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Medicinal Chemistry Optimization of Antiplasmodial Imidazopyridazine Hits from High Throughput Screening of a SoftFocus Kinase Library: Part 1

Claire Le Manach,[†,⊥] Diego Gonzàlez Cabrera,[†] Frederic Douelle,[†] Aloysius T. Nchinda,[†] Yassir Younis,[†] Dale Taylor,[∞] Lubbe Wiesner,[∞] Karen L. White,[§] Eileen Ryan,[§] Corinne March,[§] Sandra Duffy,[⌀] Vicky M. Avery,[⌀] David Waterson,[‡] Michael J. Witty,[‡] Sergio Wittlin,[◇,∫] Susan A. Charman,[§] Leslie J. Street,[†] and Kelly Chibale*[,†,#,⊥]

[†]Department of Chemistry, University of Cape Town, Rondebosch, 7701 Cape Town, South Africa

[∞]Division of Clinical Pharmacology, Department of Medicine, University of Cape Town, Observatory, 7925 Cape Town, South Africa

[⌀]Discovery Biology, Eskitis Institute, Griffith University (Nathan Campus), Don Young Road, Brisbane Qld 4111, Australia

[§]Centre for Drug Candidate Optimisation, Monash Institute of Pharmaceutical Sciences, Monash University (Parkville campus), 381 Royal Parade, Parkville VIC 3052, Australia

[‡]Medicines for Malaria Venture, ICC, Route de Pré-Bois 20, PO Box 1826, 1215 Geneva, Switzerland

[◇]Swiss Tropical and Public Health Institute, Socinstrasse 57, 4002 Basel, Switzerland

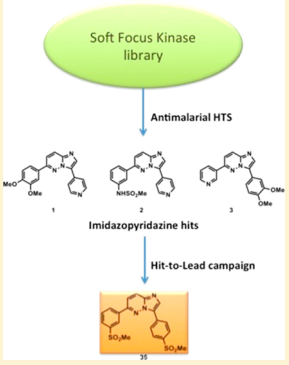
[∫]University of Basel, Socinstrasse 57, 4002 Basel, Switzerland

[#]Institute of Infectious Disease and Molecular Medicine, University of Cape Town, Rondebosch 7701 Cape Town, South Africa

[⊥]South African Medical Research Council Drug Discovery and Development Research Unit, University of Cape Town, Rondebosch, 7701 Cape Town, South Africa

Supporting Information

ABSTRACT: A novel class of imidazopyridazines identified from whole cell screening of a SoftFocus kinase library was synthesized and evaluated for antiplasmodial activity against K1 (multidrug resistant strain) and NF54 (sensitive strain). Structure−activity relationship studies led to the identification of highly potent compounds against both strains. Compound **35** was highly active (IC_{50}: K1 = 6.3 nM, NF54 = 7.3 nM) and comparable in potency to artesunate, and **35** exhibited 98% activity in the in vivo *P. berghei* mouse model (4-day test by Peters) at 4 × 50 mg/kg po. Compound **35** was also assessed against *P. falciparum* in the in vivo SCID mouse model where the efficacy was found to be more consistent with the in vitro activity. Furthermore, **35** displayed high (78%) rat oral bioavailability with good oral exposure and plasma half-life. Mice exposure at the same dose was 10-fold lower than in rat, suggesting lower oral absorption and/or higher metabolic clearance in mice.



Malaria remains a major concern for public health, especially in tropical and subtropical areas and affects 207 million people worldwide.[1] The disease is transmitted by female mosquitoes and is caused by five different species of the protozoan *Plasmodium* parasite, namely, *falciparum*, *vivax*, *malariae*, *ovale*, and *knowlesi* that infect and destroy red blood cells leading to high fever, anemia, cerebral malaria, and possibly death.[1] Of these, *falciparum* is the most prevalent species in sub Saharan Africa and the most lethal, being responsible for over 627 000 deaths a year,[1] especially among young children and pregnant women.

Malaria may be cured if diagnosed in time and treated with proper medicines. However, the rapid development of drug resistance has compromised the use of previously effective drugs such as chloroquine, sulfadoxine/pyrimethamine, and signs of artemisinin resistance have started to emerge in southeast Asia.[2,3] To overcome this, various types of drug combinations with independent modes of action have been gradually introduced; however, these present only a temporary solution.[4] The development of new antimalarial agents is thus urgently needed to counter the ever-increasing spread of drug-resistant malaria. In this regard, phenotypic whole cell high throughput screening (HTS) has been a powerful tool for identifying novel antimalarial chemotypes.[5]

Using an image-based assay,[6] HTS of a BioFocus DPI SoftFocus kinase library[7] identified a number of chemotypes

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with antimalarial activity,[8] inclusive of the diaryl-imidazopyridazine (SFK52) series (Figure 1).

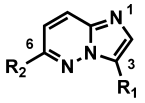


Figure 1. General structure of **SFK52** series.

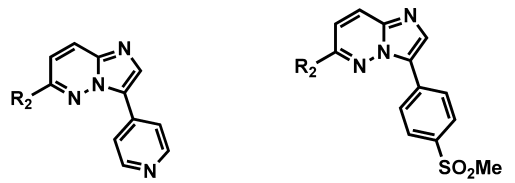
Malaria-related studies based on the imidazopyridazine scaffold have been recently reported.[9] Furthermore, several groups have been working on imidazopyridazines, focusing on other biological activities such as kinase inhibition[10] or anxiety treatment.[11] The related imidazolopiperazines with promising blood- and liver-stage activity have been identified and optimized from the whole-cell screen undertaken by Novartis.[12]

From 488 diaryl-imidazopyridazines in the SFK52 library tested in the HTS, 153 compounds displayed >80% inhibition at the screening concentration of 1.82 μM. Among these were compounds **1**, **2**, and **3** (Figure 2). In order to validate them as hits, these three compounds were first resynthesized and retested against *P. falciparum* sensitive and resistant strains (NF54 and K1 respectively) to confirm activity (IC_{50} values between 15 and 25 nM in both strains). When profiled for their in vitro ADME properties, these compounds were found to have poor metabolic stability in human liver microsomes with a predicted human hepatic extraction ratio (E_H) > 0.8. Therefore, they were not expected to perform well in vivo. In order to identify compounds with both good in vitro potency and favorable ADME properties, **1** and **2** were used as a starting point for initial SAR studies. A series of compounds based on scaffold **A** (Figure 3) was initially designed, where the aryl group at the 3-position was fixed as a pyridyl, and variations at the 6-position were introduced.

