductase/ IC_{50} rat-derived Pneumocystis dihydrofolate reductase; rl/tg = IC_{50} rat liver dihydrofolate reductase/ IC_{50} *T. gondii* dihydrofolate reductase; rl/ma = IC_{50} rat liver dihydrofolate reductase/ IC_{50} *M. avium* dihydrofolate reductase.

the form of a naphthyl ring were more potent than compounds with electron-donating groups in the side chains (**7-9**) or an unsubstituted phenyl (**10**). These results suggest that substitution on the phenyl ring of 2,4-diamino-6-methyl-5-thioarylsubstituted-7*H*-pyrrolo[2,3-*d*]pyrimidines influence both potency and selectivity for dihydrofolate reductase inhibition.

In conclusion, as an extension of our previously reported compound **1**, we designed and synthesized 2,6-diamino-6-methyl-5-thioarylsubstituted-7*H*-pyrrolo[2,3-*d*]pyrimidines **2-10** as potential inhibitors of dihydrofolate reductase from pathogenic organisms. These analogs were synthesized from the known 2-amino-6-methylpyrrolo[2,3-*d*]-pyrimidin-4(3*H*)-one (**16**) by a sequence of chlorination and amination to afford the target **14**. The most potent and selective compound in this series have a 1-naphthyl side chain, and in this series of compounds electron- withdrawing and bulky substituents in the side chain afford marginally active dihydrofolate reductase inhibitors. The single atom sulfur bridge in the side chain of these compounds is not conducive to potent dihydrofolate reductase inhibition.

EXPERIMENTAL

All evaporations were carried out *in vacuo* with a rotary evaporator. Analytical samples were dried *in vacuo* (0.2 mm Hg) in an Abderhalden drying apparatus over phosphorous pentoxide. Melting points were determined on a Mel-Temp apparatus and are uncorrected. Nuclear Magnetic Resonance Spectra (^{1}H nmr) were recorded on a Bruker WH-300 (300 MHz). The chemical shift values are expressed in ppm (parts per million) relative to tetramethylsilane as internal standard; s = singlet, d = doublet, dd = doublet of doublet, t = triplet, q = quartet, m = multiplet, bs = broad singlet. The relative integrals of peak areas agreed with those expected for the assigned structures. High Resolution Mass Spectra were recorded on a VG-7070E-HF instrument. Thin layer chromatography was performed on Aldrich silica gel plates with fluorescent indicator, and the spots were visualized under 254 and 366 nm illumination. Proportions of solvents used for thin layer chromatography are by volume. Elemental analyses were performed by Atlantic Microlabs Inc., Norcoss, GA. Analytical results indicated by element symbols are within ±0.4% of the calculated values. Fractional moles of water or organic solvents frequently found in some analytical samples of antifolates were not removed in spite of 24-48 hours of drying *in vacuo* 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-Amino-6-methyl-4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine (**17**).

To a 100 ml round bottom flask was added compound **16** (500 mg, 3 mmol), 0.2 ml *N,N*-dimethylaniline and 20 ml of phosphorous oxychloride and the resulting suspension was heated at 140 °C for 4 hours. The reaction was stopped at this point and the excess phosphorous oxychloride was evaporated under reduced pressure to give a viscous paste. To this was added ice and the solution was slowly neutralized to pH 6 with dropwise addition of ammonium hydroxide in an ice-bath. The solid that precipitated was filtered and the filtrate extracted with chloroform (5 x 100 ml). The chloroform layer was dried over magnesium sulfate for 6 hours, filtered and evaporated to dryness. To this residue was added the residue obtained on filtration. Methanol (30 ml) was added to the combined solid followed by 10 g of silica gel and the methanol was evaporated to dryness to afford a plug that was loaded on a wet (chloroform) silica gel column and eluted with a gradient of 1-3% methanol in chloroform. Fractions containing the desired spot (tlc) were collected and evaporated to afford 180 mg (32%) of **17** as an off-white solid. mp>270 °C decomposes; tlc R_f=0.63 (chloroform/methanol 5:1 with one drop of ammonium hydroxide); ^{1}H nmr (DMSO-d_6): δ 2.26(s, 3H, 6-CH_3), 5.93(s, 1H, 5-CH), 6.31(bs, 2H, 2-NH_2), 11.29(s, 1H, 7-NH), lit. [23] ^{1}H nmr (DMSO-d_6): δ 2.24(s, 3H, 6-CH_3), 5.90(s, 1H, 5-CH), 6.35(bs, 2H, 2-NH_2), 11.30(s, 1H, 7-NH).

