ACS Medicinal Chemistry Letters

Letter

pubs.acs.org/acsmedchemlett

Discovery of Antimalarial Azetidine-2-carbonitriles That Inhibit *P. falciparum* Dihydroorotate Dehydrogenase

Micah Maetani,[†,‡] Nobutaka Kato,[‡] Valquiria A. P. Jabor,[§] Felipe A. Calil,[§] Maria Cristina Nonato,[§] Christina A. Scherer,[‡] and Stuart L. Schreiber*[,†,‡,∥]

[†]Department of Chemistry and Chemical Biology, Harvard University, Cambridge, Massachusetts 02138, United States

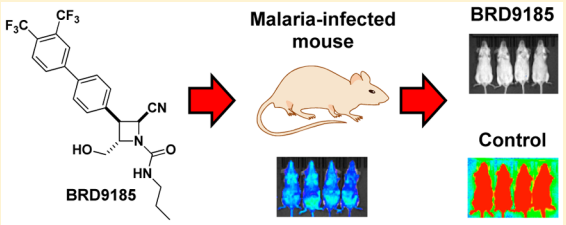
[‡]Broad Institute, Cambridge, Massachusetts 02142, United States

[§]School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Ribeirão Preto, São Paulo 14040-903, Brazil

[∥]Howard Hughes Medical Institute, Cambridge, Massachusetts 02138, United States

Supporting Information

ABSTRACT: Dihydroorotate dehydrogenase (DHODH) is an enzyme necessary for pyrimidine biosynthesis in protozoan parasites of the genus *Plasmodium*, the causative agents of malaria. We recently reported the identification of novel compounds derived from diversity-oriented synthesis with activity in multiple stages of the malaria parasite life cycle. Here, we report the optimization of a potent series of antimalarial inhibitors consisting of azetidine-2-carbonitriles, which we had previously shown to target *P. falciparum* DHODH in a biochemical assay. Optimized compound BRD9185 (**27**) has *in vitro* activity against multidrug-resistant blood-stage parasites (EC_{50} = 0.016 μM) and is curative after just three doses in a *P. berghei* mouse model. BRD9185 has a long half-life (15 h) and low clearance in mice and represents a new structural class of DHODH inhibitors with potential as antimalarial drugs.

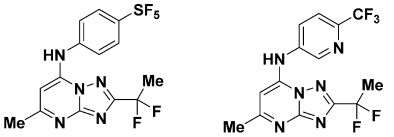


KEYWORDS: *BRD7539, BRD9185, DHODH, malaria, diversity-oriented synthesis, Plasmodium falciparum*

Malaria is a global health concern with nearly 200 million cases annually, many of which occur in sub-Saharan Africa. The disease is caused by parasitic protozoans of the genus *Plasmodium* and transmitted by female *Anopheles* mosquitos.[1] Malaria is treatable using chemotherapy, but reduced efficacy of first-line treatments artemisinin and its derivatives at the Cambodia-Thailand border underscores the need for new, safe, and effective antimalarial therapies.[2−5] Moreover, while most current antimalarial drugs target asexual blood-stage parasites, next-generation antimalarials should ideally also target the liver- and/or sexual blood-stage parasites to impede parasite replication and transmission from host-to-vector, respectively.[6] New antimalarial candidates have entered clinical trials in this regard, including one that targets dihydroorotate dehydrogenase (DHODH).

DHODH catalyzes the flavin mononucleotide (FMN)-dependent oxidation of L-dihydroorotate to orotate as the fourth step in *de novo* pyrimidine biosynthesis. While most organisms use both *de novo* and salvage pathways to generate pyrimidines, *Plasmodium* parasites lack the necessary genes for the latter, making *de novo* pyrimidine synthesis an essential pathway for the parasite.[7] One compound in the antimalarial pipeline, DSM265, has progressed to phase-II clinical trials and has activity against both asexual blood-stage and liver-stage parasites.[8] DSM265 and secondary candidate DSM421 (Chart 1) comprise a class of selective and potent antimalarial DHODH inhibitors.[9−15] These triazolopyrimidines remain the most well-studied and clinically relevant antimalarial DHODH inhibitors to date, but 5-benzimidazolyl-thiophene-2-carboxamides[16] and 7-arylaminopyrazolo[1,5-α]pyrimidines have also been reported.[17]

Chart 1. Structures of *Pf*DHODH Inhibitors in Clinical or Preclinical Development



