Triazolopyrimidine-Based Dihydroorotate Dehydrogenase Inhibitors with Potent and Selective Activity against the Malaria Parasite *Plasmodium falciparum*

Margaret A. Phillips,*[,§] Ramesh Gujjar,[#] Nicholas A. Malmquist,[§] John White,[#] Farah El Mazouni,[§] Jeffrey Baldwin,[§] and Pradipsinh K. Rathod*[,#]

Department of Pharmacology, University of Texas Southwestern Medical Center at Dallas, 6001 Forest Park Boulevard, Dallas, Texas 75390-9041, and Department of Chemistry and Global Health, University of Washington, Seattle, Washington 98195

Received January 31, 2008

A *Plasmodium falciparum* dihydroorotate dehydrogenase (*Pf*DHODH) inhibitor that is potent (K_I = 15 nM) and species-selective (>5000-fold over the human enzyme) was identified by high-throughput screening. The substituted triazolopyrimidine and its structural analogues were produced by an inexpensive three-step synthesis, and the series showed good association between *Pf*DHODH inhibition and parasite toxicity. This study has identified the first nanomolar *Pf*DHODH inhibitor with potent antimalarial activity in whole cells (EC_{50} = 79 nM).

Introduction

Malaria infects up to 900 million people and causes as many as 2.7 million deaths worldwide each year.[1,2] Nearly 40% of the world's population is at risk for contracting the disease, which has been a major cause of mortality throughout history. Antimalarial drugs have been the mainstay for managing new infections and established disease. In recent decades widespread drug resistance has been encountered for chloroquine and for almost every other available antimalarial agent.[3] Further, there are indications that drug resistance may be appearing at faster rates in some parts of the world.[4] Multidrug combinations offer temporary relief,[5,6] but given current trends, it is clear that the disease will continue to have an unacceptable impact on global health unless novel drugs are developed. A significant portion of the current arsenal of malaria drug therapies is rooted in natural remedies, starting with the discovery of quinine nearly 350 years ago.[7,8] The current challenge is to couple our knowledge of malaria genomics and biochemistry with modern platforms for drug discovery.

Pyrimidines are essential metabolites, required for DNA and RNA biosynthesis and the biosynthesis of phospholipids and glycoproteins. Unlike mammalian cells, the malaria parasite cannot salvage preformed pyrimidine bases or nucleosides, and pyrimidines must be acquired through the de novo biosynthetic pathway.[9–12] These biochemical results have been confirmed by the genome sequence showing that pyrimidine salvage enzymes are missing from the parasite.[13] Dihydrofolate reductase is a validated target for malaria treatment[14] and inhibitors of thymidylate synthase have potent antimalarial activity,[15–17] illustrating the importance of the pyrimidine biosynthetic pathway to parasite survival.

The fourth and rate-limiting step of pyrimidine biosynthesis is catalyzed by DHODH,[a] a flavin mononucleotide-dependent enzyme. The human and malarial enzymes are localized to the inner mitochondrial membrane and utilize ubiquinone (CoQ) as the physiological oxidant in the reaction.[18,19] Recent studies have suggested that the sole function of the parasite mitochondrial electron transport chain is to provide oxidized CoQ to DHODH for the synthesis of pyrimidines, confirming the essential role that DHODH plays in the biology of the parasite.[20] Inhibitors of human DHODH have proven efficacy for the treatment of rheumatoid arthritis,[21,22] with an approved compound on the market for this application (2-cyano-3-hydroxy-*N*-[4-(trifluoromethyl)phenyl]-2-butenamide **1** (A77 1726)[23] (Figure 1), the active metabolite of 5-methyl-*N*-[4-(trifluoromethyl)phenyl]isoxazole-4-carboxamide), thus demonstrating that DHODH is a "druggable" target. The X-ray structures of human and malarial DHODH have been determined.[24,25] Orotate and FMN stack against each other in the center of the β/α barrel, and the inhibitor-binding site is formed adjacent to this site by two α-helices, which lie between the predicted N-terminal transmembrane domain and the canonical β/α barrel domain (Figure 1). The inhibitor binding-pocket has extensive variation in amino acid sequence between the human and malarial enzymes, providing the structural basis for the identification of species-specific inhibitors.

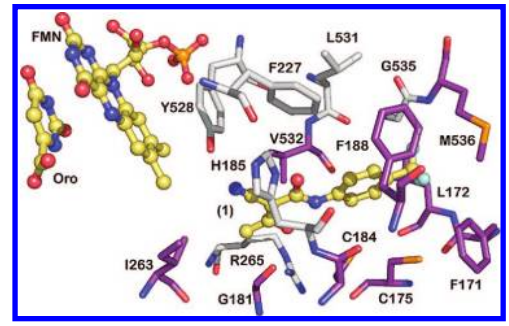


Figure 1. Structure of *Pf*DHODH active site bound to inhibitor **1**. Residues conserved between *P. falciparum* and human DHODH are displayed in gray, and variable residues are displayed in purple. Orotic acid (Oro), FMN, and **1** are displayed in yellow. The figure was generated using PyMol from the file 1TV5.pdb.

