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Article

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Identification of a potential anti-malarial drug candidate from a series of 2-aminopyrazines by optimization of aqueous solubility and potency across the parasite life-cycle.

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ABSTRACT: Introduction of water-solubilizing groups on the 5-phenyl ring of a 2-aminopyrazine series led to the identification of highly potent compounds against the blood life-cycle stage of the human malaria parasite *Plasmodium falciparum.* Several compounds displayed high in vivo efficacy in two different mouse models for malaria, *P. berghei*-infected mice and *P. falciparum*-infected NOD-*scid IL-2Rγ*^{null} mice. One of the frontrunners, compound **3**, was identified to also have good pharmacokinetics, and additionally, very potent activity against the liver and gametocyte parasite life-cycle stages.

The recent rise of resistant mutants against frontline malaria treatments in South-East Asia and South America[1] is a reminder of the constant need for novel antimalarial drugs. In order to counter new resistance mechanisms as quickly as possible when they emerge, molecules with different mechanisms of action that are not prone to resistance-development need to be continually fed into the drug pipeline. In previous papers we reported the antimalarial efficacy of aminopyridine[2,3] and aminopyrazine[4] derivatives, including the potency of 2 particular compounds, **1**[2] and **2**[4] (Figure 1). In the latter study,[4] the in vivo potency of **2** was particularly highlighted, but its low aqueous solubility at physiological pH is likely to pose challenges during development. Further optimization was therefore initiated in order to identify aminopyrazine derivatives with improved aqueous solubility at physiological pH, while retaining the good in vivo efficacy of previous analogues. Herein we describe the synthesis and lead optimization of the 2-aminopyrazine series, focusing on structure-activity relationship (SAR) studies around the 3- and 5-positions along with the introduction of water solubilizing groups. The in vitro and in vivo biological activities are described, as well as pharmacokinetic characteristics and the hERG profiles. This work led to the identification of highly potent compounds in vitro and in vivo, with significantly improved aqueous solubility, of which the piperazine amide **3** (Figure 2) proved to be particularly promising.

Chemistry: The cyclic amide derivatives (**3**, **9a$_i$-l$_i$** $_{(i= 1,2)}$, **10**, **11** and **12**) were prepared following a 6- to 7-step synthetic route (Scheme 1) from the commercially available 2-aminopyrazine. Briefly, 2-aminopyrazine was brominated at the 5-position using N-bromosuccinimide (NBS) to give bromopyrazine **4**. Subsequently, a Suzuki cross-

coupling reaction[5] with 4-methoxycarbonylphenyl boronic acid was performed at the 5-position, where after the resulting intermediate **5** was brominated at the 3-position with NBS to give intermediate **6**. After lithium hydroxide hydrolysis of the methylester, the resulting carboxylic acid **7** was subjected to amidation with a cyclic amine, mediated by EDCI, Et_3N, and HOBt, to give the amides **8a-l**. A second Suzuki cross-coupling reaction was then carried out with the desired boronic ester, either 2-(trifluoromethyl)pyridine-5-boronic pinacol ester or 4-((trifluoromethyl)phenyl)boronic acid pinacol ester, to afford compounds **$9a_i$-l_i** (i= 1,2). Finally, when applicable, the amide moiety was deprotected to give target compounds **3**, **10**, **11**, and **12**. All intermediates and target compounds were purified using column chromatography and characterized by various spectroscopic and analytical methods.

Carboxylic acids **13** and **14**, and methylester **15** were prepared using similar chemistry as shown in Scheme 2.

Likewise, for amides **16** and **17** and compounds **18-33** a similar 4-step route was followed, using the same reaction conditions. The commercially available 2-aminopyrazine was brominated at the 5-position, before the first Suzuki cross-coupling reaction was performed with the relevant boronic acid. Bromination at the 3-position was then carried out, followed by a second Suzuki coupling reaction with the appropriate boronic acid delivered the desired compounds **16-33** (scheme 3).

In vitro antiplasmodial activity: All compounds were evaluated for in vitro antiplasmodial activity against a drug sensitive (NF54) and a multi-drug resistant (K1) strain of *P. falciparum.* Chloroquine and artesunate were used as references in all

experiments. The in vitro antiplasmodial activities of the target compounds, as indicated by their IC_{50} values, are summarized in Tables 1 and 2. All compounds were equipotent against both strains, indicating that cross-resistance against chloroquine-resistant parasites is unlikely. In addition, selected compounds have been test against field resistant isolates. As shown in Table C1 of the supporting information, compounds were generally equipotent against all the strains tested, suggesting the absence of any cross-resistance.

Selected compounds were tested for cytotoxicity (Table D1 in the Supporting Information). All tested compounds displayed a selectivity index greater than 1800. Therefore, cytotoxicity was not considered to be a concern for this series.

Previous studies on related 2-aminopyridines have shown that substitution at the 4-position of the 5-phenyl ring was optimal for in vitro blood stage activity[3]. In addition, former SAR studies around the 2-aminopyrazine scaffold suggested that the SAR was conserved across the two series[2,4]. Based on these results, lead optimization in the 2-aminopyrazine series focused on exploring substitution of the 5-phenyl group with hydrophilic moieties at the para-position in order to improve solubility.

When comparing the 4-trifluoromethylpyridine derivatives with their direct phenyl analogues at the 3-position (Table 1), it appears that, in most cases, the 4-trifluorophenyl derivatives are slightly more potent against blood stage parasites than the pyridine derivatives. Incorporation of amides derived from cyclic amines on the 5-phenyl (compounds **3**, **9a$_i$**, **9d$_i$-l$_i$**, **10**-**12**) led to highly active compounds with NF54 IC_{50}s $\leq$10 nM. These amides were more active than the related carboxamides (**16** and **17**). The size of the cycloamine ring had little effect on activity as compounds with 4-, 5-, 6-, and 7-

membered rings showed comparable IC_{50}s. The N-methylated analogues of the cycloamines also maintained comparable activity to their NH counterparts. For the azetidine, pyrrolidine, and piperidine examples, substitution of the ring with water-solubilizing groups were well tolerated based on in vitro blood stage activity. Piperidines seemed to be slightly more active than pyrrolidines, while a hydroxy substituent led to more active compounds than those with an amino group. The enantiomers of **9g$_2$**, **9h** and **9i**, were separated and evaluated. The R-enantiomer **9i** was found to be the more active form with a NF54 IC_{50} of 6 nM compared to 22 nM for **9h.** Hydroxyazetidine derivative **9f** also showed potent activity (K1/NF54 IC_{50} = 7.0/9.2 nM) and was more amenable to progressing due to the lack of chirality. Carboxylic acids **14** and **13** also displayed good potency (K1/NF54 IC_{50} = 33/32 nM and 26/19 nM respectively), while methylester **15** was not active in comparison (NF54 IC_{50} = 2630 nM). The antiplasmodial activity data for the carboxylic acids generated excitement as, to date, there are no known anti-malarials in development, which contain a carboxylic acid functionality[6,7]. In addition, the introduction of a carboxylic acid moiety could potentially improve aqueous solubility at physiological pH.

The introduction of substituted pyridines at the 5-position was generally detrimental to activity, e.g. **25** NF54 IC_{50} = 48 nM compared with its phenyl analogue **2** NF54 IC_{50} = 10 nM, or **20** NF54 IC_{50} = 2708 nM compared to **9g$_2$** NF54 IC_{50} = 94. However, pyridine carboxamide **22** (NF54 IC_{50} = 5.2 nM) exhibited improved activity over the analogous phenyl compound **16** (NF54 IC_{50} = 20 nM).

An alternative strategy to improve solubility was to replace the CF_3-pyridine at the 3-position with aminopyridine or phenylcarboxamide moieties (Table 2). Although

relatively good activities were maintained, this approach generally led to a 2-6 fold drop in activity compared to the CF_3-pyridine analogues (e.g. **9l$_1$** K1/NF54 IC_{50} = 27/38 nM compared to **2** K1/NF54 IC_{50} = 8.4/10 nM).