Anal. Calcd. for $C_7H_7N_4Cl$: C, 46.04; H, 3.86; N, 30.68; Cl, 19.41. Found C, 45.98; H, 3.94; N, 30.69 Cl, 19.44.

2,4-Diamino-6-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (**14**).

To a Parr bomb apparatus was added 25 ml methanol and cooled in a dry ice-acetone bath. To this solution was added ammonia gas for 30 minutes along with continuous stirring. Compound **17** (400 mg, 2.2 mmol) was added to this saturated solution of ammonia in methanol following which the bomb was tightly sealed and placed in an oil bath at 135 °C with continuous stirring for 48 hours. At this point tlc indicated the disappearance of the starting material and formation of one major spot at R_f=0.36 (chloroform/methanol 5:1). The reaction was stopped at this point and the solution transferred to a 250 ml round bottom flask. The excess ammonia was allowed to evaporate and 4 grams of silica gel was added to this solution following which the methanol was evaporated and the plug obtained was loaded on a wet (chloroform) silica gel column and eluted with a gradient of 2-10% methanol in chloroform. Fractions containing the desired spot were collected and evaporated to afford 272 mg (76%) of **14** as an off-white solid. mp 240-245 °C; tlc R_f=0.36 (chloroform/methanol 5:1); ^{1}H nmr (DMSO-d_6): δ 2.17(s, 3H, 6-CH_3), 5.22 (bs, 2H, 4-NH_2), 5.91(s, 1H, 5-CH), 6.21(bs, 2H, 2-NH_2), 10.44(s, 1H, 7-NH).

Anal. Calcd. for $C_7H_9N_5$: C, 51.52; H, 5.56; N, 42.92. Found C, 51.35; H, 5.50; N, 42.62.

2,4-Diamino-6-methyl-5-(1-napthylthio)-7*H*-pyrrolo[2,3-*d*]-pyrimidine (**2**).

To a solution of **14** (150 mg, 0.92 mmol) in a mixture of ethanol/water (2:1, 30 ml) was added 1-napthylthiol (295 mg, 1.84 mmol) and the reaction mixture was heated to 100-110 °C, then iodine (470 mg, 1.8 mmol) was added and the heating continued with stirring for a total of 2 hours. To this mixture was added an excess of sodium thiosulfate and the reaction mixture concentrated under reduced pressure. To the resulting residue was added 10 g silica gel and 50 ml methanol and the solution evaporated to dryness to afford a plug which was loaded on top of a silica gel column and eluted with a gradient of 1-3% methanol in chloroform. Fractions containing the desired spot (tlc) were pooled and evaporated to dryness. The resulting residue was recrystallized from methanol, filtered and dried to yield 80 mg (20%) of **2**: mp >256 °C decompose; tlc R_f=0.62 (chloroform/methanol 5:1, with 2 drops of ammonium hydroxide); ^{1}H nmr (DMSO-d_6): δ 2.25 (s, 3H, 6-CH_3), 5.60 (s, 4H, 2-NH_2), 5.90 (s, 2H, 2-NH_2), 6.77-6.78 (d, 1H, $C_{10}H_7$), 7.34 (m, 1H, $C_{10}H_7$),

7.62-7.70 (m, 3H, $C_{10}H_7$), 7.94-7.97 (d, 1H, $C_{10}H_7$), 8.27-8.29 (d, 1H, $C_{10}H_7$), 11.38 (s, 1H, 7NH).

Anal. Calcd. for $C_{17}H_{15}N_5S$: C, 63.53; H, 4.70; N, 21.79; S, 9.98. Found C, 63.29; H, 4.77; N, 21.67; S, 9.96.

2,4-Diamino-6-methyl-5-(4'-chlorophenylthio)-7*H*-pyrrolo[2,3-*d*]-pyrimidine (**3**).

