Discovery of a Quinoline-4-carboxamide Derivative with a Novel Mechanism of Action, Multistage Antimalarial Activity, and Potent in Vivo Efficacy

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Supporting Information

ABSTRACT: The antiplasmodial activity, DMPK properties, and efficacy of a series of quinoline-4-carboxamides are described. This series was identified from a phenotypic screen against the blood stage of Plasmodium falciparum (3D7) and displayed moderate potency but with suboptimal physicochemical properties and poor microsomal stability. The screening hit (1, EC50 = 120 nM) was optimized to lead molecules with low nanomolar in vitro potency. Improvement of the pharmacokinetic profile led to several compounds showing excellent oral efficacy in the P. berghei malaria mouse model with ED90 values below 1 mg/kg when dosed orally for 4 days. The favorable potency, selectivity, DMPK properties, and efficacy coupled with a novel mechanism of action, inhibition of translation elongation factor 2 (PfEF2), led to progression of 2 (DDD107498) to preclinical development.

INTRODUCTION

Malaria is a devastating disease with over 214 million clinical cases in 2015.1 In that year alone, the World Health Organization (WHO) estimated 438,000 deaths, mostly among children under five in Sub-Saharan Africa. The current malaria control programs that combine preventive measures with artemisinin combination therapy (ACT) treatment have proven very effective in reducing the malaria burden. Over the past decade, the number of deaths from malaria has fallen by 4% per year, and between 2000 and 2015 the number of clinical cases of malaria has been estimated to have decreased by 40% where the disease is endemic in Africa.2 However, in recent years, parasite resistance to artemisinin has been detected in a...
number of countries in Southeast Asia. For example, in areas along the Cambodia–Thailand border, *Plasmodium falciparum*, the most deadly malaria parasite, has become resistant to most available antimalarial medicines and the spread of multidrug resistance is a major concern.

The malaria drug discovery portfolio has dramatically improved over the past 10 years. However, due to the constant battle against drug resistance and to achieve malaria elimination, new chemotypes with novel mechanisms of action are required. New drugs are needed: (1) that are not cross-resistant to existing drugs; (2) that can be given as a single dose; (3) that prevent transmission (active against the sexual stages of the parasite); (4) that can give chemoprotection (active against liver stages). Recently, we reported the discovery of translation elongation factor 2 (EF2), which is critical for *P. falciparum* replication, with excellent pharmacokinetic and antimalarial properties, and proof-of-concept on the scala of a clinical candidate (Scheme 1).

On the basis of these results, we next turned to the R2 position with the aim of reducing the number of aromatic rings. A range of nonaromatic amines able to duplicate the hydrogen bonding potential of the 3-pyridyl moiety were evaluated. Results demonstrated that basicity, lipophilicity, and linker length were all important for activity (Table 1). Compounds (17, 18, and 19) with an ethyl linked piperidine or pyrrolidine retained similar activities to the corresponding 3-pyridyl derivatives. The replacement of the cyclic amine for a dimethylamine (13) and the introduction of longer linker lengths (14) led to drop in potency. Although fluorinated compound 11 was less lipophilic than initial hit 1, it still showed poor solubility and metabolic stability.

After initial optimization of the R1 and R2 groups, we then turned our attention to the R3 substituent with the aim of reducing the number of aromatic rings and also led to improved solubility and hepatic microsomal stability. Furthermore, ligand-lipophilicity efficiency (LLE or LiPE) improved for compound 19 (LLE = 3.2) compared with the initial hit 1 (LLE = 2.6). Subsequent analogues were optimized using the ethyl linked pyrrolidine substituent on the amide at R2, which displayed the best profile in terms of lipophilicity, activity, and hepatic microsomal stability.

The initial aim of the hit to lead program was to improve potency (PfEC_{50}(3D7) < 0.1 μM), aqueous solubility (>100 μM), and metabolic stability (mouse liver microsomes Cl < 5 mL min\(^{-1}\) g\(^{-1}\)) of compound 1. Iterative rounds of drug design, synthesis, and biological evaluation were driven by the Medicines for Malaria Venture (MMV) compound progression criteria. Initial modifications were directed toward improving the physicochemical properties particularly reducing lipophilicity. The clogP of the hit was 4.3, which is higher than average for oral drugs and may contribute to the poor aqueous solubility and hepatic microsomal instability. Several points for modification on the scaffold were identified that could address the high lipophilicity: the bromine atom (R1) significantly adds to lipophilicity, as do aromatic substituents in the carboxamide (R2) and quinoline (R3) moieties. High numbers of aromatic rings are associated with unfavorable lipophilicity and poor compound developability.

The initial focus was on the R1 and R2 substituents. Quinoline-4-carboxamides 10–19 were prepared in two steps from the corresponding isatin (Scheme 1), employing the Pfitzinger reaction with 1-(p-tolyl)ethane using potassium hydroxide as a base in a mixture of ethanol and water at 125 °C under microwave irradiation to afford the quinoline-4-carboxylic acid 3,10 Coupling of 3 with the corresponding amine, using EDC and HOBt in DMF, led to compounds 10–19 (Scheme 1).

Replacement of the bromine moiety at R1 with chlorine (10) or fluorine (11) was tolerated without significant loss of activity while decreasing molecular weight and clogP (Table 1). However, removal of the halogen altogether in compound 12 led to an 8-fold drop in potency. Although fluorinated compound 11 was less lipophilic than initial hit 1, it still showed poor solubility and metabolic stability.

Results and Discussion

The initial aim of the hit to lead program was to improve potency (PfEC_{50}(3D7) < 0.1 μM), aqueous solubility (>100 μM), and metabolic stability (mouse liver microsomes Cl < 5 mL min\(^{-1}\) g\(^{-1}\)) of compound 1. Iterative rounds of drug design, synthesis, and biological evaluation were driven by the Medicines for Malaria Venture (MMV) compound progression criteria. Initial modifications were directed toward improving the physicochemical properties particularly reducing lipophilicity. The clogP of the hit was 4.3, which is higher than average for oral drugs and may contribute to the poor aqueous solubility and hepatic microsomal instability. Several points for modification on the scaffold were identified that could address the high lipophilicity: the bromine atom (R1) significantly adds to lipophilicity, as do aromatic substituents in the carboxamide (R2) and quinoline (R3) moieties. High numbers of aromatic rings are associated with unfavorable lipophilicity and poor compound developability.9

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On the basis of these results, we next turned to the R2 position with the aim of reducing the number of aromatic rings. A range of nonaromatic amines able to duplicate the hydrogen bonding potential of the 3-pyridyl moiety were evaluated. Results demonstrated that basicity, lipophilicity, and linker length were all important for activity (Table 1). Compounds (17, 18, and 19) with an ethyl linked piperidine or pyrrolidine retained similar activities to the corresponding 3-pyridyl derivatives. The replacement of the cyclic amine for a dimethylamine (13) and the introduction of longer linker lengths (14) led to drop in potency. Compounds 18 and 19 showed enhanced hepatic microsomal stability and improved solubility. Furthermore, ligand-lipophilicity efficiency (LLE or LiPE) improved for compound 19 (LLE = 3.2) compared with the initial hit 1 (LLE = 2.6). Subsequent analogues were optimized using the ethyl linked pyrrolidine substituent on the amide at R2, which displayed the best profile in terms of lipophilicity, activity, and hepatic microsomal stability.

