2-Amino-4-oxo-5-substituted-pyrrolo[2,3-*d*]pyrimidines as Nonclassical Antifolate Inhibitors of Thymidylate Synthase[1a,b]

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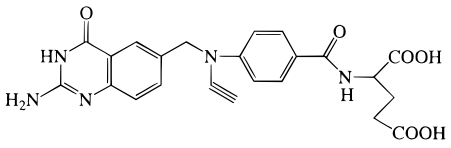
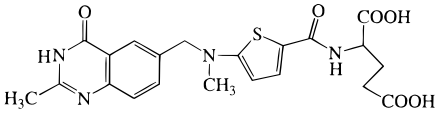
Six novel 2-amino-4-oxo-5-[(substituted phenyl)sulfanyl]pyrrolo[2,3-*d*]pyrimidines **7**−**12** were synthesized as potential inhibitors of thymidylate synthase (TS) and as antitumor and/or antibacterial agents. The analogues contain a 5-thio substituent with a phenyl, 4′-chlorophenyl, 3′,4′-dichlorophenyl, 4′-nitrophenyl, 3′,4′-dimethoxyphenyl, and 2′-naphthyl on the sulfur, and were synthesized from the key intermediate 2-(pivaloylamino)-4-oxo-6-methylpyrrolo[2,3-*d*]-pyrimidine, **17**. Appropriately substituted aryl thiols were appended to the 5-position of **17** *via* an oxidative addition reaction using iodine, ethanol, and water under conditions which also resulted in the deprotection of the 2-amino group. The compounds were evaluated against human, *Lactobacillus casei*, *Escherichia coli*, *Streptococcus faecium*, and *Pneumocystis carinii* (pc) TSs and against human, rat liver (rl), pc, and *Toxoplasma gondii* (tg) DHFRs. The nonclassical analogues with the 3′,4′-dichloro and the 4′-nitro substituents in the side chain (**9** and **10**) were more potent than *N*-[4-[*N*-[(2-amino-3,4-dihydro-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]benzoyl]-L-glutamic acid (PDDF, **1**) and *N*-[5-[*N*-[(3,4-dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-methylamino]-2-thenoyl]-L-glutamic acid (ZD1694, **2**) against human TS. Analogues with the 4′-chloro, 3′,4′-dimethoxy, and naphthyl side chains (**8, 11** and **12**) were more potent than the unsubstituted phenyl analogue (**7**) but less than **2**, **9**, and **10** by 1 order of magnitude. They were all poor inhibitors of human, rl, and pc DHFRs (IC_{50} = 10^{-5} M) but moderate inhibitors (IC_{50} = 10^{-6} M) of tg DHFR. The 4-nitro analogue, **10** (EC_{50} 1.5 μM), was comparable to PDDF in its potency as an inhibitor of the growth of the FaDu human squamous cell carcinoma cell line.

Introduction

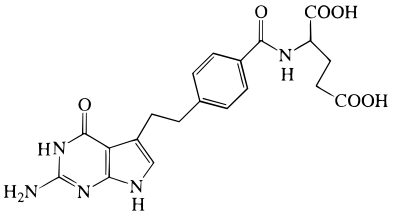
Thymidylate synthase (TS) is a crucial enzyme that catalyzes the reductive methylation of 2′-deoxyuridine-5′-monophosphate (dUMP) to 2′-deoxythymidine-5′-monophosphate (dTMP) utilizing 5,10-methylenetetrahydrofolate, a cofactor which acts as the source of the methyl group as well as the reductant.[2] This is the exclusive *de novo* source of dTMP; hence inhibition of TS activity, in the absence of salvage, leads to "thymineless death". Thus inhibition of TS has long been an attractive goal for the development of antitumor agents.[3,4] The antifolates *N*-[4-[*N*-[(2-amino-3,4-dihydro-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]benzoyl]-L-glutamic acid (PDDF, **1**)[5] and its third generation analogue *N*-[5-[*N*-[(3,4-dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-methylamino]-2-thenoyl]-L-glutamic acid, tomudex, (ZD1694, **2**)[6] are classical inhibitors of TS with antitumor activity, and **2** is currently in phase III clinical trials. These analogues possess a 6−6 ring-fused system and a benzoyl- and thenoyl-L-glutamic acid side chain at the 6-position, similar to that of natural folates. Taylor *et al.*[7] have reported the synthesis of *N*-[4-[2-(2-amino-3,4-dihydro-4-oxo-7*H*-pyrrolo-[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-L-glutamic acid, a B-ring-contracted 6−5 ring-fused classical antifolate, (LY231514, **3**), as an inhibitor of TS (K_i = 340 nM against mouse recombinant TS) and as an effective antitumor agent.



