Synthesis of Novel, Nonclassical 2-Amino-4-Oxo-6-(arylthio)ethylpyrrolo[2,3-*d*]pyrimidines as Potential Inhibitors of Thymidylate Synthase [1]

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Received September 22, 2000

A novel series of 14 nonclassical 6-substituted pyrrolo[2,3-*d*]pyrimidines **2a – 2n** were designed as potential inhibitors of thymidylate synthase, based on previously reported 2-amino-4-oxopyrrolo[2,3-*d*]-pyrimidines **1a** and **1b**. The synthesis of the target compounds **2a–2n** was accomplished by nucleophilic displacement of the mesylate **11** with appropriately substituted aromatic thiols. Most of the target compounds did not show inhibition of either *Escherichia coli* thymidylate synthase or recombinant human thymidylate synthase at the concentrations tested. However, compounds **2h** (2,4-dichloro), **2j** (3,4-dichloro) and **2m** (4-nitro) did show 25%, 40% and 35% inhibition of human thymidylate synthase at 23 μ*M*, 23 μ*M* and 24 μ*M*, respectively. These observations are in accordance with previous reports, which suggest that strong electron withdrawing substituents on the side chain aromatic ring are conducive to inhibition of thymidylate synthase.

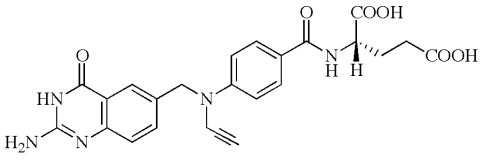
J. Heterocyclic Chem., **38**, 349 (2001).

Thymidylate synthase catalyzes the reductive methylation of 2'-deoxyuridine-5'-monophosphate to 2'-deoxythymidine-5'-monophosphate utilizing 5,10-methylenetetrahydrofolate as the source of the methyl group as well as the reductant [2]. This represents the sole *de novo* source of 2'-deoxythymidine-5'-monophosphate and hence inhibition of thymidylate synthase, in the absence of salvage, leads to "thymineless death". Therefore, the inhibition of thymidylate synthase is an attractive goal for the development of antitumor agents.

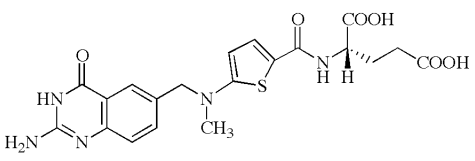
The only thymidylate synthase inhibitor approved by the Food and Drug Administration for antitumor use in the United States is 5-fluorouracil, which is a mechanism-based thymidylate synthase inhibitor. Since many types of cancer either do not respond to 5-fluorouracil or develop resistance to it, there is a pressing need to design novel analogs as potential thymidylate synthase inhibitors and antitumor agents [3].

Several thymidylate synthase inhibitors have entered clinical trials as antitumor agents, notable among them being CB3717 (PDDF) [4], ZD1694 (Tomudex) [5] and LY231514 [6]. All of these are "classical" antifolates, *i.e.* they contain a benzoyl-L-glutamate side chain, which makes them substrates for the enzyme folylpolyglutamate synthetase. Although polyglutamylation of certain classical antifolates appears necessary for cytotoxicity, it has also been implicated as a possible cause of toxicity to host cells and in the development of resistance. In addition, classical antifolates require folate uptake systems to gain access to cells. Thus, lipophilic, nonclassical thymidylate synthase inhibitors could be useful where resistance is due to decreased folylpolyglutamate synthetase activity and/or inefficient uptake systems [7].

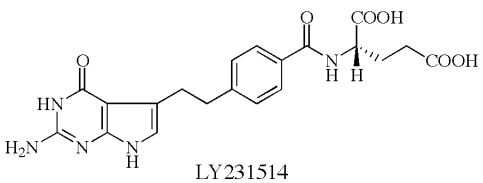
AG337 (Thymitaq) was the first nonclassical thymidylate synthase inhibitor to reach clinical trials [8]. Based on the structure of AG337, we have previously reported compound **1a** as a potent thymidylate synthase inhibitor (human thymidylate synthase IC_{50} = 3.4 x 10^{-7} *M*) [9]. Homologation of **1a** to **1b** resulted in a surprising decrease in potency against human thymidylate synthase (20% inhibition of human thymidylate synthase at 2.6 x10^{-4} *M*) [10]. Molecular modeling using SYBYL 6.5 [11] and its minimization program indicated that the 6-methyl moieties of **1a** and **1b** occupy essentially the same position as does the 6-methyl group of AG337 when bound to *Escherichia coli* thymidylate synthase, such that



