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A Novel Pyrazolopyridine with in Vivo Activity in *Plasmodium berghei*- and *Plasmodium falciparum*-Infected Mouse Models from Structure–Activity Relationship Studies around the Core of Recently Identified Antimalarial Imidazopyridazines

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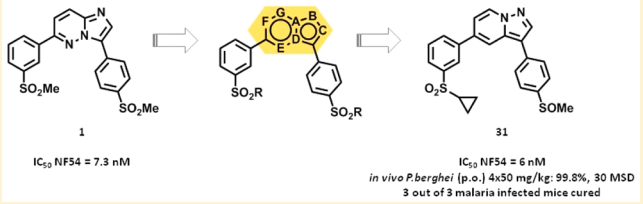
Supporting Information

ABSTRACT: Toward improving pharmacokinetics, in vivo efficacy, and selectivity over hERG, structure–activity relationship studies around the central core of antimalarial imidazopyridazines were conducted. This study led to the identification of potent pyrazolopyridines, which showed good in vivo efficacy and pharmacokinetics profiles. The lead compounds also proved to be very potent in the parasite liver and gametocyte stages, which makes them of high interest.



INTRODUCTION

Despite a notable decrease in the burden of disease between 2000 and 2012, malaria remains a major global health issue, which still accounted for about 584 000 deaths in 2013.[1] Better prevention and diagnosis, along with the use of artemisinin-based combination therapies (ACTs), have contributed to malaria-related deaths around the world being reduced by more than 40% over the last 12 years. However, signs of resistance to artemisinin have recently emerged in South-East Asia,[2−4] highlighting that the global efforts against malaria should be maintained, if not strengthened. In this context, identifying new drugs with new mechanisms of action and activity against resistant strains of the parasite remains an urgent need.

We recently reported the activity of imidazopyridazine derivatives against *Plasmodium falciparum*,[5,6] of which **1** and **2** (Figure 1) showed promising properties both in vitro and in vivo. Related antimalarial imidazopyrazines have recently been identified.[7] The solubility, in vivo efficacy, and exposure of the previously reported imidazopyridazine **1**[5] were improved with

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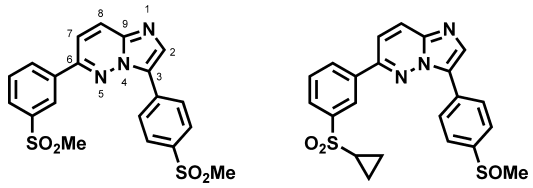
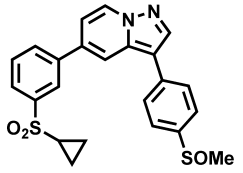


1 2

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

2,[6] which was the first compound of the imidazopyridazine series to cure mice from *Plasmodium berghei* infection at 4 × 50 mg/kg/day. However, better in vivo efficacy at lower doses has yet to be achieved, and some off-target activities, such as hERG, remain an issue. In order to extend the previous structure−activity relationship (SAR) studies and to improve pharmacokinetics (PK), in vivo efficacy, and selectivity over hERG, we focused on changes to the imidazopyridazine heterocycle. Our strategy was recently reinforced by other studies, in which imidazopyridines and imidazolopiperazines[8,9] that showed good PK properties and in vivo efficacy were identified. Herein, we describe how SAR investigations around the central core of our imidazopyridazines combined with a sulfoxide/sulfone prodrug-like strategy, which we previously adopted, led to compound **31** (Figure 2), which displays good physicochemical



31

Figure 2. Structure of compound **31**.

properties, in vivo efficacy in both *P. falciparum* SCID and *P. berghei* mouse models, and good oral pharmacokinetics. In addition to these studies, lead compounds were also profiled for activity against liver and gametocyte life cycle stages in order to obtain a broader view of the potential of this series of compounds to contribute to the malaria eradication agenda.

CHEMISTRY

Compounds **1**−**4** were prepared as previously described.[5,6]

Triazolopyridazine **8**, imidazopyrimidine **9**, imidazopyrazine **10**, imidazopyridines **11** and **12**, pyrazolopyridines **17**−**19**, and pyrazolopyrimidines **20**−**22** were synthesized in three (four for **20**) steps from the corresponding 6-halogenated heterocycles (Scheme 1). The scaffolds were either commercially available (**5a**, **13a**, **13b**) or prepared in-house (**5b**−**d**). The preparation of those that were not commercially available is described in the Supporting Information. Briefly, from the halogenated 5,6-bicyclic heterocycle, a Suzuki cross-coupling reaction was first performed with the appropriate boronic acid.[10] The resulting product was then reacted with NBS in DMF at room temperature to give the bromo derivative in high yield. Finally, a second Suzuki cross-coupling reaction with 4-(methylsulfonyl)phenyl boronic acid was performed to give the desired compounds. Compound **20** was prepared from 4-methylsulfoxide intermediate **20a**, which was then oxidized with *m*CPBA. Compounds **20** and **22** were reduced using $NaBH_4$ and a catalytic amount of $Pd(PPh_3)Cl_2$ to give their saturated analogues, **23** and **24**, respectively.

Compounds **29** and **30** were prepared following the synthetic route depicted in Scheme 2. 2-Chloro-5-iodopyridine **25** was reacted with hydrazine to give 2-hydrazinyl-5-iodopyridine **26**, which was then reacted with 4-(methylsulfonyl)benzaldehyde to give intermediate **27**. Iodo compound **28** was formed after a ring closure with (diacetoxy)-iodobenzene.[11] Finally, a Suzuki cross-coupling reaction was carried out with 3-trifluoromethylphenyl boronic acid, 3-(methylsulfonyl)phenyl boronic acid, and 3-(methylsulfinyl)-phenylboronic acid to give desired products **29**−**31**, respectively.

IN VITRO ANTIPLASMODIAL ACTIVITY

All compounds were evaluated for in vitro antiplasmodial activity against a sensitive strain (NF54), and selected compounds (12 out of 19) were tested against a multidrug resistant (K1) strain of *P. falciparum*. Chloroquine, artesunate, and **1** were used as the reference drugs in all experiments. The in vitro antiplasmodial activities of these compounds, as indicated by their IC_{50} values, are summarized in Table 1.

In general, all analogues were equipotent against both strains (K1 and NF54). Representative compounds of each series have been tested for cytotoxicity, including **1**, **3**, **12**, **18**, **19**, **22**, **24**, and **31** (Table D1 in the Supporting Information). Except for compound **12** (selectivity index = 700), all compounds tested had a selectivity index of >1000. Therefore, cytotoxicity was not considered to be an issue for this series.