Based on encouraging initial in vitro ADME data with the 4-methylsulfonylphenyl substituent, we then set the 3 position as 4-methylsulfonylphenyl instead of 4-pyridyl as reflected in scaffold **B** (Figure 3), leading to the identification of highly potent (IC_{50} <10 nM, K1 and NF54) and metabolically stable compounds (E_H <0.28) **33** and **35** (Figure 4).

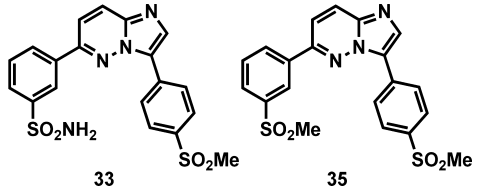
Herein we describe the synthesis of the various libraries and the results of our SAR exploration, as well as detailed profiling of the lead compound **35**.

Chemistry. Target compounds were prepared following a relatively straightforward synthetic route involving 4 steps from the commercially available 3-amino-6-chloropyridazine **4** (Scheme 1). Briefly, a quantitative ring closure using bromoacetaldehyde diethylacetal and HBr was first performed on **4** to give **5**. Iodination of **5** with NIS in DMF then led to **6** in a quantitative yield. Lastly, two successive Suzuki cross



scaffold **A** scaffold **B**

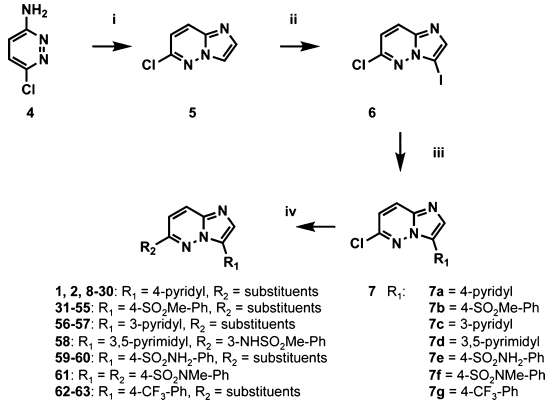
Figure 3. Structure of scaffolds **A** and **B**.



33 **35**

Figure 4. Structure of **33** and **35**.

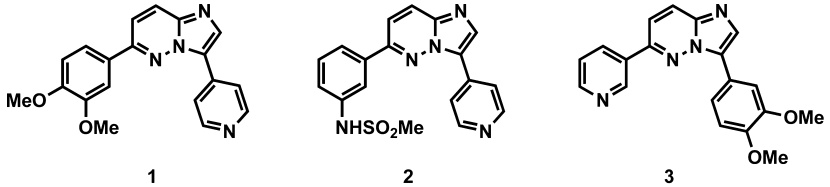
Scheme 1. Synthesis of the Imidazopyridazines 1, 2, 8−30, 31−55, 56−63[a]



[a]Reagents and conditions: (i) $BrCH_2CCH(OEt)_2$, HBr, $EtOH/H_2O$, 100 °C, quantitative; (ii) NIS, DMF, rt, 4 days, quantitative; (iii) R_1-$B(OH)_2$, $Pd(PPh_3)_2Cl_2$, aq K_2CO_3, DMF, 80 °C, 40−60%; (iv) R_1-$B(OH)_2$, $Pd(PPh_3)_2Cl_2$, aq K_2CO_3, DMF, 90 °C, 40−70%.

coupling reactions[13,14] with appropriate boronic acids gave us the desired compounds (**1, 2, 8−63**).

In Vitro Antiplasmodial Activity. To establish the SAR, we first explored aryl substitution at positions 3 and 6. All compounds were evaluated for in vitro antiplasmodial activity against the sensitive (NF54) and multidrug resistant (K1) strains of *P. falciparum*. Chloroquine and artesunate were used as the reference drugs in all experiments.[8c]



1
K1: 14.1 nM
NF54: 15.6 nM
E_H(h) = 0.86

2
K1: 15.3 nM
NF54: 15.8 nM
E_H(h) = 0.82

3
K1: 23.5 nM
NF54: 25.9 nM
E_H(h) = 0.92

Figure 2. Structures of the imidazopyridazines **1**, **2**, and **3** and associated data.

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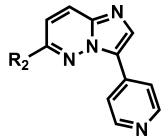
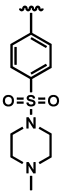
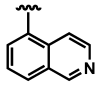
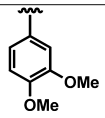
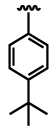
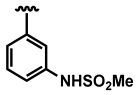
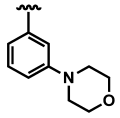
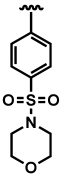
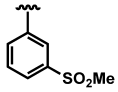
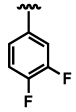
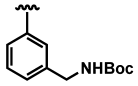
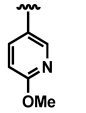
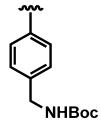
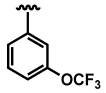
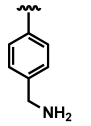
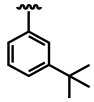
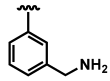
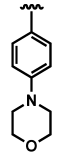
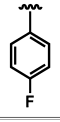
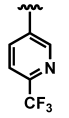
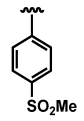
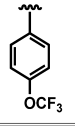
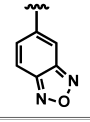
Table 1. Exploration of the 6-Position with the 3-Position Fixed as a Pyridyl



