Discovery of amodiachins, a novel class of 4-aminoquinoline antimalarials active against multi-drug resistant *Plasmodium falciparum*.

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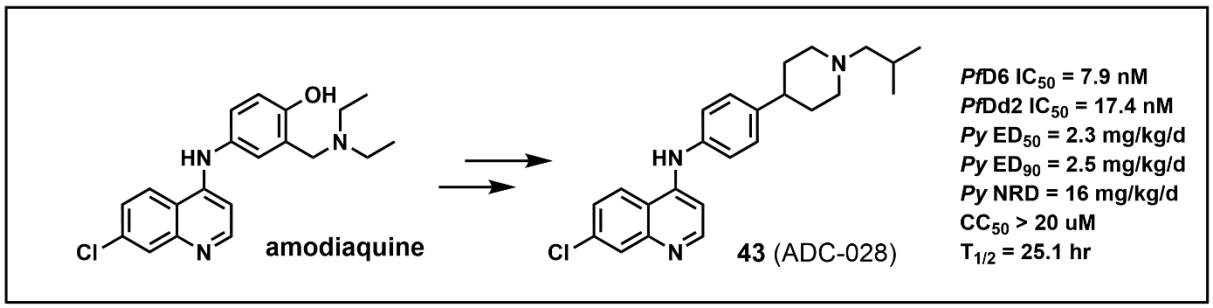


ABSTRACT:

The emergence and spread of drug-resistant strains of *Plasmodium falciparum*, the parasite responsible for the most severe, and often fatal form of malaria, have hampered worldwide prevention, treatment, and eradication efforts. This study aims to discover and develop novel antimalarial compounds to combat multidrug-resistant strains of *P. falciparum*. The research described herein builds upon optimizing the stability and activity of amodiaquine analogs, a 4-aminoquinoline antimalarial. Series of compounds were synthesized, and their efficacy was assessed against cultured *P. falciparum* and against a murine malaria model. The results indicate that the addition of a piperidine heterocycle greatly improves antiparasitic activity and varying the length and branching of a terminal alkyl attenuates metabolic stability and *in vivo* efficacy. This study highlights potential candidates for further evaluation and identifies the importance of discovering novel compounds to overcome drug resistance for treatment of malaria.

INTRODUCTION

Resistance strains of *Plasmodium falciparum* (*Pf*), the causative agent of malaria, to frontline therapies continue to emerge and spread at the cost of human lives.[1–5] Compared to pre-pandemic estimates in 2019, cases have risen by 7.9% to a staggering 247 million cases in 2022 and deaths have increased by 51.3% ultimately numbering over 627,000 people.[6] Although, the increase in case numbers and deaths is likely attributed to the effects of the impact of the Covid-19 pandemic on malaria treatment and prevention infrastructure, the emergence and continued proliferation of resistant strains of *P. falciparum*, threaten

global prevention, treatment, and eradication efforts.[6] Therefore, the continued efforts to discover and develop novel therapies that overcome resistance mechanism are desperately required.

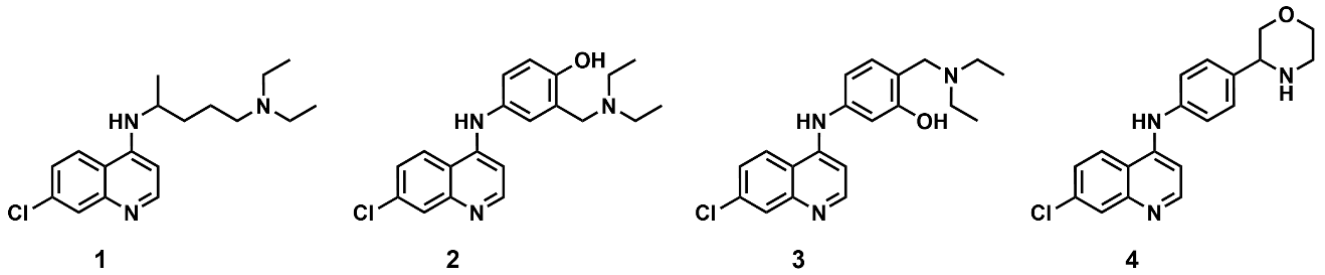


Figure 1: Chemical structures of 4-aminoquinolines chloroquine (**1**), amodiaquine (**2**), and isoquine (**3**). Representative structure (**4**) of initial rationale for the amodiachin SAR described henceforth.

Discovery of the seminal therapy quinine, inspired the continued research focused around its structure, ultimately leading to the discovery of successful class of 4-aminoquinoline antimalarials: chloroquine (**1**), amodiaquine (**2**), and piperaquine.[7] Unfortunately, chloroquine was introduced as a monotherapy in worldwide efforts to prevent and cure malaria and now chloroquine resistance in *Plasmodium falciparum* has spread to virtually all endemic areas of the globe.[8] Resistance to chloroquine and other 4-aminoquinolines is linked to the accumulation of mutations in the gene *plasmodium falciparum chloroquine resistance transporter* (*pfcrt*).[9,10] The emergence and spread of resistance to other individual and combination frontline antimalarials such as artesunate-piperaquine, and artemether-lumafantrine, as well as enduring resistance to older antimalarials only complicates the current state of available treatments for malaria in a world with widespread drug resistance.[6] It is therefore of heightened importance to the field to identify novel compounds that can overcome these multi-drug resistant (MDR) strains of *Plasmodium falciparum*.

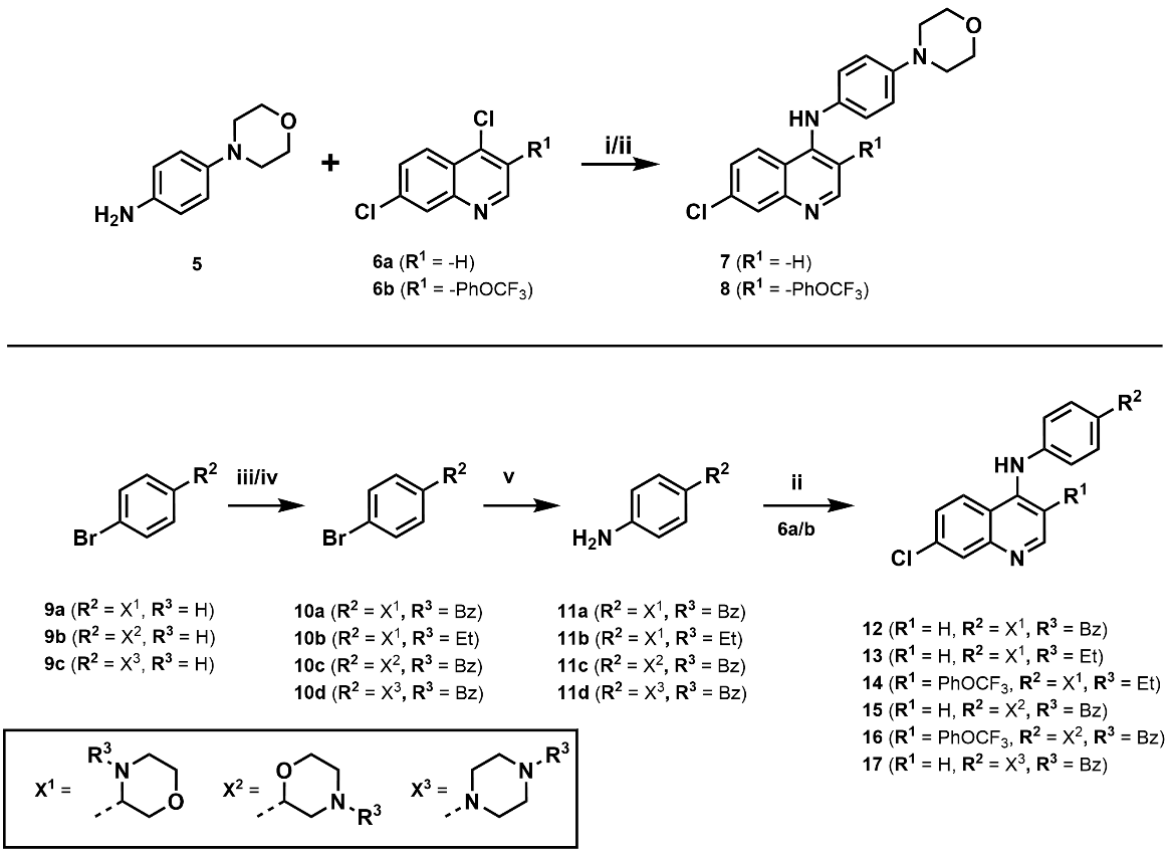
As chloroquine resistance spread, the 4-aminoquinoline amodiaquine was shown to overcome chloroquine resistance.[11,12] Approval of amodiaquine's use as a monotherapy was withdrawn due to a toxic metabolite hypothesized as a product of P-450-catalyzed oxidation of the *para*-hydroxyl of amodiaquine.[11,13] Work to eliminate this metabolic liability resulted in the compound isoquine (**3**), an amodiaquine regioisomer. When the *para*-hydroxyl and *meta*-mannich base were interchanged, isoquine exhibited a better toxicological profile, and increased activity *in vitro* and *in vivo* compared to amodiaquine; however, pharmacokinetic studies revealed that isoquine exhibited low absorption and poor oral bioavailability. Therefore, we believe that this work illuminates that there is still exploitable chemical space around the amodiquine framework. Previous work on pharmachins, a 4-aminoquinoline based on the structure of sontochin, a 3-methyl chloroquine, our group has shown that structure activity relationship (SAR) around the 3-position shows improved activity against chloroquine resistant strains of *Pf* when the 3-position alkyl was replaced by a trifluoromethoxy phenyl.[14] Furthermore, the observed increase in activity suggested that increasing rigidity at the 3-position by adding aromatic rings and/or heterocycles improves activity against MDR strains. We decided to take the same strategy to rigidify the 4-position side chain of amodiaquine as a means to overcome drug resistance and to address the toxicity issue by blocking conversion to a toxic quinoneimine metabolite. Focusing on the 4-position of amodiaquine we sought to replace the labile hydroxyl moiety and the diethylaminomethyl substituent with basic substituted heterocycles (**4**) as a countermeasure to chloroquine resistance and to increase metabolic stability. Herein, we introduce a new series termed "amodiachins" by describing the associated structure activity profile against multidrug resistant *P. falciparum* parasites and for selected molecules we provide the results from testing for metabolic stability, cytotoxicity, pharmacokinetics, and efficacy in a murine model of malaria infection.

CHEMISTRY

Initial compounds were focused on producing an amodiaquine-like structure with only a single *para*-substituted heterocycle and introducing a trifluoromethoxy phenyl at the 3-position of the quinoline ring, similar to that of the pharmachins. Compounds **7**-**21** were produced using chemical synthesis procedures

outlined in Scheme 1 and Scheme 2. Starting materials **5** and **6a** were commercially available, however **6b** was synthesized in-house using previously described methods.[15] Respective 4-aminoquinolines were prepared using either, (i) ethanol and catalytic 12 M hydrochloric acid at reflux, or (ii) 2-ethoxyethanol and phenol at 150 °C. Compounds **12**-**16** were prepared from the respective *para*-morpholine bromobenzene (**9a** and **9b**) and either alkylated with iodoethane or bromobenzyl (**10a**-**10c**). **11a**-**11c** were prepared through amination of the para-substituted bromobenzene intermediates using a Buchwald-Hartwig cross coupling reaction to afford the para-substituted anilines. Final compounds **12**-**16** were prepared through reaction conditions (ii). Using the previously described methods, compound **17** was afforded from commercially available **9c**.

Scheme 1: Synthesis of initially designed amodiachins.[a]



5

6a (R^1 = -H)
6b (R^1 = -$PhOCF_3$)

7 (R^1 = -H)
8 (R^1 = -$PhOCF_3$)

9a (R^2 = X^1, R^3 = H)
9b (R^2 = X^2, R^3 = H)
9c (R^2 = X^3, R^3 = H)

10a (R^2 = X^1, R^3 = Bz)
10b (R^2 = X^1, R^3 = Et)
10c (R^2 = X^2, R^3 = Bz)
10d (R^2 = X^3, R^3 = Bz)

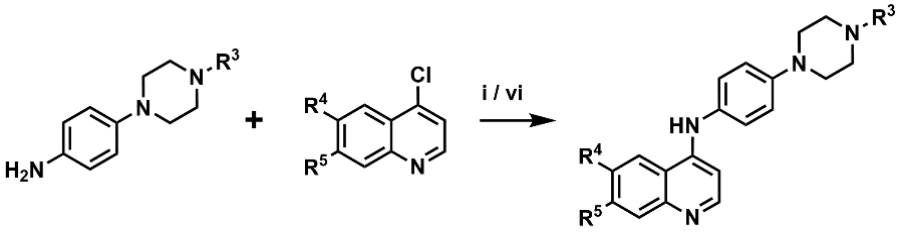
11a (R^2 = X^1, R^3 = Bz)
11b (R^2 = X^1, R^3 = Et)
11c (R^2 = X^2, R^3 = Bz)
11d (R^2 = X^3, R^3 = Bz)

12 (R^1 = H, R^2 = X^1, R^3 = Bz)
13 (R^1 = H, R^2 = X^1, R^3 = Et)
14 (R^1 = $PhOCF_3$, R^2 = X^1, R^3 = Et)
15 (R^1 = H, R^2 = X^2, R^3 = Bz)
16 (R^1 = $PhOCF_3$, R^2 = X^2, R^3 = Bz)
17 (R^1 = H, R^2 = X^3, R^3 = Bz)

[a]Reagents and conditions: (i) aniline, 4-chloroquinoline, EtOH, fuming HCl, reflux, 16 h. (ii)*:* aniline, 4-chloroquinoline, phenol, EGEE, 150 °C, 12 h. (iii): substituted bromobenzene, 2M K_2CO_3, bromobenzene, DMF, 50 °C, 12 h. (iv): substituted bromobenzene, iodoethane, DIPEA, DMF, rt, 48 h. (v): substituted bromobenzene, Pd(*t*-$Bu_3P)_2$, $Pd(dba)_2$, LiHMDS, toluene, 70 °C, 18 h, HCl.

