Synthesis of 2-Amino-4-oxo-5-substitutedbenzylthiopyrrolo[2,3-*d*]-pyrimidines as Potential Inhibitors of Thymidylate Synthase [1]

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A series of ten novel 2-amino-4-oxo-5-[(substitutedbenzyl)thio]pyrrolo[2,3-*d*]pyrimidines **2-11** were synthesized as potential inhibitors of thymidylate synthase and as antitumor agents. The analogues contain various electron withdrawing and electron donating substituents on the benzylsulfanyl ring of the side chains and were synthesized from the key intermediate 2-amino-4-oxo-6-methylpyrrolo[2,3-*d*]pyrimidine, **14**. Appropriately substituted benzyl mercaptans were appended to the 5-position of **14** *via* an oxidative addition reaction using iodine, ethanol and water. The compounds were evaluated against human, *Escherichia coli* and *Toxoplasma gondii* thymidylate synthase and against human, *Escherichia coli* and *Toxoplasma gondii* dihydrofolate reductase. The most potent inhibitor, (**6**) which has a 4'-methoxy substituent on the side chain, has an IC_{50}=25 μM against human thymidylate synthase. Contrary to analogues of general structure **1**, electron donating or electron withdrawing substituents on the side chain of **2-11** had little or no influence on the human thymidylate synthase inhibitory activity.

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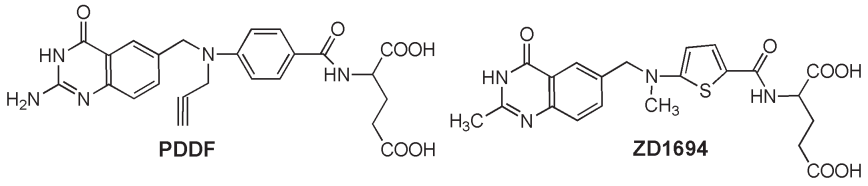
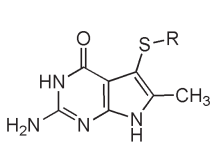
Introduction.

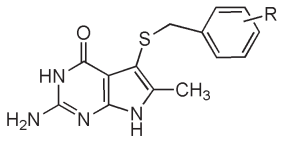
Thymidylate synthase catalyzes the reductive methylation of 2'-deoxyuridine-5'-monophosphate to 2'-deoxythymidine-5'-monophosphate utilizing 5,10-methylenetetrahydrofolate as the source of the methyl group as well as the reductant [2]. This step represents the sole *de novo* source of 2'-deoxythymidine-5'-monophosphate and hence inhibition of thymidylate synthase, in the absence of salvage, leads to "thymineless death". Thus inhibition of thymidylate synthase is an attractive goal for the development of antitumor agents [3,4].

Several thymidylate synthase inhibitors are currently undergoing clinical trials and some of them are clinically used as antitumor agents (Figure 1). Notable among these are ZD1694 [5] and LY231514 [6]. ZD1694 has been approved in Europe as an antitumor agent. LY231514 in combination with cisplatin has recently been approved for the treatment of malignant pleural mesothelioma. Classical antifolates such as PDDF [7], ZD1694 and LY231514 have