Compd	R_2	[a]Pf IC_{50} (nM)		Compd	R_2	Pf IC_{50} (nM)		Compd	R_2	Pf IC_{50} (nM)	
		K1	NF54			K1	NF54			K1	NF54
Chloroquine[b]		194	16	**13**		183	202	**23**		1247	739
Artesunate[b]		3.0	4.0	**14**		192	223				
1		14	16	**15**		203	272	**24**		1425	1876
2		15	16	**16**		246	283	**25**		1490	2242
8		28	46	**17**		415	496				
9		97	95	**18**		412	603	**26**		1606	1629
10		118	146	**19**		491	335	**27**		1670	2146
11		122	137	**20**		588	289	**28**		> 2798	> 2798
12		138	186	**21**		873	964	**29**		> 2854	> 2854
				22		980	1061	**30**		> 3181	> 3181

[a]Mean from *n* values of ≥2 independent experiments. [b]Data from Gonzalez Cabrera et al.[8c]

The in vitro antiplasmodial activities of the 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). Among the 25 evaluated compounds, seven of them (**1**, **2**, **8**–**12**) showed higher potency than chloroquine (CQ) against resistant strain K1 with an IC_{50} < 140 nM. Compounds (**1**, **2**, **8**, and **9**) exhibited high antiplasmodial activity with IC_{50} values < 100 nM, whereas the mean value for CQ in the same experiment was 194 nM; compounds **1** (IC_{50} 16 nM) and **2** (IC_{50} 16 nM) showed comparable activity to chloroquine (IC_{50} 16 nM) against NF54.

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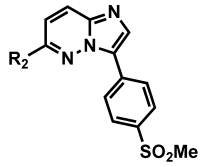
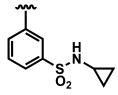
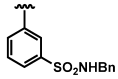
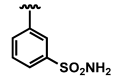
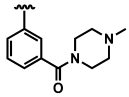
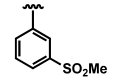
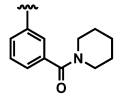
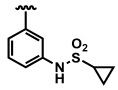
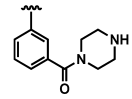
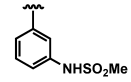
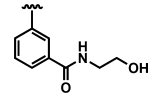
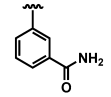
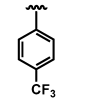
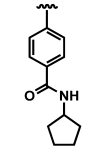
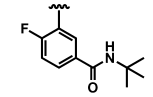
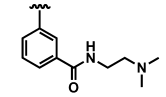
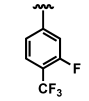
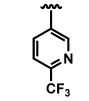
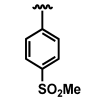
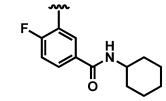
Table 2. Exploration of the 6-Position with the 3-Position Fixed as a 4-(Sulfonylmethyl) Phenyl



Compd	R	[a]Pf IC_{50} (nM) K1	[a]Pf IC_{50} (nM) NF54
	Chloroquine[b]	194	16
	Artesunate[b]	3.0	4.0
31		0.58	0.66
32		3.3	3.5
33		4.4	4.4
34		5.5	6.1
35		6.3	7.3
36		7.2	6.5
37		11	14
38		11	15
39		19	19
40		26	32
41		55	79
42		78	99
43		99	102
44		96	107
45		107	117
46		134	189
47		146	154
48		152	179
49		155	159
50		173	183
51		315	393
52		633	763
53		753	706
54		>2030	>2030
55		Solubility issue	

[a]Mean from *n* values of ≥2 independent experiments. [b]Data from Gonzalez Cabrera et al. [8c]

Regarding the SAR, *meta*-substitution at the R_2 position appeared to be optimal for potency. Replacement by a *para* substituent generally resulted in significant loss of activity: compounds **8**–**11** and **16** have *Pf* IC_{50} values ≤250 nM, whereas their 4-substituted analogues **22**, **24**, **26**, **28**, and **29** had IC_{50} values >1000 nM.

In parallel, we explored changes to the 3-position. Following encouraging initial data, we set the 3-position as a 4-methylsulfonylphenyl group based on good predicted metabolic stability of that aryl side-chain versus 4-pyridyl as reflected in scaffold **B** (Figure 3). In vitro antiplasmodial activities for this series are shown in Table 2. In this case, in comparison to chloroquine in the K1 strain, 21 of the 25 compounds (**31**–**51**) showed either higher or comparable activity. Fourteen compounds (**31**–**44**) displayed *Pf* IC_{50} <100 nM against K1, and six of them (**31, 32, 33, 34, 35, 36**) were even comparable to artesunate in both strains with *Pf* IC_{50} values ranging from 0.5 nM to 7.3 nM (Table 2).

As previously observed for the pyridyl series, electron-withdrawing *meta*-substituents on the aromatic ring at the 6-position, with a preference for 3-sulfonyl- and carbonyl derivatives, were optimal for good potency. Once again, 4-aryl substitution was generally detrimental to activity (Table 3). Compounds shown in Table 3 confirmed the importance of 3-substitution for activity.