We recently identified numerous compounds with multistage activity by growth-inhibition phenotypic screening of 100,000 compounds prepared in advance using diversity-oriented synthesis (DOS).[18] The DOS collection was synthesized using modern asymmetric organic chemistry to impart three-dimensional topographical features using the build−couple−pair strategy.[19] The success of this strategy in revealing novel therapeutic targets is illustrated by the discovery of small-molecule antimalarial inhibitors of phenylalanyl-tRNA

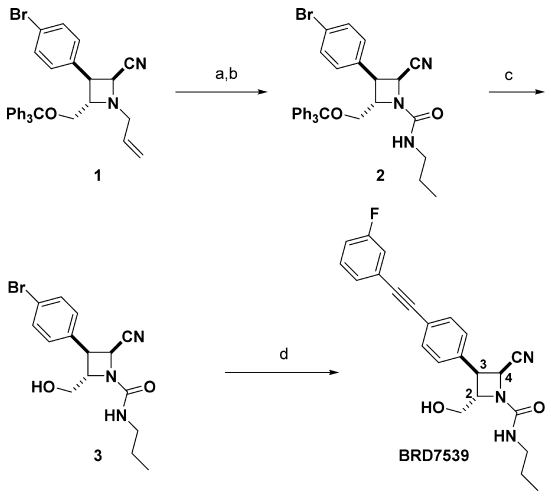
Received: January 20, 2017
Accepted: February 27, 2017
Published: February 27, 2017

DOI: 10.1021/acsmedchemlett.7b00030
ACS Med. Chem. Lett. 2017, 8, 438−442

synthetase,[18] PI4K,[18] and cytochrome bc1 Q_i,[20,21] as well as others with as-yet unidentified targets.[22] While our initial efforts focused on small-molecule inhibitors of phenylalanyl−tRNA synthetase, we became intrigued in BRD7539, which we had previously shown to target *Pf*DHODH (IC_{50} = 0.033 μM) selectively over human (*Hs*) DHODH (IC_{50} > 50 μM). BRD7539 was reported to have potent activity against both multidrug-resistant asexual blood-stage (*P. falciparum*, Dd2 strain, EC_{50} = 0.010 μM) and liver-stage (*P. berghei*, EC_{50} = 0.015 μM) parasites but no activity against sexual blood-stage (*P. falciparum*, stages IV−V, EC_{50} > 20 μM) parasites. BRD7539 is an azetidine carbonitrile with three contiguous stereocenters (2*S*,3*S*,4*S*), and stereochemistry-based structure−activity relationships (SSARs) showed that only two of eight possible stereoisomers are active.[18] The clinical relevance of *Pf*DHODH inhibitors and the selectivity and potency of BRD7539 arising directly from a high-throughput screen encouraged us to pursue this series further. Here, we report our efforts to optimize this compound and to evaluate this series *in vivo*.

To confirm the biological activity of BRD7539 and to explore structure−activity relationships (SARs) of the scaffold, we resynthesized core structure **1** (Scheme 1) as reported.[23]

Scheme 1. Elaboration of the Azetidine-2-carbonitrile Scaffold[a]



[a]Reagents and conditions: (a) $Pd(PPh_3)_4$, 1,3-dimethylbarbituric acid, 2:1 EtOH/DCM, 40 °C, 16 h, 92%; (b) propylisocyanate, DIPEA, DCM, 23 °C, 1 h, 96%; (c) trifluoroacetic acid, Et_3SiH, DCM, 23 °C, 1 h, 87%; (d) 1-ethynyl-3-fluorobenzene, XPhos Pd-G3, Et_3N, MeCN, 70 °C, 6 h, 91%.

Deallylation of the protected azetidine core and sequential capping of the nitrogen with propyl isocyanate gave urea **2**. Trityl deprotection followed by a Heck alkynylation or Suzuki reaction diversified the *para*-Br position and served as a route to most analogues. Biological activity of BRD7539 was confirmed in 20-point dose ($n \geq 2$ independent experiments in triplicate) against a multidrug-resistant strain (*P. falciparum*, Dd2 strain) using a phenotypic blood-stage growth inhibition assay that models a human blood-stage infection. Additionally, *in vitro* stability against mice and human microsomes for 1 h was used as a guide to identify analogues with potential *in vivo* stability.