After initial optimization of the R1 and R2 groups, we then turned our attention to the R3 substituent with the aim of improving potency while maintaining lipophilicity at a moderate level (clogP < 3.5). An efficient synthetic route (Scheme 2) was designed for rapid access to a variety of R3 analogues which involved reaction of 5-fluorosatin or 5-chlorosatin with malonic acid in refluxing acetic acid to provide intermediate 4. A one pot chlorination and amide formation was achieved by treating 2-hydroxyquinoline-4-carboxylic acid with thionyl chloride in the presence of DMF followed by reaction of the intermediate acid chloride with 2-pyryldin-1-yethanamine in THF at room temperature. Intermediate 5 underwent aromatic nucleophilic substitution with a range of amines under microwave irradiation in acetonitrile to afford compounds 21–30 (Table 2). This route was also used for the synthesis of derivatives with aromatic R3 substituents where a Suzuki coupling of intermediate 5 with the appropriate boronic acid or ester led to compounds 36–39.

In general, the replacement of the tolyl substituent by an array of primary and secondary amines drove lipophilicity down and also led to improved solubility and hepatic microsomal stability (Table 2). In terms of potency, small heterocycles like 4-amino-3-methyloxazole 20 and N-methylpiperazine 21 at the R3 position were not tolerated. However, introduction of the 4-morpholinopiperidine 24 (EC_{50} = 0.15 μM, LLE = 4.2) moiety...
improved both potency and ligand efficiency compared with previous lead compound 18. Compound 24 displayed good aqueous solubility and moderate mouse hepatic microsomal clearance, which is probably related to the reduction in lipophilicity (clogP = 2.9).

To improve the potency of compound 24, we investigated other aliphatic amines. The introduction of flexibility at R3 with an aliphatic amine substituent (25) led to a further improvement in potency against *P. falciparum* (EC\(_{50} = 70\) nM) and lipophilic ligand efficiency (LLE = 5.4), with excellent

Table 1. Optimization the R\(^1\) and R\(^2\) Moieties

| Comp. | R\(^1\) | R\(^2\) | clogP | P\(_{50}\) (3D7) EC\(_{50}\) (μM) | MRC-5 EC\(_{50}\) (μM) | MLM\(^a\) Clr (mL/min/g) | Sol (μM)\(^b\) |
|-------|-------|-------|-------|------------------|-----------------|------------------|-----------------|
| 1\(^b\) | Br    | 3-pyridyl | 4.3   | 0.12             | 21              | 5.3              | 39              |
| 10     | Cl    | 3-pyridyl | 4.1   | 0.29             | 21              | -                | -               |
| 11\(^b\) | F    | 3-pyridyl | 3.7   | 0.35             | 50              | 8.6              | 36              |
| 12     | H     | 3-pyridyl | 3.6   | 0.99             | >50             | -                | -               |
| 13     | Cl    |          | 4.2   | 1.2              | 9               | -                | -               |
| 14     | Cl    |          | 4.0   | 6.7              | 6               | 1.1              | 262             |
| 15     | Cl    |          | 3.3   | 3.6              | 26              | 11               | 12              |
| 16     | Cl    |          | 3.4   | 3.3              | 8               | -                | -               |
| 17     | Cl    |          | 4.7   | 0.32             | 7               | -                | -               |
| 18     | Cl    |          | 4.1   | 0.44             | 7               | 2.1              | 13              |
| 19\(^c\) | F    |          | 3.7   | 0.70             | 9               | 3.4              | 180             |

\(^a\)MLM: mouse liver microsomes. \(^b\)Sol: kinetic aqueous solubility. Data for compounds 1, 11, and 19 reported previously.\(^5\)

Scheme 1\(^a\)

Scheme 2\(^a\)

\(^a\)Conditions: (a) KOH, EtOH/water, 125 °C, microwave, 20 min, 29–58% yield; (b) amine, EDC, HOBt, DMF, room temperature, 16 h, 22–43% yield.

\(^a\)Conditions: (a) malonic acid, acetic acid, reflux, 16 h, 54%; (b) SOCl\(_2\), DMF, DCM, reflux, 3 h and then 2-pyrrolidin-1-ylethanamine, THF, room temperature, 16 h, 27–43% yield; (c) amine, acetonitrile, 170 °C, microwave, 1 h, 7–54% yield; (d) boronic acid or ester, potassium phosphate, Pd(PPh\(_3\))\(_4\), DMF/water 3/1, 130 °C, microwave, 30 min, 19–73%.
selectivity against mammalian cells. Compound 25 had good aqueous solubility and in vitro hepatic microsomal stability across a range of species (Cl\text{\textsubscript{m}} (mL min\textsuperscript{-1} g\textsuperscript{-1}): mouse 0.8; rat <0.5; human <0.5) and low plasma protein binding (59%). The good in vitro DMPK properties of compound 25 translated into reasonable in vivo pharmacokinetics in mouse (Table 7). Furthermore, compound 25 afforded oral in vivo activity (Table 8) in the \textit{P. berghei} mouse model, with a 93% reduction of parasitemia when dosed orally at 30 mg/kg once a day for four consecutive days. An in vivo pharmacokinetic study in mice for compound 25 showed low clearance, with a moderate volume of distribution and a resultant good half-life. However, oral bioavailability was poor (F = 15%). The low systemic exposure of compound 25 was not attributed to high first-pass metabolism due to the low in vitro clearance in mouse microsomes and low in vivo blood clearance but was probably due to poor permeability as highlighted by results in a PAMPA assay (Table 6). Preliminary safety profiling of compound 25 showed a weak affinity to the hERG ion channel (16% inhibition at 11 \mu M) and an oral maximum tolerated dose (MTD) greater than 300 mg/kg b.i.d. for 4 days. With an attractive overall profile, compound 25 was identified as a key molecule to declare early lead status for this series, according to the MMV compound development criteria.\textsuperscript{7}

Moving into lead optimization, our focus was to improve potency, permeability, and bioavailability through structural modifications while retaining good physicochemical properties. Reducing the flexibility of compound 25 by shortening the linker length of the aminoalkylmorpholine moiety at R\textsuperscript{3} was tolerated (26). More promising was the 17-fold improvement (EC\textsubscript{50} = 4 nM) on antiplasmodial activity obtained when the linker was extended from three to four carbons (27). Compound 27 displayed excellent lipophilic ligand efficiency (LLE = 6.2). This improvement on in vitro potency led to enhanced in vivo efficacy (Table 8) with an ED\textsubscript{90} of 2.6 mg/kg. In addition with compound 27, one out of three mice went to cure at 4 \times 30 mg/kg (q.d. po). Mouse in vivo pharmacokinetics showed a longer half-life than the early lead 25 as a result of lower in vivo clearance and a slightly higher volume of distribution (Table 7). Despite having improved in vivo potency and half-life, oral bioavailability was still poor, presumably still due to poor permeability (PAMPA \textit{P}_{\text{app}} = 2 nm/s).