Interestingly, the previously observed SAR trends in the imidazopyridazine series were conserved across the different scaffolds. Cyclopropylsulfone derivatives tend to be more potent than their methylsulfone analogues, which are, in turn, more potent than the trifluoromethyl derivatives. Moving from the imidazopyridazine to the pyrazolopyrimidine scaffold improved the activity by about 2-fold (IC_{50} K1/NF54 (nM): **3**, 0.53/0.88; **22**, 0.37/0.42). A decrease in potency was observed when the nitrogen at the 5-position was removed, yet the compounds retained good activity with IC_{50} values in the 50−150 nM range for the imidazopyridines (IC_{50} K1/NF54 (nM): **11**, 113/150; **12**, 38/46) and IC_{50} < 10 nM for the pyrazolopyridines (IC_{50} NF54 (nM): **18**, 8; **19**, 2). As before, moving the nitrogen from position 4 to position 9 resulted in an increase in activity, as the comparison between direct analogues **12** and **19** shows (IC_{50} NF54 (nM): 46 and 2, respectively). Compound **31**, the sulfoxide analogue of **19**, also showed potent activity (IC_{50} NF54 = 6 nM). Tetrahydropyrazolopyrimidines **23** and **24** were also tolerated, with IC_{50} < 100 nM.

On the other hand, adding a nitrogen at the 2 position appeared to be detrimental for activity, as **8**, **29**, and **30** all displayed IC_{50} > 1000 nM. Equally, the introduction of a nitrogen at the 7 (compound **10**) or 8 (compound **9**) position proved to be ineffective (IC_{50} NF54 > 800 nM)

IN VITRO ADME PARAMETERS

Metabolic stability of the most active compounds was assessed in vitro in human, rat, and mouse liver microsomal preparations. All active compounds were submitted for a single readout assay that determines the percentage compound remaining after a 30 min incubation in the presence of liver microsomes. The microsome-predicted hepatic extraction ratios

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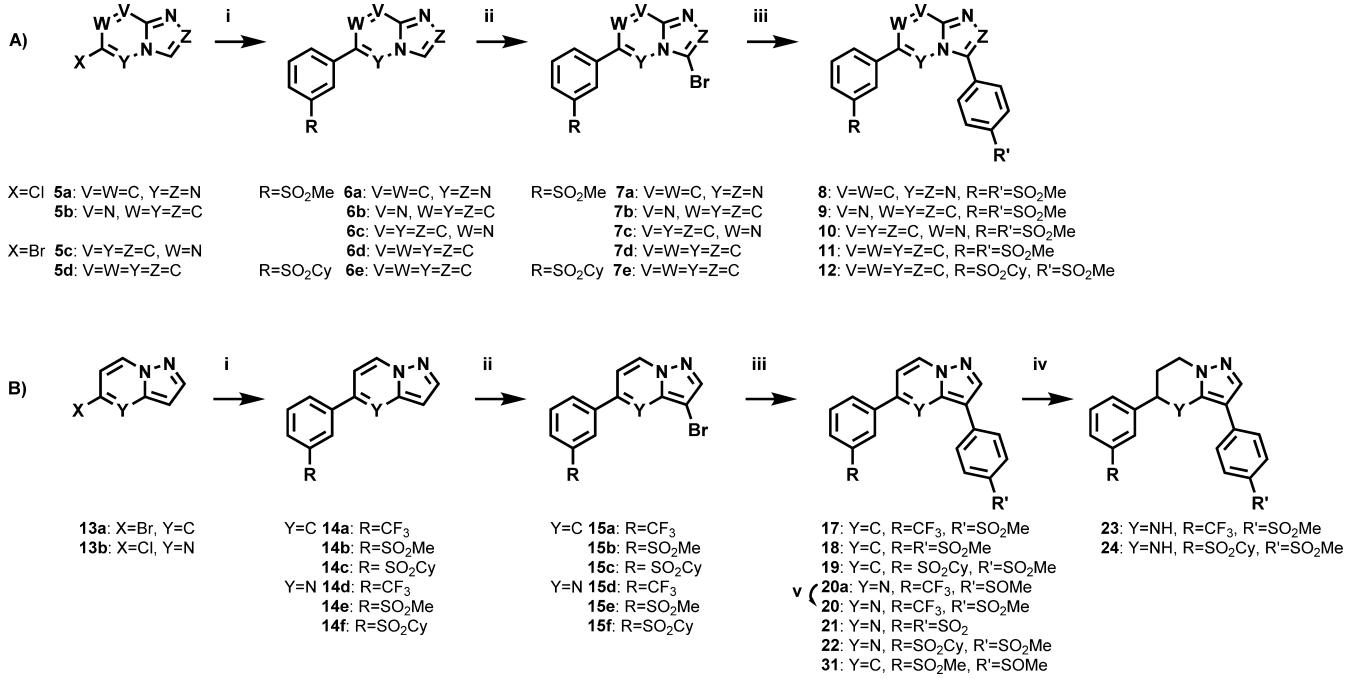
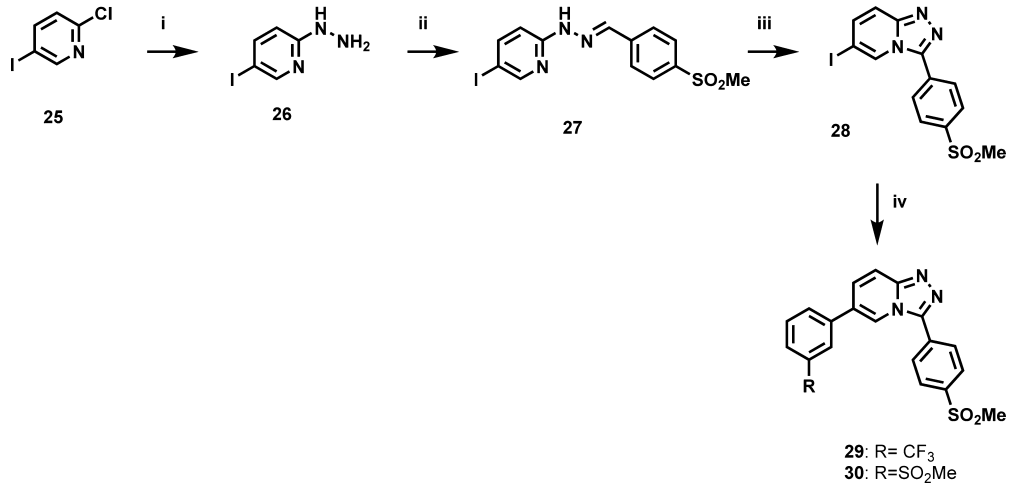
Scheme 1. Preparation of Compounds 8−12 (A) and 17−24 (B)[a]



