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Potent Antimalarial 2-Pyrazolyl Quinolone bc_1 (Q_i) Inhibitors with Improved Drug-like Properties

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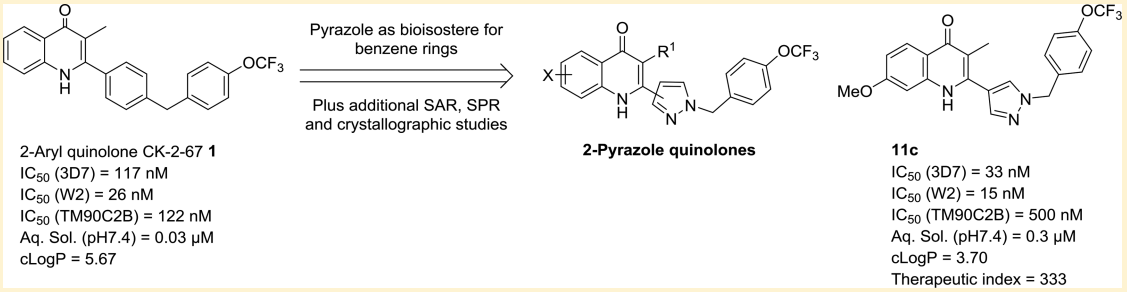
Supporting Information



ABSTRACT: A series of 2-pyrazolyl quinolones has been designed and synthesized in 5−7 steps to optimize for both *in vitro* antimalarial potency and various *in vitro* drug metabolism and pharmacokinetics (DMPK) features. The most potent compounds display no cross-resistance with multidrug resistant parasite strains (W2) compared to drug sensitive strains (3D7), with IC_{50} (concentration of drug required to achieve half maximal growth suppression) values in the range of 15−33 nM. Furthermore, members of the series retain moderate activity against the atovaquone-resistant parasite isolate (TM90C2B). The described 2-pyrazoyl series displays improved DMPK properties, including improved aqueous solubility compared to previously reported quinolone series and acceptable safety margin through *in vitro* cytotoxicity assessment. The 2-pyrazolyl quinolones are believed to bind to the ubiquinone-reducing Q_i site of the parasite bc_1 complex, which is supported by crystallographic studies of bovine cytochrome bc_1 complex.

KEYWORDS: *Quinolone, antimalarial, Plasmodium falciparum, cytochrome bc_1, atovaquone, drug resistance*

Malaria was responsible for nearly 216 million cases and an estimated 445,000 deaths in 2016.[1] Approximately half of the global population is at risk of infection, particularly in the tropical and subtropical regions where malaria is widespread.

Malaria is a disease caused by the parasite of the genus *Plasmodium* and is transmitted to people through the bites of infected female *Anopheles* mosquitoes. *Plasmodium falciparum* is the most prevalent and lethal species of the parasite to human and has developed resistance to most of the classical antimalarials.[2,3]

The quinolone scaffold is present in several antibiotics, and this chemotype possesses a wide range of biological activities including anticancer, anti-HIV, and antiviral.[4−7] The antimalarial activity of Endochin was first identified in the 1940s,[8] and recent publications have highlighted the promising potential antimalarial properties of aryl and alkyl substituted quinolones.[9−12] Studies by Nilsen and co-workers discovered the quinolone-3-diaryl ethers ELQ-300 and P4Q-391, which have excellent profiles and selectively inhibit *Plasmodium* cytochrome bc_1 complex.[13] Our group[14] and others[15] have focused on 2-aryl quinolones, and we have shown that representative 2-aryl quinolones eg. (**1**) can inhibit two mitochondrial enzymes in the electron transport chain, the cytochrome bc_1 complex and the recently identified PfNDH2 (Type II NADH:ubiquinone oxidoreductase).[16,17] This inhibition results in the collapse of the mitochondrial membrane potential, the inhibition of *de novo* pyrimidine biosynthesis, and ultimately the death of the parasite.[18]