We previously utilized a high-throughput screen (HTS) to identify a series of halogenated phenyl benzamide/naphthamides that are potent and species-selective inhibitors of *Pf*DHODH.[26] Tricyclic aromatic amines have also been reported to be parasite

* To whom correspondence should be addressed. For M.A.P.: phone, (214) 645-6164; fax, (214) 645-6166; e-mail, margaret.phillips@UTSouthwestern.edu. For P.K.R.: phone, (206) 221-6069; fax, (206) 685-8665; e-mail, rathod@chem.washington.edu.

§ University of Texas Southwestern Medical Center at Dallas.

University of Washington.

[a] Abbreviations: DHODH, dihydroorotate dehydrogenase; *Pf*DHODH, *Plasmodium falciparum* dihydroorotate dehydrogenase; CoQ, ubiquinone; FMN, flavin mononucleotide; HTS, high-throughput screen.

10.1021/jm8001026 CCC: $40.75
Published on Web 06/04/2008

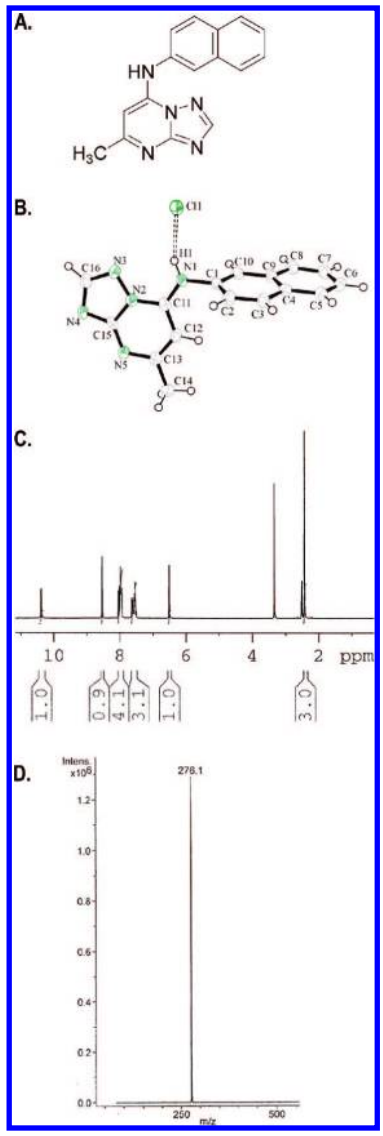


Figure 2. Compound **7**: (A) chemical structure; (B) X-ray crystal structure; (C) ^{1}H NMR in DMSO-d_6; (D) mass spectra.

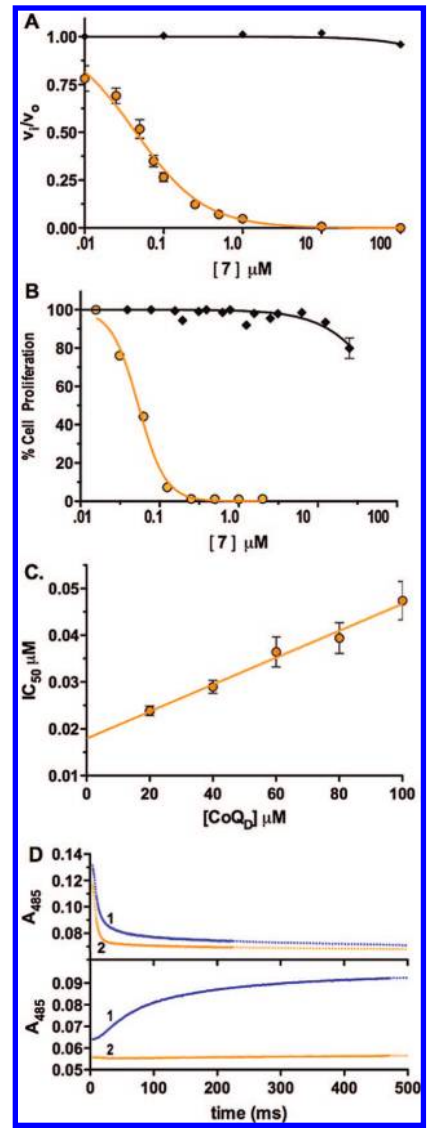


Figure 3. Selective and potent inhibition of *Pf*DHODH and *P. falciparum* cells by **7**: (A) inhibition profile for *Pf*DHODH (orange circles, $IC_{50} = 0.047 \pm 0.022$ μM, $n = 6$) compared to human DHODH (black diamonds, $IC_{50} > 200$ μM); $[E]_T = 10$ nM; (B) activity in whole cell assays against *P. falciparum* 3D7 (orange circles) or mouse L1210 (black diamonds) cells ($EC_{50} = 0.079 \pm 0.045$, $n = 9$); (C) relationship between IC_{50} and substrate concentration ($[E]_T = 5$ nM). K_I was determined by fitting the data to eq 2 ($K_I = 0.015 \pm 0.0011$ μM). (D) Rapid kinetic analysis showing **7** inhibits the CoQ_D-dependent oxidative half-reaction (bottom) but not the DHO-dependent reductive half-reaction (top): blue trace (1), no **7**; orange trace (2), **7** (50 μM).

specific inhibitors of *Pf*DHODH.[27] However, the inhibitors described in these previous studies had poor antimalarial activity in whole cell assays. Thus, while DHODH has received significant attention as a promising new target for the development of antimalarials, chemical validation of DHODH as a target in malaria had not been fully established.

