Journal of Medicinal Chemistry

Article

Medicinal Chemistry Optimization of Antiplasmodial Imidazopyridazine Hits from High Throughput Screening of a SoftFocus Kinase Library: Part 2.

Claire Le Manach, Tanya Paquet, Diego Gonzalez Cabrera, Yassir Younis, Dale Taylor, Lubbe Wiesner, Nina Lawrence, Sylva Schwager, David Waterson, Michael J. Witty, Sergio Wittlin, Leslie J. Street, and Kelly Chibale

J. Med. Chem., **Just Accepted Manuscript** • Publication Date (Web): 14 Oct 2014

Just Accepted

Journal of Medicinal Chemistry is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Medicinal Chemistry Optimization of Antiplasmodial Imidazopyridazine Hits from High Throughput Screening of a SoftFocus Kinase Library: Part 2.

Claire Le Manach[†,⊥], Tanya Paquet[†], Diego Gonzàlez Cabrera[†], Yassir Younis[†], Dale Taylor [∞], Lubbe Wiesner [∞], Nina Lawrence [∞], Sylva Schwager [#], David Waterson[‡], Michael J. Witty[‡], Sergio Wittlin[◊,∫], Leslie J. Street[†] and Kelly Chibale[,†,#,⊥]*

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

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

[‡]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, 4003 Basel, Switzerland;

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

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

ABSTRACT: Based on our recent results on a novel series of imidazopyridazine-based antimalarials, we focused on identifying compounds with improved aqueous solubility and hERG profile, while maintaining metabolic stability and *in vitro* potency. Towards this objective, 41 compounds were synthesized and evaluated for antiplasmodial activity against NF54 (sensitive) and K1 (multi-drug resistant) strains of the malaria parasite *Plasmodium falciparum*, and evaluated for both aqueous solubility and metabolic stability. Selected compounds were tested for in vitro hERG activity and *in vivo* efficacy in the *P. berghei* mouse model. Several compounds were identified with significantly improved aqueous solubility, good metabolic stability and a clean hERG profile relative to a previous frontrunner lead compound. A sulfoxide-based imidazopyridazine analog **45**, arising from a prodrug-like strategy, was completely curative in the *Plasmodium berghei* mouse model at 4 x 50 mg/kg p.o.

KEYWORDS: antiplasmodial activity, imidazopyridazines, hERG, solubility, in vivo efficacy, SoftFocus kinase library, structure activity relationships

According to the 2013 World Health Organization report[1], malaria affects 207 million people worldwide and is responsible for over 627 000 deaths a year, especially among young children and pregnant women. The disease is transmitted by female Anopheles

mosquitoes and is caused by 5 different species of the protozoan *Plasmodium* parasite. Of these *falciparum* is the most lethal and the most prevalent in sub Saharan Africa.

The rapid development of antimalarial drug resistance has compromised the use of previously effective drugs such as chloroquine, making malaria impossible to treat in some areas. Signs of artemisinin resistance emerging in southeast Asia pose an immediate and urgent challenge[2,3]. Various types of drug combinations with independent modes of action have been gradually introduced to prevent and/or slow down the emergence of resistant strains,[4] yet the need for new molecules with novel mechanisms of action remains.

Although there are several compounds in clinical or preclinical stages of development, it is noteworthy that successful development of these compounds is not guaranteed. In order to maintain a sustained pipeline of antimalarial drugs to stem the tide of drug resistance, a natural consequence of chemotherapy, the discovery and development of new molecules with novel mechanisms of action and able to circumvent antimalarial drug resistance is warranted.

We recently identified a series of imidazopyridazine derivatives[5] as a new class of antimalarial agents with *in vivo* activity in both the *P. berghei* and *P. falciparum* mouse models. The early lead and frontrunner compound, the 3,6-diarylimidazopyridazine **1** (Figure 1), showed high antiplasmodial activity *in vitro* (IC_{50} K1 = 6.3/ NF54 = 7.3 nM) and good oral efficacy (98% at 4 x 50 mg/kg p.o.) in the *in vivo* mouse *P. berghei* model. This compound has also shown activity against other lifecycle stages of the parasite (unpublished data). However, this compound did not produce an impressive *in vivo* efficacy at lower doses (4 x 10 mg/kg and 4 x 3 mg/kg), with only a mouse mean survival

of 7 days at all three doses. Moreover, compound **1** and the vast majority of the analogues in the same series displayed poor solubility (<5 μM at pH 6.5) and were found to have a serious hERG liability. The human Ether-à-go-go Related Gene (hERG) is a potassium channel associated with prolongation of the length of time between the start of the Q wave and the T wave on an electrocardiogram. Potentially life-threatening arrhythmia may result from drug-induced blockade of the hERG channel. Thus in order to address the aforementioned shortcomings and identify compounds with an improved profile, we embarked on further exploration of structure-activity relationships.

In this paper we report the strategies that were adopted in order to address both poor aqueous solubility and high hERG activity whilst retaining anti-malarial activity. In addition, a prodrug-like strategy that was successfully used to improve the anti-malarial activity *in vivo* is presented.

>>Figure 1<<

Chemistry: Most of the boronic acids used in the synthesis are commercially available. For those not commercially available, boronic esters were prepared in house and their synthesis is described in the Supporting Information.

Compounds **(6-9**, **16-18**, **21**, **22)** were prepared following a previously described synthetic route[5]. A Suzuki coupling reaction was performed with the corresponding boronic acid on 6-chloro-3-(4-sulfonylmethyl)phenyl-pyridazine to give the desired compounds (Scheme 1). An additional deprotection on Boc-protected compound **22-a** in the presence of TFA was required to obtain final compound **22**.

The synthesis of target compounds **10-15** and **25-34**, **36, 37** and **39-45** was achieved following a relatively straightforward 4-step synthetic route from the commercially available 3-amino-6-chloropyridazine **2** (Scheme 2).

Briefly, a quantitative ring closure using bromoacetaldehyde diethylacetal and HBr was performed on **2** to give **3**. A Suzuki cross coupling reaction[6,7] with suitable boronic acid gave the desired intermediates **4a-e**. Bromination of **4a-e** with NBS in DMF then led to **5a-e** in high yield. Finally, a second Suzuki cross coupling reaction with appropriate boronic acids gave the desired compounds (**10-12**, **25-34, 36, 37, 39-45**).

Tetrazole **20** was obtained from **19** using 1,4 cycloaddition with sodium azide. Amides **23** and **24** were prepared from the carboxylic acid derivatives using EDCI coupling with the corresponding amine (hydroxy-piperidine and –azetidine), Scheme 3.

Amides **35** and **38** were prepared from the corresponding acids using EDCI coupling as shown on Scheme 4.

In vitro Antiplasmodial activity and solubility: Previously disclosed antiplasmodial imidazopyridazine derivatives showed poor solubility profiles[5], which can potentially negatively impact their in vivo efficacy. In order to address this issue, we investigated various strategies to improve solubility in the imidazopyridazine series while retaining potency. To this end, several analogues were synthesized and evaluated for solubility and in vitro antiplasmodial activity against the sensitive (NF54) strain with selected compounds also being tested against the multi-drug resistant (K1) strain of *P. falciparum.* Chloroquine and artesunate were used as the reference drugs in all experiments. The in vitro antiplasmodial activities as indicated by their IC_{50} values as well as the aqueous solubility data are summarized in Table 1 and Table 2. In general, when tested in both

strains, all analogues were equipotent. The lack of cross resistance in the K1 strain suggests a distinct and novel mechanism of action of these compounds.

Selected compounds (**8**, **22**, **33**, **36**, **44** and **45**) showing good antiplasmodial activity were tested for cytotoxicity and found not to be cytotoxic at the highest concentration tested. They all showed a selectivity index of >1000. Cytotoxicity data are summarized in the Supporting Information (Table ST1). Compound **1** and its 3-(trifluoromethyl) analogue, both previously published[5], did also not show any cytotoxicity at the highest concentration tested (IC_{50} CHO cells: >200 μM, SI: >1000).