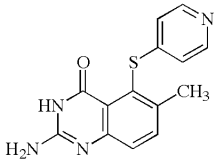
CB3717



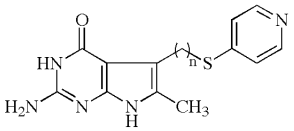
ZD1694

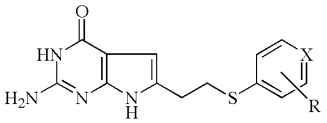


LY231514



AG337





1a: n = 0
1b: n = 1

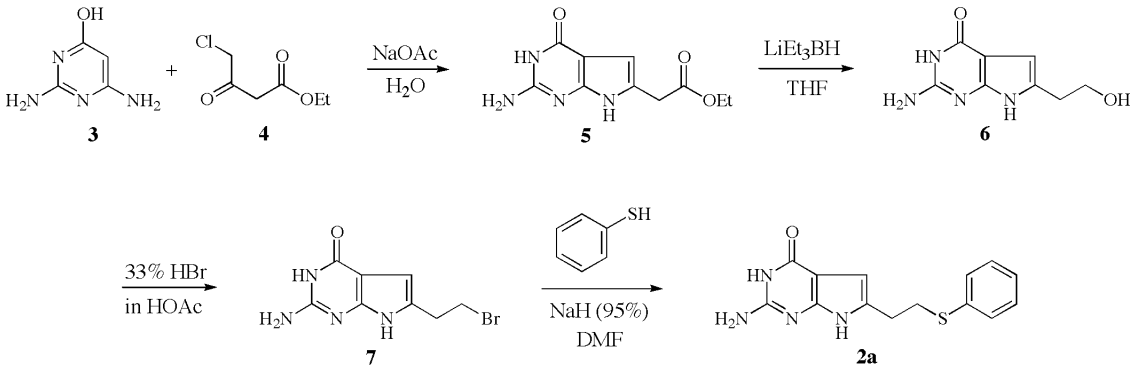
2a: X = CH, R = H
2b: X = CH, R = 2'-OCH_3
2c: X = CH, R = 4'-OCH_3
2d: X = CH, R = 2',5'-diOCH_3
2e: X = CH, R = 3',4'-diOCH_3
2f: X = CH, R = 2'-Cl
2g: X = CH, R = 4'-Cl
2h: X = CH, R = 2',4'-diCl
2i: X = CH, R = 2',5'-diCl
2j: X = CH, R = 3',4'-diCl
2k: X = CH, R = 3',5'-diCl
2l: X = CH, R = 3',4'-C_4H_4
2m: X = CH, R = 4'-NO_2
2n: X = N, R = H

it interacts with a tryptophan residue (Trp80). The 6-methyl group of AG337 also functions to orient the side chain in a conformation conducive to optimum interaction with thymidylate synthase [7]. The conformation restricting influence of the 6-methyl group may not be as significant in **1b** as it is in **1a**, which results in the observed decrease in potency against thymidylate synthase.

Molecular modeling studies further indicated that replacing the 6-methyl group with an extended 3-atom side chain could also enable the side chain to access the same region of thymidylate synthase as does the 5-position substituent. Thus, we designed compounds **2a–2n** to study the effects on thymidylate synthase inhibition and to determine selectivity against thymidylate synthase from various sources.

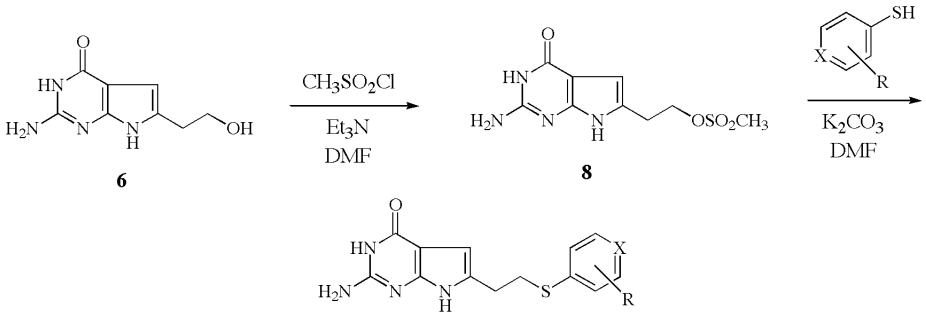
The synthesis of target compounds **2a-2n** commenced from commercially available 2,4-diamino-6-hydroxypyrimidine, **3**, and ethyl-4-chloroacetoacetate, **4** (Scheme 1). In a procedure similar to the one reported by Secrist and Liu [12], cyclocondensation in sodium acetate-water resulted in exclusive formation of the pyrrolopyrimidine **5** in 55% yield. Reduction of the ester was accomplished using 8 equivalents of a 1 *M* solution of lithium triethylborohydride (Super-Hydride) in tetrahydrofuran. The resulting alcohol **6** was thus obtained in 78% yield. The alcohol function of **6** was converted to the bromide by reaction with 33% hydrogen bromide in glacial acetic acid. This afforded the bromide **7** in 88% crude yield, which was used for the subsequent step without further purification. Nucleophilic displacement of the bromide in **7** with benzenethiol in the presence of sodium hydride in *N*,*N*-dimethylformamide at room temperature afforded the desired compound **2a** in extremely poor yield (6%); most of the starting bromide remained unreacted. Attempts to drive the reaction forward by increasing the temperature only resulted in the formation of degradation products, as evidenced by thin layer chromatography, which could not be isolated. Thus, the alcohol **6** was converted to a mesylate, which is a better leaving group than bromide. Reaction of **6** with methanesulfonyl chloride in *N*,*N*-dimethylformamide, using triethylamine as base, indeed afforded the mesylate **8** in 80% yield (Scheme 2). Nucleophilic displacement of the mesylate in **8** with benzenethiol in *N*,*N*-dimethylformamide, using potassium carbonate as base, afforded compound **2a** in improved yield (26%). Similarly, using the appropriate aromatic thiols,