[a]Reagents and conditions: (i) 3-R-Ph-B(OH)$_2$ (1.1 equiv), Pd(PPh$_3$)$_2$Cl$_2$ (0.05 equiv), aq. K$_2$CO$_3$ (1 M) (1.05 equiv), DMF, 90 °C; (ii) NBS (1.1 equiv), DMF, rt, 1 h; (iii) 3-R′-Ph-B(OH)$_2$ (1.1 equiv), Pd(PPh$_3$)$_2$Cl$_2$ (0.05 equiv), aq. K$_2$CO$_3$ (1 M) (1.05 equiv), DMF, 90 °C; (iv) NaBH$_4$ (10 equiv), Pd(PPh$_3$)$_2$Cl$_2$ (20 wt %), DCM/EtOH; (v) *m*CPBA (1.2 equiv), DCM, 0 °C to rt, 1 h.

Scheme 2. Preparation of Compounds 29 and 30[a]



[a]Reagents and conditions: (i) NH$_2$-NH$_2$ (5 equiv), EtOH; (ii) 4-SO$_2$-benzaldehyde (1 equiv), EtOH; (iii) PhI(OAc)$_2$ (1.4 equiv), DCM, rt; (iv) 3-R-Ph-B(OH)$_2$, (1.2 equiv), Pd(PPh$_3$)$_2$Cl$_2$ (0.07 equiv), K$_2$CO$_3$ (1) (1.5 equiv), dioxane/water (25:10), 110 °C.

(E_H) were determined for selected compounds using a five-point assay (60 min). All results are summarized in the Supporting Information (Table E1). All compounds proved to be stable across the three species (human, rat, and mouse).

The solubility of most compounds remained poor. Compounds **10** and **24** had improved solubility at pH 2, but, generally, none of these core changes improved solubility.

■ HERG ACTIVITY

The activity against the hERG potassium channel was determined using in vitro IonWorks patch-clamp electrophysiology.[12] Selected compounds were evaluated for hERG activity in order to determine if any of the core changes would facilitate mitigating cardiotoxicity risks. Generally, hERG liability remains high, with IC$_{50}$'s less than 10 μM. Pyrazolopyrimidine **22** (hERG IC$_{50}$: 0.5 μM) showed similar activity against hERG as that of its imidazopyridazine analogue, **3** (hERG IC$_{50}$: 0.4 μM). In the imidazopyridine series, **11** (hERG IC$_{50}$: 4.8 μM) showed a slight improvement compared to **1** (hERG IC$_{50}$: 0.9 μM). Excitingly, compound **18** proved to be devoid of hERG activity with an IC$_{50}$ > 33 μM, as compared to 0.9 for the analogous imidazopyridazine **1**. The lack of hERG activity in the pyrazolopyridine series, however, appears to hold only for the methylsulfone, as the more active cyclopropyl-

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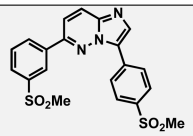
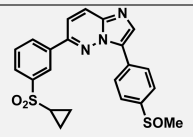
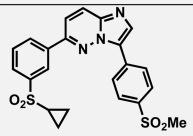
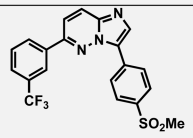
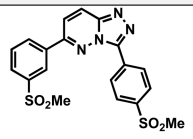
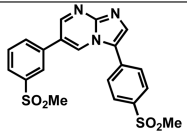
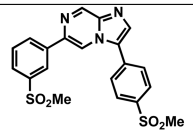
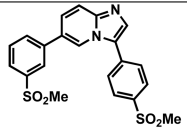
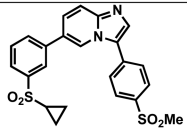
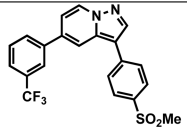
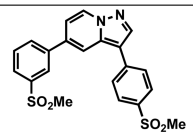
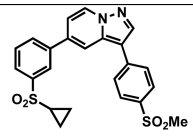
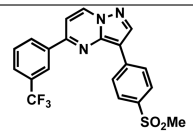
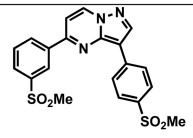
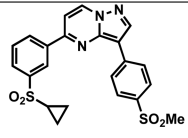
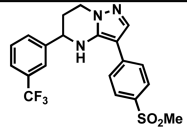
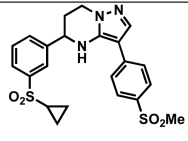
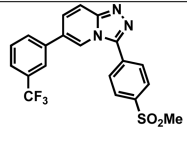
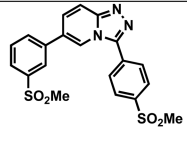
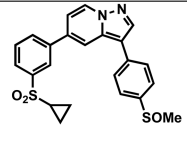
Table 1. Antiplasmodial Activities

Compd	Structure	Pf IC_{50} (nM)[a,b]	
		K1	NF54
1		6.3	7.3
2		-	1.1
3		0.53	0.88
4		46	60
8		-	>2336
9		>1000	>1000
10		-	877
11		113	150
12		38	46
17		-	98
18		-	8
19		2.4	2.7
20		43	48
21		2.2	2.6
22		0.37	0.42
23		-	818
24		46	44
29		>1000	>1000
30		>1000	>1000
31		-	6

[a]Mean from *n* values of ≥2 independent experiments with multidrug resistant (K1) and sensitive (NF54) strains of *P. falciparum*. The majority of the individual values varied by less than 2× (maximum 3×). [b]Chloroquine and artesunate were used as reference drugs in all experiments. Against NF54 and K1, our laboratory standard IC_{50} values for chloroquine and artesunate are 16 nM/194 nM and 4.0 nM/3.0 nM (mean from ≥10 independent assays). IC_{50} values that differed more than 3× from laboratory standard values were not included in the analysis.