Previously compound **1** was identified by us as one of the lead compounds with good antimalarial activity in a drug discovery program[14] (Figure 1). While compound **1** demonstrated good antimalarial activities against various strains of *P. falciparum*, it required further optimization of its physiochemical properties, especially lipophilicity (ClogP) and aqueous solubility. In this Letter we describe the further optimization and the synthesis of a

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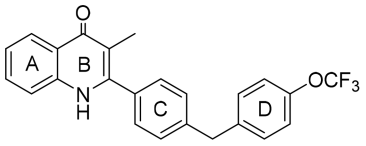


2-Aryl quinolone CK-2-67 **1**

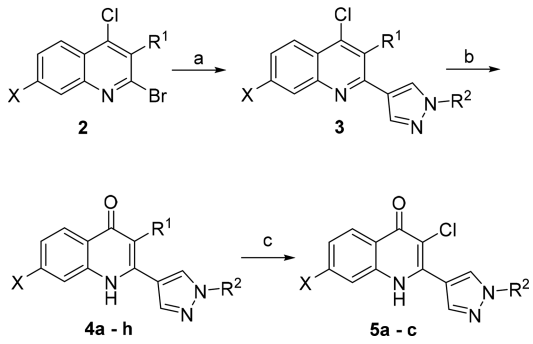
IC_{50} (3D7) = 117 nM
IC_{50} (W2) = 26 nM
IC_{50} (TM90C2B) = 122 nM
Aq. Sol. (pH7.4) = 0.03 μM
cLogP = 5.67

Figure 1. Initial lead **1** and its antimalarial activities and physiochemical properties.

series of 2-pyrazolyl quinolone with the aim of reducing ClogP and improving the aqueous solubility while maintaining/improving the antimalarial activity. It has been well documented that pyrazole is a bioisostere for benzene ring and can improve physiochemical properties (i.e., aqueous solubility) by reducing CLogP.[19] This strategy was applied to compound **1** by replacing the C-ring with a pyrazolyl ring. Different substituents on other parts of the molecule such as A-ring, B-ring, and D-ring were also explored. In addition to medicinal chemistry optimization, we were also interested in probing the effect of chemical substitution on bc_1 (Q_i) site binding by comparing our previously published bc_1 enzyme−inhibitor complexes with lead pyrazoles prepared in this work.

The 2-pyrazolyl quinolone analogues were prepared by three different synthetic routes. The synthesis of quinolones **4a−h** is depicted in Scheme 1. 2-Bromo-4-chloroquinoline **2**, synthesized from oxidation of corresponding 4-chloroquinoline followed by bromination, was coupled with readily available pyrazole boronic acid pinacol ester, giving the quinoline **3** in 38−93% yields. Upon hydrolysis using acetic acid or formic acid, quinoline **3** provided quinolones **4a−h** in excellent yields. Some selected 3*H*-quinolones were further chlorinated by sodium dichloroisocyanurate to give the 3-Cl analogue **5a−c** in 56−72% yields.

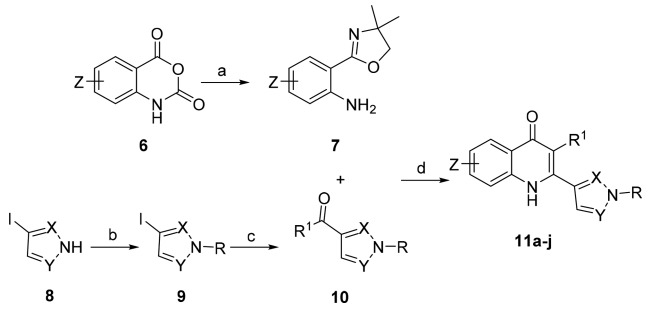
Scheme 1. General Route 1 for Synthesis of Pyrazole Quinolones[a]



[a]Conditions and reagents: (a) pyrazole boronic acid pinacol ester, 10 mol % $PdCl_2$(dppf), K_2CO_3·1.5H_2O, dioxane, reflux, 24 h; (b) AcOH, H_2O, 120 °C, 24−48 h or HCl(aq), dioxane, reflux, 48 h or HCOOH/H_2O, DMF, 140 °C, 4 h; (c) sodium dichloroisocyanurate, MeOH, NaOH(aq), r.t., o/n.