Assessment for hERG activity: The activity against the hERG potassium channel was determined using in vitro IonWorks patch-clamp electrophysiology[8]. Selected compounds were evaluated for hERG activity with the goal of identifying compounds with activity greater than 10 μM or with a selectivity index (SI) > 1000 in order to minimize cardiotoxicity risks. N-methyl 1,4-diazepane derivative **9d$_2$** and N-*tert*-butyl piperazine **9e$_2$** had low micromolar activity against the potassium channel with IC_{50}s of 5 μM and 2 μM respectively. Piperazine amide **3** and amino-pyrrolidine amide **9j$_2$** had IC_{50}s around 10 μM and selectivity indexes of 1900 and 526 respectively. Based on their relatively low hERG activity and high selectivity indexes, cardiotoxicity is not likely a major risk for these two compounds. 4-Hydroxypiperidine amides **9l$_2$** and **9l$_1$**, hydroxypyrrolidine amide **9k$_1$**, and carboxylic acid **14** were all considered inactive against the hERG potassium channel since they displayed IC_{50}'s >33 μM and SI greater than 1000 (>868 for **9l$_1$**).

Aqueous solubility: As previously stated, the aim of this work was to identify compounds with potent activity against blood stage malaria parasites whilst improving aqueous solubility over the phenylsulfone-based analogues, exemplified by **2**. The strategy employed was to lower the logD (based on calculated values) by introducing hydrophilic water solubilizing groups. Several approaches were accordingly investigated.

The first strategy involved replacement of the hydrophobic trifluoromethylpyridine group of **2** with an aminopyridine group as in compound **29** improved solubility at pH 2 and at the more relevant physiological pH 6.5 (200/149 μM, respectively). However, this trend was not consistent across the analogues made with an aminopyridine group at the 3-position (Table 2), as illustrated by compounds **27** and **30**, which remained highly insoluble at pH 6.5. In addition, such a change negatively impacted in vivo efficacy (see below).

A second strategy to reduce logD and improve aqueous solubility was to use a pyridine instead of a phenyl substituent at the 5-position. As with the aminopyridine analogues at the 3-position, some enhancement of solubility was observed at lower pH (**24:** 199 μM at pH 2), but a lack of solubility at pH 6.5 (**24:** 34 μM) proved the phenylpyridine change not to be effective.

A further strategy was to substitute the 5-phenyl ring with carboxamides and cyclic amides at the 4-position. A range of cyclic amides, including amides derived from substituted and unsubstituted azetidines, pyrrolidines, piperazines, piperidines, and 1,4-diazepanes, was assessed. Carboxylic acids were also investigated at the 4-position. As shown in Table 1, the acids and most amides had lower logD than **2.**

The carboxamides remained relatively insoluble (pH 2/pH 6.5: **17**: 78/<5 μM; **16**: <5/<5 μM). Generally, pendent primary amines resulted in good solubility at pH 2 and moderate to good solubility at pH 6.5. Replacing the amine with a hydroxy group resulted in a similar improvement in aqueous solubility. Indeed, hydroxypyrrolidines **9i** (pH 2/pH 6.5: 103/90 μM) and **9h** (pH 2/pH 6.5: 93/68 μM) displayed moderate to good solubility at both acidic and physiological pH's, as did the hydroxyazetidine derivative **9f** (pH 2/pH

6.5: 110/55 μM), and the hydroxypiperazine **9-l$_1$** (pH 2/pH 6.5: 200/184 μM). The methylated or NH 1,4-diazepane and piperazine amides showed good solubility, with the NH-piperazine amide derivatives **3**, and **10** standing out with > 150 μM solubility at both pH's (pH 2/pH 6.5: 192/158 μM; 200/198 μM, respectively).

Finally, the two carboxylic acids **14** and **13** had good solubility at physiological pH (pH 6.5: 95 and 178 μM, respectively).

Metabolic stability in hepatic liver microsomes: Metabolic stability of the lead compounds was assessed in vitro in human, rat, and mouse liver microsomal preparations. All active compounds were submitted for a turnover assay that determines the percentage compound remaining after a 30-minute incubation in the presence of liver microsomes. Subsequently, microsome-predicted hepatic extraction ratios (E_H) were determined for selected compounds using a 5-point assay (60 minutes). All the results are summarized in the Supporting Information (Table F1). All of the tested compounds proved to be stable across the 3 species with >90% of compound remaining after 30 minutes, except for the N-tert-butyl piperazine derivatives **9e$_2$** and **9e$_1$**, which was expected, as one can anticipate metabolism on the *tert*-butyl group.

In vivo efficacy and pharmacokinetic studies: A selection of compounds with suitable in vitro properties were tested in vivo in the *P. berghei*-infected and *P. falciparum*-infected NOD-*scid IL-2Rγ^{null}* (SCID)[9,10] mouse models for malaria. Efficacy in the *P. berghei* assay was determined following oral administration (p.o.) of 10 mg/kg/day and/or 3 mg/kg/day for 4 consecutive days (4-day Peters' test[11]). The parasitemia was

measured at day 7 and the mice were monitored for symptoms for up to 30 days. The efficacy results are summarized in Table 3. The N-methylpiperazines **9a$_1$** and **9a$_2$** were efficacious at 4x10 mg/kg with >99.8% reduction in parasitemia. For **9a$_2$** average mouse survival days (MSD) was 26 days, and 2 mice out of 3 were cured (defined as having no detectible parasites at day 30). **9a$_2$** was also active at 4x3 mg/kg, but the MSD was only 12 days. Four compounds afforded a complete cure (3/3 mice cured and MSD >30 days) with ≥99.9% activity at 4x10 mg/kg, namely NH-piperazine **3**, carboxamide **17**, 2-hydroxy-azetidine **9f,** and carboxylic acid **14**. These four compounds were also very potent at 4x3 mg/kg with >99% reduction in parasitemia at day 7. However, the MSD remained lower than 20 days and no complete cure was achieved at this dose. Other compounds of interest include the 3-hydroxypyrrolidines **9i** and **9h**, 4-hydroxypiperidine **9l$_2$**, and carboxylic acid **13** that cleared >99% of parasites at 4x10 mg/kg and >90% at 4x3 mg/kg as measured on day 7.

Interestingly, neither of the 3-aminopyrrolidines (**9j$_2$** and **9j$_1$**) or the 4-aminopiperidines (**9h** and **9i**) were active at 4x3 mg/kg, which suggests that the amino substituent is sub-optimal for in vivo efficacy in these instances at this dosing regimen. The pyridine analogue of **3**, namely **10**, was not efficacious at 4x10 mg/kg (<40% parasite reduction), and neither were the NH 1.4-diazepane derivatives **12** and **11**. Lastly, it is interesting to note that the pyridine derivatives were generally less active than the related phenyl analogues. In an attempt to rationalize the varying in vivo efficacies, drug plasma levels from the in vivo efficacy studies were measured for **3**, **9a$_2$**, **9e$_1$**, **9g$_2$**, **9i**, **13**, **14**, **16**, and **17**. As shown in Figure 3, all compounds with >90% reduction in parasitemia at 4x3 mg/kg had a good plasma exposure, especially compounds **13**, **14**, **16** and **17**. Although

the plasma drug levels of **3** were not as high as for that of primary amides **16** and **17** or carboxylic acids **13** and **14**, the compound remained very active in vivo. The intrinsic potency and plasma protein binding may play a role in the in vivo efficacy. On the other hand, for compound **9e$_1$**, the reduced in vivo efficacy could be due to a higher clearance rate as the drug levels dropped drastically after 4h.

In addition, the ED_{90}s (the dose that reduces parasitemia at day 7 after infection by 90% with respect to vehicle-treated mice) for compounds **3**, **17**, **9i**, and **14** were determined in the *P. berghei* model. All four compounds were highly potent with ED_{90}'s ≤ 1mg/kg (1.0, 0.3-1.0, 1.1, and 0.56 mg/kg respectively).