### Table 2. SAR Study of the R\textsuperscript{3} Substituent: Amines

| Comp | R\textsuperscript{1} | R\textsuperscript{3} | clog\textsubscript{\text{P}} | \text{Pf}(3DT) \text{EC}_{50} (\mu M) | MRC-5 \text{EC}_{50} (\mu M) | MLM Cl\text{\textsubscript{m}} (mL/min/g) | Sol (\mu M) | \text{MTD} (mg/kg) |
|------|-----------------|-----------------|-----------------|----------------|----------------|----------------|-----------------|----------------|
| 20 | F | | 2.4 | >50 | >50 | - | - |
| 21 | F | | 2.0 | 34 | 20 | - | - |
| 22 | F | | 2.2 | 5.8 | >50 | - | - |
| 23 | F | | 2.6 | 0.68 | 25 | 0.5 | 220 |
| 24 | CI | | 2.9 | 0.15 | 13 | 2.3 | 210 |
| 25 | F | | 2.1 | 0.07 | 41 | 0.8 | 230 |
| 26 | F | | 1.8 | 0.19 | 50 | <0.5 | |
| 27 | CI | | 2.7 | 0.004 | 13 | 2.0 | 220 |
| 28 | F | | 2.9 | 8.9 | 19 | - | - |
| 29 | F | | 2.8 | 0.002 | >50 | - | - |
| 30 | CI | | 3.1 | 0.006 | 31 | 2.0 | 230 |

\textsuperscript{a}MLM: mouse liver microsomes. \textsuperscript{b}Kinetic aqueous solubility.
Modifications of our lead compound (25) focused on modulation of basicity, with the aim of improving permeability. Specifically, we envisaged that lowering basicity would reduce protonation at physiological pH, increase passive permeability, and ultimately improve bioavailability. Early lead 25 has two basic groups, a pyrrolidine (R²) and a morpholine (R³) with calculated pKₐ values of 8.5 and 7.0 respectively. We first investigated the effect that modifications on the morpholine group at R³ had on in vitro activity and permeability. We found that the morpholine oxygen was crucial for antiparasitic activity and replacement of the morpholine group by piperidine (28) was not tolerated. In contrast, the morpholine nitrogen was not essential for potency and removal, as exemplified by compounds 29 and 30, was well tolerated leading to single digit nanomolar potency against *P. falciparum*. Moreover, the removal of the basic group at R³ had not only improved activity but increased permeability more than 20-fold (30, Pᵢ = 48 nm/s). Furthermore, improved in vitro permeability also translated in vivo, with an increase in oral bioavailability in mice (F = 23%).

After establishing a link between basicity, PAMPA permeability, and oral bioavailability for the series, we focused on modulating the pKₐ of the pyrrolidine group, the stronger of the two basic groups on early lead 25. Taking into account previous SAR showing that basicity and linker length were important for activity, we designed a short array of analogues with decreasing basicity. Predicted pKₐ ranged from 5.0 for 3-difluoropyrrolidine 35 to 7.9 for 4-fluoropiperidine 31 compared with a predicted pKₐ of 8.5 for the lead pyrrolidine 25 (Table 3). As expected, a reduction of basicity resulted in up to 50-fold improvement in permeability. However, potency was dramatically reduced, highlighting the importance of the basic pyrrolidine nitrogen at the R² position.

Our attention then turned back to the R³ position, with the aim of further improving permeability and bioavailability across species. Previous SAR had shown that a methyl group at the para position of an aromatic ring at C-2 was tolerated, so we therefore expanded the range of substituents to include larger groups while maintaining moderate lipophilicity. This strategy also had the advantage of reducing the number of H-bond donors and molecular flexibility, two key factors that can modulate permeability. Early examples of substitution at R³ showed that introduction of amines, such as dimethylamine (36) and morpholine (37), reduced lipophilicity and was tolerated in terms of potency compared to compounds 11 and 19 (Table 4). Taking into account the leap in potency observed for 25 with a morpholine attached through a flexible linker at C-2, we prepared compound 2 with a carbon spacer between the phenyl ring and the morpholine to allow improved rotation. The introduction of the benzyl morpholine at R³ (compound 2) resulted in a 70-fold increase in potency against Pf (3D7) (EC₅₀ = 1 nM) while retaining good ligand efficiency (LLE = 5.9), more than 30 fold increase in permeability (PAMPA Pᵢ = 73 nm/s) and excellent bioavailability in mice (F = 74%).

Furthermore, in vivo efficacy studies with compound 2 demonstrated complete cure at 4 × 30 mg/kg po q.d. in a *P. berghei* mouse model, with an ED₉₀ of 0.1–0.3 mg/kg (Table 8).

We developed short, four-step synthetic routes for synthesis of 2 and 42 which did not involve the use of palladium catalysis (Scheme 3). Two approaches were employed to synthesize the methyl ketone 8 depending on the availability of commercial starting materials. In the first route, nucleophilic displacement of commercially available 4-[(bromomethyl)]benzonitrile with morpholine using trimethylamine as a base in DCM gave intermediate 6 which was then converted into the desired methyl ketone 8 by reaction with methylmagnesium bromide followed by an acidic quench. In the second route, radical bromination of commercially available 1-(2-chloro-4-methylphenyl)ethanone with NBS using catalytic amounts of benzoyl peroxide in dichlorobenzene afforded compound 7, which was subsequently reacted with morpholine to yield the methyl ketone 8. As described earlier, a Pf/zipper reaction of 5-fluoroisatin with the appropriate methyl ketone led to acid 9. The final amides were prepared using 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT) as coupling agent and N-methylmorpholine in DCM at room temperature. Details of other synthetic routes for individual compounds are described in the Supporting Information.

