# Inhibitors of Dihydrofolate Reductase: Design, Synthesis and Antimicrobial Activities of 2,4-Diamino-6-methyl-5-ethynylpyrimidines



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Novel 2,4-diamino-6-methyl-5-ethynylpyrimidines were prepared *via* palladium catalyzed coupling of 2,4-diamino-5-iodo-6-methyl-pyrimidine with terminal acetylenes. The compounds were inhibitors of dihydrofolate reductase and showed *in vitro* activity against several species of opportunistic fungi and the protozoan *Toxoplasma gondii*.

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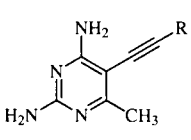
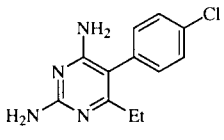
Life-threatening systemic infections of opportunistic organisms such as *Candida albicans*, *Cryptococcus neoformans*, *Aspergillus fumigatus*, and *Toxoplasma gondii* are of increasing clinical importance [1]. *C. albicans* has become the fifth leading cause of microbial infection in the hospital setting [2], and opportunistic pathogens present a serious problem to immunocompromised patients, especially those infected with human immunodeficiency virus [3]. The available therapies for these diseases are limited, and the need for safer and more effective drugs has prompted increased efforts in the search for novel antifungal and antiprotozoal agents [4].

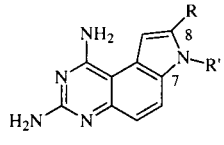
This paper describes the preparation of 5-ethynyl-2,4-diaminopyrimidines **1** as inhibitors of dihydrofolate reductase and their evaluation as potential antimicrobial agents. Dihydrofolate reductase is an established target for antimicrobial activity [5,6], and inhibitors of the enzyme have shown clinical effectiveness against opportunistic infections [7,8]. For example, pyrimethamine (**2**), in combination with sulfonamides, is clinically effective against toxoplasmosis [9]. In addition, a series of 2,4-diaminopyrroloquinazolines, represented by compounds **3a** and **b,** are high affinity inhibitors of *C. albicans* dihydrofolate reductase and display potent activity against fungi and protozoa [10]. The novel 5-ethynylpyrimidines **1** were good inhibitors of dihydrofolate reductase, and members of the series showed activity against *T. gondii* up to 20-fold greater than that of pyrimethamine. The compounds also significantly inhibited the growth of *C. albicans*, *C. neoformans*, and *A. fumigatus*.




**1**




**2**




**3a**, R,R' = $CH_3$
**b**, R = $CH_3$, R' = $CHEt_2$

The design of compounds **1** arose from molecular modeling and X-ray crystallographic studies of *C. albicans* dihydrofolate reductase and its complexes with the series of pyrroloquinazolines, *e.g.*, **3a** and **b**. Branched alkyl substituents at the 7- and 8-positions of the pyrroloquinazoline ring system fit into a hydrophobic region of the enzyme and impart significant affinity for the protein. The biological activities of the pyrroloquinazolines suggested that small molecular size may confer enhanced antimicrobial properties. Thus, the ethynylpyrimidines **1** were designed with the intent of keeping molecular size to a minimum and utilizing the hydrophobic pocket of dihydrofolate reductase that is exploited by the 7- and 8-substituents of compounds **3a** and **b**. Superposition of molecular models of **1** and **3** and modeling of **1** in the active site of dihydrofolate reductase suggested that the R-substituent of **1** would interact favorably with the enzyme hydrophobic pocket of interest. For example, Figure 1 illustrates the superposition of molecular models of compounds **1d** and **3a** and shows that the methyl groups of the t-butyl moiety of **1d** are spatially similar to the 7,8-dimethyl substituents of compound **3a**.