Further to the *P. berghei* efficacy studies, promising compounds (**3**, **9i**, **9f**, **9l$_2$**, **13**, **14**, and **17**) were also evaluated in the *P. falciparum* SCID mouse model. Efficacy was assessed following administration of one oral dose per day for four consecutive days. The parameter estimated in this model were the ED_{90} in mg/kg and the estimated average daily exposure in whole blood necessary to achieve the ED_{90}, denoted as AUC_{ED90}. The results are summarized in Table 3. All tested compounds were efficacious in the *P. falciparum* SCID mouse model with ED_{90}'s <0.25 mg/kg and related AUC_{ED90}'s between 0.02 (**9f**) and 0.20 (**14**). ED_{90}'s from the in vivo *P.b.* and *P.f.* SCID studies are comparable, showing good correlation between the two different in vivo efficacy models of malaria.

Blood concentrations were measured following the first oral administration of compound to all *P. falciparum*-infected SCID mice in the efficacy studies. PK parameters are summarized in Table I1 of the Supporting Information. All compounds showed increasing exposure with increasing dose. Remarkably, for all compounds, the dose level

of 1 mg·kg^{-1} gave higher parasite clearance than that at 10 mg·kg^{-1} even though mice treated at 10 mg·kg^{-1} showed the highest exposure in blood. Further detailed PK/PD studies should be performed to elucidate whether this phenomenon is caused by decreased compound exposure upon multiple dosing at the highest dose levels (pharmacokinetics) or unexpected reduction of parasite clearance (pharmacodynamics).

In addition to the efficacy studies, the pharmacokinetics of the aforementioned seven compounds in rats was determined. The data is shown in Table 4. **3** and **14** clearly stood out with a bioavailability of 98% and 94% respectively. They also proved to have a good plasma half-life (5.2 h and 4.8 h respectively) and low clearance and volume of distribution. **9i** also had a good bioavailability of 71% in rat and long half-life (7.2h). However its plasma clearance rate and volume of distribution appeared to be higher than for **3** and **14**. The other compounds in the study only achieved 20 to 38 % oral biovailability and were generally cleared from plasma at a higher rate.

In vitro evaluation in the *P. berghei (P.b.)* liver and *P. falciparum* gametocyte malaria parasite lifecycle stages: The lead compounds **3**, **9i**, **9f**, **9l$_2$**, **13**, **14** , and **17** were profiled against the liver and gametocyte parasite life-cycle stages to evaluate their potential further. Activities are reported in Table 5. IC$_{50}$'s were obtained for the *P.b*. liver stage[12] and *P. falciparum* early and late stage gametocytes[13] (EG and LG respectively). The piperazine amide **3** was highlighted as the most active compound in the series against the *P.b.* liver assay and in the gametocyte assays. Excitingly, it demonstrated highly potent activity against the liver stage with a subnanomolar IC$_{50}$ (0.92 nM), and displayed good activity against the early and late stage gametocytes (IC$_{50}$ = 134/66 nM

respectively). **9f**, **9l$_2$**, and **9i** also had activities below 15 nM (2.7, 4.1, and 14 nM respectively) against the liver stage. However, they were less active against early stage gametocytes (IC_{50} >200 nM). They remained active against late stage gametocytes, especially compound **9f**, which had a 45 nM IC_{50}. The two carboxylic acids **13**, and **14**, and carboxamide **19** were less active in these assays.

Conclusion: Several strategies were adopted to improve aqueous solubility and developability of compounds in the 2-aminopyrazine series. The most successful approach was to incorporate amides containing a water solubilizing group at the 4-position of the 5-phenyl ring. Cyclic amides proved to be highly efficacious in vivo in both the *P. falciparum* SCID and *P. berghei* mouse models for malaria, with ED_{90}s ≤ 1 mg/kg.

The piperazine amide **3** in particular was shown to be one of the most potent compounds in vitro against asexual blood, liver, and gametocyte stages of the malaria parasite. With the aid of its good pharmacokinetics properties, including a long half-life and bioavailability of 98%, in vitro activity translated into potent in vivo efficacy. Good selectivity over inhibition of the hERG channel was also achieved. The overall profiles of compound **3** demonstrates its potential to become a drug candidate for the treatment of malaria and further profiling is in progress. It is an attractive potential follow-on compound to the previously described 2-aminopyridine **1**[2] a novel *Plasmodium* PI4K inhibitor that is currently in clinical trials for the treatment of malaria.

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. ^{13}C NMR spectra were recorded at 75 MHz on a Varian Mercury Spectrometer or at 100 MHz on Varian Unity Spectrometer. High-resolution mass spectra were recorded on a VG70 SEQ micromass spectrometer. Melting points (M.P.) were determined by Differential Scanning Calorimetry (DSC) using TA Q200/Q2000 DSCfrom TA Instruments. 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.

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

General procedure for the bromination at the 3-position: To a suspension of 2-aminopyrazine (1 eq) in dry DCM (1 M) was added N-bromosuccinimide (1 eq) in portion at 0º C under nitrogen. The reaction mixture was stirred at RT for 1.5 h, before being concentrated under *vacuum*. Water (30 mL) was added and the resulting solid was filtered off. The filtrate was extracted with DCM:MeOH (9:1, x 4), and the combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 and concentrated

under *vacuum*. The crude solid was purified by column chromatography over silica gel (230-400 mesh) by using 25-28% of ethyl acetate in petroleum ether as an eluent to afford product **4** (52%) as a yellow solid.

^{1}H NMR (400 MHz, DMSO-d_6): δ 8.03 (d, J = 1.3 Hz, 1H), 7.68 (d, J = 1.3 Hz, 1H), 6.66 (s, 2H). LC-MS APCI: found m/z = 176.0 $[M+H]^+$, (calculated for $C_4H_4BrN_3$: 172.95, 174.95); Purity by LC-MS: 99.8%.

General procedure for the first Suzuki cross-coupling reaction: To a solution of **4** (1 eq) in 1,4-dioxane (resulting solution at 0.6 M) of was added 4-methoxycarbonylphenylboronic acid (1.1 eq) followed by potassium phosphate tribasic (1.2 eq) and water (0.068 $Vol_{dioxane}$ mL) at RT. The reaction mixture was purged with nitrogen for 1 h. Bis(triphenylphosphine)palladium(II)chloride (0.07 eq) was then added to the reaction mixture. The reaction mixture was heated to reflux for 16h, cooled to RT and concentrated under *vacuum* to remove dioxane. 50 mL of water was added and the resulting solid was filtered off, washed with water (20 mL x 3), dried and again washed with MeOH (10 mL x 4), and dried to afford compound **5**.

Methyl 4-(5-aminopyrazin-2-yl)benzoate **5**: 91%, pale yellow solid.

^{1}H NMR (400 MHz, DMSO-d_6): δ 8.62 (d, J = 1.20 Hz, 1H), 8.07 (d, J = 8.56 Hz, 2H), 7.98-8.00 (m, 3H), 6.78 (s, 2H), 3.86 (s, 3H). LC-MS APCI: found m/z = 230.2 $[M+H]^+$, (calculated for $C_{12}H_{11}N_3O_2$: 229.23), purity by LC-MS: 84.5%.

General procedure for the second bromination: To a suspension of compound **5** at 0ºC (1 eq) in dry DCM (0.5 M) was added N-bromosuccinimide (1.1 eq) in portions under nitrogen. The resulting mixture was stirred at RT for 1 h. To the reaction mixture was

added 20 mL of water and the resulting mixture was extracted with DCM:MeOH. Combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 and concentrated under *vacuum*. The crude material was purified by column chromatography over silica gel 230-400 mesh using 24% of ethyl acetate and 4% of DCM in petroleum ether as an eluent to afford compound **6**.