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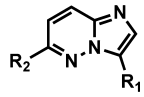
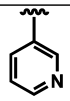
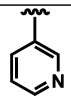
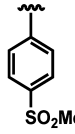
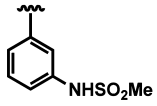
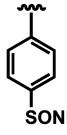
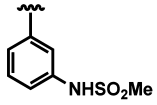
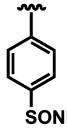
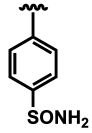
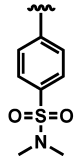
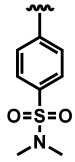
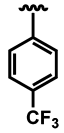
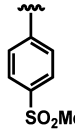
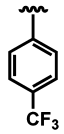
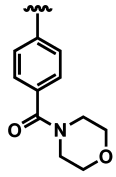
Table 3



Compd	R_1	R_2	Pf IC_{50} (nM)		E_H values Human
			K1	NF54	
56			1072	1273	-
57			>2854	>2854	-
58			76	93	0.75
59			20	18	0.42
60			>2328	>2328	-
61			>2059	>2059	-
62			668	764	< 0.28
63			732	763	0.59

In Vitro Metabolic Stability. Metabolic stability of the most active compounds was assessed in vitro in human (and rat) microsomal preparations[15] to help in guiding the choice toward metabolically stable substituents. The microsome-predicted hepatic extraction ratios (E_H) are summarized in the Supporting Information (Table ST1). In general, the metabolic stability values were consistent across rat and human microsomes. The results showed that none of the pyridyl derivatives of the first library were metabolically stable except **29**. This is not surprising because the pyridine nitrogen of these compounds is likely to be oxidized to the N-oxide in liver microsomes. Among the second library where the 3-position was set as a 4-methylsulfonylphenyl, nine compounds (**33, 35, 38, 41, 42, 43, 44, 46, 48**) were found to have good metabolic stability (E_H values <0.28). Very encouraging were compounds **33**, **35**, and **38**, which showed both high potency (IC_{50} ≤10 nM) along with good metabolic stability (E_H value <0.28).

Physicochemical Properties. Physicochemical properties of the most active compounds were assessed using a combination of in silico and experimental techniques. Most of the compounds displayed poor kinetic solubility at pH 6.5 but increased solubility under acidic conditions, consistent with

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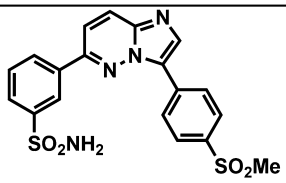
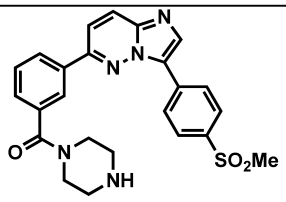
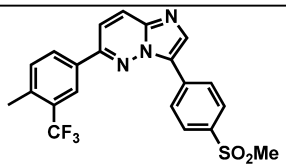
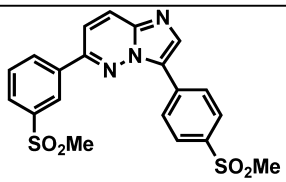
Table 4. In Vitro and in Vivo Oral Antimalarial Efficacy of Selected Compounds[a−d]

Compound	Structure	Pf IC_{50} (nM)		% reduction in parasitemia in *P. berghei* infected mice (MSD)[a] at 4x50 mg/kg
		K1	NF54	
33		4.4	4.4	<40 (4)[c]
38		10.8	14.9	<40 (4)[c,d]
40		25.5	32.4	79 (7)
35		6.3	7.3	98 (7)
Chloroquine 4x30 mg/kg		194	16	99.9 (24)
Artesunate 4x30 mg/kg		3.4	4.2	99 (10)
Mefloquine 4x30 mg/kg		8.4	12	99.9 (29)

[a]MSD = mean survival time (in days). [b]Artesunate and mefloquine were dissolved or suspended in a nonsolubilizing, standard suspension vehicle called SSV (0.5% [wt/vol] carboxymethylcellulose, 0.5% [vol/vol] benzyl alcohol, 0.4% [vol/vol] Tween 80, and 0.9% [wt/vol] sodium chloride in water. [c]Mice were euthanized on day 4 in order to prevent death otherwise occurring at day 6. [d]Mean from *n* values of 2 independent experiments.

expected ionization behavior. The pyridine derivatives from the first library displayed high solubility (>100 μg/mL) at pH 2. As for the library derived from scaffold **B**, compounds generally showed poor aqueous solubility at neutral pH, with a slight improvement under acidic conditions but the solubility still remained very low. Only compounds **34**, **42** and **50** showed good aqueous solubility at both pH 2.0 and 6.5, with values ranging between 50 and 100 μg/mL or higher. **38** showed good solubility (>100 μg/mL) at pH 2.5. Regarding the partition coefficients, all the values were in the range of 1.9 − 5.3 at pH 7.4. The physicochemical data are summarized in the Supporting Information (Table ST2).

In Vivo Efficacy Studies. Compounds **33**, **40**, **38**, and **35**, each of which displayed good in vitro antiplasmodial activity and metabolic stability, were tested for in vivo efficacy in *P. berghei*-infected mice. The in vivo activity was evaluated

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following oral administration (p.o.) of 50 mg/kg/day for 4 days. The results are summarized in Table 4.

Compounds **33** and **38** were ineffective in vivo with less than 40% reduction in parasitemia compared to untreated infected animals. Mouse exposure studies after po dosing showed very low blood levels for these two compounds suggesting poor oral absorption and/or high clearance. **40** showed a reduction in parasitemia of 79% with a mean survival time of 7 days. Interestingly, **35** showed a 98% suppression of parasitemia at this dose level with a mean survival time of 7 days. Efficacy of **35** was evaluated at lower doses in *P.berghei*-infected mice and the effective doses where 50% and 90% reduction in parasitemia was observed were 1.4 and 15 mg/kg, respectively.

Even though **35** displayed high potency in vitro versus *P. falciparum*, this did not translate into high potency in vivo versus *P. berghei*. This is despite the plasma concentrations being maintained at quite high levels (Table 5). The in vivo

Table 5. Plasma Concentrations after First Oral Administration of 50 mg/kg in *P.berghei* Mouse Model (Total Dosing Regimen Was 4 × 50 mg/kg)

	dose (mg/kg)	app. AUC (μM·h)	app. C_{max} (μM)	app. $C_{av_{24}}$ (μM)
35	50	27.8	2.2	1.2
33	50	c.n.c.	0.02	c.n.c.
38	50	c.n.c.	0.01	c.n.c.

efficacy of **35** was also assessed against *P. falciparum* in a SCID mouse model.[16] The compound was found to be very potent (ED_{90} = 1.5 mg/kg) and comparable to marketed antimalarial drugs (Table ST3 in Supporting Information). This result appears to be more consistent with the in vitro activity, and therefore, the *P.berghei* data are likely to reflect a parasite strain difference.