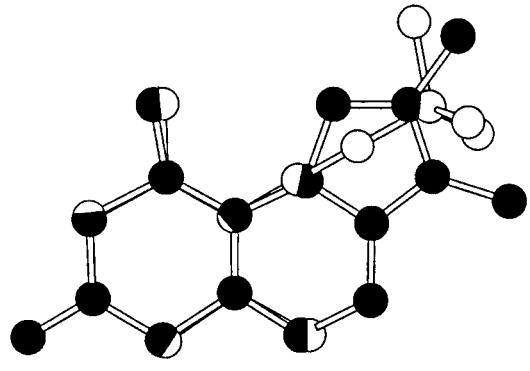


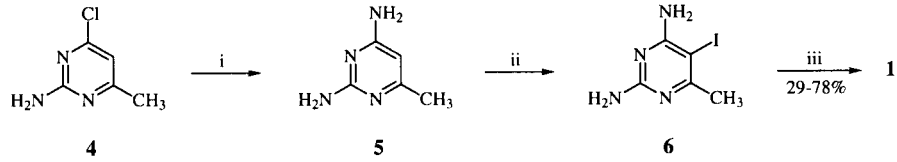
Figure 1. Overlay of molecular models of **1d** (atoms represented by open circles) and **3a** (atoms represented by filled circles) to illustrate the spatial relationship between the alkynyl substituent of compounds **1** and the 7,8-substituents of compounds **3**.

Although a plethora of 2,4-diaminopyrimidines have been prepared [11-13], 5-ethynyl derivatives are relatively rare. Such compounds might be prepared from the corresponding uracils, since it is known that 5-iodouracils can be converted to 5-ethynyl derivatives *via* palladium-catalyzed coupling with alkynyl stannanes [14,15] and terminal acetylenes [16]. However, conversion of uracils into diaminopyrimidines is sometimes troublesome and limited in scope [17,18]. Therefore, we developed a more convenient and efficient route to the title compounds involving the direct coupling of terminal acetylenes with a 5-iodo-2,4-diaminopyrimidine. As shown in Scheme 1, commercially available 2-amino-4-chloro-6-methylpyrimidine was treated with ammonia in ethanol under pressure to give the 2,4-diaminopyrimidine **5** [19]. Compound **5** was iodinated in 75% yield using iodine monochloride [20-22] to provide the iodopyrimidine **6**. Coupling of pyrimidine **6** with various terminal acetylenes using palladium catalysis [23,24] gave the compounds **1** listed in Table 1. Subsequent to the completion of this work, a patent describing a similar preparation of a series of 5-(arylethynyl)-2,4-diaminopyrimidines appeared [25].

moderately potent inhibitors of *C. albicans* dihydrofolate reductase with $K_i$ values ranging from 10-200 n*M* and were significantly more active as inhibitors of human dihydrofolate reductase ($K_i$ 0.08-5.9 n*M*). Consistent with the structural comparison of compounds **1d** and **3a** shown in Figure 1, inhibition of *C. albicans* and human dihydrofolate reductase was similar for the two compounds. However, the other ethynyl substituents examined in this study did not provide increased enzyme inhibition, in contrast to the greatly enhanced potency furnished by branched alkyl substituents at the 7- and 8-positions of the pyrroloquinazoline system [10].

For the majority of the alkynylpyrimidines, the *C. albicans*-to-human dihydrofolate reductase $K_i$ ratio was in the range of 30-225, similar to that observed for the pyrroloquinazoline series of inhibitors, *e.g.*, **3a,b,** and significantly less than the ratio of 1600 found for pyrimethamine (**2**). Compound **1f** was much less active against human dihydrofolate reductase than other members of the series and its *C. albicans*-to-human dihydrofolate reductase $K_i$ ratio was 4.7. This difference in dihydrofolate reductase species selectivity of compound **1f** compared to **1a-e,g**

Scheme I*




*Reagents: (i) Excess ammonia in ethanol, 160°; (ii) ICl, methanol; (iii) HC≡CR, bis(triphenylphosphine)palladium(II) chloride, Cu(I)I, $Et_3N$, dimethylformamide