As highlighted above, a basic group is not required for activity at R³. Thus, derivatives of 2 with either reduced basicity (40, 41, and 42) or a nonbasic substituent (38, 43, 44) at R³ showed excellent potency with the exception of amide 39. The introduction of a conformationally restricted bridge amide as exemplified by compound 39 is detrimental for potency, possibly because it does not allow rotation of the morpholine group to adopt the optimal orientation for binding. The importance of the orientation of the morpholine substituent for activity is also highlighted by compound 45. In this case, a change of the methylmorpholine group from para to the meta position led to a weakly active compound.

### Table 3. Modulating Basicity of the Amide Substituent

| Comp   | R      | clogP | R group pkₐ | PAMPA Pe (nm/s) | Pf (3D7) EC₅₀ (μM) |
|--------|--------|-------|-------------|-----------------|---------------------|
| 25     | ![R²](2) | 2.1   | 8.5         | 2.3             | 0.07                |
| 31     | ![R²](2) | 2.3   | 7.9         | 58              | 0.9                 |
| 32     | ![R²](2) | 1.4   | 7.0         | 24              | 2.5                 |
| 33     | ![R²](2) | 2.5   | 7.5         | 94              | 15                  |
| 34     | ![R²](2) | 2.5   | 5.1         | 120             | 36                  |
| 35     | ![R²](2) | 2.2   | 5.0         | 89              | 18                  |

*Calculated pKₐ using ChemAxon software. Controls: atenolol, 0.7 nm/s; propanolol, 159 nm/s.*
Table 4. SAR Study of the R² Substituent: Aromatic Groups

| Comp | R²   | MW | clogP | R² group pKa a | P½ (3D7) EC₅₀ (µM) | MRC-5 EC₅₀ (µM) |
|------|------|----|-------|----------------|---------------------|-----------------|
| 36   |     | 406| 3.3   | 4.3           | 0.81                | 10              |
| 37   |     | 448| 3.3   | 0.4           | 0.60                | 17              |
| 38   |     | 463| 3.3   | 7.2 (6.8)     | 0.001               | 24              |
| 39   |     | 476| 2.5   | Non basic     | 0.14                | >50             |
| 40   |     | 480| 3.4   | 6.1 (6.1)     | 0.0006              | 19              |
| 41   |     | 480| 3.4   | 6.4 (6.3)     | 0.002               | 41              |
| 42   |     | 497| 3.8   | 6.1 (6.3)     | 0.003               | >50             |
| 43   |     | 511| 2.4   | 3.4           | 0.0006              | 41              |
| 44   |     | 460| 3.2   | Non basic     | 0.007               | -               |
| 45   |     | 463| 3.3   | 6.9           | 11                  | -               |

aCalculated pKₐ using ChemAxon software. Experimental pKₐ using potentiometric titration is shown in parentheses. bData for this compound reported previously.5

On the basis of their excellent potency, good permeability, microsomal stability, and solubility (Table 6), compounds 2, 40, 41, 43, and 44 were progressed for efficacy studies in mice dosing at 1 mg/kg for 4 days (Table 8). Compounds 40, 43, and 44 showed excellent activity at this low dose, with reductions of parasitemia above 99%. Compound 41 showed the best survival time (14 days) comparable with 2.

Once an optimal R² substituent had been identified, we turned our attention back to the R² substituent to see if it was possible to improve the profile of compound 2 (Table 5). As highlighted before, lowering basicity at R² results in a reduction in potency. The replacement of the pyrrolidine for a morpholine in compound 46 led to a 12-fold drop in potency. The amide NH is also important for activity, as capping with a methyl group resulted in an 87-fold decrease in potency against

P. falciparum (3D7) (47, EC₅₀ = 87 nM). Finally, it is possible to reduce the size of the ring on the R² substituent and retain activity as shown by compounds 48 and 49. Compound 49 showed excellent in vivo activity in the P. berghei mouse model at 4 × 10 mg/kg and 4 × 3 mg/kg. (Table 8). However, none of these compounds offered a particular advantage to compound 2.

Although in vitro DMPK data and in vivo efficacy in the P. berghei model were comparable for compound 2 and the fluorinated derivative 41, oral bioavailability in rat (33% for 41 vs 84% for 2) was lower and rat intravenous elimination half-life (4 h for 41 vs 10 h for 2) was shorter for 41 (Table 9). Therefore, compound 2 showed the best overall profile for further progression from this novel quinoline-4-carboxamide series (Table 9 and Figure 2). Compound 2 fulfilled the efficacy and DMPK requirements for a late lead according to the MMV criteria and, following further profiling, was selected as a preclinical candidate by MMV. The studies required to profile 2 for candidate selection have been described elsewhere.5

We also profiled key compounds of this series for their activity against different life stages of the malaria parasite life cycle (Table 10). Following a mosquito bite (blood meal), sporozoites are injected into the skin and migrate in the bloodstream to the liver, where they invade liver hepatocytes and then develop into liver schizonts. Compounds active against liver schizonts can potentially prevent disease development and be used in chemoprotection. Compounds 2, 27, 30, and 38 showed low nanomolar activity against the liver schizont forms of Plasmodium yoelii.14 Several compounds were also tested in vitro against P. falciparum late stage (IV–V) gametocytes. Stage V gametocytes, typically insensitive to antimalarial drugs, are infectious to mosquitoes and responsible for the transmission of the disease. 4-Quinolinecarboxamides, in particular compounds 2 and 30, are potent antigametocytic (stage IV–V) with nanomolar activities.15 The ability of compounds of this series to block transmission was further tested in the P. berghei ookinete development assay, which simulates in vitro the first 24 h of parasite development in the mosquito midgut, from mature gametocyte transformation into gametes, through fertilization and to mature ookinete development. Compounds 2, 27, 30, and 38 showed nanomolar potency in this assay.14

Finally, compounds 2 and 30 were tested against several Plasmodium falciparum drug resistant strains (K1, W2, 7G8, TM90C2A, D6, and V1/S) showing similar levels of activity across strains14b (Table 11).