In Vivo Pharmacokinetic Studies. The rat pharmacokinetic profile of compound **35** was determined following dosing at 3.6 mg/kg iv and po. The results are shown in Figure 5 and Table 6. Compound **35** showed good oral bioavailability in rats (78%) and a relatively long half-life (7 h). The in vivo plasma clearance in rats after iv dosing was low (5.8 mL/min/kg) with a volume of distribution of 3.0 L/kg.

CONCLUSION

A novel class of compounds has been identified that combines good in vitro potency against *P. falciparum* with oral efficacy in vivo in a *P. berghei* mouse model. Despite the high rat oral bioavailability and microsomal stability, in vivo efficacy of the lead compound **35** in the *P. berghei* mouse model remains weak (MSD = 7). However, the in vivo activity was found to be high against *P. falciparum* in the SCID mouse model, which is more consistent with the in vitro activity and suggesting a parasite strain difference.

The lead optimization campaign is focused on addressing this disconnect partly through SAR studies aimed at improving solubility to address low blood levels and in vivo clearance in the mouse model and partly through exploring other routes of clearance.

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 were anhydrous. ^{1}H

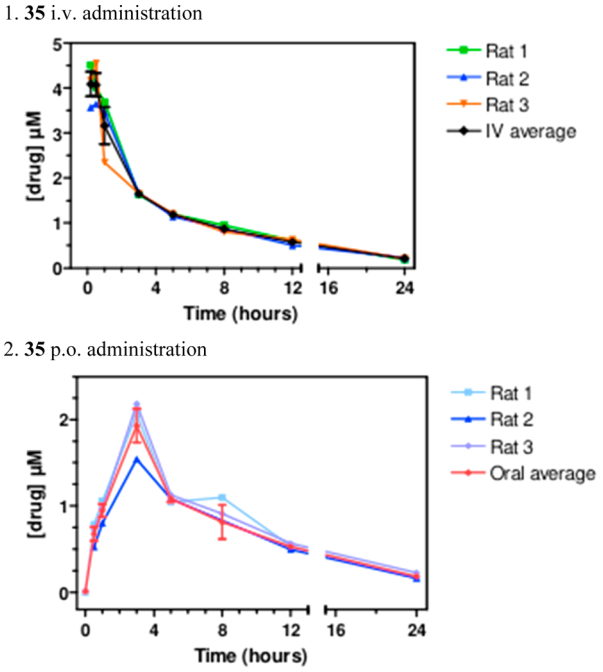
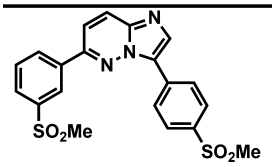


Figure 5. Pharmacokinetics data of **35** in Sprague–Dawley rats after iv and po administration.

Table 6. Pharmacokinetics Data of 35 in Sprague–Dawley Rats



	IV	Oral
Dose (mg/mL)	3.6	3.6
Apparent $t_{½}$ (h)	7.15	7.08
Plasma CL_{total} (mL/min/kg)	5.77	-
V_{ss} (L/kg)	3.01	-
AUC $_{0-\infty}$ (µM.h)	24.3	18.9
C_{max} (µM)	-	1.90
T_{max} (h)	-	3.0
BA (%)	-	77.7

NMR spectra were recorded on a Varian Mercury spectrometer at 300 MHz or a Varian Unity spectrometer at 400 MHz with Me_4Si as 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.

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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 HPLC method is described in the Supporting Information.

6-Chloroimidazo[1,2-b]pyridazine (5). To a solution of 3-amino-6-chloropyridazine **4** (1 g, 7.7 mmol, 1 equiv) in EtOH (15 mL) and water (10 mL) was added bromoacetaldehyde diethylacetal (2.2 mL, 14.1 mmol, 2 equiv) and HBr (0.7 mL). The solution cleared up after the addition of HBr. The resulting mixture was refluxed at 103 °C overnight. After completion of the starting material, the solution was diluted in EtOAc and washed with saturated Na_2CO_3. The solvents were removed in vacuo, and the crude was used as is for the next step.

6-Chloro-3-iodoimidazo[1,2-b]pyridazine (6). Compound **5** (948 mg, 6.2 mmol, 1 equiv) was dissolved in DMF, and the resulting mixture was flushed with nitrogen. NIS (1.5 g, 6.8 mmol, 1.1 equiv) was added in one portion, and the solution was stirred at room temperature for 4 days. DMF was removed in vacuo. The residue was dissolved in DCM and washed with a saturated solution of $Na_2SO_2O_5$. The organic phase was concentrated, and the resulting compound was crystallized in Et_2O to give **6** in quantitative yield.

General Procedure for the First Suzuki Cross-Coupling Reaction. Compound **6** (500 mg, 1.79 mmol, 1 equiv) was dissolved in DMF (5 mL) with the corresponding boronic acid (1.97 mmol, 1.1 equiv) and $Pd(PPh_3)_2Cl_2$ (63 mg, 0.09 mmol, 0.05 equiv). The resulting mixture was flushed with nitrogen for 15 min after which aqueous K_2CO_3 (1M) (1.9 mL, 1.88 mmol, 1.05 equiv) was added. The solution was heated to 80 °C and stirred for 12 h at this temperature. After dilution in DCM and water, the solution was extracted with DCM 3 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 product in 52 to 59% yield.