Scheme 2 depicts the chemical synthesis of compounds **20**-**26** using methods (i) and (vi) to examine the contribution of the quinoline ring substituents to activity. Commercially available **18a** and **18b** were used in the preparation of compounds through **20**-**26** using previously described methods (i), and an alternative method (vi) where the appropriate 4-chloro containing quinoline was mixed with the aniline in the presence of phenol in DMF, and heated to 150 °C for 45 minutes in a microwave reactor.

Scheme 2: Synthesis of compounds that explored the contribution of substituents around benzenoid ring of quinoline.[b]



18a = (**R**^{3} = Et)
18b = (**R**^{3} = Me)

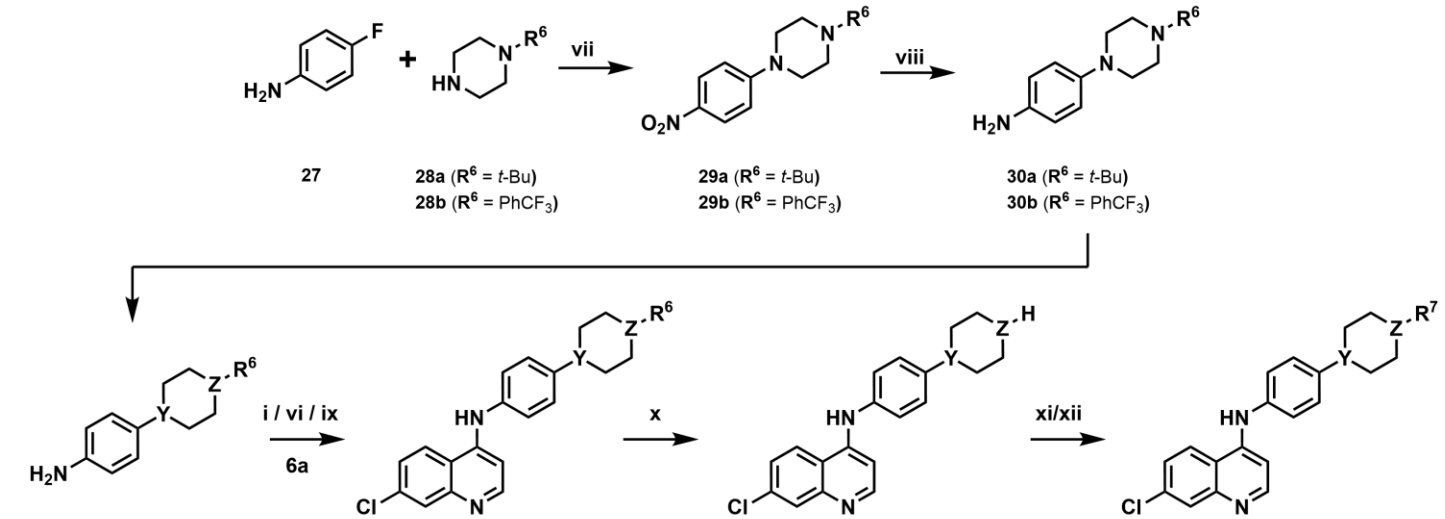
6a = (**R**^{4} = H, **R**^{5} = Cl)
19a = (**R**^{4} = Cl, **R**^{5} = H)
19b = (**R**^{4} = H, **R**^{5} = H)
19c = (**R**^{4} = Cl, **R**^{5} = Cl)
19d = (**R**^{4} = H, **R**^{5} = Br)
19e = (**R**^{4} = H, **R**^{5} = CF_3)

20 = (**R**^{3} = Et, **R**^{4} = H, **R**^{5} = Cl)
21 = (**R**^{3} = Me, **R**^{4} = H, **R**^{5} = Cl)
22 = (**R**^{3} = Et, **R**^{4} = H, **R**^{5} = H)
23 = (**R**^{3} = Et, **R**^{4} = Cl, **R**^{5} = H)
24 = (**R**^{3} = Et, **R**^{4} = Cl, **R**^{5} = Cl)
25 = (**R**^{3} = Et, **R**^{4} = H, **R**^{5} = Br)
26 = (**R**^{3} = Et, **R**^{4} = H, **R**^{5} = CF_3)

[b]Reagents and conditions: (i) aniline, 4-chloroquinoline, EtOH, fuming HCl, reflux, 16 h. (vi) aniline, 4-chloroquinoline, phenol, DMF, 150 °C, 2 h.

Target compounds **31**-**45** were prepared through the synthesis outlined in Scheme 3 to assess the contribution of the heterocycle to antimalarial activity and later the contribution of the tertiary amines alkyl tail to *in vivo* antimalarial efficacy. Compounds **31** and **32** were synthesized from commercially available alkyl piperazine (**28a** and **28b**) and **27** with trimethylamine in DMSO and heated to 120 °C for 16 hours (vii). Resulting intermediates were reduced to the appropriate aniline, and then used in previously described procedure (i) with **6a** to afford the final products. Commercially available **30c**-**30e** and **6a** were used in previously described procedures (vi) to produce compounds **33**-**35**. Compounds **36** and **37** were synthesized from **30f** and **30g** in the presence of **6a** in THF and heated to 120 °C for 20 minutes (ix). To afford compounds **40**-**45**, **36** and **37** were deprotected in the presence of trifluoroacetic acid in dichloromethane (x) and then used with the appropriate alkyl halide and either (xi) DIPEA in DMF at room temperature for 16 hours, or (xii) potassium carbonate in DMF heated to 85 °C for 16 hours.

Scheme 3: Synthesis of amodiachins that explored the contribution of the heterocycle and its alkyl substituents to activity.[c]



27

28a (**R**^{6} = *t*-Bu)
28b (**R**^{6} = $PhCF_3$)

29a (**R**^{6} = *t*-Bu)
29b (**R**^{6} = $PhCF_3$)

30a (**R**^{6} = *t*-Bu)
30b (**R**^{6} = $PhCF_3$)

30a (**Y** = N, **Z** = N, **R**^{6} = *t*-Bu)
30b (**Y** = N, **Z** = N, **R**^{6} = $PhCF_3$)
30c (**Y** = N, **Z** = N, **R**^{6} = cycPr)
30d (**Y** = N, **Z** = C, **R**^{6} = H)
30e (**Y** = C, **Z** = N, **R**^{6} = *i*-Pr)
30f (**Y** = N, **Z** = N, **R**^{6} = Boc)
30g (**Y** = C, **Z** = N, **R**^{6} = Boc)

31 (**Y** = N, **Z** = N, **R**^{6} = *t*-Bu)
32 (**Y** = N, **Z** = N, **R**^{6} = $PhCF_3$)
33 (**Y** = N, **Z** = N, **R**^{6} = *c*-Pr)
34 (**Y** = N, **Z** = C, **R**^{6} = H)
35 (**Y** = C, **Z** = N, **R**^{6} = *i*-Pr)
36 (**Y** = N, **Z** = N, **R**^{6} = Boc)
37 (**Y** = C, **Z** = N, **R**^{6} = Boc)

38 (**Y** = N, **Z** = N)
39 (**Y** = C, **Z** = N)

40 (**Y** = C, **Z** = N, **R**^{7} = Et)
41 (**Y** = C, **Z** = N, **R**^{7} = Pr)
42 (**Y** = C, **Z** = N, **R**^{7} = Bu)
43 (**Y** = C, **Z** = N, **R**^{7} = *i*-Bu)
44 (**Y** = C, **Z** = N, **R**^{7} = *s*-Bu)
45 (**Y** = N, **Z** = N, **R**^{7} = *i*-Bu)

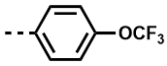
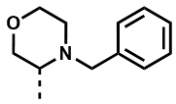
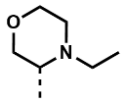
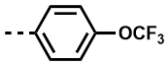
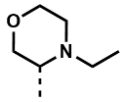
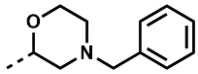
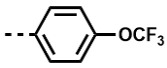
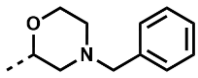
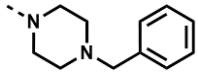
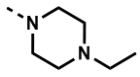
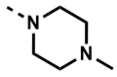
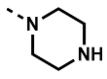
[c]Reagents and conditions: (vii): **27**, substituted piperazine, TEA, DMSO, 120 °C, 16 h. (viii) substituted nitrobenzene, Pd/C, MeOH, rt, 12 h. (i) aniline, 4-chloroquinoline, EtOH, fuming HCl, reflux, 16 h. (vi) aniline, 4-chloroquinoline,

phenol, DMF, 150 °C, 2 h. (vi) aniline, 4-chloroquinoline, THF, 120 °C, 20 m. (x): 4-analinoquinoline, TFA, DCM, 2 h. (xi): 4-analinoquinoline, iodoethane, DIPEA, DMF, rt, 16 h. (xii): 4-analinoquinoline, alkyl halide (1.2 equiv.), K_2CO_3, DMF, 85 °C, 16 h.

RESULTS AND DISCUSSION

Compounds were assessed for antiplasmodial activity against drug-sensitive (D6) and multidrug-resistant (Dd2) strains of *Plasmodium falciparum* in a fluorescence-based antiplasmodial assay.[16,17]

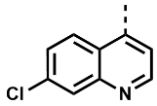
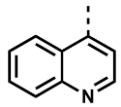
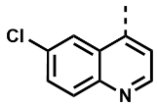
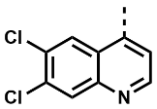
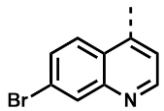
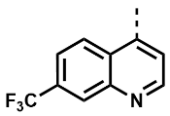
Table 1. Activity against *Pf* D6 and Dd2 and cytotoxicity of amodiachins.

Compound	R_1	R_2	IC_{50} v D6 (nM)	IC_{50} v Dd2 (nM)	CC_{50} v HepG2 (μM)	cLogP
7	--H		≥ 250	≥ 250	> 200.0	4.60
8			≥ 250	≥ 250	> 200.0	7.61
12	--H		100	≥ 250	> 200.0	6.59
13	--H		≥ 250	≥ 250	> 200.0	5.25
14			≥ 250	≥ 250	> 200.0	8.26
15	--H		107	≥ 250	38.0	6.45
16			≥ 250	≥ 250	> 200.0	9.46
17	--H		16.1	16.0	> 200.0	6.88
20	--H		16.1	16.0	> 200.0	5.69
21	--H		13.0	14.0	185.7	5.16
38	--H		21.8	65.8	53.5	4.58

Compounds **7, 8,** and **12**-**16** had limited activity (*Pf*D6 and *Pf*Dd2 IC_{50} > 250 nM – Table 1), and the only activity to note was **12** and **15** where we observed IC_{50} values of 100 nM and 107 nM, respectively vs. *Pf*D6. However this level of activity wasn't maintained against the multidrug resistant Dd2 strain of *P.*

falciparum (Table 1). Moving away from the morpholine heterocycle, a piperazine ring was next implemented as **17**. Promising activity against drug resistant *Pf*Dd2 IC_{50} = 16.1 nM and drug sensitive *Pf*Dd2 IC_{50} = 16.0 nM, was observed with this compound. Concerned that this molecule might be unstable to microsomal metabolism it was decided to replace the terminal aromatic ring with an ethyl (**20**) and methyl alkyl (**21**). The dependency of antiplasmodial activity was further investigated by varying the substituents at the 6 and 7 positions of the quinoline ring (Table 2). Interestingly, loss of the 7-position chlorine atom as in **22** reduces potency in combination with decreased susceptibility against drug resistant Dd2 strain. However, introducing the chlorine at the 6 position (**23**) rescues desensitization, but loss of potency is still maintained. It was not until the 7-position chlorine was reintroduced, as in **24**, that we observe the potency return to the low nanomolar (≤ 20 nM) range. Clearly, it was apparent that the 7 position chlorine was essential for the most potent activity, and therefore substitutions were varied at the critical 7-position to include the slightly larger halogen, bromine **(25)**, as well as trifluoromethyl **(26)**. Herein we can see that although activity against *Pf*D6 and *Pf*Dd2 is maintained with both derivatives, however **25** was more toxic to mammalian cells (CC_{50} = 47.5) than its progenitor **20**. Furthermore, the trifluoromethyl derivative, **26**, exhibited a slight decrease in antiplasmodial activity against the drug-sensitive *Pf*D6 strain.