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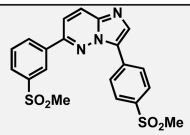
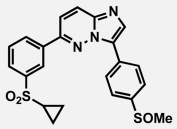
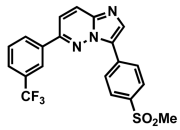
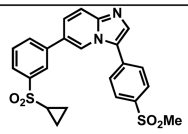
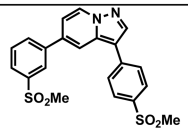
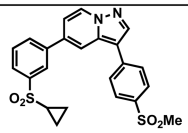
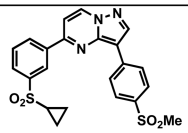
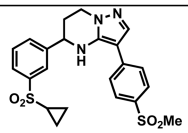
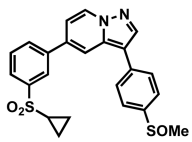
Table 2. In Vivo Antimalarial Oral Efficacy of Selected Compounds in the *P. berghei* Mouse Model

Compound	Structure	Oral dose (mg/kg)[a]	% reduction parasitemia (MSD)[b,c,d]
1		4 x 50	99.8 (7)
2		4 x 50	99.8 (>30)
			3 out of 3 mice cured
		4 x 10	99.6 (8)
		4 x 3	98 (7)
4		4 x 50	99.8 (>30)
			3 out of 3 mice cured
		4 x 10	70(7)
		4 x 3	<40 (4)
12		4 x 50	99.5 (10)
18		4 x 50	60 (4)
19		4 x 50	71 (7)
22		4 x 50	86 (7)
24		4 x 50	78 (7)
31		4 x 50	99.8 (>30)
			3 out of 3 mice cured
		4 x 10	99.9 (8)
		4 x 3	75 (7)
Chloroquine[e]		4 x 30	99.9 (24)
Artesunate[e]		4 x 30	99 (10)
Mefloquine[e]		4 x 30	99.9 (29)

[a]Once per day on 4 consecutive days (4, 24, 48, and 72 h after infection). [b]MSD = mean survival time (in days). [c]Mice with <40 and 60% parasitemia reduction were euthanized on day 4 in order to prevent death otherwise occurring at day 6. [d]Artesunate and mefloquine were dissolved or suspended in a nonsolubilizing, standard suspension vehicle called SSV (0.5% [w/v] carboxymethylcellulose, 0.5% [v/v] benzyl alcohol, 0.4% [v/v] Tween 80, and 0.9% [w/v] sodium chloride in water). [e]Data from Le Manach et al.[21]

sulphone analogue **19** has an IC_{50} for hERG of 1.4 μM. We reasoned that the nitrogen at the 5-position of the imidazopyridazine ring might be involved in an interaction with the hERG channel, but the activity for hERG appears to be

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more complex given the difference between the IC_{50}'s of **18** and **19**.

IN VIVO EFFICACY, PHARMACOKINETIC STUDIES, AND METABOLITE IDENTIFICATION

A representative compound of each series with good in vitro antiplasmodial activity and microsomal metabolic stability, e.g., imidazopyridine **12**, pyrazolopyridine **18**, pyrazolopyrimidine **22**, and tetrahydro-pyrazolopyrimidine **24**, was evaluated for in vivo activity in *P. berghei*-infected mice. The in vivo activity was determined following oral administration (p.o.) of 50 mg/kg/day for 4 consecutive days. If the mice were cured at 4 × 50 mg/kg, then further studies were carried out at lower doses of 4 × 10 mg/kg and 4 × 3 mg/kg. The efficacy results are summarized in Table 2. Previously published results for **1** and **2** are also included in the table for comparison. For each compound, a mouse snapshot PK was performed using blood samples from the in vivo efficacy experiments and a complementary rat PK was independently carried out. Generally, there was a good correlation between the observed in vivo efficacy and the pharmacokinetic parameters.

Even though **22** displayed similar physicochemical properties as those of **1** and was 10 times more active in vitro, the potency did not translate to in vivo efficacy, providing only 86% activity and 7 mean survival days (MSD). The reduced analogue of pyrazolopyrimidine **22** (the tetrahydropyrazolopyrimidine **24**) also failed to show good activity in vivo, with only 78% of the parasitemia cleared after treatment. Compound **12** showed a similar activity in vivo to that of **1**, with 99.5% activity but with no major improvement, albeit the MSD was slightly better, increasing from 7 to 10 days. Pyrazolopyridines **18** and **19** performed poorly, with 60 and 71% of parasitemia cleared after treatment, respectively, and less than 7 days survival (4 days for **18**; 7 days for **19**).

In all cases, plasma exposure was poor following oral dosing and bioavailability was very low, even close to null in the cases of **18** and **22** (Tables 3 and 4).

Table 3. Mouse Snapshot Pharmacokinetic Profiles for Compounds 18, 22, 24, and 31 after the First Oral Administration of 50 mg/kg

Compound	Whole-Blood Concentration (μM)		
	1 h	4 h	24 h
18	7.6	0.486	0.29
19	0.697	1.061	BLQ[a]
22	0.169	0.145	<0.01
24	0.456	0.177	BLQ[a]
31	2.96	1.78	0.03
metabolized into **19**	7.9	10.3	3.1

[a]Below detection limits.

On the other hand, **31** displayed 99.8% parasitemia reduction at 4 × 50 mg/kg with >30 survival days, and 3 out of 3 mice were cured. This compound also displayed good activity at the lower multiple doses of 4 × 10 mg/kg (99.9% activity) and 4 × 3 mg/kg (75% activity), albeit there was no improvement in mean survival days, with 8 MSD at 4 × 10 mg/kg and 7 MSD at 4 × 3 mg/kg.

Pharmacokinetic and metabolite identification studies in vitro after incubation with liver microsomes and in vivo using blood samples from the rat PK experiment to confirm that **19** was, indeed, the major metabolite of **31** validated our strategy, as they clearly showed that **31** was metabolized mainly to the active sulfone **19** (Figure 3). In rats, further oxidation of the sulfone (as a metabolite of **31** or as direct parent) occurred on the pyridine core or phenyl rings (M1, M3, M4) and, more unexpectedly, on the pyrazole part of the core (M2). Metabolically liable sites of pyrazolo[1,5-*a*]pyridines are known to be mainly on the pyridine core (positions 5, 7, and 8),[13] which are electron-rich due to electron-donating resonance effects of bridge-head nitrogens, or on alkyl side chains when present at positions 2 and 3[14] (see Supporting Information for chromatograms and fragmentation patterns).