Scheme 1



3 **4** **5** **6** **7** **2a**

Scheme 2



2a: X = CH, R = H
2b: X = CH, R = 2'-OCH_3
2c: X = CH, R = 4'-OCH_3
2d: X = CH, R = 2',5'-diOCH_3
2e: X = CH, R = 3',4'-diOCH_3
2f: X = CH, R = 2'-Cl
2g: X = CH, R = 4'-Cl
2h: X = CH, R = 2',4'-diCl
2i: X = CH, R = 2',5'-diCl
2j: X = CH, R = 3',4'-diCl
2k: X = CH, R = 3',5'-diCl
2l: X = CH, R = 3',4'-C_4H_4
2m: X = CH, R = 4'-NO_2
2n: X = N, R = H

compounds **2b-n** were obtained in yields ranging from 15-38%. ^{1}H nmr spectra and elemental analyses of compounds **2a-2n** were in accordance with their expected structures.

Compounds **2a-2n** were evaluated as inhibitors of *Escherichia coli* thymidylate synthase and recombinant human thymidylate synthase. None of the target compounds inhibited *Escherichia coli* thymidylate synthase at the concentrations tested. However, with human thymidylate synthase, compound **2h**, with a 2,4-dichloro substitution, compound **2j**, with a 3,4-dichloro substitution, and compound **2m**, with a 4-nitro substitution, showed 25% at 23 μ*M*, 40% at μ*M*, and 35% at 24 μ*M* inhibition of human thymidylate synthase respectively. Webber *et al.* [7] and Gangjee *et al.* [13] have reported that strong electron withdrawing groups on the aromatic ring result in better thymidylate synthase inhibitors, and our results seem to be in accord with these observations.

EXPERIMENTAL

All evaporations were carried out *in vacuo* with a rotary evaporator. Melting points were determined on a Mel-Temp II melting point apparatus and are uncorrected. Proton nuclear magnetic resonance (^{1}H nmr) spectra were recorded on a Bruker WH-300 (300MHz) spectrometer. Chemical shifts are expressed as δ values (parts per million) relative to tetramethylsilane as an internal standard: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, bs = broad singlet. Thin layer chromatography (tlc) was performed on silica gel plates with fluorescent indicator, and visualized with light at 254 nm and 366 nm. Column chromatogrphy was performed on 230-400 mesh silica gel purchased from Aldrich Chemical Co., Milwaukee, WI. Solvents routinely used for reactions and purification were purchased from Aldrich or Fisher Scientific Co., Pittsburgh, PA and used without further purification. Proportions of solvents used for thin layer chromatography and column chromatography are by volume. Samples for elemental analysis were dried *in vacuo* in an Abderhalden drying apparatus over phosphorous pentoxide and refluxing ethanol. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA. Elemental compositions are 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 prevented despite drying *in vacuo* at 80 °C and were confirmed, where possible, by their presence in the ^{1}H nmr spectra.

2-Amino-4-oxo-6-carbethoxymethylpyrrolo[2,3-*d*]pyrimidine (**5**).

To a solution of 2,4-diamino-6-hydroxypyrimidine, **3**, (7.20 g, 50 mmole) and sodium acetate (4.10 g, 50 mmole) in water (150 ml) at reflux was added, dropwise, ethyl-4-chloroaceto-acetate, **4** (7.41 ml, 55 mmole). Within an hour, a thick white precipitate appeared. The mixture was refluxed for 18 hours. The suspension was cooled to room temperature, filtered, washed with water (2 x 50 ml), acetone (2 x 50 ml) and dried to afford 7.31 g (54%) of **5** as a buff colored solid: mp >250 °C (lit. mp: 260 °C [12]); ^{1}H nmr (DMSO-d_6) δ 1.16-1.21 (t, 3H, -COOCH$_2$C*H*$_3$), 3.57 (s, 2H, -C*H*$_2$COOEt), 4.04-4.11 (q, 2H, -COOC*H*$_2$CH$_3$), 6.00 (s, 1H, 5-H), 6.04(bs, 2H, 2-NH_2), 10.20 (bs, 1H, 3-H), 10.90 (bs, 1H, 7-H).