Our first strategy towards addressing the solubility issue involved reducing lipophilicity of the compounds by replacing phenyl rings with pyridyl rings. In this context, compounds **6-15** were prepared. Antiplasmodial activity and aqueous solubility were assessed and the results are listed in Table 1. Unfortunately most of the pyridine derivatives showed little improvement in solubility, not unexpected given the weak basicity of the methylsulfonyl-, and trifluoromethyl substituted pyridines, and in some cases antiplasmodial activity decreased dramatically. Compounds **7**, **13-15** turned out to be poorly active with IC_{50}'s ≥ 470 nM. Compound **6** was moderately active (IC_{50} ≈ 100 nM) but its solubility was still poor. Compounds **8** and **11** remained active (IC_{50} ≈ 50 nM) but solubility still remained an issue. The more basic amino-pyridine compounds **9** and **12** retained high potency and showed improved solubility ≥ 100 μM at pH 2 although solubility at pH 6.5 remained poor.

A second strategy, which involved introducing water-solubilizing H-bonding groups onto the phenyl and pyridine rings was also implemented. In this regard, analogues based on piperazine amides, sulfonamides, tetrazoles, benzotriazoles, hydroxy-pyrrolidine, -

piperidine or –azetidine were synthesized accordingly. The structures, in vitro antiplasmodial activities and solubility values for the best derivatives are shown in Table 2. The rest of the data is in the Supporting Information (Tables ST2). An improvement in solubility was observed for the hydroxyl-containing amides **17**, **35** and **23** and the piperazine sulfone **26** at both pH 2 and pH 6.5. In all cases solubility appeared to be close to or above 100 μM, whilst compounds remained highly active with NF54 IC_{50}'s below 35 nM.

In a third strategy, the sulfonylmethyl substituent was also replaced by a more strongly H-bonding sulfinylmethyl group at position 6 and/or 3 (**16**, **32, 37** and **40**) or by various alkylsulfonyl chains substituted with terminal amine (**22**, **36**) or alcohol groups (**21**). Replacement of the sulfone group with sulfoxide led to a significant increase in aqueous solubility from <5 μM for reference compound **1** to >100 μM for the four sulfoxide derivatives (**16**, **32, 37** and **40)**. It was pleasing to observe that all compounds proved to be highly potent (IC_{50}'s ≤ 35 nM), except compound **37**, which showed moderate activity (NF54 IC_{50} = 103 nM). Regarding compounds with alkyl-sulfones, the two compounds with a terminal amino group **22** and **36** also proved to be highly soluble and active (NF54 IC_{50} = 3.0 nM and 10 nM respectively). The exception was compound **37**, which showed moderate activity (NF54 IC_{50} = 103 nM).

Additional compounds containing a cyclopropylsulfone instead of a methylsulfone at the 6-position were also prepared and tested (Table 3). Interestingly, the cyclopropyl substituent imparted a significant improvement in potency. Compounds **42**, **44** and **45** were about 5 times more active than their sulfonylmethyl analogues **10**, **29** and **32**. Tetrazole derivative **43** was 13 times more active than its corresponding sulfone whereas

41 was only slightly more active than **11** (1.6 times). Solubility remained unchanged for compounds **41-43** compared to their methylsulfonyl analogues. For compounds **44** and **45** a drop in solubility was observed.

hERG activity: The activity against the hERG potassium channel was determined using in vitro IonWorks patch-clamp electrophysiology[8]. Sulfoxides **32**, **16** and **40**, hydroxyl-cycloalkyl amides **35** and **22**, and tetrazoles **43** and **45** were evaluated for hERG activity. The metabolized form of sulfoxide **45,** sulfone **46,** proved to be highly active in the hERG assay with an IC_{50} of 0.4 (0.3 – 0.5) μM. On the other hand compounds **32** and **16** were found to have - hERG IC_{50}'s of 4.0 (2.6 - 6.2) μM and 4.2 (3.3 – 5.2) μM respectively, while activity was moderate for **40** with an IC_{50} of 12.0 (8.0 – 18.0) μM-. Gratifyingly, the introduction of hydrophilic groups such as amines, tetrazoles and hydroxyl-cycloalkyl amides improves selectivity over hERG and the analogues with improved solubility (**35**, **22** and **43**) lacked activity against hERG at the highest (IC_{50}'s > 33 μM) concentration tested. Unfortunately, all of the sulfones and consequently the pro-drug sulfoxides retained a hERG risk due to anticipated oxidation to the corresponding sulfones in vivo.

In vitro metabolic stability: Metabolic stability of the most active compounds was assessed in vitro in human, rat (and mouse) microsomal preparations[9]. A quick (1 point) assay determines the percentage remaining after 30 minutes incubation in the presence of liver microsomes. Most of our compounds were assayed for metabolic stability using this method and the results are shown in Table ST3 of the Supporting Information. The microsome-predicted hepatic extraction ratios (E_H) were determined using a more precise assay (5 points – 60 minutes) and the results of the few compounds that have been

evaluated for metabolic stability using this assay are shown in Table ST4 of the Supporting Information. In general there is a good correlation between the results obtained with the one-point assay and those resulting from the five-point assay. The metabolic stability values were most often consistent across rat and human microsomes. However, in some cases species differences were observed.

Compounds were generally designed to provide good metabolic stability by ensuring electron deficient aryl rings and avoiding metabolically labile substituents. An exception are the compounds with sulfoxides side chains which were expected to behave as pro-drugs and to be rapidly metabolized to the corresponding active sulfones even in the in vitro microsomal assays.

Amongst the compounds showing good solubility and high potency against *P. falciparum*, **22**, **35** and **36** showed good in vitro metabolic stability in liver microsomes. On the other hand, compounds **23**, **34** and **32** exhibited moderate metabolic stability while **16**, **17**, **40** and **26** had poor to moderate stability with species differences being observed. All cyclopropyl derivatives proved stable across the 3 species except the bis-sulfoxide **45**.

In vivo efficacy studies: Compounds that displayed good in vitro antiplasmodial activity and metabolic stability together with improved solubility were tested for in vivo efficacy in *P. berghei* infected mice. A few compounds with less metabolic stability, such as the sulfoxide "pro-drugs" were also evaluated in vivo. The in vivo activity was determined following oral administration (p.o.) of 50 mg/kg/day for 4 days. The results are summarized in Table 4. Compounds **22, 34**, **35** and **43** showed poor (<40% reduction in parasitemia) despite showing good in vitro potency, solubility and microsomal

metabolic stability. The three mono- or bissulfoxide derivatives **16, 32** and **40** showed improved in vivo efficacy at 4 x 50 mg/kg p.o. relative to the previous frontrunner compound **1**. The mean survival days (MSD) improved from 7 to 14 days for **32** and **16**. An encouraging result (25 MSD) was delivered by compound **40** with 2 out of 3 mice treated with this compound experiencing a complete cure. The most impressive activity, however, was demonstrated by compound **45**, the cyclopropylsulfoxide analog of **32**. This compound was completely curative at 4 x 50 mg/kg p.o. It is noteworthy that this is the first compound in the imidazopyridazine series to demonstrate a complete cure. It remains unclear if the very last parasites were actually eliminated by the drug itself or removed by the host immune system. However, at 4 x 10 mg/kg and 4 x 3 mg/kg a fast recrudescence of parasites was observed, the number of MSD were 8 and 7 days respectively. Such fast recrudescence of parasites indicates that the immune system was at least during that time window not having an influence on parasite proliferation. In addition a fast recrudescence was also observed with the reference compound artesunate, which is known to have a short half-life. The in vivo activity of **45** remained high with 99.6 % inhibition of parasitemia at 4 x 10 mg/kg and 98% at 4 x 3 mg/kg. Disappointingly, none of the potent compounds with water solubilizing side-chains in the amide or sulfone series showed in vivo activity, and poor absorption or high non-microsomal clearance was considered the likely cause.

In vivo pharmacokinetic studies: Mouse snapshot pharmacokinetics were performed in parallel to *P. berghei* in vivo efficacy studies in an attempt to rationalize the observed efficacy. Infected mice were treated with the various compounds at 50 mg/kg (p.o.) for 4 days. Blood samples were taken 1 hour, 4 hours and 24 hours after the first dose. These

samples were then analyzed by HPLC in order to determine the pharmacokinetic profiles (Table 5). Profiles of **22** and **35** showed that very little compound was present after 4 hours and unusual plasma profiles, suggesting poor absorption. Compounds **16** and **40** were metabolized into **1**. The solubility of the initial compounds **16** and **40** was improved compared to our original compound **1**. The increase in in vivo potency can be explained by the increased plasma exposure of compound **1** at t = 4h. As expected **40** gave better in vivo efficacy results than **16**, which in turn gave better results than **1**. Considering all the data, it is most likely that **32** behaves similarly to **16**.