Our initial SAR focused primarily on the acetylene (R_1) region of BRD7539 as this was the most facile point of diversification to explore and a possible toxicity concern (Table 1).[24] Activity was assessed using the phenotypic blood-stage growth inhibition assay. The activity of BRD7539 was reconfirmed in dose, and *in vitro* activity was maintained with a wide variety of hydrophobic acetylenes (**4**−**6**, **9**, **10**). Heteroaromatic 2- and 3-pyridyl analogues (**7**−**8**) showed significant loss in activity compared to aromatic analogues. Interestingly, *cis*-alkene (**11**) and alkane (**12**) derivatives of BRD7539 showed only a slight loss in activity, suggesting that the acetylene was not necessary. Indeed, unsubstituted biaryl **13** is essentially equipotent to BRD7539 while removing the distal ring (**17**, Scheme S1) abolished activity, indicating the need for a large hydrophobic region in the scaffold. Having removed the acetylenic toxicity concern, we decided to use this region to modulate mouse and human microsomal stability while maintaining activity. Improved stability should correlate with favorable pharmacokinetic (PK) properties. Analogues bearing a 4-pyridyl (**22**) and 4-methanesulfonyl (**23**) distal aryl were synthesized in an effort to improve solubility but were inactive *in vitro*. CF_3-substitution (**24**−**26**) was found to impart greater *in vitro* microsomal stability than methoxy substituents (**28**−**30**). Ultimately, we found that the addition of two $-CF_3$ groups on the distal phenyl ring (BRD9185, **27**) to be comparable in activity and microsomal stability to BRD7539.

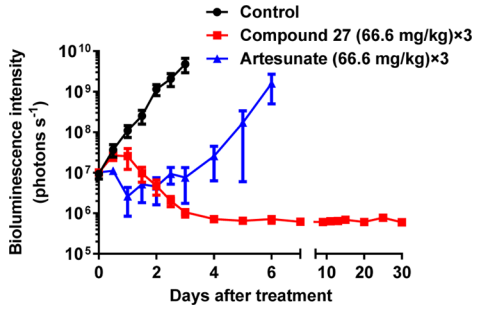


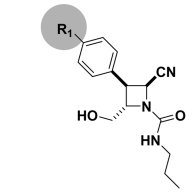
Figure 1. *In vivo* efficacy studies of BRD9185 (**27**) using *P. berghei* mouse model. CD-1 mice were infected with *P. berghei* (ANKA GFP-luc) at −24 h (Day −1) and dosed orally at 0, 24, and 48 h with compound **27**, artesunate, or vehicle solution. Infections were monitored using the *in vivo* imaging system (IVIS), and bioluminescence intensity was quantified from each mouse and plotted against time. Animals with parasitemia exceeding 25% were humanely euthanized. No recrudescence was observed after 3 × 66.6 mg/kg doses of **27** (n = 4). By contrast, recrudescence is observed quickly after treatment with 3 × 66.6 mg/kg doses of artesunate (n = 2).

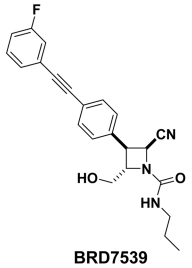
We briefly sought to evaluate the role of the primary alcohol (R_2) and secondary nitrile (R_3) on activity (Table 2). Analogues were synthesized from BRD7539 in 1−3 steps or from commercially available starting materials (Schemes S2−S7). Modifying the nitrile to a methyl ester (**32**) or alcohol (**33**) abolished activity. This is unsurprising given our previous result that the 2*S*,3*S*,4*R* diastereomer, which only differs from BRD7539 at the nitrile-bearing stereocenter, is inactive, hinting at the importance of this functional group. This also illustrates the subtle but significant role stereochemistry can have on small molecule−protein interactions and highlights the strength of diversity-oriented synthesis in identifying these key interactions. Any modification of the primary alcohol, including methylation (**34**) or conversion to a primary amine (**36**), resulted in large loss in activity.

To confirm that our lead compound **27** inhibits *Pf*DHODH despite removal of the acetylene motif, we performed biochemical assays against both recombinant *P. falciparum*

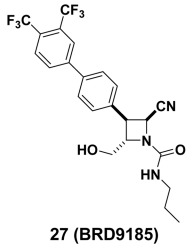
DOI: 10.1021/acsmedchemlett.7b00030
ACS Med. Chem. Lett. 2017, 8, 438−442

Table 1. Activity of BRD7539 Analogues at R_1 Position against Dd2 Parasites[a]

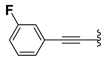
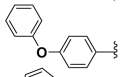
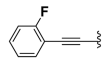
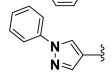
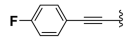
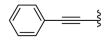
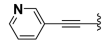
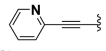
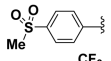
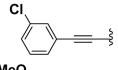
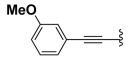
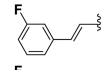
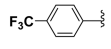
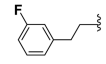
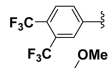
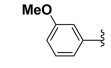
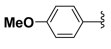
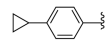