Methyl 4-(5-amino-6-bromopyrazin-2-yl)benzoate **6**: 47%, pale yellow solid

^{1}H NMR (300 MHz, DMSO-d_6): δ 8.70 (s, 1H), 7.98-8.05 (m, 4H), 7.08 (s, 2H), 3.85 (s, 3H). LC-MS APCI: found *m/z* = 309.8 $[M+H]^+$, (calculated for $C_{12}H_{10}BrN_3O_2$: 306.99, 308.99), purity by LC-MS: 95.2%.

General procedure for the ester hydrolysis: To a solution of compound **6** (4.76 g, 15.44 mmol) in THF (50 mL) was added lithium hydroxide monohydrate (1.5 eq) at RT and the resulting mixture was stirred for 16 h. It was then concentrated under *vacuum*, before 10 mL of water were added. Concentrated HCl was added dropwise until the pH of the solution was acidic. The solid was filtered off, washed with water, dried and again washed with DCM to give compound **7**.

4-(5-amino-6-bromopyrazin-2-yl)benzoic acid **7**: 90%, pale yellow solid.

^{1}H NMR (300 MHz, DMSO-d_6): δ 13.00 (bs, 1H), 8.68 (s, 1H), 7.95-8.02 (m, 4H), 7.04 (s, 2H). LC-MS APCI: found *m/z* = 295.6 $[M+H]^+$, (calculated for $C_{11}H_8BrN_3O_2$: 292.98, 294.98), purity by LC-MS: 94.7%.

General procedure for the amide coupling: To a suspension of compound **7** (1 eq) in dry THF (0.2M) was added EDCI.HCl (1.2 eq), HOBt (0.1 eq) and triethylamine (2.5 eq)

at RT under nitrogen. The resulting mixture was stirred for 1 h. N-tert-butyl piperazine was then added (532 mg, 3.74 mmol) at RT and stirring was allowed for 16 h. Water was added and the solution was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 and concentrated under *vacuum*. The crude material was purified by column chromatography over neutral alumina using 3% MeOH in DCM as an eluent to yield the desired compounds **8a-l**.

Second Suzuki coupling: To a solution of compound **8** (1 eq) in 1, 4-dioxane (0.2 M) was added the appropriate boronic acid pinacol ester (1.1 eq) at RT and the solution was purged with nitrogen for 30 minutes. Bis(triphenylphosphine)palladium(II)chloride (0.07 eq) and 1 M aqueous solution of potassium carbonate (1.2 eq, pre-purged with nitrogen) were added to the reaction mixture. The reaction mixture was heated to reflux for 16 h and cooled to RT. Brine solution was added to the reaction mixture and extraction with ethyl acetate was performed. Combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under *vacuum*. The crude material was purified by column chromatography over silica gel 230-400 mesh by using 3.5% of MeOH in DCM as an eluent to afford the desired compounds **$9a_i$-l_i**$_{(i = 1,2)}$

Boc-deprotection: The protected amides were stirred in neat TFA for 12 hours, at which time TFA was removed under pressure. The residue was dissolved in DCM/MeOH (9:1) and stirred with Amberlyst A21 for 1 h. The resin was filtered off, the solvents were removed under pressure and the residue was purified by column chromatography.

(4-(5-amino-6-(4-(trifluoromethyl)phenyl)pyrazin-2-yl)phenyl)(piperazin-1-yl)methanone **3**:

^{1}H NMR (300 MHz, DMSO-d_6): δ 8.69 (s, 1H), 8.09 (d, *J* = 8.20 Hz, 2H), 8.03 (d, *J* = 8.04 Hz, 2H), 7.88 (d, *J* = 8.16 Hz, 2H), 7.53 (d, *J* = 8.24 Hz, 2H), 6.63 (s, 2H), 3.60-3.40 (m, 5H), 2.81 (brs, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 45.3 (2C), 65.4, 123.4, 125.3 (2C), 126.0 (2C), 128.0 (2C), 129.4 (q), 129.6 (2C), 135.3, 136.7, 138.2, 139.3, 139.7, 142.0, 153.0, 169.5. HPLC/MS: (ESI)$^+$: found *m/z* = 428.2 $[M+H]^+$, (calculated for $C_{22}H_{20}F_3N_5O$: 427.16), purity by HPLC: 98.8% ; M.P.: 195.4 ^{o}C (DSC)

(4-(5-amino-6-(4-(trifluoromethyl)phenyl)pyrazin-2-yl)phenyl)(3-hydroxyazetidin-1-yl)methanone **9f:**

^{1}H NMR (400 MHz, DMSO-d_6): δ 8.70 (s, 1H), 8.08 (d, *J* = 8.8 Hz, 2H), 8.03 (d, *J* = 8.7 Hz, 2H), 7.88 (d, *J* = 8.7 Hz, 2H), 7.70 (d, *J* = 8.8 Hz, 2H), 6.60 (s, 2H), 5.74 (d, *J* = 5.6 Hz,1H), 4.57-4.45 (m, 2H), 4.27 (brs, 1H), 4.08 (brs, 1H), 3.81 (brs, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 58.9, 63.3, 123.4, 125.1 (2C), 126.0, 126.0, 128.7 (2C), 129.3 (q), 129.6 (2C), 132.7, 136.9, 139.2, 139.4, 139.8, 142.0, 153.0, 169.3. LC-MS APCI: found *m/z* = 415.2 $[M+H]^+$, (calculated for $C_{22}H_{19}F_3N_4O_2$: 414.13), purity by HPLC: >99.0%.

(R)-(4-(5-amino-6-(4-(trifluoromethyl)phenyl)pyrazin-2-yl)phenyl)(3-hydroxypyrrolidin-1-yl)methanone **9i:**

^{1}H NMR (400 MHz, DMSO-d_6): δ 8.69 (s, 1H), 8.03-8.07 (m, 4H), 7.88 (d, *J* = 8.20 Hz, 2H), 7.58-7.61 (m, 2H), 6.62 (s, 2H), 4.95-5.04 (m, 1H), 4.24-4.33 (m, 1H), 3.40-3.63 (m, 4H), 1.81-1.97 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 32.6, 34.9, 44.6, 47.4, 54.9, 57.6, 68.5, 69.9, 123.4, 125.0 (2C), 126.1, 126.2, 128.1, 128.2, 129.1 (q), 129.6 (2C), 136.4, 136.8, 138.4, 139.4, 139.7, 142.0, 152.9, 168.7, 168.7. LC-MS APCI: found *m/z* = 429.2 $[M+H]^+$, (calculated for $C_{22}H_{19}F_3N_4O_2$: 428.15), purity by HPLC: 98.0%.

(4-(5-amino-6-(4-(trifluoromethyl)phenyl)pyrazin-2-yl)phenyl)(4-hydroxypiperidin-1-yl)methanone **9l$_2$:**

^{1}H NMR (400 MHz, DMSO-d$_6$): δ 8.68 (s, 1H), 8.03-8.07 (m, 4H), 7.88 (d, *J* = 8.28 Hz, 2H), 7.45 (d, *J* = 8.20 Hz, 2H), 6.61 (s, 2H), 4.81 (d, *J* = 3.92 Hz, 1H), 4.01 (bs, 1H), 3.72-3.76 (m, 1H), 3.54 (bs, 1H), 3.18 (bs, 1H), 3.16 (bs, 1H), 1.73-1.78 (m, 2H), 1.36 (bs, 2H). ^{13}C NMR (100 MHz, DMSO-d$_6$) δ: 33.9 (2C), 44.2(2C), 65.6, 123.5, 124.8 (2C), 125.6, 125.6, 127.3 (2C), 128.9 (q), 129.1(2C), 135.4, 136.3, 137.6, 139.0, 139.2, 141.6, 152.5, 168.8. LC-MS APCI: found *m/z* = 443.2 $[M+H]^+$, (calculated for $C_{23}H_{21}F_3N_4O_2$: 442.16), purity by HPLC: 98.1%.