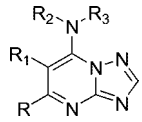
Results and Discussion

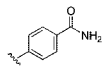
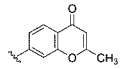
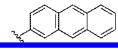
HTS-Based Discovery of an Antimalarial. Here, we describe the identification of a *Pf*DHODH inhibitor, (5-methyl-[1,2,4]triazolo[1,5-*a*]pyrimidin-7-yl)naphthalen-2-ylamine **7** (DSM 1)[28] (Figure 2A), that shows potent and species-selective antiproliferative effects against the *P. falciparum* malaria parasite. Compound **7** was discovered by HTS of a 220 000 compound library of "druglike" molecules using a colorometric enzyme assay.[26] *P. falciparum* DHODH was inhibited by **7** with $IC_{50} = 0.047 \pm 0.022$ μM, and it is >5000-fold selective when compared to the human enzyme (Figure 3A, Table 1). It inhibits the proliferation of *P. falciparum* parasites in whole cell assays with similar potency ($EC_{50} = 0.079 \pm 0.048$ μM for clone 3D7, Figure 3B) and it does not inhibit the growth of a mouse cell line (L1210) ($EC_{50} > 10$ μM). It is also highly active against drug resistant strains of *P. falciparum*, including the multiple-drug-resistant Dd2 ($EC_{50} = 0.14 \pm 0.05$ μM).

The initial structural annotation of **7** in the HTS chemical library database was incorrect as judged by high-resolution mass spectroscopy. The chemical identity of **7** was elucidated using NMR analysis of the original material, and these data were consistent with the structure depicted in Figure 2A. Compound **7** was resynthesized using a simple three-step synthetic method (Scheme 1). The absolute structure of resynthesized **7** was determined by X-ray crystallography (Figure 2B) and further confirmed by mass spectrometry and NMR (Figure 2C,D).

Structure–Activity Relationships (SARs). A series of triazolopyrimidine analogues of **7** were synthesized and tested against the enzyme and against parasites in whole cell assays (Table 1). These compounds show a wide range of IC_{50} values

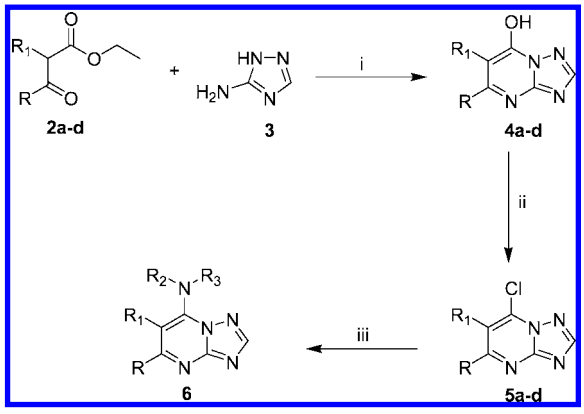
Table 1. Structure and Activity of the Triazolopyrimidine-Based Series against *Pf*DHODH and *P. falciparum* in Whole Cell Assays[a]



compd	R	R_1	R_2	R_3	IC_{50} (μM) *Pf*DHODH	EC_{50}(μM) *Pf* 3D7 cells
7	CH_3	H	H		0.047±0.022	0.079±0.045
8	CF_3	H	H		0.21±0.07	3.3±0.0
9	C_2H_5	H	H		0.19±0.073	0.31±0.32
10	CH_3	CH_3	H		0.16±0.096	0.55±0.22
11	CH_3	H	CH_3		3.0±0.84	16±4.0
12	CH_3	H	$C_6H_5CH_2$		93±9	35±35
13	CH_3	H	H		>200	>50
14	CH_3	H	H		1.7±0.49	1.6±0.35
15	CH_3	H	H		1.2±0.28	2.2±0.5
16	CH_3	H	H		0.33 ±0.1	1.7±0.56
17	CH_3	H	H		2.0±0.07	0.41±0.18
18	CH_3	H	H		45±6.0	4.4±1.8
19	CH_3	H	H		>100	>100
20	CH_3	H	H		0.056±0.024	0.19±0.12

[a] Errors represent the standard error of the mean. The IC_{50} for inhibition of human DHODH was >200 μM for all listed compounds. Enzyme data were collected with the DCIP assay. Growth inhibition by **7** was also tested on additional *P. falciparum* cell lines. EC_{50} values for FCR3, K1, Dd2, HB3, and D6 were 0.18 ± 0.018, 0.14 ± 0.008, 0.14 ± 0.05, 0.10 ± 0.044, and 0.058 ± 0.001, respectively.