2-Amino-4-oxo-6-(2-hydroxyethyl)pyrrolo[2,3-*d*]pyrimidine (**6**).

To a suspension of the ester **5** (1.0 g, 4.24 mmole) in anhydrous tetrahydrofuran (25 ml) at 0 °C was added a 1 *M* solution of lithium triethylborohydride (Super-Hydride) in tetrahydrofuran (33 ml, 33.92 mmole). The solution was stirred for 30 minutes, after which water (30 ml) was added carefully, followed by acidification of the mixture to pH 5.0 with 5 *N* hydrochloric acid. Tetrahydrofuran was evaporated under vacuum, and the white precipitate obtained was refrigerated overnight, filtered and dried under vacuum over phosphorous pentoxide to afford 0.615 g (75%) of **6** as a white solid:

mp >275 °C; ^{1}H nmr (DMSO-d_6) δ 2.60 – 2.64 (t, 2H, -CH_2CH$_2$OH), 3.55 -3.61 (q, 2H, -CH$_2$CH_2OH), 4.61 – 4.64 (t, 1H, -OH), 5.88 (s, 1H, 5-H), 5.96 (bs, 2H, 2-NH$_2$), 10.12 (bs, 1H, 3-H), 10.76 (bs, 1H, 7-H).

2-Amino-4-oxo-6-(2-bromoethyl)pyrrolo[2,3-*d*]pyrimidine (**7**).

A mixture of the alcohol **6** (1.15 g, 6.0 mmole) and 30% hydrogen bromide in glacial acetic acid (30 ml) was stirred at room temperature for 18 hours. The acetic acid was evaporated under reduced pressure. Ether (50 ml) was added, and the solid obtained was filtered, washed with ether and dried to afford 1.6 g (88%) of **7** as a pale brown solid: mp 229-233 °C; ^{1}H nmr (DMSO-d_6) δ 2.77-2.81 (t, 2H, -CH_2CH$_2$Br), 4.17-4.21 (t, 2H, -CH$_2$CH_2Br), 5.94(s, 1H, 5-H), 6.00 (bs, 2H, 2-NH$_2$), 10.15 (bs, 1H, 3-H), 10.88 (bs, 1H, 7-H).

2-Amino-4-oxo-6-(2-methanesulfonyloxyethyl)pyrrolo[2,3-*d*]pyrimidine (**8**).

To a solution of the alcohol **6** (0.25 g, 1.29 mmole) in *N*,*N*-dimethylformamide (20 ml) at 0 °C was added triethylamine (0.27 ml, 1.93 mmole) and methanesulfonyl chloride (0.16 g, 1.42 mmole) and the solution stirred under nitrogen for 2 hours. The reaction mixture was filtered and the filtrate was evaporated to dryness. The residue was suspended in acetone, silica gel (0.50 g) was added to the suspension and the acetone evaporated to form a plug, which was loaded on top of a silica gel column (20 cm x 2 cm) and eluted using a 5:1 mixture of chloroform:methanol. Fractions containing the product (tlc: R_f = 0.41, chloroform:methanol, 3:1) were pooled and the solvent evaporated to afford 0.28 g (80%) of **8** as a white solid: mp >250 °C (dec.); ^{1}H nmr (DMSO-d_6) δ 2.89-2.93 (t, 2H, -CH_2CH$_2$OSO$_2$CH$_3$), 3.13 (s, 3H, -OSO$_2$CH$_3$), 4.36 – 4.41 (t, 2H, -CH$_2$CH_2OSO$_2$CH$_3$), 6.01 (bs, 3H, 2-NH$_2$ & 5-H), 10.17 (bs, 1H, 3-H), 10.91 (bs, 1H, 7-H).

Anal. Calcd. for C$_9$H$_{12}$N$_4$SO$_4$•0.2HCON(CH$_3$)$_2$: C, 40.19; H, 4.71; N, 20.50; S, 11.18. Found: C, 40.26; H, 4.50; N, 20.17; S, 11.27.

2-Amino-4-oxo-6-[2-(phenylthio)ethyl]pyrrolo[2,3-*d*]pyrimidine (**2a**).