Compound **3** was synthesized as described for **2** using 4-chlorophenylthiol (355 mg, 2.45 mmol) and **14** (200 mg, 1.23 mmol): yield 21%; mp >280 °C; tlc R_f=0.57 (chloroform/methanol 5:1, with 2 drops of ammonium hydroxide); ^{1}H nmr (DMSO-d_6): δ 2.29 (s, 3H, 6-CH_3), 5.60 (s, 2H, 4-NH_2), 5.98 (s, 2H, 2-NH_2), 7.00-7.02 (d, 2H, C_6H_4), 7.29-7.32 (d, 2H, C_6H_4), 11.33 (s, 1H, 7NH); HRMS (EI): *m/e* calculated for (M^+) $C_{13}H_{12}ClN_5S$ 305.0501; found m/z = 305.0500.

2,4-Diamino-6-methyl-5-(2-napthylthio)-7*H*-pyrrolo[2,3-*d*]-pyrimidine (**4**).

Compound **4** was synthesized as described for **2** using 2-naphthylthiol (295 mg, 1.84 mmol) and **14** (150 mg, 0.92 mmol): yield 22%; mp >278 °C; tlc R_f=0.56 (chloroform/methanol 5:1, with 2 drops of ammonium hydroxide); ^{1}H nmr (DMSO-d_6): δ 2.29 (s, 3H, 6-CH_3), 5.61 (s, 2H, 4-NH_2), 6.01 (s, 2H, 2-NH_2), 7.20-7.85 (m, 7H, $C_{10}H_7$), 11.35 (s, 1H, 7NH).

Anal. Calcd. for $C_{17}H_{15}N_5S\times0.2H_2O$: C, 63.53; H, 4.70; N, 21.79; S, 9.98. Found C, 62.83; H, 4.78; N, 21.55; S, 9.87.

2,4-Diamino-6-methyl-5-(3'-chlorophenylthio)-7*H*-pyrrolo[2,3-*d*]-pyrimidine (**5**).

Compound **5** was synthesized as described for **2** using 3-chlorophenylthiol (266 mg, 1.84 mmol) and **14** (150 mg, 0.92 mmol): yield 32%; mp >267 °C; tlc R_f=0.52 (chloroform/methanol 5:1, with 2 drops of ammonium hydroxide); ^{1}H nmr (DMSO-d_6): δ 2.22 (s, 3H, 6-CH_3), 5.65 (s, 2H, 4-NH_2), 5.92 (s, 2H, 2-NH_2), 6.58-6.60 (d, 1H, C_6H_3), 7.28-7.31 (d, 2H, C_6H_3), 7.63 (s, 1H, C_6H_3), 11.44 (s, 1H, 7NH); HRMS (EI): *m/e* calculated for (M^+) $C_{13}H_{12}ClN_5S$ 305.0501; found m/z = 305.0504.

2,4-Diamino-6-methyl-5-(2',4'-dichlorophenylthio)-7*H*-pyrrolo-[2,3-*d*]pyrimidine (**6**).

Compound **6** was synthesized as described for **2** using 2,4-dichlorophenylthiol (330 mg, 1.84 mmol) and **14** (150 mg, 0.92 mmol): yield 25%; mp >286 °C; tlc R_f=0.59 (chloroform/methanol 5:1, with 2 drops of ammonium hydroxide); ^{1}H nmr (DMSO-d_6): δ 2.22 (s, 3H, 6-CH_3), 5.65 (s, 2H, 4-NH_2), 5.92 (s, 2H, 2-NH_2), 6.58-6.60 (d, 1H, C_6H_3), 7.28-7.31 (d, 2H, C_6H_3), 7.63 (s, 1H, C_6H_3), 11.44 (s, 1H, 7NH).

Anal. Calcd. for $C_{13}H_{11}Cl_2N_5S$: C, 45.89; H, 3.26; N, 20.58; S, 9.42; Cl, 20.84. Found C, 46.11; H, 3.30; N, 20.57; S, 9.47; Cl, 20.84.

2,4-Diamino-6-methyl-5-(4'-dimethoxyphenylthio)-7*H*-pyrrolo[2,3-*d*]pyrimidine (**7**).