Table 4. Rat PK Parameters for Compounds 18, 22, 24, and 31

Compound	Method	Dose	$t_{1/2}$ (h)	Plasma Cl (mL/min/kg)	V_d (L/kg)	*B* (%)
12	p.o.	20	3.9			51
	i.v.	5	3.0	8.7	2.3	
18	p.o.	15	2.6			1
	i.v.	5	4.1	13.9	4.8	
19	p.o.	10	5.7			61
	i.v.	3	2.7	3.5	0.8	
22	p.o.	20	7.8			1
	i.v.	5	2.8	1.1	0.3	
31	p.o.	15	1.0			14
	i.v.	5	2.7	2.9	6.8	

The mouse snapshot PK from the in vivo experiment of **19** made it clear that exposure was poor following oral administration, which correlates with the poor in vivo efficacy of **19**. On the other hand, the mouse snapshot PK from the in vivo efficacy study of **31** showed a very good exposure of **19** following administration of **31**, with a significant amount of the drug still present in the blood after 24 h (3 μM). These findings confirm the advantage of using the sulfoxide over the sulfone to improve solubility and bioavailability.

In addition to the mouse snapshot PK, full rat PK studies of both **31** and **19** were carried out. The parent sulfoxide, **31**, was rapidly absorbed and converted to metabolite **19**, therefore yielding a low oral bioavailability for the sulfoxide of 14%. The bioavailability of the sulfone metabolite **19** was calculated to be 25% in this experiment. PK studies with **19** showed a bioavailability of 61%, which does not correlate with the poor in vivo efficacy. Moreover, it does not seem to reflect the benefits of the sulfoxide for in vivo activity. The disconnect between the results may be due to species differences between rats and mice.

The therapeutic efficacy of **31** was also assessed against *P. falciparum* in a SCID mouse model[15] following administration of one oral dose per day for four consecutive days and measuring the effect on blood parasitemia. Blood samples were also collected for a corresponding oral exposure evaluation. Blood levels of both **31** and its metabolite, **19**, were measured because these compounds are equipotent against *P. falciparum* and levels of metabolite **19** were observed to be higher than those of parent compound **31**. Under these conditions, **31** (considered to be the sum of parent compound **31** and its main metabolite, **19**) was efficacious against *P. falciparum* upon once-a-day for 4 consecutive days administration, with a 90% effective dose of ED_{90} = 0.5 mg/kg and exposure at ED_{90} of AUC_{ED90} = 1 μg·h/mL per day, which is better than the standard marketed antimalarials artesunate (11.1 mg/kg),

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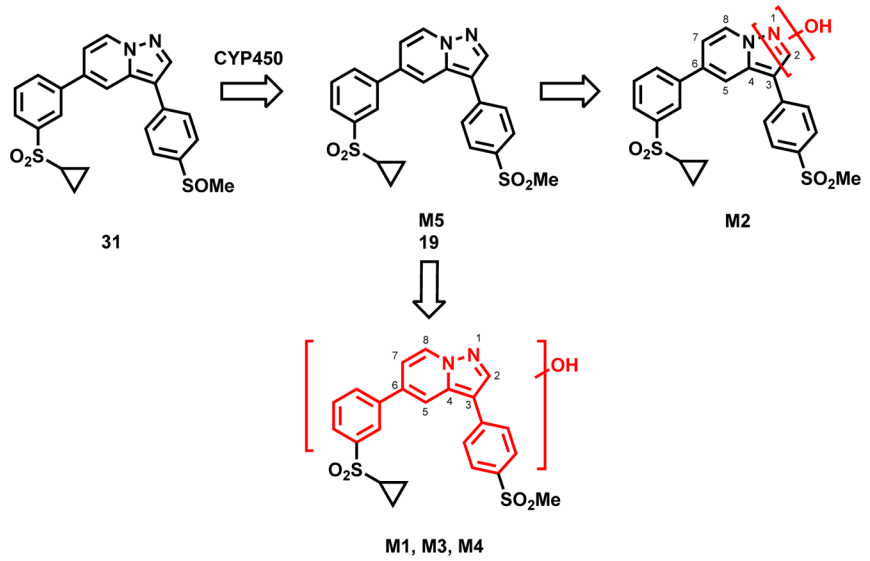

Figure 3. Proposed metabolic pathway of **31** and **19** in rats.

Table 5. Exposure of 31 and 19 in *P. falciparum*-Infected Humanized Mouse after Oral Administration of 31

Compound	Dose ($mg \cdot kg^{-1}$)	C_{max} ($\mu g \cdot mL^{-1}$)	t_{max} (h)	$AUC_{(0-t)}$ ($\mu g \cdot h/mL^{-1}$)
31	0.1	0.002	0.25	0.0021[a]
	0.5	0.004	0.25	0.0121[b]
	1.5	0.030	0.5	0.0405[b]
	10	0.050	2	0.293[b]
	30	0.226	6	1.2354[c]
19	0.1	0.025	2	0.139[b]
	0.5	0.106	2	1.012[c]
	1.5	0.467	2	3.196[c]
	10	2.600	4	24.78[c]
	30	7.43	8	78.28[c]

[a]t = 2 h. [b]t = 8 h. [c]t = 23 h.

chloroquine (4.3 mg/kg), pyrimethamine (0.9 mg/kg),[16] and mefloquine (7.7 mg/kg)[17] in the same model. Exposure data is summarized in Table 5.

IN VITRO EVALUATION IN THE *P. BERGHEI* LIVER AND GAMETOCYTE MALARIA PARASITE LIFE CYCLE STAGES

Our lead compounds **1**, **18**, **19**, and **22** were also profiled against different parasite life cycle stages to evaluate their potential as dual- and/or triple-action antimalarials. Activities are reported in Table 6. IC_{50}'s were obtained for the *P. berghei* liver stage and the early and late stage gametocytes (EG and LG, respectively). Excitingly, all compounds proved to be very potent, with activities less than 20 nM for the liver stage and less than 100 nM for the gametocytes, both in the early and late stages. Pyrazolopyrimidine **22** stands out among the lead compounds, with activities in the single-digit nanomolar range (targeting EG and LG equally at ~2 nM). **31** was also potent against EG (IC_{50} = 72 nM) and LG (IC_{50} = 163 nM), which makes it very interesting as a prodrug, as this activity will be reinforced by the activity of **19**.

