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# Identification, Design and Biological Evaluation of Heterocyclic Quinolones Targeting *Plasmodium falciparum* Type II NADH:Quinone Oxidoreductase (PfNDH2)

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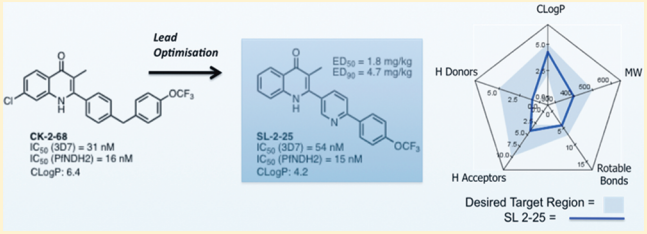
Supporting Information

**ABSTRACT:** Following a program undertaken to identify hit compounds against NADH:ubiquinone oxidoreductase (PfNDH2), a novel enzyme target within the malaria parasite *Plasmodium falciparum*, hit to lead optimization led to identification of CK-2-68, a molecule suitable for further development. In order to reduce ClogP and improve solubility of CK-2-68 incorporation of a variety of heterocycles, within the side chain of the quinolone core, was carried out, and this approach led to a lead compound SL-2-25 (**8b**). **8b** has $IC_{50}$s in the nanomolar range versus both the enzyme and whole cell *P. falciparum* ($IC_{50}$ = 15 nM PfNDH2; $IC_{50}$ = 54 nM (3D7 strain of *P. falciparum*) with notable oral activity of $ED_{50}/ED_{90}$ of 1.87/4.72 mg/kg versus *Plasmodium berghei* (NS Strain) in a murine model of malaria when formulated as a phosphate salt. Analogues in this series also demonstrate nanomolar activity against the $bc_1$ complex of *P. falciparum* providing the potential added benefit of a dual mechanism of action. The potent oral activity of 2-pyridyl quinolones underlines the potential of this template for further lead optimization studies.




## INTRODUCTION

Drug resistance to established antimalarials such as chloroquine is driving the rise in global mortality due to malaria.[1] Malaria is responsible for roughly one million deaths annually,[2] and as such novel inhibitors active against new parasite targets are urgently required in order to sustain and develop treatments against malaria.[3] As described in the previous companion paper in this issue (DOI: 10.1021/jm201179h),[4] a program was undertaken to identify hit compounds against NADH:ubiquinone oxidoreductase (PfNDH2), a novel enzyme target within the malaria parasite *Plasmodium falciparum*.[5] A number of these compounds were also found to target the $bc_1$ complex of *P. falciparum* giving these drugs a dual mode of action.

PfNDH2 has only one known inhibitor, N-hydroxy-2-dodecyl-4(1H)quinolone (HDQ),[6] and this was used along with a range of chemoinformatics methods in the rational selection of 17 000 compounds for high-throughput screening (HTS).[7] Several distinct chemotypes were identified and briefly examined leading to the selection of the quinolone core as the key target for structure−activity relationship (SAR) development and subsequent identification of CK-2-68 as a lead for further development.

Our initial studies focused on compounds with mono aryl groups at the 2-position; however it became rapidly apparent that activity below 500 nM against the 3D7 strain of *P. falciparum* was not possible. A progression toward the close HDQ analogues where a longer biaryl/phenoxy biaryl replaced the metabolically vulnerable HDQ-side chain improved both antimalarial and PfNDH2 activity. A series of structural modifications including the introduction of a methyl substituent at the 3-position led to the generation of over 60 compounds as exemplified by **2** (CK-2-68) with an activity of 31 nM against 3D7 and 16 nM against PfNDH2 (Figure 1). It was apparent from preliminary animal studies that ClogP needed to be reduced and aqueous solubility needed to be enhanced in order to administer the drug in a suitable vehicle without the need for a pro-drug approach. Introduction of various heterocycles into the quinolone side chain led to the selection of a series of compounds containing a pyridine group within the side chain. Incorporation of a pyridine group reduces ClogP, improves aqueous solubility, and allows the possibility of salt formation. Further strategies investigated included

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the use of polar heterocycles in the side chain, use of protonatable groups within the side chain, extending the terminal group using polar heterocycles and the placement of a polar group centrally in the side chain with a lipophilic group at the terminal end.

## RESULTS AND DISCUSSION

Investigations into possible solutions to reduce ClogP revealed that the incorporation of a heterocycle into the side chain was imperative to achieving this.[8] It was apparent from literature searches that the chemistry used to achieve this would be more easily facilitated if there was no linker between the two rings within the side chain. With this in mind, we undertook the synthesis of some of the key bisaryl compounds known to have good activity (see previous companion paper[4] in this issue) but with no linker between the aryl rings rather than a $CH_2$ or O linker to check activity was maintained. It can be seen from Table 5 that antimalarial activity is maintained. The synthesis of these compounds is described in the following schemes along with the heterocyclic compounds.

Initially, the incorporation of a pyridine ring into the side chain was targeted, and the optimal A ring and terminal aryl ring substituents investigated. The methods used to synthesize these compounds can be seen in Schemes 1−3. For measured solubility values of select compounds, please see Table S1 in Supporting Information.

Aldehyde **3** was used in a Grignard reaction to give alcohol **4** in 69−88% yields. Where aldehyde **3** was not commercially available, the aldehydes were synthesized in house (see Supporting Information). Alcohol **4** was oxidized using PCC to give ketone **5** in 66−90% yields. Oxazoline **7** was prepared from the respective isatoic anhydride **6** in yields of 30−60%. In the majority of compounds the isatoic anhydrides were commercially available; when this was not the case they were synthesized (see Supporting Information). Reaction of oxazoline **7** with ketone **5** in the presence of PTSA gave the desired **8a−z and 9a−c** in 23−69% yields. Quinolones **9b** and **9c** were synthesized as precursors to quinolones **12a−f** as seen in Scheme 3. As can be seen in Table 1 this methodology was used not only to insert a pyridine into the side chain, it was also used to incorporate a small number of bipyridyl, O-linked, C linked, and other heterocyclic groups into the side chain.[9]