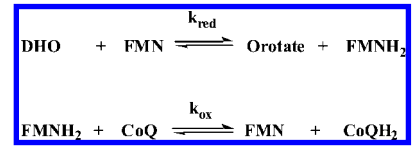
Scheme 1. Synthesis of the Triazolopyrimidine-Based Series[a]



[a] Reagents and conditions: (i) AcOH, 3.5–8 h, reflux, 40–58%; (ii) $POCl_3$, 30–60 min, reflux, 43–65%; (iii) R_2R_3NH, EtOH, 8–15 h, room temp, 80–87%.

against *Pf*DHODH (0.05 to >200 μM), and importantly the inhibitory activity against the enzyme shows strong association with potency on the parasites in whole cell assays. These results are consistent with DHODH being the cellular target of **7** and its derivatives. The compound series also allowed us to establish

Scheme 2



an emerging and preliminary SAR for inhibitory activity on *Pf*DHODH and parasites. From these data we now know that (i) R and R_1 alkyl substituients can be modified with only a modest decrease in activity (**8**−**10**), (ii) substitutions at R_2 resulted in a loss of potency (**11**, **12**), (iii) the introduction of heteroatoms on, or in, the napthyl ring reduced activity (**13**−**17**), (iv) the naphthalene attached at the 2-position is optimal, whereas naphthalene attached at the 1-position showed reduced activity (**18**); (v) replacement of naphthalene with the smaller aniline group significantly reduced activity (**19**), while the larger anthracene moiety was well tolerated (**20**).

Binding Mode and Species Selectivity. The biochemical mechanism of inhibition by **7** was studied in detail to gain insight into the structural basis for the selective and potent binding of this compound to *Pf*DHODH. Steady-state kinetic analysis shows that the IC_{50} for **7** increases linearly with increasing CoQ_D concentration (Figure 3C) as expected for a competitive tight binding inhibitor.[29] These data were fitted to eq 2 yielding a K_I in the low nanomolar range ($K_I = 0.015 \pm 0.001$ μM). Pre-steady-state stopped flow spectroscopy was performed to characterize the effect of **7** on the individual oxidative and reductive half-reactions catalyzed by *Pf*DHODH. Compound **7** inhibited the oxidative half-reaction (k_{ox}), preventing the transfer of electrons from $FMNH_2$ to CoQ, while it did not affect the DHO dependent reductive half-reaction (k_{red}) (Scheme 2, Figure 3D). We previously observed a similar pattern of inhibition for **1** and the biphenylamide inhibitors from our HTS screen.[30] Thus, these data suggest that all three inhibitor classes utilize the same mechanism to inhibit DHODH. Finally, site-directed mutagenesis of residues in the inhibitor binding-site (F227A and R265A) of *Pf*DHODH increased the IC_{50} of **7** by 940-fold and 130-fold, respectively ($IC_{50} = 44 \pm 10$ μM for F227A; $IC_{50} = 6.1 \pm 1.1$ μM for R265A), providing strong evidence that **7** is also bound in this site. As the inhibitor binding site is not conserved in amino acid composition between *P. falciparum* and human DHODH (Figure 1), this binding mode explains the profound species-selective binding of **7** and its derivatives.

Significance. Malaria is one of the most pressing medical problems in the developing world. Target-based drug discovery has been put forth as a promising mechanism for the discovery of new drugs; however, it is often difficult to translate potency on the enzyme target to activity in whole cell assays. Our discovery of DHODH inhibitors by HTS that have potent antimalarial activity provides a successful example of this approach. The triazolopyrimidine-based series exhibits good association between *Pf*DHODH inhibitory activity and antimalarial potency in the infected erythrocyte model, while showing no appreciable activity against the human enzyme or a mouse cell line. These data provide the first example of a DHODH inhibitor with low nanomolar activity in malaria whole cell assays, and the SAR analysis provides the best chemical evidence to date validating *Pf*DHODH as a target for the discovery of new antimalarial compounds. Compound **7** is druglike (as defined by Lipinski's rule of five),[31] simple, and inexpensive to synthesize. Thus, our study has identified a highly

promising candidate for a lead optimization program to develop an antimalarial drug that exploits this new target.

Experimental Section

HTS Screen. Compound **7** was identified by HTS based on a colorimetric end point assay of *Pf*DHODH activity. The details of the HTS assay, the compound library, and the screen method have been previously published.[26]

Protein Purification and Steady-State Kinetic Analysis. *P. falciparum* and human DHODH were expressed and purified and steady-state kinetic assays were performed as previously described.[26,32,33] Reaction details are provided in Supporting Information. Data were fitted to eq 1 to determine IC_{50} values and to eq 2 to determine K_I for a tight-binding competitive inhibitor[29] using Prism (GraphPad).

$$v_i = \frac{v_o}{1 + \frac{[I]}{IC_{50}}} \quad (1)$$

$$IC_{50} = K_i\left(1 + \frac{[S]}{K_m}\right) + 0.5[E]_T \quad (2)$$

Pre-Steady-State Kinetic Analysis by Stopped Flow Spectroscopy. The transition of FMN between the oxidized and reduced state was monitored at 485 nm on a Bio-Logic SFM-3 stopped-flow instrument as described.[30]

***P. falciparum* Cell Culture.** Parasite clones were propagated[34] and [^{3}H]-hypoxanthine uptake was measured in drug-treated *P. falciparum* infected erythrocytes and L1210 cells as described.[15] Data were fitted to eq 3 to determine EC_{50}.

$$\%\ \text{cell proliferation} = \frac{100\%}{1 + 10^{(\log EC_{50} - \log [I])\text{HillSlope}}} \quad (3)$$

Curve Fitting and Error Analysis. Enzyme IC_{50} and parasite EC_{50} data were determined over a range of inhibitor concentrations using triplicate data points at each concentration. IC_{50} values were determined using the graphing program Prism (GraphPad). Data reported in Table 1 represent the average of at least two independent experiments.