Table 6. *P. berghei* Liver and Gametocyte Activities for 1, 18, 19, 22, and 31

		Gametocytes IC_{50} (nM)	
Compound	*P. berghei* Liver IC_{50} (nM)	Early Stage	Late Stage
1	4.1	60	27
18	6.6	94	66
19	20	4.5	29
22	0.61	2.0	1.4
31		163	72

CONCLUSIONS

The SAR investigations around the central core of imidazopyridazine **1** led to the identification of highly potent compounds against *P. falciparum*, with in vitro activities <50 nM and as low as 0.5 nM for imidazopyrimidine **22** and 2 nM for pyrazolopyridine **19**. In vitro potency did not translate to in vivo efficacy due to the poor pharmacokinetic profiles of compounds **18** and **22**. However, a prodrug approach applied to methylsulfone **19** led to the preparation of the methylsulfoxide **31**, which cured mice infected with *P. berghei* parasites at 4 × 50 mg/kg. Metabolite identification studies showed that **31** rapidly generates **19** in vivo, leading to higher plasma exposure compared to the direct use of **19**. The lack of hERG liability for **18** was gratifying, but, unfortunately, it did not translate across all compounds in the pyrazolopyridine series. Our lead compounds also proved to be highly potent against *P. berghei* liver stage as well as early and late stage gametocytes. This finding makes them highly desirable in the

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antimalarial pipeline. Of particular interest is the activity of **31** against both blood and gametocyte stages, which will be enhanced by the activity of its metabolite, **19**.

EXPERIMENTAL SECTION

All commercially available chemicals were purchased from either Sigma-Aldrich or Combi-Blocks. All solvents were dried by appropriate techniques. Unless otherwise stated, all solvents used were anhydrous. Infrared (IR) absorptions were measured on a PerkinElmer Spectrum One FT-IR spectrometer. ^{1}H NMR spectra were recorded on a Varian Mercury spectrometer at 300 MHz or a Varian Unity spectrometer at 400 MHz, with Me_4Si as the internal standard. ^{13}C NMR spectra were recorded at 75 MHz on a Varian Mercury spectrometer or at 100 MHz on Varian Unity spectrometer, with Me_4Si as internal standard. High-resolution mass spectra were recorded on a VG70 SEQ micromass spectrometer. Melting points were determined using a Reichert-Jung Thermovar hot-stage microscope and are uncorrected. Analytical thin-layer chromatography (TLC) was performed on aluminum-backed silica-gel 60 F_{254} (70−230 mesh) plates. Column chromatography was performed with Merck silica-gel 60 (70−230 mesh). Chemical shifts (δ) are given in ppm downfield from TMS as the internal standard. Coupling constants, *J*, are recorded in hertz (Hz).

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

The data that is not shown below is supplied in the Supporting Information.

General Procedure for the First Suzuki Cross-Coupling Reaction. Compounds **5a−d** (respectively, **13a−b**) (1 equiv) were dissolved in DMF with the corresponding boronic acid (1.1 equiv) and $Pd(PPh_3)_2Cl_2$ (0.05 equiv). The resulting mixture was flushed with nitrogen for 15 min, after which aqueous K_2CO_3 (1 M) (1.05 equiv) was added. The solution was heated at 90 °C and stirred for 12 h at this temperature. After dilution in DCM and water, the solution was extracted with DCM three times. The combined organic phases were rinsed with brine and dried over Na_2SO_4. The solvents were removed in vacuo, and the residue was purified by column chromatography and recrystallized in an adequate solvent system to give the desired intermediate, **6a−e** (respectively, **14a−f**).

6-(3-(Cyclopropylsulfonyl)phenyl)imidazo[1,2-*a*]pyridine (6e). 35% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.41 (s, 1H), 8.10 (t, 1H, *J* = 2.0), 7.95−7.90 (m, 1H), 7.85, 7.82 (m, 1H), 7.76 (d, 1H, *J* = 9.2 Hz), 7.73−7.65 (m, 3H), 7.45 (dd, 1H, *J* = 2.0 and 9.2 Hz), 2.56−2.48 (m, 1H), 1.44−1.37 (m, 2H), 1.12−1.04 (m, 2H).

5-(3-(Methylsulfonyl)phenyl)pyrazolo[1,5-*a*]pyridine (14b). 77% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.45 (d, 1H, *J* = 7.6 Hz), 8.10 (t, 1H, *J* = 2.0 Hz), 7.88 (d, 1H, *J* = 2.4 Hz), 7.85 (ddd, 1H, *J* = 2.0, 2.0, and 8.0 Hz), 7.80 (ddd, 1H, *J* = 2.0, 2.0, and 8.0 Hz), 7.68 (dd, 1H, *J* = 0.8 and 2.0 Hz), 7.57 (t, 1H, *J* = 8.0 Hz), 6.91 (dd, 1H, *J* = 7.6 and 2.0 Hz), 6.50 (dd, 1H, *J* = 0.8 and 2.4 Hz), 3.02 (s, 3H).

5-(3-(Cyclopropylsulfonyl)phenyl)pyrazolo[1,5-*a*]pyridine (14c). 75% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.59 (d, 1H, *J* = 6.4 Hz), 8.16 (s, 1H), 8.00 (s, 1H), 7.96−7.84 (m, 2H), 7.79 (s, 1H), 7.66 (t, 1H, *J* = 7.6 Hz), 7.04 (d, 1H, *J* = 6.4 Hz), 6.62 (s, 1H), 2.59−2.47 (m, 1H), 1.42−1.34 (m, 1H), 1.09−1.03 (m, 1H).

5-(3-(Cyclopropylsulfonyl)phenyl)pyrazolo[1,5-*a*]pyrimidine (14f). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.78 (d, 1H, *J* = 7.6 Hz), 8.63 (t, 1H, *J* = 1.6 Hz), 8.42−8.36 (m, 1H), 8.18 (d, 1H, *J* = 2.4 Hz), 8.03−8.00 (m, 1H), 7.74 (t, 1H, *J* = 8.0 Hz), 7.33 (d, 1H, *J* = 7.2 Hz), 6.77 (dd, 1H, *J* = 0.8 and 2.4 Hz), 2,59−2.51 (m, 3H), 1.44−1.39 (m, 2H), 1.11−1.06 (m, 2H).