The synthesis of quinolones **11a−j** was accomplished in 3−6 steps from commercially available starting materials according to the synthetic methodology showed in Scheme 2. Oxazoline **7** was synthesized from the corresponding isatoic anhydride **6** in 60−75% yields. Substituted pyrazole **9**, synthesized from corresponding iodopyrazole **8** and benzyl bromide in excellent yield (see Supporting Information), was converted to ketone[20] **10** in 26−55% yields. Cyclization of oxazoline **7** with ketone **10** in the presence of catalytic trifluoromethanesulfonic acid afforded the desired quinolones **11a−j** in 42−84% yields.

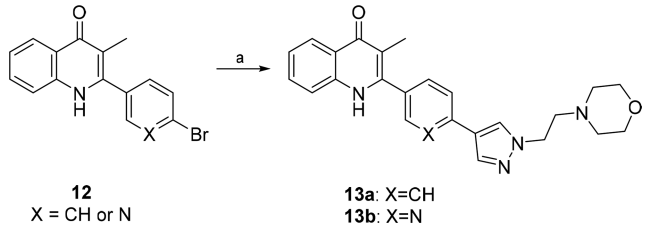
Scheme 2. General Route 2 for Synthesis of Pyrazole Quinolones[a]



[a]Conditions and reagents: (a) 2-amino-2-methyl-propanol, $ZnCl_2$, PhCl, 135 °C, 24 h; (b) corresponding benzyl bromide, K_2CO_3, acetone, reflux, 3 h; (c) Pd_2(dba)$_3$, dppp, pyrrolidine, 4 Å M.S., DMF, 110 °C, 6 h; (d) CF_3SO_3H, *n*-BuOH, N_2, 130 °C, 24 h.

Investigations also focused on the possibility of formulating the series as salts and improving the solubility by extending the side chain and introducing the morpholine group at the terminal as illustrated by **13a** and **13b**. The synthesis of the extended side chain quinolones **13a** and **13b** was shown in Scheme 3. Quinolone **12** (see Supporting Information) was coupled with the corresponding boronic acid pinacol ester to provide quinolones **13a** and **13b**.

Scheme 3. General Route 3 for Synthesis of Pyrzaole Quinolones with Extended Side-Chains[a]



[a]Conditions and reagents: (a) 5 mol % $PdCl_2$(dppf), K_2CO_3, H_2O/dioxane, 100 °C, 5 h.

In vitro antimalarial activity of the quinolone analogues was assessed against the 3D7 strain (chloroquine sensitive) of *Plasmodium falciparum* (Table 1). Several analogues exhibit improved antimalarial activity compared with the original lead **1**. As observed from previous work, a *p*-OCF_3 substituent on the D-ring in the 2-pyrazolyl series provides better antimalarial activity than *p*-F. The terminal phenyl ring is more favorable than a pyridinyl or morpholine ring. Longer side chains, as seen in **11j**, **13a**, and **13b**, results in a significant loss in antimalarial activity. A clear trend is seen in the nature of the A-ring substituent X. In general, the presence of F, Cl, and OMe on the A-ring is well tolerated and often improves the activity as shown when comparing **4e** (100 nM), **11c** (33 nM), **11g** (80 nM), and **11h** (50 nM). A small electron withdrawing substituent on the 6-position of quinolone is more favorable (see **11d** and **11e**). While F and Cl at the 7-position of quinolone exhibit potent activity, 7-CF_3 is less tolerated and a 8-fold drop in activity is observed. Among the substituents on the A-ring, 7-OMe enhances the activity greatly. The position of the pyrazolyl ring that links to the quinolone core also effects the activity. When the 3-position of