1a R=4-pyridine
1b R=3',4'-diClPh
1c R=4'-NO_2Ph



2-11

2 R=H
3 R=2'-CH_3
4 R=2'-Cl
5 R=4'-CH_3
6 R=4'-OCH_3
7 R=4'-Cl
8 R=4'-F
9 R=4'-Br
10 R=2',4'-diCl
11 R=3',4'-diCl

Figure 1

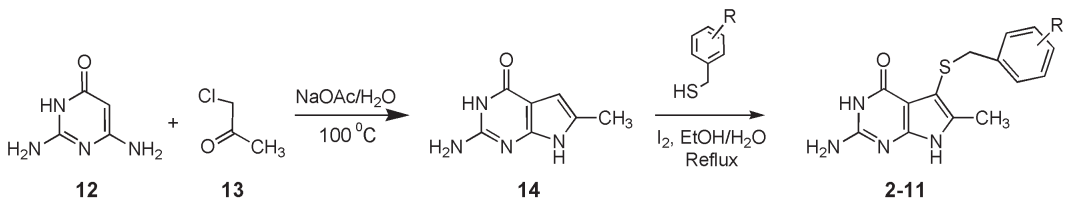
a benzoyl-L-glutamic acid side chain which often makes them substrates for the enzyme folylpoly-γ-glutamate synthetase (FPGS). Folylpoly-γ-glutamate synthetase catalyzes the formation of poly-γ-glutamates which lead to high intracellular concentrations of these antitumor agents and increases thymidylate synthase inhibitory activity for certain antifolates (ZD1694 and LY231514). Although polyglutamation of some antifolates is necessary for cytotoxicity, it is also responsible for toxicity to host cells. Additionally, to gain access into the cell, classical antifolates depend on a reduced folate carrier system, the impairment of which can lead to drug resistance [8]. These problems, associated with classical antifolates can be overcome by nonclassical lipophilic analogues which lack the γ-glutamate moiety. Such analogues would passively diffuse into cells and would not depend on folylpoly-γ-glutamate synthetase for their potency [8].

Based on the structure of AG337 (thymitaq), the first nonclassical thymidylate synthase inhibitor to reach clinical trials [10], we have previously reported compounds **1a-c** as potent inhibitors of human thymidylate synthase [11,12]. Compounds **1b-c**, which have electron withdrawing nitro- and chloro- substituents in either the 3'- and/or 4'- position of the phenyl ring were much more potent than the unsubstituted phenyl or analogues containing methoxy substituents. Compounds **1b-c** are more potent than the classical, clinically used analogs ZD1694 and LY231514 against human thymidylate synthase.

sterically restrict the rotation of the 5-position side-chain so that it adopts the most favorable conformation for binding to thymidylate synthase. Molecular modeling using Sybyl 6.8 [13] and superimposition of compounds **2-11** onto the ternary complex of *Escherichia coli* TS-FdUMP-AG337 [14] indicates that the pyrrolo[2,3-*d*]pyrimidine with a 6-methyl moiety mimics the 6-methyl of the quinazoline of AG337 and, as reported previously by Gangjee *et al*. [11,12] for compounds **1a-c**, provides potent thymidylate synthase inhibition. Thus we elected to retain the 6-methyl group in the design of compounds **2-11** as inhibitors of thymidylate synthase.

The key intermediate in the synthesis of analogues **2-11** was the 2-amino-4-oxo-6-methylpyrrolo[2,3-*d*]pyrimidine, **14** (Scheme 1), to which various side chains could be conveniently appended. Compound **14** was obtained in a one step reaction by the addition of chloroacetone, **13**, to a solution of 2,6-diamino-4-hydroxypyrimidine, **12**, in sodium acetate and water as reported previously [12]. Analogues **2-11** were obtained by oxidative addition of appropriately substituted benzyl mercaptans to the 5-position of **14** (Scheme 1). Thus heating a mixture of **14** with the substituted benzyl mercaptans in a mixture of ethanol/water (2:1) with two equivalents of iodine at reflux for a period of 4-6 hours afforded the desired target compounds **2-11** in reasonably good yields without the necessity of protecting the 2-amino group of **14**, as reported previously [12]. This method had the advantages