6-Chloro-3-(pyridin-4-yl)imidazo[1,2-b]pyridazine (7a). Column DCM/MeOH (98:2, 95:5, 90:10), recrystallization in Et_2O, 59%. ^{1}H NMR (400 MHz, $CDCl_3$): δ **8.72** (d, 2H; J = 6.4); **8.23** (s, 1H); **8.01, 7.99** (m, 3H; J = 6.4); **7.16** (d, 1H; J = 9.2). ^{13}C NMR (100 MHz, $CDCl_3$): δ 150.8, 150.4, 147.5, 140.1, 135.5, 135.2, 127.7, 126.4, 121.5, 120.1, 119.6. MS (EI+): m/z = 230.0

6-Chloro-3-(4-(methylsulfonyl)phenyl)imidazo[1,2-b]-pyridazine (7b). Column EtOAc/Hex (3:1), (5:1), EtOAc, EtOAc/MeOH (9:1, 8:2), recrystallization AcOEt/Hex, 52%. ^{1}H NMR (400 MHz, $CDCl_3$): δ **8.29** (d, 2H; J = 8.9); **8.19** (s, 1H); **8.07** (d, 2H; J = 8.9); **8.01** (d, 1H; J = 9.3); **7.17** (d, 1H; J = 9.3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 147.7, 140.1, 134.8, 133.4, 132.0, 128.0, 127.6, 127.0, 125.9, 119.4, 44.6. MS (EI+): m/z = 306.9

General Procedure for the Second Suzuki Cross-Coupling Reaction. Compound **7** (100 mg, 1 equiv) was dissolved in DMF (2 mL) 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 (1M) (1.05 equiv) was added. The solution was heated to 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, the residue was purified by column chromatography and recrystallized in an adequate solvent system to give the desired product in 22 to 78% yield.

General Procedure for Boc-Deprotection. Boc-protected compound (1 equiv) was dissolved in DCM. Ten equivalents of TFA was added, and the resulting mixture was stirred overnight at room temperature. After evaporation of the solvents, the residue was dissolved in DCM/MEOH (1:1) and Amberlyst A21 was added. The mixture was stirred for 30 min, and the resin was filtered and rinsed with MEOH. The filtrate was concentrated and the residue purified on silica gel.

6-(3,4-Dimethoxyphenyl)-3-(pyridin-4-yl)imidazo[1,2-b]-pyridazine (1). Column DCM/MeOH (95:5, 90:10), recrystallization in AcOEt/Hex, 47%. ^{1}H NMR (300 MHz, $CDCl_3$): δ **8.73** (d, 2H; J = 6.3); **8.24** (s, 1H); **8.15** (d, 2H; J = 6.3); **8.08** (d, H; J = 9.6); **7.65, 7.56** (m, 3H); **7.04** (d, 1H; J = 8.4); **4.02−3.99** (2s, 2 × 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 151.9, 151.4, 150.4, 149.8, 140.8, 136.4, 134.8, 128.2, 126.3, 126.0, 120.4, 120.2, 116.6, 111.6, 110.0, 56.3, 56.2. MS (EI+): m/z = 331.9 (exact Mass =332.1273). mp = 157 °C.

***N*-(3-(3-(Pyridin-4-yl)imidazo[1,2-b]pyridazin-6-yl)phenyl)-methanesulfonamide (2).** Column DCM/MeOH (95:5, 90:10), recrystallization in EtOH/DCM, 45%. ^{1}H NMR (300 MHz, $CDCl_3$): δ **8.71** (d, 2H; J = 6.0); **8.56** (s, 1H); **8.35** (d, 1H; J = 9.6); **8.30** (d, 2H; J = 6.0); **8.12** (s, 1H); **7.90** (d, 1H; J = 9.6); **7.85** (d, 1H; J = 7.8); **7.56** (dd, 1H; J = 7.8, J = 8.4); **7.39** (d, 1H; J = 8.4); **3.10** (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 151.0, 150.1, 140.5, 139.5, 135.9; 135.6, 135.5, 130.2, 126.6, 124.9, 122.3, 121.2, 119.4, 117.1, 116.8, 40.0. MS (EI+): m/z = 364.8 (exact mass = 365.0946). mp =253 °C.

6-(3-(Methylsulfonyl)phenyl)-3-(pyridin-4-yl)imidazo[1,2-b]-pyridazine (8). Column DCM/MeOH (98:2, 95:5), recrystallization in DCM/AcOEt, 60%. ^{1}H NMR (400 MHz, $CDCl_3$): δ **8.74** (d, 2H; J = 6.0); **8.53** (d, 1H; J = 1.8); **8.34** (d, 1H; J = 8.8); **8.28** (s, 1H); **8.17** (d, 1H; J = 9.2); **8.10, 8.07** (m, 3H; J = 6.4); **7.79** (t, 1H; J = 8.8); **7.65** (d, 1H; J = 9.2). ^{13}C NMR (100 MHz, $CDCl_3$): 151.3, 141.8, 140.3, 136.9; 135.7, 135.4, 131.0, 130.4, 128.8, 126.8, 125.9, 119.9, 115.9, 44.4. MS (EI+): m/z = 349.9 (exact mass = 350.0837). mp =240 °C.

***tert*-Butyl(3-(3-(pyridin-4-yl)imidazo[1,2-b]pyridazin-6-yl)-phenyl)methylcarbamate (9).** Column DCM/MeOH (98:2, 95:5), recrystallization in DCM/AcOEt, 67%. ^{1}H NMR (400 MHz, $CDCl_3$): δ **8.73** (d, 2H; J = 6.4); **8.24** (s, 1H); **8.12** (d, 2H; J = 6.4); **8.09** (d, 1H; J = 9.4); **7.91** (s, 1H); **7.90** (d, 1H; J = 6.8); **7.59** (d, 1H; J = 9.4); **7.57, 7.51** (m, 1H); **7.45** (d, 1H). MS (EI+): m/z = 401.0 (exact mass = 401.1852)

***N*-Cyclopropyl-3-(3-(4-(methylsulfonyl)phenyl)imidazo[1,2-b]pyridazin-6-yl)benzenesulfonamide (31).** Column DCM/MeOH (98:2), recrystallization in DCM/MeOH, 22%. ^{1}H NMR (400 MHz, dMSO-d_6): δ **8.55, 8.49** (m, 4H); **8.43, 8.38** (m, 2H); **8.07, 8.02** (m, 3H); **7.99, 7.95** (m, 2H); **7.83** (dd, 1H; J = 8.0, J = 7.6); **3.25** (s, 3H); **0.53, 0.45** (m, 2H); **0.43, 0.37** (m, 2H); **2.24, 2.11** (m, 1H). ^{13}C NMR (100 MHz, $CD_3OD/CDCl_3$): 151.3, 141.4, 136.2, 134.2, 133.7, 130.9, 130.1, 128.8, 127.8, 127.1, 126.4, 125.9, 116.9, 44.2, 24.0, 5.6. MS (EI+): m/z = 467.7 (exact mass = 468.0926). mp =235 °C.