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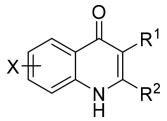
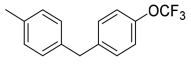
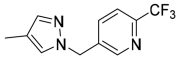
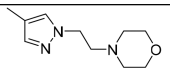
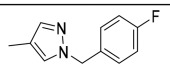
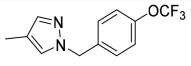
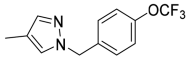
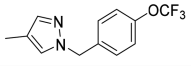
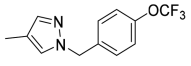
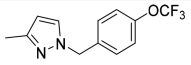
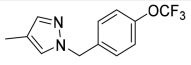
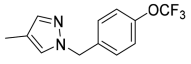
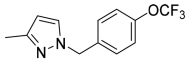
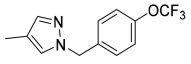
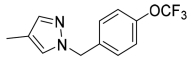
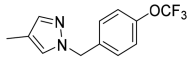
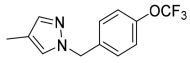
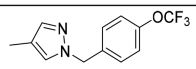
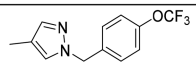
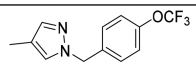
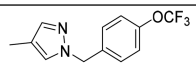
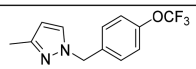
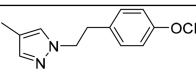
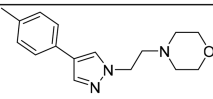
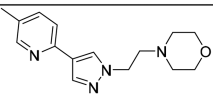
Table 1. *In Vitro* Antimalarial Activities of Quinolones versus the 3D7 Strain$^+$ of *Plasmodium falciparum*[a]



	R^1	R^2	X	IC_{50} 3D7 (nM) ± SD
1	Me		-	117 ± 27
4a	H		-	580 ± 90
4b	H		-	690 ± 160
4c	H		-	160 ± 20
4d	H		-	74 ± 14
4e	Me		-	100 ± 12
4f	CO_2Et		-	89 ± 5
4g	H		7-OMe	419 ± 47
4h	H		7-OMe	541 ± 35
5a	Cl		-	166 ± 19
5b	Cl		7-OMe	91 ± 17
5c	Cl		7-OMe	376 ± 54
11a	Et		-	88 ± 6

	R^1	R^2	X	IC_{50} 3D7 (nM) ± SD
11b	^{i}Pr		-	299 ± 69
11c	Me		7-OMe	33 ± 5
11d	Me		6-F	54 ± 7
11e	Me		6-Cl	110 ± 16
11f	Me		7-CF_3	810 ± 96
11g	Me		7-F	80 ± 13
11h	Me		7-Cl	50 ± 4
11i	Me		-	270 ± 57
11j	Me		-	>1000
13a	Me		-	>1000
13b	Me		-	>1000

[a]50% inhibitory concentration *in vitro* against *P. falciparum* chloroquine-sensitive (3D7) lines.

pyrazolyl ring is linked to the quinolone core (**4h**, **5c**, and **11i**) (instead of the 4-position), there is a reduction in potency. Looking into the substituents at the 3-position of the quinolone, most of substituents, except isopropyl group, are well-tolerated. In contrast to previous SAR studies, 3-chloro analogues are less potent than the 3-Me analogue (as seen in **5b** and **11c**), which is the most active in this series.

A selection of compounds was tested against the chloroquine resistant strain of *P. falciparum*, W2, and atovaquone resistant TM90C2B containing the Y268S mutation in the quinol oxidation Q_o site of the parasite mitochondrial cytochrome bc_1 complex[21−23] (Table 2). The SAR trends observed from the 3D7 data are similar to the W2 data with the presence of a 7-methoxy (**11c**) enhancing activity when compared to unsubstituted analogue (**4e**). Interestingly, unlike the activity data against 3D7 strain, the presence of 3-Cl in the quinolone core enhances activity compared with 3-methylation. In a confirmatory study that assessed antimalarial potency against the transgenic *P. falciparum* TX13 strain,[24] expressing yeast dihydroorotate dehydrogenase,[25] **5b** showed no inhibition at >1000 nM, further supporting that the series is targeting the respiratory chain of the parasite mitochondrion. To determine if the antimalarial activity is a result of on-target plasmodial bc_1 inhibition, the enzymatic activity was determined by monitoring cytochrome *c* reduction