Similar studies were conducted with compound **45** and similar behavior was observed, in which both the parent compound and metabolite are active. The sulfoxide was rapidly metabolized to the very active sulfone **46** (IC_{50} K1/NF54 = 0.5/0.9 nM) (Figure 2). The superior solubility of the sulfoxide allowed the sulfone biotransformation product to achieve a high circulating concentration *in vivo*. This is analogous to a prodrug approach albeit the sulfoxide is strictly speaking not a classical prodrug.

Further to the mouse snapshot studies, pharmacokinetic studies with **45** and **46** were performed in male Sprague-Dawley rats for comparison (Table 6). Sulfone **46** achieved a maximum plasma concentration of 1.5 μM with a bioavailability of 24%. As expected, sulfoxide **45** had a short half-life (0.55 h) and high clearance (46 mL/min/kg), only reaching a plasma concentration of 0.04 μM. Yet, an oral bioavailability of 11% was observed. On the other hand, the plasma concentration of **46**, achieved through an equivalent dose of **45**, was 2.3 μM with a proportional increase in bioavailability to 36%. Presumably the increased exposure of sulfone **46** achieved through administering

sulfoxide **45**, together with the superior potencies of both parent and metabolite compounds, contributes to the in vivo efficacy observed.

Conclusion: In our quest for compounds with better solubility and an improved hERG profile, we have identified two molecules (**22** and **35**) with improved properties. In addition, sulfoxide derivatives **16**, **32** and **40** were shown to have greatly improved solubility compared to the original early lead sulfone compound **1** but retain the hERG liability. This improvement in solubility was accompanied by improvement in in vivo efficacy. Cyclopropyl analog **45** was completely curative in the *P. berghei* mouse model at 4 x 50 mg/kg and becomes only the first compound in this series to achieve this feat**.** The curative action is in contrast with artesunate and chloroquine, which, when given orally at 4 x 30 mg/kg, showed mean survival times of 10 and 24 days, respectively. Following the same dosing regimen, mefloquine prolonged survival up to 29 days[5]. For the 3 reference compounds to achieve cure in the *P. berghei* mouse model, four daily oral doses of 100 mg/kg of artesunate or chloroquine are required, whereas mefloquine induces strong acute toxicity symptoms at this high dose.

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. ^{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 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. Analytical thin-layer chromatography (TLC) was performed on aluminium-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.

6-Chloroimidazo[1,2-b]pyridazine (3): To a solution of 3-Amino-6-Chloropyridazine **2** (1 g, 7.7 mmol, 1 eq) in EtOH (15 mL) and water (10 mL) was added bromoacetaldehyde diethylacetal (2.2 mL, 14.1 mmol, 2 eq) 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. δ (ppm): ^{1}H NMR (300 MHz, $CDCl_3$), 7.91, 7.96 (m, 3H); 7.75 (s, 1H); 7.03 (d, 1H: J= 9.2).

6-(3-(Methylsulfonyl)phenyl)imidazo[1,2-b]pyridazine (4a): Compound **3** (2 g, 13 mmol, 1 eq) was dissolved in DMF (15 mL) with 3-(methylsulfonyl)phenyl boronic acid (2.8 g, 14.3 mmol, 1.1 eq) and $Pd(PPh_3)_2Cl_2$ (456 mg, 0.65 mmol, 0.05 eq). The resulting mixture was flushed with nitrogen for 15 min, after which aqueous K_2CO_3 (1M) (13 mL, 13.7 mmol, 1.05 eq) 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 (DCM/MeOH 98:2, 95:5) and cristallized in ethyl acetate to give the desired product **4** in 50 % yield. δ (ppm): ^{1}H NMR (300 MHz, $CDCl_3$), 8.59 (t, 1H; J = 1.8); 8.26 (d, 2H; J = 8.1); 8.10, 8.07 (m, 3H); 7.85 (d, 1H; J = 1.2); 7.77 (t, 1H; J = 7.8); 7.53 (d, 1H; J = 9.6); 3.14 (s, 3H).

Compounds **4b, 4c, 4d** and **4e** were obtained following the same procedure using 3-trifluomethylphenyl boronic acid, 3-(cyclopropylsulfonyl)phenyl boronic acid, 3-(methylsulfonyl)phenyl boronic acid and (5-(methylsulfonyl)pyridin-3-yl)boronic acid respectively.

6-(3-(Trifluoromethyl)phenyl)imidazo[1,2-b]pyridazine (4b): 58%, δ (ppm): ^{1}H NMR (400 MHz, $CDCl_3$), 8.21 (s, 1H); 8.13, 7.94 (m, 3H); 7.80 (s, 1H); 7.71 (d, 1H; J = 8.8); 7.61 (t, 1H; J = 8.0); 7.48 (d, 1H; J = 9.6).

6-(3-(Cyclopropylsulfonyl)phenyl)imidazo[1,2-b]pyridazine (4c): 51%, δ (ppm): ^{1}H NMR (400 MHz, $CDCl_3$), 8.52 (t, 1H; J = 1.8); 8.24 (d, 2H; J = 8.0); 8.10, 8.01 (m, 3H); 7.85 (s, 1H); 7.73 (t, 1H; J = 7.8); 7.54 (d, 1H; J = 9.6); 2,57, 2.51 (m, 1H); 1.44, 1.41 (m, 2H); 1.09 1.06 (m, 2H).

6-(3-(Methylsulfinyl)phenyl)imidazo[1,2-b]pyridazine (4d): 89%, MS (EI+): m/z 257.1 (exact Mass = 257.0623).

6-(5-(Methylsulfonyl)pyridin-3-yl)imidazo[1,2-b]pyridazine (4e): 42%, MS (EI+): m/z 274.1 (exact Mass = 274.0525).

3-Bromo-6-(3-(methylsulfonyl)phenyl)imidazo[1,2-b]pyridazine (5a): Compound **4a** (2 g, 7.8 mmol, 1 eq) was dissolved in DMF (5 mL) and the resulting mixture was flushed with nitrogen. NBS (1.5 g, 8.3 mmol, 1.1 eq) was added in one portion and the

solution was stirred at r.t. for 1 hour, after which the compound crashed out. DMF was removed *in vacuo.* The residue was cristallized with EtOAc and the resulting solid was filtered off and rinsed with hexane to give **5a**. R = SO_2Me, 85%, δ (ppm): ^1H NMR (300 MHz, $CDCl_3$), 8.57 (t, 1H; J = 1.8); 8.39 (dd, 1H; J = 1.5; J = 8.1); 8.09, 8.05 (m, 2H); 7.84 (d, 1H; J = 1.2); 7.79 (t, 1H; J = 7.8); 7.59 (d, 1H; J = 9.6); 3.14 (s, 3H).

Compounds **5b, 5c, 5d** and **5e** were obtained following the same procedure using **4b**, **4c**, **4d** and **4e**, respectively.

3-Bromo-6-(3-(trifluoromethyl)phenyl)imidazo[1,2-b]pyridazine (5b): R = CF_3, 85%, δ (ppm): ^1H NMR (300 MHz, $CDCl_3$), 8.33, 8.22 (m, 2H); 8.07 (d, 1H; J = 9.6); 7.85 (s, 1H); 7.80 (d, 1H; J = 7.8); 7.70 (t, 1H; J = 7.8); 7.59 (d, 1H; J = 9.6).

3-Bromo-6-(3-(cyclopropylsulfonyl)phenyl)imidazo[1,2-b]pyridazine (5c): R = SO_2(cyclopropyl), 86%, δ (ppm): ^1H NMR (300 MHz, $CDCl_3$), 8.57 (t, 1H; J = 1.8); 8.39 (dd, 1H; J = 1.5; J = 8.0); 8.09, 8.05 (m, 2H); 7.84 (d, 1H; J = 1.2); 7.79 (t, 1H; J = 7.8); 7.59 (d, 1H; J = 9.6); 3.14 (s, 3H); 2,57, 2.50 (m, 1H); 1.44, 1.41 (m, 2H); 1.10 1.07 (m, 2H).