BRD7539

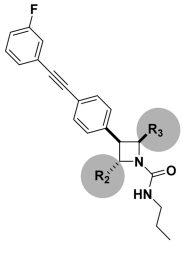


27 (BRD9185)

Cmpd	R_1	EC_{50} (μM)	M/H[b]	Cmpd	R_1	EC_{50} (μM)	M/H[b]
BRD7539		0.010	86/99	17		8.375	-
3		0.249	-	18		0.005	8/51
4		0.083	85/85	19		0.010	34/74
5		0.013	100/100	20		12.630	-
6		0.016	-	21		0.139	-
7		5.640	-	22		5.541	-
8		0.427	-	23		4.390	91/90
9		0.020	85/96	24		0.097	0/16
10		0.016	45/94	25		0.012	39/96
11		0.035	56/87	26		0.039	81/91
12		0.046	0/14	27		0.016	95/90
13		0.019	-	28		0.352	-
14		0.051	-	29		0.015	0/74
15		0.106	-	30		0.015	0/7
16		0.019	-	31		0.041	50/66

[a]EC_{50} values are the mean of at least two independent experiments. [b]Mouse (M) and human (H) microsomal stability (% remaining after 1 h). Data were obtained from a single experiment performed in duplicate and calculated from a six-point curve over 1 h.

Table 2. Activity of BRD7539 Analogues at R_2 and R_3 Positions against Dd2 Parasites[a]



Cmpd	R_2	R_3	EC_{50} (μM)
32			1.629
33	"		16.200
34			1.613
35		"	0.390
36		"	4.440
37		"	0.102
38		"	0.307

[a]EC_{50} values are the mean of at least two independent experiments.

and human DHODH enzyme (Table 3, Figure S1). Similar to hit compound BRD7539, **27** is a potent inhibitor of *Pf*DHODH (IC_{50} = 0.012 μM) but not *Hs*DHODH (IC_{50} > 50 μM), suggesting that this class of DHODH inhibitors provides selectivity between orthologues. The IC_{50} of selected analogues against *Pf*DHODH was also shown to track well with Dd2 EC_{50} (Table S1). To assess the suitability of BRD9185 further for *in vivo* use, we measured plasma protein binding and obtained mouse PK data. Lead compound **27** is highly protein bound in both mouse and human plasma (>99%) and is a highly bioavailable (94%), long half-life (15 h) compound in mice (PO 5 mg/kg; IV 1 mg/kg) with low clearance (0.40 mL/min/kg). Notably, the $DNAUC_{0\text{-inf}}$ of **27** is >54.3 μM, higher than the EC_{50} *in vitro*.

Based on the promising PK properties, we were interested in evaluating the efficacy of **27** *in vivo* (Figure 1). We used a blood-stage model with the rodent malaria parasite *P. berghei* that expresses luciferase and treated infected CD-1 mice for 3 days with 66.6 mg/kg of **27** or vehicle (70% PEG300, 30% solution of 5% dextrose in H_2O). Bioluminescence intensity was used to measure parasite growth, and artesunate was used as a positive control. No parasites were detected after 30 days in mice treated with **27**, suggesting that the analogue achieved a sterile cure for *P. berghei*. These results are particularly interesting in light of the properties of DSM265 and the triazolopyrimidine series, which are not effective in the *P. berghei* (*Pb*) model due to poor binding to the *Pb*DHODH enzyme.[9,14] This raises the possibility that **27** has a different mechanism of action from DSM265 on *Pf*DHODH. However, **27** binds competitively with decylubiquinone (Figure S2), the same proposed binding site as DSM265.[8] X-ray crystallography studies are underway to gain insights into binding features of these compounds.

DOI: 10.1021/acsmedchemlett.7b00030
ACS Med. Chem. Lett. 2017, 8, 438−442

Table 3. Key Properties of Lead Compound 27

in vitro enzyme inhibition, IC_{50}[a]	
*Pf*DHODH (μM)	0.012
*Hs*DHODH (μM)	>50
plasma protein binding[b]	
mouse (%)	99.3
human (%)	>99.0
mouse PK[c]	
$t_{1/2}$ (h)	15.2
C_0 (μM)	4.9
C_{max} (μM)	16.9
$DNAUC_{0\text{-}inf}$ (μM·h)	>54.3
V_{ss} (L/kg)	0.37
CL (mL/min/kg)	0.40
F (%)	94

[a]Mean of a single experiment in triplicate. [b]Single experiment, calculated from a six-point curve over 1 h. [c]$t_{1/2}$, terminal half-life; C_0, initial serum concentration at $t = 0$; C_{max}, peak serum concentration; $DNAUC_{0\text{-}inf}$, dose-normalized area under the plasma concentration vs time curve following PO dosing; V_{ss}, volume of distribution at steady state; CL, plasma clearance; *F*%, bioavailability. IV dosing in 5% DMSO/10% cremophor/85% H_2O at 0.25 mg/mL (1 mg/kg). PO dosing in 70% PEG300/30% (5% dextrose in H_2O) at 0.50 mg/mL (5 mg/kg). $n = 3$ mice in both IV and PO groups.