Scheme 1



Molecular modeling using SYBYL 6.8 [13] and superimposition of the 6-5 pyrrolo[2,3-*d*]pyrimidine onto the 6-6-quinazoline (AG337) indicated that the smaller 6-5 system has its 5- and 6-atoms closer to the pyrimidine ring than the 5- and 6-atoms of the quinazoline in AG337. In compounds **1a-c** the sulfur bridge is directly attached to the 5-position of the pyrrolo[2,3-*d*]pyrimidine, thus shortening the distance between the pyrimidine ring and the side chain substituent as compared to the quinazolines. We reasoned that increasing the length of the bridge, as in compounds **2-11**, by one atom may compensate for the contraction of the B-ring in 6-5 systems. Webber *et al*. [9] suggested that the 6-methyl moiety of AG337 serves two important functions, one being its ability to make hydrophobic contact with a Trptophan (Trp80) of *Escherichia coli* thymidylate synthase and the other was to

over our previously reported method [12] of shorter reaction times (4-6 hours vs 16 hours) and not requiring protecting and deprotecting the 2-amino group, which occurs in poor yields (20-30%) over two steps. Another significant modification of the reported method [12] was the use of sodium thiosulfate to remove the excess iodine. Further, evaporation of the solvent under reduced pressure afforded a residue which was purified by column chromatography to afford the target compounds **2-11** in 25-46% yields. The ^{1}H nmr of **2-11** indicated the absence of the 5-aromatic proton as well as the presence of methylene protons between 3.90-3.99 ppm and the appropriate aromatic protons of the side-chain phenyl ring, confirmed that substitution had occurred.

Compounds **2-11** were evaluated [15,16] as inhibitors of *Escherichia coli* and human thymidylate synthase along

with PDDF, ZD1694, and LY231514 and as inhibitors of *Escherichia coli* and human dihydrofolate reductase along with methotrexate. The IC_{50} values were all >20 μM, and **2-11** were 10-1000 times less potent than PDDF, ZD1694 or LY231514 against the TS enzymes and 1000 times less potent than methotrexate against the DHFR enzymes. Surprisingly, the most potent compound in this series has an electron donating 4'-methoxy (IC_{50} = 25 μM) substitution in the side chain. Additionally, the presence of electron donating or electron withdrawing substituents in the side chain had little or no influence on human thymidylate synthase inhibition. On the basis of the analogues reported in this study, along with the previous reports of Gangjee *et al*. [11,12], it appears that for 5-substituted pyrrolo[2,3-*d*]pyrimidines the 6-methyl moiety is conducive for activity when the bridge at the 5-position is a single sulfur atom. However, if the bridge is homologated to a two-atom S8-C9 chain with a 6-methyl substituent, the analogue loses activity as compared with the single-atom sulfur bridge (compare **1a-c** [11,12] with **2-11**).

EXPERIMENTAL

All evaporations were carried out *in vacuo* with a rotary evaporator. Analytical samples were dried *in vacuo* (0.2 Torr) in an Abderhalden drying apparatus over phosphorous pentoxide. Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Nuclear magnetic resonance spectra for proton (^{1}H nmr) were recorded on a Bruker WH-300 (300 MHz) spectrometer. Data was accumulated by 16K size with a 0.5 s delay time and 70° tip angle. 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 (HRMS) were recorded on a VG-7070E-HF instrument. Thin layer chromatography (tlc) was performed on POLYGRAM Sil G/UV_{254} 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-3,4-dihydro-4-oxo-7*H*-pyrrolo[2,3-*d*]pyrimidine (**14**).

A suspension of 2,6-diamino-4-hydroxypyrimidine (**12**) (1.26 g, 10 mmol) in 25 ml of water containing sodium acetate (0.82 g, 10 mmol) was heated to 100 °C until it formed a clear solution. Chloroacetone (**13**) (0.79 ml, 10 mmol) was added to this solution in one lot, following which a precipitate began to form within 10 minutes. The reaction mixture was heated with stirring at 100 °C for an additional 4 hours, cooled to 0 °C, and filtered to afford 1.15 g (70%) of **14** as a slight pink colored solid: mp >260 °C (dec) tlc; R_f 0.37 (chloroform/methanol 4:1, silica gel); ^{1}H nmr (Me_2SO-d_6): δ 2.14(s, 3 H, 6-CH_3), 5.83(s, 1 H, 5-CH), 5.98(bs, 2 H, 2-NH_2), 10.15(bs, 1 H, 7-NH), 10.78 (bs, 1 H, 3-NH).