### CONCLUSION

We have evolved a malaria phenotypic hit series, which started with moderate in vitro activity and selectivity but suboptimal metabolic stability and physicochemical properties, into an early lead compound 25 with improved potency, ligand efficiency, metabolic stability, and in vivo efficacy. The lead optimization phase focused on improving low oral bioavailability caused by the poor permeability of the early lead. The most advanced quinoline-4-carboxamides showed exceptional in vitro and in vivo activities, with a reduction of parasitemia of more than 99% when administered at low doses (4 × 1 mg/kg, 4 days, po) in the P. berghei mouse model. In addition to potent intraerythrocyte activity, compounds in this series showed similar potency against liver schizonts, gametocytes, and ookinetes in vitro. Furthermore, a combination of in vitro and in vivo activities across the different stages of the malaria...
parasite life cycle demonstrated the potential of this novel quinoline-4-carboxamide series to meet a number of the MMV’s malaria target candidate profiles (TCPs), as previously described. Compound 2 has been extensively profiled in vitro and in vivo and displays activity against multiple life-cycle stages of the parasite with a long half-life in preclinical animal studies. With this profile, compound 2 has the potential for single dose treatment of malaria as part of a combination therapy, together with transmission blocking potential (TCP2 and TCP3b). It also has the potential for chemoprotection (TCP4). Compound 2 was active against parasites that show resistance to currently used antimalarials and has a novel mode of action through inhibition of elongation factor 2 (PfeEF2), which is involved in protein synthesis. The favorable potency, selectivity, DMPK properties, efficacy, safety profile, and novel mechanism of action support the progression of 2 toward clinical development.

**EXPERIMENTAL SECTION**

**Chemistry. General.** Solvents and reagents were purchased from commercial suppliers and used without further purification. Dry solvents were purchased in Sure/Seal bottles stored over molecular sieves. Reactions using microwave irradiation were carried out in a Biotage Initiator microwave. Normal phase TLCs were carried out on precoated silica plates (Kieselgel 60 F254, BDH) with visualization via UV light (UV 254/365 nm) and/or ninhydrin solution. Flash chromatography was performed using CombiFlash Companion Rf (Teledyne ISCO) and prepacked silica gel columns purchased from Grace Davison Discovery Science or SiliCycle. Mass-directed preparative HPLC separations were performed using a Waters HPLC (2545 binary gradient pumps, 515 HPLC make-up pump, 2767 sample manager) connected to a Waters 2998 photodiode array detector and a Waters 3100 mass detector. Preparative HPLC separations were performed with a Gilson HPLC (321 pumps, 819 injection module, 215 liquid handler/injector) connected to a Gilson 155 UV/vis detector. On both instruments, HPLC chromatographic separations were conducted using Waters XBridge C18 columns, 19 mm × 100

| Comp | R² | MW  | clogP | pKᵣ group | Pf (3D7) EC₅₀ (µM) | HepG2 EC₅₀ (µM) |
|------|----|-----|-------|------------|-------------------|-----------------|
| 46   | H  | 479 | 2.7   | 6.3        | 0.012             | -               |
| 47   | F  | 477 | 3.3   | 8.6        | 0.087             | >50             |
| 48   | H  | 448 | 3.0   | 9.3        | 0.002             | >50             |
| 49   | N  | 448 | 3.0   | 7.8        | 0.004             | 41              |

*Calculated pKᵣ using ChemAxon software.

**Figure 2.** Mean blood concentration time profile of compounds 2 and 41 following intravenous or oral administration to male Sprague Dawley rats.

**Table 5. SAR Study of the R² Substituent**

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Scheme 3"
mm, 5 μm particle size, using 0.1% ammonia in water (solvent A) and acetonitrile (solvent B) as mobile phase. 1H NMR, 19F NMR spectra were recorded on a Bruker Avance DPX 500 spectrometer (1H at 500.1 MHz, 19F at 470.5 MHz) or a Bruker Avance DPX 300 (1H at 300 MHz). Chemical shifts (δ) are expressed in ppm recorded using the residual solvent as the internal reference in all cases. Signal splitting patterns are described as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), broadened (br), or a combination thereof. Coupling constants (J) are quoted to the nearest 0.1 Hz. Low resolution mass spectroscopy (HRMS) was performed using a Bruker Daltonics MicroTOF mass spectrometer. LC–MS analysis and chromatographic separation were conducted with a Bruker Daltonics MicroTOF mass spectrometer or an Agilent Technologies 1200 series HPLC connected to an Agilent Technologies 6130 quadrupole LC/MS, where both instruments were connected to an Agilent diode array detector. The column used was a Waters XBridge column (50 mm × 2.1 mm, 3.5 μm particle size), and the compounds were eluted with a gradient of 5%–95% acetonitrile/water + 0.1% ammonia. All final compounds showed chemical purity of ≥95% as determined by the

Table 6. Key in vitro DMPK Data for Selected Analogues

| compd | PAMPA P (nm/s) | Sol. (μM) | MLM Ch (mL min⁻¹ g⁻¹) | RLM Ch (mL min⁻¹ g⁻¹) | HLM Ch (mL min⁻¹ g⁻¹) | PPB (%) | hERG IC₅₀ (μM) |
|-------|----------------|-----------|-----------------------|-----------------------|------------------------|--------|----------------|
| 25    | 2.3            | 232       | 0.8                   | <0.5                  | <0.5                   | 59     | >11            |
| 27    | 2              | 217       | 2.0                   | <1                    | 49                     | 11     |
| 30    | 48             | 232       | 2                     | 0.8                   | 1                      | 77     | >11            |
| 38    | 29             | 109       | 1.9                   | <0.8                  | ≤1.1                   | 63     | 16             |
| 40    | 63             | >208      | 1.0                   | <0.5                  | 75                     | 11     |
| 41    | 132            | 208       | <0.5                  | <0.5                  | 1.5                    |        |
| 42    | >201           | 0.5       |                       |                       |                        | 56     |
| 43    | 14             | 171       | <0.5                  | 0.8                   |                        |        |
| 44    | 49             | >217      | 2.0                   | 0.6                   | 2                      |        |
| 46    | >210           | <0.5      |                       |                       |                        |        |
| 47    | 137            | >210      | <0.5                  |                       |                        |        |
| 48    | 49             |           |                       |                       |                        |        |
| 49    | 75             | 71        | 0.5                   |                       |                        |        |

“Controls: atenolol, 0.2–4.6 nm/s; propanolol, 103–159 nm/s. **MLM: mouse liver microsome. RLM: rat liver microsome. HLM: human liver microsome. **Mouse plasma protein binding. **Measured using IonWorks Patch Clamp electrophysiology. **Data for this compound reported previously.

Table 7. In vivo Pharmacokinetic Profile in Mice of Key Compounds

| compd | Clb (mL min⁻¹ kg⁻¹) | Vd (L/kg) | T₁/₂ (h) | C₀ (ng/mL) | AUC (ng·min/mL) | T₀.₀ (h) | F (%) |
|-------|---------------------|-----------|----------|------------|-----------------|---------|-------|
| 25    | 14                  | 3         | 3.2      | 315        | 92922           | 2       | 15    |
| 27    | 4                   | 3.5       | 12.5     | 579        | 17615           | 2       | 16    |
| 30    | 34                  | 7.4       | 2.9      | 193        | 72728           | 0.5     | 23    |
| 2⁰    | 12³                 | 15⁴       | 16⁵      | 90⁶        | 179272°         | 1⁷      | 74    |

“Data for this compound reported previously. °iv dose: 1 mg/kg, po dose: 3 mg/kg.

