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Structure–Activity Relationship Studies of Orally Active Antimalarial 3,5-Substituted 2-Aminopyridines

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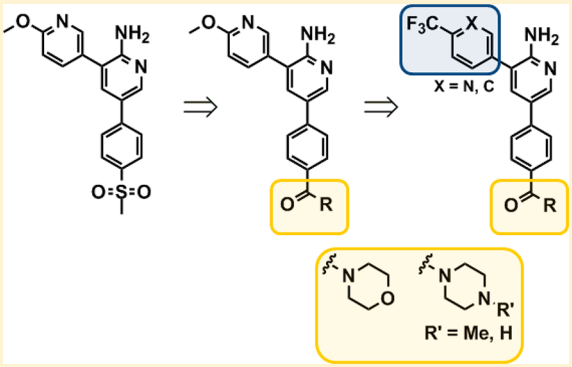
Supporting Information

ABSTRACT: In an effort to address potential cardiotoxicity liabilities identified with earlier frontrunner compounds, a number of new 3,5-diaryl-2-aminopyridine derivatives were synthesized. Several compounds exhibited potent antiplasmodial activity against both the multidrug resistant (K1) and sensitive (NF54) strains in the low nanomolar range. Some compounds displayed a significant reduction in potency in the hERG channel inhibition assay compared to previously reported frontrunner analogues. Several of these new analogues demonstrated promising in vivo efficacy in the *Plasmodium berghei* mouse model and will be further evaluated as potential clinical candidates. The SAR for in vitro antiplasmodial and hERG activity was delineated.



INTRODUCTION

Malaria, a parasitic disease caused in humans primarily by *Plasmodium falciparum* and *Plasmodium vivax*,[1] has been recently listed as the fifth of the top ten causes of death in low-income countries. According to the World Health Organization 2011 report, malaria was responsible for 216 million clinical cases and 655000 deaths in 2010, especially among children and pregnant women.[2]

Despite the effectiveness that standard antimalarial agents have had in recent years,[3] rapid widespread emergence of drug resistance has dramatically compromised the therapeutic options for the treatment of malaria.[4] Thus, the search for novel, structurally diverse, and affordable drugs has become an urgent necessity to control and eventually eradicate this parasitic disease.[1]

Recently, we demonstrated the potential of 3,5-diaryl-2-aminopyridines **1** and **2** (Figure 1) as clinical candidate antimalarials based on mice in vivo efficacy data. One of the liabilities identified with the aminopyridine **1** is potential cardiotoxicity risks as signaled by the moderate hERG activity (IC_{50} = 5.5 μM) in the IonWorks patch clamp electrophysiology assay.[5]

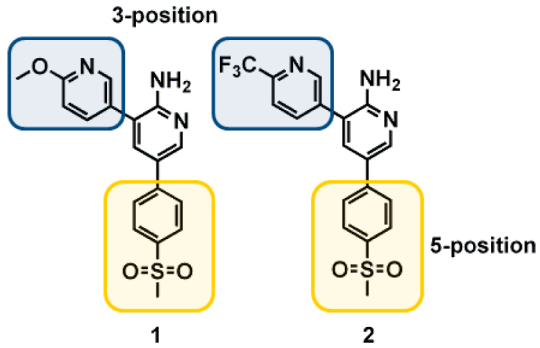


Figure 1. Chemical Structures of **1** and **2**.

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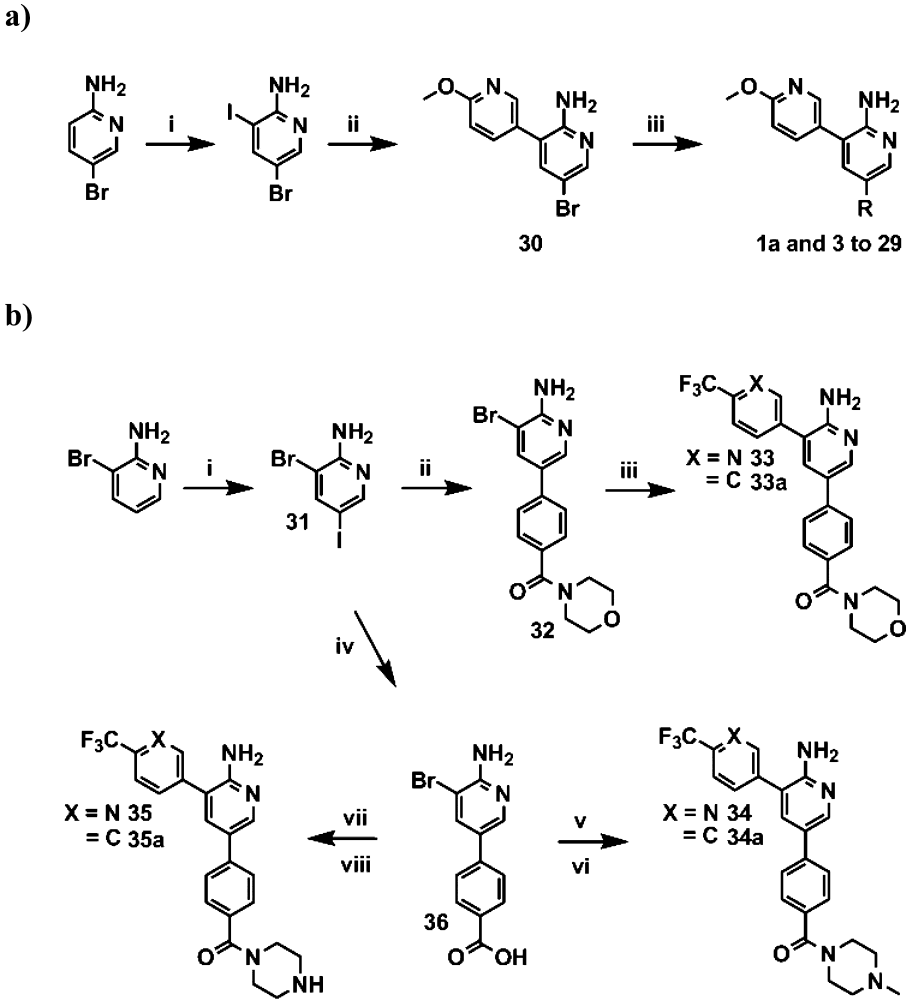
Scheme 1. Synthetic Approaches to 3,5-Diaryl-2-aminopyridines Analogues[a]