3-(3-(4-(Methylsulfonyl)phenyl)imidazo[1,2-b]pyridazin-6-yl)benzenesulfonamide (32). Column DCM/MeOH (9:1, 85:15), recrystallization in DCM/MeOH, 43%. ^{1}H NMR (400 MHz, $CDCl_3$): δ **8.59** (t, 1H; J = 1.2); **8.44** (d, 2H; J = 8.8); **8.27** (ddd, 1H; J = 1.6, J = 7.6, J = 1.2); **8.25** (s, 1H); **8.19** (d, 1H; J = 9.2); **8.09** (d, 2H; J = 8.8); **8.10, 8.05** (m, 1H); **7.84** (d, 1H; J = 9.2); **7.72** (d, 1H; J = 7.6); **3.15** (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 150.8, 146.8, 140.6, 139.7, 136.0, 133.5, 130.8, 130.6, 128.0, 127.6, 126.9, 124.6, 117.1, 44.1. MS (EI+): m/z = 467.9 (exact mass = 428.0613).

6-(3-(Aminosulfonyl)phenyl)-3-(4-(methylsulfonyl)phenyl)-imidazo[1,2-b]pyridazine (33). Column DCM/MeOH (90:10), recrystallization in DCM/MeOH, 43%. ^{1}H NMR (300 MHz, $CDCl_3$): δ **8.59** (t, 1H; J = 2.0); **8.44** (d, 2H; J = 8.4); **8.27** (t, 1H; J = 6.0); **8.25** (s, 1H); **8.19** (d, 1H; J = 9.6); **8.09** (d, 2H; J = 8.7); **8.07** (m, 1H); **7.84** (d, 1H; J = 9.6); **7.72** (d, 1H; J = 8.1); **3.15** (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 150.8, 146.8, 140.6, 139.7, 136.0, 133.5, 130.8, 130.6, 128.0, 127.6, 126.9, 124.6, 117.1, 44.1. MS (EI+): m/z = 428.1 (exact mass = 428.0613). mp =237 °C

(4-Methylpiperazin-1-yl)(3-(3-(4-(methylsulfonyl)phenyl)-imidazo[1,2-b]pyridazin-6-yl)phenyl)methanone (34). Column DCM/MeOH (98:2), recrystallization in DCM/MeOH, 50%. ^{1}H NMR (300 MHz, $CDCl_3$): δ **8.37** (d, 1, J = 8.7); **8.21** (s, 3H); **8.15** (d, 1H, J = 9.6); **8.10** (d, 2H, J = 8.7); **8.04** (dd, 1H; J = 1.5, J = 9.0); **7.64** (d, 1H, J = 7.2); **7.61** (d, 1H, J = 9.6); **7.56** (dd, 1H, J = 7.8, J = 1.5); **4.06, 3.55** (m, 4H); **3.14** (s, 3H); **2.79, 2.51** (m, 4H); **2.47** (s,

3H). ^{13}C NMR (100 MHz, $CDCl_3$): 169.4, 151.4, 140.1, 139.0, 136.9, 135.8, 134.9, 134.0, 129.3, 128.7, 128.2, 127.8, 126.8, 126.5, 125.9, 116.3, 55.8, 47.6, 45.9, 44.5, 42.2. MS (EI+): m/z = 475.1 (exact mass = 475.1678). mp =215 °C.

6-(3-(Methylsulfonyl)phenyl)-3-(4-(methylsulfonyl)phenyl)-imidazo[1,2-b]pyridazine (35). Column DCM/MeOH (98:2, 95:5), recrystallization in DCM/MeOH, 48%. ^{1}H NMR (400 MHz, $CDCl_3$): δ **8.44** (d, 1H; J = 1.6); **8.27** (d, 2H; J = 8.6); **8.24** (d, 1H: J = 8.4); **8.10** (s, 1H); **8.08** (d, 1H; J = 9.6); **7.99, 7.93** (m, 3H); **7.69** (dd, 1H; J = 8.0, J = 8.4); **7.65** (d, 1H; J = 9.6); **3.04 − 3.02** (2s, 2 × 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 150.2, 141.9, 140.2, 139.4, 136.9, 135.2, 133.7 131.9, 130.4, 128.8, 127.9, 126.9, 125.9, 115.9, 44.5, 44.4. MS (EI+): m/z = 427.0 (exact mass = 427.0660). mp =135 °C.

(3-(3-(4-(Methylsulfonyl)phenyl)imidazo[1,2-b]pyridazin-6-yl)phenyl)(piperidin-1-yl)methanone (36). Column DCM/MeOH (98:2, 95:5), recrystallization in DCM/MeOH, 48%. ^{1}H NMR (300 MHz, CD_3OD): δ **8.39** (d, 2H; J = 8.7); **8.22** (s, 1H); **8.15**(d, 1H; J = 9.3); **8.10, 8.02** (m, H; J = 8.7); **8.03** (m, 4H); **7.63** (dd, 1H; J = 9.3); **7.59, 55** (m, 1H); **3.13** (s, 3H); **3.87, 3.64** (m, 2H); **3.57, 3.29** (m, 2H); **1.84, 1.47** (m, 2H). ^{13}C NMR (100 MHz, $CD_3OD/CDCl_3$): 169.3, 151.5, 140.3, 139.1, 137.5, 135.7, 134.8, 134.0, 129.3, 128.5, 127.9, 127.8, 126.8, 126.5, 125.6, 116.4, 44.5, 43.2, 26.5, 25.6, 24.4. MS (EI+): m/z 460.0 (exact mass = 460.1569).