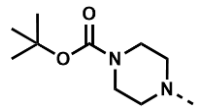
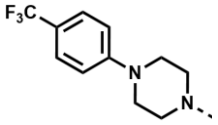
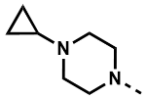
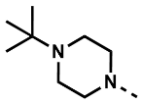
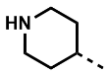
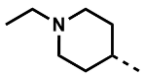
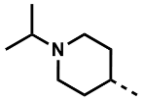
Table 2. Activity against *Pf*D6 and *Pf*Dd2 and cytotoxicity of amodiachins.

Compound	R_3	IC_{50} v D6 (nM)	IC_{50} v Dd2 (nM)	CC_{50} v HepG2 (µM)	cLogP
20		16.1	16.0	> 200.0	5.69
22		71.7	131	105.6	4.88
23		69.8	66.0	> 200.0	5.69
24		16.0	21.3	> 200.0	6.31
25		10.3	19.3	47.5	5.84
26		19.6	37.9	> 200.0	5.93

Having established that the 7-position chlorine atom was an essential element for antiplasmodial activity in this 4-aminoquinoline SAR campaign, we returned our focus to the 4-position sidechain. Indeed, from the results presented in Table 1 it would appear that the attachment of a piperazine ring to the aromatic phenyl ring greatly improves the antiparasitic activity, we therefore decided to explore structural variation of the N-terminal group. The corresponding N-Boc analog (**36**) exhibits diminished antiplasmodial activity which may related to the weakened basicity of the terminal nitrogen existing as a carbamate. This is consistent with the poor activity of **38** where the same nitrogen atom carries only a hydrogen atom. Use of a large aromatic trifluoromethylphenyl group (**38**) also diminished the activity relative to **20**. In hopes of enhancing basicity and metabolic stability at the outermost ring nitrogen we introduced cyclopropyl (**33**) and tertiary-butyl (**31**) groups. While the results for **33** showed relatively modest antiplasmodial activity, we found that the potency

of **31** was in the very low nanomolar range with sub-10nM IC_{50} values against both tested *P. falciparum* strains. Following these results, we explored the contribution of the individual piperazine nitrogen atoms on the observed antiplasmodial activity. This led to the design and synthesis of two novel piperidine derivatives with one positioning the nitrogen atom attached directly to the aromatic ring (**34**) while the other one places the nitrogen at the outermost position (**39**). The activity of the **34** was severely reduced in both strains of *Pf*, but if the piperadine's nitrogen is placed at the terminal position as a secondary amine (**39)**, activity in *Pf* D6 is maintained compared to **20**, but susceptibility of *Pf*Dd2 was decreased to 79.7 nM. A similar result observed in the similar compound **38** (Table 1). Next, we decided to explore the effect of converting the outer secondary amine of **39** to a tertiary amine by addition of an ethyl group. That strategy was proven to be effective, for when adorned with an ethyl substituent, compound **40** provided impressively low nM IC_{50}'s with a modest ~2-fold decrease in susceptibility against multidrug resistant *Pf*Dd2. It is noteworthy that **40** exhibits a modest level of cytotoxicity against mammalian cells in culture (CC_{50} = 111 µM). We therefore decided to quickly examine the impact of increasing length and branching of the terminal alkyl group as the addition of the ethyl substituent in **40**, appeared to decrease the cytotoxicity compared to **39**. Compound **35** achieved just that, while maintaining activity against both *Pf*D6 and *Pf*Dd2 strains. The terminal nitrogen leads to greater potency, but the addition of a terminal alkane rescues the compounds cytotoxicity while delivering the killing blow in the resistant strain of *P. falciparum*.

Table 2. Activity against *Pf* D6 and Dd2 and cytotoxicity of amodiachins.

Compound	R_4	IC_{50} v D6 (nM)	IC_{50} v Dd2 (nM)	CC_{50} v HepG2 (µM)	cLogP
36		70.3	153	> 200.0	6.57
32		44.4	123	> 200.0	7.77
33		39.2	84.4	> 200.0	5.53
31		5.55	9.20	> 200.0	6.39
34		145	150	> 200.0	5.98
39		10.2	79.7	58.0	5.34
40		4.56	13.2	111.4	6.32
35		4.94	14.5	> 200.0	6.63

It was at this point that select amodiachins were chosen based on potent antiplasmodial activity (D6 IC_{50} < 20 nM), limited decreased susceptibility in the drug resistant strain (D6 IC_{50}/ Dd2 IC_{50} < 2.5), and low cytotoxicity (CC_{50} > 100 µM), and where then used in the next phase of evaluating their potential.

Compounds were first evaluated for metabolic stability in pooled liver murine microsomes (Table 4), from which metabolic stability ($t_{1/2}$) were calculated. The difference of either 1 or 2 carbons in chain length (**21** and **20**) from the terminal nitrogen of piperazine did not enhance metabolic stability ($t_{1/2}$ = 1.44 and 1.86 min respectively). However, when capped with a benzyl moiety as in **17**, stability was noticeably increased ($t_{1/2}$ = 13.9 min), and interestingly when adorned with a heavily branched tert-butyl group found in **31**, no detectable amount of compound was metabolized over the course of the experiment. Evaluating piperidine containing compounds led to a similar observation; increasing the branching of the alpha carbon by a single carbon in **40**, let to a dramatic increase in stability ($t_{1/2}$ = 11.0 to 44.1 min) as seen in **35**. All compounds except **21**, were downselected for *in vivo* assessment of antiplasmodial activity in a modified Peter's 4-day murine malaria model against P. yoelii.[18] Each analog was tested at a fixed dose of 2.5 mg/kg/d and parasitemia on the final day was determined from direct microscopic analysis of blood smears. Percent parasite suppression (Table 4) was calculated from average parasitemia of experimental groups compared to average parasitemia of vehicle only control group. All compounds except **17** exhibited relatively modest suppression at this dose, and were selected as candidates for further evaluation to establish the effective dose to reduce parasitemia by 50% (ED_{50}) and 90% (ED_{90}) as well as attempting to determine a nonrecrudescence dose (NRD). Compounds **20** and **40** exhibited a relatively large gap between ED_{50} and ED_{90} suggesting rapid metabolism as previously indicated by the results of the microsomal stability assay, or possibly due to poor adsorption. The most effective antimalarial from this screen was **35,** with an ED_{50} and ED_{90} of 1.1 and 4.1 mg/kg/d. This finding coupled with high metabolic stability and excellent *in vitro* antiplasmodial activity, inspired the next round of SAR.

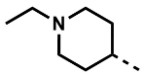
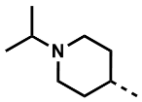
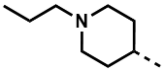
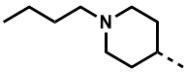
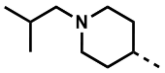
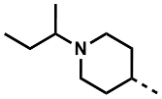
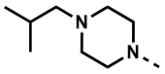
Table 4: Microsomal stability, parasite burden suppression, and *in vivo* efficacy of select amodiachins.

Compound	$t_{1/2}$ (min), CL_{int} (mL/min/kg)	% *Py* Suppression (2.5 mg/kg/d)	ED_{50} v *Py* (mg/kg/d)	ED_{90} v *Py* (mg/kg/d)	NRD (mg/kg/d)
17	13.92, 391.9	0%	-	-	-
20	1.86, 2930	60%	1.5	8.4	ND (10)
21	1.44, 3784	-	-	-	-
31	∞, 0.00	30%	4.0	7.6	ND (20)
40	11.0, 496.7	13%	1.8	13	ND (10)
35	44.1, 123.6	48%	1.1	4.1	ND (10)

Compound **35**'s superior *in vitro* activity against both strains of *Pf* and the striking *in vivo* efficacy against *Py*, compelled us to evaluate the *in vivo* contribution of the terminal alkyl that adorned the piperidine nitrogen. Structural isomers of 3 and 4 carbon alkyl chains were synthesized to explore this space.

Table 5: Activity against *Pf* D6 and Dd2 and cytotoxicity of amodiachins.

Compound	R_4	IC_{50} v D6 (nM)	IC_{50} v Dd2 (nM)	CC_{50} v HepG2 (µM)	cLogP

40		4.56	13.2	111.4	6.32
35		4.94	14.5	> 200.0	6.63
41		9.25	19.3	> 200.0	6.85
42		9.03	20.4	> 200.0	7.37
43		7.90	17.4	> 200.0	7.24
44		13.8	19.1	> 200.0	7.15
45		9.79	17.8	> 200.0	6.61

All compounds exhibited similar *in vitro* activities against D6 and Dd2 (Table 5). When examined for murine microsomal stability (Table 6), **41** demonstrated superior stability ($t_{1/2}$ = 70 minutes) and compounds **42**, **43**, and **44** displayed similar stability to **35**. These results allowed us to evaluate all these compounds for *Py* parasite suppression in the modified 4-Day Peter's test (Table 6). Surprisingly, **41** produced the lowest degree of parasitemia suppression among the tested analogs with 39% suppression compared to control, whereas **43** reduced parasitemia to 91% compared to control. Although, all other compounds in this set met the downselection thresholds, **43** was selected for full assessment of parasite suppression and elimination *in vivo* (Table 4) due to this powerful result. Compound **43** revealed impressive parasite suppression, albeit higher ED_{50} (2.1 mg/kg/d) than compounds **20**, **40**, and **35** (ED_{50} = 1.5, 1.8, and 1.1 mg/kg/d respectively), but a 2.5 mg/kg/d ED_{90} was the lowest from all previous ED_{90}s highlighting the high performance of **43** at a low dose. When **43** was given at 16 mg/kg for 4 days, the parasite burden was completely eliminated by Day 5, and recrudescence was prevented for the remainder of the experiment (Day 30). I should be noted that 3 out of 4 mice were cured at the end of the 30-day experiment when dosed at 8 mg/kg for 4 days.

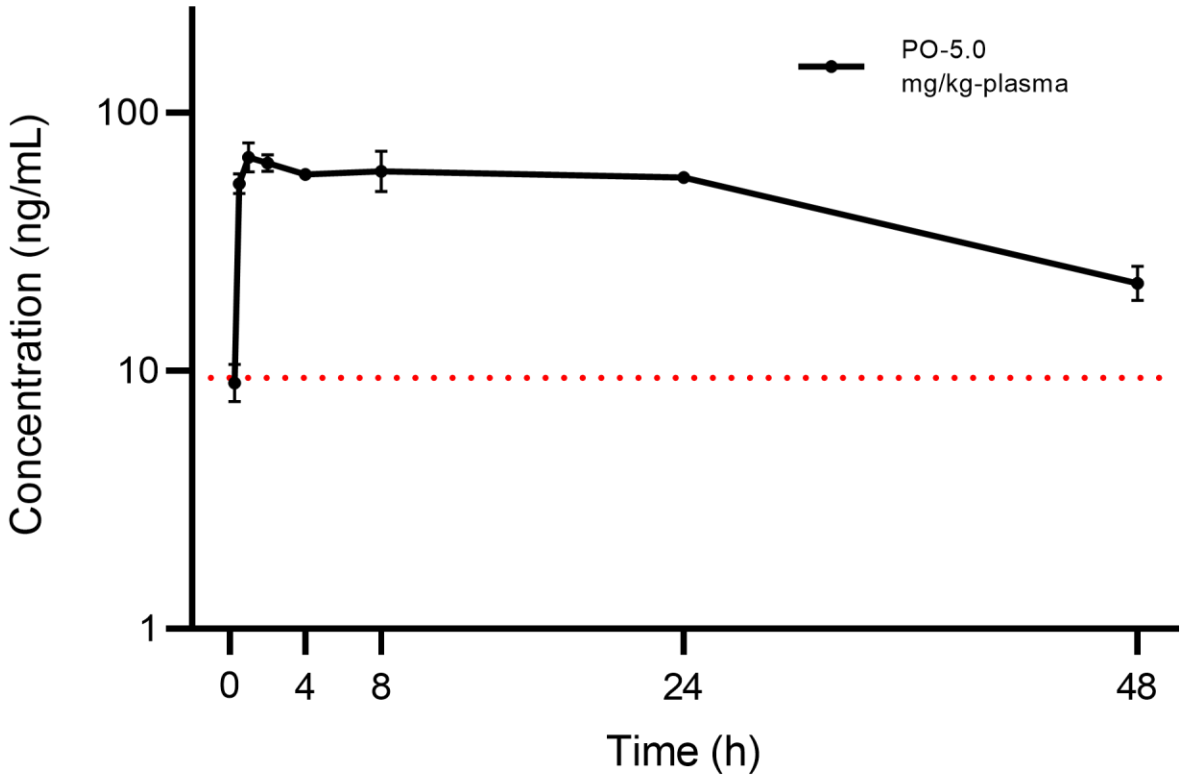
Table 6: Microsomal stability, parasite burden suppression, and *in vivo* efficacy of select amodiachins

Compound	$t_{1/2}$ (min), CL_{int} (mL/min/kg)	% *Py* Suppression (2.5 mg/kg/d)	ED_{50} v *Py* (mg/kg/d)	ED_{90} v *Py* (mg/kg/d)	NRD (mg/kg/d)
35	44.1, 123.6	48%	-	-	-
41	70.0, 78.0	39%	-	-	-
42	42.1, 129.7	53%	-	-	-

43	48.2, 113.3	91%	2.3	2.5	16
44	55.5, 98.3	71%	-	-	-
45	6.47, 842.9	30%	-	-	-

Having demonstrated that the iso-butyl group unlocks superior *in vivo* efficacy in the amodiachin series, we prepared a piperazine mimetic of **43** for the purpose of direct comparison (**44**). Although **44** exhibited similar antiplasmodial activity in cultured parasites (*Pf*D6 and *Pf*Dd2 IC_{50} = 9.79 and 17.8 nM), the compound displayed relatively poor metabolic stability ($t_{1/2}$ = 6.47 min) which was observed for the other piperazine containing amodiachin analogs. When evaluated for parasitemia suppression at 2.5 mg/kg in the modified Peters 4-day assay, parasite burden was only reduced to 30% compared to control. This result compares poorly to the 91% parasite burden suppression observed in piperidine analog **43**. Taken together, our data suggests that **43** has superior *in vivo* efficacy compared to all other compounds in this series.