3-Bromo-6-(3-(methylsulfinyl)phenyl)imidazo[1,2-b]pyridazine (5d): R = SOMe, 92%, δ (ppm): ^1H NMR (400 MHz, DMSO-d_6, 8.32 (t, 1H; J = 1.6); 8.25 (d, 1H; J = 9.6); 8.20 (d, 1H; J = 7.6); 7.96, 7.87 (m, 2H); 7.81 (d, 1H; J = 7.6); 7.72 (t, 1H; J = 7.6); 2.78 (s, 3H).

3-Bromo-6-(5-(methylsulfonyl)pyridin-3-yl)imidazo[1,2-b]pyridazine (5e): 92%, δ (ppm): ^1H NMR (400 MHz, DMSO-d_6), 9.60 (d, 1H; J = 2.0); 9.25 (d, 1H; J = 2.4); 8.93 (dd, 1H, J = 2.0, J = 2.4); 8.39 (d, 1H; J = 9.6); 8.13 (d, 1H; J = 9.6); 8.04 (s, 1H); 3.44 (s, 3H).

General procedure for the first Suzuki cross-coupling reaction: Compounds **5a-e** (1 eq) were dissolved in DMF (1 mL/100mg of **5a-e**) with the corresponding boronic acid (1.1 eq) and $Pd(PPh_3)_2Cl_2$ (0.05 eq). The resulting mixture was flushed with nitrogen for 15 min, after which aqueous K_2CO_3 (1M) (1.05 eq) was added. The solution was heated to 90°C (up to 120°C for compound **33**) 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 recristallized in an adequate solvent system to give the desired product in 30 to 68 % yield.

6-(3-(Methylsulfonyl)phenyl)-3-(4-(methylsulfonyl)phenyl)imidazo[1,2-b]pyridazine (16): Column DCM/MeOH (98:2, 95:5), crystallization in AcOEt, 30**%**. ^{1}H (400 MHz, $CDCl_3$), 8.38 (d, 2H; J = 8.8); 8.32, 8.24 (m, 3H); 8.21, 8.17 (m, 1H); 8.11 (d, 2H; J = 8.8); 7.77, 7.73 (m; 2H); 3.13 (s, 3H); 2.82 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): 150.6, 147.7, 140.1, 139.2, 135.9, 135.4, 133.2, 130.2, 129.2, 127.5, 126.8, 126.4, 126.1, 125.3, 122.2, 116.9, 43.5, 43.3. MS (EI+): m/z = 411.0 (exact Mass = 411.0711).

3-((3-(3-(4-(Methylsulfonyl)phenyl)imidazo[1,2-b]pyridazin-6-yl)phenyl)sulfonyl)propan-1-amine (22): The suzuki coupling was performed with the boronic ester *tert*-butyl (3-((3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)sulfonyl)propyl)carbamate, which synthesis is described in the Supporting Information. The desired compound was obtained after purification on silica gel with DCM/MeOH (98:2) to (95:5) in 40% yield. This compound was then deprotected with TFA (general procedure in the Supporting Information). After evaporation of the solvent,

the product was crystallized in MeOH/Et_2O**.** Filtration gave the desired product in 79% yield. ^1H (400 MHz, dmso-d_6), 8.61 (s, 1H); 8.60, 8.56 (m, 4H); 8.46 (d, 1H; J = 9.6); 8.11, 8.07 (m, 3H); 7.95 (t, 1H; J = 7.8); 7.66 (s, 2H); 3.58 (t, 2H; J = 7.4); 3.35 (s, 3H); 2.91 (t, 2H; J = 7.4); 1.91, 1.68 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): 150.0, 140.1,139.7, 139.3, 136.3, 135.6, 133.1, 132.4, 130.7, 129.2, 127.4, 126.9, 126.4, 126.1, 116.8, 51.6, 41.5, 374, 20.8. MS (EI+): m/z = 470.1 (exact Mass = 470.1082).

(4-Hydroxypiperidin-1-yl)(3-(3-(4-(methylsulfonyl)phenyl)imidazo[1,2-b]pyridazin-6-yl)phenyl)methanone (23): 6-chloro-3-(4-Sulfonylmethyl)phenyl-chloropyridazine was reacted with 3-carboxyphenylboronic acid following the general Suzuki procedure described above. The isolated carboxylic acid derivative (40 mg, 01.102 mmol, 1eq) was then dissolved in DMF (1 mL) with EDCI.HCl (30 mg, 0.143 mmol, 1.4 eq), Et_3N (57 µL, 0.408 mmol, 4 eq) and HOBt (2 mg, 0.01 mmol, 0.1 eq). After stirring at r.t. for 1h, hydroxypiperidine (11 mg, 0.112 mmol, 1.1 eq) was added. The solution was stirred at r.t. overnight. Extra hydropiperidine (1.1 eq), EDCI.HCl (1.4 eq), Et_3N (4 eq) and HOBt (0.1 eq) were added and the resulting mixture was stirred for an extra 48h. DMF was then removed and the residue was purified on silica gel DCM/MeOH (100:0, 98:2, 95:5, 90:10). White powder, 37**%**. δ (ppm): ^1H NMR (400 MHz, dmso-d_6), 8.55 (d, 2H; J = 8.4); 8.50 (s, 1H); 8.36 (d, 1H ; J = 9.2); 8.23 (d, 1H; J = 7.6); 8.11, 8.06 (m, 3H); 8.00 (d, 1H; J = 9.2); 7.69 (t, 1H; J = 7.6); 7.56 (d, 1H; J = 7.6); 4.81 (d, 1H; J = 4.0); 3.84, 3.75 (m, 4H); 3.25 (s, 3H), 3.19, 3.14 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6): 168.0, 150.7, 139.8, 139.0, 137.1, 135.1, 134.9, 133.0, 129.2, 128.2, 127.7, 127.2, 126.4, 126.2, 125.9, 124.9, 116.7, 65.1, 43.3. MS (EI+): m/z = 476.1 (exact Mass = 476.1518).

3-(4-(Methylsulfinyl)phenyl)-6-(3-(methylsulfonyl)phenyl)imidazo[1,2-b]pyridazine (32): Column DCM/MeOH (98:2, 96:4), crystallization in AcOEt, 68**%**. δ (ppm): ^{1}H NMR (400 MHz, $CDCl_3$), 8.56 (t, 1H; J = 2.0); 8.37, 8.31 (m, 3H); 8.21 (s, 1H); 8.18 (d, 1H; J = 9.6); 8.10 (dd, 1H; J = 1.6, J = 7.2); 7.83 (d, 2H; J = 8.8); 7.79 (t; 1H; J = 8.0); 7.64 (d, 1H; J = 9.6); 3.13 (s, 3H); 2.81 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): 150.1, 145.3, 141.9, 139.7, 137.1, 134.5, 132.0, 131.2, 130.5, 128.8, 127.4, 126.8, 126.0, 124.1, 115.6, 44.5, 44.0. MS (EI+): m/z = 411.0 (exact Mass = 411.0711).

(3-Hydroxypyrrolidin-1-yl)(4-(6-(3-(methylsulfonyl)phenyl)imidazo[1,2-b]pyridazin-3-yl)phenyl)methanone (35): Column DCM/MeOH (98:2, 95:5), crystallization in AcOEt, 33**%**. δ (ppm): (400 MHz, $CDCl_3$), 8.51 (s, 1H); 8.27 (t, 1H; J = 2.0); 8.17, 7.96 (m, 5H); 7.77, 7.69 (m, 1H); 7.68,7.60 (m, 2H); 7.59, 7.53 (m, 1H); 4.58 (s, 0.5H); 4.47 (s, 0.5H); 3.97, 3.47 (m, 4H); 3.11 (s, 3H); 2.15, 1.96 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): 169.5, 149.7, 141.8, 137.0, 136.1, 134.1, 134.0, 132.0, 130.4, 130.0, 129.8, 128.7, 128.2, 127.8, 126.5, 125.9, 115.3, 70.8, 69.5, 57.5, 55.0, 47.4, 44.5, 34.8, 32.9, 29.7, 24.8. MS (EI+): m/z = 461.9 (exact Mass = 462.1362).