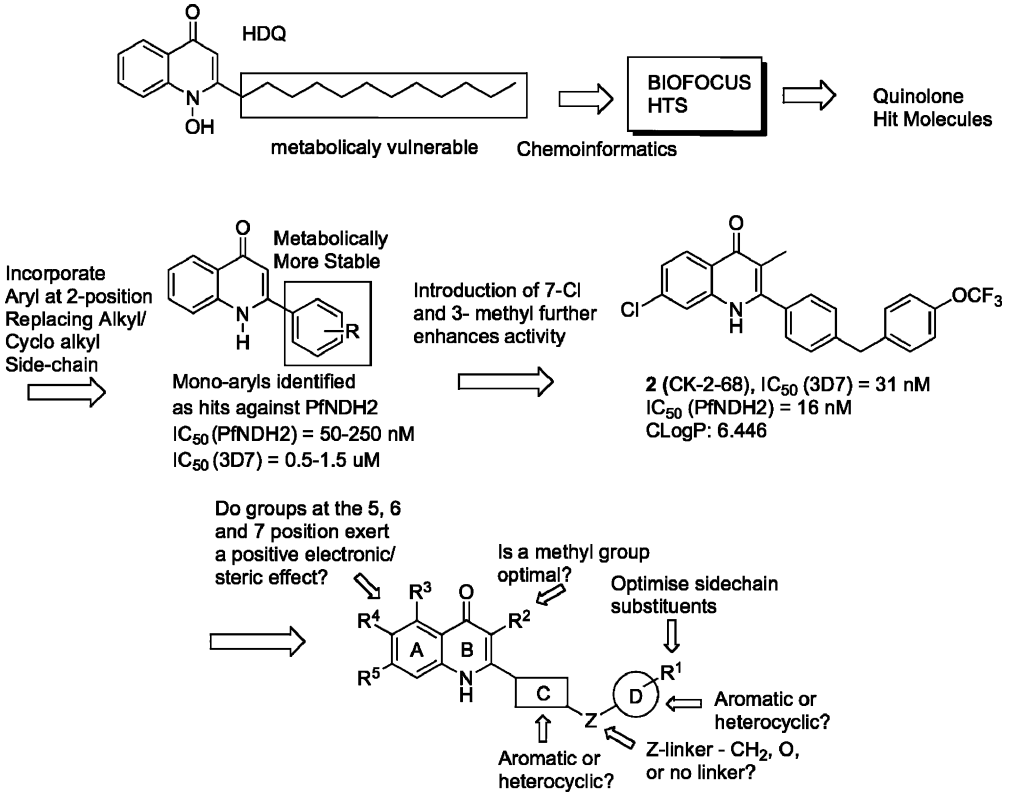


**Figure 1.** Mono aryl quinolones identified as hits from high-throughput screen and initial SAR work.

**Scheme 1. Synthesis of Quinolones 8a−z and 9a−c**

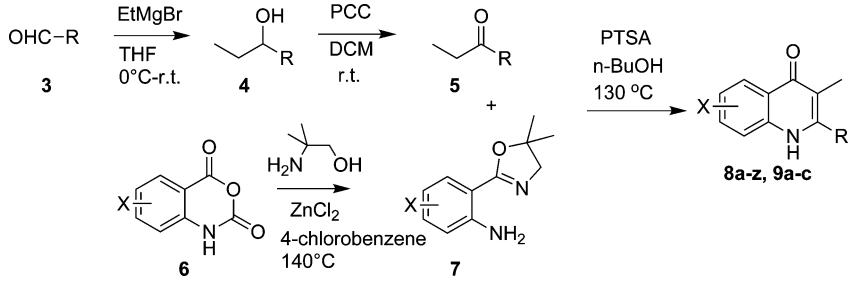



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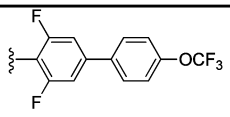
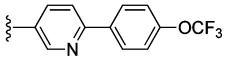
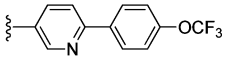
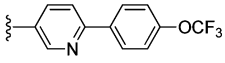
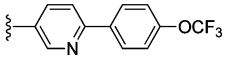
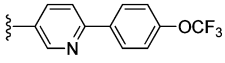
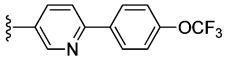
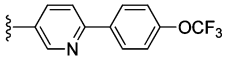
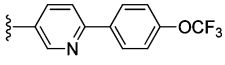
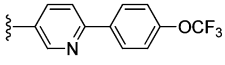
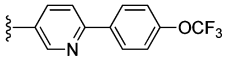
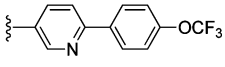
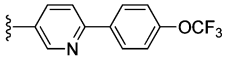
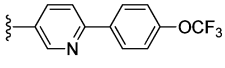
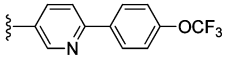
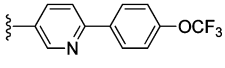
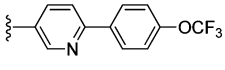
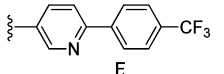
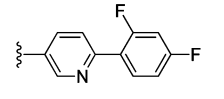
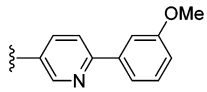
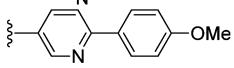
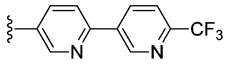
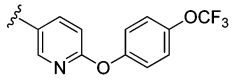
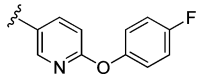
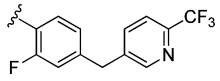
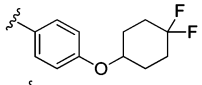
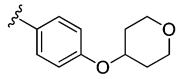
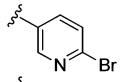
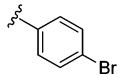
Table 1. Yields for the Synthesis of Compounds 8a−z and 9a−c[a]