Table 2. *In Vitro* Antimalarial Activities of Selected Quinolones versus W2 and TM90C2B and *Pf*NDH2 Enzyme Inhibition Data[a]

compound	IC_{50} W2 (nM)	IC_{50} TM90C2B (nM)	IC_{50} *Pf*NDH2 (nM)
chloroquine	12.3	14.5	ND[b]
atovaquone	0.3	9908	10,000
1	26	122	20
4e	33	ND	837
5a	14	ND	ND
5b	11	110	ND
11c	15	500	1,000
11i	49	300	68

[a]50% inhibitory concentration *in vitro* against *P. falciparum* chloroquine-resistant W2 strain (Indochina), Atovaquone resistant TM90C2B strain, and PfNDH2 enzyme inhibition data.[24] [b]ND, not determined.

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using decylubiquinol as electron donor as previously reported.[26] This enzymatic study confirmed **11c** as a potent *Pf* bc_1 inhibitor with an IC_{50} of 0.75 nM (Figure S1). However, it is noteworthy that, although relative to atovaquone, some of the selected compounds in this series are active against the TM90C2B strain, and reduced potency is seen compared to 3D7 and W2. A possible explanation for this observation could be that for this series, there is a contributing element of Q_o site inhibition; it has been noted by Riscoe and co-workers that minor modifications to the quinolone core of a series of related endochin quinolone analogues can subtly affect both *Pf* bc_1 Q_o/Q_i sites binding.[27] This observation may well explain in part the reduced potency of **11c** versus the Q_o site mutated TM90C2B strain.

One of the major aims in this lead optimization process was to improve the physiochemical properties of compound **1**, especially its aqueous solubility. Aqueous solubility of molecules is related to lipophilicity (CLogP) and crystal packing via π-stacking of aromatic ring systems (as reflected in the melting point).[28] Replacement of the benzene C-ring to a pyrazole ring and incorporating various substitutions at the 3-position of the quinolone can dramatically change both CLogP and melting point of the analogues in this series, and thus improve the aqueous solubility profile (Table 3). Replacement of the benzene

Table 3. CLogP Value, Melting Point, and Aqueous Solubility at pH 7.4 for Selected 2-Pyrazolyl Quinolones

compound	CLogP	melting point (°C)	solubility[a] (μM)
1	5.67	213	0.03
4e	3.71	194	0.1
5a	4.04	256	0.01
11a	4.24	143	0.2
11c	3.70	172	0.3
11i	3.92	64	0.4

[a]Solubility in pH 7.4 PBS buffer.

C-ring with pyrazole reduced CLogP by between 1.5 to 2 units. Incorporation of a substituent, such as Me or Et, at the 3-position of the quinolone ring likely reduces the planarity of the side-chain, reducing packing, and this reduces the melting point. The most significant reduction in melting point came as the result of modification of the linkage of the pyrazole heterocycle from a 1,4 to 1,3 arrangement. The combination of reduction in both lipophilicity and aggregation via π-stacking of aromatic ring systems resulted in over 10-fold improvement in aqueous solubility for some selected analogues in this series (**11c** and **11i**).

To further examine the DMPK properties, selected compounds in the series have also been screened for metabolic stability and plasma protein binding *in vitro* (Table S1). From the human microsomal stability and rat hepatocyte stability data, all selected representatives in the 2-pyrazolyl quinolone series had very low clearance and good metabolic stability. Most of the tested compounds, except **5a**, had high human plasma protein binding level, but below 99.9% bound, which is comparable with other antimalarial quinolones.