1: PDDF



2: ZD1694



3: LY231514

A potential drawback of classical antifolates, such as **1**, **2**, and **3**, is their requirement for the reduced folate carrier system for transport into cells,[8] the impairment of which can lead to drug resistance.[9−12] An additional

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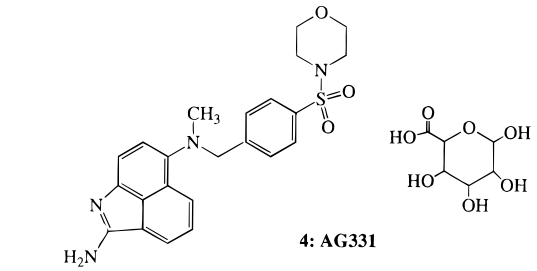
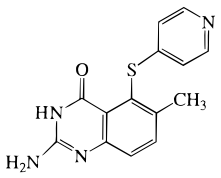
cause of resistance to classical antifolates, particularly of TS inhibitors, is that their antitumor activity is in part determined by their ability to function as substrates for the enzyme folylpoly-γ-glutamate synthetase (FPGS).[6,7,13,14] FPGS catalyzes the formation of poly-γ-glutamates which are long-acting, noneffluxing forms of the classical antifolates that lead to high intracellular concentrations of these antitumor agents.[6,7,15−18] Cells that poorly polyglutamylate these antifolates are resistant to these drugs.[13,19] For both **2** and **3** it has been shown that conversion of the monoglutamate forms to their pentaglutamates produces a 60-fold (for **2**)[6] to a 130-fold (for **3**)[7] increase in TS inhibitory potency. Although polyglutamylation is necessary for the cytotoxicity to tumor cells, it has also been implicated as a possible cause of detrimental side effects, such as renal and hepatic toxicities, in the host.[20,21]

The disadvantages of resistance to classical antifolate TS inhibitors could be overcome by lipophilic nonclassical antifolates. Such compounds would diffuse into cells passively and circumvent drug resistance in cells due to defective active transport. Further, the detrimental effects, of resistance and toxicity, associated with polyglutamylation would also be eliminated. One such lipophilic antifolate, which is incapable of being polyglutamylated and bears little structural resemblance to the normal folate cofactor, is N^6-[4-(*N*-morpholinosulfonyl)benzyl]-N^6-methyl-2,6-diaminobenz[*cd*]indole glucuronate (AG331, **4**).[22] Compound **4** is currently in phase I clinical trials as an antitumor agent.[23] Webber *et al.*[24] recently reported a series of classical and nonclassical 5-substituted quinazolines as inhibitors of TS of which 2-amino-6-methyl-5-(pyridin-4-ylsulfanyl)-3*H*-quinazolin-4-one (AG337, **5**), was the most promising and is currently in phase II clinical trials as an antitumor agent. On the basis of these reports and with the goal of developing new cancer chemotherapeutic agents, we designed, synthesized, evaluated, and reported[25] a nonclassical compound **6** which incorporates the pyrrolo[2,3-*d*]pyrimidine ring of **3** and the side chain of **5**. Analogue **6** was active against human TS and moderately cytotoxic against human CCRF-CEM leukemic cells. Compound **6** also exhibited selectivity for human *vs* bacterial TS, indicating a species difference in TS from different sources.

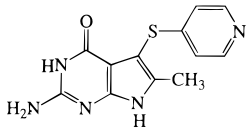
Species differences in bacterial *vs* human dihydrofolate reductases (DHFR) have led to clinically useful lipophilic inhibitors of DHFR, such as trimethoprim and pyrimethamine, which are selective against bacteria and fungi. Selectivity of DHFR inhibitors for different species has been attributed to their differences in inhibition and also to the fact that some microbes lack cellular transport mechanism(s) for folate and reduced folates and are, thus, unable to utilize preformed folates. Two such organisms of concern that lack the transport mechanism for classical folates and antifolates are *Pneumocystis carinii* (pc) and *Toxoplasma gondii* (tg). Infections caused by these organisms are the principal cause of death in patients with the acquired immunodeficiency syndrome (AIDS). We reasoned that since a difference in inhibitory potency exists between TS from different sources, nonclassical, lipophilic inhibitors of TS may also provide for selective inhibition of bacteria and/or fungal TS. Thus the target nonclassical TS inhibitors, **7**−**12**, in addition to their antitumor effects,