| Compound | R | X | % Yield **4** | % Yield **5** | % Yield **7** | % Yield **8/9** |
|---|---|---|---|---|---|---|
| **8a** |  | H | 82 | 85 | 45 | 33 |
| **8b** |  | H | 73 | 85 | 45 | 69 |
| **8c** |  | 5-OMe | 73 | 85 | 98 | 4 |
| **8d** |  | 6-Cl | 73 | 85 | 47 | 34 |
| **8e** |  | 6-OMe | 73 | 85 | 52 | 32 |
| **8f** |  | 6-$OCF_3$ | 73 | 85 | 54 | 33 |
| **8g** |  | 6-$CF_3$ | 73 | 85 | 51 | 33 |
| **8h** |  | 7-F | 73 | 85 | 60 | 30 |
| **8i** |  | 7-OMe | 73 | 85 | 60 | 23 |
| **8j** |  | 7-Cl | 73 | 85 | 58 | 30 |
| **8k** |  | 7-$CF_3$ | 73 | 85 | 56 | ? |
| **8l** |  | 7-$SO_2Me$ | 73 | 85 | 57 | 40 |
| **8m** |  | 8-OMe | 73 | 85 | 30 | 25 |
| **8n** |  | 5-F, 7-F | 73 | 85 | 52 | 33 |
| **8o** |  | 6-F, 7-F | 73 | 85 | 55 | 41 |
| **8p** |  | 6-Cl, 7-F | 73 | 85 | 41 | 40 |
| **8q** |  | 6-F, 7-Cl | 73 | 85 | 30 | 50 |
| **8r** |  | H | 68 | 78 | 45 | 48 |
| **8s** |  | H | 65 | 75 | 45 | 20 |

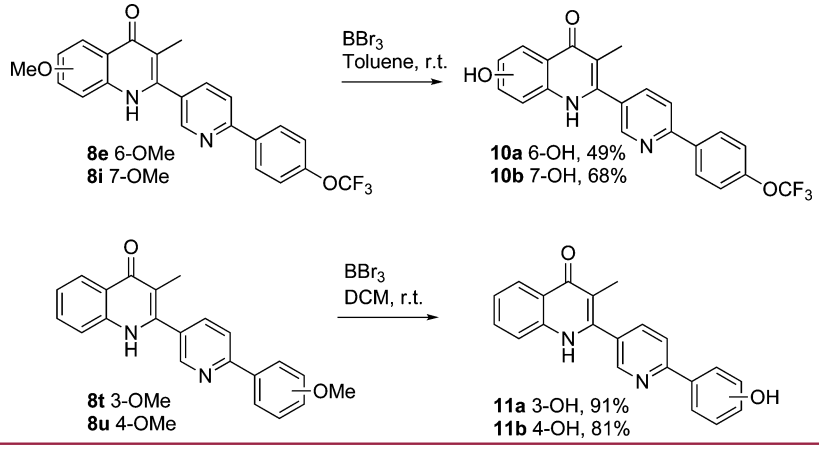

dx.doi.org/10.1021/jm201184h | J. Med. Chem. 2012, 55, 1844−1857

**Table 1. continued**

| Compound | R | X | % Yield **4** | % Yield **5** | % Yield **7** | % Yield **8/9** |
|---|---|---|---|---|---|---|
| **8t** |  | H | 75 | 85 | 45 | 58 |
| **8u** |  | H | 69 | 82 | 45 | 52 |
| **8v** |  | H | 70 | 90 | 45 | 52 |
| **8w** |  | H | 72 | 80 | 45 | 25 |
| **8x** |  | H | 80 | 80 | 45 | 28 |
| **8y** |  | H | 88 | 66 | 45 | 52 |
| **8z** |  | H | - | *30 | 45 | 39 |
| **9a** |  | H | - | *52 | 45 | 33 |
| **9b** |  | H | 68 | 80 | 45 | 30 |
| **9c** |  | H | - | - | 45 | 30 |

[a]Asterisk indicates alternative route; please see Supporting Information.

**Scheme 2. Synthesis of Hydroxyl Quinolones 10a–b and 11a–b**




The presence of an alcohol both in the A ring of the quinolone core and in the terminal aryl group of the side chain was investigated. In the case of the A ring of the quinolone core compounds with a hydroxyl group at the 6(**10a**) and 7(**10b**) positions were synthesized from their respective methoxy compounds using boron tribromide in 49% and 68% yields (Scheme 2). Hydroxyl groups at the 3(**11a**) and 4(**11b**) positions on the terminal phenyl ring were achieved using a similar method in 91% and 81% yields, respectively.

Where synthesis of the bicyclic side chain was not possible prior to the quinolone formation step, it was often possible to carry out the Suzuki reaction at the final step (Scheme 3). This was advantageous when investigating the scope of the Z group attached to the terminal ring. Quinolones **9a** or **9b** were reacted with boronic acid under the Suzuki conditions described below to give quinolones **12a–f** in 50–70% yields (Table 2).

The nature of the group at the 3-position was also investigated for both bisaryl no-linker compounds and heterocyclic quinolones. Ester and methyl alcohol groups were synthesized by reaction of amine ester **13** (for synthesis, please see Supporting Information) with oxalyl chloride. The product from this reaction was used crude and heated in Dowtherm to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give iodo quinolone **15**, and subsequent Suzuki reaction gave bisaryl ester quinolone compounds **16a** and **16b** in 53% and 75% yields. Reduction of quinolone **16b** to methyl alcohol **17** was achieved using $LiBH_4$ in 66% yield.

In the case of the heterocyclic side chain, ester **19** was synthesized with the side chain fully in place. Cyclisation to the quinolone **21** was achieved by reaction with NaH in DMF in 28% yield. $LiBH_4$ reduction gave methyl alcohol **22** in 40% yield (Scheme 5).

The presence of a methoxy group at the 3-position was also investigated. Aniline was reacted with 2-chloroacetonitrile to give $\beta$-chloro ketone **23** in 54% yield. Reaction of **23** with acetic anhydride gave amide **24** in 97% yield. Cyclization to **25** was achieved using NaH in 61% yield. Reaction of **25** with aldehyde **5** in the presence of catalytic piperidine gave alkene **26** in 93% yield. Conversion to quinolone **27** was achieved by reaction with bromine at room temperature, followed by refluxing with sodium methoxide in MeOH/dioxane, and subsequently refluxing in concentrated HCl to give 3-methoxyquinolone **27** in 6% yield (Scheme 6).