Table 8. In vivo Oral Activity in the P. berghei Mouse Model Peter’s Test

| compd | activity (%) | survival (days) | cure | activity (%) | survival (days) | cure | activity (%) | survival (days) | cure | activity (%) | survival (days) | cure |
|-------|--------------|-----------------|------|--------------|-----------------|------|--------------|-----------------|------|--------------|-----------------|------|
| 25    | 93.0         | 7               | 1/3  | 99.7         | 15              | 3/3  | 96.0         | 9               | 48.0 | 6.0          | 48.0            | 6.0  |
| 27    | 99.8         | 22              | 1/3  | 99.9         | 8               | 3/3  | 98.0         | 6               | 90.0 | 7            | 90.0            | 7    |
| 40    | 99.8         | >30             | 3/3  | 99.9         | >30             | 3/3  | 99.9         | 25              | 99.9 | 14           | 99.9            | 14   |
| 41    | 99.9         | 10              | 8    | 99.8         | 6               | 3/3  | 99.9         | 25              | 99.9 | 14           | 99.9            | 14   |
| 44    | 99.1         | 20.3            | 1/3  | 99.2         | 9.3             |      |              |                 |      |              |                 |      |

Table 9. In vivo Pharmacokinetic Parameters in Male Sprague Dawley Rat

| compd | Clb (mL min⁻¹ kg⁻¹) | Vd (L/kg) | T₁/₂ (h) | C₀ (ng/mL) | AUC (ng·min/mL) | T₀.₀ (h) | F (%) |
|-------|---------------------|-----------|----------|------------|-----------------|---------|-------|
| 2⁰    | 18                  | 15        | 10       | 180⁰       | 4⁴           | 20054²   | 84    |
| 41    | 32                  | 10        | 4        | 29         | 8              | 30400    | 33    |

“Data for this compound reported previously. °po dose: 5 mg/kg.
Table 10. Activity against Plasmodium Life Cycle Stages

| compd | P{sub}F (IC{sub}50) (nM) | P{sub}F liver stage (IC{sub}50) (nM) | P{sub}F GAM IV–V (IC{sub}50) (nM) | P{sub}F plokistone (IC{sub}50) (nM) |
|-------|-----------------|--------------------------------------|----------------------------------|----------------------------------|
| 27    | 4               | 18                                   | 104                              | 5                                |
| 30    | 6               | 1                                     | 39                               | 14                               |
| 2     | 1               | 1                                     | 1                                | 1                                |
| 38    | 4               | 4                                     | 152                              | 15                               |

"Run in duplicate. Reference controls: pyronaridine EC{sub}50 = 3108 nM, tafenoquine EC{sub}50 = 5250 nM, artemisinin EC{sub}50 = 0.8 nM. Data reported previously."

Table 11. Activity against Plasmodium falciparum Resistant Strains

| compd | NF5 | K1 | W2 | 7G8 | TM90 | CA | D6 | V1 | S |
|-------|-----|----|----|-----|------|----|----|----|----|
| 30    | 0.5 | 0.8| 0.6| 0.7 | 0.5  | 0.7| 0.9| 0.7| 0.7 |
| 2     | 0.5 | 0.8| 0.6| 0.7 | 0.5  | 0.7| 0.9| 0.7| 0.7 |

"Data for compound 2 have been previously reported."
Featured Article

To a stirred suspension of 6-chloro-2-((4-morpholinobutyl)amino)quinoline-4-carboxamide (20) (8.50 g, 38 mmol) in anhydrous DCM (250 mL), was added anhydrous DMF (2 mL) followed by 96 mg of BINAP (10% mol, 0.05 mmol) in dioxane (4 mL). Reaction was heated at 170 °C overnight in a sealed tube. Reaction crude was filtered through Celite, and the filtrate was partitioned between water (5 mL) and DCM (25 mL). Organic phase was dried over MgSO4 and solvents were removed under reduced pressure. Product was purified by column chromatography on a 120 g silica gel cartridge. Gradient: 2 with concentrated NH3 in DCM. Gradient: 20–100% B. The relevant fractions were combined and concentrated to dryness under reduced pressure to obtain the desired amine. Yield, 13% (4 mg, 0.02 mmol), yellow solid. Yield, 22% (20 mg); 1H NMR (500 MHz, CDCl3) δ 7.71–7.64 (m, 2H), 7.39–7.34 (m, 1H), 7.23 (s, 1H), 7.13–7.11 (m, 1H), 7.04–7.03 (m, 1H), 6.88–6.87 (m, 1H), 4.88 (s, 2H), 4.28 (t, J = 5.4 Hz, 2H), 2.93–2.92 (m, 2H), 2.77 (t, J = 6.0 Hz, 2H), 2.60–2.57 (m, 4H), 1.82–1.77 (m, 4H) ppm; 13C NMR (125 MHz, CDCl3) δ –117.16 ppm; LC–MS m/z 409 (M + 1).

6-Fluoro-2-(4-morpholinopiperidin-1-yl)-N-(2-(pyrrolidin-1-yl)ethyl)quinoline-4-carboxamide (24). Prepared using general procedure C starting from 5 (R1 = F) (0.2 mmol, 65 mg), yellow solid. Yield, 25% (15 mg); 1H NMR (500 MHz, CDCl3) δ 7.43–7.40 (m, 2H), 7.23–7.19 (m, 1H), 7.14–7.11 (m, 1H), 7.04–7.03 (m, 1H), 6.90–6.89 (m, 1H), 4.84 (s, 2H), 4.27 (t, J = 5.4 Hz, 2H), 2.93–2.92 (m, 2H), 2.77 (t, J = 6.0 Hz, 2H), 2.60–2.57 (m, 4H), 1.82–1.77 (m, 4H) ppm; 13C NMR (125 MHz, CDCl3) δ –117.16 ppm; LC–MS m/z 409 (M + 1).

6-Fluoro-2-(3-morpholinopropylamino)-N-(2-(pyrrolidin-1-yl)ethyl)quinoline-4-carboxamide (25). Prepared using general procedure C starting from 5 (R1 = Cl) (0.2 mmol, 70 mg), yellow solid. Yield, 31% (28 mg); 1H NMR (500 MHz, CDCl3) δ 7.97 (t, J = 13.2 Hz, 2H), 3.74 (t, J = 4.7 Hz, 4H), 3.66–3.62 (m, 2H), 2.98–2.93 (m, 2H), 2.77 (t, J = 6.0 Hz, 2H), 2.61–2.57 (m, 4H), 1.96–1.94 (m, 4H) ppm; 13C NMR (125 MHz, CDCl3) δ –118.31 ppm; LC–MS m/z 456 (M + 1).