4-(5-amino-6-(6-(trifluoromethyl)pyridin-3-yl)pyrazin-2-yl)benzoic acid **13:**

^{1}H NMR (400 MHz, DMSO-d$_6$) δ 9.16 (d, *J* = 2.1 Hz, 1H), 8.74 (s, 1H), 8.47 (dd, *J* = 8.1, 2.1 Hz, 1H), 8.13 (d, *J* = 8.5 Hz, 2H), 8.04-8.00 (m, 3H), 6.64 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d$_6$) δ 167.5, 153.5, 150.1, 141.0, 140.6, 139.6, 138.3, 137.1, 134.6, 130.5, 130.3, 125.4 (2C), 121.1(2C). HPLC/MS: $(ESI)^+$: found *m/z* = 361.1 $[M+H]^+$, (calculated for $C_{17}H_{11}F_3N_4O_2$: 360.08), purity = 99%

4-(5-amino-6-(4-(trifluoromethyl)phenyl)pyrazin-2-yl)benzoic acid **14**: ^{1}H NMR (400 MHz, DMSO-d$_6$): δ 12.91 (1H, broad s), 8.68 (1H, s), 8.10 (2H, d, *J* = 8.2 Hz), 7.99 (4H, m), 7.86 (2H, d, *J* = 8.2Hz), 6.69 (2H, s). ^{13}C NMR (100 MHz, DMSO-d$_6$): δ 167.5, 153.1, 141.9, 141.3, 140.1, 139.0, 137.1, 130.3 (2C), 130.1, 129.6 (2C), 129.3 (q), 126.1, 126.0, 125.3 (2C), 123.4. LC-MS: $(ESI)^+$: found *m/z* = 360.1 $[M+H]^+$, (calculated for $C_{18}H_{12}F_3N_3O_2$: 359.08), purity = 99.9% ; M.P.: 267.6 ^oC (DSC)

4-(5-amino-6-(4-(trifluoromethyl)phenyl)pyrazin-2-yl)benzamide) **17:**

^{1}H NMR (400 MHz, DMSO-d_6): δ 8.71 (s, 1H), 8.01-8.08 (m, 5H), 7.94 (d, J = 8.40 Hz, 2H), 7.87 (d, J = 8.24 Hz, 2H), 7.37 (s, 1H), 6.62 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 123.4, 125.0 (2C), 126.0, 126.0, 128.5 (2C), 129.1 (q), 129.6 (2C), 133.6, 136.9, 139.3, 139.8, 139.9 142.0, 153.0, 168.0. LC-MS APCI: found *m/z* = 359.2 $[M+H]^+$, (calculated for $C_{18}H_{13}F_3N_4O$: 358.10), purity by HPLC: 99.6%.

In vitro *P. falciparum* assay and in vivo *P. berghei* 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[14]. In vivo *P. berghei* efficacy was conducted as previously described[3], 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 parasitemia 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 administered orally as four consecutive daily doses (4, 24, 48 and 72 h after infection). Blood samples for the quadruple-dose regimens were collected on day 4 (96 h after infection) on 2 of the 3 mice.

In vitro gametocytocidal activity:

In vitro gametocytocidal activity was determined using luciferase reporter lines specifically enabling screening against early stage gametocytes (>90% stage II/III) and late stage gametocytes (>95% stage IV/V) as previously reported[13]. More details are given in section J of the Supporting Information.

***In vitro P.b.* liver assays:**

This assay is based on the murine malaria parasite *Plasmodium berghei* transformed with Luciferase. Hepatic human transformed cells (HepG2), pretreated for 18 hours with the compound to investigate, are infected with freshly dissected *P. berghei* Luciferase sporozoites. After another 48 hours of incubation with the compound to investigate, the viability of *P. berghei* exoerythrocytic forms (EEF) and the HepG2 host cells is measured by bioluminescence.

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

ASSOCIATED CONTENT:

Supporting Information Available: 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.

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ABBREVIATIONS USED: SAR, structure-activity relationships; ADME, absorption, distribution, metabolism and excretion; CQ, chloroquine; p.o., oral administration; i.v., intraveneous administration; MSD, mean survival days; PK, pharmacokinetics; NMR, nuclear magnetic resonance; M.P.,melting point; DSC, differential scanning calorimetry; RT, room temperature; MMV, Medicines for Malaria Venture.

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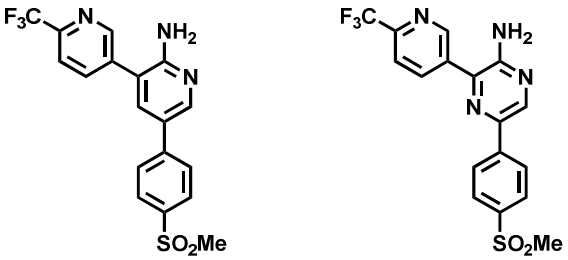
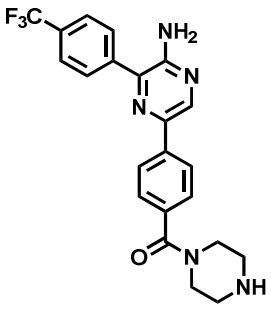
Figure 1. Structures of compounds **1** and **2**:



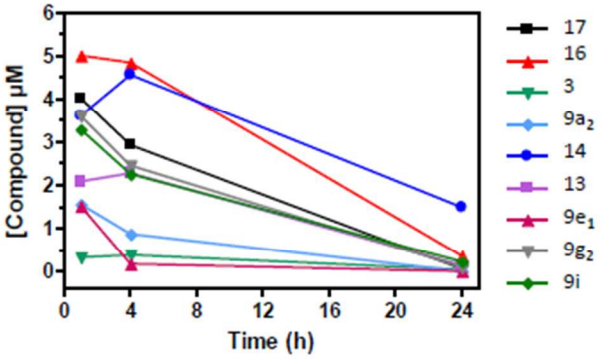
1 **2**

Figure 2.