Figure 2: Mean plasma concentration-time profile of 43 after PO doses at 5 mg/kg in male CD1 mice (n=3).[d]



[d]Red dotted line indicates the IC_{90} of **43** against D6 determined from the non-linear regression analysis of florescence data used to determine the IC_{50} of **43**.

To further explore the developmental potential of **43** we investigated its pharmacokinetic profile in mice following a single oral dose of 5.0 mg/kg in PEG-400 (Figure 2) for 48 hours. It is noteworthy that **43** appeared to be well adsorbed with maximum bloodstream concentrations reached very quickly (T_{max} = 1.0 h), achieving a maximum concentration of 67.7 ng/mL. Notably, exposure was maintained at relatively high

levels (AUC_{last} = 2362 hr•ng/mL) compared to maximum level of adsorption (C_{max} = 67.7 ng/mL). Over the 48 hour experiment, blood stream concentrations of **43** remained well above the lower limit of quantification (LLOQ = 1 ng/mL) with an AUC_{last} = 2362 hr•ng/mL. As that is the case, $T_{1/2}$ and AUC_{INF} were instead predicted from the experimental data, and therefore are likely larger than their predicted values, 25.1 h and 3156 hr•ng/mL respectively. Future PK experiments will be performed and monitored for 120 hours in tandem with a separate intravenously injected experiment to fully realize exposure, half-life, and bioavailability of **43**. These results taken together with the ability of **43** to overcome multi-drug resistance mechanisms present in the Dd2 strain of *Pf*, its relatively long metabolic stability in murine microsomes, and its ability to completely cure a *Py* infection – identify **43** as a potent compound with untapped potential as a novel antimalarial against multi-drug resistant *plasmodium falciparum* parasites.

SUMMARY AND CONCLUDING REMARKS

The ongoing struggle with malaria and the ever-changing landscape of antimalarial drug resistance compel our efforts to discover new therapeutics to fill gaps in current therapeutic options for treatment of acute and severe infections. Our interest in revisiting amodiaquine, relates to it rapid schizonticidal effect and the relatively unexplored chemical space surrounding its 4-aminophenyl side chain. For the present study we synthesized compounds that contain different heterocycles connected through the 4-aminophenyl side chain and found that addition of a piperazine ring in this position unlocked potent antiplasmodial activity. Further exploration into the quinoline ring, confirmed the superiority of a 7-position chlorine atom as it contributed to excellent low nanomolar activity against both drug sensitive and multidrug resistant strains of *P. falciparum* with limited cytotoxicity. Ultimately, the observation that piperazine containing amodiachins encouraged us to expand the structure-activity profiling to replace it with the structurally related piperidine. When assessed for metabolic stability and parasite suppression *in vivo*, the piperidine containing derivative **35** hinted at the potential of this new subseries. Structural isomers of **35** were prepared containing 3 and 4 linear and branched carbon alkyl chains. *In vitro* and *in vivo* assessment of these derivatives showcased **43** with impressive *in vitro* antiplasmodial IC_{50} values and superior efficacy *in vivo* compared to the other members of this series. *In vivo* efficacy of **43** was remarkable with a very sharp action curve against murine malaria as evidenced by ED_{50} and ED_{90} values of 2.3 and 2.5 mg/kg/day, respectively, following 4 days of oral dosing. A nonrecrudescence dose of 16 mg/kg/day for **43** over the same time interval was also a distinguishing attribute over other derivatives. Pharmacokinetic analysis of **43** revealed rapid oral absorption and extended pharmacokinetics. Taken together, these findings highlight the potential of amodiachins like **43** as a potential 4-aminoquinoline replacement drug.

Finally, the struggle to contain and eradicate malaria continues as the parasite seems to evolve counter moves to develop resistance to new antimalarial drugs seemingly as quickly as they are developed. This means that we need to redouble our efforts to develop novel antimalarials that can be used in combination with other drug to treat or prevent malaria infection. We feel that the work described herein will someday yield a new drug to help in this fight against one of the oldest diseases ever faced by the human race.

EXPERIMENTAL SECTION

Chemistry

Materials and Instruments. All solvents, starting materials, and reagents were acquired from commercial sources, including (but not limited to): Fisher Scientific, TCI Chemicals, Combi Blocks, Enamine, Sigma Aldrich. Reaction progress was monitored by TLC, GCMS, or HPLC when permitted. Both reverse phase and normal phase flash chromatography was performed using Biotage Isolera and columns including Sfär Silica, Sfär Silica HC, Sfär Silica-Duo, Sfär Silica-KP Amino, and Sfär C18-Duo. ^{1}H NMR spectra were taken on a Bruker 400 MHz instrument, and chemical shifts are reported relative to TMS (0.0 ppm) and NMR Solvent ($CDCl_3$, DMSO-d_6, or CD_3OD). Final compounds were measured to be >95% pure by high performance liquid chromatography (HPLC) using an Agilent Technologies 1260 Infinity II system (unless otherwise noted). High-resolution mass spectrometry (HRMS) using electrospray ionization was performed by the Portland State University BioAnalytical Mass Spectrometry Facility for additional structure verification.

General Procedure (i)**:** To a round bottom flask a mixture of aniline (1.1 equiv.) and 4-chloroquinoline (1 equiv.) was dissolved in ethanol (0.1 M) and catalytic fuming HCl (0.05 equiv.) added. The resulting mixture was refluxed for 16 h, where upon the resulting 4-analinoquinoline precipitates as an HCl salt. The reaction mixture was condensed *in vacuo* and the resulting 4-analinoquinoline was resuspended in methylene chloride and aqueous 2 M sodium hydroxide. The resulting organic layer was extracted with water, brine, dried with magnesium sulfate, concentrated in vacuo, and purified by flash chromatography.

General Procedure (ii)*:* To a carius tube a mixture of aniline (3 equiv.), 4-chloroquinoline (1 equiv.), and phenol (0.05 equiv.) was dissolved in 2-ethoxyethanol (0.1 M). The resulting mixture was heated to 150 °C for 12 hours. The reaction mixture was condensed *in vacuo* and was resuspended in methylene chloride and aqueous 2 M sodium hydroxide. The resulting organic layer was extracted with water, brine, dried with magnesium sulfate, concentrated in vacuo, and purified by flash chromatography.

General Procedure (iii): To a round bottom flask equipped with a stir bar para substituted bromobenzene (1 equiv.) was dissolved in DMF followed by the addition of 2M potassium carbonate (2 equiv.) and bromobenzene (1.1 equiv.). The flask was fixed with a septa and stirred at 50 °C for 12 h. Reaction mixture was diluted with water and extracted with ethyl acetate. Organic layers were combined and washed with water and brine, and dried with magnesium sulfate. Organic filtrate was condensed in vacuo and purified by flash chromatography.

General Procedure (iv): To a round bottom flask equipped with a stir bar para substituted bromobenzene (1 equiv.) was dissolved in DMF (0.5 M) followed by the addition of iodoethane (1.1 equiv.) and N,N-diisopropylethylamide. The flask was fixed with a septa and stirred at room temperature for 48 h. Reaction mixture was diluted with water and extracted with ethyl acetate. Organic layers were combined and washed with water and brine, and dried with magnesium sulfate. Organic filtrate was condensed in vacuo and purified by flash chromatography.

General Procedure (v): To a dry round bottom flask equipped with a stir bar under inert conditions toluene (0.4 M) was added and degassed with argon for 10 m. bis(tri-tert-butylphosphine)palladium(0) (2.5% molar equiv.) and bis(dibenzylideneacetone)palladium(0) (2.5% molar equiv.) was added to the flask and stirred for 5 m. The substituted aryl halide (1 equiv.) was added and the reaction vessel was capped with reflux condenser and septa and was placed under argon atmosphere. 1M lithium bis(trimethylsilyl)amide in toluene (1.5 equiv.) was added under inert conditions and the reaction was heated to 70 °C for 18 h. Upon consumption of substituted aryl halide, the crude reaction mixture was diluted in ethyl acetate and the newly formed silylamide was deprotected with 1 drop of 1 M hydrochloric acid. The mixture was washed with 2 M sodium hydroxide, water, and brine, and dried with magnesium sulfate. Dried organic layer was filter and condensed in vacuo, and purified by flash chromatography.

General Procedure (vi): To a microwave reactor flask a mixture of aniline (1 equiv.), 4-chloroquinoline (1.2 equiv.) and phenol (2.0 equiv.) were dissolved in DMF (1.0 M). The flask was sealed and heated to 150 °C for 2 h on high adsorption. Reaction mixture was condensed in vacuo and resuspended in methylene chloride and aqueous 2 M sodium hydroxide. Resulting organic layer was extracted with water, brine, dried with magnesium sulfate, concentrated in vacuo, and purified via flash chromatography to afford desired 4-analinoquinoline.

General Procedure (vii): To a round bottom flask equipped with magnetic stir bar was charged with 4-fluoronitrobenzene (1 equiv.), substituted piperazine (1.1 equiv.). Solids were dissolved in dimethyl sulfoxide (1.0 M) and trimethylamine (3.5 equiv.) was added the reaction vessel was heated at 120 °C for 16 hours. The resulting mixture was allowed to cool to room temperature and filtered over vacuum filtration using minimal amounts of DMSO to transfer. Resulting solid was recrystallized using ethanol and ethyl acetate, and filtered over vacuum filtration. Desired product was air dried.

General Procedure (viii): A mixture of piperazine substituted nitrobenzene (1.0 equiv.) and wet palladium on carbon (0.1 equiv.) were dissolved in methanol (0.025 M) in a shaker flask. Shaker flask sparged with hydrogen at 45 psi and shaken for 12 hours in Parr Hydrogenator. Pd/C filtered over celite with excess

methanol and resulting filtrate condensed in vacuo. Solid resuspended in ethyl acetate and basified with 2 M sodium hydroxide. Resulting organic layer was washed with water and brine, and dried with magnesium sulfate. The mixture was condensed in vacuo, and purified via flash chromatography to afford piperazine substituted aniline.

General Procedure (ix): To a microwave reactor flask a mixture of aniline (1 equiv.) and 4-chloroquinoline (1.2 equiv.) were dissolved in THF (1.0 M). The flask was sealed and heated to 120 °C for 20 min on high adsorption. Reaction mixture was condensed in vacuo and resuspended in methylene chloride and aqueous 2 M sodium hydroxide. Resulting organic layer was extracted with water, brine, dried with magnesium sulfate, concentrated in vacuo, and purified via flash chromatography to afford desired 4-analinoquinoline.

General Procedure (x): Boc-protected 4-analinoquinoline (1 equiv.) dissolved in methylene chloride (1.0 M) and trifluoroacetic acid added (20 equiv.), reaction stirred at room temperature for 2 hours. Reaction mixture diluted in methylene chloride and aqueous 2 M sodium hydroxide. Resulting precipitate filtered over vacuum filtration and washed with additional methylene chloride and water. Solid air dried to yield desired 4-analinoquinoline, no further purification needed.

General Procedure (xi): A solution of 4-analinoquinoline (1.0 equiv.), iodoethane (1.1 equiv.), and N,N-diisopropylethylamine (2.0 equiv.) in dimethylformamide (0.2 M) was stirred at room temperature for 16 hr. Upon completion, reaction mixture was poured over ice and resulting solid separated by vacuum filtration. Solid was dissolved in methylene chloride, dried with magnesium sulfate, condensed in vacuo, and purified using flash chromatography to afford alkylated 4-analinoquinoline.

General Procedure (xii): A stirred solution of 4-analinoquinoline (1.0 equiv.), alkyl halide (1.2 equiv.), and potassium carbonate (5.0 equiv.) in dimethylformamide (0.2 M) was heated to 85 °C for 16 hr. Upon completion, reaction mixture condensed in vacuo. Resulting solid dissolved in methylene chloride and filtered using vacuum filtration. Filtrate extracted with 2 M sodium hydroxide and brine, condensed in vacuo, dry loaded onto celite and purified via flash chromatography. Fractions containing desired product were condensed in vacuo, and resulting solid upon the addition of aqueous 2 M sodium hydroxide was filtered via vacuum filtration and dried in a vacuum oven to afford alkylated 4-analinoquinoline.

Synthetic Descriptions of Presented amodiachins.

7-chloro-N-(4-morpholinophenyl)quinolin-4-amine (**7**). Prepared using general procedure (i) with 7.22 mmol **5**, and 21.6 mmol **6a**. Yield 80% (2.452 g). ^{1}H-NMR (400 MHz; MeOD): δ 8.56 (dd, *J* = 9.1, 0.5 Hz, 1H), 8.35 (d, *J* = 7.1 Hz, 1H), 7.95 (dd, *J* = 2.1, 0.5 Hz, 1H), 7.80 (dd, *J* = 9.1, 2.1 Hz, 1H), 7.41-7.37 (m, 2H), 7.26-7.22 (m, 2H), 6.83 (d, *J* = 7.1 Hz, 1H), 3.92-3.90 (m, 4H), 3.32-3.29 (m, 4H). HRMS (ESI) - *m/z* of [$C_{19}H_{19}ClN_3O^+$]: 340.1216, actual: 340.1207.