Table 1

Biological Data for Dihydrofolate Reductase Inhibitors [a]

| Compound | R | Dihydrofolate Reductase $K_i$ (n*M*) [b] | | Minimum Inhibitory Concentration (μg/ml) | | | |
|---|---|---|---|---|---|---|---|
| | | *C. albicans* | Human | *C. albicans* | *C. neoformans* | *T. gondii* | *A. fumigatus* |
| **1a** | $CH(CH_3)_2$ | 51 | 0.88 | 0.8 | 1.6 | - | 12.5 |
| **b** | $C_5H_9$ | 11 | 0.33 | 0.2 | 0.1 | 0.25 | 6.2 |
| **c** | $CH(CH_3)(C_2H_5)$ | 29 | 0.30 | 0.4 | 0.2 | 0.1 | 6.2 |
| **d** | $C(CH_3)_3$ | 18 | 0.080 | 0.4 | 0.2 | 0.025 | 12.5 |
| **e** | $C(C_2H_5)_2OH$ | 198 | 1.7 | 12.5 | 12.5 | - | >50 |
| **f** | $C_6H_5$ | 28 | 5.9 | 0.4 | 0.4 | - | 6.2 |
| **g** | $CH_2CH(CH_3)_2$ | 24 | 0.44 | - | - | - | - |
| **2** | - | 274 | 0.17 | 50 | - | 0.5 | - |
| **3a** | - | 2.0 | 0.087 | 1.6 | 6.2 | - | >50 |
| **b** | - | 0.03* | 0.0003* | 0.025 | 0.05 | 0.00025 | 6.2 |

[a] See reference 10 for testing procedures. Assays for *C. neoformans* and *A. fumigatus* followed the protocol described for *C. albicans*. [b] Values marked by an asterisk were measured directly. Otherwise, values were calculated from the $IC_{50}$ value.

The 5-ethynylpyrimidines **1a-g** were evaluated as inhibitors of *C. albicans* and human dihydrofolate reductase and for growth inhibition of selected microorganisms. As shown in Table 1, the compounds were found to be

may be related to the rigid nature of the phenyl substituent and the differences in active site geometry observed for the two enzymes [26]. The inhibitor binding site of *C. albicans* dihydrofolate reductase is wider than that of the

human protein and appears to be better able to accommodate the extended geometry of compound **1f**.

Broad-spectrum antimicrobial activity was observed for the ethynylpyrimidines **1**. Compound concentrations effective against whole cells ranged as low as 0.025 μg/ml (*T. gondii*) for **1d** and 0.1 μg/ml (*C. neoformans*) for **1b**. The activity of compound **1d** against *T. gondii* was 20-fold greater than that of pyrimethamine, a clinically effective therapy for toxoplasmosis. Compounds **1** showed minimum inhibitory concentration values as low as 0.2 μg/ml (compound **1b**) against *C. albicans* and were generally more efficient inhibitors of *C. albicans* growth, compared to the pyrroloquinazolines **3**, as measured by the ratio of *C. albicans* minimum inhibitory concentration to *C. albicans* dihydrofolate reductase $K_i$. For example, compound **1b** was 5-fold less active than **3a** as an inhibitor of *C. albicans* dihydrofolate reductase but was 8-fold more potent as an inhibitor of *C. albicans* cell growth. Compounds **1b**,**c** and **f** were as effective as the pyrroloquinazoline **3b** in the growth inhibition of *A. fumigatus*.

In summary, the series of 5-ethynylpyrimidines reported here represents a novel class of dihydrofolate reductase inhibitor with broad-spectrum antimicrobial activity. The ethynyl substituents appear to take good advantage of a hydrophobic pocket of dihydrofolate reductase, and this class of inhibitor may offer the potential for differentiation between the fungal and human enzymes. Members of this series showed significantly greater activity against *T. gondii* than the clinically effective agent pyrimethamine.