[a]Reagents and conditions: (a) (i) I_2, DMSO, 100 °C, 4 h, rt, 12 h, 38%; (ii) 6-methoxypyridin-3-yl boronic acid, $Pd(PPh_3)_2Cl_2$, K_2CO_3, 1,4-dioxane, 90 °C, 14 h, 72%; (iii) R = appropriate boronic acid, $Pd(PPh_3)_2Cl_2$, K_2CO_3, 1,4-dioxane or DMF, 90 °C, 14 h, 28−76%; (b) (i) I_2, DMSO, 100 °C, 4 h, rt, 12 h, 50%; (ii) 4-(morpholine-4-carbonyl)phenylboronic acid, $Pd(PPh_3)_2Cl_2$, K_2CO_3, 1,4-dioxane, 90 °C, 14 h, 63%; (iii) R_1 = appropriate boronic acid, $Pd(PPh_3)_2Cl_2$, K_2CO_3, 1,4-dioxane, 90 °C, 14 h, 62% and 76%; (iv) 4-carboxyphenylboronic acid, $Pd(PPh_3)_2Cl_2$, K_2CO_3, DMF, 90 °C, 14 h, 59%; (v) $SOCl_2$, CH_2Cl_2, rt, 3h, 1-methyl piperazine, triethylamine, 0 °C to rt, 6h, 94%; (vi) appropriate boronic acid, $Pd(PPh_3)_2Cl_2$, K_2CO_3, 1,4-dioxane or DMF, 90 °C, 14 h, 45% and 42%; (vii) 1-Boc piperazine, EDCI, HOBt, DMF, rt, 12 h, 59%, appropriate boronic acid, $Pd(PPh_3)_2Cl_2$, K_2CO_3, 1,4-dioxane or DMF, 90 °C, 14 h, 58% and 50%; (viii) trifluoroacetic acid, CH_2Cl_2, rt, 2 h, Amberlyst A-21, CH_2Cl_2/CH_3OH, 30 min, 90%.

Drug-induced blockade of the hERG potassium channel is associated with prolongation of the length of time between the start of the Q wave and the T wave on an electrocardiogram which may, under certain circumstances, lead to potentially life-threatening arrhythmia.[6] Thus accordingly we embarked on a campaign aimed at identifying compounds that would either minimize or eliminate the hERG liability associated with **1** while retaining high in vitro potency along with good ADME properties and in vivo efficacy.

RESULTS AND DISCUSSION

Chemistry. Target compounds (**1a** and **3**−**29**) in which the 5-aryl substituent was systematically varied, were prepared using key intermediate **30** and the appropriate commercially available boronic acid as shown in Scheme 1a. The synthesis described in Scheme 1b was then utilized to optimize the 3-aryl substituent of the most potent 5-aryl-2-aminopyridine derivatives. Suzuki cross-coupling[7] between starting material **31**[5] and commercially available 4-(morpholine-4-carbonyl)phenylboronic acid yielded key intermediate **32**. This was in turn subjected to a second Suzuki reaction to afford analogues **33** and **33a**. Derivatives **34**−**35a** were accessed via amidation of intermediate **36** in the presence of the relevant piperazine amine followed by a Suzuki cross-coupling reaction with commercially available boronic acids. Deprotection of the *N*-Boc moiety of precursors to **35** and **35a** was accomplished using trifluoroacetic acid followed by its removal with Amberlyst A-21.[8]

Biology. *In Vitro Antiplasmodial Activity.* Table 1 summarizes the IC_{50} values against multidrug resistant (K1) and sensitive (NF54) strains of *P. falciparum*[9] along with physicochemical properties. In general, the dose−response curves for the highly active compounds were steep and the IC_{90} values ∼2× higher than the IC_{50} values. These compounds were designed with a view to investigating the structural and electronic properties of the 5-aryl substituent necessary for modulating the antiplasmodial, ADME, and hERG activity. As previously noted, potent activity was associated with the presence of a methylsulfonyl group at the *para* position.[5] Potent

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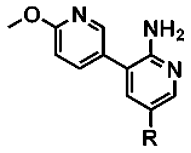
Table 1. In Vitro Activity against Sensitive and Multidrug-Resistant Strains of *Plasmodium falciparum*, Solubility, and Partition Coefficients Values



compd	R	IC50 (*n*M)[a]		solubility (*μ*g/mL)[b]		logD[c]
		K1	NF54	pH 2.0	pH 6.5	pH 7.4
chloroquine[d]		194	16			
artesunate[d]		3	4			
1[e]		31	48			2.2
1a		2527	2102	>100	25-50	2.3
3		44	42	>100	6.3-12.5	2.8
4		68	68	>100	12.5-25	2.2
5		1059	1019	>100	12.5-25	1.8
6		109	111	>100	6.3-12.5	2.7
7		33	33	>100	6.3-12.5	2.7
8		166	180	>100	25-50	2.7
9		812	791	>100	25-50	2.2
10		107	107	>100	6.3-12.5	2.5
11		942	980	>100	12.5-25	2.7
12		8898	9285	>100	1.6-3.1	4.3
13		10576	10043	>100	1.6-3.1	4.2
14		1508	1336	>100	25-50	2.8
15		480	346	>100	6.3-12.5	2.4
16		2018	2050	>100	3.1-6.3	3.1
17		2140	2226	>100	12.5-25	2.6
18		98	104	>100	12.5-25	2.4
19		3018	3018	>100	6.3-12.5	3
20		408	463	>100	6.3-12.5	2.9
21		283	268	12.5-25	6.3-12.5	0.8