To a dispersion of 95% dry sodium hydride (50 mg, 1.99 mmole) in *N*,*N*-dimethylformamide (10 ml) was added thiophenol (182 mg, 1.66 mmole), and the mixture stirred under nitrogen at room temperature. After 1 hour, the bromide **7** (200 mg, 0.77 mmole) was added all at once, and the mixture stirred at room temperature for a further 6 hours. The solvent was evaporated under reduced pressure. The residue was dissolved in methanol, silica gel (0.50 g) was added and the solvent evaporated to form a plug that was loaded on top of a silica gel column (20 cm x 2 cm) and eluted using a gradient of 5%-10% methanol in chloroform. Fractions containing the product (tlc: R_f = 0.26, chloroform:methanol, 9:1) were pooled and the solvent was evaporated to afford 0.014 g (6%) of **2a** as an off white solid: mp >260 °C (dec.); ^{1}H nmr (DMSO-d_6) δ 2.75-2.80 (t, 2H, -CH_2CH$_2$S-), 3.19-3.23 (t, 2H, -CH$_2$CH_2S-), 5.96 (s, 1H, 5-H), 6.01 (bs, 2H, 2-NH$_2$), 7.18-7.33 (m, 5H, phenyl), 10.16 (bs, 1H, 3-H), 10.88 (bs, 1H, 7-H).

Anal. Calcd. for C$_{14}$H$_{14}$N$_4$SO•0.09CHCl$_3$: C, 56.96; H, 4.78; N, 18.86; S, 10.79. Found: C, 57.27; H, 4.88; N, 18.54; S, 10.85.

General Method for the Synthesis of Compounds **2a**-**2n**.

To a solution of the appropriate aromatic thiol in *N*,*N*-dimethylformamide was added potassium carbonate, and the mixture was stirred at room temperature under nitrogen. After 1 hour, the mesylate **8** was added all at once, and the mixture was stirrred for a further 6 hours at room temperature. The solvent was evpaorated under reduced pressure and the residue was dissolved in methanol. Silica gel was added, and the solvent evaporated to form a plug which was dried, loaded on top of a silica gel column (20 cm x 2 cm) and eluted using a gradient of 5%-10% methanol in chloroform. Fractions containing the product were pooled and the solvent was evaporated to afford the product.

2-Amino-4-oxo-6-[2-(phenylthio)ethyl]pyrrolo[2,3-*d*]pyrimidine (**2a**).

Compound **2a** was synthesized using thiophenol (100 mg, 0.92 mmole), potassium carbonate (190 mg, 1.37 mmole) and **8** (125 mg, 0.46 mmole), which afforded 35 mg (26%) of **2a** as an off-white solid, which was identical in all respects to that synthesized by the method described above.

2-Amino-4-oxo-6-[2-(2'-methoxyphenylthio)ethyl]pyrrolo-[2,3-*d*]pyrimidine (**2b**).

Compound **2b** was synthesized using 2-methoxybenzenethiol (256 mg, 1.83 mmole), potassium carbonate (380 mg, 2.75 mmole) and **8** (250 mg, 0.92 mmole), which afforded 68 mg (23%) of **2b** as a white solid: mp 233 °C (dec.); ^{1}H nmr (DMSO-d_6) δ 2.75-2.80 (t, 2H, -CH_2CH$_2$S-), 3.10-3.15 (t, 2H, -CH$_2$CH_2S-), 3.80 (s, 3H, 2'-OCH$_3$), 5.97 (bs, 2H, 2-NH$_2$), 5.98 (s, 1H, 5-H), 6.94-6.98 (q, 2H, aromatic), 7.15-7.20 (t, 1H, aromatic), 7.26-7.29 (dd, 1H, aromatic), 10.14 (bs, 1H, 3-H), 10.88 (bs, 1H, 7-H).

Anal. Calcd. for C$_{15}$H$_{16}$N$_4$SO$_2$: C, 56.95; H, 5.10; N, 17.71; S, 10.13. Found: C, 56.70; H, 5.10; N, 17.53; S, 10.02

2-Amino-4-oxo-6-[2-(4'-methoxyphenylthio)ethyl]pyrrolo-[2,3-*d*]pyrimidine (**2c**).

Compound **2c** was synthesized using 4-methoxybenzenethiol (256 mg, 1.83 mmole), potassium carbonate (380 mg, 2.75 mmole) and **8** (250 mg, 0.92 mmole), which afforded 72 mg (24%) of **2c** as a white solid: mp >230 °C (dec.); ^{1}H nmr (DMSO-d_6) δ 2.68-2.73 (t, 2H, -CH_2CH$_2$S-), 3.06-3.12 (t, 2H, -CH$_2$CH_2S-), 3.74 (s, 3H, 4'-OCH$_3$), 5.93 (s, 1H, 5-H), 5.98 (bs, 2H, 2-NH$_2$), 6.90-6.93 (d, 2H, 3'-H & 5'-H), 7.32-7.35 (d, 2H, 2'-H and 6'-H), 10.13 (bs, 1H, 3-H), 10.83 (bs, 1H, 7-H).