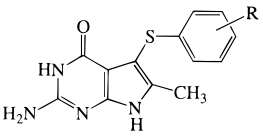
4: AG331



5: AG337



6



7: R = H
8: R = 4'-Cl
9: R = 3',4'-Cl_2
10: R = 4'-NO_2
11: R = 3',4'-$(OCH_3)_2$
12: R = 3',4'-$(CH)_4$

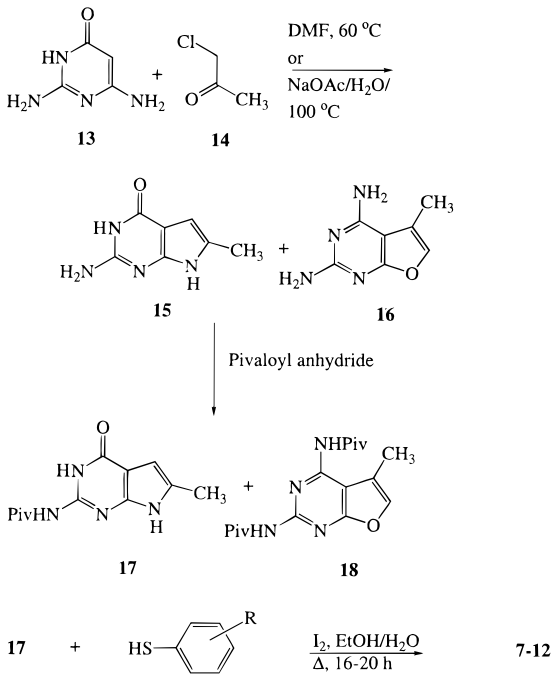
were also of interest as selective antibacterial and antifungal agents.

The potent nonclassical TS inhibitors in the literature possess different electron-withdrawing groups in place of the CO-glutamate of classical antifolates. Electron-donating groups para to N^{10} have been postulated to diminish the magnitude and alter the direction of the dipole−dipole interaction between TS and the substituted phenyl ring of the inhibitor.[24,26,27] The analogues **7**−**12** were synthesized as an extension of our previous work[25] to explore the effects on TS inhibition of electron-withdrawing and -donating groups on the side chain phenyl ring.

Chemistry

The key intermediate in the synthesis of analogues **7**−**12** was the 2-(pivaloylamino)-4-oxo-6-methylpyrrolo-[2,3-*d*]pyrimidine, **17** (Scheme 1), to which various side chains could be conveniently attached. Compound **17** was obtained *via* protection of the 2-amino group of **15** which we[25] initially synthesized utilizing the method reported by Secrist and Liu.[28] This method involves heating a mixture of 2,6-diamino-4-hydroxypyrimidine, **13**, with chloroacetone, **14**, in dimethylformamide at 60 °C for 2 days, which affords two products on TLC at R_f 0.36 and 0.56 corresponding to the pyrrolo[2,3-*d*]pyrimidine **15** (55%) and the furo[2,3-*d*]pyrimidine **16**, respectively. In an attempt to improve the yield of the desired pyrrolo[2,3-*d*]pyrimidine **15**, in this study we used a modification of the method reported by Ramasamy *et al.*[29] which involved addition of chloroacetone, **14**, to a solution of 2,6-diamino-4-hydroxypyrimidine, **13**, in sodium acetate and water. Although the product begins to fall out of solution within 10 min, the heating was continued at 100 °C for 4 h for completion of the reaction. This method has the advantage of a

Scheme 1



shorter reaction time (4 *vs* 48 h[28]) and higher yields of the pyrrolo[2,3-*d*]pyrimidine (70% *vs* 55%[25,28]). The mixture of the pyrrolo[2,3-*d*]pyrimidine **15** and the furo-[2,3-*d*]pyrimidine **16** was not separated but rather converted to their more lipophilic pivaloyl-protected derivatives **17** and **18** using pivaloyl anhydride and potassium carbonate. The protected mixture was separated by boiling in ethyl acetate. The highly lipophilic 2,4-bis(pivaloylamino)-5-methylfuro[2,3-*d*]pyrimidine (**18**)-was soluble and was separated by filtration. The desired compound 2-(pivaloylamino)-4-oxo-3,4-dihydro-7*H*-pyrrolo[2,3-*d*]pyrimidine (**17**) was obtained as an ethyl acetate insoluble solid in sufficiently pure form for use in further reactions.