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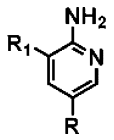
Table 1. continued

compd	R	IC$_{50}$ (nM)[a]		solubility (μg/mL)[b]		logD[c]
		K1	NF54	pH 2.0	pH 6.5	pH 7.4
22		119	119	>100	12.5-25	1.7
22a		1598	1963	>100	12.5-25	1.7
23		161	161	>100	>100	2.1
24		290	275	50-100	50-100	1.8
25		209	225	>100	12.5-25	1.7
26		302	328	>100	>100	1.9
27		347	347	>100	>100	2.5
28		57	52	>100	>100	2
29		74	74	25-50	25-50	2

[a]Mean from n values of ≥2 independent experiments. [b]Estimated using nephelometry. [c]Estimated using the chromatographic glogD technique. [d]Data from Gonzalez Cabrera et al.[11] [e]Data from Younis et al.[5]

Table 2. In Vitro Activity against Sensitive and Multidrug-Resistant Strains of *Plasmodium falciparum*, Solubility and Partition Coefficients Values



compd	R	R$_1$	IC$_{50}$ (nM)[a]		solubility (μg/mL)[b]		logD[c]
			K1	NF54	pH 2.0	pH 6.5	pH 7.4
chloroquine[d]			194	16			
artesunate[d]			3	4			
33			12	15	>100	25-50	2.4
33a			19	20	>100	6.3-12.5	3.1
34			11	15	>100	>100	2.9
34a			17	20	>100	25-50	3.9
35			10	11	>100	50-100	2.4
35a			7.3	9.4	>100	25-50	3.3

[a]Mean from n values of ≥2 independent experiments. [b]Estimated using nephelometry. [c]Estimated using the chromatographic glogD technique. [d]Data from Gonzalez Cabrera et al.[11]

activity was retained with other polar, electron-withdrawing groups such as N-substituted sulphonamides (**3**, **4**, and **7** but not the primary sulphonamide **5**), and primary, secondary, or tertiary carboxamides (**22**, **24**−**29**, and **23**, respectively). Small polar amides such as the primary carboxamide or morpholino, piperazine, or methyl piperazine amides gave compounds with excellent potency, e.g., **28** (IC$_{50}$ NF54 = 52 nM) and **29** (IC$_{50}$ NF54 = 74 nM), which were followed up for further optimization (see below).

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While other polar groups such as $MeSO_2NH-$ (**9**) and $-CO_2H$ (**21**) retained some activity, lipophilic electron withdrawing groups such as $-OCF_3$ (**12**) or $-CF_3$ (**13**) were much less active.

As was observed in the original screening of the BioFocus DPI library set, substitution at the *para* position was strongly favored over the corresponding *meta* and/or *ortho* position as was confirmed in some of the most active compounds (compare **1** with **1a** and **22** with **22a**).[5] Introduction of substituent groups onto the aryl ring *ortho* or *meta* to the 4-methanesulfonyl group also reduced the activity **10** (IC_{50} NF54 = 107 nM) and **11** (IC_{50} NF54 = 980 nM). However, some examples retained activity when the adjacent substituent was sterically constrained as part of an electron deficient bicycle, e.g., **18** and **20** with IC_{50} 104 nM (NF54) and 463 nM (NF54), respectively.

On the basis of the previously reported SAR for the frontrunner analogues **1** and **2** (Figure 1),[5] the 2-methoxypyridine substituent in the 3-aryl group of the most active 5-substituted amide analogues was replaced by 6-trifluoromethylpyridin-3-yl or 4-trifluoromethylphenyl moieties. The critical role played by these molecular fragments in the antiplasmodial activity can be observed in Table 2. In general, IC_{50} values of the prepared analogues were 20 or below 20 nM and represent the most potent compounds of the series.

In Vitro ADME Profiling. All compounds were assessed for their physicochemical properties and in vitro metabolic stability in human microsomal preparations.[10] The estimated spartition coefficients were generally low to moderate, with $LogD_{7.4}$ values ranging from 0.8 to 4.3 (Tables 1 and 2).

The kinetic solubility at pH 6.5 was quite varied, with 17 analogues displaying poor solubility and the remaining compounds showing moderate solubility. Good solubility was only observed for **23**, **26**, **27**, **28**, and **34**. Under acidic conditions (pH 2), all the compounds displayed improved solubility, which supports their predicted basic characteristics.

Generally, most of the derivatives were metabolically stable in human liver microsomes (Table 3) and based on the in vitro intrinsic clearance values, low or intermediate in vivo hepatic clearance would be expected. In addition, the microsome-predicted hepatic extraction ratios (E_H) of each compound were comparable across three species: human, rat, and mouse (data not shown).