To gain insight into the key protein/ligand binding interactions of 2-pyrazole quinolones within a bc_1 complex, we cocrystallized bovine heart-derived cytochrome bc_1[29] with compound **11c**. Clear and defined omit F_o−F_c electron density within the Q_i pocket near heme b_H (Figure 2A,B) showed unambiguous binding of the quinolones to the Q_i site. The carbonyl of quinolone core forms H-bonds with His201 side chain, and the aromatic tail is positioned within the hydrophobic

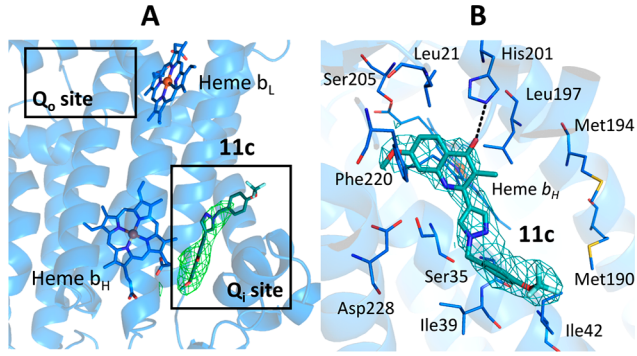

Figure 2. Cytochrome bc_1 Q_i site (bovine heart derived) bound inhibitor **11c**. (A) The omit F_o−F_c map (green) contoured at 3σ level around **11c** (teal) compounds shown as sticks. The cartoon representation of cytochrome *b* subunit is shown in blue. The Q_i and Q_o sites are marked by black boxes. (B) The $2F_o$−F_c electron density map (cyan) contoured at 1σ level around the inhibitors. Surrounding residues are drawn as blue lines and hydrogen bonds as black dashed lines.

region. The planar quinolone ring of **11c** makes an aromatic stacking with the phenyl ring of Phe220, and its amine points to the side chain of Ser35. The aromatic tail is packed in the hydrophobic cavity conferred by Ile39 and Ile42.

As there is no structure of *P. falciparum* cytochrome bc_1, its homology model was generated by SWISS-MODEL online tool[30] based on the primary sequence (Q02768) and the bovine cytochrome *b* (PDB: 5OKD) template. The *Pf* model was aligned to the bovine crystal structure to visualize inhibitor interactions within the *Pf* Q_i site (Figure S2). The parasite's Q_i binding pocket appears to be smaller than bovine, and there could be a steric contact of Phe30 (Ser35 in bovine) side chain with the pyrazole ring of **11c**. The inhibitors had to adopt different poses in the *Pf* Q_i site because of steric clashes with the calculated protein model. To predict possible binding poses in the parasite enzyme, *in silico* docking was performed by SwissDock[31] with defined interest region of Q_i site. The final solution for **11c** was determined based on the compound pose in bovine crystal structure with the highest *FullFitness* scoring of −858.01 kcal/mol. As the absence of 7-methoxy group on the A-ring often reduces antimalarial activities, compound **4e**, which is the unsubstituted analogue of **11c**, was docked into the *Pf* Q_i site with *FullFitness* score of −854.04 kcal/mol. The molecular docking results are shown in Figure 3. Both compounds can form a hydrogen bond with His192, but the presence of 7-methoxy group in **11c** causes a shift in binding location away from the **4e** position with stronger binding explained by π-stacking interaction of D-ring with Phe30 and Phe37 side chains. This observation provides insight as to how 7-methoxy quinolone analogues have improved potency over other derivatives. Future work will utilize the homology *Pf* bc_1 model with the mammalian bovine structures described here to guide chemical substitution that enhances parasite potency and selectivity further.

Finally, given that members of this series have the propensity to bind to mammalian bc_1, we examined the cytotoxicity profiles in the Hep G2 cell line (Table 4). From this *in vitro* toxicity assessment, the tested 2-pyrazolyl quinolone analogues showed similar or higher IC_{50} values than the negative control, Tamoxifen, which indicate low cytotoxicity for the analogues tested. Based on the 3D7 IC_{50} data, there is a sufficient safety windows for the tested analogues with **11c** expressing the highest therapeutic index ratio of 333.