Analogues **7**−**12** were obtained in a one-step oxidative addition of appropriately substituted aryl thiols to the 5-position of **17**. Thus heating a mixture of **17** with the substituted aryl thiols in a mixture of ethanol/water (2:1) with 2 equiv of iodine at reflux for a period of 16−20 h afforded the desired target compounds **7**−**12**. Within 1 h TLC of the reaction mixtures showed the formation of a new spot at R_f 0.66−0.68 with the starting material spot at R_f 0.64. On continued reflux an additional new spot was detected at R_f 0.15−0.19 which after 16−20 h was the major spot observed. Evaporation of the solvents under reduced pressure, followed by cooling and addition of cold distilled water to the residue, resulted in the formation of a precipitate which was collected by filtration. Washing with diethyl ether removed unreacted aryl thiol and afforded the target compounds **7**−**12** in 36−77% yields. The large upfield singlet corresponding to the pivaloyl group protons was absent in the ^{1}H NMR spectra of these compounds which showed instead a singlet exchangeable with deuterium oxide at 6.08−6.21 ppm that integrated for the two protons of the 2-amino group. This along with the absence of the 5-aromatic proton and the presence of the appropriate protons of the side chain for **7**−**12** confirmed that both substitution and deprotection had occurred.

On the basis of TLC data it was apparent that the initial substitution step was rapid in all cases and was complete within 4−5 h, indicating that the electron-donating or -withdrawing effects of substituents in the aryl thiol ring did not influence the oxidative addition reaction for pyrrolo[2,3-*d*]pyrimidines, which is in agreement with the observations of Beveridge and Harris[30] for indoles and pyrroles. The deprotection step, however, varied from 16 to 20 h; analogues **7**−**10** required 16 h while analogues **11** and **12** required 20 h for complete deprotection.

Table 1. TS Inhibition IC_{50} (μM)[31−33]

compd	human	*L. casei*	*E. coli*	*S. faecium*	*P. carinii*
1 (PDDF)	0.18	0.095	0.037	0.037	0.09
2 (ZD1694)	0.88	8.8	5.3	8.8	0.53
6[25]	0.34	100	>29 (30)[a]	29	ND
7	30	>30 (0)	>30 (30)	>30 (20)	60
8	1.0	>2.6 (0)	>26 (40)	30	5.3
9	0.13	45	45	>45 (31)	2.2
10	0.15	5.1	13	15	2.0
11	2.4	>24 (33)	>24 (30)	>24 (12)	ND
12	2.0	>25 (32)	>25 (36)	>25 (0)	ND

[a] Number in parentheses indicates percent inhibition obtained at that concentration.

Biological Evaluation and Discussion

Compounds **7**−**12** were evaluated[31−33] as inhibitors of human, *Lactobacillus casei*, *Escherichia coli*, *Streptococcus faecium*, and *Pneumocystis carinii* TS (Table 1) along with PDDF and ZD1694. Replacing the thiopyridine ring of **6** with a phenyl ring, as in **7**, diminished inhibition for human TS, *L. casei* TS, and *S. faecium* TS. For human TS, analogues with substitutions on the phenyl ring were more inhibitory than the unsubstituted phenyl analogue. The electronic nature of the substitutent on the side chain, however, was an important factor in determining inhibitory potency. Electron-withdrawing 4′-chloro, 3′,4′-dichloro, and 4′-nitro substituents in analogues **8**, **9**, and **10**, respectively, showed the most potent inhibition against isolated human TS, and **9** and **10** were equipotent with PDDF and were better than ZD1694 (**2**), which, as mentioned above, is currently in phase III clinical trials as an antitumor agent. A comparison of the inhibitory potencies of **6** and **7** against human TS indicates a significant loss of potency in going from an electron-withdrawing substituent in **6** to an unsubstituted phenyl ring in **7**. This deficiency in **7** is more than overcome by the introduction of one or more electron-withdrawing groups as illustrated by the greater than 30-fold increase in potency of **8** and a greater than 200-fold increase in potency of **9** and **10** compared to that of **7**. Electron-donating groups, as in **11**, or the replacement of the phenyl ring with a naphthalene ring, as in **12**, increases activity against human TS compared to the unsubstituted phenyl ring in **7**, but are 1 order lower than that of **9** and **10** which contain strong electron-withdrawing groups. Thus for human TS, electron-withdrawing groups are highly conducive to potency. For *L. casei* TS a similar trend, as observed for human TS, was apparent in that strong electron-withdrawing groups present in **9** and **10** rendered these analogues more potent than the unsubstituted phenyl analogue **7** and electron-donating groups in **11** and a naphthyl ring instead of a phenyl ring in **12** were more potent than **7**. Compound **10** was similar in potency to ZD1694 against *L. casei* TS. With the exception of PDDF, all the analogues tested were severalfold less potent against