Compound **7** was synthesized as described for **2** using 4-methoxyphenylthiol (430 mg, 3.06 mmol) and **14** (250 mg, 1.53 mmol): yield 43%; mp >257 °C; tlc R_f=0.57 (chloroform/methanol 5:1, with 2 drops of ammonium hydroxide); ^{1}H nmr (DMSO-d_6): δ 2.27 (s, 3H, 6-CH_3), 3.69 (s, 3H, 4'-OCH_3), 5.57 (s, 2H, 4-NH_2), 6.04 (s, 2H, 2-NH_2), 6.84-6.87 (d, 2H, C_6H_4), 7.00-7.03 (d, 2H, C_6H_4), 11.21 (s, 1H, 7NH).

Anal. Calcd. for $C_{14}H_{15}N_5OS$: C, 55.80; H, 5.02; N, 23.24; S, 10.64. Found C, 55.75; H, 5.03; N, 23.11; S, 10.70.

2,4-Diamino-6-methyl-5-(2',5'-dimethoxyphenylthio)-7*H*-pyrrolo[2,3-*d*]pyrimidine (**8**).

Compound **8** was synthesized as described for **2** using 2,5-dimethoxyphenylthiol (419 mg, 2.46 mmol) and **14** (200 mg, 1.23 mmol): yield 41%; mp >270 °C; tlc R_f=0.58 (chloroform/methanol 5:1, with 2 drops of ammonium hydroxide); ^{1}H nmr (DMSO-d_6): δ 2.24 (s, 3H, 6-CH_3), 3.57 (s, 3H, 2'/5'-OCH_3), 3.82 (s, 3H, 2'/5'-OCH_3), 6.08 (s, 1H, C_6H_3), 6.70-6.73 (m, 2H, C_6H_3), 6.94-6.97 (d, 1H, C_6H_3), 7.18 (s, 2H, 4-NH_2), 7.30-7.42 (br s, 2H, 2-NH_2), 12.20 (s, 1H, 7NH).

Anal. Calcd. for $C_{15}H_{17}N_5O_2S$: C, 54.37; H, 5.17; N, 21.13; S, 9.68. Found C, 53.97; H, 5.12; N, 20.92; S, 9.70.

2,4-Diamino-6-methyl-5-(2'-methoxyphenylthio)-7*H*-pyrrolo-[2,3-*d*]pyrimidine (**9**).

Compound **9** was synthesized as described for **2** using 2-methoxyphenylthiol (430 mg, 3.06 mmol) and **14** (250 mg, 1.53 mmol): yield 36%; mp >252 °C; tlc R_f=0.58 (chloroform/methanol 5:1, with 2 drops of ammonium hydroxide); ^{1}H nmr (DMSO-d_6): δ 2.21 (s, 3H, 6-CH_3), 3.87 (s, 3H, 2'-OCH_3), 5.61 (s, 2H, 4-NH_2), 5.99 (s, 2H, 2-NH_2), 6.50-6.52 (d, 1H, C_6H_4), 6.79 (t, 1H, C_6H_4), 6.97-7.00 (d, 1H, C_6H_4), 7.07-7.09 (t, 1H, C_6H_4), 11.29 (s, 1H, 7NH).

Anal. Calcd. for $C_{14}H_{15}N_5OS\times0.4H_2O$: C, 55.80; H, 5.02; N, 23.24; S, 10.64. Found C, 54.49; H, 5.16; N, 22.70; S, 10.39.

2,4-Diamino-6-methyl-5-phenylthio-7*H*-pyrrolo[2,3-*d*]pyrimidine (**10**).

Compound **10** was synthesized as described for **2** using phenylthiol (136 mg, 1.23 mmol) and **14** (100 mg, 0.61 mmol): yield 25%; mp >280 °C; tlc R_f=0.59 (chloroform/methanol 5:1, with 2 drops of NH_4OH); ^{1}H nmr (DMSO-d_6): δ 2.25 (s, 3H, 6-CH_3), 5.58 (s, 2H, 4-NH_2), 5.98 (s, 2H, 2-NH_2), 7.01-7.04 (m, 2H, C_6H_5), 7.11-7.13 (m, 1H, C_6H_5), 7.26 (m, 2H, C_6H_5), 11.28 (s, 1H, 7NH).

Anal. Calcd. for $C_{13}H_{13}N_5S$: C, 57.54; H, 4.83; N, 25.81; S, 11.82. Found C, 57.33; H, 4.81; N, 25.64; S, 11.78.

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