6-(3-(Methylsulfinyl)phenyl)-3-(4-(methylsulfinyl)phenyl)imidazo[1,2-b]pyridazine (40): Column DCM/MeOH (98:2, 95:5), crystallization in AcOEt, 38**%**. δ (ppm): ^{1}H (400 MHz, $CDCl_3$), 8.34, 8.28 (m, 3H); 8.19, 8.12 (m, 3H); 7.80 (d, 2H; J = 8.4); 7.76, 7.68 (m; 2H); 7.65 (d, 1H; J = 9.6); 2.80 (s, 3H); 2.79 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): 150.4, 147.7, 145.3, 139.6, 136.1, 134.6, 130.6, 130.1, 129.1, 126.6, 125.2, 124.1, 122.1, 116.4, 43.2. MS (EI+): m/z = 395.1 (exact Mass = 395.0762).

3-(4-(1H-Tetrazol-5-yl)phenyl)-6-(3-(cyclopropylsulfonyl)phenyl)imidazo[1,2-b]pyridazine (43): Column DCM/MeOH (98:2) (95:5) (90:10) (85:15), 30%. δ (ppm):

^{1}H NMR (300 MHz, $CDCl_3$), 8.50 (d, 2H; J = 8.7); 8.34 (s, 1H); 8.28, 8.20 (m, 3H); 8.10 (d, 2H; J = 8.7); 7.92 (d, 1H; J = 9.6); 7.73 (t; 1H; J = 7.2); 7.70, 7.66 (m, 1H); 2.82 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): 155.3, 149.7, 141.5, 139.5, 136.2, 134.5, 131.7, 130.4, 128.4, 127.0; 126.5, 126.4, 125.3, 123.6, 116.1, 31.7, 5.3. MS (EI+): m/z 443.0755 (exact Mass = 443.1164).

6-(3-(Cyclopropylsulfonyl)phenyl)-3-(4-(methylsulfinyl)phenyl)imidazo[1,2-b]pyridazine (45): Column: DCM/MeOH (100:0) (98:2) (95:5), 44%. δ (ppm): ^{1}H NMR (400 MHz, $CDCl_3$), 8.49 (t, 1H; J = 2.0); 8.32, 8.29 (m, 3H); 8.18 (s, 1H); 8.15 (d, 1H; J = 9.6); 8.05, 8.00 (m, 1H); 7.80 (d, 2H; J = 8.8); 7.75 (t, 1H; J = 8.0); 7.62 (d, 1H; J = 9.6); 2.79 (s, 3H); 2.57, 2.47 (m, 1H); 1.43, 1.37 (m, 2H); 1.12, 1.06 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$), 150.3, 145.3, 142.2, 140.0, 137.0, 134.7, 131.8, 131.4, 130.4, 129.1, 128.4, 127.8, 127.5, 126.9, 126.3, 124.2, 115.7, 44.1, 33.1, 21.1, 6.3. MS (EI+): m/z 437.0 (exact Mass = 437.0868).

6-(3-(Cyclopropylsulfonyl)phenyl)-3-(4-(methylsulfonyl)phenyl)imidazo[1,2-b]pyridazine (46): ^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 8.51 (t, 1H; J = 1.6); 8.40 (d, 2H; J = 8.4); 8.32 (dd, 1H; J = 7.8); 8.26 (s, 1H); 8.20 (d, 1H; J = 9.6); 8.13, 8.04 (m, 3H); 7.79 (t, 1H; J = 7.8); 7.66 (d, 1H; J = 9.6); 3.13 (s, 3H); 2.60, 2.48 (m, 1H); 1.48, 1.40 (m, 2H); 1.16, 1.07 (m, 2H). MS (EI+): m/z 453.1 (exact Mass = 453.0817).

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[10]. In vivo efficacy was conducted as previously described[11], 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 1,000 erythrocytes (that is, 0.1%). Activity was calculated as the difference between the mean per cent parasitaemia for the control and treated groups expressed as a per cent relative to the control group. Compounds were dissolved or suspended in a non-solubilizing, 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), and orally administered once per day on four consecutive days (4, 24, 48 and 72 h after infection). Blood samples for the quadruple-dose regimens were collected on day 4 (96 h after infection).

Pharmacokinetic studies in Male Sprague-Dawley Rats

Adult male Sprague-Dawley rats were starved overnight prior to exposure with the test compounds. Compounds were freshly prepared to 3.6 mg/kg body weight immediately prior to dosing. Compounds were dissolved in a mixture of water, 0.01 M hydrochloric acid solution, propylene glycol, ethanol and Tween 80 (1:2:1:0.1:0.1). All compounds were dosed orally in 1mL volumes. For the intravenous administration, compounds were dosed in 350 µL. Water was provided ad libitum throughout the exposure and food was returned to the animals 5 hours post-dose.

Blood samples were collected into heparinized microcentrifugation tubes at specific time-points and spun down at 12000 G for three minutes. Plasma was removed and held at -80°C until analysis by LC/MS/MS methods.

Compounds were extracted and quantified by transferring 20 µL of plasma to a separate vessel and adding 100 µL of cold acetonitrile to precipitate plasma proteins.

Samples were vortexed vigorously for 30s and then pelleted at 12000 G for three minutes. A volume of 50 µL of the supernatant was transferred to a 96-well plate and 50 µL of 0.1% (vol/vol) formic acid solution added to each well. 5µL from each well was used for LC/MS/MS analysis.

Chromatographic separation was achieved on a Phenomenex Hydro-RP column using a varying gradient of 0.1% (vol/vol) formic acid and acetonitrile. Detection was carried out on an ABSciex API 3200 mass spectrometer. Pharmacokinetic parameters were determined using non-compartmental analysis methods in PK Solutions 2.0 (Summit Research Services, Montrose CO, USA).

ASSOCIATED CONTENT:

Supporting Information. Additional details of the characterization of selected compounds and the procedures used for metabolism studies. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION:

Corresponding author

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

ACKNOWLEDGMENT:

We thank MEDICINES FOR MALARIA VENTURES (MMV) for financial support of this research (Project MMV09/0002). The University of Cape Town, South African Medical Research Council, and South African Research Chairs Initiative of the

Department of Science and Technology, administered through the South African National Research Foundation are gratefully acknowledged for support (K.C). We thank Christian Scheurer, Sibylle Sax and Ursula Lehmann (Swiss Tropical and Public Health Institute) for assistance in performing antimalarial assays. We also thank Prof Peter J. Smith and Dr Carmen de Kock (Division of Pharmacology, University of Cape Town) for antiplasmodial data as well as Nina Lawrence and Sylva Schwager (Division of Pharmacology, University of Cape Town) for in vitro microsomal stability data.

ABBREVIATIONS USED:

CQ, chloroquine; p.o., oral administration; i.v., intraveneous administration; MSD, mean survival days; PK, pharmacokinetics; NMR, nuclear magnetic resonance; TLS, thin layer chromatography; MMV, medicines for malaria ventures; r.t., room temperature, LC/MS/MS, liquid chromatography – tandem mass spectrometry.

REFERENCES:

1. World Health Organization (WHO), World Malaria Report 2013; http://www.who.int/malaria/publications/world_malaria_report_2013/report/en/. Date accessed June 12.2014.

2. Burrows, N. J.; Chibale, K.; Wells, T.N.C. The state of the art in anti-malarial drug discovery and development. *Curr. Top. Med. Chem.* **2011**, *11*, 1226-1254.

3. Dondorp, A. M.; Nosten, F.; Yi, P.; Das, D.; Phyo, A. P.; Tarning, J.; Lwin, K. M.; Ariey, F.; Hanpithakpong, W.; Lee, S. J.; Ringwald, P.; Silamut, K.; Imwong, M., Chotivanich, K.; Lim, P.; Herdman, T.; An, S. S.; Yeung, S.; Singhsivanon, P.; Day, N.

P.; Lingegardh, N.; Socheat, D.; White, N. J. Artemisinin resistance in *Plasmodium falciparum* malaria. *N. Engl. J. Med.* **2009**, *361*, 455-467.