2-Amino-6-methyl-5-benzylthio-3,4-dihydro-4-oxo-7*H*-pyrrolo-[2,3-*d*]pyrimidine (**2**).

To a solution of **14** (1.0 g, 6.0 mmol) in a mixture of ethanol/water (2:1, 90 ml) was added benzyl mercaptan (1.5 g, 12.0 mmol) and the reaction mixture was heated to 100-110 °C. Iodine (3.0 g, 12.0 mmol) was added and the heating continued with stirring for a total of 4 hours. To this mixture was added an excess of sodium thiosulfate and the solvent was removed under reduced pressure. To the residue was added 10 g of silica gel and 50 ml of methanol, and the mixture was evaporated to dryness to afford a plug which was loaded on top of a silica gel column. The column was eluted with a gradient of 1-5% methanol in chloroform and fractions containing the desired spot (tlc) were pooled and evaporated to dryness. The resulting residue was recrystallized from methanol, filtered and dried to yield 0.7 g (40%) of **2**: mp 278-282 °C; tlc R_f 0.50 (chloroform/methanol 5:1, with 2 drops of ammonium hydroxide); ^{1}H nmr (DMSO-d_6): δ 1.82 (s, 3H, 6-CH_3), 2.23 (s, 3H, 6-CH_3), 3.95 (s, 2H, -CH_2), 6.07 (s, 2H, 4-NH_2), 7.04-7.25 (m, 5H, C_6H_5), 10.23 (s, 1H, 7-NH), 10.95 (s, 1H, 3-NH).

Anal. Calcd. for $C_{14}H_{14}N_4OS\bullet 1.0H_2O$: C, 58.72; H, 4.93; N, 19.57; S, 11.20. Found C, 55.25; H, 5.30; N, 18.41; S, 10.53.

2-Amino-6-methyl-5-(2'-methylbenzyl)thio-3,4-dihydro-4-oxo-7*H*-pyrrolo[2,3-*d*]pyrimidine (**3**).

Compound **3** was synthesized as described for **2**: Yield 41%; mp 268-270 °C; tlc R_f 0.49 (chloroform/methanol 5:1, with 2 drops of ammonium hydroxide); ^{1}H nmr (DMSO-d_6): δ 1.80 (s, 3H, 6-CH_3), 2.33 (s, 3H, 2'-CH_3), 3.94 (s, 2H, -CH_2), 6.07 (s, 2H, 4-NH_2), 6.81-6.83 (d, 1H, C_6H_4), 6.97 (m, 1H, C_6H_4), 7.07 (d, 2H, C_6H_4), 10.22 (s, 1H, 7-NH), 10.94 (s, 1H, 3-NH).

Anal. Calcd. for $C_{15}H_{16}N_4OS$: C, 59.98; H, 5.37; N, 18.65; S, 10.67. Found C, 59.80; H, 5.37; N, 18.58; S, 10.70.

2-Amino-6-methyl-5-(2'-chlorobenzyl)thio-3,4-dihydro-4-oxo-7*H*-pyrrolo[2,3-*d*]pyrimidine (**4**).

Compound **4** was synthesized as described for **2**: Yield 43%; mp 269-270.5 °C; tlc R_f 0.45 (chloroform/methanol 5:1, with 2 drops of ammonium hydroxide); ^{1}H nmr (DMSO-d_6): δ 1.72 (s, 3H, 6-CH_3), 3.99 (s, 2H, -CH_2), 6.08 (s, 2H, 4-NH_2), 6.81-6.84 (d, 1H, C_6H_4), 7.09 (t, 1H, C_6H_4), 7.17-7.19 (t, 1H, C_6H_4), 7.35-7.38 (d, 1H, C_6H_4), 10.25 (s, 1H, 7-NH), 10.95 (s, 1H, 3-NH).