General Chemistry. 7-Amino substituted [1,2,4]triazolo[1,5-*a*]pyrimidine compounds (Table 1) were prepared by Scheme 1. Briefly, 3-amino-[1,2,4]triazole **3** was condensed with the substituted ethyl acetoacetates **2a–d** to form the substituted 7-hydroxy[1,2,4]triazolo[1,5-*a*]pyrimidines **4a–d**. Chlorination with phosphorous oxychloride gave the corresponding 7-chloro-[1,2,4]triazolo[1,5-*a*]pyrimidines **5a–d**,[35] which upon treatment with substituted aryl amines in ethanol resulted in products (**7–20**).

Compounds 4a–d. A mixture of 3-amino-1,2,4-triazole **3** (20 mmol) and substituted ethyl acetoacetate **2a–d** (20 mmol) was heated under reflux in acetic acid (10 mL) for 3.5–8 h. The product was then cooled to RT, filtered, washed with water, and dried under vacuum to give a white solid with 40–58% yield.

Compounds 5a–d. [1,2,4]triazolo[1,5-*a*]pyrimidin-7-ol (**4a–d**) (6.5 mmol) was added to 1.82 ml (19.5 mmol) of phosphorous oxychloride and heated under reflux for 30–60 min in a round bottom flask, during which the solid dissolved and hydrogen chloride was evolved. Excess phosphorous oxychloride was removed by distillation at reduced pressure on a steam-bath and the residue triturated with ice water. Product was extracted from the aqueous mixture with methylene chloride, evaporated, and purified by column chromatography using 60% EtOAc/Hexane at a yield of 43–65%. Compound **5b** was used without purification.

Compounds 7–20. The appropriate aryl substituted amine (1 mmol) was added to compound **5a–d** (1 mmol) in absolute ethanol (10 mL) and stirred at RT for 8–15 h. Products were purified by column chromatography with CH_2Cl_2/MeOH/NH_4OH (23:1:1). Yields ranged from 80–87%.

Analysis. As a test of purity, compounds were subjected to HPLC analysis. Compounds eluted as a single peak and the activity of the peak fraction was confirmed by demonstrating *Pf*DHODH inhibitory activity. General analytical and chemical methods, and representative HPLC peaks for the most active compounds, compounds **7** and **20**, are provided as Supporting Information.

Physical Properties. 5-Methyl[1,2,4]triazolo[1,5-*a*]pyrimidin-7-ol (4a). Mp 287 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.15 (s, 1H), 5.82 (s, 1H), 2.30 (s, 3H). MS *m*/*z* 151.1 $(M + H)^+$.

5-Trifluoromethyl[1,2,4]triazolo[1,5-*a*]pyrimidin-7-ol (4b). Mp 263 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.40 (s, 1H), 8.04 (s, 1H, OH), 6.14 (s, 1H). MS *m*/*z* 202.9 $(M - H)^-$.

5-Ethyl[1,2,4]triazolo[1,5-*a*]pyrimidin-7-ol (4c). Mp 215 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.18 (s, 1H), 5.82 (s, 1H), 2.60 (m, 2H), 1.21 (m, 3H). MS *m*/*z* 162.9 $(M - H)^-$.

5,6-Dimethyl[1,2,4]triazolo[1,5-*a*]pyrimidin-7-ol (4d). Mp 308 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.15 (s, 1H), 2.29 (s, 3H), 1.92 (s, 3H). MS *m*/*z* 163.0 $(M - H)^-$.

7-Chloro-5-methyl[1,2,4]triazolo[1,5-*a*]pyrimidine (5a). Mp 150 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.50 (s, 1H), 7.15 (s, 1H), 2.75 (s, 3H). MS *m*/*z* 169.1 $(M + H)^+$.

7-Chloro-5-ethyl[1,2,4]triazolo[1,5-*a*]pyrimidine (5c). Mp 184 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.52 (s, 1H), 7.13 (s, 1H), 3.04 (m, 2H), 1.40 (m, 3H). MS *m*/*z* 183.1 $(M + H^+)$.

(7-Chloro-5,6-dimethyl[1,2,4]triazolo[1,5-*a*]pyrimidine (5d). Mp 147 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.59 (s, 1H), 2.63 (s, 3H), 2.40 (s, 3H). MS *m*/*z* 183.1 $(M + H^+)$.

(5-Methyl[1,2,4]triazolo[1,5-*a*]pyrimidin-7-yl)naphthalen-2-ylamine DSM1 (7). Mp 220 °C (lit.[36] 216–217 °C). ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.35 (brs, NH, exchangeable), 8.50 (s, 1H), 7.85–8.05 (m, 4H), 7.45–7.60 (m, 3H), 6.50 (s, 1H), 2.40 (s, 3H). MS *m*/*z* 276.1 $(M + H^+)$.