These data collectively show that azetidine-2-carbonitriles comprise a promising, potent, and selective new class of inhibitors of *Pf*DHODH. In contrast to other antimalarial DHODH inhibitors to date, compound **27** exhibits a sterile cure in an *in vivo P. berghei* model after just three doses. Additional efforts assessing the inhibition of azetidine-2-carbonitriles against DHODH from other *Plasmodium* species and evaluating efficacy of this series in the humanized NSG mouse *P. falciparum* model are underway.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.7b00030.

Supplementary figures, experimental details, compound characterization, and abbreviations (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: stuart_schreiber@harvard.edu.

ORCID

Felipe A. Calil: 0000-0001-7503-4078

Christina A. Scherer: 0000-0002-1659-2827

Stuart L. Schreiber: 0000-0003-1922-7558

Funding

This work was supported in part by the Bill & Melinda Gates Foundation (grant OPP1032518 awarded to S.L.S.). S.L.S. is an Investigator at the Howard Hughes Medical Institute. M.M. was supported by a fellowship from the National Science Foundation (DGE1144152) and from Harvard University's Graduate Prize Fellowship. Biochemical and mechanistic studies for DHODH were supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), grant number 2012/25075-0 (to M.C.N.).

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Leslie N. Aldrich, Jennifer A. Beaudoin, Christopher J. Gerry, and Jingqiang Wei (Broad Institute) for advice regarding compound synthesis; Eamon Comer and Marshall Morningstar (Broad Institute) for helpful discussions; and Broad Institute Compound Management and analytical teams for assistance with compound access and characterization.

■ REFERENCES

(1) WHO. World Malaria Report 2015. http://who.int/malaria/publications/world-malaria-report-2015/report/en/.

(2) Dondorp, A. M.; Nosten, F.; Yi, P.; Das, D.; Phyo, A. P.; Tarning, J.; Lwin, K. M.; Ariey, F.; Hanpithakpong, W.; Lee, S. J.; Ringwald, P.; Silamut, K.; Imwong, M.; Chotivanich, K.; Lim, P.; Herdman, T.; An, S. S.; Yeung, S.; Singhasivanon, P.; Day, N. P. J.; Lindegardh, N.; Socheat, D.; White, N. J. Artemisinin resistance in Plasmodium falciparum malaria. *N. Engl. J. Med.* **2009**, *361*, 455−467.

(3) O'Brien, C.; Henrich, P. P.; Passi, N.; Fidock, D. A. Recent clinical and molecular insights into emerging artemisinin resistance in Plasmodium falciparum. *Curr. Opin. Infect. Dis.* **2011**, *24*, 570−577.

(4) Flannery, E. L.; Chatterjee, A. K.; Winzeler, E. A. Antimalarial drug discovery: approaches and progress towards new medicines. *Nat. Rev. Microbiol.* **2013**, *11*, 849−862.

(5) Miotto, O.; Almagro-Garcia, J.; Manske, M.; MacInnis, B.; Campino, S.; Rockett, K. A.; Amaratunga, C.; Lim, P.; Suon, S.; Sreng, S.; Anderson, J. M.; Duong, S.; Nguon, C.; Chuor, C. M.; Saunders, D.; Se, Y.; Lon, C.; Fukuda, M. M.; Amenga-Etego, L.; Hodgson, A. V.; Asoala, V.; Imwong, M.; Takala-Harrison, S.; Nosten, F.; Su, X.-Z.; Ringwald, P.; Ariey, F.; Dolecek, C.; Hien, T. T.; Boni, M. F.; Thai, C. Q.; Amambua-Ngwa, A.; Conway, D. J.; Djimde, A. A.; Doumbo, O. K.; Zongo, I.; Ouedraogo, J. B.; Alcock, D.; Drury, E.; Auburn, S.; Koch, O.; Sanders, M.; Hubbart, C.; Maslen, G.; Ruano-Rubio, V.; Jyothi, D.; Miles, A.; O'Brien, J.; Gamble, C.; Oyola, S. O.; Rayner, J. C.; Newbold, C. I.; Berriman, M.; Spencer, C. C.; McVean, G.; Day, N. P.; White, N. J.; Bethell, D.; Dondorp, A. M.; Plowe, C. V.; Fairhurst, R. M.; Kwiatkowski, D. P. Multiple populations of artemisinin-resistant Plasmodium falciparum in Cambodia. *Nat. Genet.* **2013**, *45*, 648−655.