L. casei TS than against human TS, clearly indicating a difference in the two enzymes. Against *E. coli* TS, similar inhibition profiles were observed as mentioned above for *L. casei* TS. Here, as in human TS, electron-withdrawing groups (**9** and **10**) were more conducive to inhibition than the unsubstituted analogue **7**. However, similar to *L. casei* TS, the potencies of all the compounds evaluated, except PDDF, were severalfold less than against human TS. Analogues **7**−**10** along with PDDF and ZD1694 were also evaluated as inhibitors of *P. carinii* TS. Compounds **8**, **9**, and **10** with electron withdrawing groups were from 12- to 30-fold more potent than the unsubstituted analogue **7**, indicating, as with human TS, *E. coli* TS, and *L. casei* TS, that compounds with electron-withdrawing groups were better than the unsubstituted analogue **7**.

Against *S. faecium* TS, the results were somewhat different. Compounds **8** and **10** with electron-withdrawing groups were more potent than **7**. However, compound **9** with 3′,4′-dichlorophenyl substitution was not as inhibitory as **8** or **10** and was similar to **7**. Since the electronic nature of the phenyl substituent of **8**, **9**, and **10** are similar, the only difference of **9** is the additional *m*-chloro substituent. Further, compound **11** with electron-donating groups and compound **12** with a naphthyl ring were less inhibitory than the unsubstituted phenyl analogue **7**. These results of **9**, **11**, and **12** are in contrast to those observed for human TS, *L. casei* TS, and *E. coli* TS, where **9**, **11**, and **12** were consistently better inhibitors than **7**. Comparison of **9**, **11**, and **12** at the same concentrations in *S. faecium* TS, as in the other two bacterial enzymes *L. casei* TS and *E. coli* TS, indicates a decrease in potency for *S. faecium* TS. One plausible explanation for the lower potency of **9**, **11**, and **12** may be steric hindrance which is not well tolerated by *S. faecium* TS. All three analogues (**9**, **11**, and **12**) contain a meta substitution in addition to the para substitution on the phenyl ring. This additional meta substitution could provide for steric hindrance to the attachment of **9**, **11**, and **12** to *S. faecium* TS, resulting in the observed lower potency. Compound **10**, the most potent analogue of the series (**6**−**12**), was again about half as potent as ZD1694.

In summary, for 5-substituted-2-amino-4-oxopyrrolo-[2,3-*d*]pyrimidines, strong electron-withdrawing groups (NO_2 and diCl) on the side chain phenyl ring afforded significantly better inhibitors of human TS, *L. casei* TS, *E. coli* TS, and *P. carinii* TS compared to the unsubstituted phenyl ring. These results are similar to the result observed for the quinazolines reported by Webber *et al.*[24] For human TS and *L. casei* TS, electron-donating groups in the phenyl ring and the substitution of a naphthyl ring for a phenyl also afforded better inhibitors than the unsubstituted phenyl, but the difference in inhibitory potency was not as pronounced as for strong electron-withdrawing groups. Against *S. faecium* TS, though electron-withdrawing groups were beneficial for inhibition, the presence of a meta substituent on the phenyl ring, irrespective of its electronic nature, decreased activity. Further, none of the analogues were significant inhibitors of bacterial TS and shared this property with ZD1694. PDDF was the most potent inhibitor of bacterial and fungal TS of the compounds evaluated, and compound **9** and **10** along with PDDF were the most potent inhibitors of human TS.