Anal. Calcd. for $C_{14}H_{13}N_4OSCl$: C, 52.42; H, 4.08; N, 17.46; S, 9.99; Cl, 11.05. Found C, 52.28; H, 4.14; N, 17.58; S, 10.09; Cl, 11.00.

2-Amino-6-methyl-5-(4'-methylbenzyl)thio-3,4-dihydro-4-oxo-7*H*-pyrrolo[2,3-*d*]pyrimidine (**5**).

Compound **5** was synthesized as described for **2**: Yield 46%; mp 244-245 °C; tlc R_f 0.49 (chloroform/methanol 5:1, with 2 drops of ammonium hydroxide); ^{1}H nmr (DMSO-d_6): δ 1.85 (s, 3H, 6-CH_3), 2.22 (s, 3H, 4'-CH_3), 3.92 (s, 2H, -CH_2), 6.06 (s, 2H, 4-NH_2), 6.93-7.00 (m, 4H, C_6H_4), 10.22 (s, 1H, 7-NH), 10.94 (s, 1H, 3-NH).

Anal. Calcd. for $C_{15}H_{16}N_4OS\bullet0.8H_2O$: C, 59.98; H, 5.37; N, 18.65; S, 10.67. Found C, 57.23; H, 5.64; N, 17.80; S, 10.19.

2-Amino-6-methyl-5-(4'-methoxybenzyl)thio-3,4-dihydro-4-oxo-7*H*-pyrrolo[2,3-*d*]pyrimidine (**6**).

Compound **6** was synthesized as described for **2**: Yield 33%; mp 243-245 °C; tlc R_f 0.50 (chloroform/methanol 5:1, with 2 drops of ammonium hydroxide); ^{1}H nmr (DMSO-d_6): δ 1.86 (s, 3H, 6-CH_3), 3.68 (s, 3H, 4'-OCH_3), 3.90 (s, 2H, -CH_2), 6.07 (s, 2H, 4-NH_2), 6.74-6.76 (d, 2H, C_6H_4), 6.97-6.99 (d, 2H, C_6H_4), 10.22 (s, 1H, 7-NH), 10.95 (s, 1H, 3-NH). HRMS (EI): *m/e* calculated for (M^+) $C_{15}H_{16}N_4O_2S$ 316.0994; found m/z = 316.0990.

2-Amino-6-methyl-5-(4'-chlorobenzyl)thio-3,4-dihydro-4-oxo-7*H*-pyrrolo[2,3-*d*]pyrimidine (**7**).

Compound **7** was synthesized as described for **2**: Yield 28%; mp 274-275.5 °C; tlc R_f 0.49 (chloroform/methanol 5:1, with 2 drops of ammonium hydroxide); ^{1}H nmr (DMSO-d_6): δ 1.82 (s, 3H, 6-CH_3), 3.94 (s, 2H, -CH_2), 6.08 (s, 2H, 4-NH_2), 7.03-7.05 (d, 2H, C_6H_4), 7.22-7.25 (d, 2H, C_6H_4), 10.24 (s, 1H, 7-NH), 10.96 (s, 1H, 3-NH).

Anal. Calcd. for $C_{14}H_{13}N_4OSCl\bullet0.6H_2O$: C, 52.42; H, 4.08; N, 17.46; S, 9.99; Cl, 11.05. Found C, 50.71; H, 4.32; N, 16.90; S, 9.67; Cl, 10.69.

2-Amino-6-methyl-5-(4'-fluorobenzyl)thio-3,4-dihydro-4-oxo-7*H*-pyrrolo[2,3-*d*]pyrimidine (**8**).