Incorporation of a morpholine group as the terminal ring in side chain was pursued to allow the possibility of salt formation.[10] Bromo benzyl compound **28** was reacted with morpholine in the presence of potassium carbonate to give benzyl morpholine **29** in 96% yield. Reaction with EtMgBr and CuI, then subsequently HCl, gave ethyl ketone **30** in 77% yield. This was then coupled with oxazoline **7** in 30% yields to give quinolones **31a** and **31b** (Scheme 7).

Other functionality in the terminal ring has been investigated such as pyridine ring (**8y**), difluoro cyclohexyl ring (**8z**), and a tetrahydropyran (**9a**). Quinolone **35** was synthesized as a direct analogue of **8** containing a nitrogen in the A ring. Ethyl ketone **32** was reacted with trimethyl orthoformate in the presence of PTSA to give dimethyl acetal **33** in 70% yield. Heating acetal **33** with pyridyl amine **34** in Dowtherm A gave quinolone **35** in 32% yield (Scheme 8).

Synthesis of quinolones with an extended morpholine side chains were also prepared using chemistry similar to that employed in Scheme 3. Reaction of **9b/c** with benzyl morpholine borane ester gave morpholine quinolones **36a** and **36b** in 61% and 50% yields (Scheme 9).

A series of quinolones were then prepared containing an aryl group with an ethoxy linkage to a heterocyclic ring. This was to further explore the side chain SAR and provide us with further

**Scheme 3. Synthesis of Quinolones 12a–f**

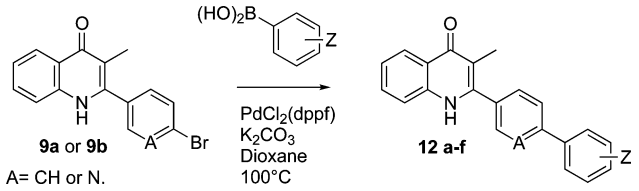
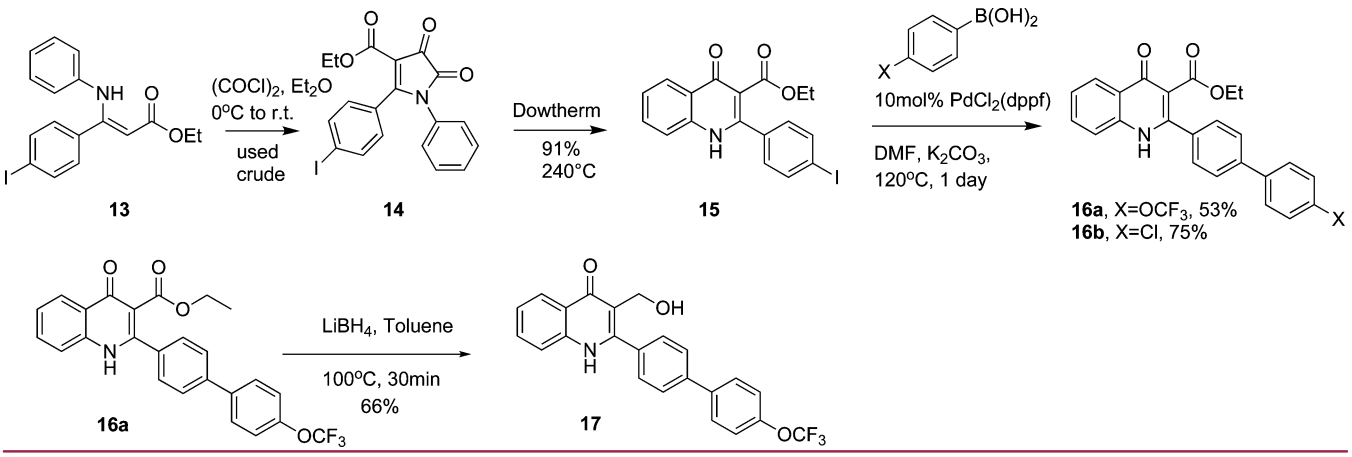


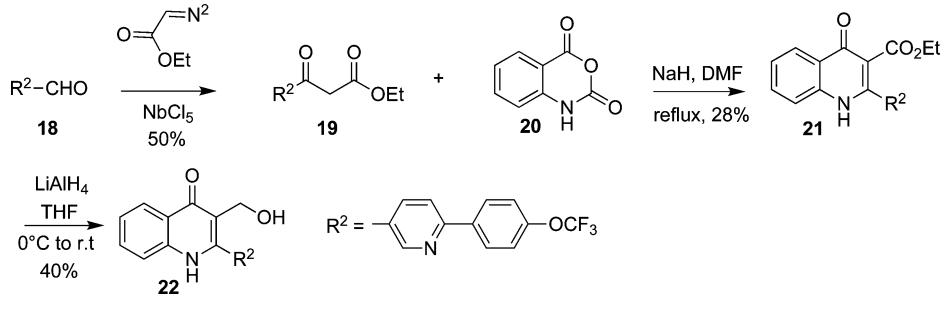

**Table 2. Yields for the Synthesis of Compounds 12a–f**

| compound | A | Z | % yield **12** |
|---|---|---|---|
| **12a** | CH | 4-$OCF_3$ | 60 |
| **12b** | CH | 2-$CF_3$ | 50 |
| **12c** | CH | 2-F, 4-F | 65 |
| **12d** | CH | 2-F | 69 |
| **12e** | N | 2-$CF_3$ | 70 |
| **12f** | N | 2-F | 65 |