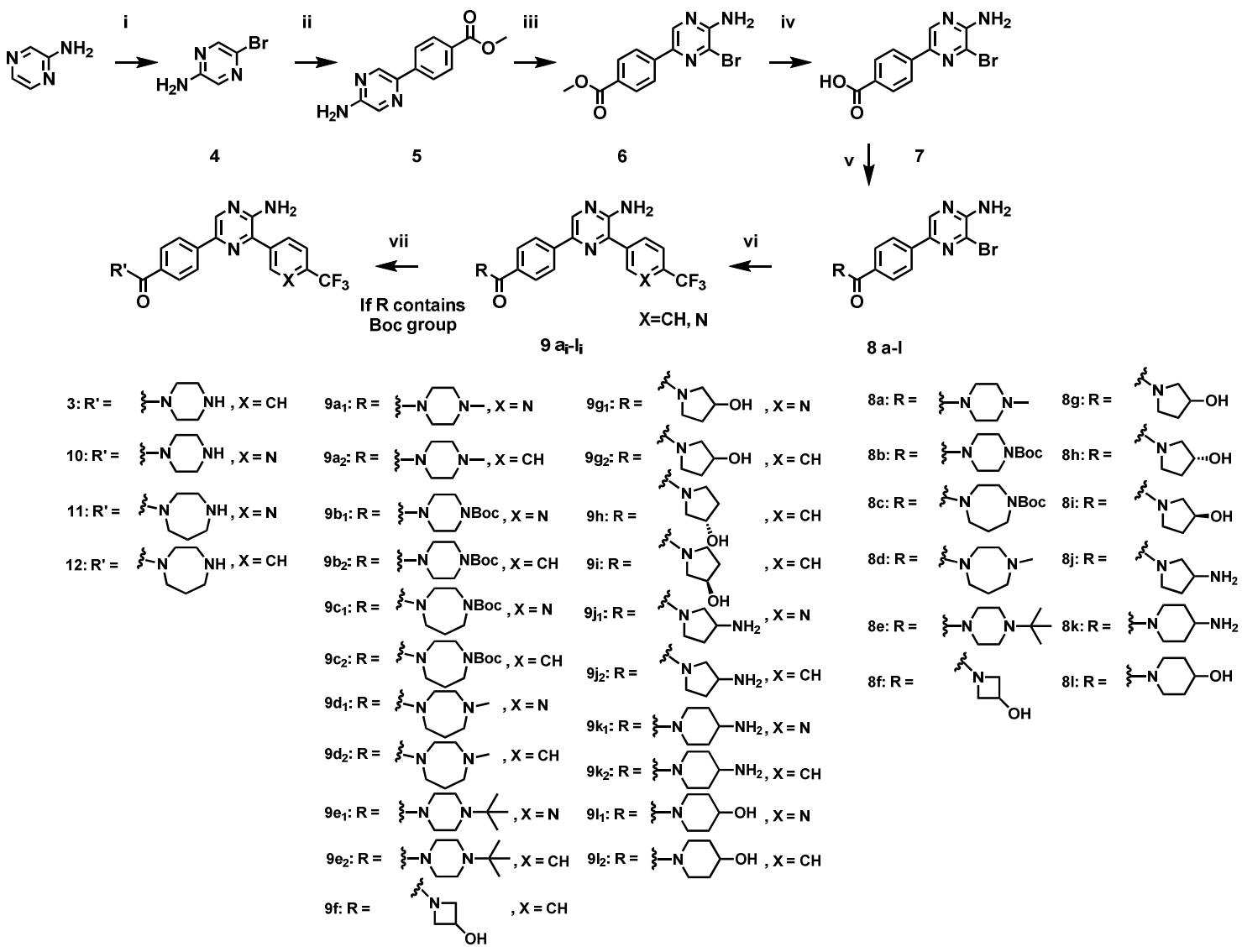
3

Figure 3. Exposure and PK parameters after oral administration (4x3 mg/kg) for representative compounds (snapshot mouse PK).



MMV #	Dose (mg/kg)	App. AUC (µM.h)	App. Cmax (µM)	App. Cav_{24} (µM)
17	3	42.3	4	1.8
16	3	69.3	5	2.9
3	3	5.4	0.4	0.2
$9a_2$	3	c.n.c.*	1.6	c.n.c.*
14	3	79.7	5	3.5
13	3	30.81	2.29	1.48
$9e_1$	3	4.34	1.52	0.57
$9g_2$	3	34.7	3.61	2.06
9i	3	33.19	3.29	1.93

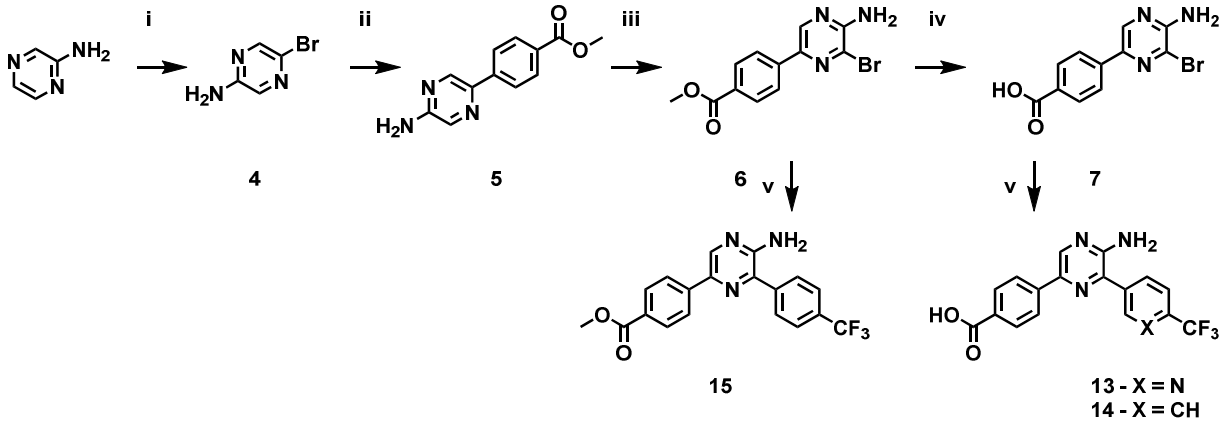
Scheme 1: Preparation of compounds **3**, **9a$_i$-l$_i$** (i= 1,2), **10**,**11** and**12**.



Reagents and conditions: (i) NBS (1.1 eq), DCM, RT, 1.5h, 52% yield; (ii) 4-COOMe-Ph-B(OH)$_2$ (1.1 eq), Pd(PPh$_3$)$_2$Cl$_2$ (0.07 eq), aq. K_2CO_3 (1M) (1.2 eq), dioxane, reflux,16h, 91% yield; (iii) NBS (1.1 eq), DCM, RT, 1h, 47% yield; (iv) LiOH (1.5 eq), THF, 16h, 90% yield; (v) NHR'R (eq), EDCI.HCl (1.2 eq), Et_3N (eq), HOBt (0.1eq), THF, RT, 16h, 63-88% yield; (vi) 4-CF_3-Ph-B(OH)$_2$,pinacol ester or 4-CF_3-Pyridine-

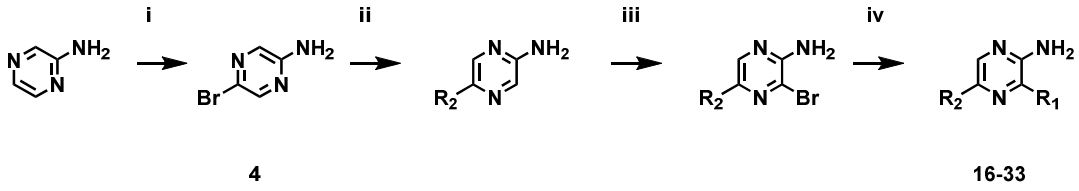
$B(OH)_2$, pinacol ester (1.1 eq), $Pd(PPh_3)_2Cl_2$ (0.07 eq), aq. K_2CO_3 (1M) (1.2 eq), dioxane, reflux, 16h, 58-68% yield; (vii) TFA, RT, 16h, 59-71%

Scheme 2: Preparation of carboxylic acids **13**, **14** and methylester **15**.



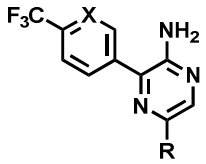
Reagents and conditions: (i) NBS (1.1 eq), DCM, RT, 1.5h, 52% yield; (ii) 4-COOMe-Ph-$B(OH)_2$ (1.1 eq), $Pd(PPh_3)_2Cl_2$ (0.07 eq), aq. K_2CO_3 (1M) (1.2 eq), dioxane, reflux,16h, 91% yield; (iii) NBS (1.1 eq), DCM, RT, 1h, 47% yield; (iv) LiOH (1.5 eq), THF, 16h, 90% yield; (v) 4-CF_3-Ph-$B(OH)_2$, pinacol ester or 4-CF_3-Pyridine-$B(OH)_2$, pinacol ester (1.1 eq), $Pd(PPh_3)_2Cl_2$ (0.07 eq), aq. K_2CO_3 (1M) (1.2 eq), dioxane, reflux, 16h, 58-68% yield.

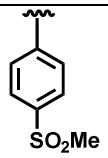
Scheme 3: Preparation of compounds **18-33**.

