Brief Articles

Design, Synthesis, and Biological Evaluation of 2,4-Diamino-5-methyl-6-substituted-pyrrolo[2,3-*d*]pyrimidines as Dihydrofolate Reductase Inhibitors[1]

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2,4-Diamino-5-methyl-6-(substituted-phenyl)thiopyrrolo[2,3-*d*]pyrimidines **4**−**11** were synthesized as dihydrofolate reductase (DHFR) inhibitors against opportunistic pathogens that afflict patients with AIDS. Synthesis was achieved from 2,4-diamino-5-methypyrrolo[2,3-*d*]pyrimidine and substituted phenylthiols under modified conditions reported by Gangjee et al. Some of these compounds were potent and selective against DHFR from both *Toxoplasma gondii* and *Mycobacterium avium* compared to mammalian DHFR. Compound **11** with a 1-naphthyl substituent is 16-fold more potent and equally selective against *Toxoplasma gondii* DHFR as the clinically used trimethoprim.

Introduction

Patients with acquired immune deficiency syndrome (AIDS) suffer from and succumb to opportunistic infections caused by *Pneumocystis carinii* (*P. carinii*) and *Toxoplasma gondii* (*T. gondii*).[2] Current therapy involves different dihydrofolate reductase (DHFR) inhibitors including selective but weakly potent agents trimethoprim (TMP) and pyrimethamine, in combination with sulfonamides to enhance potency,[2] and the potent and toxic antifolates trimetrexate (TMQ) and piritrexim (PTX) that are coadministered with leucovorin for host rescue.[2] Serious toxicities often force the cessation of treatment.[2] *Mycobacterium avium* (*M. avium*) Complex or MAC also adversely affects the quality of life of AIDS patients.[2] Thus, it is of considerable interest to incorporate selectivity and potency into a single agent that can be used alone to treat these infections.

X-ray crystal structures[3] have shown that most catalytic site residues in DHFR, involved in binding antifolates, are conserved in both human (h) DHFR and pathogen DHFR. The hydrophilic Asn64 in hDHFR, located just outside the binding site, is replaced by hydrophobic Phe69 in pcDHFR, Phe91[4] in tgDHFR, and Val58[5] in maDHFR. Therefore, it should be possible to design selective DHFR inhibitors that interact hydrophobically with pcDHFR, tgDHFR, and maDHFR.

Compound **1**[6] (Figure 1) displays good selectivities for both pcDHFR and tgDHFR compared to mammalian rat liver (rl) DHFR. The crystal structure of **1** revealed a close hydrophobic interaction of the 2-naphthyl moiety of **1** with Phe69 of pcDHFR that is believed to be responsible for its selectivity.[6] Enhancing this hydrophobic interaction could increase the selectivity for DHFR from opportunistic pathogens. We designed and synthesized 6-5 bicyclic antifolates **4**−**11** (Figure 1) with the side chain appended at the 6-position rather than the 5-position as in **1**. This positional variation provides a closer proximity of the hydrophobic 6-substituent to the hydrophobic residues on the pathogen DHFR and should provide greater potency and selectivity. Rosowsky et al.[7,8] and Donkor et al.,[9] using a different design rationale, reported compounds of general structure **3** with a carbon atom bridge that afforded fair tgDHFR potency (IC_{50} = 3.2 μM) and selectivity (25-fold). The inclusion of a sulfur atom bridge rather than a carbon is expected to further increase the proximity of the 6-aryl ring to the hydrophobic residues on the pathogen enzymes because of an increase in the size of the sulfur as well as a decrease in the C−S−C angle (98°) compared to a C−C−C angle (109°) in **3**. This would allow a side chain phenyl distance between a one- and two-carbon-atom bridge as in **3** (n = 2). Gangjee et al.[10] reported a 5-desmethylpyrido[2,3-*d*]pyrimidine analogue of TMQ, **2**, whose potency was decreased compared to the 5-methyl analogue, suggesting that the 5-methyl group in TMQ and perhaps PTX is important for high potency. X-ray crystal structure,[3,11] multiple sequence alignment,[4,5] and molecular modeling (SYBYL 6.8[12]) indicate that the 5-methyl group interacts with Ile123 in pcDHFR, Val151 in tgDHFR, and Ile102 in maDHFR. The 5-methyl group also affects the conformations of the 6 side chain in these molecules and could contribute to the potency. Molecular modeling suggested that a 5-methyl group of a 6−5 bicyclic antifolate has similar interaction and conformational effects.