In Vitro hERG Activity. Several derivatives of the series were tested for their activity against the hERG potassium channel using in vitro IonWorks patch clamp electrophysiology. As can be observed in Table 4, compounds displayed varying degrees of activity. Derivatives, which displayed poor solubility, as evidenced by precipitation under the assay conditions, were tested at a maximum concentration of 11 μM. Analogues with better solubility were tested at 33 μM. Compounds where the methanesulfonyl group is replaced by sulphonamide or *N*,*N*-dimethylacetamide groups showed an improved hERG profile in comparison to the parent compound **1** (IC_{50} = 5.5 μM), excluding **6**. Furthermore, when more polar substituents were introduced in the 5-aryl group, as in for example methyl piperazine **28**, an improvement in the hERG profile was observed (IC_{50} = 20.5 μM). Improvements in the hERG liability were also observed by the introduction of 6-trifluoromethylpyridin-3-yl groups at the 3-aryl position. However, derivatives containing the 4-trifluoromethylphenyl moiety, **33a** (IC_{50} = 3 μM) and **35a** (IC_{50} = 5.5 μM), showed no improvement relative to the parent compound **1** (IC_{50} = 5.5 μM).

Table 3. In Vitro Metabolic Stability Assessed in Human Liver Microsomes

compd	degradation half-life (min)	in vitro CL_{int} (μL/min/mg protein)	microsome-predicted E_H[a]
1a	227	7.6	0.3
3	17.5	98.8	0.85
4	181	9.6	0.45
5	>250	<7	<0.28[b]
6	114	15	0.46
7	67.2	25.8	0.59
8	49.1	35.3	0.66
9	>250	<7	<0.28[b]
10	>250	<7	<0.28[b]
11	>250	<7	<0.28[b]
12	76	22.8	0.56
13	285	6.1	0.25
14	87	19.9	0.53
15	210	8	0.32
16	203	9	0.32
17	23	76	0.81
18	146	12	0.4
19	143	12	0.4
20	181	10	0.35
21	>250	<7	<0.28[b]
22	>250	<7	<0.28[b]
22a	>250	<7	<0.28[b]
23	156	11.1	0.38
24	>250	<7	<0.28[b]
25	>250	<7	<0.28[b]
26	151	12	0.39
27	>250	<7	<0.28[b]
28	>250	<7	<0.28[b]
29	>250	<7	<0.28[b]
33	>250	<7	<0.28[b]
33a	186	9	0.34
34	120	14	0.45
34a	56	31	0.63
35	>250	<7	<0.28[b]
35a	123	14	0.44

[a]Predicted hepatic extraction ratio based on in vitro intrinsic clearance. [b]No measurable degradation of the parent compound was observed, hence, the clearance parameters could not be determined. E_H considered to be <0.28.

In Vivo Efficacy Studies. On the basis of in vitro potency, metabolic stability, and structural diversity, nine compounds were tested for in vivo oral activity in the *P. berghei* infected mouse model.[9] Parasitemia reduction and mean survival days (MSD) for single- or multi-dose regimens are reported in Table 5.

The in vivo antimalarial effect for compounds **22** and **23** at the 4 × 50 mg/kg multidose was high (99.6% and 99.7% inhibition), albeit no cure was observed as evidenced by a MSD of 12 days. On the other hand, the in vivo efficacy was modest at a single oral dose of 30 mg/kg (73% inhibition for **22**). In the multi (4 × 50 mg/kg) oral dosing regimen, differences between **29** (MSD = 13), **33** (MSD = 24), and **33a** (MSD = 12) became more apparent, where **33** proved to be superior.

Despite the potent in vitro activity against *P. falciparum*, compounds **34**, **34a**, and **35** started to lose in vivo activity at the 1 × 10 mg/kg oral dose (73%, 92%, and 43%). Only **35a** showed better efficacy, where dosing of 3, 10, and 30 mg/kg were able to suppress parasitemia by 71%, 98%, and 99.2%, respectively.

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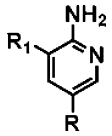
Table 4. hERG Inhibition Data



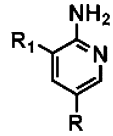
compd	R	R_1	hERG (μM)
3			9.7
4			>11[a]
6			2.1
7			>11[a]
8			10.4
23			>11[a]
28			20.5
29			>11[a]
33			20.2
33a			3[b]
34			8.2
34a			16.3
35			19.7
35a			5.5

[a]No hERG inhibition at the highest measured concentration (11 μM). [b]Average of two independent experiments.

Unfortunately, none of the compounds produced a complete cure of mice. This is in contrast to previously reported **1** and **2** derivatives.[5]

Mouse Exposure Studies. In an attempt to rationalize the observed in vivo efficacy, mouse exposure studies were conducted on five representative analogues. These analogues were chosen on the basis of their varying degrees of in vivo efficacy. The plasma concentrations in mice were measured after a single oral dose of 30 mg/kg and followed for 24 h using a limited sampling schedule. As observed in Table 6, the plasma concentrations of all tested compounds, with the exception of **33a**, were higher than those for **33**. It was also shown that this analogue exhibited a relatively short duration of exposure (plasma concentrations were not detectable 24 h after administration), but when administered orally to mice at multiple doses of 4 × 50 mg/kg, it displayed 99.7% inhibition of *P. berghei* and prolonged mean mouse survival to 24 days compared to 12, 12, 13, and 12 days exhibited by **22**, **23**, **29**, and **33a**, respectively. Thus, even the relatively low systemic exposure of **33** was sufficient to significantly clear parasites in blood given the significantly higher in vitro potency (IC_{50} = 12 nM (K1), 15 nM (NF54)) and the dose regime utilized. Moreover, the lower in vivo activity exhibited by **33a** compared to **33**, regardless of its apparently longer duration of exposure in vivo and similar antiplasmodial activity, may be rationalized on the basis of the lower overall plasma concentrations achieved for **33a**.