7-chloro-N-(4-morpholinophenyl)-3-(4-(trifluoromethoxy)phenyl)quinolin-4-amine (**8**). Prepared using General Procedure (ii) with 3.00 mmol **5**, 1.02 mmol **6b**. Yield 38% (0.195 g). ^{1}H-NMR (400 MHz; $CDCl_3$): δ 8.71 (s, 1H), 8.08 (d, *J* = 2.1 Hz, 1H), 7.73 (dd, *J* = 9.1, 0.4 Hz, 1H), 7.49-7.45 (m, 2H), 7.32-7.27 (m, 3H), 6.81 (s, 4H), 6.02 (s, 1H), 3.89-3.86 (m, 4H), 3.13-3.10 (m, 4H). HRMS (ESI) - *m/z* of [$C_{26}H_{22}ClF_3N_3O_2^+$]: 500.1354, actual: 500.1347.

4-benzyl-3-(4-bromophenyl)morpholine (10a). Prepared using General Procedure (iii) with 9.89 mmol **9a**. Yield 85% (2.780 g). ^{1}H-NMR (400 MHz; DMSO-d_6): δ 7.53-7.50 (m, 2H), 7.35-7.26 (m, 7H), 4.50 (dd, *J* = 10.2, 2.3 Hz, 1H), 3.94-3.90 (m, 1H), 3.66 (td, *J* = 11.4, 2.4 Hz, 1H), 3.55-3.47 (m, 2H), 2.85-2.82 (m, 1H), 2.70-2.67 (m, 1H), 2.15 (td, *J* = 11.5, 3.3 Hz, 1H), 1.94 (dd, *J* = 11.3, 10.3 Hz, 1H).

3-(4-bromophenyl)-4-ethylmorpholine (10b). Prepared using General Procedure (iv) with 20.6 mmol **9a**. Yield 60% (4.123 g). ^{1}H-NMR (400 MHz; $CDCl_3$): δ 7.47 (d, *J* = 8.6 Hz, 2H), 7.27 (d, *J* = 8.1 Hz, 2H), 3.98-3.94 (m, 1H), 3.80-3.74 (m, 1H), 3.69 (ddd, *J* = 10.7, 2.8, 1.1 Hz, 1H), 3.38-3.28 (m, 2H), 3.00 (d, *J* = 11.8 Hz, 1H), 2.60-2.51 (m, 1H), 2.37 (td, *J* = 11.7, 3.4 Hz, 1H), 2.03 (dq, *J* = 13.0, 6.6 Hz, 1H), 0.97 (t, *J* = 7.2 Hz, 3H).

4-benzyl-2-(4-bromophenyl)morpholine (10c). Prepared using General Procedure (iii) with 3.99 mmol **9b**. Yield 70% (2.809 g). ^{1}H-NMR (400 MHz; DMSO-d_6): δ 7.59 (d, J = 8.6 Hz, 2H), 7.48 (d, J = 8.3 Hz, 2H), 7.33-7.21 (m, 5H), 3.78 (dt, J = 11.3, 1.5 Hz, 1H), 3.68-3.64 (m, 1H), 3.60 (d, J = 13.4 Hz, 1H), 3.53 (td, J = 11.5, 2.3 Hz, 1H), 3.40 (dd, J = 10.1, 3.3 Hz, 1H), 3.27 (d, J = 11.0 Hz, 1H), 2.93 (d, J = 13.4 Hz, 1H), 2.64 (d, J = 11.8 Hz, 1H), 2.20 (td, J = 11.8, 3.3 Hz, 1H).

1-benzyl-4-(4-bromophenyl)piperazine (10d). Prepared using General Procedure (iii) with 4.00 mmol **9c**. Yield 35% (0.461 g). ^{1}H-NMR (400 MHz; DMSO-d_6): δ 7.35-7.31 (m, 6H), 7.29-7.25 (m, 1H), 6.90-6.86 (m, 2H), 3.52 (s, 2H), 3.14-3.11 (m, 4H), 2.51-2.48 (m, 4H).

4-(4-benzylmorpholin-3-yl)aniline (11a). Prepared using General Procedure (v) with 7.75 mmol **10a**. Yield 81% (1.638 g) ^{1}H-NMR (400 MHz; DMSO-d_6): δ 7.33-7.30 (m, 4H), 7.27-7.23 (m, 1H), 6.95 (d, *J* = 8.3 Hz, 2H), 6.48 (d, *J* = 8.5 Hz, 2H), 4.99 (s, 2H), 4.26 (dd, *J* = 10.2, 2.1 Hz, 1H), 3.87-3.84 (m, 1H), 3.61 (td, *J* = 11.4, 2.3 Hz, 1H), 3.49 (s, 2H), 2.70-2.65 (m, 2H), 2.12 (td, *J* = 11.5, 3.3 Hz, 1H), 1.96 (dd, *J* = 11.2, 10.5 Hz, 1H).

4-(4-ethylmorpholin-3-yl)aniline (11b). Prepared using General Procedure (v) with 9.30 mmol **10b**. Yield 76% (1.467 g) ^{1}H-NMR (400 MHz; DMSO-d_6): δ 6.97 (d, *J* = 8.3 Hz, 2H), 6.51 (d, *J* = 8.5 Hz, 2H), 4.97 (s, 2H), 3.82 (dt, *J* = 11.1, 1.6 Hz, 1H), 3.56 (td, *J* = 11.4, 2.3 Hz, 1H), 3.50-3.46 (m, 1H), 3.17 (t, *J* = 10.6 Hz, 1H), 3.02 (dd, *J* = 10.2, 3.4 Hz, 1H), 2.91-2.87 (m, 1H), 2.49-2.45 (m, 1H), 2.17 (td, *J* = 11.7, 3.4 Hz, 1H), 1.90 (dq, *J* = 12.9, 6.6 Hz, 1H), 0.86 (t, *J* = 7.2 Hz, 3H).

4-(4-benzylmorpholin-2-yl)aniline (11c). Prepared using General Procedure (v) with 2.72 mmol **10c**. Yield 86% (0.625 g) ^{1}H-NMR (400 MHz; $CDCl_3$): δ 8.51 (d, *J* = 5.3 Hz, 1H), 8.03 (d, *J* = 2.1 Hz, 1H), 7.85 (d, *J* = 8.9 Hz, 1H), 7.46 (dd, *J* = 8.9, 2.2 Hz, 1H), 7.23-7.20 (m, 2H), 7.03-6.99 (m, 2H), 6.72 (d, *J* = 5.3 Hz, 1H), 6.52 (s, 1H), 3.25 (t, *J* = 5.1 Hz, 4H), 2.60 (t, *J* = 5.0 Hz, 4H), 2.18 (d, *J* = 7.4 Hz, 2H), 1.91-1.81 (m, 1H), 0.96 (d, *J* = 6.6 Hz, 6H).

4-(4-benzylpiperazin-1-yl)aniline (11d). Prepared using General Procedure (v) with 1.50 mmol **10d**. Yield 69% (0.278 g). ^{1}H-NMR (400 MHz; MeOD): δ 7.40-7.33 (m, 4H), 7.30 (dd, J = 5.4, 3.5 Hz, 1H), 6.89-6.79 (m, 4H), 3.61 (s, 2H), 3.15 (t, J = 5.0 Hz, 4H), 2.66 (t, J = 5.0 Hz, 4H).

N-(4-(4-benzylmorpholin-3-yl)phenyl)-7-chloroquinolin-4-amine (12). Prepared using General Procedure (ii) with 0.930 **11a** and 0.606 mmol **6a**. Yield 24% (0.062 g). ^{1}H-NMR (400 MHz; $CDCl_3$): δ 8.59 (d, *J* = 5.3 Hz, 1H), 8.06 (d, *J* = 2.1 Hz, 1H), 7.87 (d, *J* = 9.0 Hz, 1H), 7.57 (d, *J* = 7.9 Hz, 2H), 7.49 (d, *J* = 2.2 Hz, 1H), 7.34-7.32 (m, 5H), 7.31-7.30 (m, 1H), 7.28-7.26 (m, 1H), 7.00 (d, *J* = 5.3 Hz, 1H), 6.63 (d, *J* = 0.2 Hz, 1H), 3.91-3.87 (m, 2H), 3.83 (d, *J* = 8.6 Hz, 1H), 3.77-3.71 (m, 1H), 3.49 (q, *J* = 9.6 Hz, 2H), 2.97 (d, *J* = 13.4 Hz, 1H), 2.84-2.80 (m, 1H), 2.33 (td, *J* = 11.8, 3.4 Hz, 1H). HRMS (ESI) - *m/z* of [$C_{26}H_{25}ClN_3O^+$]: 430.1687, actual: 430.1679.

7-chloro-N-(4-(4-ethylmorpholin-3-yl)phenyl)quinolin-4-amine (13). Prepared using General Procedure (ii) with 0.507 mmol **11b** and 1.52 mmol **6a**. Yield 18% (0.062 g). ^{1}H-NMR (400 MHz; $CDCl_3$): δ 8.60 (d, *J* = 5.3 Hz, 1H), 8.06 (d, *J* = 2.1 Hz, 1H), 7.87 (d, *J* = 9.0 Hz, 1H), 7.49 (dd, *J* = 9.0, 2.2 Hz, 1H), 7.44 (d, *J* = 8.1 Hz, 2H), 7.28 (d, *J* = 8.5 Hz, 6H), 7.01 (d, *J* = 5.3 Hz, 1H), 6.60 (d, *J* = 0.3 Hz, 1H), 4.01-3.97 (m, 1H), 3.83-3.74 (m, 2H), 3.43 (t, *J* = 10.6 Hz, 1H), 3.37-3.33 (m, 1H), 3.04-3.01 (m, 1H), 2.69-2.60 (m, 1H), 2.40 (td, *J* = 11.7, 3.4 Hz, 1H), 2.13-2.05 (m, 1H), 1.01 (t, *J* = 7.2 Hz, 3H). HRMS (ESI) - *m/z* of [$C_{21}H_{23}ClN_3O^+$]: 368.1529, actual: 368.1522.

7-chloro-N-(4-(4-ethylmorpholin-3-yl)phenyl)-3-(4-(trifluoromethoxy)phenyl)quinolin-4-amine (14). Prepared using General Procedure (ii) with 0.946 **11b** and 0.605 mmol **6b**. Yield 18% (0.057 g). ^{1}H-NMR (400 MHz; $CDCl_3$): δ 8.77 (s, 1H), 8.13-8.12 (m, 1H), 7.80-7.77 (m, 1H), 7.45 (d, *J* = 8.6 Hz, 2H), 7.39-7.36 (m, 1H), 7.30-7.28 (m, 2H), 7.18-7.16 (m, 2H), 6.74-6.72 (m, 2H), 6.11 (d, *J* = 0.2 Hz, 1H), 3.97-3.93 (m, 1H), 3.79-3.72 (m, 1H), 3.69-3.66 (m, 1H), 3.38-3.32 (m, 1H), 3.24-3.20 (m, 1H), 3.00-2.96 (m, 1H), 2.59-2.53 (m, 1H), 2.38-2.31 (m, 1H), 2.04-1.98 (m, 1H), 0.98 (t, *J* = 7.1 Hz, 3H). HRMS (ESI) - *m/z* of [$C_{28}H_{26}ClF_3N_3O_2^+$]: 528.1667, actual: 528.1662.

N-(4-(4-benzylmorpholin-2-yl)phenyl)-7-chloroquinolin-4-amine (15). Prepared using General Procedure (ii) with 0.904 **11c** and 0.602 mmol **6a**. Yield 21% (0.054 g). ^{1}H-NMR (400 MHz; $CDCl_3$): δ 8.05 (d, *J* = 2.1 Hz, 1H), 7.87 (d, *J* = 9.0 Hz, 1H), 7.47 (dd, *J* = 8.9, 2.2 Hz, 1H), 7.43 (d, *J* = 8.3 Hz, 2H), 7.39-7.34 (m, 4H), 7.31 (dt, *J* = 4.7, 2.2 Hz, 1H), 7.28-7.26 (m, 2H), 6.95 (d, *J* = 5.3 Hz, 1H), 6.61 (s, 1H), 4.62 (dd, *J* = 10.2, 2.3 Hz, 1H), 4.06 (ddd, *J* = 11.3, 3.3, 1.4 Hz, 1H), 3.89 (td, *J* = 11.4, 2.5 Hz, 1H), 3.59 (dd, *J* = 18.6, 8.0 Hz, 2H), 2.97 (dt, *J* = 11.5, 2.0 Hz, 1H), 2.82-2.79 (m, 1H), 2.34 (td, *J* = 11.5, 3.4 Hz, 1H), 2.20-2.15 (m, 1H). HRMS (ESI) - *m/z* of [$C_{26}H_{25}ClN_3O^+$]: 430.1687, actual: 430.1682.