## EXPERIMENTAL

Melting points were determined using a Thomas-Hoover apparatus and are uncorrected. Chromatography was carried out on E. Merck silica gel 60 (particle size 0.040-0.063 mm) using the solvents listed under the individual experiments. The acetylenes were obtained from Lancaster Synthesis and Farchan Labs. The iodine monochloride solution and phenylacetylene were obtained from Aldrich. $^1$H nmr spectra were measured at 200 MHz with a Varian XL-200 spectrometer. Chemical shifts are in parts per million (δ), relative to the observed solvent resonance (dimethyl sulfoxide, 2.50). Multiplicities are designated as singlet (s), doublet (d), triplet (t), multiplet (m), and broad (br). High-resolution mass spectra were obtained on a VG70SQ with a VG11-250J data system using Fast Atom Bombardment ionization and a resolution of 10,000 (10% valley definition). Low resolution chemical ionization mass spectra were obtained at Oneida Research Services, Whitesboro, NY, using methane as the reagent gas. Elemental analyses were performed by Atlantic Microlab, Inc., Atlanta, GA.

2,4-Diamino-5-iodo-6-methylpyrimidine (**6**) [25].

To a solution of **5**•hydrochloride [11] (5.0 g, 31 mmoles) in 100 ml of methanol was added 100 ml of 1 *M* iodine monochloride in dichloromethane in one portion, and the mixture was stirred 18 hours at room temperature. The solvent was removed *in vacuo* to leave a brown paste. Diethyl ether (500 ml) was added and the mixture was rapidly stirred for 1 hour. The resulting solid was isolated by vacuum filtration, washed with ether and dried *in vacuo* to give 10.5 g of a solid. The solid was sonicated with 500 ml 1.0 *N* sodium hydroxide, filtered and dried to provide 5.8 g (73% yield) of the expected product as a yellow solid, mp 169-170°; ms: (chemical ionization) m/z 251 ($MH^+$) 100%; $^1$H nmr (dimethyl-$d_6$ sulfoxide): δ 2.3 (s, 3H); 6.0 (br s, 2H); 6.3 (br s, 2H).

*Anal.* Calcd. for $C_5H_7N_4I$: C, 24.02; H, 2.82; N, 22.41; I, 50.75. Found: C, 24.19; H, 2.87; N, 22.28; I, 50.62.

General Procedure for the Palladium-catalyzed Preparation of 2,4-Diamino-5-ethylnylpyrimdines **1**.

2,4-Diamino-6-methyl-5-(2-phenylethynyl)pyrimidine (**1f**) [25].

To a mixture of compound **6** (1.5 g, 6.0 mmoles) in 150 ml of dry dimethylformamide was added triethylamine (150 ml), bis(triphenylphosphine) palladium(II) chloride (0.28 g, 0.4 mmole), and copper(I) (0.08 g, 0.4 mmole). Phenylacetylene (2.6 ml, 24 mmoles) was added slowly *via* syringe, and the mixture was stirred 18 hours at room temperature. The solvent was evaporated *in vacuo*, and the residue was subjected to flash chromatography (silica gel, 98/2:ethyl acetate/ethanol) to give 1.2 g of a pale yellow solid. The solid was stirred 3 hours with 500 ml of 1.0 *N* sodium hydroxide and isolated by filtration. The solid was washed with water and dried *in vacuo* to furnish 0.80 g (59%) of compound **1f**, mp 159-160°.

*Anal.* Calcd. for $C_{13}H_{12}N_4$•0.8 $H_2O$: C, 65.42; H, 5.74; N, 23.47. Found: C, 65.65; H, 5.65; N, 23.52.

Recrystallization (acetonitrile) and extensive drying (80°, 0.1 mm Hg, 48 hours) provided a dry sample, mp 160-161°; hrms: calcd. for $C_{13}H_{12}N_4$ ($MH^+$): 225.1140, found: 225.1132; $^1$H nmr (dimethyl-$d_6$ sulfoxide): δ 2.3 (s, 3H), 6.3 (br s, 2H), 6.5 (br s, 2H), 7.4 (m, 3H), 7.6 (m, 2H).