**Scheme 4. Synthesis of 3-Ester Quinolones 16a, 16b, and 3-Methyl Alcohol Quinolone 17**



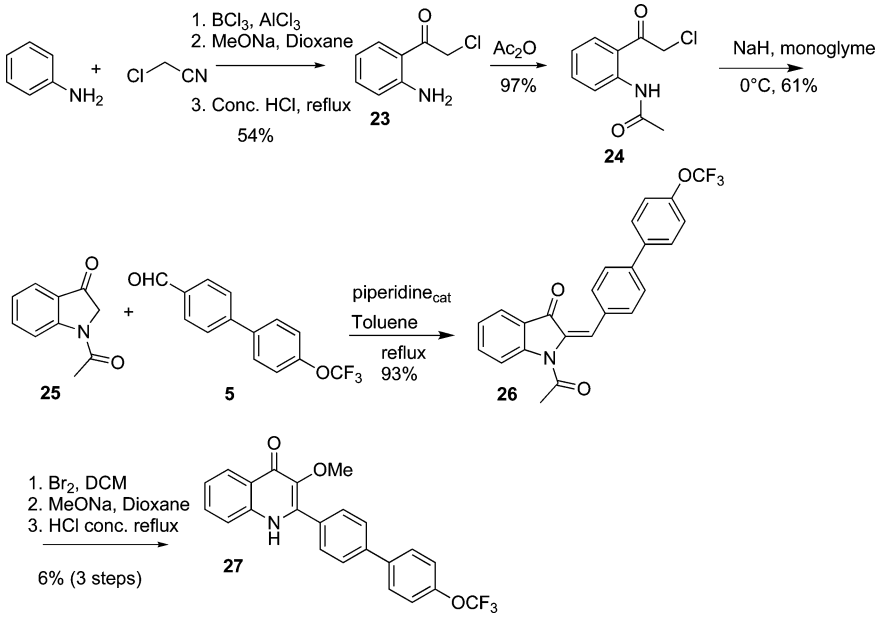

**Scheme 5. Synthesis of 3-Ester Quinolone 21 and 3-Methyl Alcohol Quinolone 22**



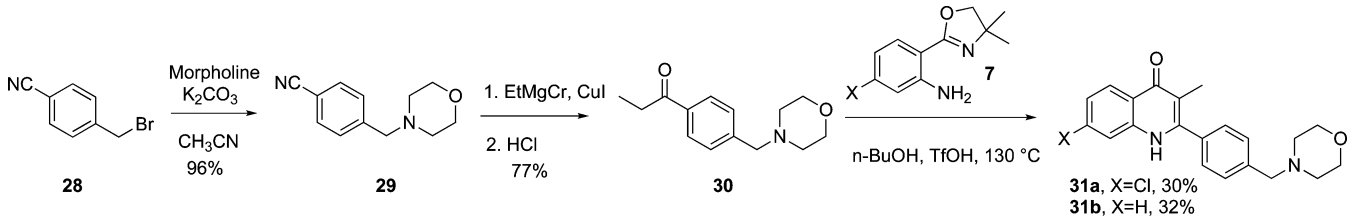


dx.doi.org/10.1021/jm201184h | J. Med. Chem. 2012, 55, 1844–1857

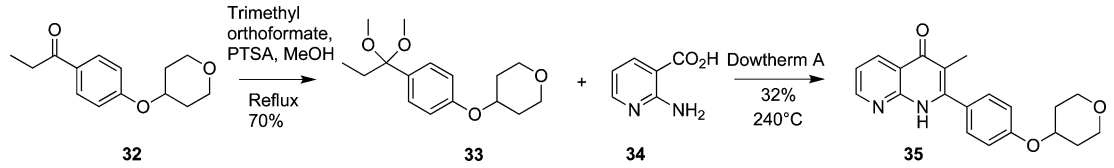
**Scheme 6. Synthesis of 3-Methoxy Quinolone 27**



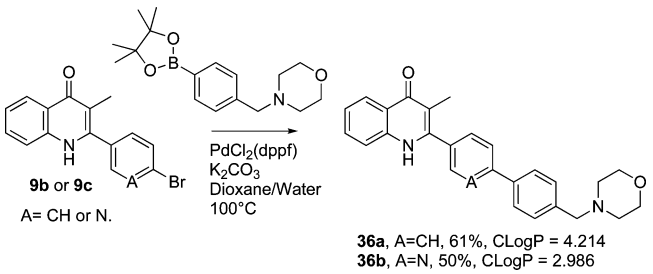

**Scheme 7. Synthesis of Morpholino Quinolones 31a and 31b**




**Scheme 8. Synthesis of Quinolone 35**




**Scheme 9. Synthesis of Extended Side Chain Morpholino Compounds 36a and 36b**




information about the length of side chain tolerated. These compounds also have a ClogP of 3−4. Phenol **37** was reacted with chloro ethyl amine **38** in the presence of potassium carbonate to give ethoxy morpholine **39**. Morpholine **39** was then reacted with oxazoline **7** as described previously to give quinolones **40a−g** (Scheme 10 and Table 3).

Crystals of quinolones **40a** (see Figure 2, CCDC 833299) and **40c** (see Figure 3, CCDC 833897) were grown and their structures confirmed by X-ray crystallography.

Replacement of the phenyl group in the side chain with a pyrazole group provides a further dramatic reduction in ClogP. Compounds **42a** and **42b** were synthesized using chemistry described previously (Scheme 11).

A series of compounds with an extended ethoxy morpholine and piperazine side chains were also synthesized. To incorporate the oxyl-linked side chain $BBr_3$ was the used to demethylate methoxy ketone **43** to give alcohol **44** in 60−64% yields. Addition of the ethyl morpholine/piperazine subunit was achieved using potassium carbonate to give side chain **45** in 69−77% yields. Reaction with oxazoline **7** in the presence of triflic acid gave quinolones **46a−d** in 40−50% yields (Scheme 12).

Incorporation of protonatable nitrogens into the middle of the side chain with a lipophilic group at the terminal end


dx.doi.org/10.1021/jm201184h | J. Med. Chem. 2012, 55, 1844−1857

**Scheme 10. Synthesis of Ethoxy Amine Quinolones 40a−g**

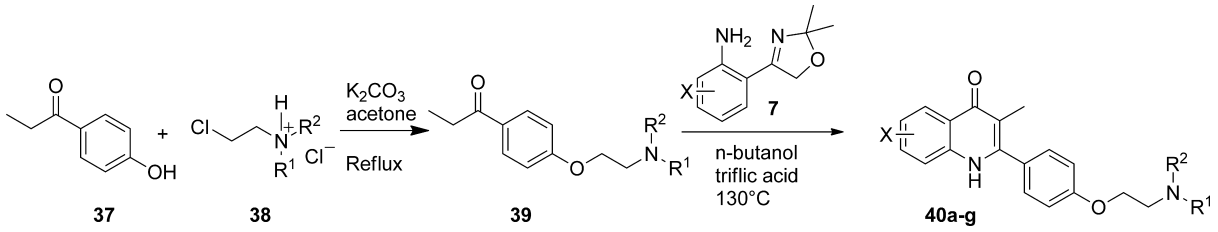
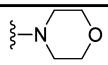
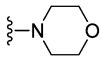
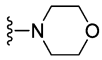
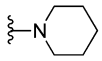
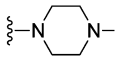