4. Pink, R.; Hudson, A.; Mouries, M. A.; Bendig, M. Opportunities and challenges in antiparasitic drug discovery. *Nat. Rev. Drug Dicovery* **2005**, *4*, 727-740.

5. Le Manach, C.; Gonzalez Cabrera, D.; Douelle, F.; Nchinda, A.; Younis, Y.; Taylor, D.; Wiesner, L.; White, K. L.; Ryan E.; March, C.; Duffy, S.; Avery, V. M.; Waterson, D.; Witty, M. J.; Wittlin, S.; Charman, S. A.; Street, L. J.; Chibale, K. Medicinal chemistry optimization of antiplasmodial imidazopyridazines hits from high throughput screening of a SoftFocus Kinase Library: Part 1. *J. Med. Chem.* **2014**, 57, 2789-2798.

6. Miyaura, N.; Suzuki, A. Palladium-catalyzed cross-coupling reactions of organoboron compounds. *Chem. Rev.* **1995**, *95,* 2457-2483.

7. Thompson, A. E.; Hughes, G.; Batsanov, A. S.; Bryce, M. R.; Parry, P. R.; Tarbit, B. Palladium-catalyzed cross-coupling reactions of pyridylboronic acids with heteroaryl halides bearing a primary amine group: synthesis of highly substituted bipyridines and pyrazinopyridines. *J. Org. Chem*. **2005**, *70,* 388-390.

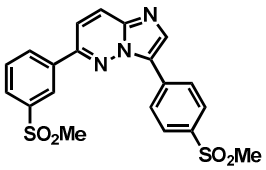
8. (a) Bridglang-Taylor, M. H.; Hargreaves, A. C.; Easter, A.; Orme, A.; Henthorn, D. C.; Ding, M.; Davis, A. M.; Small, B. G.; Heapy, C. G.; Abi-Gerges, N.; Persson, F.; Jacobson, I.; Sullivan, M.; Albertson, N.; Hammond, T. G.; Sullivan, E.; Valentin, J.-P.; Pollard, C. E. Optimisation and validation of a medium-throughput electrophysiology-based hERG assay using IonWorks TM HT. *J. Pharmacol. Toxicol. Methods* **2006**, *54*, 189-199; (b) Essen BioScience, hERG IC_{50} IonWorks Assay Example Report; http://www.essenbioscience.com/media/uploads/files/hERG_10xcpd_Exemplar_Report.pdf. Date accessed April 25.2014

9. Obach, R. S. Prediction of Human Clearance Data: An examination of *in vitro* half-life approach and nonspecific binding to microsomes. *Drug. Metab. Dispos.* **1999**, *27*, 1350-1359.

10. Snyder, C.; Chollet, J.; Santo-Tomas, J.; Scheurer, C.; Wittlin, S. In vitro and in vivo interaction of synthetic peroxide RBx11160 (OZ277) with piperaquine in Plasmodium models. *Exp Parasitol.* 2007 ***115*****,** 296-300.

11. González Cabrera, D.; Douelle, F.; Younis, Y.; Feng, T.-S.; Le Manach, C.; Nchinda, A. T.; Street, L. J.; Scheurer, C.; Kamber, J.; White, L. K.; Montagnat, O. D.; Ryan, E.; Katneni, K. M.; Joseph, J. T.; Bashyam, S.; Waterson D.; Witty, M. J.; Wittlin, S.; Charman, S. A.; Chibale, K. Structure-activity relanshionship studies of orally active antimalarial 3,5-substituted 2-aminopyridines. *J. Med. Chem.* **2012**, *55*, 11022-11030.