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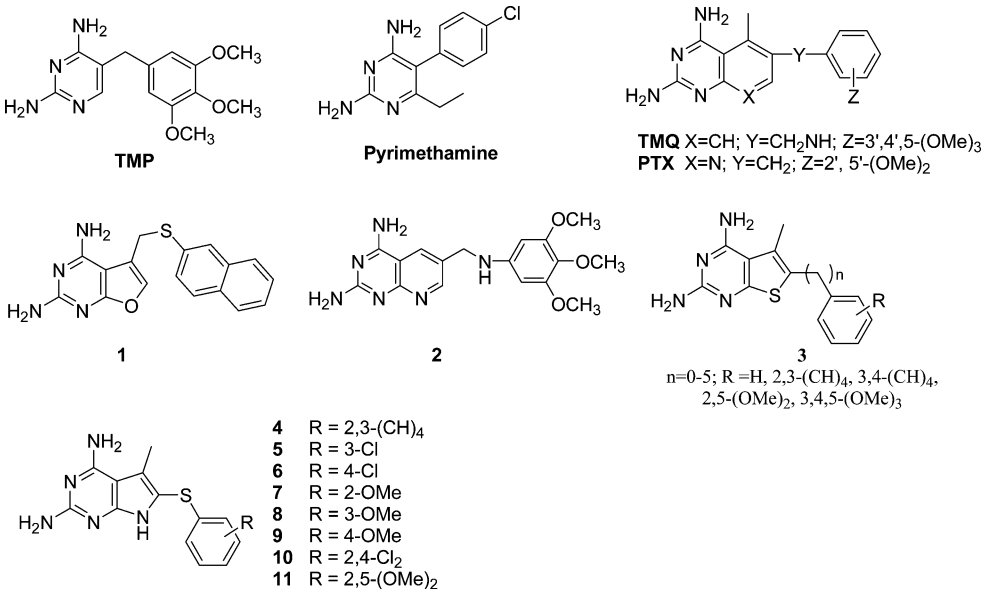

Figure 1. Structures of antifolates.