Table 5. In Vivo Antimalarial Efficacy Using Single- and Multidose of Selected Compounds in *Plasmodium berghei*-Infected Mice[a]



compd	R	R_1	Oral dose (mg/kg)	% reduction parasitemia (MSD)[b]
			4x30	99.9 (24)[d]
			100	>99.9 (12)[d]
chloroquine			30	99.7 (9)[d]
			10	99.5 (7)[d]
			3	83 (7)[d]
			4x50	99.6 (12)
22			100	99 (7)
			30	73 (7)
23			4x50	99.7 (12)
29			4x50	99.7 (13)
			4x50	99.7 (24)
33			30	98 (8)
			10	86 (7)
			4x50	99.3 (12)
33a			30	95 (7)
			10	67 (7)
			30	92 (10)
34			10	74 (7)
			3	<40 (3)[c]
			30	98 (7)
34a			10	92 (7)
			3	51 (8)
			30	64 (6)
35			10	43 (7)
			3	<40 (3)[c]
			30	99.2 (8)
35a			10	98 (7)
			3	71 (8)

[a]Compounds were dissolved or suspended in 70/30 Tween 80/ethanol and diluted 10× with water, except for **34**, **34a**, **35**, and **35a**, which were dosed in a nonsolubilizing, standard suspension vehicle (HPMC). [b]MSD = mean survival time (in days). [c]Mice were euthanized on day 3 in order to prevent death otherwise occurring at day 6. [d]Data from Gonzalez Cabrera et al.[11]

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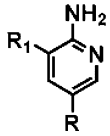
Table 6. Plasma Concentrations of Compounds 22, 23, 29, 33, and 33a in Mice Following Administration of a Single 30 mg/kg Oral Dose



compd	R	R_1	plasma concentration (μM)[a] 0.5 h[b]	8 h[b]	24 h[b]	C_{av0-8h}[d]	$C_{av0-24h}$[d]
22			10.6	1.3	0.01	8.2	2.4
23			30.8	6.4	<0.001[c]	25.1	c.n.c.
29			10.3	0.8	ND	7.8	c.n.c.
33			4.11	0.84	ND	3.4	c.n.c.
33a			0.3	0.05	0.001	0.2	0.1

[a]Values are the mean from two animals. [b]Sample time postdose in hours. ND = not detected. [c]Based on the lower limit of quantitation. [d]Avarage plasma concentration over the time period (t) calculated as AUC_{0-t}/t. c.n.c. = could not be calculated as plasma concentrations at 24h were not available.

CONCLUSION

A series of 3,5-diaryl-2-aminopyridine derivatives has been synthesized and evaluated for SAR in vitro against K1 and NF54 strains of *P. falciparum*. Most of the synthesized compounds showed IC_{50} values in the low nanomolar range and good to moderate metabolic stability in human liver microsomes. From among the several derivatives synthesized, **28**, **33**, **34a**, and **35** also exhibited a dramatic reduction in hERG activity compared to the parent compound **1**.[5] However, improvements of in vitro antiplasmodial and hERG activity was not accompanied by exceptional curative oral efficacy in the *P. berghei* mouse model.

Despite the improved in vitro antiplasmodial and hERG activities that some of these compounds exhibited, further optimization to ameliorate in vivo efficacy against *P. berghei* is essential prior to selection of a clinical candidate.

EXPERIMENTAL SECTION

General Comments on Experimental Data. Chloroform ($CHCl_3$) and tetrahydrofuran (THF) solvents were analytical grade, without stabilizer; ethyl acetate, hexane, and dichloromethane were distilled. Unless stated otherwise, all other reagents were purchased from commercial sources and used without further purification. Column chromatography was carried out using silica gel 60 (Fluka) particle size 0.063−0.2 mm (70−230 mesh ASTM) as the stationary phase. Analytical TLC was performed on silica on TLC aluminum foils, H × W 20 cm ×20 cm, with fluorescent indicator (200 μm thick, Fluka) and visualized under UV light. Melting points were determined on a Reichert−Jung Thermovar hotstage microscope and are uncorrected. Routine ^1H and ^{13}C NMR spectra were recorded on either a Varian Mercury-300 (^1H 300.1, ^{13}C 75.5 MHz) or 400 MHz on a Bruker AV 400 (^1H 400.0, ^{13}C 100.6 MHz) instrument. Spectra were recorded at ambient temperature unless otherwise stated. Chemical shifts (δ) are reported in parts per million from low to high field and referenced to residual solvent. Standard abbreviations indicating multiplicity are used as follows: br s = broad singlet, d = doublet, m = multiplet, q = quartet, quint. = quintet, s = singlet, t = triplet. In many cases. DMSO-d_6 was used as a solvent, and the ^1H was referenced to 2.500 ppm for the quintuplet downfield methyl signal. ^{13}C was reference to the methyl carbon septuplet at 39.52 ppm. Atmospheric pressure chemical ionization (APCI) mass spectrometry was carried out by the services at the Centre for Drug Candidate Optimisation and Syngene. LC purity traces were performed using one of the methods shown in Supporting Information.

Purity was determined by HPLC, and all compounds were confirmed to have >95% purity.