Anal. Calcd. for C$_{15}$H$_{16}$N$_4$SO$_2$•0.2H$_2$O: C, 56.30; H, 5.17; N, 17.51; S, 10.02. Found: C, 56.29; H, 5.11; N, 17.48; S, 10.07.

2-Amino-4-oxo-6-[2-(2',5'-dimethoxyphenylthio)ethyl]pyrrolo-[2,3-*d*]pyrimidine (**2d**).

Compound **2d** was synthesized using 2,5-dimethoxybenzenethiol (313 mg, 1.83 mmole), potassium carbonate (380 mg, 2.75 mmole) and **8** (250 mg, 0.92 mmole), which afforded 49 mg (15%) of **2d** as a white solid: mp 243 °C (dec.); ^{1}H nmr (DMSO-d_6) δ 2.76-2.81 (t, 2H, -CH_2CH$_2$S-), 3.12-3.17 (t, 2H, -CH$_2$CH_2S-), 3.71 (s, 3H, 5'-OCH$_3$), 3.73 (s, 3H, 2'-OCH$_3$), 5.96 (bs, 2H, 2-NH$_2$), 5.98 (s, 1H, 5-H), 6.68-6.71 (dd, 1H, aromatic), 6.79-6.80 (d, 1H, aromatic), 6.87-6.90 (d, 1H, aromatic), 10.14 (bs, 1H, 3-H), 10.87 (bs, 1H, 7-H).

Anal. Calcd. for $C_{16}H_{18}N_4SO_3 \cdot 0.2H_2O$: C, 54.91; H, 5.30; N, 16.01; S, 9.16. Found: C, 54.77; H, 5.20; N, 15.97; S, 9.10.

2-Amino-4-oxo-6-[2-(3',4'-dimethoxyphenylthio)ethyl]pyrrolo-[2,3-*d*]pyrimidine (**2e**).

Compound **2e** was synthesized using 3,4-dimethoxybenzenethiol (313 mg, 1.83 mmole), potassium carbonate (380 mg, 2.75 mmole) and **8** (250 mg, 0.92 mmole), which afforded 80 mg (25%) of **2e** as a white solid: mp 212 °C (dec.); ^{1}H nmr (DMSO-d_6) δ 2.70-2.75 (t, 2H, -CH_2CH$_2$S-), 3.10-3.15 (t, 2H, -CH$_2$CH_2S-), 3.74 (s, 3H, 4'-OCH$_3$), 3.75 (s, 3H, 3'-OCH$_3$), 5.94 (s, 1H, 5-H), 5.98 (bs, 2H, 2-NH$_2$), 6.93-6.94 (m, 3H, aromatic), 10.13 (bs, 1H, 3-H), 10.84 (bs, 1H, 7-H).

Anal. Calcd. for $C_{16}H_{18}N_4SO_3$: C, 55.48; H, 5.24; N, 16.17; S, 9.26. Found: C, 55.52; H, 5.28; N, 16.08; S, 9.15.

2-Amino-4-oxo-6-[2-(2'-chlorophenylthio)ethyl]pyrrolo[2,3-*d*]-pyrimidine (**2f**).

Compound **2f** was synthesized using 2-chlorobenzenethiol (318 mg, 2.20 mmole), potassium carbonate (456 mg, 3.30 mmole) and **8** (300 mg, 1.10 mmole), which afforded 134 mg (38%)of **2f** as a pale yellow solid: mp 247 °C (dec.); ^{1}H nmr (DMSO-d_6) δ 2.82-2.87 (t, 2H, -CH_2CH$_2$S-), 3.24-3.29 (t, 2H, -CH$_2$CH_2S-), 6.01 (bs, 3H, 2-NH$_2$ and 5-H), 7.16-7.21 (t, 1H, aromatic), 7.32-7.37 (t, 1H, aromatic), 7.43-7.46 (d, 2H, aromatic), 10.15 (bs, 1H, 3-H), 10.91 (bs, 1H, 7-H).

Anal. Calcd. for $C_{14}H_{13}N_4ClSO$: C, 52.42: H, 4.08; N, 17.46; Cl, 11.05; S, 9.99. Found: C, 52.11; H, 4.11; N, 17.34; Cl, 11.02; S, 9.91.

2-Amino-4-oxo-6-[2-(4'-chlorophenylthio)ethyl]pyrrolo[2,3-*d*]-pyrimidine (**2g**).