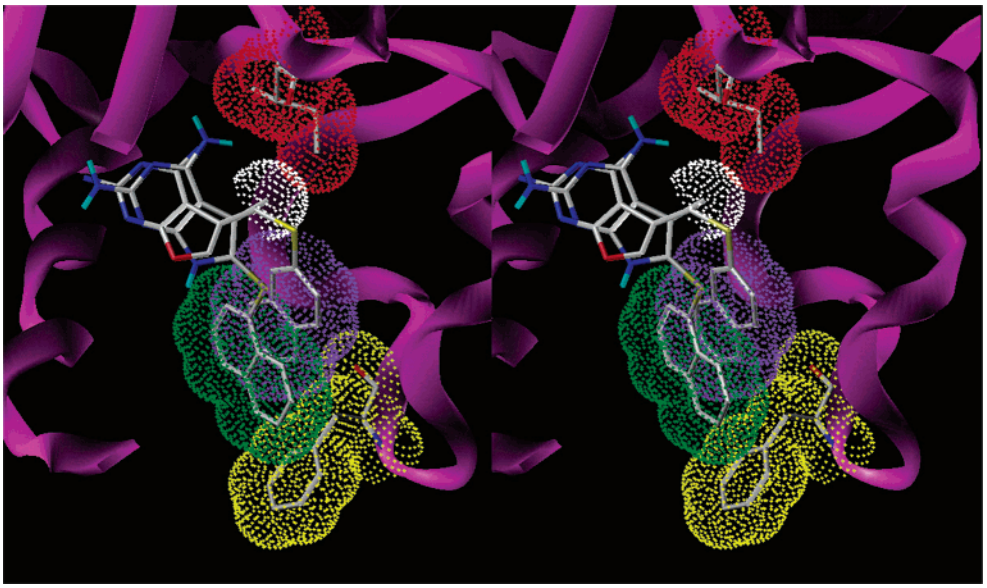

Figure 2. Comparison between the docked structure of **4** and the crystallographic structure of **1** in pcDHFR in stereoview. The van der Waals surface of the 6 side chain of **4** (green) is closer to the van der Waals surface of Phe69 (yellow) compared to that of the 5 side chain of **1** (purple). The van der Waals surface of the 5-methyl of **4** (white) also makes hydrophobic contacts with that of Ile123 (red).

A comparison between the docked structure (FLO96[13]) of **4** and the crystallographic structure of **1** in pcDHFR (Figure 2) indicated that the 6 side chain of **4** could indeed enhance the proximity of the 6-phenyl substituent in hydrophobic contact with Phe69 and the 5-methyl group with the hydrophobic Ile123. Similar interactions were anticipated with tgDHFR and maDHFR.

Chemistry

A two-step procedure reported by Taylor et al.[14] afforded **14** (Scheme 1). Compounds **4**−**11** were synthesized from **14** via an oxidative thiolation reported by Gangjee et al.[15] The original procedure required pivaloyl protection of the amino group(s), which significantly lowered the overall yield (25% on average over two steps[15]). After several variations of time, temperature, and the sequence of addition of the reactants, it was discovered that the addition of iodine after the thiophenols rather than before followed by a workup under basic conditions with ammonium hydroxide afforded the target compounds without the necessity of protection and an improvement in the overall yield to 42%. Thus, **14** was treated with 2 equiv of the appropriate ben-

Scheme 1

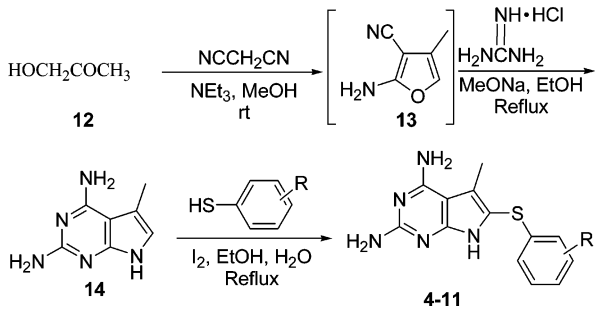

Table 1. Inhibition Concentrations against Dihydrofolate Reductases from *P. carinii*, *T. gondii*, *M. avium*, and Rat Liver[a] and Selectivity Ratios [(IC_{50} rlDHFR)/(IC_{50} pcDHFR), (IC_{50} rlDHFR)/(IC_{50} tgDHFR), and (IC_{50} rlDHFR)/(IC_{50} maDHFR)]

	inhibition concn (IC_{50}, μM)			selectivity ratio (IC_{50}/IC_{50})			
compd	pcDHFR	tgDHFR	maDHFR	rlDHFR	rl/pc	rl/tg	rl/ma
1	0.65	11.6		12.3	18.9	1.1	
4	37.3	0.16	0.7	4.57	0.12	27.9	6.92
5	42.9	6.94	4.4	47.7	1.11	6.87	10.9
6	16.7	8.93	4	19	1.14	2.13	4.77
7	22	1.02	0.3	2.32	0.11	2.27	6.82
8	60.2	4.76	1.37	2.06	0.03	0.43	1.50
9	112	2.69	0.6	31.3	0.28	11.6	56.4
10	12 (14[b])	20	19	110	ND	5.5	5.79
11	14.6	0.17	0.1	7.8	0.5	45.9	78
TMQ	0.042	0.010	0.0015	0.003	0.07	0.3	2.0
TMP	12	2.8	0.3	180	14	65	610

[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.[10] [b] Number in parentheses is the percent inhibition at the given concentration.

zenethiol followed by the addition of 2 equiv of iodine in 2:1 ethanol/water to afford **4**−**11** after workup.