**Table 3. Yields for the Synthesis of Compounds 40a−g**

| Compound | X | $NR^1R^2$ | % Yield **39** | % Yield **40** |
|---|---|---|---|---|
| **40a** | H |  | 80 | 48 |
| **40b** | 6-Cl |  | 80 | 44 |
| **40c** | 7-Cl |  | 80 | 43 |
| **40d** | H |  | 76 | 48 |
| **40e** | H |  | 72 | 42 |
| **40f** | H |  | 60 | 48 |
| **40g** | H |  | 59 | 44 |

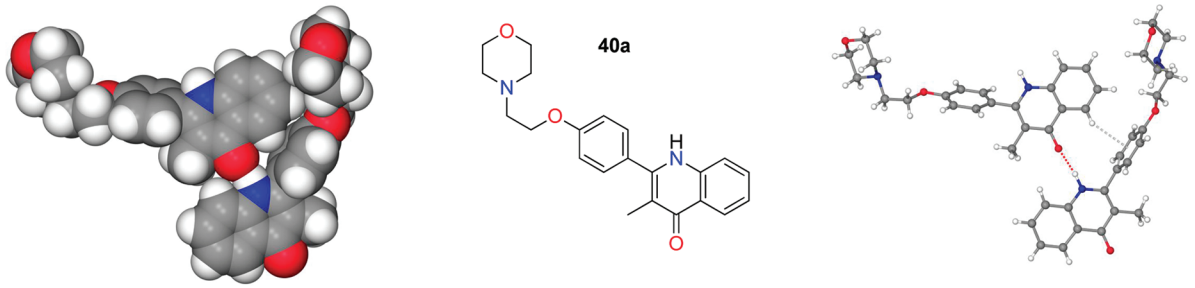



**Figure 2.** X-ray crystal structure of quinolone **40a**.

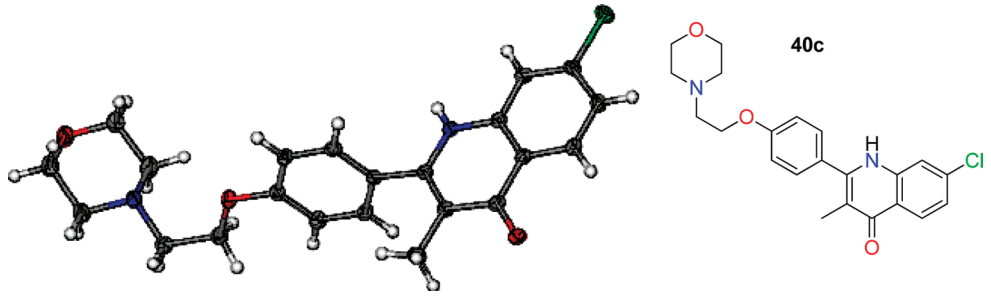



**Figure 3.** X-ray crystal structure of quinolone **40c**.

(a strategy employed in GSKs pyridone $bc_1$ program) has also briefly been investigated. In house models of similar enzymatic systems (e.g., $bc_1$ complex) have shown that charge is not tolerated close to the headgroup so this would provide side chain extension and possibly improve binding. Chloro, bromo quinoline **47** was reacted with boronic ester **48** to give 2-substituted quinoline **49** in 79% yield. Bromination of the methyl group of quinolone **49** using NBS and AIBN gave **50** in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44% yield. Subsequent reaction of this with piperazine **51** gave quinoline **52** in 70% yield. Conversion to quinolone **53** was achieved using formic acid in 70% yield (Scheme 13).

While our primary focus was to use medicinal chemistry manipulation of the core template to maximize solubility and activity, pro-drug approaches were also briefly examined as detailed in the previous paper.[11] Quinolone **8b** was reacted with tetrabenzyl pyrophosphate in the presence of NaH to give the phosphonate ester **54** in 80% yield. Hydrogenation using Pd/C gave phosphate pro-drug **55** in 71% yield (Scheme 14).

Morpholine carbamate[12] pro-drug **56** was made by reacting quinolone **8b** with morpholine carbonyl chloride in the presence of potassium *tert*-butoxide to give the pro-drug in 78% yield (Scheme 15). It was however found that the best activity was achieved with the phosphoric acid salt of lead compounds **8b** and **8h**.

**Antimalarial Activity.** Tables 5, 6, and 7 show the antimalarial activity of all quinolones synthesized against the 3D7 strain of *P. falciparum*. Table 5 shows all data pertaining to the bisaryl no linker compounds. This data clearly demonstrate that no linker is tolerated. Other SAR details of note are that a high degree of substitution in the side chain close to the quinolone core is poorly tolerated as exemplified by **8a** (940 nM) suggesting that flexibility of the side chain is key to the activity of these compounds. As seen with previous side chain variations, the 4-$OCF_3$ group is the optimal terminal group (**12a**, 59 nM). The methyl group is favored as a 3-substituent with Me (**12a**, 59 nM) > $CH_2OH$ (**17**, 162 nM) > $CO_2Et$ (**16a**, 347 nM) in terms of activity.

Table 6 shows the antimalarial data for all bicyclic pyridine quinolones. Variations in the X group show that smaller groups such as Cl, F, and OH are well tolerated with larger groups such as $CF_3$, $OCF_3$, and $SO_2Me$ not tolerated as exemplified by **8h** (75 nM) compared to **8l** (>1000 nM). Investigations into the nature of the Y group have shown that the presence of an OH group results in loss of activity as seen with **11a** and **11b**. As a general rule substitution at the 4-position is favored over the 2 and 3 position. The methyl group again is found to be optimal as a substituent at the 3-position with Me (**8b**, 54 nM) > $CH_2OH$ (**21**, 103 nM) > $CO_2Et$ (**22**, 987 nM) in terms of activity.