Figure 1. Structure of compound **1**



IC_{50} (nM): K1 = 6.3 / NF54 = 7.3

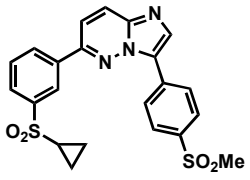
Kin. Sol. (µM): pH 2: 78 / pH 6.5: <5

E_H (h/r/m): <0.42/<0.30/<0.33

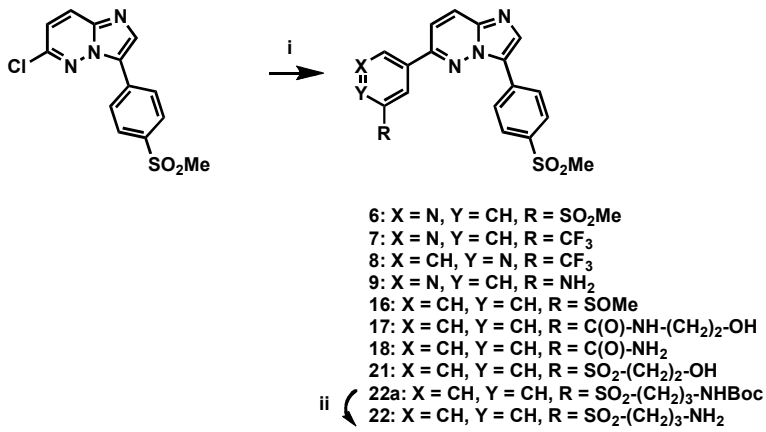
hERG (µM): 0.9

in vivo *P. berghei* (p.o.) at 4 x 50 mg/kg: 98%, 7 MSD

Figure 2. Structure of **46**

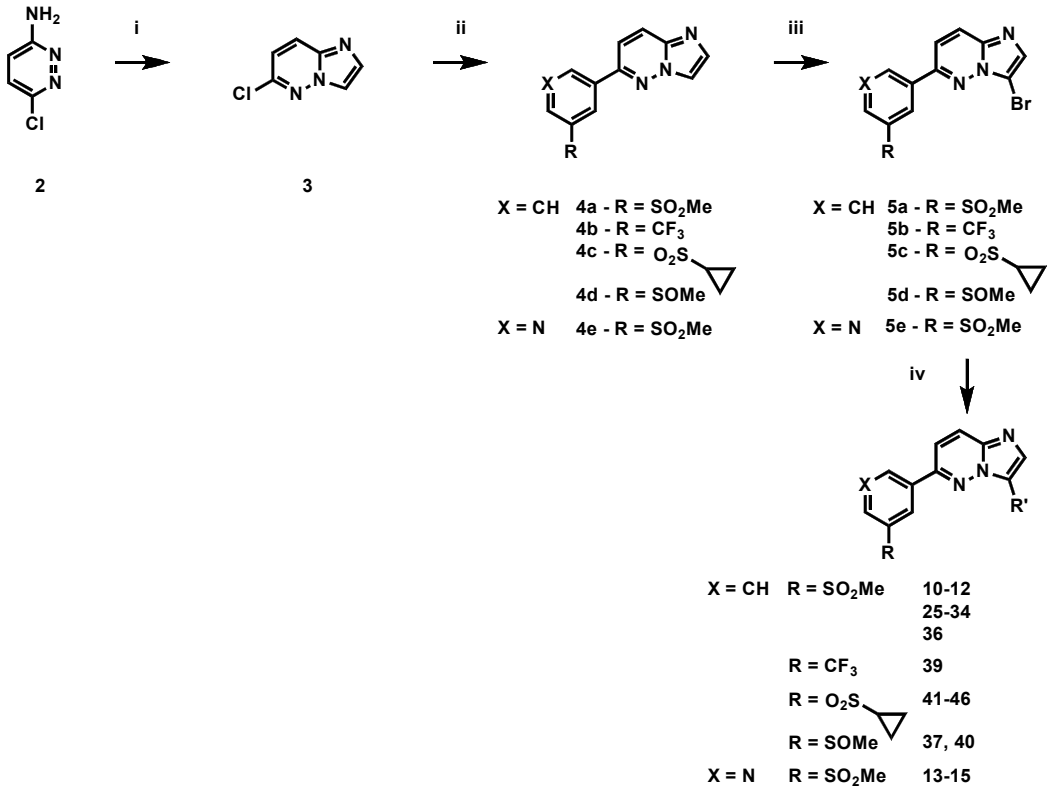


Scheme 1. Synthetic route for compounds **6-9, 16-18, 21, 22**



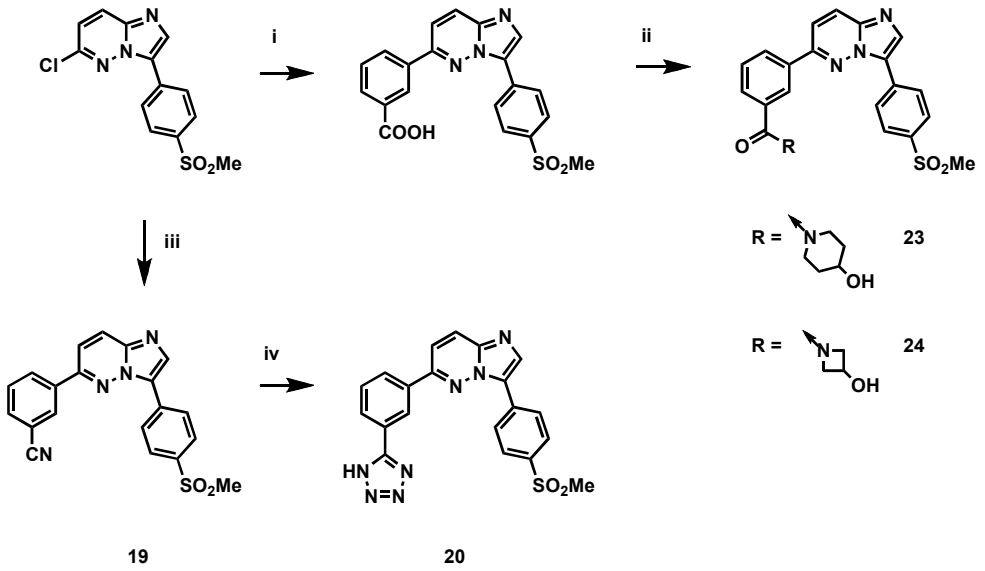
Reagents and conditions: (i) boronic acid (1.1 eq), $Pd(PPh_3)_2Cl_2$ (0.05 eq), aq. K_2CO_3 (1.05 eq), DMF, 90°C; (ii) 20% TFA:DCM (v:v), 0°C, 1h

Scheme 2. Synthetic route for compounds **10-15**, **25-34**, **36**, **37**, **39-45**



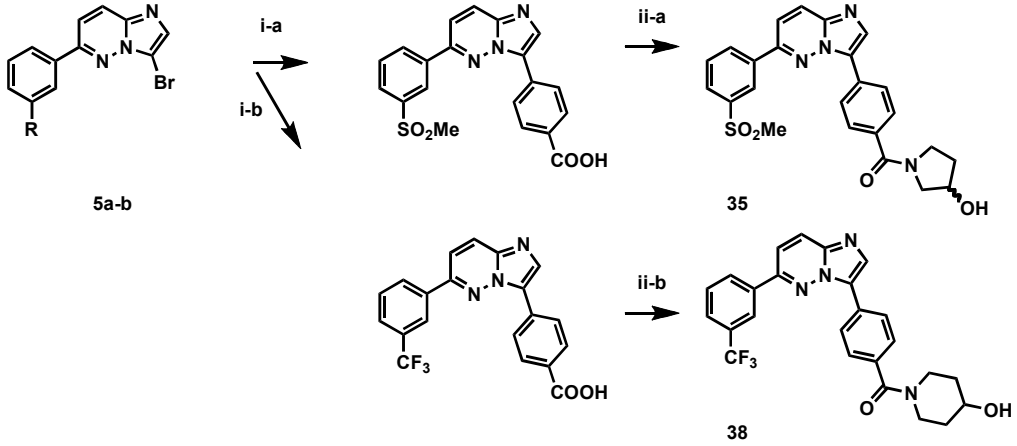
Reagents and conditions: (i) $BrCH_2CCH(OEt)_2$ (1.3 eq), HBr, $EtOH/H_2O$, 100°C, quantitative; (ii) Boronic acid (1.1 eq), $Pd(PPh_3)_2Cl_2$ (0.05 eq), aq. K_2CO_3 (1.05 eq), DMF, 80°C, 50-58 %; (iii) NBS (1.1 eq), DMF, r.t., 1h, 85%; (iv) R'-$B(OH)_2$ (1.1 eq), $Pd(PPh_3)_2Cl_2$ (0.05 eq), aq. K_2CO_3 (1.05 eq), DMF, 90°C, 40-70%

Scheme 3. Synthetic route for compounds **19**, **20**, **23** and **24**



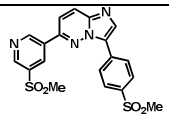
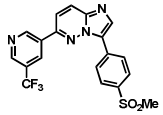
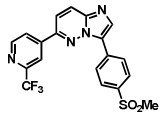
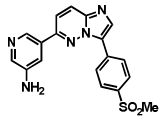
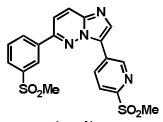
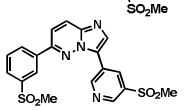
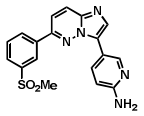
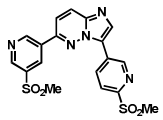
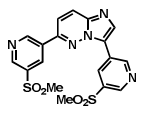
Reagents and conditions: (i) 3-(COOH)Ph-$B(OH)_2$, (1.1 eq) $Pd(PPh_3)_2Cl_2$ (0.05 eq), aq. K_2CO_3 (1.05 eq), DMF, 100°C, 71 %; (ii) Hydroxy-piperidine (**23**) or Hydroxy-azetidine (**24**) (1.1 eq), EDCI.HCl (1.4 eq), Et_3N (4 eq), HOBt (0.1 eq), DMF, r.t. (**23**) or 90°C (**24**), 37-51%; (iii) 3-(CN)Ph-$B(OH)_2$ (1.1 eq), $Pd(PPh_3)_2Cl_2$ (0.05 eq), aq. K_2CO_3 (1.05 eq), DMF, 90°C, 54 %; (iv) NaN_3 (6 eq), NH_4Cl (6 eq), 120°C, 5h, 86%

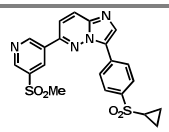
Scheme 4. Synthetic route for amide compounds **35** and **38**



Reagents and conditions: (i) 4-(COOH)-Ph-B(OH)$_2$ (1.1 eq), Pd(PPh$_3$)$_2$Cl$_2$ (0.05 eq), aq. K$_2$CO$_3$ (1.05 eq), DMF, 100°C, 68% (**a**), 54% (**b**); (ii) (a) Hydroxy-pyrrolidine (1.1 eq), EDCI.HCl (1.4 eq), Et$_3$N (4 eq), HOBt (0.1 eq), DMF, r.t., 33%; (ii) (b) Hydroxy-piperidine (1.1 eq), EDCI.HCl (1.4 eq), Et$_3$N (4 eq), HOBt (0.1 eq), DMF, r.t., 30%

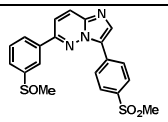
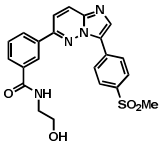
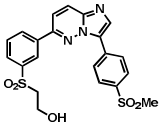
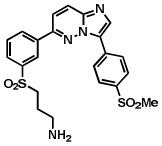
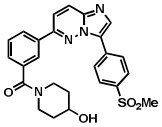
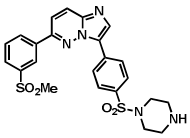
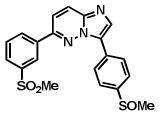
Table 1. Effect of pyridine rings on solubility and antiplasmodial activity

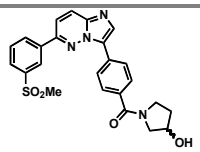
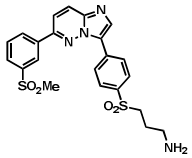
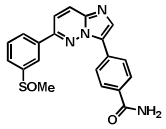
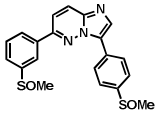
Compd	Structure	Solubility (µM)		Pf IC$_{50}$ (nM)[a,b]	
		pH 2 (STD)	pH 6.5 (STD)	K1	NF54
6		<5	<5	82	96
7		<5	<5	-	1050
8		5.4 (0.1)	<5	26	45
9		174(0.4)	15 (0.2)	55	69
10		<5	<5	9.9	12
11		16 (0.1)	<5	42	58
12		192 (3.6)	<5	19	23
13		52 (0.9)	13 (0.3)	610	808
14		183 (0.6)	53 (1.4)	473	636

15		<5	<5	484	662

[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 differed less than 2x (maximum 3x).
[b] Chloroquine and artesunate were used as the reference drugs in all experiments. Against NF54 and K1, our laboratory standard IC_{50} values for chloroquine and artesunate are 16/194 nM and 4.0/3.0 nM (mean from ≥ 10 independent assays). IC_{50} values, which differed more than 3x from the laboratory standard values were not included in the analysis.