Biological Activity and Discussion

Compounds **4**−**11** were evaluated as inhibitors of pcDHFR, tgDHFR, maDHFR, and rlDHFR, which served as the mammalian standard (Table 1). Several compounds displayed 10-fold or higher selectivity ratios for tgDHFR or maDHFR compared to rlDHFR. Compounds with electron-donating methoxy substitutions in the phenyl ring (e.g., **11**) displayed higher activities against both tgDHFR and maDHFR compared to those with electron-withdrawing chloro substitutions (e.g., **10**). The position of the chloro substitution did not influence the activity, however, against tgDHFR and maDHFR; the inhibitory activities of the monomethoxy-substituted analogues followed the trend ortho > para > meta. Compound **4**, the 1-naphthyl-substituted analogue, also showed good potency and selectivity against tgDHFR. Compound **11**, the 2,5-dimethoxyphenyl analogue, is 16-fold more potent and equally selective compared to TMP against tgDHFR and is 19-fold more potent and nearly 2-fold more selective than the most selective compound of the 6-carbon-bridged analogues **3**.[7−9] These data support the hypothesis that the 6 side chain of these compounds interacts more favorably with Phe91 in tgDHFR and Val58 in maDHFR and that the sulfur bridge increases activity and selectivity. The 1-naphthyl analogue **4** and the methoxy substituents on the phenyl ring in **11** also contribute to the potency and selectivity and are superior to other substitutions.

The relative inactivity of these compounds against pcDHFR was surprising and may reflect a lack of simultaneous accommodation of both the 5-methyl and the 6-substituent with a combination of the longer Ile123 and the larger Phe69, respectively, in pcDHFR. In contrast, tgDHFR has a shorter Val151 and a Phe91 while maDHFR has Ile102 but a shorter Val58 and both are thus better able to accommodate the 5-methyl and 6-substituent, resulting in higher potencies. The crystal structures of **4** and **11** with pcDHFR, tgDHFR, rlDHFR, and hDHFR are currently in progress and should afford insight into the potency and selectivity of **4** and **11**.

Experimental Section

2,4-Diamino-5-methyl-6-(1-naphthylthio)pyrrolo[2,3-*d*]-pyrimidine (4). To a solution of **14** (0.10 g, 0.61 mmol) in a mixture of ethanol/water (2:1, 50 mL) was added 1-naphthylthiol (0.20 g, 1.22 mmol), and the reaction mixture was heated to reflux followed by the addition of iodine (0.31 g, 1.22 mmol). After 4 h, the mixture was cooled to room temperature and concentrated under reduced pressure. The residue was washed with 20 mL of saturated sodium thiosulfate solution in water and chromatographed on silica gel with 10% MeOH/$CHCl_3$ as the eluent. Fractions containing the product (TLC) were combined and evaporated to give **4** (0.08 g, 41%): mp 288.7−293 °C (dec); TLC R_f = 0.52 ($CHCl_3$/MeOH, 5:1, silica gel); ^{1}H NMR (Me_2SO-d_6) δ 2.34 (s, 3H, 5-CH_3), 5.63 (s, 2H, 2-NH_2), 6.29 (s, 2H, 4-NH_2), 6.80−8.26 (m, 7H, $C_{10}H_7$), 11.00 (s, 1H, 7-H). Anal. ($C_{17}H_{15}N_5S\cdot 0.20CH_3OH$) C, H, N, S.