6-Fluoro-2-(3-morpholinobutylamino)-N-(2-(pyrrolidin-1-yl)ethyl)quinoline-4-carboxamide (26). Prepared using general procedure C starting from 5 (R1 = F) (0.3 mmol, 88 mg), yellow solid. Yield, 29% (33 mg); 1H NMR (500 MHz, CDCl3) δ 7.99 (t, J = 13.2 Hz, 2H), 7.19–7.16 (m, 1H), 6.36–6.35 (m, 1H), 6.02 (s, 1H), 3.64 (br s, 4H), 3.56 (q, J = 6.6 Hz, 2H), 3.22 (br s, 2H), 2.70 (t, J = 5.9 Hz, 2H), 2.53 (br s, 4H), 2.35 (br s, 4H), 1.71 (br s, 4H), 1.65 (br s, 4H) ppm; 13C NMR (125 MHz, CDCl3) δ –119.32 ppm; LC–MS m/z 430 (M + 1).

6-Fluoro-2-(3-morpholinopropionic acid)-N-(2-(pyrrolidin-1-yl)ethyl)quinoline-4-carboxamide (27). Prepared using general procedure C starting from 5 (R1 = F) (0.3 mmol, 88 mg), yellow solid. Yield, 25% (20 mg); 1H NMR (500 MHz, CDCl3) δ 7.92–7.89 (m, 2H), 7.48–7.44 (m, 2H), 7.23–7.20 (m, 2H), 2.77–2.74 (m, 2H), 2.57–2.60 (m, 4H), 1.78–1.81 (m, 4H) ppm; 13C NMR (125 MHz, CDCl3) δ –118.87 ppm; LC–MS m/z 416 (M + 1).
To a solution of [R1 = F] (0.25 mmol, 80 mg), white solid. Yield, 7% (60 mg); ¹H NMR (500 MHz, CDCl₃) δ 8.18–8.14 (m, 3H), 7.96–7.94 (m, 2H), 7.54–7.50 (m, 3H), 7.10 (dd, J = 4.9, 9.9 Hz, 1H), 3.76–3.72 (m, 6H), 3.69–3.66 (m, 4H), 2.78 (t, J = 6.0, 9.9 Hz, 2H), 2.61–2.59 (m, 4H), 1.81–1.79 (m, 4H), 0.83 (s, 1H, 1H), 1.40–1.32 (m, 2H, 2H) ppm; LC–MS m/z 415 [M + H]⁺.

7-Fluoro-4-(morpholin-4-yl)phenyl]-N-(2-(pyrrolidin-1-yl)ethyl)quinoline-4-carboxamide (39). Prepared using general procedure D starting from [R1 = F] (0.25 mmol, 80 mg), white solid. Yield, 73% (87 mg); ¹H NMR (500 MHz, CDCl₃) δ 5.14, 5.11, 5.05 ppm; LC–MS m/z 477 [M + H]⁺.

2-Chloro-4-(morpholinomethyl)phenyl]-6-fluoroquinoline-4-carboxylic Acid (9, X = Cl). A mixture of 1-(2-chloro-4-methylphenyl)ethanone (1.65 g, 6.65 mmol) and acetonitrile (25 mL) was prepared at rt and stirred under nitrogen. Potassium carbonate (1.10g 7.98 mmol) was then added and the mixture was stirred in vacuo for 2 h. After 2 h, TLC showed presence of product and starting material. The mixture was stirred at rt for 1 h. Then, the resulting precipitate was filtered, washed with dichloromethane (2× 50 mL), and concentrated under reduced pressure. The mixture was then diluted with dichloromethane (50 mL), filtered through a phase separator and the filtrate was concentrated under reduced pressure. The mixture was purified by column chromatography (40–100% ethyl acetate/hexane) to afford a mixture of products (9.8 g, 4.25 mmol, 64%) as a yellow oil. ¹H NMR (500 MHz, CDCl₃) δ 2.64, 2.60, 2.58, 2.56, 2.54, 2.52, 2.50, 2.48, 2.46, 2.38, 2.36, 2.34, 2.32, 2.30, 2.28, 2.26, 2.24, 2.22, 2.20, 2.18, 2.16, 2.14, 2.12, 2.10, 2.08, 2.06, 2.04, 2.02, 2.00, 1.98, 1.96, 1.94, 1.92, 1.90, 1.88, 1.86, 1.84, 1.82, 1.80, 1.78, 1.76, 1.74, 1.72, 1.70, 1.68, 1.66, 1.64, 1.62, 1.60, 1.58, 1.56, 1.54, 1.52, 1.50, 1.48, 1.46, 1.44, 1.42, 1.40, 1.38, 1.36, 1.34, 1.32, 1.30, 1.28, 1.26, 1.24, 1.22, 1.20, 1.18, 1.16, 1.14, 1.12, 1.10, 1.08, 1.06, 1.04, 1.02, 1.00, 0.98, 0.96, 0.94, 0.92, 0.90, 0.88, 0.86, 0.84, 0.82, 0.80, 0.78, 0.76, 0.74, 0.72, 0.70, 0.68, 0.66, 0.64, 0.62, 0.60, 0.58, 0.56, 0.54, 0.52, 0.50, 0.48, 0.46, 0.44, 0.42, 0.40, 0.38, 0.36, 0.34, 0.32, 0.30, 0.28, 0.26, 0.24, 0.22, 0.20, 0.18, 0.16, 0.14, 0.12, 0.10, 0.08, 0.06, 0.04, 0.02, 0.00 ppm; LC–MS m/z 477 [M + H]⁺.
then diluted with DCM (5 mL), and the organic layers were washed with water (2 × 3 mL) and filtered through a phase separator and the organic layers concentrated under reduced pressure and purified by column chromatography (0–10% MeOH in MeOH/DCM) to afford an off-white solid. Analysis by 1H NMR showed impurities, and the mixture was further purified by MDAP to produce 2-(2-chloro-(morpholinomethyl)phenyl)-6-fluoro-N-(2-(pyrrolidin-1-yl)ethyl)-quinoline-4-carboxamide (228 mg, 0.46 mmol, 61%) as an off-white solid. 1H NMR (500 MHz; CDCl3) δ 7.95–7.90 (m, 2H), 7.82–7.75 (m, 5H), 6.78 (d, J = 7.9 Hz, 1H), 7.89 (s, 1H), 8.05 (dd, J = 2.8, 10.0 Hz, 1H), 7.19 (d, J = 4.3 Hz, 4H) ppm; LC/MS m/z 497 [M + H]+.