6-(3-(Cyclopropanesulfonamido)phenyl)-3-(4-(methylsulfonyl)phenyl)imidazo[1,2-b]pyridazine (37). Column DCM/MeOH (99:1, 98:2), recrystallization in DCM/MeOH, 50%. ^{1}H NMR (300 MHz, CD_3OD): δ **8.43** (d, 2H; J = 8.7); **8.23** (s, 1H); **8.15** (d, 1H; J = 9.3); **8.12, 8.09** (m, 3H); **7.79** (ddd, 1H; J = 1.2, J = 1.6, J = 7.8); **7.62** (d, 1H; J = 9.3); **7.53** (t, 1H; J = 7.8); **7.33** (ddd, 1H; J = 1.2, J = 1.8, J = 7.8); **6.62** (s, 1H); **3.13** (s, 3H); **2.64, 2.51** (m, 2H); **1.32, 1.22** (m, 2H); **1.06, 0.96** (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): 151.6, 138.8, 136.0, 133.6, 129.8, 127.5, 126.8, 125.7, 122.6, 122.2, 118.8, 116.8, 43.9, 29.7, 5.0. MS (EI+): m/z = 467.9 (exact mass = 468.0926).

(3-(3-(4-(Methylsulfonyl)phenyl)imidazo[1,2-b]pyridazin-6-yl)phenyl)(piperazin-1-yl)methanone (38). Suzuki reaction was performed with homemade boc-protected [3-(1-piperazinylcarbonyl)-phenyl]-boronic acid, pinacol ester. After deprotection following the Boc deprotection procedure, compound **38** was obtained. Column DCM/MeOH (95:5, 90:10), recrystallization in DCM/MeOH, 90%. ^{1}H NMR (300 MHz, CD_3OD): δ **8.52** (d, 2H; J = 8.7); **8.34** (s, 1H); 8.26, **8.22** (m, 2H); **8.18** (s, 1H); **8.12** (d, 2H; J = 8.7); 7.**95** (d, 1H; J = 9.6); **7.71** (t, 1H; J = 7.8); **7.63** (d, 1H; J = 7.8); **3.92, 3.49** (m, 4H); **3.21** (s, 3H); **3.06, 2.82** (m, 4H). ^{13}C NMR (100 MHz, dmso-d_6): 169.2, 151.5, 140.6, 139.7, 136.4, 135.9, 133.8, 130.0, 129.4, 129.0, 130.0, 127.2, 126.9, 126.6, 126.4, 117.6, 44.1, 43.1. MS (EI+): m/z = 460.9 (exact Mass =461.1522)

(3-(3-(4-(Methylsulfonyl)phenyl)imidazo[1,2-b]pyridazin-6-yl)phenyl)methanesulfonamide (39). Column AcOEt/MeOH (1:0, 9:1), recrystallization MeOH/AcOEt, 40%. ^{1}H NMR (400 MHz, DMSO-d_6): **8.65** (d, 2H; J = 7.6); **8.58** (s, 1H); **8.41** (d, 1H; J = 9.6); **8.14** (s, 1H); **8.12** (d, 2H; J = 7.6); **7.96** (d, 1H; J = 9.6); **7.87** (d, 1H; J = 7.6); 7.**59** (t, 1H; J = 7.6); **7.39** (d, 1H; J = 7.6); **3.31** (s, 3H); **3.12** (s, 3H). ^{13}C NMR (100 MHz, CD_3OD): 150.9, 140.0, 139.6, 139.0, 135.9, 135.1, 133.2, 130.0, 127.2, 126.4, 126.1, 125.8, 122.0, 121.1, 117.3, 116.4, 43.4, 38.4. MS (EI+): m/z = 441.9 (exact mass = 442.0769). mp =135 °C.

6-(4-Methyl-3-(trifluoromethyl)phenyl)-3-(4-(methylsulfonyl)phenyl)imidazo[1,2-b]pyridazine (40). Column DCM/MeOH (98:2), recrystallization DCM/MeOH, 63%. ^{1}H NMR (300 MHz, $CDCl_3$): **8.41** (d, 2H; J = 8.4); **8.24** (d, 1H; J = 6.0); **8.23** (s, 1H); **8.16** (d, 1H; J = 9.6); **8.13, 8.08** (m, 1H); **8.12** (d, 2H; J = 8.7); **7.63** (d, 1H; J = 9.6); **7.50** (d, 1H; J = 8.1); **3.13** (s, 3H); **2.60** (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 151.0, 147.6, 140.5, 139.4, 135.7, 135.2, 134.2, 133.4, 133.2, 130.3, 130.1, 128.1, 127.0, 126.9, 124.6, 124.6, 123.0, 116.2, 44.8, 19.5. MS (EI+): m/z = 431.0 (exact mass = 431.0915).

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 as described by González Cabrera et al.[8c] In vivo efficacy was conducted as previously described,[8c] 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 determined using standard flow cytometry techniques. Compounds were dissolved or suspended in a nonsolubilizing, standard suspension vehicle called HPMC (0.5% [wt/vol] hydroxypropylmethylcellulose, 0.5% [vol/vol] benzyl alcohol, 0.4% [vol/vol] Tween 80, and 0.9% [wt/vol] sodium chloride in water). Blood samples for the quadruple-dose regimens were collected on day 4 (96 h after infection).

■ 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 PK and metabolism studies. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: Kelly.Chibale@uct.ac.za. Fax: +27-21-6505195. Tel.: +27-21-6502557.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED:

HTS, high throughput screening; SAR, structure−activity relashionships; ADME, absorption, distribution, metabolism, and excretion; CQ, chloroquine; po, oral administration; iv, intraveneous administration; MSD, mean survival days; PK, pharmacokinetics; NMR, nuclear magnetic resonance; TLC, thin layer chromatography; MMV, Medicines for Malaria Ventures

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