Compound **8** was synthesized as described for **2**: Yield 30%; mp 264-266 °C; tlc R_f 0.50 (chloroform/methanol 5:1, with 2 drops of ammonium hydroxide); ^{1}H nmr (DMSO-d_6): δ 1.83 (s, 3H, 6-CH_3), 3.94 (s, 2H, -CH_2), 6.08 (s, 2H, 4-NH_2), 7.00-7.06 (m, 4H, C_6H_4), 10.24 (s, 1H, 7-NH), 10.96 (s, 1H, 3-NH).

Anal. Calcd. for $C_{14}H_{13}N_4OSF\bullet1.0H_2O$: C, 55.25; H, 4.31; N, 18.41; S, 10.54; F, 6.24. Found C, 52.16; H, 4.69; N, 17.38; S, 9.95; F, 5.89.

2-Amino-6-methyl-5-(4'-bromobenzyl)thio-3,4-dihydro-4-oxo-7*H*-pyrrolo[2,3-*d*]pyrimidine (**9**).

Compound **9** was synthesized as described for **2:** Yield 36%; mp 254-256 °C; tlc R_f 0.48 (chloroform/methanol 5:1, with 2 drops of ammonium hydroxide); ^{1}H nmr (DMSO-d_6): δ 1.81 (s, 3H, 6-CH_3), 3.92 (s, 2H, -CH_2), 6.09 (s, 2H, 4-NH_2), 6.96-6.99 (d, 2H, C_6H_4), 7.36-7.38 (d, 2H, C_6H_4), 10.25 (s, 1H, 7-NH), 10.97 (s, 1H, 3-NH). HRMS (EI): *m/e* calculated for (M^+) $C_{14}H_{13}N_4OSBr$ 363.9993; found m/z = 363.9980

2-Amino-6-methyl-5-(2',4'-dichlorobenzyl)thio-3,4-dihydro-4-oxo-7*H*-pyrrolo[2,3-*d*]pyrimidine (**10**).

Compound **10** was synthesized as described for **2:** Yield 25%; mp 274-275.5 °C; tlc R_f 0.50 (chloroform/methanol 5:1, with 2 drops of NH_4OH); ^{1}H nmr (DMSO-d_6): δ 1.74 (s, 3H, 6-CH_3), 3.98 (s, 2H, -CH_2), 6.09 (s, 2H, 4-NH_2), 6.79-6.81 (d, 1H, C_6H_3), 7.18-7.21 (d, 1H, C_6H_3), 7.52 (s, 1H, C_6H_3), 10.27 (s, 1H, 7-NH), 10.98 (s, 1H, 3-NH).

Anal. Calcd. for $C_{14}H_{12}N_4OSCl_2$: C, 47.33; H, 3.40; N, 15.77; S, 9.03; Cl, 19.96. Found C, 47.27; H, 3.49; N, 15.75; S, 9.01; Cl, 19.85.

2-Amino-6-methyl-5-(3',4'-dichlorobenzyl)thio-3,4-dihydro-4-oxo-7*H*-pyrrolo[2,3-*d*]pyrimidine(11).

Compound 11 was synthesized as described for **2**: Yield 30%; mp 274-276.5 °C; tlc R_f 0.50 (chloroform/methanol 5:1, with 2 drops of ammonium hydroxide); ^{1}H nmr (DMSO-d_6): δ 1.84 (s, 3H, 6-CH_3), 3.95 (s, 2H, -CH_2), 6.10 (s, 2H, 4-NH_2), 6.97-7.00 (d, 1H, C_6H_3), 7.25 (s, 1H, C_6H_3), 7.42-7.45 (d, 1H, C_6H_3), 10.27 (s, 1H, 7-NH), 11.02 (s, 1H, 3-NH).

Anal. Calcd. for $C_{14}H_{12}N_4OSCl_2\bullet1.0H_2O$: C, 47.33; H, 3.40; N, 15.77; S, 9.03; Cl, 19.96. Found C, 45.05; H, 3.78; N, 15.01; S, 8.59; Cl, 19.00.

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