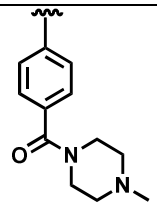
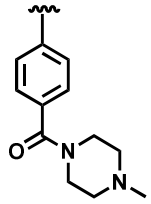
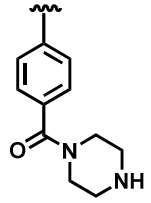
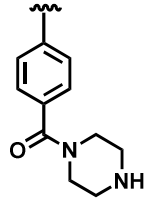
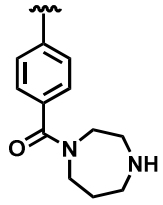
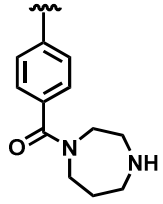


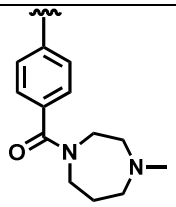
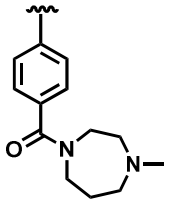
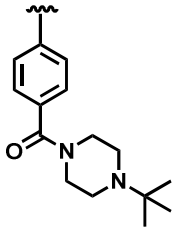
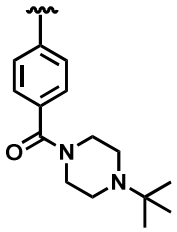
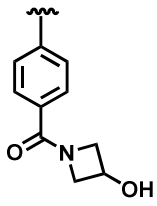
Reagents and conditions: (i) NBS (1.1 eq), DCM, RT, 1.5h, 52% yield; (ii) R_2-$B(OH)_2$ (1.1 eq), $Pd(PPh_3)_2Cl_2$ (0.07 eq), aq. K_2CO_3 (1M) (1.2 eq), dioxane, reflux,16h, 51-67% yield; (iii) NBS (1.1 eq), DCM, RT, 1h; 47-69% yield (iv) R_1-$B(OH)_2$ (1.1 eq), $Pd(PPh_3)_2Cl_2$ (0.07 eq), aq. K_2CO_3 (1M) (1.2 eq), dioxane, reflux, 16h, 49-63% yield.

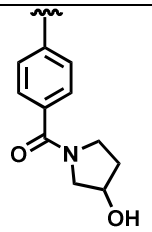
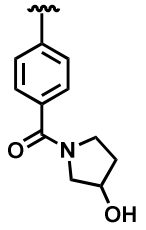
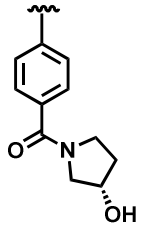
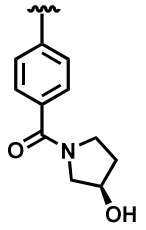
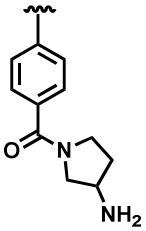
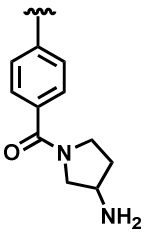
Table 1. Antiplasmodial activity, solubility and calculated ADME properties for compounds **3**, **9a$_i$-l$_i$**, **10-25**

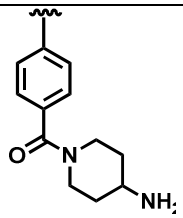
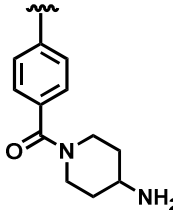
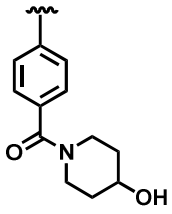
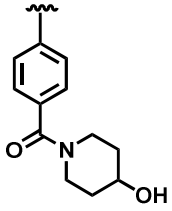
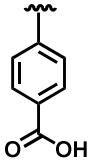
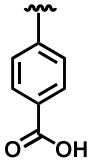


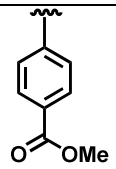
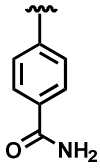
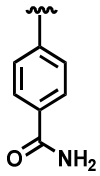
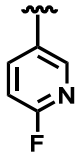
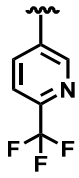
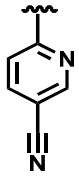
Compound	X	R	IC$_{50}$ (nM)[a,b]		Solubility (µM)		cLogD$_{7.4}$
			K1	NF54	pH2	pH6.5	
2	**N**		8.4	10	<5	<5	2.97

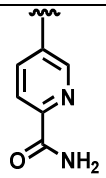
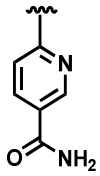
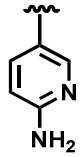
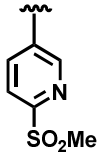
9a$_1$	**N**		9.9	9.9	200	182	1.25
9a$_2$	**CH**		6.8	7.9			1.97
10	**N**		7.7	9.1	200	198	1.04
3	**CH**		5.2	5.4	192	158	2.54
11	**N**		10.2	12.7	200	146	1.19
12	**CH**		6.3	7.7	190	128	1.87

9d$_1$	**N**		9.9	15	200	85	1.31
9d$_2$	**CH**		5.7	6.8	200	152	2.04
9e$_1$	**N**		6.4	8.9	200	113	2.18
9e$_2$	**CH**		11	17	200	95	2.90
9f	**CH**		7.0	9.2	110	55	2.88

9g$_1$	**N**		12	18	200	155	2.22
9g$_2$	**CH**		6.8	9.6	129	77	2.95
9h	**CH**		-	6	93	68	2.95
9i	**CH**		20	22	103	90	2.95
9j$_1$	**N**		26	40	200	116	0.93
9j$_2$	**CH**		12	19	165	102	1.65

9k$_1$	**N**		18	20	200	163	0.99
9k$_2$	**CH**		7.5	10	200	88	1.71
9l$_1$	**N**		9	10	200	184	2.29
9l$_2$	**CH**		5.0	5.4	119	19	3.01
13	**N**		26	19	191	178	1.60
14	**CH**		32	33	<5	95	2.32

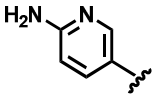
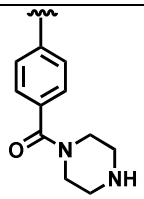
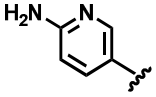
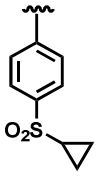
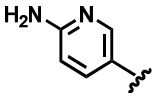
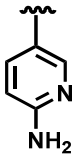
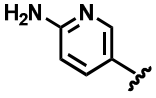
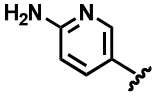
15	**CH**		-	2630	<5	<5	4.02
16	**N**		16	20	<5	<5	2.44
17	**CH**		13	14	80	<5	3.16
18	**N**		182	282	<5	<5	2.88
19	**N**		-	680	<5	<5	3.66
20	**N**		-	2708	-	-	2.60
21	**N**		73	94	<5	5	3.32

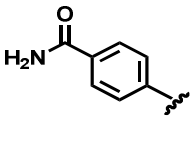
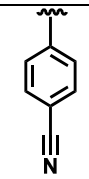
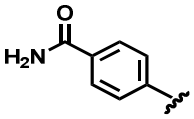
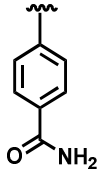
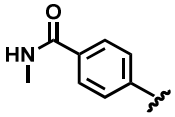
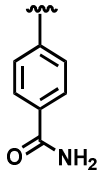
22	N		7.2	5.2	7	5	1.72
23	N		-	1583	123	<5	1.72
24	N		30	42	199	34	2.21
25	N		40	48	79	37	2.35

[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 varied less than 2x (maximum 3x)

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

Table 2. Antiplasmodial activity, solubility and calculated ADME properties for compounds 26-33

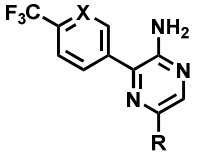
Compound	R_1	R_2	IC_{50} (nM)[a,b]		Solubility (µM)		$cLogD_{7.4}$
			K1	NF54	pH2	pH6.5	
26			37	51	189	186	0.11
27			44	38	212	13	2.01
28			143	203	-	-	0.59
29			27	38	206	149	1.52
30			149	222	208	<5	1.87