Table 2. Inhibitory Concentrations (IC_{50}, μM) against DHFRs and Selectivity Ratios[a]

compd	*P. carinii* (pc)	rat liver (rl)	selectivity ratio rl/pc	*T. gondii* (tg)	selectivity ratio rl/tg
8	40	24.6	0.62	3.1	7.9
9	>20	>20	ND	11.7	>1.7
10	>15	7470	ND	244	30.6
11	>21	>21	ND	20	>1

[a] These assays were carried out at 37 °C under conditions of substrate (90 μM dihydrofolic acid) and cofactor (119 μM NADPH) in the presence of 150 mM KCl.[35,36]

Table 3. Growth Inhibition of the FaDu Human Squamous Cell Carcinoma Cell Line by Continuous (120 h) Exposure to the Inhibitors[a]

Compound	EC_{50}, μM	n
7	>10	2
8	6.7 ± 1.5	3
10	1.5 ± 0.4	3
11	>10	2
PDDF[b]	1.7 ± 0.2	4
AG331[b]	1.0 ± 0.1	6

[a] Compound **9** was poorly soluble and was not tested. [b] McGuire *et al.*, manuscript in preparation.

Inspite of the homology between TS isolated thus far,[34] the selectivity of these analogues for human over bacterial TS (Table 1) implies that a difference does exist, which we are currently exploring with a variety of different analogues in an attempt to provide agents which are selective toward bacterial TS. In preliminary experiments, analogue **7** gave 54% inhibition of *P. carinii* growth in culture when tested at a concentration of 36 μM. These results indicate that growth inhibition by **7** occurs at concentrations comparable to the IC_{50} for *P. carinii* TS (60 μM).

Analogues **8**, **9**, **10**, and **11** were also evaluated[35,36] as inhibitors of *T. gondii*, *P. carinii*, and rat liver DHFR (Table 2). Although all the analogues were poor inhibitors of *P. carinii* and rat liver DHFR, they were moderate inhibitors of *T. gondii* DHFR. Compounds **8** and **10** were 8 and 31 times more selective, respectively, for *T. gondii* DHFR compared to rat liver DHFR. This parallels the trend we recently reported[37] for nonclassical 5-substituted-2,4-diaminopyrrolo[2,3-*d*]pyrimidine inhibitors of DHFR.

The potent human TS inhibitory activity of compounds **7**, **8**, **10**, and **11** prompted the evaluation of these analogues as inhibitors of the growth of the FaDu human squamous cell carcinoma cell line in culture (Table 3). Compound **9** could not be evaluated due to its poor solubility. The growth inhibitory potency of **10** was comparable to that of PDDF and AG331 (Table 3). Thus nonclassical analogues such as **10** could be useful antitumor agents against tumors resistant to classical antifolates[24] such as PDDF, **2**, and **3**. These classical analogues require active transport into tumors and need polyglutamylation to exert their antitumor effects, both of which are sources of tumor resistance[8,11−14,17,20,21] and neither of which is necessary for the cytotoxicity of **10**. We are currently exploring analogues of **10** as potential antitumor agents, the results of which will be the topic of future communications.

Experimental Section

All evaporations were carried out *in vacuo* with a rotary evaporator. Analytical samples were dried *in vacuo* (0.2 mmHg) in an Abderhalden drying apparatus over P_2O_5 and refluxing ethanol or toluene. 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. 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 TLC 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 h 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 (15). Method A. To a suspension of 2,6-diamino-4-hydroxypyrimidine (**13**) (5.04 g, 40.0 mmol) in *N*,*N*-dimethylformamide (70 mL) was added chloroacetone (**14**) (4.44 g, 48.0 mmol), and the mixture was stirred at 60 °C for 48 h under nitrogen. Two new spots at R_f 0.37 (corresponding to **15**) and R_f 0.56 (corresponding to **16**) were seen on TLC (CHCl$_3$/MeOH, 4:1).[28] The mixture was directly converted to **17** without separation of **15** and **16**.

Method B. A suspension of 2,6-diamino-4-hydroxypyrimidine (**13**) (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 (**14**) (0.79 mL, 10 mmol) was added to this solution in one lot, following which a precipitate began to form within 10 min. The reaction mixture was heated with stirring at 100 °C for an additional 4 h, cooled to 0 °C, and filtered to afford 1.15 g (70%) of **15**: TLC R_f 0.37 (CHCl$_3$/MeOH, 4:1); ^{1}H NMR (Me$_2$SO-d_6) δ 2.15 (s, 3 H, 6-CH$_3$), 5.84 (s, 1 H, 5-CH), 5.97 (bs, 2 H, 2-NH$_2$), 10.14 (bs, 1 H, 7-NH), 10.79 (bs, 1 H, 3-NH).