(6) Anthony, M. P.; Burrows, J. N.; Duparc, S.; Moehrle, J. J.; Wells, T. N. The global pipeline of new medicines for the control and elimination of malaria. *Malar. J.* **2012**, *11*, 316.

(7) Phillips, M. A.; Rathod, P. K. Plasmodium dihydroorotate dehydrogenase: a promising target for novel anti-malarial chemotherapy. *Infect. Disord.: Drug Targets* **2010**, *10*, 226−239.

(8) Phillips, M. A.; Lotharius, J.; Marsh, K.; White, J.; Dayan, A.; White, K. L.; Njoroge, J. W.; El Mazouni, F.; Lao, Y.; Kokkonda, S.; Tomchick, D. R.; Deng, X.; Laird, T.; Bhatia, S. N.; March, S.; Ng, C. L.; Fidock, D. A.; Wittlin, S.; Lafuente-Monasterio, M.; Benito, F. J.; Alonso, L. M.; Martinez, M. S.; Jimenez-Diaz, M. B.; Bazaga, S. F.; Angulo-Barturen, I.; Haselden, J. N.; Louttit, J.; Cui, Y.; Sridhar, A.; Zeeman, A. M.; Kocken, C.; Sauerwein, R.; Dechering, K.; Avery, V. M.; Duffy, S.; Delves, M.; Sinden, R.; Ruecker, A.; Wickham, K. S.; Rochford, R.; Gahagen, J.; Iyer, L.; Riccio, E.; Mirsalis, J.; Bathhurst, I.; Rueckle, T.; Ding, X.; Campo, B.; Leroy, D.; Rogers, M. J.; Rathod, P. K.; Burrows, J. N.; Charman, S. A. A long-duration dihydroorotate dehydrogenase inhibitor (DSM265) for prevention and treatment of malaria. *Sci. Transl. Med.* **2015**, *7*, 296ra111.

(9) Coteron, J. M.; Marco, M.; Esquivias, J.; Deng, X.; White, K. L.; White, J.; Koltun, M.; El Mazouni, F.; Kokkonda, S.; Katneni, K.; Bhamidipati, R.; Shackleford, D. M.; Angulo-Barturen, I.; Ferrer, S. B.; Jimenez-Diaz, M. B.; Gamo, F. J.; Goldsmith, E. J.; Charman, W. N.; Bathurst, I.; Floyd, D.; Matthews, D.; Burrows, J. N.; Rathod, P. K.; Charman, S. A.; Phillips, M. A. Structure-guided lead optimization of triazolopyrimidine-ring substituents identifies potent Plasmodium falciparum dihydroorotate dehydrogenase inhibitors with clinical candidate potential. *J. Med. Chem.* **2011**, *54*, 5540−5561.

(10) Gujjar, R.; El Mazouni, F.; White, K. L.; White, J.; Creason, S.; Shackleford, D. M.; Deng, X.; Charman, W. N.; Bathurst, I.; Burrows, J.; Floyd, D. M.; Matthews, D.; Buckner, F. S.; Charman, S. A.; Phillips,