General Procedure 1 for the Synthesis of 3-(6-Methoxypyridin-3-yl)-5-(3-(methylsulfonyl)phenyl)pyridin-2-amine 1a. To a solution of 5-bromo-3-(6-methoxypyridin-3-yl)pyridin-2-amine (100 mg, 0.36 mmol)[5] in 1,4-dioxane (2 mL), 3-(methylsulfonyl)-phenylboronic acid (79 mg, 0.39 mmol) was added. The mixture was thoroughly degassed with nitrogen for 15 min, at which time $Pd(PPh_3)_2Cl_2$ (13 mg, 0.02 mmol) was added under an atmosphere of nitrogen, followed by (1M) aqueous K_2CO_3 (0.38 mL, 0.38 mmol). The reaction mixture was stirred at 90 °C for 14 h, poured into H_2O (15 mL), and extracted with ethyl acetate (3 × 10 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The remaining residue was subjected to column chromatography on silica gel using hexane/ethyl acetate in a 8:2 v/v ratio as eluent to furnish **1a** as a white solid (78 mg, 62%); mp 216−218 °C. ^1H NMR (400.0 MHz, DMSO-d_6): δ 8.42 (d, J = 2.4 Hz, 1H), 8.31 (d, J = 2.4 Hz, 1H), 8.15 (t, J = 1.7 Hz, 1H), 8.03 (dt, J = 1.7 Hz, 7.8 Hz, 1H), 7.86 (dd, J = 2.4 Hz, 8.4 Hz, 1H), 7.82−7.78 (m, 1H), 7.77 (d, J = 2.4 Hz, 1H), 7.68 (t, J = 8.4 Hz, 1H), 6.93 (d, J = 8.4 Hz, 1H), 5.97 (br s, 2H), 3.92 (s, 3H), 3.28 (s, 3H). ^{13}C NMR (100.6 MHz, DMSO-d_6): δ 163.4, 157.5, 147.1, 146.1, 142.2, 140.0, 139.5, 136.6, 130.9, 130.4, 127.4, 125.0, 124.2, 123.9, 117.7, 111.0, 53.7, 43.9. Anal. RP-HPLC t_R = 11.90 min (method 1A, purity 95.53). LRMS (APCI): m/z = 356.2 [(M + H)$^+$] (anal. calcd for $C_{18}H_{17}N_3O_3S^+$: m/z = 355.10).

General Procedure 2 for the Synthesis of (4-(6-Amino-5-(6-(trifluoromethyl)pyridin-3-yl)pyridin-3-yl)phenyl)-(morpholino)methanone 33. To a solution of **32** (0.25 g, 0.70 mmol) in 1,4-dioxane (5 mL), 2-(trifluoromethyl)pyridine-5-boronic acid pinacol ester (0.21 g, 0.76 mmol) was added. The mixture was thoroughly degassed with nitrogen for 15 min, at which time $Pd(PPh_3)_2Cl_2$ (0.03 g, 0.04 mmol) was added under an atmosphere of nitrogen, followed by (1M) aqueous K_2CO_3 (0.73 mL, 0.73 mmol). The reaction mixture was stirred at 90 °C for 14 h and extracted with ethyl acetate (4 × 10 mL). The combined organic layers were washed with brine (3 × 5 mL), dried over Na_2SO_4, and concentrated in vacuo. The remaining residue was subjected to column chromatography on silica gel using dichloromethane/methanol in a 9.7:0.3 v/v ratio as eluent to furnish **33** as a white solid. Yield 0.19 g, 62%; mp 122−124 °C. ^1H NMR (300.1 MHz, DMSO-d_6): δ 8.90 (d, J = 1.8 Hz, 1H), 8.43 (d, J = 2.4 Hz, 1H), 8.23 (dd, J = 2.4 Hz, 8.1 Hz, 1H), 7.96 (d, J = 8.4 Hz, 1H), 7.81 (d, J = 2.4 Hz, 1H), 7.75 (d, J = 8.4 Hz, 2H), 7.46 (d, J = 8.4 Hz, 2H), 6.06 (br s, 2H), 3.62 (br s, 4H), 3.52 (br s, 4H). ^{13}C NMR (100.6 MHz, DMSO-d_6): δ 168.9, 156.5, 150.0, 146.6, 138.6, 138.3, 137.4, 136.5, 133.5, 127.8, 125.4, 124.2, 123.2, 120.7, 115.7, 66.1, 47.8. Anal. RP-HPLC t_R = 4.90 min (method 1B, purity 99.2%). LR-MS (APCI): m/z = 429.1 [(M + H)$^+$] (anal. calcd for $C_{22}H_{19}F_3N_4O_2^+$: m/z = 428.15).

General Procedure 3 for the Synthesis of (4-(6-Amino-5-(6-(trifluoromethyl)pyridin-3-yl)pyridin-3-yl)phenyl)(4-methylpiperazin-1-yl)methanone 34. Thionyl chloride (0.74 mL, 10.23 mmol) was slowly added to a solution of **36** (0.6 g, 2.05 mmol) in dry dichloromethane (5 mL) and refluxed for 2 h. The reaction mixture was concentrated under reduced pressure and dissolved in dichloromethane (2 mL). A solution of 1-methyl piperazine (0.25 mL, 2.25 mmol) in triethylamine (0.86 mL, 6.14 mmol) was then added to the reaction mixture at 0 °C and allowed to stir for 6 h. The mixture was extracted with dichloromethane (2 × 5 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The remaining residue was subjected to column chromatography on silica gel using dichloromethane/methanol in a 9.7:0.3 v/v ratio as eluent to furnish (4-(6-amino-5-bromopyridin-3-yl)phenyl)(4-methylpiperazin-1-yl)-