N-(4-(4-benzylmorpholin-2-yl)phenyl)-7-chloro-3-(4-(trifluoromethoxy)phenyl)quinolin-4-amine (16). Prepared using General Procedure (ii) with 0.933 mmol **11c** and 0.602 mmol **6b**.Yield 33% (0.117 g). ^{1}H-NMR (400 MHz; DMSO-d_6): δ 8.76 (s, 1H), 8.71 (s, 1H), 8.24-8.22 (m, 1H), 8.05 (d, *J* = 2.2 Hz, 1H), 7.61 (dd, *J* = 9.1, 2.2 Hz, 1H), 7.47-7.43 (m, 2H), 7.33-7.26 (m, 5H), 7.22-7.20 (m, 2H), 6.92 (d, *J* = 8.5 Hz, 2H), 6.58 (d, *J* = 8.6 Hz, 2H), 4.27 (dd, *J* = 10.1, 1.9 Hz, 1H), 3.87-3.83 (m, 1H), 3.59 (td, *J* = 11.3, 2.2 Hz, 1H), 3.47 (s, 2H), 2.65 (d, *J* = 11.6 Hz, 2H), 2.13-2.06 (m, 1H), 1.84 (dd, *J* = 11.4, 10.4 Hz, 1H). HRMS (ESI) - *m/z* of [$C_{33}H_{28}ClF_3N_3O_2^+$]: 590.1825, actual: 590.1816.

N-(4-(4-benzylpiperazin-1-yl)phenyl)-7-chloroquinolin-4-amine (17). Prepared using General Procedure (ii) with 0.904 mmol **11d** and 0.642 mmol **6a**. Yield 46% (0.125 g). ^{1}H-NMR (400 MHz; DMSO-d_6): δ 8.19 (d, J = 5.5 Hz, 1H), 7.71 (d, J = 2.2 Hz, 1H), 7.38 (dd, J = 9.0, 2.2 Hz, 1H), 7.35 (t, J = 4.3 Hz, 4H), 7.35 (d, J = 4.3 Hz, 4H), 7.30-7.25 (m, 1H), 7.10 (d, J = 8.9 Hz, 2H), 6.96 (d, J = 9.0 Hz, 2H), 6.48 (d, J = 5.5 Hz, 1H), 3.54 (s, 2H), 3.13 (t, J = 4.9 Hz, 4H), 2.54 (t, J = 4.9 Hz, 4H).

7-chloro-N-(4-(4-ethylpiperazin-1-yl)phenyl)quinolin-4-amine (20). Prepared using General Procedure (i) with 1.50 mmol **18a** and 1.68 mmol **6a**. Yield 42% (0.287 g). 1H-NMR (400 MHz; CDCl3): δ 8.48 (d, J = 5.3 Hz, 1H), 8.00 (d, J = 2.1 Hz, 1H), 7.83 (d, J = 8.9 Hz, 1H), 7.43 (dd, J = 8.9, 2.2 Hz, 1H), 7.22-7.18 (m, 2H), 7.01-6.97 (m, 2H), 6.70 (d, J = 5.3 Hz, 1H), 6.50 (s, 1H), 3.26 (t, J = 5.1 Hz, 4H), 2.64 (t, J = 5.0 Hz, 4H), 2.50 (q, J = 7.2 Hz, 2H), 1.15 (t, J = 7.2 Hz, 3H). HRMS (ESI) - *m/z* of [$C_{21}H_{24}ClN_4^+$]: 367.1689, actual: 367.1687.

7-chloro-N-(4-(4-methylpiperazin-1-yl)phenyl)quinolin-4-amine (21). Prepared using General Procedure (i) with 1.50 mmol **18b** and 1.65 mmol **6a**. Yield 57% (0.300 g) ^{1}H-NMR (400 MHz; $CDCl_3$): δ 8.50 (d, J = 5.3 Hz, 1H), 8.03 (d, J = 2.1 Hz, 1H), 7.86 (d, J = 9.0 Hz, 1H), 7.45 (dd, J = 8.9, 2.2 Hz, 1H), 7.24-7.20 (m, 2H), 7.03-6.99 (m, 2H), 6.72 (d, J = 5.3 Hz, 1H), 6.55 (s, 1H), 3.27 (t, J = 5.0 Hz, 4H), 2.63 (t, J = 5.0 Hz, 4H), 2.40 (s, 3H). HRMS (ESI) - *m/z* of [$C_{20}H_{22}ClN_4^+$]: 353.1533, actual: 353.1526.

6**-chloro-N-(4-(4-ethylpiperazin-1-yl)phenyl)quinolin-4-amine (22).** Prepared using General Procedure (vi) with 1.01 mmol **18a** and 1.21 mmol **19a**. Yield 62% (0.232 g). ^{1}H-NMR (400 MHz; $CDCl_3$): δ 8.51 (dd, J = 5.3, 0.4 Hz, 1H), 7.98 (d, J = 9.0 Hz, 1H), 7.91 (d, J = 2.2 Hz, 1H), 7.63 (dd, J = 9.0, 2.2 Hz, 1H), 7.24-7.20 (m, 2H), 7.04-7.00 (m, 2H), 6.75 (d, J = 5.3 Hz, 1H), 6.43 (d, J = 0.2 Hz, 1H), 3.28 (t, J = 5.1 Hz, 4H), 2.67 (t, J = 5.1 Hz, 4H), 2.52 (q, J = 7.2 Hz, 2H), 1.17 (t, J = 7.2 Hz, 3H). HRMS (ESI) - *m/z* of [$C_{21}H_{25}N_4^+$]: 333.2079, actual: 333.2074.

N-(4-(4-ethylpiperazin-1-yl)phenyl)quinolin-4-amine (23). Prepared using General Procedure (i) with 1.01 mmol **18a** and 1.21 mmol **19b**. Yield 11% (0.036 g). ^{1}H-NMR (400 MHz; $CDCl_3$): δ 8.51 (dd, *J* = 5.3, 0.4 Hz, 1H), 7.98 (d, *J* = 9.0 Hz, 1H), 7.91 (d, *J* = 2.2 Hz, 1H), 7.63 (dd, *J* = 9.0, 2.2 Hz, 1H), 7.24-7.20 (m, 2H), 7.04-7.00 (m, 2H), 6.75 (d, *J* = 5.3 Hz, 1H), 6.43 (d, *J* = 0.2 Hz, 1H), 3.28 (t, *J* = 5.1 Hz, 4H), 2.67 (t, *J* = 5.1 Hz, 4H), 2.52 (q, *J* = 7.2 Hz, 2H), 1.17 (t, *J* = 7.2 Hz, 3H). HRMS (ESI) - *m/z* of [$C_{21}H_{24}ClN_4^+$]: 367.1689, actual: 367.1686.

6,7-dichloro-N-(4-(4-ethylpiperazin-1-yl)phenyl)quinolin-4-amine (24). Prepared using General Procedure (i) with 1.01 mmol **18a** and 1.02 mmol **19c**. Yield 6.9% (0.028 g). ^{1}H-NMR (400 MHz; DMSO-d_6): δ 8.99 (s, 1H), 8.75 (s, 1H), 8.40 (d, *J* = 5.3 Hz, 1H), 8.07 (s, 1H), 7.20 (d, *J* = 9.0 Hz, 2H), 7.03 (d, *J* = 9.1 Hz, 2H), 6.66 (d, *J* = 5.4 Hz, 1H), 3.18-3.15 (m, 4H), 2.55-2.53 (m, 4H), 2.42-2.33 (m, 2H), 1.05 (t, *J* = 7.2 Hz, 3H).

7-bromo-N-(4-(4-ethylpiperazin-1-yl)phenyl)quinolin-4-amine (25). Prepared using General Procedure (vi) with 1.00 mmol **18a** and 1.30 mmol **19d**. Yield 22% (0.093 g). ^{1}H-NMR (400 MHz; $CDCl_3$): δ 8.59 (d, *J* = 5.3 Hz, 1H), 8.34 (s, 1H), 8.04 (d, *J* = 8.9 Hz, 1H), 7.68 (dd, *J* = 8.8, 1.7 Hz, 1H), 7.26-7.22 (m, 2H), 7.05-7.01 (m, 2H), 6.81 (d, *J* = 5.3 Hz, 1H), 6.59 (s, 1H), 3.29 (t, *J* = 5.1 Hz, 4H), 2.67 (t, *J* = 5.0 Hz, 4H), 2.53 (q, *J* = 7.2 Hz, 2H), 1.18 (t, *J* = 7.2 Hz, 3H). HRMS (ESI) - *m/z* of [$C_{21}H_{24}BrN_4^+$]: 413.1160, actual: 413.1160.

N-(4-(4-ethylpiperazin-1-yl)phenyl)-7-(trifluoromethyl)quinolin-4-amine (26). Prepared using General Procedure (vi) with 0.63 mmol **18a** and 0.64 mmol **19e**. Yield 46% (0.141 g). ^{1}H-NMR (400 MHz; $CDCl_3$): δ 8.59 (d, *J* = 5.3 Hz, 1H), 8.34 (s, 1H), 8.04 (d, *J* = 8.9 Hz, 1H), 7.68 (dd, *J* = 8.8, 1.7 Hz, 1H), 7.26-7.22 (m, 2H), 7.05-7.01 (m, 2H), 6.81 (d, *J* = 5.3 Hz, 1H), 6.59 (s, 1H), 3.29 (t, *J* = 5.1 Hz, 4H), 2.67 (t, *J* = 5.0 Hz, 4H), 2.53 (q, *J* = 7.2 Hz, 2H), 1.18 (t, *J* = 7.2 Hz, 3H). HRMS (ESI) - *m/z* of [$C_{22}H_{24}F_3N_4^+$]: 401.1953, actual: 401.1948.

1-(tert-butyl)-4-(4-nitrophenyl)piperazine (29a). Prepared using General Procedure (vii) with 5.5 mmol **27** and 6.0 mmol **28a**. Yield 53% (0.773 g). ^{1}H-NMR (400 MHz; $CDCl_3$): δ 8.14-8.10 (m, 2H), 6.83-6.79 (m, 2H), 3.42-3.40 (m, 4H), 2.73-2.70 (m, 4H), 1.11 (s, 9H).

1-(4-nitrophenyl)-4-(4-(trifluoromethyl)phenyl)piperazine (29b). Prepared using General Procedure (vii) with 18.0 mmol **27** and 19.8 mmol **28b**. Yield 20% (1.254 g). ^{1}H-NMR (400 MHz; CDCl3): δ 8.21-8.17 (m, 2H), 7.55 (d, J = 8.6 Hz, 2H), 6.97 (d, J = 8.7 Hz, 2H), 6.91-6.87 (m, 2H), 3.64 (dd, J = 6.5, 4.1 Hz, 4H), 3.51 (dd, J = 6.5, 4.1 Hz, 4H).

4-(4-(tert-butyl)piperazin-1-yl)aniline (30a). Prepared using General Procedure (viii) with 2.62 mmol **29a.** Yield 56%. ^{1}H-NMR (400 MHz; $CDCl_3$): δ 6.82 (d, J = 8.8 Hz, 2H), 6.65 (d, J = 8.8 Hz, 2H), 3.41 (s, 1H), 3.06 (t, J = 4.9 Hz, 4H), 2.74 (t, J = 4.9 Hz, 4H), 1.11 (s, 9H).

4-(4-(4-(trifluoromethyl)phenyl)piperazin-1-yl)aniline (30b). Prepared using General Procedure (viii) with 3.57 mmol **29b**. Yield 53% (0.773 g). ^{1}H-NMR (400 MHz; $CDCl_3$): δ 7.54-7.51 (m, 2H), 7.02-6.98 (m, 2H), 6.90-6.87 (m, 2H), 6.72-6.69 (m, 2H), 3.50 (s, 1H), 3.46-3.43 (m, 4H), 3.22-3.19 (m, 4H).

N-(4-(4-(tert-butyl)piperazin-1-yl)phenyl)-7-chloroquinolin-4-amine (31). Prepared using General Procedure (vi) with 0.88 mmol **30a** and 1.01 mmol **6a**. Yield 18% (0.072 g). ^{1}H-NMR (400 MHz; $CDCl_3$): δ 8.51 (d, J = 5.3 Hz, 1H), 8.03 (d, J = 2.1 Hz, 1H), 7.85 (d, J = 9.0 Hz, 1H), 7.46 (dd, J = 8.9, 2.2 Hz, 1H), 7.23-7.20 (m, 2H), 7.03-6.99 (m, 2H), 6.72 (d, J = 5.3 Hz, 1H), 6.52 (d, J = 0.2 Hz, 1H), 3.26 (t, J = 4.9 Hz, 4H), 2.80 (t, J = 4.9 Hz, 4H), 1.16 (s, 9H). HRMS (ESI) - *m/z* of [$^+C_{23}H_{28}ClN_4$]: 395.2003, actual: 395.2000.

7-chloro-N-(4-(4-(4-(trifluoromethyl)phenyl)piperazin-1-yl)phenyl)quinolin-4-amine (32). Prepared using General Procedure (vi) with 0.63 mmol **30b** and 0.64 mmol **6a**. Yield 46% (0.141 g). ^{1}H-NMR (400 MHz; $CDCl_3$): δ 8.52 (d, J = 5.3 Hz, 1H), 8.04 (d, J = 2.1 Hz, 1H), 7.87 (d, J = 9.0 Hz, 1H), 7.55 (d, J = 8.6 Hz, 2H), 7.47 (dd, J = 8.9, 2.2 Hz, 1H), 7.25 (s, 2H), 7.08-7.04 (m, 2H), 7.02 (d, J = 8.7 Hz, 2H), 6.75 (d, J = 5.3 Hz, 1H), 6.56 (s, 1H), 3.51-3.48 (m, 4H). HRMS (ESI) - *m/z* of [$C_{26}H_{23}ClF_3N_4^+$]: 483.1564, actual: 483.1562.