General Procedure for the Synthesis of Compounds 5−11. Compounds **5**−**11** were synthesized from **14**, appropriately substituted thiophenols, and iodine as described for **4**. After evaporation of the solvent under reduced pressure, the residue was washed with ethyl acetate followed by the addition of 200 mL of methanol and the pH was adjusted to 10 with concentrated NH_4OH. The suspension was left at room temperature for 30 min and filtered. The residue was washed well with methanol and air-dried.

2,4-Diamino-5-methyl-6-(3′-chlorophenylthio)pyrrolo-[2,3-*d*]pyrimidine (5). Compound **5** (0.43 g, 46%) was obtained from **14** (0.5 g, 3.06 mmol), 3-chlorobenzenethiol (0.89 g, 6.12 mmol), and iodine (1.56 g, 6.12 mmol): mp 298.7−304.3 °C (dec); TLC R_f = 0.47 ($CHCl_3$/MeOH, 5:1, silica gel); ^{1}H NMR (Me_2SO-d_6) δ 2.31 (s, 3H, 5-CH_3), 5.68 (s, 2H, 2-NH_2), 6.33 (s, 2H, 4-NH_2), 6.95 (d, 2H, aromatic), 7.18 (d, 1H, aromatic), 7.30 (t, 1H, aromatic), 11.00 (s, 1H, 7-H). Anal. ($C_{13}H_{12}N_5ClS$) C, H, N, Cl, S.

2,4-Diamino-5-methyl-6-(4′-chlorophenylthio)pyrrolo-[2,3-*d*]pyrimidine (6). Compound **6** (0.68 g, 73%) was obtained from **14** (0.5 g, 3.06 mmol), 4-chlorobenzenethiol (0.89 g, 6.12 mmol), and iodine (1.56 g, 6.12 mmol): mp 316.8−319.4 °C (dec); TLC R_f = 0.47 ($CHCl_3$/MeOH, 5:1, silica gel); ^{1}H NMR (Me_2SO-d_6) δ 2.30 (s, 3H, 5-CH_3), 5.64 (s, 2H, 2-NH_2), 6.30 (s, 2H, 4-NH_2), 6.98 (d, 2H, 3′,5′-2H), 7.33 (d, 2H, 2′,6′-2H), 10.98 (s, 1H, 7-H). Anal. ($C_{13}H_{12}N_5ClS$) C, H, N, Cl, S.

2,4-Diamino-5-methyl-6-(2′-methoxyphenylthio)pyrrolo-[2,3-*d*]pyrimidine (7). Compound **7** (0.39 g, 42%) was obtained from **14** (0.5 g, 3.06 mmol), 2-methoxybenzenethiol (0.86 g, 6.12 mmol), and iodine (1.56 g, 6.12 mmol): mp 306.7−313.7 °C (dec); TLC R_f = 0.50 ($CHCl_3$/MeOH, 5:1, silica gel); ^{1}H NMR (Me_2SO-d_6) δ 2.27 (s, 3H, 5-CH_3), 3.85 (s, 3H, 2-OCH_3), 5.61 (s, 2H, 2-NH_2), 6.25 (s, 2H, 4-NH_2), 6.37 (d, 1H, 3′-H), 6.79 (t, 1H, 4′-H), 6.97 (d, 1H, 4′-H), 7.09 (t, 1H, 6′-H), 10.88(s, 1H, 7-H). Anal. ($C_{14}H_{15}N_5OS$) C, H, N, S.