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methanone as a colorless solid (0.65 g, 94%), which was used without further purification. To a solution of (4-(6-amino-5-bromopyridin-3-yl)phenyl)(4-methylpiperazin-1-yl)methanone (0.25 g, 0.67 mmol) in 1,4-dioxane (4 mL), 2-(trifluoromethyl)pyridine-5-boronic acid pinacol ester (0.20 g, 0.73 mmol) was added. The mixture was thoroughly degassed with nitrogen for 15 min, at which time $Pd(PPh_3)_2Cl_2$ (23 mg, 0.03 mmol) was added under an atmosphere of nitrogen, followed by (1M) aqueous K_2CO_3 (0.70 mL, 0.70 mmol). The reaction mixture was stirred at 90 °C for 14 h and extracted with ethyl acetate (4 × 10 mL). The combined organic layers were washed with brine (3 × 5 mL), dried over Na_2SO_4, and concentrated in vacuo. The remaining residue was subjected to column chromatography on silica gel using dichloromethane/methanol in a 9.7:0.3 v/v ratio as eluent to furnish **34** as a white solid (0.13 g, 45%); mp 82−84 °C. ^{1}H NMR (400.0 MHz, DMSO-d_6): δ = 8.92 (s, 1H), 8.43 (d, J = 2.4 Hz, 1H), 8.25 (d, J = 8.4 Hz, 1H), 7.99 (d, J = 8.4 Hz, 1H), 7.82 (d, J = 2.4 Hz, 1H), 7.75 (d, J = 8.4 Hz, 2H), 7.43 (d, J = 8.2 Hz, 2H), 6.22 (s, 2H), 3.60 (br s, 4H), 2.33 (br s, 4H), 2.20 (s, 3H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 168.8, 156.5, 150.0, 146.6, 138.5, 138.3, 137.4, 136.6, 133.9, 127.6, 125.4, 123.2, 120.7, 115.7, 54.5, 46.9, 45.5, 41.6. Anal. RP-HPLC t_R = 12.63 min (method 2A, purity 96%). LRMS (APCI): m/z = 442.2 [(M + H)$^+$] (anal. calcd for $C_{23}H_{22}F_3N_5O^+$: m/z = 441.18).

General Procedure 4 for the Synthesis of (4-(6-Amino-5-(6-(trifluoromethyl)pyridin-3-yl)pyridin-3-yl)phenyl)(piperazin-1-yl)methanone 35. EDCI (1.24 g, 6.49 mmol) was added in small batches to a solution of **36** (1.73 g, 5.90 mmol) and HOBt (0.88 g, 6.49 mmol) in dry DMF (15 mL). The reaction mixture was stirred for 10 min, at which time a solution of N-Boc piperazine (1.21 g, 6.49 mmol) and triethylamine (2.46 mL, 17.71 mmol) in dry DMF (2 mL) was added and the solution was stirred for an additional 12 h at room temperature. H_2O (10 mL) was added to the mixture and extracted with ethyl acetate (4 × 10 mL). The combined organic layers were washed, dried over Na_2SO_4, and concentrated in vacuo. The remaining residue was subjected to column chromatography on silica gel using ethyl acetate to yield (4-(6-amino-5-bromopyridin-3-yl)phenyl)(N-Boc piperazin-1-yl)methanone (1.61 g, 59%), which was used without further purification. To a solution of (4-(6-amino-5-bromopyridin-3-yl)phenyl)(N-Boc piperazin-1-yl)methanone (0.25 g, 0.54 mmol) in 1,4-dioxane (4 mL), 2-(trifluoromethyl)pyridine-5-boronic acid pinacol ester (0.16 g, 0.60 mmol) was added. The mixture was thoroughly degassed with nitrogen for 15 min, at which time $Pd(PPh_3)_2Cl_2$ (19 mg, 0.03 mmol) was added under an atmosphere of nitrogen, followed by aqueous K_2CO_3 (0.57 mL, 0.57 mmol). The reaction mixture was stirred at 90 °C for 14 h, poured into H_2O (10 mL), and extracted with ethyl acetate (4 × 10 mL). The combined organic layers were washed with brine (3 × 5 mL), dried over Na_2SO_4, and concentrated in vacuo. The remaining residue was subjected to column chromatography on silica gel using dichloromethane/methanol in a 9.7:0.3 v/v ratio as eluent to furnish (4-(6-amino-5-(6-(trifluoromethyl)pyridin-3-yl)pyridin-3-yl)-phenyl)(N-Boc piperazin-1-yl)methanone (0.17 g, 58%) as a white solid, which was used without further purification. To a suspension of 4-(6-amino-5-(6-(trifluoromethyl)pyridin-3-yl)pyridin-3-yl)phenyl)(N-Boc piperazin-1-yl)methanone (0.17 g, 0.32 mmol) in dichloromethane (2 mL) was added trifluoroacetic acid (0.12 mL, 1.61 mmol). The pale-yellow solution was stirred vigorously for 12 h. The reaction mixture was concentrated under reduced pressure to give a pale-yellow solid. The resulting powder was stirred with Amberlyst A-21 resin in a dichloromethane/methanol 1:1 v/v ratio (15 mL) for 1 h. The reaction mixture was then filtered, and the solvent was removed under reduced pressure to yield **35** as hygroscopic powder (0.12 g, 90%); mp 92−94 °C. ^{1}H NMR (400.0 MHz, DMSO-d_6): δ 8.91 (d, J = 1.8 Hz, 1H), 8.42 (d, J = 2.4 Hz, 1H), 8.24 (dd, J = 1.9 Hz, 8.4 Hz, 1H), 7.98 (d, J = 8.4 Hz, 1H), 7.81 (d, J = 2.4 Hz, 1H), 7.74 (d, J = 8.4 Hz, 2H), 7.41 (d, J = 8.4 Hz, 2H), 6.19 (s, 2H), 3.38 (br s, 2H), 3.26 (br s, 2H), 2.67 (br s, 4H). ^{13}C NMR (100.6 MHz, DMSO-d_6): δ 168.8, 156.5, 150.1, 146.6, 138.6, 137.4, 136.6, 133.9, 127.7, 125.4, 124.3, 123.2, 120.7, 115.7, 47.6, 45.3, 42.5. Anal. RP-HPLC t_R = 5.66 min (method 2B, purity 98%). LRMS (APCI): m/z = 428.2 [(M + H)$^+$] (anal. calcd for $C_{22}H_{20}F_3N_5O^+$: m/z = 427.16).