DOI: 10.1021/acsmedchemlett.7b00030
ACS Med. Chem. Lett. 2017, 8, 438−442

M. A.; Rathod, P. K. Lead-optimization of aryl and aralkyl amine based triazolopyrimidine inhibitors of Plasmodium falciparum dihydroorotate dehydrogenase with anti-malarial activity in mice. *J. Med. Chem.* **2011**, *54*, 3935−3949.
(11) Phillips, M. A.; Gujjar, R.; Malmquist, N. A.; White, J.; El Mazouni, F.; Baldwin, J.; Rathod, P. K. Triazolopyrimidine-based dihydroorotate dehydrogenase inhibitors with potent and selective activity against the malaria parasite, Plasmodium falciparum. *J. Med. Chem.* **2008**, *51*, 3649−3653.
(12) Deng, X.; Kokkonda, S.; El Mazouni, F.; White, J.; Burrows, J. N.; Kaminsky, W.; Charman, S. A.; Matthews, D.; Rathod, P. K.; Phillips, M. A. Fluorine modulates species selectivity in the triazolopyrimidine class of Plasmodium falciparum dihydroorotate dehydrogenase inhibitors. *J. Med. Chem.* **2014**, *57*, 5381−5394.
(13) Kokkonda, S.; Deng, X.; White, K. L.; Coteron, J. M.; Marco, M.; de Las Heras, L.; White, J.; El Mazouni, F.; Tomchick, D. R.; Manjalanagara, K.; Rudra, K. R.; Chen, G.; Morizzi, J.; Kaminsky, W.; Leroy, D.; Martinez-Martinez, M. S.; Jimenez-Diaz, M. B.; Bazaga, S. F.; Angulo-Barturen, I.; Waterson, D.; Burrows, J. N.; Matthews, D.; Charman, S. A.; Phillips, M. A.; Rathod, P. K. Tetrahydro-2-naphthyl and 2-indanyl triazolopyrimidines targeting Plasmodium falciparum dihydroorotate dehydrogenase display potent and selective antimalarial activity. *J. Med. Chem.* **2016**, *59*, 5416−5431.
(14) Phillips, M. A.; White, K. L.; Kokkonda, S.; Deng, X.; White, J.; El Mazouni, F.; Marsh, K.; Tomchick, D. R.; Manjalanagara, K.; Rudra, K. R.; Wirjanata, G.; Noviyanti, R.; Price, R. N.; Marfurt, J.; Shackleford, D. M.; Chiu, F. C; Campbell, M.; Jimenez-Diaz, M. B.; Bazaga, S. F.; Angulo-Barturen, I.; Martinez, M. S.; Lafuente-Monasterio, M.; Kaminsky, W.; Silue, K.; Zeeman, A.-M.; Kocken, C.; Leroy, D.; Blasco, B.; Rossignol, E.; Rueckle, T.; Matthews, D.; Burrows, J. N.; Waterson, D.; Palmer, M. J.; Rathod, P. K.; Charman, S. A. A triazolopyrimidine-based dihydroorotate dehydrogenase inhibitor (DSM421) with improved drug-like properties for treatment and prevention of malaria. *ACS Infect. Dis.* **2016**, *2*, 945−957.
(15) Deng, X.; Gujjar, R.; El Mazouni, F.; Kaminsky, W.; Malmquist, N. A.; Goldsmith, E. J.; Rathod, P. K.; Phillips, M. A. Structural plasticity of malaria dihydroorotate dehydrogenase allows selective binding of diverse chemical scaffolds. *J. Biol. Chem.* **2009**, *284*, 26999−27009.
(16) Booker, M. L.; Bastos, C. M.; Kramer, M. L.; Barker, R. H., Jr; Skerlj, R.; Sidhu, A. B.; Deng, X.; Celatka, C.; Cortese, J. F.; Guerrero-Bravo, J. E.; Crespo-Llado, K. N.; Serrano, A. E.; Angulo-Barturen, I.; Jimenez-Diaz, M. B.; Viera, S.; Garuti, H.; Wittlin, S.; Papastogiannidis, P.; Lin, J. W.; Janse, C. J.; Khan, S. M.; Duraisingh, M.; Coleman, B.; Goldsmith, E. J.; Phillips, M. A.; Munoz, B.; Wirth, D. F.; Klinger, J. D.; Wiegand, R.; Sybertz, E. Novel inhibitors of Plasmodium falciparum dihydroorotate dehydrogenase with anti-malarial activity in the mouse model. *J. Biol. Chem.* **2010**, *285*, 33054−33064.
(17) Azeredo, L. F.; Coutinho, J. P.; Jabor, V. A.; Feliciano, P. R.; Nonato, M. C.; Kaiser, C. R.; Menezes, C. M.; Hammes, A. S.; Caffarena, E. R.; Hoelz, L. V.; de Souza, N. B.; Pereira, G. A.; Ceravolo, J. P.; Krettli, A. U.; Boechat, N. Evaluation of 7-arylaminopyrazolo[1,5-α]pyrimidines as anti-Plasmodium falciparum, antimalarial, and *Pf*-dihydroorotate dehydrogenase inhibitors. *Eur. J. Med. Chem.* **2017**, *126*, 72−83.