Table 7 shows the antimalarial activity of the other heterocyclic quinolones investigated. From the results below it can be seen that one pyridine ring **8b** (54 nM) is favored over two pyridine rings **8v** (370 nM). Again 4-$OCF_3$ is the optimal terminal substituent as seen with **8w** (40 nM) vs **8x** (279 nM). Incorporation of a morpholine/piperazine group generally leads to a loss of activity.

Having established the whole cell activity of all quinolone compounds they were then tested against the PfNDH2 enzyme (see Tables 5 and 6). Because of the time-consuming nature of the assay[13] and large volume of parasites needed only a small selection of the most active compounds were then tested against P*fbc*$_1$ (see footnote, Tables 5 and 6). A large number of the quinolones tested demonstrate nanomolar activity against both PfNDH2 and P*fbc*$_1$ confirming the dual mechanism of action that these compounds possess.

From these compounds a selection was tested against the atovaquone resistant TM90C2B strain of *P. falciparum* ($IC_{50}$ for atovaquone is 12 $\mu$M in this strain) containing the cytochrome *b* mutation Y268S.

Additionally a more select range of compounds were tested against the chloroquine resistant strain of *P. falciparum*, W2. The SAR trends identified from the 3D7 data largely hold true for the W2 data with the presence of a group other than a *para* $OCF_3$ group causing a reduction in activity **12e** (88 nM) and **12f** (120 nM) groups reducing activity when compared to **8b** (50 nM).

The initial lead from the set of heterocyclic compounds was **8b**. **8b**, the phosphate salt of **8b**, and both the morpholine and phosphate pro-drugs were tested for in vivo activity using Peters' Standard 4-day test.[14] With **8b** some solubility problems were encountered with the use of standard suspension vehicle (SSV) as the vehicle; the compound had to

**Scheme 11. Synthesis of Morpholino Quinolones 42a and 42b**

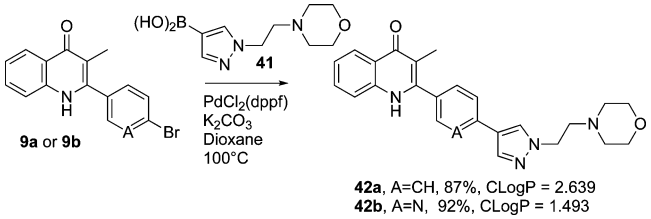
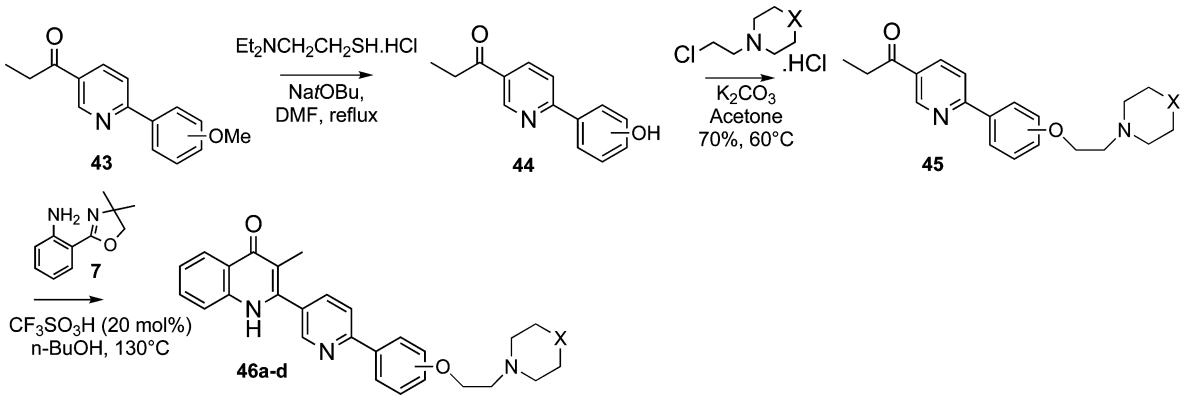



**Table 4. Yields for the Synthesis of Compounds 46a–d**

| compound | X | side chain | % yield **44** | % yield **45** | % yield **46** |
|---|---|---|---|---|---|
| **46a** | O | 3-O- | 60 | 70 | 50 |
| **46b** | $CH_2$ | 3-O- | 60 | 69 | 42 |
| **46c** | O | 4-O- | 64 | 74 | 49 |
| **46d** | $CH_2$ | 4-O- | 64 | 77 | 40 |

**Scheme 12. Synthesis of Extended Side Chain Ethoxy Morpholine Quinolones 46a–d**





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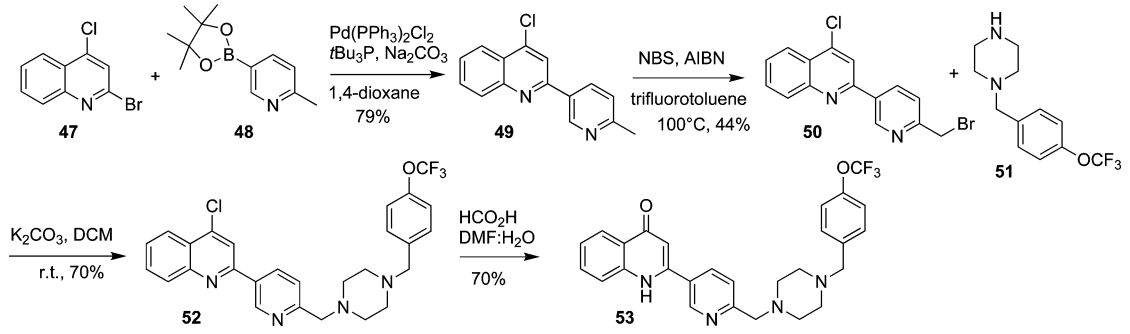
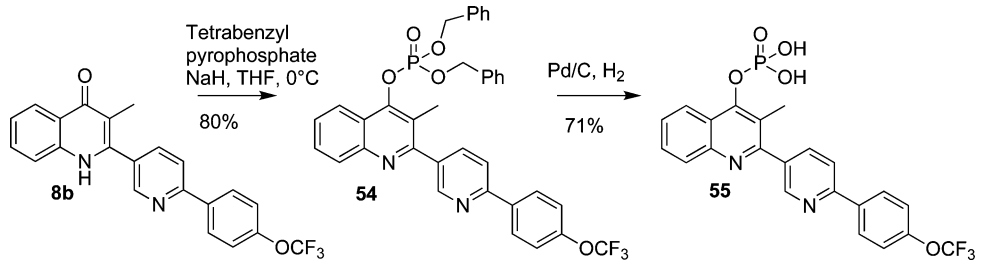
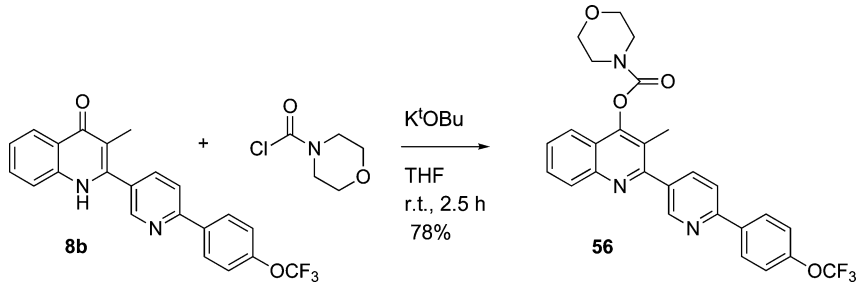
**Scheme 13. Synthesis of Quinolone 53**