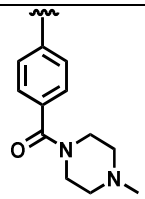
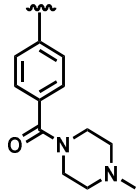
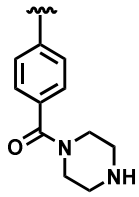
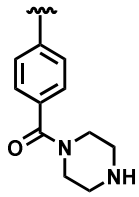
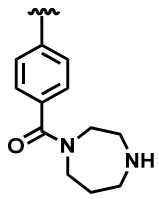
31			44	57	-	-	2.10
32			21	25	-	-	1.22
33			52	52	186	178	1.43

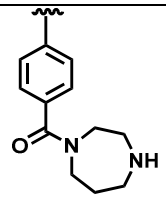
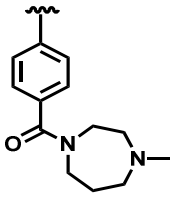
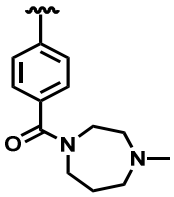
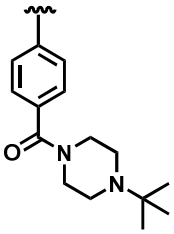
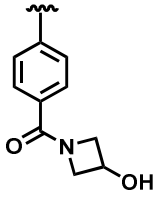
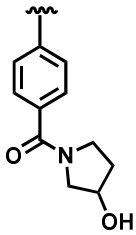
[a] Mean from n values of ≥2 independent experiments with multidrug resistant (K1) and sensitive (NF54) strains of *P. falciparum*. The majority of the individual values varied less than 2x (maximum 3x)

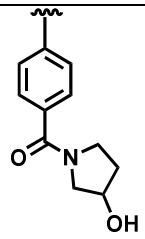
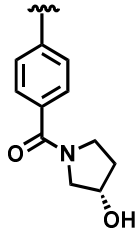
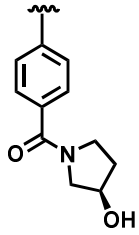
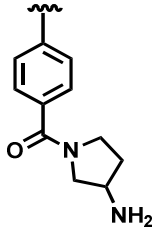
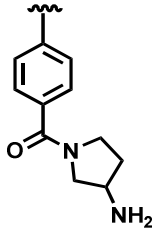
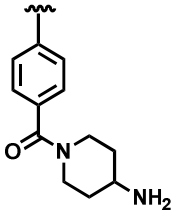
[b] Chloroquine and artesunate were used are reference drugs in all experiments. Against NF54 and K1, our laboratory standard IC_{50} values for chloroquine and artesunate are 16 nM / 194 nM and 4.0 nM / 3.0 nM (mean from ≥10 independent assays). IC_{50} values that differed more than 3x from laboratory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

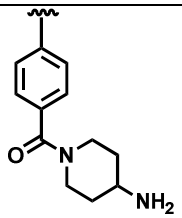
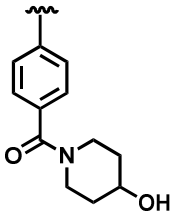
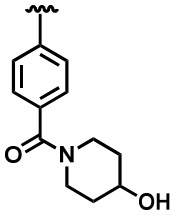
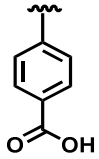
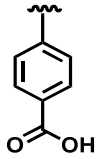
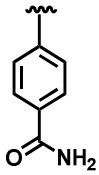
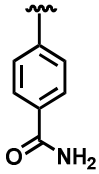
Table 3. In vivo antimalarial oral efficacy of selected compounds in the *P.berghei* mouse model and the *P. falciparum* SCID mouse model

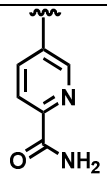


Compound	X	R	*P. berghei mouse model*			*P.falciparum SCID mouse model*	
			Oral dose (mg/kg)[a]	**% reduction parasitemia (MSD)[b,c,d]**	**ED_{90} (mg/kg)**	**ED_{90} (mg/kg)**	**AUC_{ED90} (µg.h/mL^{-1})**
$9a_1$	**N**		4x10 4x3	99.8 (8) 81 (7)	-	-	-
$9a_2$	**CH**		4x10 4x3	99.9 (26 -2/3 cured) 99.9 (12)	-	-	-
10	**N**		4x3	<40 (eut.)	-	-	-
3	**CH**		4x10 4x3	>99.9 (>30 – 3/3 cured) >99.9 (10)	1.0	0.25	<0.065
11	**N**		4x3	55 (7)	-	-	-

12	**CH**		4x3	88 (11)	-	-	-
9d$_1$	**N**		4x3	99.9 (12)	-	-	-
9d$_2$	**CH**		4x3	90 (7)	-	-	-
9e$_1$	**N**		4x3	88 (14)	-	-	-
9f	**CH**		4x10 4x3	99.9 (>30 – 3/3 cured) 99.7 (10)	-	0.17	0.022
9g$_1$	**N**		4x3	61 (7)	-	-	-

9g$_2$	**CH**		4x3	99.6 (8)	-	-	-
9h	**CH**		4x10 4x3	100 (16) 99.6 (8)	-	-	-
9i	**CH**		4x10 4x3	100 (26) 99.7 (8)	1.1	0.14	0.060
9j$_1$	**N**		4x3	47 (7)	-	-	-
9j$_2$	**CH**		4x3	71 (7)	-	-	-
9k$_1$	**N**		4x3	41 (6)	-	-	-

9k$_2$	**CH**		4x3	65 (7)	-	-	-
9l$_1$	**N**		4x10 4x3	85 (7) 64 (7)	-	-	-
9l$_2$	**CH**		4x10 4x3	99.9 (11) 98.7 (7)	-	0.11	0.060
13	**N**		4x10 4x3	99.8 (10) 93 (7)	-	<0.50	<0.44
14	**CH**		4x10 4x3	99.9 (>30 – 3/3 cured) 99.9 (14)	0.56	<0.10	0.20
16	**N**		4x10 4x3	99.9 (14) 99.4 (7.7)	-	-	-
17	**CH**		4x10 4x3	99.9 (>30 – 3/3 cured) 99.9 (20 – 1/3 cured)	0.3-1.0	<0.10	<0.075

22	N		4x10 4x3	99.9 (14) 97.5 (7)	-	-	-
Chloroquine[e]			4 x 30	99.9 (24)			
Artesunate[e]			4 x 30	99 (10)			
Mefloquine[e]			4 x 30	99.9 (29)			

[a] Once per day on 4 consecutive days (4, 24, 48 and 72 h after infection).

[b]MSD = mean survival time (in days).

[c] Mice with <40 parasitemia reduction were euthanized on day 4 in order to prevent death otherwise occurring at day 6.

[d] Artesunate and mefloquine were dissolved or suspended in a 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.[15]

Table 4. Rat PK parameters for compounds

Compound	Method	Dose	$t_{1/2}$ (h)	Plasma Cl (mL/min/kg)	Vd (L/kg)	B (%)
3	p.o.	20	5.3	-	-	98
	i.v.	5	7.8	10.3	5.5	

9f	p.o.	20	5.2	-	-	20
	i.v.	5	3	22	5.7	-
9i	p.o.	20	7.2	-	-	71
	i.v.	5	5.3	13.5	8.2	-
9l$_2$	p.o.	10	5.4	-	-	25
	i.v.	3	8.1	7.1	5.1	-
13	p.o.	20	3.9	-	-	38
	i.v.	5	4.4	16.7	6.4	-
14	p.o.	15	4.8	-	-	94
	i.v.	5	4.9	10.1	4.3	-
17	p.o.	20	7.0	-	-	25
	i.v.	5	1	39	3.5	-

Table 5. *Pb.* liver and gametocyte activities for compounds **3**, **9f**, **9i**, **9l$_2$**, **13**, **14**, **and 17**

Compound	***Pb.* Liver IC$_{50}$ (nM)**	**Gametocytes IC$_{50}$ (nM)**	
		Early stage	Late Stage
3	0.92	134	66

	9f	2.7	415	45
	9i	14	323	137
	9l$_2$	4.1	603	179
	13	528	168	845
	14	51	649	729
17		-	754	237

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