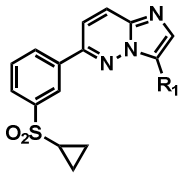
Table 2. Introduction of water solubilizing groups to improve solubility

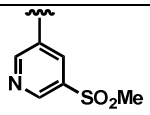
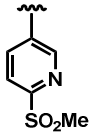
Compd	**Structure**	**Solubility (µM)**		**Pf IC_{50} (nM)[a,b]**	
		pH 2 (STD)	pH 6.5 (STD)	K1	NF54
16		188 (2.7)	157 (4.1)	36	39
17		>200	>100	78	99
21		190 (8.2)	8.6 (0.1)	-	2.2
22		>200	>200	2.6	2.7
23		>200	178 (1.1)	-	6.5
26		166 (8.8)	162 (0.4)	8.8	14
32		207 (1.2)	98 (1.6)	-	6.5

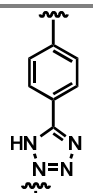
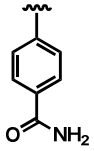
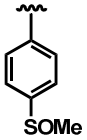
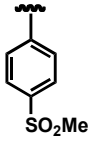
35		191 (0.8)	177 (11)	21	18
36		>200	180 (7.5)	9.4	11
37		112 (7.5)	<5	-	103
40		205 (1.3)	192 (2.4)	30	35

[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 differed less than 2x (maximum 3x).
[b] Chloroquine and artesunate were used as the reference drugs in all experiments. Against NF54 and K1, our laboratory standard IC_{50} values for chloroquine and artesunate are 16/194 nM and 4.0/3.0 nM (mean from ≥ 10 independent assays). IC_{50} values, which differed more than 3x from the laboratory standard values were not included in the analysis.

Table 3. Increasing potency with 3-cyclopropylsulfonylphenyl

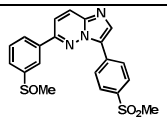
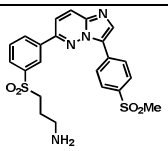


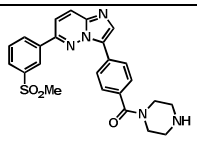
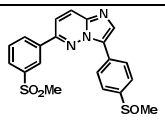
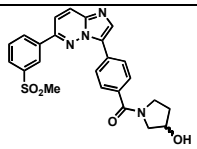
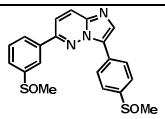
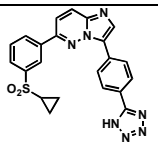
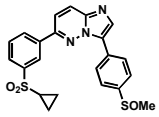
Compd	**R_1**	**Solubility (μM)**		**Pf IC_{50} (nM)[a,b]**	
		pH 2 (STD)	pH 6.5 (STD)	K1	NF54
41		18 (0.3)	<5	31	37
42		<5	<5	2.0	3.3

43		<5	183 (3.4)	25	43
44		6.3 (0.3)	<5	1.2	1.5
45		61 (2.4)	<5	-	1.1
46		19 (6.0)	<5	0.53	0.88

[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 differed less than 2x (maximum 3x).
[b] Chloroquine and artesunate were used as the reference drugs in all experiments. Against NF54 and K1, our laboratory standard IC_{50} values for chloroquine and artesunate are 16/194 nM and 4.0/3.0 nM (mean from ≥ 10 independent assays). IC_{50} values, which differed more than 3x from the laboratory standard values were not included in the analysis.

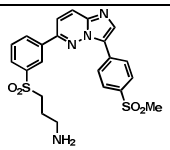
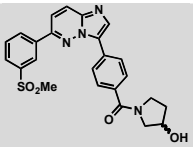
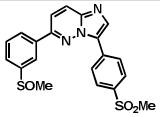
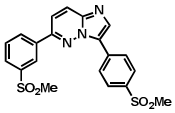
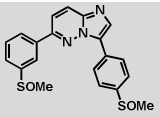
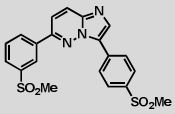
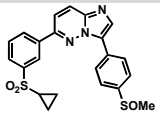
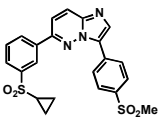
Table 4. In vivo antimalarial oral efficacy of selected compounds in the *P.berghei* mouse model

Compound	Structure	Oral dose (mg/kg)[a]	% reduction parasitemia (MSD)[b,c,d]
16		4 x 50	99.8 (14)
22		4 x 50	<40 (4)

34		4 x 50	<40 (4)
32		4 x 50	99.7 (14)
35		4 x 50	<40 (4)
40		4 x 50	99.8 (25) 2 out of 3 mice cured
43		4 x 50	<40 (4)
45		4 x 50 4 x 10 4 x 3	99.8 (>30) 3 out of 3 mice cured 99.6 (8) 98 (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 four consecutive days (4, 24, 48 and 72 hours after infection).
[b]MSD = mean survival time (in days).
[c]Mice 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 non-solubilizing, 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).
[e] data from Le Manach et al.[5]

Table 5. Mouse snapshot Pharmacokinetic profiles for compounds **16, 22**, **35**, **40** and **45** after the first oral administration of 50 mg/kg

Compound	Structure	Plasma concentration (μM)		
		1h	4h	24h
22		-[a]	<0.01	<0.01
35		-[a]	<0.01	<0.01
16		0.59	0.49	0
metabolized into				
1		6.27	8.44	0.01
40		2.44	0.76	0
metabolized into				
1		3.18	6 .78	0.01
45		0.08	0.02	0
metabolized into				
46		0.71	0.42	0.01

[a] Below detection limits.

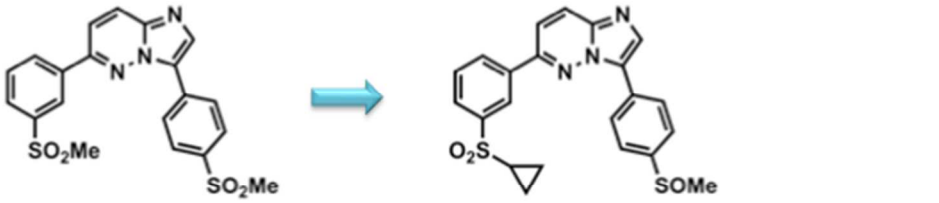
Table 6. Pharmacokinetic Parameters for **1**, **45**, and **46** in Male Sprague-Dawley Rats Following Intravenous and Oral Administration

Parameter	1[a]	46	46	45

					from 45		
	IV	**Oral**	**IV**	**Oral**	**Oral**	**IV**	**Oral**
Dose (mg/mL)	3.6	3.6	3.6	3.6	3.6[b]	3.6	3.6
Apparent $t_{½}$ (h)	7.2	7.1	6.7	4.8	5.8	0.55	4.8
Plasma CL_{total} (mL/min/kg)	5.8	-	1.3	-	-	46	-
V_{ss} (L/kg)	3.0	-	0.73	-	-	2.3	-
$AUC_{0-\infty}$ (µM·h)	24	19	121	27	-	3.3	0.36
C_{max} (µM)	-	1.9	-	1.5	2.3	-	0.04
T_{max} (h)	-	3.0	-	8.0	5.0	-	12
Bioavailability (%)	-	78	-	24	36	-	11

[a] As previously reported.[5]
[b] Measured and calculated parameters for sulfone **46** from an oral dose of sulfoxide **45**.

TABLE OF CONTENTS GRAPHIC



IC_{50} : NF54 = 7.3 nM
in vivo *P. berghei* (p.o.) at 4x50 mg/kg: 98%, 7 MSD

IC_{50} : NF54 = 1.1 nM
in vivo *P. berghei* (p.o.) at 4x50 mg/kg: 99%, 30 MSD

3 out of 3 malaria infected mice cured