(5-Trifluoromethyl[1,2,4]triazolo[1,5-*a*]pyrimidin-7-yl)naphthalen-2-ylamine (8). Mp 239 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.80 (s, 1H), 8.0–8.20 (m, 4H), 7.55–7.70 (m, 3H), 6.70 (s, 1H). MS *m*/*z* 330.1 $(M + H^+)$.

(5-Ethyl[1,2,4]triazolo[1,5-*a*]pyrimidin-7-yl)naphthalen-2-ylamine (9). Mp 238 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.40 (brs, NH, exchangeable), 8.54 (s, 1H), 7.94–8.03 (m, 4H), 7.50–7.64 (m, 3H), 6.50 (s, 1H), 2.70 (m, 2H), 1.20 (m, 3H). MS *m*/*z* 290.2 $(M + H^+)$.

(5,6-Dimethyl[1,2,4]triazolo[1,5-*a*]pyrimidin-7-yl)naphthalen-2-ylamine (10). Mp 262 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.0 (brs, NH, exchangeable), 8.82 (s, 1H), 7.75–7.95 (m, 3H), 7.35–7.60 (m, 4H), 2.65 (s, 3H), 2.05 (s, 3H). MS *m*/*z* 290.1 $(M + H^+)$.

Methyl-(5-methyl[1,2,4]triazolo[1,5-*a*]pyrimidin-7-yl)naphthalen-2-ylamine (11). Mp 158 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.23 (s, 1H), 7.82–7.93 (m, 3H), 7.69 (s, 1H), 7.52 (m, 2H), 7.40 (d, J = 8.7 Hz, 1H), 6.64 (s, 1H), 3.73 (s, 3H), 2.51 (s, 3H). MS *m*/*z* 290.2 $(M + H^+)$.

Benzyl-(5-methyl[1,2,4]triazolo[1,5-*a*]pyrimidin-7-yl)naphthalen-2-ylamine (12). Mp 172 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.35 (s, 1H), 7.75–7.90 (m, 4H), 7.40–7.50 (m, 5H), 7.15–7.30 (m, 3H), 6.53 (s, 1H), 5.63 (s, 2H), 2.45 (s, 3H). MS *m*/*z* 366.3 $(M + H^+)$.

4-(5-Methyl[1,2,4]triazolo[1,5-*a*]pyrimidin-7-ylamino)benzamide (13). Mp 294 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.60 (s, 1H), 7.90–8.04 (m, 2H and NH, exchangeable), 7.55 (m, 2H), 7.40 (brs, NH, exchangeable), 6.60 (s, 1H), 2.51 (s, 3H). MS *m*/*z* 269.1 $(M + H^+)$.

(5-Methyl[1,2,4]triazolo[1,5-*a*]pyrimidin-7-yl)quinolin-3-ylamine (14). Mp 243 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.00 (s, 1H), 8.45 (s, 1H), 8.20–8.30 (m, 2H), 8.10 (brs, NH, exchangeable), 7.88 (d, J = 7.5 Hz, 1H), 7.75 (m, 1H), 7.70 (m, 1H), 6.44 (s, 1H), 2.62 (s, 3H). MS *m*/*z* 277.1 $(M + H^+)$.

(5-Methyl[1,2,4]triazolo[1,5-*a*]pyrimidin-7-yl)quinolin-6-ylamine (15). Mp 280 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.00 (s, 1H), 8.45 (s, 1H), 8.20–8.25 (m, 2H), 8.12 (brs, NH, exchangeable), 7.80 (s, 1H), 7.75 (d, J = 7.6 Hz, 1H), 7.54–7.60 (m, 1H), 6.54 (s, 1H), 2.55 (s, 3H). MS *m*/*z* 277.1 $(M + H^+)$.

3-(5-Methyl[1,2,4]triazolo[1,5-*a*]pyrimidin-7-ylamino)naphthalen-2-ol (16). Mp 301 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.51 (s, 1H), 7.95 (s, 1H), 7.87 (m, 1H), 7.78 (m, 1H), 7.41–7.45 (m, 1H), 7.31–7.35 (m, 2H), 6.29 (s, 1H), 2.42 (s, 3H). MS *m*/*z* 292.1 $(M + H^+)$.

2-Methyl-7-(5-methyl[1,2,4]triazolo[1,5-*a*]pyrimidin-7-ylamino)chromen-4-one (17). Mp 306 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.41 (s, 1H), 8.30 (d, J = 8.52 Hz, 1H), 8.15 (brs, NH, exchangeable), 7.44 (s, 1H), 7.40 (d, J = 8.0 Hz, 1H), 6.69 (s, 1H), 6.22 (s, 1H), 2.66 (s, 3H), 2.44 (s, 3H). MS *m*/*z* 308.1 ($M + H^+$).

(5-Methyl[1,2,4]triazolo[1,5-*a*]pyrimidin-7-yl)naphthalen-1-ylamine (18). Mp 192 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.43 (brs, NH, exchangeable), 8.55 (s, 1H), 8.05 (m, 2H), 7.88 (m, 1H), 7.50–7.65 (m, 4H), 5.69 (s, 1H), 2.26 (s, 3H). MS *m*/*z* 276.1 ($M + H^+$).

