

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

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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 treatment¹⁴ 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

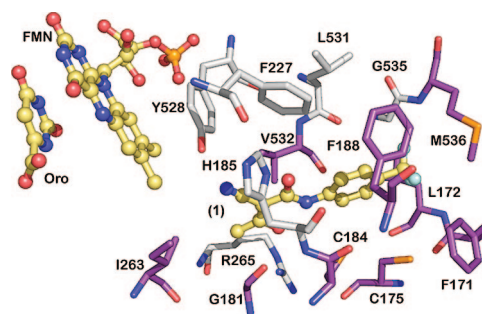


Figure 1. Structure of *Pf*DHODH active site bound to inhibitor **1**. Residues conserved between *P. falciparum* and human DHODH are displayed in gray, and variable residues are displayed in purple. Orotic acid (Oro), FMN, and **1** are displayed in yellow. The figure was generated using PyMol from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

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.

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

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

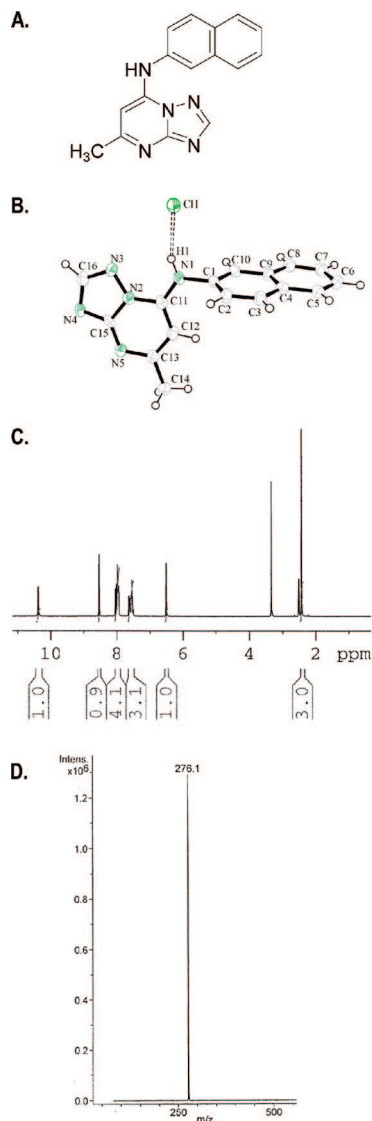


Figure 2. Compound **7**: (A) chemical structure; (B) X-ray crystal structure; (C) ^1H NMR in $\text{DMSO}-d_6$; (D) mass spectra.

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 colorimetric enzyme assay.²⁶ *P. falciparum* DHODH was inhibited by **7** with $\text{IC}_{50} = 0.047 \pm 0.022 \mu\text{M}$, 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 ($\text{EC}_{50} = 0.079 \pm 0.048 \mu\text{M}$ for clone 3D7, Figure 3B) and it does not inhibit the growth of a mouse cell line (L1210) ($\text{EC}_{50} > 10 \mu\text{M}$). It is also highly active against