General Procedure for the Bromination. Intermediates **6a−e** (respectively, **14a−f**) (1 equiv) were dissolved in DMF at 0 °C, and NBS (1.1 equiv) was added. The resulting mixture was allowed to reach rt and stirred for 2 h. After the DMF was removed, the residue was dissolved in DCM and washed with a saturated solution of bicarbonate. The organic phase was dried over Na_2SO_4, and DCM was removed. The residue was then crystallized in EtOAc.

3-Bromo-6-(3-(cyclopropylsulfonyl)phenyl)imidazo[1,2-*a*]pyridine (7e). 87% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.66−8.54 (m, 1H), 8.19 (s, 1H), 8.00 (s, 1H), 7.98−7.91 (m, 2H), 7.80−7.66 (m, 2H), 7.12 (d, 1H, *J* = 9.6 Hz), 2.58−2.47 (m, 1H), 1.45−1.37 (m, 2H), 1.13−1.04 (m, 2H).

3-Bromo-5-(3-(methylsulfonyl)phenyl)pyrazolo[1,5-*a*]pyridine (15b). 78% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.52 (d, 1H, *J* = 7.6 Hz), 8.25 (t, 1H, *J* = 1.6 Hz), 8.04−7.91 (m, 3H), 7.77−7.67 (m, 2H), 7.06 (d, 1H, *J* = 7.6 Hz), 3.13 (s, 3H).

3-Bromo-5-(3-(cyclopropylsulfonyl)phenyl)pyrazolo[1,5-*a*]pyridine (15c). 81% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.52 (d, 1H, *J* = 7.6 Hz), 8.19 (t, 1H, *J* = 1.6 Hz), 7.98 (s, 1H), 7.97−7.91 (m, 2H), 7.74−7.66 (m, 2H), 7.08 (d, 1H, *J* = 7.6 Hz), 2.57−2.48 (m, 1H), 1.44−1.38 (m, 1H), 1.11−1.05 (m, 1H).

3-Bromo-5-(3-(cyclopropylsulfonyl)phenyl)pyrazolo[1,5-*a*]pyrimidine (15f). 86% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.73 (d, 1H, *J* = 7.6 Hz), 8.61 (t, 1H, *J* = 2.0 Hz), 8.51−8.48 (m, 1H), 8.15 (s, 1H), 8.00−8.05 (m, 1H), 7.74 (t, 1H, *J* = 8.0 Hz), 7.37 (d, 1H, *J* = 7.6 Hz), 2.60−2.50 (m, 3H), 1.44−1.39 (m, 2H), 1.11−1.06 (m, 2H).

Compounds **8−12** and **17−22** were prepared from intermediates **7a−e** and **15a−f** following the same procedure as that for intermediates **6a−e** and **14a−f**.

6-(3-(Cyclopropylsulfonyl)phenyl)-3-(4-(methylsulfonyl)-phenyl)imidazo[1,2-*a*]pyridine (12). Column chromatography: DCM/MeOH (98:2) (95:5). 20% yield after crystallization in AcOEt (light yellow powder). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.53 (s, 1H), 8.11 (d, 2H, *J* = 8.8 Hz), 8.05 (s, 1H), 7.93 (d, 1H, *J* = 8.0 Hz), 7.86 (s, 1H), 7.85−7.78 (m, 3H), 7.68 (t, 1H, *J* = 8.0 Hz), 7.53 (d, 1H, *J* = 9.6 Hz), 3.13 (s, 3H), 2.56−2.46 (m, 1H), 1.42−1.34 (m, 2H), 1.12−1.03 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 146.1, 142.0, 140.2, 138.6, 134.7, 134.5, 131.9, 130.3, 128.7, 127.2, 126.6, 126.0, 125.7, 124.7, 121.0, 118.9, 44.5, 32.9, 6.1. MS (EI+): *m*/*z* 452.0 (exact mass = 452.0864).

5-(3-(Methylsulfonyl)phenyl)-3-(4-(methylsulfonyl)phenyl)-pyrazolo[1,5-*a*]pyridine (18). 50% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.93 (d, 1H, *J* = 8.0 Hz), 8.59 (s, 1H), 8.35−8.32 (m, 2H), 8.27 (d, 1H, *J* = 8.0 Hz), 8.07 (d, 2H, *J* = 8.8 Hz), 8.01−7.96 (m, 3H), 7.81 (t, 1H, *J* = 8.0 Hz), 7.47 (dd, 1H, *J* = 2.0 and 7.6 Hz), 3.33 (s, 3H), 3.24 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 149.2, 149.6, 142.3, 139.5, 138.3, 138.2, 137.0, 136.4, 132.7, 130.7, 130.5, 128.3, 127.5, 127.2, 125.8, 115.3, 112.8, 111.8, 44.2, 43.9. MS (ESI)$^+$: *m*/*z* = 427.0 $[M + H]^+$, 465.0 $[M + K]^+$ (exact mass = 426.0708).

5-(3-(Cyclopropylsulfonyl)phenyl)-3-(4-(methylsulfonyl)-phenyl)pyrazolo[1,5-*a*]pyridine (19). 41% yield. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.94 (dd, 1H, *J* = 0.9 and 7.5 Hz), 8.61 (s, 1H), 8.36−8.25 (m, 3H), 8.09 (d, 2H, *J* = 8.7 Hz), 8.01−7.73 (m, 3H), 8.81 (t, 1H, *J* = 7.5 Hz), 7.46 (dd, 1H, *J* = 0.9 and 7.2 Hz), 3.26 (s, 3H), 3.10−2.99 (m, 1H), 1.24−1.14 (m, 2H), 1.12−1.02 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 142.6, 142.1, 139.6, 138.34, 133.28, 137.0, 136.4, 132.7, 130.8, 130.5, 128.3, 127.5, 125.9, 115.3, 112.8, 111.8, 44.2, 32.5, 6.0. MS (ESI)$^+$: *m*/*z* = 453.1 $[M + H]^+$ (exact mass = 452.0864).

5-(3-(Cyclopropylsulfonyl)phenyl)-3-(4-(methylsulfonyl)-phenyl)pyrazolo[1,5-*a*]pyrimidine (22). Column chromatography: DCM/MeOH (98:2), 31% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.83 (d, 1H, *J* = 7.2 Hz), 8.64 (dd, 1H, *J* = 1.6 and 2.0 Hz), 8.57 (s, 1H), 8.48 (d, 1H, *J* = 8.4 Hz), 8.37 (d, 2H, *J* = 8.4 Hz), 8.10−8.04 (m, 3H), 7.80 (dd, 1H, *J* = 8.0 Hz), 7.46 (d, 1H, *J* = 7.2 Hz), 2.58−2.52 (m, 1H), 1.47−1.43 (m, 2H), 1.15−1.09 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.6, 155.4, 144.9, 144.8, 142.2, 138.1, 137.8, 132.8, 131.0, 129.8, 128.1, 126.3, 108.3, 107.0, 44.2, 32.4, 6.1. MS (EI+): *m*/*z* = 453.0 (exact mass = 453.0817).