Compound **2g** was synthesized using 4-chlorobenzenethiol (307 mg, 2.13 mmole), potassium carbonate (442 mg, 3.12 mmole) and **8** (290 mg, 1.06 mmole), which afforded 125 mg (36%) of **2g** as a pale green solid: mp 230 °C (dec.); ^{1}H nmr (DMSO-d_6) δ 2.75-2.80 (t, 2H, -CH_2CH$_2$S-), 3.20-3.25 (t, 2H, -CH$_2$CH_2S-), 5.97 (s, 1H, 5-H), 5.99 (bs, 2H, 2-NH$_2$), 7.36 (s, 4H, aromatic), 10.14 (bs, 1H, 3-H), 10.87 (bs, 1H, 7-H).

Anal. Calcd. for $C_{14}H_{13}N_4ClSO \cdot H_2O$: C, 49.63; H, 4.46; N, 16.54; Cl, 10.46; S, 9.46. Found: C, 49.42; H, 4.36; N, 16.35; Cl, 10.71; S, 9.33.

2-Amino-4-oxo-6-[2-(2',4-dichlorophenylthio)ethyl]pyrrolo-[2,3-*d*]pyrimidine (**2h**).

Compound **2h** was synthesized using 2,4-dichlorobenzenethiol (393 mg, 2.20 mmole), potassium carbonate (456 mg, 3.30 mmole) and **8** (300 mg, 1.10 mmole), which afforded 99 mg (25%) of **2h** as a white solid: mp 230 °C (dec.); ^{1}H nmr (DMSO-d_6) δ 2.81-2.86 (t, 2H, -CH_2CH$_2$S-), 3.25-3.30 (t, 2H, -CH$_2$CH_2S-), 6.01 (bs, 3H, 2-NH$_2$ and 5-H), 7.43-7.47 (m, 2H, aromatic), 7.63 (s, 1H, aromatic), 10.16 (bs, 1H, 3-H), 10.90 (bs, 1H, 7-H).

Anal. Calcd. for $C_{14}H_{12}N_4Cl_2SO$: C, 47.33; H, 3.40; N, 15.77; Cl, 19.96; S, 9.03. Found: C, 47.58; H, 3.44; N, 15.49; Cl, 20.01; S, 8.94.

2-Amino-4-oxo-6-[2-(2',5'-dichlorophenylthio)ethyl]pyrrolo-[2,3-*d*]pyrimidine (**2i**).

Compound **2i** was synthesized using 2,5-dichlorobenzenethiol (393 mg, 2.20 mmole), potassium carbonate (456 mg, 3.30 mmole) and **8** (300 mg, 1.10 mmole), which afforded 115 mg (29%) of **2i** as a white solid: mp 236 °C (dec.); ^{1}H nmr (DMSO-d_6) δ 2.83-2.88 (t, 2H, -CH_2CH$_2$S-), 3.31-3.36 (t, 2H, -CH$_2$CH_2S-), 6.00 (bs, 2H, 2-NH$_2$), 6.02 (s, 1H, 5-H), 7.22-7.25 (dd, 1H, aromatic), 7.41-7.42 (d, 1H, aromatic), 7.46-7.48 (d, 1H, aromatic), 10.16 (bs, 1H, 3-H), 10.91 (bs, 1H, 7-H).

Anal. Calcd. for $C_{14}H_{12}N_4Cl_2SO$: C, 47.33; H, 3.40; N, 15.77; Cl, 19.96; S, 9.03. Found: C, 47.17; H, 3.53; N, 15.50; Cl, 19.90; S, 8.98.

2-Amino-4-oxo-6-[2-(3',4'-dichlorophenylthio)ethyl]pyrrolo-[2,3-*d*]pyrimidine (**2j**).

Compound **2j** was synthesized using 3,4-dichlorobenzenethiol (362 mg, 2.02 mmole), potassium carbonate (418 mg, 3.03 mmole) and **8** (275 mg, 1.01 mmole), which afforded 80 mg (22%) of **2j** as a white solid: mp 215 °°C (dec.); ^{1}H nmr (DMSO-d_6) δ 2.77-2.82 (t, 2H, -CH_2CH$_2$S-), 3.25-3.30 (t, 2H, -CH$_2$CH_2S-), 5.98 (bs, 3H, 2-NH$_2$ and 5-H), 7.30-7.33 (m, 1H, aromatic), 7.53-7.57 (m, 2H, aromatic), 10.14 (bs, 1H, 3-H), 10.87 (bs, 1H, 7-H).

Anal. Calcd. for $C_{14}H_{12}N_4Cl_2SO$: C, 47.33; H, 3.40; N, 15.77; Cl, 19.96; S, 9.03. Found: C, 47.24; H, 3.43; N, 15.69; Cl, 20.08; S, 9.02.

2-Amino-4-oxo-6-[2-(3',5'-dichlorophenylthio)ethyl]pyrrolo-[2,3-*d*]pyrimidine (**2k**).