7-chloro-N-(4-(4-cyclopropylpiperazin-1-yl)phenyl)quinolin-4-amine (33). Prepared using General Procedure (i) with 1.50 mmol **30c** and 0.78 mmol **6a**. Yield 6% (0.019 g). ^{1}H-NMR (400 MHz; DMSO-d_6): δ 8.91 (s, 1H), 8.41 (d, J = 9.1 Hz, 1H), 8.37 (d, J = 5.4 Hz, 1H), 7.85 (d, J = 2.2 Hz, 1H), 7.53 (dd, J = 9.0, 2.3 Hz, 1H), 7.22-7.18 (m, 2H), 7.03-6.99 (m, 2H), 6.63 (d, J = 5.4 Hz, 1H), 3.11 (t, J = 5.0 Hz, 4H), 2.69 (t, J = 5.0 Hz, 4H), 1.67 (tt, J = 6.7, 3.4 Hz, 1H), 0.48-0.43 (m, 2H), 0.37-0.34 (m, 2H). HRMS (ESI) - *m/z* of [$C_{22}H_{24}ClN_4^+$]: 379.1690, actual: 379.1685.

7-chloro-N-(4-(piperidin-1-yl)phenyl)quinolin-4-amine (34). Prepared using General Procedure (i) with 1.00 mmol **30d** and 1.14 mmol **6a**. Yield 15% (0.052 g). ^{1}H-NMR (400 MHz; DMSO-d_6): δ 8.91 (s, 1H), 8.42 (d, *J* = 9.0 Hz, 1H), 8.37 (d, *J* = 5.4 Hz, 1H), 7.85 (d, *J* = 2.2 Hz, 1H), 7.53 (dd, *J* = 9.0, 2.3 Hz, 1H),

7.19 (d, *J* = 9.0 Hz, 2H), 7.01 (d, *J* = 9.0 Hz, 2H), 6.62 (d, *J* = 5.4 Hz, 1H), 3.15 (t, *J* = 5.4 Hz, 4H), 1.67-1.62 (m, 4H), 1.58-1.53 (m, 2H). HRMS (ESI) - *m/z* of [$C_{20}H_{21}ClN_3^+$]: 338.1424, actual: 338.1419.

7-chloro-N-(4-(1-isopropylpiperidin-4-yl)phenyl)quinolin-4-amine (35). Prepared using General Procedure (i) with 1.10 mmol **30e** and 1.0 mmol **6a**. Yield 29% (0.111 g). ^{1}H-NMR (400 MHz; CD_3OD): δ 8.37 (d, J = 5.6 Hz, 1H), 8.31 (dd, J = 9.0, 0.4 Hz, 1H), 7.87 (d, J = 1.9 Hz, 1H), 7.51 (dd, J = 9.0, 2.2 Hz, 1H), 7.37-7.32 (m, 4H), 6.88 (d, J = 5.6 Hz, 1H), 3.12 (d, J = 11.6 Hz, 2H), 2.90-2.84 (m, 1H), 2.64 (tt, J = 12.0, 3.9 Hz, 1H), 2.48-2.42 (m, 2H), 1.95 (dd, J = 12.8, 1.7 Hz, 2H), 1.89-1.79 (m, 2H), 1.17 (d, J = 6.6 Hz, 6H). HRMS (ESI) - *m/z* of [$C_{23}H_{27}ClN_3^+$]: 380.1894, actual: 380.1890.

tert-butyl 4-(4-((7-chloroquinolin-4-yl)amino)phenyl)piperazine-1-carboxylate (36). Prepared using General Procedure (ix) with 5.52 mmol **30f** and 5.53 mmol **6a**. Yield 69% (1.672 g). ^{1}H-NMR (400 MHz; $CDCl_3$): δ 8.51 (d, J = 5.3 Hz, 1H), 8.03 (d, J = 2.1 Hz, 1H), 7.87 (d, J = 9.0 Hz, 1H), 7.46 (dd, J = 9.0, 2.2 Hz, 1H), 7.25-7.22 (m, 2H), 7.03-6.99 (m, 2H), 6.73 (d, J = 5.3 Hz, 1H), 6.57 (s, 1H), 3.63 (t, J = 5.2 Hz, 4H), 3.18 (t, J = 5.1 Hz, 4H), 1.52 (s, 9H). HRMS (ESI) - *m/z* of [$C_{24}H_{28}ClN_4O_2^+$]: 439.1901, actual: 439.1899.

tert-butyl 4-(4-((7-chloroquinolin-4-yl)amino)phenyl)piperidine-1-carboxylate (37). Prepared using General Procedure (ix) with 5.80 mmol **30f** and 5.81 mmol **6a**. Yield 87% (2.210 g). ^{1}H-NMR (400 MHz; DMSO-d_6): δ 9.04 (s, 1H), 8.45-8.41 (m, 2H), 7.89 (d, J = 2.2 Hz, 1H), 7.57 (dd, J = 9.0, 2.3 Hz, 1H), 7.33-7.28 (m, 4H), 6.87 (d, J = 5.4 Hz, 1H), 4.09 (d, J = 11.7 Hz, 2H), 2.83-2.67 (m, 3H), 1.79 (d, J = 13.2 Hz, 2H), 1.56-1.45 (m, 2H), 1.43 (s, 9H).

7-chloro-N-(4-(piperazin-1-yl)phenyl)quinolin-4-amine (38). Prepared using general procedure (x) with 3.42 mmol **36**. Yield 40% (0.464 g) ^{1}H-NMR (MHz; $CDCl_3$): δ 8.54 (d, J = 5.3 Hz, 1H), 8.03 (d, J = 2.1 Hz, 1H), 7.85 (d, J = 9.0 Hz, 1H), 7.46 (dd, J = 9.0, 2.2 Hz, 1H), 7.30-7.27 (m, 2H), 7.25-7.22 (m, 2H), 6.91 (d, J = 5.3 Hz, 1H), 6.55 (dd, J = 2.6, 0.3 Hz, 1H), 3.24-3.20 (m, 2H), 2.80-2.74 (m, 2H), 2.66-2.63 (m, 1H), 1.89-1.84 (m, 2H), 1.72-1.62 (m, 2H). HRMS (ESI) - *m/z* of [$C_{19}H_{20}ClN_4^+$]: 339.1376, actual: 339.1372.

7-chloro-N-(4-(piperidin-4-yl)phenyl)quinolin-4-amine (39). Prepared using general procedure (x) with 3.51 mmol **37**. Yield 69%. ^{1}H-NMR (400 MHz; $CDCl_3$): δ 8.54 (d, *J* = 5.3 Hz, 1H), 8.03 (d, *J* = 2.1 Hz, 1H), 7.85 (d, *J* = 9.0 Hz, 1H), 7.46 (dd, *J* = 9.0, 2.2 Hz, 1H), 7.30-7.27 (m, 2H), 7.25-7.22 (m, 2H), 6.91 (d, *J* = 5.3 Hz, 1H), 6.55 (dd, *J* = 2.6, 0.3 Hz, 1H), 3.24-3.20 (m, 2H), 2.80-2.74 (m, 2H), 2.66-2.63 (m, 1H), 1.89-1.84 (m, 2H), 1.72-1.62 (m, 2H). HRMS (ESI) - *m/z* of [$C_{20}H_{21}ClN_3^+$]: 338.1424, actual: 338.1419.

7-chloro-N-(4-(1-ethylpiperidin-4-yl)phenyl)quinolin-4-amine (40). Title compound prepared using General Procedure (xi) with 1.00 mmol **39** and 1.12 mmol iodoethane. Yield: 30% (0.108 g). ^{1}H-NMR (400 MHz; DMSO-d_6): δ 9.02 (s, 1H), 8.44-8.41 (m, 2H), 7.88 (d, J = 2.2 Hz,1H), 7.56 (dd, J = 9.0, 2.3 Hz, 1H), 7.29 (d, J = 2.1 Hz, 4H), 6.85 (d, J = 5.3 Hz, 1H), 3.01-2.98 (m, 2H), 2.36 (ddd, J = 5.5, 1.2, 0.6 Hz, 2H), 1.97-1.97 (m, 2H), 1.79-1.76 (m, 2H), 1.65 (qd, J = 12.3, 3.2 Hz, 2H), 1.03 (t, J = 7.2 Hz, 3H). HRMS (ESI) - *m/z* of [$C_{22}H_{25}ClN_3^+$]: 366.1737, actual: 366.1732.

7-chloro-N-(4-(1-propylpiperidin-4-yl)phenyl)quinolin-4-amine (41). Title compound was prepared using General Procedure (xii) with 1.00 mmol **39** and 1.2 mmol iodopropane. Yield: 26% (0.099 g). ^{1}H-NMR (400 MHz; DMSO-d_6): δ 9.04 (s, 1H), 8.43 (dd, J = 7.2, 5.3 Hz, 2H), 7.89 (d, J = 2.2 Hz, 1H), 7.57 (dd, J = 9.0, 2.3 Hz, 1H), 7.30 (d, J = 2.5 Hz, 4H), 6.86 (d, J = 5.4 Hz, 1H), 2.97 (d, J = 11.3 Hz, 2H), 2.26 (dd, J = 8.1, 6.8 Hz, 2H), 2.00-1.94 (m, 2H), 1.79-1.75 (m, 2H), 1.71-1.60 (m, 2H), 1.47 (sextet, J = 7.4 Hz, 2H), 0.88 (t, J = 7.4 Hz, 3H). HRMS (ESI) - *m/z* of [$C_{24}H_{29}ClN_3^+$]: 394.2051, actual: 394.2046.

N-(4-(1-butylpiperidin-4-yl)phenyl)-7-chloroquinolin-4-amine (42). Title compound was prepared using General Procedure (xii) with 1.00 mmol **39** and 1.2 mmol iodobutane. Yield: 63% (0.249 g). ^{1}H-NMR (400 MHz; DMSO-d_6): δ 9.05 (s, 1H), 8.43 (dd, J = 7.2, 5.2 Hz, 2H), 7.89 (d, J = 2.2 Hz, 1H), 7.57 (dd, J = 9.0, 2.2 Hz, 1H), 7.32-7.27 (m, 4H), 6.86 (d, J = 5.4 Hz, 1H), 2.97 (d, J = 11.5 Hz, 2H), 2.29 (t, J = 7.4 Hz, 2H), 1.96 (td, J = 11.6, 2.2 Hz, 2H), 1.77 (dd, J = 12.6, 1.6 Hz, 2H), 1.65 (qd, J = 12.2, 3.3 Hz, 2H), 1.47-

1.40 (m, 2H), 1.31 (dq, J = 14.9, 7.4 Hz, 2H), 0.90 (t, J = 7.3 Hz, 3H). HRMS (ESI) - *m/z* of [$C_{24}H_{29}ClN_3^+$]: 394.2051, actual: 394.2046.

7-chloro-N-(4-(1-isobutylpiperidin-4-yl)phenyl)quinolin-4-amine (43). Title compound was prepared using General Procedure (xii) with 1.00 mmol **39** and 2.03 mmol 1-iodo-2-methylpropane. Yield: 43% (0.168 g). ^{1}H-NMR (400 MHz; DMSO-d_6): δ 9.05 (t, J = 0.2 Hz, 1H), 8.44-8.41 (m, 2H), 7.89 (d, J = 2.2 Hz, 1H), 7.57 (dd, J = 9.0, 2.3 Hz, 1H), 7.32-7.27 (m, 4H), 6.86 (d, J = 5.4 Hz, 1H), 2.93 (d, J = 11.4 Hz, 2H), 2.06 (d, J = 7.4 Hz, 2H), 1.99-1.93 (m, 2H), 1.81-1.75 (m, 3H), 1.71-1.61 (m, 2H), 0.88 (d, J = 6.6 Hz, 6H). HRMS (ESI) - *m/z* of [$C_{24}H_{29}ClN_3^+$]: 394.2051, actual: 394.2045.

N-(4-(1-(sec-butyl)piperidin-4-yl)phenyl)-7-chloroquinolin-4-amine (44). Title compound was prepared using General Procedure (xii) with 1.01 mmol **39** and 1.2 mmol 2-iodobutane. Yield: 50% (0.201 g). ^{1}H-NMR (400 MHz; DMSO-d_6): δ 9.04 (s, 1H), 8.43 (dd, J = 7.2, 4.9 Hz, 2H), 7.89 (d, J = 2.2 Hz, 1H), 7.57 (dd, J = 9.0, 2.3 Hz, 1H), 7.31-7.27 (m, 4H), 6.86 (d, J = 5.4 Hz, 1H), 2.80 (d, J = 8.1 Hz, 2H), 2.48-2.37 (m, 3H), 2.23-2.18 (m, 1H), 1.78 (d, J = 12.9 Hz, 2H), 1.69-1.48 (m, 3H), 1.28 (dt, J = 13.5, 7.4 Hz, 1H), 0.94 (d, J = 6.6 Hz, 3H), 0.88 (t, J = 7.4 Hz, 3H). HRMS (ESI) - *m/z* of [$C_{24}H_{29}ClN_3^+$]: 394.2051, actual: 394.2046.