5-(3-(Cyclopropylsulfonyl)phenyl)-3-(4-(methylsulfinyl)-phenyl)pyrazolo[1,5-*a*]pyridine (31). Column chromatography: DCM/MeOH (100:0) (98:2) (95:5), 39% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.91 (d, 1H, *J* = 9.6 Hz), 8.54 (s, 1H), 8.33−8.24 (m, 3H), 8.02 (d, 2H, *J* = 8.8 Hz), 7.99−7.94 (m, 1H), 7.83 (d, 1H, *J* = 7.6 Hz), 7.79 (d, 2H, *J* = 8.8 Hz), 7.43 (dd, 1H, *J* = 2.0 and 7.6 Hz), 3.08−2.98 (m, 1H), 2.79 (s, 3H), 1.26−1.18 (m, 2H), 1.13−1.07 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 144.1, 142.2, 142.1, 139.7, 136.8, 135.9, 135.5, 132.6, 130.8, 130.4, 127.7, 127.4, 125.8, 124.9, 115.3, 112.6, 112.4, 43.8, 32.5, 6.0. MS (ESI)$^+$: *m*/*z* = 437.1 $[M + H]^+$ (exact mass = 436.0915).

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Tetrahydro-pyrazolopyrimidines 23 and 24. Compound **20** (respectively, **22**) was dissolved in a mixture of DCM/EtOH (3:2) with $Pd(PPh_3)_2Cl_2$ (20 wt %). $NaBH_4$ (10 equiv) was then added, and the reaction mixture was stirred overnight at rt or until consumption of the starting material was complete. The solvents were removed, and the residue was purified by column chromatography using DCM/MeOH (100:0) (98:2) to give **23** (respectively, **24**) in 74% (respectively, 79%) yield.

5-(3-(Cyclopropylsulfonyl)phenyl)-3-(4-(methylsulfonyl)-phenyl)-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyrimidine (24). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.93 (s, 1H), 7.85−7.75 (m, 4H), 7.71−7.66 (m, 4H), 6.97 (s, 1H), 4.80−4.71 (m, 1H), 4.23−4.12 (m, 1H), 3.97−3.87 (m, 1H), 3.17 (s, 3H), 2.88−2.80 (m, 1H), 2.37−2.28 (m, 1H), 2.27−2.16 (m, 1H), 1.16−1.09 (m, 2H), 1.08−1.00 (m, 2H). ^{13}C (100 MHz, DMSO-d_6): δ 144.8, 142.7, 140.7, 139.0, 137.1, 135.5, 131.5, 129.6, 127.3, 126.1, 125.1, 124.6, 100.6, 52.5, 43.8, 43.7, 32.0, 29.4, 5.4. MS (EI+): m/z 457.1 (exact mass = 457.1130).

In Vitro *P. falciparum* Assay and in Vivo Antimalarial Efficacy Studies. Compounds were screened against multidrug resistant (K1) and sensitive (NF54) strains of *P. falciparum* in vitro using the modified [^{3}H]-hypoxanthine incorporation assay.[18] In vivo efficacy was conducted as previously described,[19] with the modification that mice (n = 3) were infected with a GFP-transfected *P. berghei* ANKA strain (donated by A. P. Waters and C. J. Janse, Leiden University, The Netherlands), and parasitemia was determined using standard flow cytometry techniques. The detection limit was 1 parasite in 1000 erythrocytes (that is, 0.1%). Activity was calculated as the difference between the mean percent parasitemia for the control and treated groups expressed as a percent relative to the control group. Compounds were dissolved or suspended in a nonsolubilizing, standard suspension vehicle called HPMC (0.5% [w/v] hydroxypropylmethylcellulose, 0.5% [v/v] benzyl alcohol, 0.4% [v/v] Tween 80, and 0.9% [w/v] sodium chloride in water) and administered orally as four consecutive daily doses (4, 24, 48, and 72 h after infection). Blood samples for the quadruple-dose regimens were collected on day 4 (96 h after infection).

In Vitro Gametocytocidal Activity. In vitro gametocytocidal activity was determined using luciferase reporter lines specifically enabling screening against early stage gametocytes (>90% stage II/III) and late stage gametocytes (>95% stage IV/V) as previously reported.[20] All gametocytocidal activities were additionally confirmed in an orthogonal assay by measuring ATP production in gametocytes treated with the compounds,[20] with resultant activities that can be compared across assay platforms.

In Vitro *P. berghei* Liver Assays. This assay is based on the murine malaria parasite *P. berghei* transformed with luciferase. Hepatic human transformed cells (HepG2), pretreated for 18 h with the compound to be investigated, are infected with freshly dissected *P. berghei* luciferase sporozoites. After another 48 h of incubation with the compound of interest, the viability of *P. berghei* exoerythrocytic forms (EEF) and the HepG2 host cells is measured by bioluminescence.

This assay allows us to identify compounds with an eventual activity against sporozoite infection of liver cells as well the viability of liver schizonts. More details are provided in the Supporting Information.

In Vitro and in Vivo Metabolites Identification Methods. Metabolite identification of **31** and **19** was performed by LC-ESI-MS/MS using in vivo blood samples from rat PK and in vitro rat liver microsomal incubations. Details of the metabolite identification methods are presented in the Supporting Information.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.5b01605.

Additional details of the characterization of selected compounds and the procedures used for the in vitro and in vivo antimalarial studies as well as PK and metabolism studies (PDF)

SMILES nomenclature and K1 and NF54 IC_{50} values for compounds **1**−**31** (CSV)

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

SAR, structure−activity relationships; ADME, absorption, distribution, metabolism, and excretion; CQ, chloroquine; p.o., oral administration; i.v., intraveneous administration; MSD, mean survival days; PK, pharmacokinetics; NMR, nuclear magnetic resonance; TLC thin-layer chromatography; rt, room temperature; MMV, Medicines for Malaria Venture

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