(18) Kato, N.; Comer, E.; Sakata-Kato, T.; Sharma, A.; Sharma, M.; Maetani, M.; Bastien, J.; Brancucci, N. M.; Bittker, J. A.; Corey, V.; Clarke, D.; Derbyshire, E. R.; Dornan, G. L.; Duffy, S.; Eckley, S.; Itoe, M. A.; Koolen, K. M.; Lewis, T. A.; Lui, P. S.; Lukens, A. K.; Lund, E.; March, S.; Meibalan, E.; Meier, B. C.; McPhail, J. A.; Mitasev, B.; Moss, E. L.; Sayes, M.; VanGessel, Y.; Wawer, M. J.; Yoshinaga, T.; Zeeman, A.-M.; Avery, V. M.; Bhatia, S. N.; Burke, J. E.; Catteruccia, F.; Clardy, J. C.; Clemons, P. A.; Dechering, K. J.; Duvall, J. R.; Foley, M. A.; Gusovsky, F.; Kocken, C. H.; Marti, M.; Morningstar, M. L.; Munoz, B.; Neafsey, D. E.; Sharma, A.; Winzeler, E. A.; Wirth, D. F.; Scherer, C. A.; Schreiber, S. L. Diversity-oriented synthesis yields novel multistage antimalarial inhibitors. *Nature* **2016**, *538*, 344−349.
(19) Dancík, V.; Seiler, K. P.; Young, D. W.; Schreiber, S. L.; Clemons, P. A. Distinct biological network properties between the targets of natural products and disease genes. *J. Am. Chem. Soc.* **2010**, *132*, 9259−9261.
(20) Heidebrecht, R. W., Jr.; Mulrooney, C.; Austin, C. P.; Barker, R. H., Jr.; Beaudoin, J. A.; Cheng, K. C.; Comer, E.; Dandapani, S.; Dick, J.; Duvall, J. R.; Ekland, E. H.; Fidock, D. A.; Fitzgerald, M. E.; Foley, M.; Guha, R.; Hinkson, P.; Kramer, M.; Lukens, A. K.; Masi, D.; Marcaurelle, L. A.; Su, X.-Z.; Thomas, C. J.; Weïwer, M.; Wiegand, R. C.; Wirth, D.; Xia, M.; Yuan, J.; Zhao, J.; Palmer, M.; Munoz, B.; Schreiber, S. L. Diversity-oriented synthesis yields a novel lead for the treatment of malaria. *ACS Med. Chem. Lett.* **2012**, *3*, 112−117.
(21) Lukens, A. K.; Heidebrecht, R. W., Jr.; Mulrooney, C.; Beaudoin, J. A.; Comer, E.; Duvall, J. R.; Fitzgerald, M. E.; Masi, D.; Galinsky, K.; Scherer, C. A.; Palmer, M.; Munoz, B.; Foley, M.; Schreiber, S. L.; Wiegand, R. C.; Wirth, D. F. Diversity-oriented synthesis probe targets Plasmodium falciparum cytochrome b ubiquinone reduction site and synergizes with oxidation site inhibitors. *J. Infect. Dis.* **2015**, *211*, 1097−1103.
(22) Swann, J.; Corey, V.; Scherer, C. A.; Kato, N.; Comer, E.; Maetani, M.; Antonova-Koch, Y.; Reimer, C.; Gagaring, K.; Ibanez, M.; Plouffe, D.; Zeeman, A.-M.; Kocken, C. H.; McNamara, C. W.; Schreiber, S. L.; Campo, B.; Winzeler, E. A.; Meister, S. High-throughput luciferase-based assay for the discovery of therapeutics that prevent malaria. *ACS Infect. Dis.* **2016**, *2*, 281−293.
(23) Lowe, J. T.; Lee, M. D., IV; Akella, L. B.; Davoine, E.; Donckele, E. J.; Durak, L.; Duvall, J. R.; Gerard, B.; Holson, E. B.; Joliton, A.; Kesavan, S.; Lemercier, B. C.; Liu, H.; Marie, J.-C.; Mulrooney, C. A.; Muncipinto, G.; Welzel-O'Shea, M.; Panko, L. M.; Rowley, A.; Suh, B.-C.; Thomas, M.; Wagner, F. F.; Wei, J.; Foley, M. A.; Marcaurelle, L. A. Synthesis and profiling of a diverse collection of azetidine-based scaffolds for the development of CNS-focused lead-like libraries. *J. Org. Chem.* **2012**, *77*, 7187−7211.
(24) Peukert, S.; Nunez, J.; He, F.; Dai, M.; Yusuff, N.; DiPesa, A.; Miller-Moslin, K.; Karki, R.; Lagu, B.; Harwell, C.; Zhang, Y.; Bauer, D.; Kelleher, J. F.; Egan, W. A method for estimating the risk of drug-induced phototoxicity and its application to smoothened inhibitors. *MedChemComm* **2011**, *2*, 973−976.

DOI: 10.1021/acsmedchemlett.7b00030
ACS Med. Chem. Lett. 2017, 8, 438−442