7-chloro-N-(4-(4-isobutylpiperazin-1-yl)phenyl)quinolin-4-amine (45). Title compound was prepared using General Procedure (xii) with 0.50 mmol **38** and 1.20 mmol 1-iodo-2-methylpropane. Yield: 19% (0.037 g). ^{1}H-NMR (400 MHz; $CDCl_3$): δ 8.51 (d, *J* = 5.3 Hz, 1H), 8.03 (d, *J* = 2.1 Hz, 1H), 7.85 (d, *J* = 8.9 Hz, 1H), 7.46 (dd, *J* = 8.9, 2.2 Hz, 1H), 7.23-7.20 (m, 2H), 7.03-6.99 (m, 2H), 6.72 (d, *J* = 5.3 Hz, 1H), 6.52 (s, 1H), 3.25 (t, *J* = 5.1 Hz, 4H), 2.60 (t, *J* = 5.0 Hz, 4H), 2.18 (d, *J* = 7.4 Hz, 2H), 1.91-1.81 (m, 1H), 0.96 (d, *J* = 6.6 Hz, 6H). HRMS (ESI) - *m/z* of [$C_{23}H_{28}ClN_4^+$]: 395.2003, actual: 395.1992.

Biology

Plasmodium falciparum Drug Sensitivity: The following parasite strains were used in this study and obtained through BEI Resources, NIAID, NIH. *Plasmodium falciparum*, Strain D6 (MRA-285, originally from Sierra Leone, has modest resistance to mefloquine). (46) Strain Dd2 (MRA-150, originated from Indochina; derived from W2-mef and is resistant to chloroquine, pyrimethamine, and mefloquine).

Parasite Culture: *P. falciparum* parasites were thawed from frozen stock and cultured in suspended human erythrocytes (Lampire Biological Laboratories, Pipersville, PA) not more than 28 days old at 2% hematocrit. The culture medium used: RPMI-1640 supplemented with 25 mM HEPES buffer, 25 mg/L gentamicin sulfate, 45 mg/L hypoxanthine, 10 mM glucose, 2 mM glutamine, and 0.5% Albumax II (complete medium).[19] Cultures were maintained in a standard low oxygen atmosphere (5% O_2, 5% CO_2, 90% N_2) in an environmental chamber and incubated at 37 °C. Cultures were subpassaged every 3–4 days into a fresh culture flask containing complete media and erythrocytes.

In vitro antiplasmodial activity (IC_{50}) against P. falciparum: Final compounds were assessed for *in vitro* antiplasmodial activity against D6 and Dd2 strains of *P. falciparum* using the previously described fluorescence-based SYBR Green assay.[16] In short, compounds were prepared as 10 mM stocks in DMSO. Compounds were evaluated in quadruplicate in flat-bottomed clear 96-well plates and plated at a 2-fold serial dilution with the final column left untreated to span a range of 0.25-250 nM. Asynchronous *P. falciparum* inoculated erythrocytes in growth media were added in order of increasing drug concentration to each well for a total volume of 100 µL, final hematocrit of 2%, and initial parasitemia of 0.2%.Controlls included; non-infected red blood cells, chloroquine and amodiaquine. Plates were incubated in controlled low oxygen atmosphere (5% O_2, 5% CO_2, 90% N_2) at 37 °C. 72 h post inoculation, all wells are simultaneously lysed and dyed using 100 µL of a SYBR Green I dye-detergent solution. Plates were incubated at ambient temperature and atmosphere in the dark for at least 1 h. Fluorescence was read at 497 nm excitation and 520 nm emission bands with a Spectramax iD3 plate reader. Fluorescence values were normalized with respect to the untreated control wells representing normal parasite growth and plotted against the logarithm of drug concentration. An IC_{50} was determined for each compound by fitting this data to a variable slope nonlinear regression curve using Graphpad Prism software (v. 9).

HepG2 Cytotoxicity Assay: Final compounds were assessed for mammalian cytotoxicity using an immortalized human liver carcinoma cell line (HepG2) using previously described methods.[20] In short, final compounds were prepared in DMSO as 10 mM stock solutions. Human hepatocarcinoma (HepG2) cells were cultured in RPMI-1640 medium containing 10% fetal bovine serum at 37 °C in a humidified 5% CO_2 atmosphere. To 96-well flat-bottomed tissue culture plates, HepG2 cells were added at 2 x 10^4 density with an additional 160 µL of complete culture media per well and were incubated overnight at 37 °C to allow for adherence. Compound stocks aliquots were applied as 40 µL solutions in complete media to each well in a serial dilution series that ranged from 200-0.2 µL as duplicates. A 10 mM DMSO stock of mefloquine was used as a positive control and prepared as previously described. After drug-treated plates were incubated in 5% CO_2 atmosphere at 37 °C for 24 h, they were aspirated and 200 µL of complete media was added to each well for an additional 24 h incubation in same conditions as previously mentioned. 20 µL of resazurin (Alamar Blue) in PBS buffer was added to each well to a final concentration of 10 µM, and the plates were incubated for 3 h. Fluorescence was measured at 560 nm excitation and 590 nm emission bands using a Spectramax iD3 plate reader. Fluorescence output values were normalized to the untreated control wells and plotted against the logarithm of drug concentration. Cytotoxicity (CC_{50}) was determined for each compound by fitting this data to a variable slope nonlinear regression curve using Graphpad Prism software (v. 9).

In Vivo Parasite Suppression against murine Plasmodium yoelii: The parasite suppression of select compounds at a fixed dose was measured using a modified 4-day Peters test. Female CF1 mice from Charles River Laboratories were inoculated intravenously with approximately 2.5–5.0 × 10^4 parasitized erythrocytes (murine malaria P. yoelii, Kenya strain MR4 MRA-428) from a donor mouse (experiment day zero). On the following 4 days (Days 1–4), solutions of the test compounds in PEG-400 (PEG-400 only for control mice) were administered by oral gavage once daily. Parasitemia of each mouse was determined by microscopic examination of Giemsa stained blood smears on day 5. Percent parasite suppression assessed by comparing parasitemia of treated mice relative to untreated controls using Graphpad Prism (v. 9). The procedures involved, together with all matters relating to the care, handling, and housing of the animals used in this study, were approved by the Portland VA Medical Center Institutional Animal Care and Use Committee.

In Vivo Efficacy (ED_{50}, ED_{90}, and NRD) against murine Plasmodium yoelii: The *in vivo* efficacy of select compounds was measured using a modified 4-day Peters test. Female CF1 mice from Charles River Laboratories were inoculated intravenously with approximately 2.5–5.0 × 10^4 parasitized erythrocytes (murine malaria P. yoelii, Kenya strain MR4 MRA-428) from a donor mouse (experiment day zero). On the following 4 days (Day 1–4), solutions of the test compounds in PEG-400 (PEG-400 only for control mice) were administered by oral gavage once daily. Select compounds were assessed at 1.0, 2.5, 5.0, and 10 mg/kg/d, additional experiments outside of the previous range were added if necessary to obtain an interpolated ED_{50} and ED_{90} value. Parasitemia of each mouse was determined by microscopic examination of Giemsa stained blood smears on Day 5. Mice with no observable parasitemia by microscopic analysis on day 5 were monitored twice weekly for parasitemia until parasites were observed, or until day 30. *In vivo* efficacy against infection (ED_{50} and ED_{90}) assessed by generating dose–response curves of parasitemia of treated mice relative to untreated controls using Graphpad Prism (v. 9). Non-recrudescence dose was identified as minimum dose required to maintain a 0% parasitemia by microscopic analysis until day 30. The procedures involved, together with all matters relating to the care, handling, and housing of the animals used in this study, were approved by the Portland VA Medical Center Institutional Animal Care and Use Committee.

Murine Microsomal Stability: Select compounds were assessed for murine microsomal stability in pooled liver microsomes performed at ChemPartner, Shanghai, China. Compounds were incubated at 37 °C and 1 µM concentration in murine liver microsomes (Corning) for 1 h at a protein concentration of 0.5 mg/mL in potassium phosphate buffer at pH 7.4 containing 1.0 mM EDTA. The metabolic reaction was initiated by NADPH and quenched with ice-cold acetonitrile at 15 min increments up to 1 h. The progress of compound metabolism was followed by LC-MS/MS (ESI positive ion, LC-MS/MS-034(API-6500+) using a C18 stationary phase (ACQUITY UPLC BEH C18 (2.1 × 50 mm, 1.7 µm)) and a MeOH/water mobile phase

containing 0.25% FA and 1 mM NH_4OAc. Imipramine or Osalmid were used as internal standards, ketanserin was used as a metabolically unstable control compound. Concentration versus time data for each compound were fitted to an exponential decay function to determine the first-order rate constant for substrate depletion, which was then used to calculate the degradation half-life ($t_{1/2}$) and predicted intrinsic clearance value (Cl_{int}) from an assumed murine hepatic blood flow of 90 mL/min/kg.

*Pharmacokinetic Study of **43** in Mice.* The title compound was selected for pharmacokinetic analysis in mice at a dose of 5 mg/kg performed at ChemPartner in Shanghai, China. Three groups of three male CF1 mice (JH Laboratory Animal) were administer the drug in PEG-400 at 5.0 mg/kg by oral gavage. At the following time points: 0.25, 0.5, 1, 2, 4, 8, 24, and 48 hr post dose administration a single group of mice were manually restrained and approximately 110 µL of blood were taken from the animals via facial vein for semi-serial bleeding into K_2EDTA tubes. Samples were put on ice and centrifuged (2000 G, 5 min at under 4 °C) within 15 minutes of collection. An aliquot of 3 µL sample was added to 200 µL internal standard (Diclofenac, 40 ng/mL) in ACN. The mixture was vortexed for 1 m, and centrifuged at 5800 rpm for 10 min. 100 µL supernatant was transferred to a new tube. 0.5 µL of solvent was injected into LC-MS/MS and ran on a Waters ACQUITY UPLC HSS T3 (2.1 x 50 mm, 1.8 µm) column. Pharmacokinetic analysis as a best-fit curve was prepared from drug concentration in plasma as a function of time using WinNonlin software (Pharsight – Mountain View, CA). The exposure (AUC_{last}), half-life ($T_{1/2}$), maximum concentration (C_{max}) and time of maximum concentration (T_{max}) will be determined form data. Goodness-of-fit was assessed by the r^2 (linear regression coefficient) of the drug concentration on the terminal phase.

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ACKNOWLEDGEMENTS:

The authors would like to acknowledge Dr. Aaron Nilsen, Dr. Sovitj Pou, and Dr. Rolf Winter for their support and guidance during the synthesis of these compounds.

Funding Sources

This project was supported with funds from the United States Department of Veterans Affairs, Veterans Health Administration, Office of Research and Development Program, with additional supplemental funding from VA Technology Transfer, Merit Review Grant award number i01 BX003312 (M.K.R.). M.K.R. is a recipient of a VA Research Career Scientist Award (14S-RCS001). Research reported in this publication was also supported by the US National Institutes of Health under award numbers R01AI100569 and R01AI141412 (M.K.R.) and by the US Department of Defense Peer Reviewed Medical Research Program (Log # PR181134) (M.K.R.). The National Science Foundation provided instrument funding for the BioAnalytical Mass Spectrometry Facility at Portland State University (NSF, MRI 1828573).

Conflicts

The authors have no conflicts of interest to declare. A patent application on the intellectual property described herein has been filed with Oregon Health and Science University.

Abbreviations

ACN	acetonitrile
AUC_{INF}	area under the curve extrapolated to infinity
AUC_{last}	area under the curve from last time point
Boc	*tert*-butyloxycarbonyl
Bu	butyl
Bz	benzyl
CD_3OD	deuterated methanol
$CDCl_3$	deuterated chloroform
cLogP	calculated partition coefficient
cyc-Pr	*cyclo*-propyl
DCM	dichloromethane
DIPEA	N,N-diisopropylethylamide
DMF	dimethylformamide
DMSO	dimethyl sulfoxide
DMSO-d_6	deuterated dimethyl sulfoxide
EGEE	2-ethoxyethanol
Et	ethyl
EtOH	ethanol
FA	formic acid
GCMS	gas chromatography mass spectroscopy
HCl	hydrochloric acid
HPLC	high pressure liquid chromatography
HRMS	high resolution mass spectroscopy
i-Bu	*iso*-butyl
i-Pr	*iso*-propyl
K_2EDTA	dipotassium ethylenediaminetetraacetic acid
LC-MS/MS	liquid chromatography tandem mass spectroscopy
LiHMDS	lithium bis(trimethylsilyl)amide
LLOQ	lower limit of quantification
MDR	multi-drug resistant
MeOH	methanol

NMR	nuclear magnetic resonance
NRD	nonrecrudescence dose
PEG-400	polyethylene glycol - 400
Pd/C	palladium on carbon
Pf	*Plasmodium falciparum*
pfcrt	*Plasmodium falciparum chloroquine resistant transport*
PfD6	*Plasmodium falciparum D6 strain*
PfDd2	*Plasmodium falciparum Dd2*
$PhCF_3$	phenyl trifluoromethyl
$PhOCF_3$	phenyl trifluoromethoxide
PK	pharmacokinetic
PO	per os (by mouth)
Pr	propyl
Py	*Plasmodium yoelii*
rt	room temperature
s-Bu	*sec*-butyl
SAR	structure activity relationship
t-Bu	*tert*-butyl
$T_{1/2}$	half-life
TEA	trimethylamine
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	tetramethylsilane
UPLC	Ultra-performance liquid chromatography