2-(Pivaloylamino)-6-methyl-3,4-dihydro-4-oxo-7*H*-pyrrolo[2,3-*d*]pyrimidine (17). To a solution of **15** (2.00 g, 12.3 mmol) in *N*,*N*-dimethylformamide (50 mL) was added anhydrous K_2CO_3 (1.70 g, 12.3 mmol) followed by pivaloyl anhydride (7.99 g, 42.9 mmol). The suspension was stirred under nitrogen at 100 °C for 12 h. The reaction mixture was cooled to room temperature and filtered, and the filtrate was evaporated under reduced pressure. The residue was dissolved in methylene chloride (200 mL) and washed with water (2 × 150 mL). The organic layer was dried (MgSO$_4$) and filtered. After evaporation of the filtrate, the residue was stirred in boiling ethyl acetate (300 mL) and filtered. The residue was washed with hot ethyl acetate (100 mL) and dried to afford 1.30 g (43%) of **17** as an off-white solid: mp 290−294 °C; TLC R_f 0.54 (CHCl$_3$/MeOH, 9:1, with 2 drops of concentrated NH$_4$OH); ^{1}H NMR (Me$_2$SO-d_6) δ 1.23 (s, 9 H, C(CH$_3$)$_3$), 2.25 (s, 3 H, 6-CH$_3$), 6.07 (s, 1 H, 5-CH), 10.75 (s, 1 H, 7-NH), 11.35 (s, 1 H, 3-NH), 11.78 (s, 1 H, CONH).

2-Amino-6-methyl-5-(phenylsulfanyl)-3,4-dihydro-4-oxo-7*H*-pyrrolo[2,3-*d*]pyrimidine (7). To a solution of **17** (0.10 g, 0.43 mmol) in a mixture of ethanol/water (2:1, 30 mL) was added iodine (0.22 g, 0.86 mmol) followed by thiophenol (0.10 g, 0.86 mmol), and the reaction mixture was heated to 120−130 °C for 16 h. The mixture was cooled to room temperature and concentrated under reduced pressure. Cold water (100 mL) was added to the residue and the pH adjusted to 8 with concentrated NH$_4$OH with stirring. The suspension was left at 4 °C for 6 h and filtered. The residue was washed well with water and air-dried. The residue was then stirred with diethyl ether (25 mL), filtered, washed with additional amounts of ether to remove the unreacted thiophenol, and dried to yield 0.09 g (77%) of **7**: mp 275−280 °C dec; TLC R_f 0.40 (CHCl$_3$/MeOH, 5:1, with 2 drops of NH$_4$OH); ^{1}H NMR (Me$_2$SO-d_6) δ 2.18 (s, 3 H, 6-CH$_3$), 6.11 (bs, 2 H, 2-NH$_2$), 6.99 (m, 3 H, C$_6$H$_5$), 10.22 (s, 1 H, 7-NH), 11.41 (s, 1 H, 3-NH). Anal. Calcd for (C$_{13}$H$_{11}$N$_4$OS·0.3H$_2$O) C, H, N, S.

2-Amino-6-methyl-5-[(4′-chlorophenyl)sulfanyl]-3,4-dihydro-4-oxo-7*H*-pyrrolo[2,3-*d*]pyrimidine (8). Compound **8** was synthesized as described for **7**. **8**: yield 40%; mp 255−265 °C dec; TLC R_f 0.41 (CHCl$_3$/MeOH, 5:1, with 2 drops of NH$_4$OH); ^{1}H NMR (Me$_2$SO-d_6) δ 2.18 (s, 3 H, 6-CH$_3$), 6.13 (bs, 2 H, 2-NH$_2$), 6.99 (d, 3 H, C$_6$H$_5$), 10.22 (s, 1 H, 7-NH), 11.41 (s, 1 H, 3-NH). Anal. Calcd for (C$_{13}$H$_{11}$N$_4$OS·0.3H$_2$O) C, H, N, S.