(5-Methyl[1,2,4]triazolo[1,5-*a*]pyrimidin-7-yl)phenylamine (19). Mp 187 °C (lit.[37] 188 °C). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.32 (s, 1H), 8.01 (brs, NH, exchangeable), 7.48–7.53 (m, 2H), 7.28–7.40 (m, 3H), 6.38 (s, 1H), 2.55 (s, 3H). MS *m*/*z* 226.1 ($M + H^+$).

(5-Methyl[1,2,4]triazolo[1,5-*a*]pyrimidin-7-yl)anthracen-2-ylamine (20). Mp 231 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.45 (brs, NH, exchangeable), 8.61–8.55 (m, 3H), 8.08–8.20 (m, 4H), 7.51–7.65 (m, 3H), 6.63 (s, 1H), 2.45 (s, 3H). MS *m*/*z* 326.2 $(M + H)^+$.

Acknowledgment. The authors gratefully acknowledge Amgen for analytical chemistry support during the structural identification of DSM1 (**7**). This work was supported by the U.S. National Institutes of Health Grants AI053680 (to M.A.P. and P.K.R.), MG00203 (to N.A.M.), and AI26912 and AI67670 (to P.K.R.). M.A.P. also acknowledges support from the Welch Foundation (Grant I-1257), and P.K.R. acknowledges support from a Senior Scholar Award in Global Infectious Diseases from the Ellison Medical Foundation and from the UW Keck Center.