*Anal.* Calcd. for $C_{13}H_{12}N_4$: C, 69.62; H, 5.39; N, 24.98. Found: C, 69.67; H, 5.43; N, 24.88.

2,4-Diamino-6-methyl-5-(3-methyl-1-butynyl)pyrimidine (**1a**).

This compound was obtained in 29% yield as a white solid, mp 142-143°; $^1$H nmr (dimethyl-$d_6$ sulfoxide): δ 1.2 (d, J = 7.5 Hz, 6H), 2.15 (s, 3H), 2.8 (m, 1H), 6.1 (br s, 4H); ms: (chemical ionization) m/z 191 (MH+) 100%.

*Anal.* Calcd. for $C_{10}H_{14}N_4$: C, 63.13; H, 7.42; N, 29.45. Found: C, 63.04; H, 7.41; N, 29.37.

2,4-Diamino-5-(cyclopentylethynyl)-6-methylpyrimidine (**1b**).

This compound was obtained in 46% yield as a white solid, mp 162-163°; $^1$H nmr (dimethyl-$d_6$ sulfoxide): δ 1.5-1.7 (m, 6H), 1.8-2.3 (m, 2H), 2.2 (s, 3H), 2.9 (m, 1H), 6.1 (br s, 4H); ms: (electrospray) 217 (MH+) 100%.

*Anal.* Calcd. for $C_{12}H_{16}N_4$•0.1$H_2O$: C, 66.09; H, 7.49; N, 25.69. Found: C, 65.84; H, 7.49; N, 25.44.

2,4-Diamino-6-methyl-5-(3-methyl-1-pentynyl)pyrimidine (**1c**).

This compound was obtained in 78% yield as a white solid, mp 170-171°; $^1$H nmr (dimethyl-$d_6$ sulfoxide): δ 1.0 (t, J = 7.5, 3H), 1.2 (d, J = 7.5, 3H), 1.5 (m, 2H), 2.2 (s, 3H), 2.65 (m, 1H), 6.2 (br s, 4H); ms: (chemical ionization) 205 (MH+) 100%.

*Anal.* Calcd. for $C_{11}H_{16}N_4$: C, 64.68; H, 7.89; N, 27.43. Found: C, 64.59; H, 7.90; N, 27.41.

2,4-Diamino-6-methyl-5-(3,3-dimethyl-1-butynyl)pyrimidine (**1d**).

This compound was obtained in 74% yield as a white solid, mp 184-185°; $^1$H nmr (dimethyl-$d_6$ sulfoxide): δ 1.3 (s, 9H), 2.15 (s, 3H), 6.1 (br s, 4H); ms: (chemical ionization) 205 (MH+) 100%.

*Anal.* Calcd. for $C_{11}H_{16}N_4$: C, 64.68; H, 7.89; N, 27.43. Found: C, 64.61, H, 7.86; N, 27.50.

1-(2,4-Diamino-6-methyl-5-pyrimidinyl)-3-ethyl-1-pentyn-3-ol (**1e**).

This compound was obtained in 64% yield as a white solid, mp 162-163°; $^1$H nmr (dimethyl-$d_6$ sulfoxide): δ 0.9 (t, J = 6.6 Hz, 6H), 1.6 (m, 4H), 2.15 (s, 3H), 5.15 (s, 1H), 6.2 (br s, 4H); ms: (chemical ionization) 235 (MH+) 100%.

*Anal.* Calcd. for $C_{12}H_{18}N_4O$: C, 61.52; H, 7.74; N, 23.91. Found: C, 61.44; H, 7.72; N, 23.83.

2,4-Diamino-5-(2-methylpropyl)ethynylpyrimidine (**1g**).

This compound was obtained in 27% yield as a white solid, mp 149-150°; $^1$H nmr (dimethyl-$d_6$ sulfoxide): δ 1.0 (d, J = 8.5 Hz, 6H), 1.8 (m, 1H), 2.1 (s, 3H), 2.3 (d, J = 6.6 Hz, 2H), 6.1 (br s, 4H).

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