2,4-Diamino-5-methyl-6-(3′-methoxyphenylthio)pyrrolo-[2,3-*d*]pyrimidine (8). Compound **8** (0.46 g, 50%) was obtained from **14** (0.5 g, 3.06 mmol), 3-methoxybenzenethiol (0.86 g, 6.12 mmol), and iodine (1.56 g, 6.12 mmol): mp 264.4−268.3 °C (dec); TLC R_f = 0.50 ($CHCl_3$/MeOH, 5:1, silica gel); ^{1}H NMR (Me_2SO-d_6) δ 2.30 (s, 3H, 5-CH_3), 3.66 (s, 3H, 3-OCH_3), 5.70 (s, 2H, 2-NH_2), 6.36 (s, 2H, 4-NH_2), 6.53 (d, 2H, 2′, 4′-2H), 6.70 (t, 1H, 6′-H), 7.18 (d, 1H, 5′-H), 11.00 (s, 1H, 7-H). Anal. ($C_{14}H_{15}N_5OS$) C, H, N, S.

2,4-Diamino-5-methyl-6-(4′-methoxyphenylthio)pyrrolo-[2,3-*d*]pyrimidine (9). Compound **9** (0.20 g, 21%) was obtained from **14** (0.5 g, 3.06 mmol), 4-methoxybenzenethiol (0.86 g, 6.12 mmol), and iodine (1.56 g, 6.12 mmol): mp 291.7−295.8 °C (dec); TLC R_f = 0.50 ($CHCl_3$/MeOH, 5:1, silica gel); ^{1}H NMR (Me_2SO-d_6) δ 2.33 (s, 3H, 5-CH_3), 3.68 (s, 3H, 4-OCH_3), 5.60 (s, 2H, 2-NH_2), 6.25 (s, 2H, 4-NH_2), 6.86 (d, 2H, 3′, 5′-2H), 7.04 (d, 2H, 2′, 6′-2H), 10.93 (s, 1H, 7-H). Anal. ($C_{14}H_{15}N_5OS$) C, H, N, S.

2,4-Diamino-5-methyl-6-(2′,4′-dichlorophenylthio)-pyrrolo[2,3-*d*]pyrimidine (10). Compound **10** (0.12 mg, 20%) was obtained from **14** (0.3 g, 1.84 mmol), 2,4-dichlorobenzenethiol (0.66 g, 3.68 mmol), and iodine (0.93 g, 3.68 mmol) (**10**): mp 331.0−334.9 °C (dec); TLC R_f = 0.50 ($CHCl_3$/MeOH, 5:1, silica gel); ^{1}H NMR (Me_2SO-d_6) δ 2.29 (s, 3H, 5-CH_3), 5.69 (s, 2H, 2-NH_2), 6.35 (s, 2H, 4-NH_2), 6.48 (d, 1H, 5′-H), 7.32 (t,

1H, 6′-H), 7.62 (d, 1H, 3′-H), 11.02 (s, 1H, 7-H). Anal. ($C_{13}H_{11}N_5Cl_2S$) C, H, N, Cl, S.

2,4-Diamino-5-methyl-6-(2′,5′-dimethoxyphenylthio)-pyrrolo[2,3-*d*]pyrimidine (11). Compound **11** (0.08 g, 37%) was obtained from **14** (0.10 g, 0.61 mmol), 2′,5′-dimethoxyphenylthiol (0.21 g, 1.22 mmol), and iodine (0.31 g, 1.22 mmol): mp 248.3−252.0 °C (dec); TLC $R_f = 0.52$ ($CHCl_3$/MeOH, 5:1, silica gel); ^{1}H NMR (Me_2SO-d_6) δ 2.34 (s, 3H, 5-CH_3), 3.35 (s, 3H, OCH_3), 3.68 (s, 3H, OCH_3), 5.58 (s, 2H, 2-NH_2), 6.22 (s, 2H, 4-NH_2), 6.55 (d, 1H, 6′-CH), 6.81−6.88 (m, 2H, 3′, 4′-CH), 10.92 (s, 1H, 7 −H). Anal. ($C_{15}H_{17}N_5OS$) C, H, N, S.

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Supporting Information Available: Additional experimental details and elemental analysis results. This material is available free of charge via the Internet at http://pubs.acs.org.

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