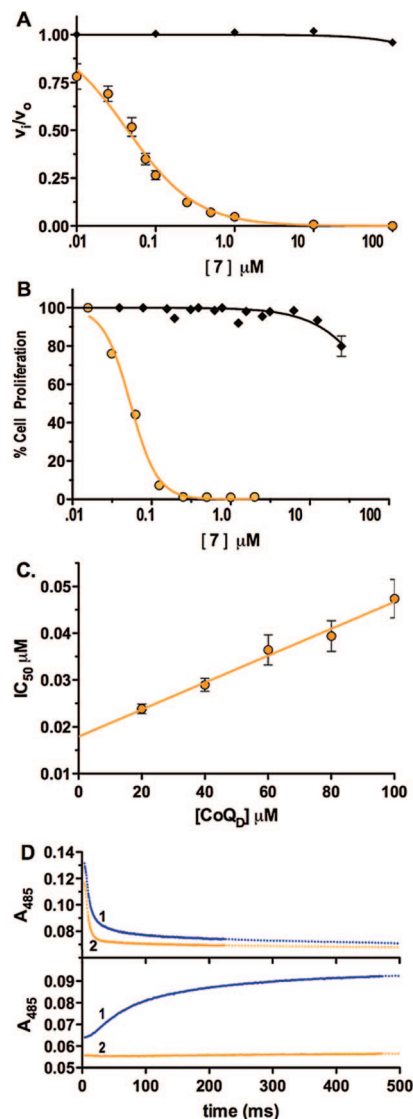


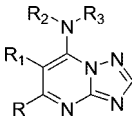














Figure 3. Selective and potent inhibition of *Pf*DHODH and *P. falciparum* cells by **7**: (A) inhibition profile for *Pf*DHODH (orange circles, $\text{IC}_{50} = 0.047 \pm 0.022 \mu\text{M}$, $n = 6$) compared to human DHODH (black diamonds, $\text{IC}_{50} > 200 \mu\text{M}$); $[\text{E}]_T = 10 \text{ nM}$; (B) activity in whole cell assays against *P. falciparum* 3D7 (orange circles) or mouse L1210 (black diamonds) cells ($\text{EC}_{50} = 0.079 \pm 0.045$, $n = 9$); (C) relationship between IC_{50} and substrate concentration ($[\text{E}]_T = 5 \text{ nM}$). K_1 was determined by fitting the data to eq 2 ($K_1 = 0.015 \pm 0.0011 \mu\text{M}$). (D) Rapid kinetic analysis showing **7** inhibits the CoQD -dependent oxidative half-reaction (bottom) but not the DHO-dependent reductive half-reaction (top): blue trace (1), no **7**; orange trace (2), **7** ($50 \mu\text{M}$).

drug resistant strains of *P. falciparum*, including the multiple-drug-resistant Dd2 ($\text{EC}_{50} = 0.14 \pm 0.05 \mu\text{M}$).

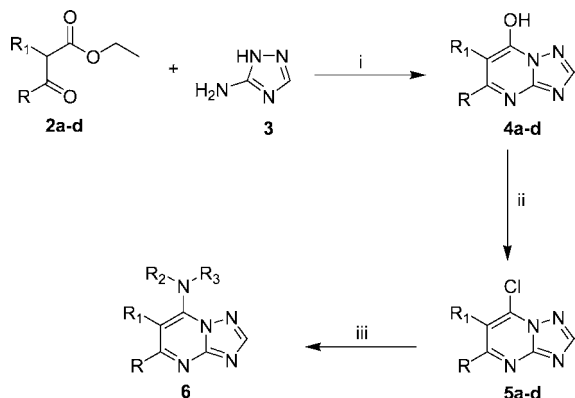
The initial structural annotation of **7** in the HTS chemical library database was incorrect as judged by high-resolution mass spectroscopy. The chemical identity of **7** was elucidated using NMR analysis of the original material, and these data were consistent with the structure depicted in Figure 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

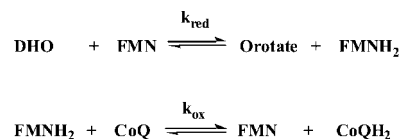
						
compd	R	R ₁	R ₂	R ₃	IC ₅₀ (μM) <i>Pf</i> /DHODH	EC ₅₀ (μM) <i>Pf</i> /3D7 cells
7	CH ₃	H	H		0.047±0.022	0.079±0.045
8	CF ₃	H	H		0.21±0.07	3.3±0.0
9	C ₂ H ₅	H	H		0.19±0.073	0.31±0.32
10	CH ₃	CH ₃	H		0.16±0.096	0.55±0.22
11	CH ₃	H	CH ₃		3.0±0.84	16±4.0
12	CH ₃	H	C ₆ H ₅ CH ₂		93±9	35±35
13	CH ₃	H	H		>200	>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 IC₅₀ 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. EC₅₀ 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) POCl₃, 30–60 min, reflux, 43–65%; (iii) R₂R₃NH, 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 R₁ alkyl substituents can be modified with only a modest decrease in activity (**8–10**), (ii) substitutions at R₂ resulted in a loss of potency (**11, 12**), (iii) the introduction of heteroatoms on, or in, the naphthyl 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 IC₅₀ 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 ± 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 FMNH₂ 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 IC₅₀ of **7** by 940-fold and 130-fold, respectively (IC₅₀ = 44 ± 10 μM for F227A; IC₅₀ = 6.1 ± 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 IC₅₀ values and to eq 2 to determine K_i for a tight-binding competitive inhibitor²⁹ 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 propagated³⁴ and [³H]-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 EC₅₀.

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

Curve Fitting and Error Analysis. Enzyme IC₅₀ and parasite EC₅₀ data were determined over a range of inhibitor concentrations using triplicate data points at each concentration. IC₅₀ 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 CH₂Cl₂/MeOH/NH₄OH (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. ¹H 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. ¹H 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. ¹H 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. ¹H 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. ¹H NMR (300 MHz, CDCl₃): 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. ¹H 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. ¹H 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). ¹H 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. ¹H 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. ¹H 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. ¹H 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. ¹H 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. ¹H 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. ¹H 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. ¹H NMR (300 MHz, CDCl₃): 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. ¹H NMR (300 MHz, CDCl₃): 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. ¹H 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. ¹H NMR (300 MHz, CDCl₃): 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. ¹H 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). ¹H NMR (300 MHz, CDCl₃): 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. ¹H 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⁺).

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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>.

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