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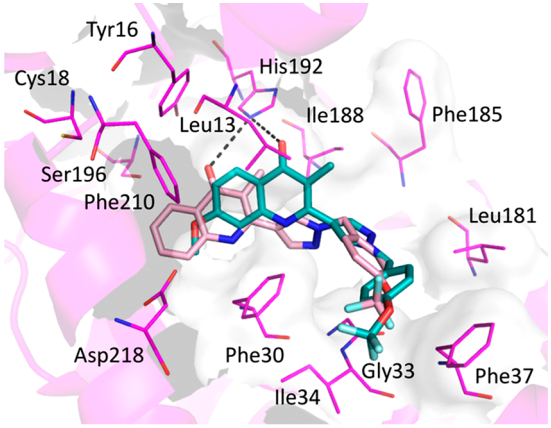

Figure 3. *In silico* docking of **4e** (pink) and **11c** (teal) into the *Plasmodium falciparum* Q_i site. The protein structure and residues shown in magenta. The binding surface shown in gray. Hydrogen bonds are indicated by black dashed lines.

Table 4. *In Vitro* Cytotoxicity Assessment Using Hep G2 Cells for Selected 2-Pyrazolyl Quinolones

compound	Hep G2 toxicity IC_{50} (μM) ± SEM	therapeutic index[a]
4e	13.0 ± 1.7	130
5a	28.4 ± 8.3	171
11a	19.3 ± 3.3	219
11c	11.0 ± 0.7	333
11i	21.2 ± 0.8	79
rotenone	1.52 ± 0.24	
tamoxifen	12.0 ± 0.5	

[a]Therapeutic index is determine by comparing the HepG2 IC_{50} values with the corresponding 3D7 IC_{50} values.

To conclude, a series of 2-pyrazolyl quinolones with potent antimalarial activity against the 3D7 strain and W2 strain of *P. falciparum* have been identified. Representative analogue **11c** has improved antimalarial activity, physiochemical, and DMPK properties in comparison to previously reported lead molecules in addition to low cytotoxicity. While the series on a whole have improved solubility compared with previous quinolone derivatives, further work is required to find quinolone derivatives with solubility in a more desired range (>50 μM). Crystallography and homology based modeling of mammalian and parasite bc_1 complexes have now been produced that may allow rational drug design approaches to be initiated for more selective *Pf* bc_1 Q_i inhibitors. It is noteworthy that, despite the enzymatic and crystallographic data described above, we cannot rule out that this series of 2-pyrazolyl quinolones may potentially target other components of the electron transport chain of the parasite mitochondrion.

Further work also is in progress to investigate the *in vivo* PK profiles and efficacy of this series and to profile the lead compounds for their activity against liver and sexual stage of the parasites.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.8b00371.

Synthetic methods, procedures, and chemical analysis data of all final compounds (except compound **1**) and the intermediates; biological testing methods and procedures; and cytochrome bc_1 preparation and crystallography (PDF)

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Author Contributions

W.D.H., S.C.L., K.A., S.V.A., N.B., G.A.B., and P.M.O. contributed to writing of the manuscript; P.M.O., S.A.W., S.V.A., S.S.H., and G.A.B. conceived this work; W.D.H. and G.N. designed, synthesized, and characterized chemical compounds; J.D. and R.S.P. conducted biological studies; K.A. and S.V.A. performed crystallographic studies. All authors have given approval to the final version of the manuscript.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

CCR2, CC chemokine receptor ;; CCL2, CC chemokine ligand 2; CCR5, CC chemokine receptor 5

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NOTE ADDED AFTER ASAP PUBLICATION

This paper published ASAP on November 1, 2018 with errors in Table S2 in the Supporting Information file. The corrected paper reposted to the Web on November 7, 2018.

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