5-Fluoro-N-(2-morpholinomethyl)-2-(4-morpholinomethyl)phenyl)quinoline-4-carboxamide (46). A solution of 6-fluoro-2-(4-(morpholinomethyl)phenyl)quinoline-4-carboxylic acid (9, X = H) (0.1 g, 0.27 mmol) and NaHCO3 sat aq solution (10 mL). Organic phase was dried over MgSO4 and solvents were removed under reduced pressure. Product precipitated from acetonitrile (15 mL) as a white solid. Yield, 63% (85 mg); 1H NMR (500 MHz, CDCl3) δ 8.22 (dd, J = 5.4, 9.2 Hz, 1H), 8.12 (d, J = 8.2 Hz, 2H), 8.00–7.97 (m, 2H), 7.58–7.52 (m, 3H), 6.67 (t, J = 4.8 Hz, 1H), 3.77–3.70 (m, 10H), 3.61 (s, 2H), 2.70 (t, J = 5.9 Hz, 2H), 2.57 (brs, 4H), 2.75 (t, J = 4.3 Hz, 4H) ppm; 19F NMR (407.5 MHz; CDCl3) δ −111.23; LC–MS m/z 479 [M + H]+.

5-Fluoro-N-methyl-2-(4-(morpholinomethyl)phenyl)-N-(2-(pyrrolidin-1-yl)ethyl)quinoline-4-carboxamide (47). To a suspension of 6-fluoro-2-(4-(morpholinomethyl)phenyl)quinoline-4-carboxylic acid (9, X = H) (100 mg, 0.27 mmol) in anhydrous DCM (10 mL) were added N-methylmorpholine (0.06 mL, 0.54 mmol, 2 equiv) and 2-chloro-4,6-dimethoxy-1,3,5-triazine CDMT (57 mg, 0.32 mmol, 1.2 equiv). The reaction mixture was stirred at room temperature overnight. Reaction was partitioned between DCM (50 mL) and NaHCO3 sat aq solution (10 mL). Organic phase was dried over MgSO4 and solvents were removed under reduced pressure. Product precipitated from acetonitrile (15 mL) as a white solid. Yield, 69% (85 mg); 1H NMR (500 MHz, CDCl3) δ 8.32 (dd, J = 4.3, 4.3 Hz, 4H), 3.53 (s, 2H), 3.27 (s, 2H), 2.57 (brs, 4H), 2.32 (s, 1H), 2.90 (s, 3H), 2.43 (s, 4H), 2.11–2.06 (m, 4H), 1.81–1.51 (m, 4H) ppm; LC–MS m/z 477 [M + H]+.

N-(2-(Cyclopropylamino)ethyl)-6-fluoro-2-(4-(morpholinomethyl)phenyl)quinoline-4-carboxamide (48). To a suspension of 6-fluoro-2-(4-(morpholinomethyl)phenyl)quinoline-4-carboxylic acid (9, X = H) (0.1 g, 0.27 mmol) in anhydrous DCM (10 mL) were added N-methylmorpholine (0.06 mL, 0.54 mmol, 2 equiv) and 2-chloro-4,6-dimethoxy-1,3,5-triazine CDMT (57 mg, 0.32 mmol, 1.2 equiv). The reaction mixture was stirred at room temperature overnight. Reaction was partitioned between DCM (50 mL) and water (10 mL) and the layers were separated. The aqueous portion was extracted with further DCM (10 mL). The combined DCM extracts were evaporated in vacuo. The residue was dissolved in DCM and purified by silica gel (12 g), eluting with 0–100% EtOAc/hexane and then 0–50% (10% MeOH in DCM/DCM) to afford N-(2,2-dimethoxyethyl)-6-fluoro-2-(4-(morpholinomethyl)phenyl)quinoline-4-carboxamide as a yellow gum (109 mg, 79% yield). A solution of N-(2,2-dimethoxyethyl)-6-fluoro-2-(4-(morpholinomethyl)phenyl)quinoline-4-carboxamide (109 mg, 0.24 mmol) in 1,4-dioxane (5 mL) was treated with conc. HCl (1 mL) and stirred at room temperature for 1.5 h. The mixture was neutralized with saturated sodium bicarbonate solution and then extracted with EtOAc (2 × 20 mL). The combined EtOAc extracts were evaporated in vacuo to afford 6-fluoro-2-(4-(morpholinomethyl)phenyl)-N-(2-oxoethyl)quinoline-4-carboxamide as a yellow gum (78 mg, 71% yield). A mixture of 6-fluoro-2-(4-(morpholinomethyl)phenyl)-N-(2-oxoethyl)quinoline-4-carboxamide (78 mg, 0.19 mmol), azetidine (32 mg, 0.57 mmol) in DCM (5 mL) was stirred for 15 min in a stoppered flask at room temperature. Sodium triacetoxoboryl-dride (56 mg, 0.26 mmol) was then added, and the reaction mixture was stirred at room temperature overnight. The reaction mixture was partitioned between water (10 mL) and DCM (10 mL) and the aqueous fraction was extracted with further DCM (10 mL). The combined DCM extracts were evaporated in vacuo. The residue was dissolved in DMF and purified by mass directed autoprep 5–95% MeCN, basic, to afford a yellow gum. The sample was freeze-dried to afford 49 as a cream colored solid (18 mg, 14% yield); 1H NMR (500 MHz, CDCl3) δ 8.21 (dd, J = 5.4, 9.2 Hz, 1H), 8.13 (d, J = 8.4 Hz, 2H), 7.90–7.97 (m, 2H), 7.58–7.52 (m, 3H), 6.73–6.72 (m, 1H), 3.76 (t, J = 4.6 Hz, 4H), 3.61 (s, 2H), 3.57–3.53 (m, 2H), 3.28 (t, J = 7.0 Hz, 4H), 2.72 (t, J = 5.8 Hz, 2H), 2.51 (s, 4H), 2.14–2.07 (m, 2H); LC–MS m/z 449 [M + H]+. Details of other synthetic routes for individual compounds are described in the Supporting Information.

Biological Materials and Methods. This is included in the Supporting Information.

Ethical Statements. In vivo antimalarial efficacy studies in P. berghei carried out at the Swiss Tropical and Public Health Institute (Basel, Switzerland) adhere to local and national regulations of laboratory animal welfare in Switzerland (awarded Permit No. 1731). Protocols are regularly reviewed and revised following approval by the local authority (Veterinäramt Basel Stadt).

Mouse and rat pharmacokinetics were carried out at the University of Dundee. All regulated procedures on living animals were carried out with the permission of the University’s highest governing body.
Molecular formula strings and some data (CSV)

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