■ ASSOCIATED CONTENT

Supporting Information

Additional details of the characterization of selected compounds and the procedures used for the in vitro and in vivo antimalarial studies as well as in vitro metabolism and mouse exposure studies. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

DMF, dimethylformamide; po, oral administration; HPLC, high pressure liquid chromatography; HPMC, hydroxypropylmethyl cellulose; MSD, mean survival time; SAR, structure−activity relationship; PK, pharmacokinetics; TLC, thin layer chromatography; TMS, tetramethylsilane; MMV, Medicines for Malaria Venture

■ REFERENCES

(1) (a) Miller, L. H.; Baruch, D. I.; Marsh, K.; Doumbo, O, K. The pathogenic basis of malaria. *Nature* **2002**, *415*, 673−679. (b) Burrows, N. J.; Chibale, K.; Wells, T. N. C. The state of the art in anti-malarial drug discovery and development. *Curr. Top. Med. Chem.* **2011**, *11*, 1226−1254. (c) Biot, C.; Chibale, K. Novel approaches to antimalarial drug discovery, Infect. *Disord. Drug Targets* **2006**, *6*, 173−204.

(2) *World Malaria Report 2011*; World Health Organization: Geneva, 2011.

(3) Wells, T. N. C.; Alonso, P. L.; Gutteridge, W. E. New medicines to improve control and contribute to the eradication of malaria. *Nature Rev. Drug Discovery* **2009**, *8*, 879−891.

(4) (a) Olliaro, P. L. and Boland, P. B. Clinical Public Health Implications of Antimalarial Drug Resistance. *Antimalarial Chemotherapy: Mechanisms of Action, Resistance, and New Directions in Drug Discovery*; Rosenthal, P. J., Ed.; Humana Press: Totowa, NJ, 2001; pp 65−83; (b) Ridley, R. G. Medical need, scientific opportunity and the drive for antimalarial drugs. *Nature* **2002**, *415*, 686−693.

(5) Younis, Y.; Douelle, F.; Feng, T-. S.; Gónzalez Cabrera, D.; Le Manach, C.; Nchinda, A. T.; White, K.; Shackleford, D. M.; Morizzi, J.; Mannila, J.; Kasiram Katneni, K. L.; Bhamidipati, R.; Zabiulla, K. M.; Joseph, J. T.; Bashyam, S.; Waterson, D.; Witty, M. J.; Hardick, D.; Wittlin, S.; Avery, V.; Charman, S. A.; Chibale, K. 3,5-Diaryl-2-aminopyridines as a novel class of orally active antimalarials demonstrating single dose cure in mice and clinical candidate potential. *J. Med. Chem.* **2012**, *55*, 3479−3487.

(6) (a) Redfern, W. S.; Carlsson, L.; Davis, A. S.; Lynch, W. G.; MacKenzie, I.; Palethorpe, S.; Siegl, P. K. S.; Strang, I.; Sullivan, A. T.; Wallis, R.; Camm, A. J.; Hammond, T. G. Relationships between preclinical cardiac electrophysiology, clinical QT interval prolongation and torsade de pointes for a broad range of drugs: evidence for a provisional safety margin in drug development. *Cardiovasc. Res.* **2003**,

58, 32−45. (b) Jamieson, C.; Moir, E. M.; Rankovic, Z. Medicinal chemistry of hERG optimizations: highlights and hang-ups. *J. Med. Chem.* **2006**, *49*, 5030−5046.
(7) (a) Miyaura, N.; Suzuki, A. Palladium-Catalyzed Cross-Coupling Reactions of Organoboron Compounds. *Chem. Rev.* **1995**, *95*, 2457−2483. (b) Thompson, A. E.; Hughes, G.; Batsanov, A. S.; Bryce, M. R.; Parry, P. R.; Tarbit, B. Palladium-Catalyzed Cross-Coupling Reactions of Pyridylboronic Acids with Heteroaryl Halides Bearing a Primary Amine Group: Synthesis of Highly Substituted Bipyridines and Pyrazinopyridines. *J. Org. Chem.* **2005**, *70*, 388−390.
(8) Srinivasan, N.; Yurek-George, A.; Ganesan, A. Rapid deprotection of *N*-Boc amines by TFA combined with freebase generation using basic ion-exchange resins. *Mol. Diversity* **2005**, *9*, 291−293.
(9) Charman, S. A.; Arbe-Barnes, S.; Bathurst, I. C.; Brun, R.; Campbell, M.; Charman, W. N.; Chiu, F. C. K.; Chollet, J.; Craft, J. C.; Creek, D. J.; Dong, Y.; Matile, H.; Maurer, M.; Morizzi, J.; Nguyen, T.; Papastogiannidis, P.; Scheurer, C.; Shackleford, D. M.; Sriraghavan, K.; Stingelin, L.; Tang, Y.; Urwyler, H.; Wang, X.; White, K. L.; Wittlin, S.; Zhou, L.; Vennerstrom, J. L. Synthetic ozonide drug candidate OZ439 offers new hope for a single-dose cure of uncomplicated malaria. *Proc. Natl. Acad. Sci. U. S. A.* **2011**, *108*, 4400−4405.
(10) Obach, R. S. Prediction of Human Clearance of Twenty-Nine Drugs from Hepatic Microsomal Intrinsic Clearance Data: An Examination of In Vitro Half-Life Approach and Nonspecific Binding to Microsomes. *Drug Metab. Dispos.* **1999**, *27*, 1350−1359.
(11) González Cabrera, D.; Douelle, F.; Feng, T.-S.; Nchinda, A. T.; Younis, Y.; White, K. L.; Wu, Q.; Ryan, E.; Burrows, J. N.; Waterson, D.; Witty, M. J.; Wittlin, S.; Charman, S. A.; Chibale, K. Novel Orally Active Antimalarial Thiazoles. *J. Med. Chem.* **2011**, *54*, 7713−7719.