Supporting Information Available: Detailed assay and analytical methods and HPLC traces for **7** and **20**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Breman, J.; Egan, A.; Keusch, G. The intolerable burden of malaria: a new look at the numbers. *Am. J. Trop. Med. Hyg.* **2001**, *64* (Suppl. 1–2), iv–vii.
(2) Snow, R. W.; Craig, M.; Deichmann, U.; Marsh, K. Estimating mortality, morbidity and disability due to malaria among Africa's non-pregnant population. *Bull. W. H. O.* **1999**, *77*, 624–640.
(3) White, N. J. Antimalarial drug resistance. *J. Clin. Invest.* **2004**, *113*, 1084–1092.
(4) Rathod, P. K.; McErlean, T.; Lee, P. C. Variations in frequencies of drug resistance in *Plasmodium falciparum*. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 9389–9393.
(5) Ridley, R. G. Medical need, scientific opportunity and the drive for antimalarial drugs. *Nature* **2002**, *415*, 686–693.
(6) Pink, R.; Hudson, A.; Mouries, M. A.; Bendig, M. Opportunities and challenges in antiparasitic drug discovery. *Nat. Rev. Drug Discovery* **2005**, *4*, 727–740.
(7) Bray, P. G.; Ward, S. A.; O'Neill, P. M. Quinolines and artemisinin: chemistry, biology and history. *Curr. Top. Microbiol. Immunol.* **2005**, *295*, 3–38.
(8) Rosenthal, P. *Antimalarial Chemotherapy: Mechanisms of Action, Resistance, and New Directions in Drug Discovery*; Humana Press Inc: Totowa, NJ, 2001.
(9) Gero, A.; O'Sullivan, W. Purines and pyrimidines in malarial parasites. *Blood Cells* **1990**, *16*, 467–484.
(10) Gutteridge, W.; Trigg, P. Incorporation of radioactive precursors into DNA and RNA of *Plasmodium knowlesi* in vitro. *J. Protozool.* **1970**, *17*, 89–96.
(11) Reyes, P.; Rathod, P.; Sanchez, D.; Mrema, J.; Rieckmann, K.; Heidrich, H. Enzymes of purine and pyrimidine metabolism from the human malaria parasite, *Plasmodium falciparum*. *Mol. Biochem. Parasitol.* **1982**, *5*, 275–290.
(12) Sherman, I. Biochemistry of Plasmodium. *Microbiol. Rev.* **1979**, *43*, 453–495.
(13) Gardner, M. J.; et al. Genome sequence of the human malaria parasite *Plasmodium falciparum*. *Nature* **2002**, *419*, 498–511.
(14) White, N. Drug resistance in malaria. *Br. Med. Bull.* **1998**, *54*, 703–715.
(15) Jiang, L.; Lee, P.; White, J.; Rathod, P. Potent and selective activity of a combination of thymidine and 1843U89, a folate-based thymidylate synthase inhibitor, against *Plasmodium falciparum*. *Antimicrob. Agents Chemother.* **2000**, *44*, 1047–1050.
(16) Rathod, P. K.; Khatri, A.; Hubbert, T.; Milhous, W. K. Selective activity of 5-fluoroorotic acid against *Plasmodium falciparum* in vitro. *Antimicrob. Agents Chemother.* **1989**, *33*, 1090–1094.
(17) Rathod, P. K.; Reshmi, S. Susceptibility of *Plasmodium falciparum* to a combination of thymidine and ICI D1694, a quinazoline antifolate directed at thymidylate synthase. *Antimicrob. Agents Chemother.* **1994**, *38*, 476–480.
(18) Nagy, M.; Lacroute, F.; Thomas, D. Divergent evolution of pyrimidine biosynthesis between anaerobic and aerobic yeasts. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 8966–8970.
(19) Jones, M. Pyrimidine nucleotide biosynthesis in animals: genes, enzymes, and regulation of UMP biosynthesis. *Annu. Rev. Biochem.* **1980**, *49*, 253–279.
(20) Painter, H. J.; Morrisey, J. M.; Mather, M. W.; Vaidya, A. B. Specific role of mitochondrial electron transport in blood-stage *Plasmodium falciparum*. *Nature* **2007**, *446*, 88–91.
(21) Goldenberg, M. Leflunomide, a novel immunomodulator for the treatment of active rheumatoid arthritis. *Clin. Ther.* **1999**, *21*, 1837–1852.
(22) Herrmann, M.; Schleyerbach, R.; Kirschbaum, B. Leflunomide: an immunomodulatory drug for the treatment of rheumatoid arthritis and other autoimmune diseases. *Immunopharmacology* **2000**, *47*, 273–289.
(23) John, W. P.; Paul, S. C.; Michael, J. E. 3-Carboxy-5-methyl-*N*-[4-(trifluoromethyl)phenyl]-4-isoxazolecarboxamide, a new prodrug for the antiarthritic agent, 2-cyano-3-hydroxy-*N*-[4-(trifluoromethyl)phenyl]-2-butenamide. *J. Med. Chem.* **1992**, *35*, 507–510.
(24) Liu, S.; Neidhardt, E.; Grossman, T.; Ocain, T.; Clardy, J. Structures of human dihydroorotate dehydrogenase in complex with antiproliferative agents. *Structure* **2000**, *8*, 25–33.
(25) Hurt, D. E.; Widom, J.; Clardy, J. Structure of *Plasmodium falciparum* dihydroorotate dehydrogenase with a bound inhibitor. *Acta Crystallogr., Sect. D: Biol. Crystallogr.* **2006**, *62*, 312–323.
(26) Baldwin, J.; Michnoff, C. H.; Malmquist, N. A.; White, J.; Roth, M. G.; Rathod, P. K.; Phillips, M. A. High-throughput screening for potent and selective inhibitors of *Plasmodium falciparum* dihydroorotate dehydrogenase. *J. Biol. Chem.* **2005**, *280*, 21847–21853.
(27) Heikkila, T.; Ramsey, C.; Davies, M.; Galtier, C.; Stead, A. M.; Johnson, A. P.; Fishwick, C. W.; Boa, A. N.; McConkey, G. A. Design and synthesis of potent inhibitors of the malaria parasite dihydroorotate dehydrogenase. *J. Med. Chem.* **2007**, *50*, 186–191.
(28) Phillips, M.; Rathod, P. K.; Baldwin, J.; Gujjar, R. Dihydroorotate Dehydrogenase Inhibitors with Selective Anti-Malarial Activity. WO Patent 2007149211 A1, 2007; U.S. Patent 20080027079 A1, 2008.
(29) Copeland, R. A. *Evaluation of Enzyme Inhibitors in Drug Discovery*; Wiley: Hoboken, NJ, 2005; pp 185−192.
(30) Malmquist, N. A.; Gujjar, R.; Rathod, P. K.; Phillips, M. A. Analysis of flavin oxidation and electron transfer inhibition in *Plasmodium falciparum* dihydroorotate dehydrogenase. *Biochemistry* **2008**, *47*, 2466–2475.
(31) Lipinski, C. A. Drug-like properties and the causes of poor solubility and poor permeability. *J. Pharmacol. Toxicol. Methods* **2000**, *44*, 235–249.
(32) Baldwin, J.; Farajallah, A.; Malmquist, N.; Rathod, P.; Phillips, M. Malarial dihydroorotate dehydrogenase: substrate and inhibitor specificity. *J. Biol. Chem.* **2002**, *277*, 41827–41834.
(33) Malmquist, N. A.; Baldwin, J.; Phillips, M. A. Detergent-dependent kinetics of truncated *Plasmodium falciparum* dihydroorotate dehydrogenase. *J. Biol. Chem.* **2007**, *282*, 12678–12686.
(34) Desjardins, R. E.; Canfield, C. J.; Haynes, J. D.; Chulay, J. D. Quantitative assessment of antimalarial activity in vitro by a semiautomated microdilution technique. *Antimicrob. Agents Chemother.* **1979**, *16*, 710–718.
(35) Chambers, V. C. The structure and reactivity of 5-hydroxy-7-methyl-*s*-triazolo[2,3-*a*]pyrimidine. *J. Am. Chem. Soc.* **1960**, *82*, 605–609.
(36) Levin, Y. A.; Sergeeva, E. M.; Kukhtin, V. A. Condensed heterocycles. V. Reaction of 4-chloro-6-methyl-1,2,4-triazolo[2,3-α]pyrimidine with some nitrogenous bases. *Zh. Obshch. Khim.* **1964**, *34*, 205–209.
(37) Reynolds, G. A.; VanAllan, J. A. Structure of certain polyazaindenes. VII. 4-Amino-6-methyl-1,3,3a,7-tetraazaindene and its derivatives. *J. Org. Chem.* **1961**, *26*, 115–117.

JM8001026