2-Amino-6-methyl-5-[(3′,4′-dichlorophenyl)sulfanyl]-3,4-dihydro-4-oxo-7*H*-pyrrolo[2,3-*d*]pyrimidine (9). Compound **9** was synthesized as described for **7**. The residue was recrystallized with ethyl acetate/acetic acid to afford **9** in 55% yield: mp 235−245 °C dec; TLC R_f 0.41 (CHCl$_3$/MeOH, 5:1, with 2 drops of NH$_4$OH); ^{1}H NMR (Me$_2$SO-d_6) δ 2.19 (s, 3 H, 6-CH$_3$), 6.16 (bs, 2 H, 2-NH$_2$), 6.96 (m, 1 H, 5′-CH), 7.16 (s, 1 H, 2′-CH), 7.44 (m, 1 H, 6′-CH), 10.26 (bs, 1 H, 7-NH), 11.51 (bs, 1 H, 3-NH). Anal. Calcd for (C$_{13}$H$_{10}$N$_4$OSCl$_2$·0.5H$_2$O·0.1HI) C, H, N, S, Cl.

2-Amino-6-methyl-5-[(4′-nitrophenyl)sulfanyl]-3,4-dihydro-4-oxo-7*H*-pyrrolo[2,3-*d*]pyrimidine (10). Compound **10** was synthesized as described for **7**. **10**: yield 60%; mp 245−255 °C dec; TLC R_f 0.42 (CHCl$_3$/MeOH, 5:1, with 2 drops of NH$_4$OH); ^{1}H NMR (Me$_2$SO-d_6) δ 2.19 (s, 3 H, 6-CH$_3$), 6.21 (bs, 2 H, 2-NH$_2$), 7.17 (d, 2 H, 2′,6′-CH), 8.06 (d, 2 H, 3′,5′-CH), 10.35 (bs, 1 H, 7-NH), 11.58 (bs, 1 H, 3-NH). Anal. Calcd for (C$_{13}$H$_{11}$N$_5$O$_3$S·0.5H$_2$O) C, H, N, S.

2-Amino-6-methyl-5-[(3′,4′-dimethoxyphenyl)sulfanyl]-3,4-dihydro-4-oxo-7*H*-pyrrolo[2,3-*d*]pyrimidine (11). Compound **11** was synthesized as described for **7**. **11**: yield 75%; mp 235−245 °C dec; TLC R_f 0.41 (CHCl$_3$/MeOH, 5:1, with 2 drops of NH$_4$OH); ^{1}H NMR (Me$_2$SO-d_6) δ 2.21 (s, 3 H, 6-CH$_3$), 3.68 (s, 3 H, OCH$_3$), 3.75 (s, 3 H, OCH$_3$), 6.08 (bs, 2 H, 2-NH$_2$), 6.57 (d, 1 H, 5′-CH), 6.89 (s, 1 H, 2′-CH), 10.23 (s, 1 H, 7-NH), 11.32 (s, 1 H, 3-NH). Anal. Calcd for (C$_{15}$H$_{16}$N$_4$O$_3$S·0.5H$_2$O) C, H, N, S.

2-Amino-6-methyl-5-(2′-naphthylsulfanyl)-3,4-dihydro-4-oxo-7*H*-pyrrolo[2,3-*d*]pyrimidine (12). Compound **12** was synthesized as described for **7**. **12**: yield 36%; mp 245−255 °C dec; TLC R_f 0.39 (CHCl$_3$/MeOH, 5:1, with 2 drops of NH$_4$OH); ^{1}H NMR (Me$_2$SO-d_6) δ 2.22 (s, 3 H, 6-CH$_3$), 6.13 (bs, 2 H, 2-NH$_2$), 7.20 (d, 1 H, C$_{10}$H$_7$), 7.38 (m, 3 H, C$_{10}$H$_7$), 7.74 (m, 3 H, C$_{10}$H$_7$), 10.23 (bs, 1 H, 7-NH), 11.46 (bs, 1 H, 3-NH). Anal. Calcd for (C$_{17}$H$_{14}$N$_4$OS·0.3H$_2$O·0.3HI) C, H, N, S.

Cell Lines and Methods for Measuring Growth Inhibitory Potency. The FaDu human squamous cell carcinoma monolayer cell line was subcultured in RPMI 1640/10% fetal calf serum in 100 mm cell culture dishes (Falcon) as described.[25] Inhibition of growth of these cell lines during continuos drug exposure was measured as described.[25] EC$_{50}$ values were determined visually from plots of percent control growth versus the logarithm of drug concentration. The cell line was verified to be negative for mycoplasma contamination using GenProbe test kit during the course of these studies.

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