Compound **2k** was synthesized using 3,5-dichlorobenzenethiol (362 mg, 2.02 mmole), potassium carbonate (418 mg, 3.03 mmole) and **8** (275 mg, 1.01 mmole), which afforded 110 mg (30%) of **2k** as a white solid: mp 223 °C (dec.); ^{1}H nmr (DMSO-d_6) δ 2.78-2.83 (t, 2H, -CH_2CH$_2$S-), 3.29-3.33 (t, 2H, -CH$_2$CH_2S-), 5.99 (bs, 3H, 2-NH$_2$ and 5-H), 7.36 (s, 3H, aromatic), 10.15 (bs, 1H, 3-H), 10.89 (bs, 1H, 7-H).

Anal. Calcd. for $C_{14}H_{12}N_4Cl_2SO$: C, 47.33; H, 3.40; N, 15.77; Cl, 19.96; S, 9.03. Found: C, 47.32; H, 3.42; N, 15.64; Cl, 20.09; S, 9.03.

2-Amino-4-oxo-6-[2-(2'-naphthylthio)ethyl]pyrrolo[2,3-*d*]-pyrimidine (**2l**).

Compound **2l** was synthesized using 2-naphthalenethiol (293 mg, 1.83 mmole), potassium carbonate (380 mg, 2.75 mmole) and **8** (250 mg, 0.92 mmole), which afforded 72 mg (23%) of **2l** as a white solid: mp 240 °C (dec.); ^{1}H nmr (DMSO-d_6) δ 2.83-2.88 (t, 2H, -CH_2CH$_2$S-), 3.31-3.36 (t, 2H, -CH$_2$CH_2S-), 6.01 (bs, 3H, 2-NH$_2$ and 5-H), 7.43-7.53 (m, 3H, aromatic), 7.84-7.89 (m, 4H, aromatic), 10.15 (bs, 1H, 3-H), 10.91 (bs, 1H, 7-H).

Anal. Calcd. for $C_{18}H_{16}N_4SO \cdot 0.2H_2O$: C, 63.58; H, 4.86; N, 16.48; S, 9.43. Found: C, 63.62; H, 4.82; N, 16.49; S, 9.45.

2-Amino-4-oxo-6-[2-(4'-nitrophenylthio)ethyl]pyrrolo[2,3-*d*]-pyrimidine (**2m**).

Compound **2m** was synthesized using 4-nitrobenzenethiol (342 mg, 2.20 mmole), potassium carbonate (456 mg, 3.30 mmole) and **8** (300 mg, 1.10 mmole), which afforded 95 mg (26%) of **2m** as a pale orange solid: mp 237 °C (dec.); ^{1}H nmr (DMSO-d_6) δ 2.84-2.89 (t, 2H, -CH_2CH$_2$S-), 3.38 (t, 2H, -CH$_2$CH_2S-), 6.00 (bs, 3H, 2-NH$_2$ and 5-H), 7.51-7.54 (d, 2H, 2'-H and 6'-H), 8.11-8.14 (d, 2H, 3'-H and 5'-H), 10.15 (bs, 1H, 3-H), 10.91 (bs, 1H, 7-H).

Anal. Calcd. for $C_{14}H_{13}N_5SO_3 \cdot 0.7H_2O$: C, 48.89; H, 4.22; N, 20.36; S, 9.32. Found: C, 48.72; H, 4.04; N, 20.13; S, 9.54.

2-Amino-4-oxo-6-[2-(4'-pyridylthio)ethyl]pyrrolo[2,3-*d*]-pyrimidine (**2n**).

Compound **2n** was synthesized using 4-mercaptopyridine (245 mg, 2.20 mmole), potassium carbonate (456 mg, 3.30 mmole) and **8** (300 mg, 1.10 mmole), which afforded 120 mg (38%) of **2n** as a white solid: mp >260 °C (dec.); ^{1}H nmr (DMSO-d_6) δ 2.82-2.87 (t, 2H, -CH_2CH$_2$S-), 3.31 (t, 2H, -CH$_2$CH_2S-), 6.00 (bs, 3H, 2-NH$_2$ and 5-H), 7.28-7.29 (d, 2H, 2'-H and 6'-H), 8.36-8.37 (d, 2H, 3'-H and 5'-H), 10.15 (bs, 1H, 3-H), 10.91 (bs, 1H, 7-H).

Anal. Calcd. for $C_{13}H_{13}N_5SO$•0.4H_2O: C, 53.01; H, 4.72; N, 23.78; S, 10.89. Found: C, 52.97; H, 4.57; N, 23.89; S, 10.68.

Acknowledgement.

This work was supported in part by grants from the National Institute of Allergy and Infectious Diseases AI41743(AG) and AI44661(AG), and from the National Cancer Institute CA10914 (RLK).

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