**Scheme 14. Synthesis of Phosphate Pro-Drug 55**




**Scheme 15. Synthesis of Morpholine Pro-Drug 56**




be dosed as a suspension and a reduced % parasite clearance was seen. The use of 5% DMSO and 5% EtOH in tetraglycol (DET), where **8b** fully dissolved provided proof of concept that **8b** clears the parasite in vivo with 100% parasite kill being achieved at 20 mg/kg. However, for full $ED_{50}/ED_{90}$ determination the use of SSV was preferred. With both the phosphate salt of **8b** and the morpholine prodrug **56** 100% parasite kill was seen in SSV. As salt formulation was preferable over a pro-drug approach, formulation as the phosphate salt was employed for full $ED_{50}/ED_{90}$ determination. The phosphate pro-drug of **8b**, compound **55** was successfully dosed in a sodium carbonate solution and 100% parasite kill was also seen at 20 mg/kg.

Compound **8b** was selected to determine the in vivo activity in terms of $ED_{50}/ED_{90}$. Formulation as the phosphate salt offers greater in vivo activity as is demonstrated by the marked improvement in activities when comparing the parent compound **8b** ($ED_{50}/ED_{90}$ = 12.75/27.30 mg/kg) to its phosphate salt ($ED_{50}/ED_{90}$ = 1.87/4.72 mg/kg). Against the same strain, chloroquine had an $ED_{50}/ED_{90}$ of 3.3 mg/kg/4.6 mg/kg and artemether was active at 3.1 mg/kg/5.8 mg/kg in terms of $ED_{50}/ED_{90}$ indicating that these molecules have potency similar to proven antimalarials in this model. Measured solubility values as depicted in Table S1 (Supporting Information) confirm that while solubility is a challenge we still face, the incorporation of a heterocyclic ring does improve solubility at low pH and that solubility is further enhanced when compounds are formulated as the phosphate salt.

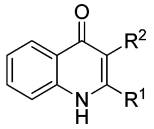
*Cytotoxicity.* No significant cytotoxicity was observed for **8b** at any concentration ($CC_{50}$ > 50 $\mu$M) in HEPG2 cells.

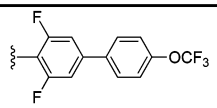
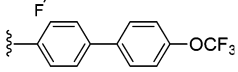
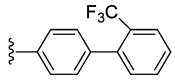
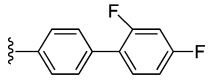
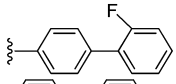
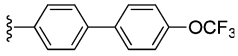
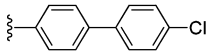
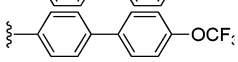
*Human Liver Microsomal Incubations.* **8b** was incubated at a concentration of 1 $\mu$M with human liver microsomes (1 mg/mL) in the presence of NADPH for 0, 10, 30, and 60 min. Greater than 60% of the parent compound was present after 60 min incubation. The in vitro half-life for **8b** was shown to be 96.7 min, with an intrinsic clearance value of 1.78 mL/min/kg.

*In Vivo Pharmacokinetic Parameters (Rat).* Upon oral dosing in the rat of the morpholine pro-drug (20 mg/kg), the observed pharmacokinetic parameters in plasma for the parent compound, **8b**, were $C_{max}$ 8.1 $\mu$g/mL, $T_{max}$ 7.0 h, half-life ($T_{1/2}$) of 20.3 h, a volume of distribution $V_d$ of 2875.6 mL/kg, an area under the curve of AUC0-t 167.2 $\mu$g.h/mL and a calculated total clearance ClT was 98.0 mL/h/kg (see Supporting Information). The pharmacokinetic features of the **8b** salt were also favorable; the observed parameters for the parent


dx.doi.org/10.1021/jm201184h | J. Med. Chem. 2012, 55, 1844–1857

Table 5. In Vitro Antimalarial Activities of Bisaryl Quinolones versus 3D7 *Plasmodium falciparum*[a]



| Compound | $R^1$ | $R^2$ | $IC_{50}$ (nM) 3D7 ± SD/ ($IC_{50}$ (nM) $PfNDH_2$) |
|---|---|---|---|
| **8a** |  | Me | 940 ± 50 |
| **12a** |  | Me | 59 ± 9/(<1) |
| **12b** |  | Me | 96 ± 17/(8.1) |
| **12c** |  | Me | 213 ± 33 |
| **12d** |  | Me | 471 ± 75 |
| **16a** |  | $CO_2Et$ | 347 ± 80 |
| **16b** |  | $CO_2Et$ | 220 ± 40 |
| **17** |  | $CH_2OH$ | 162 ± 19 (69) |

[a]P*fbc*$_1$ $IC_{50}$ data(nM): **12a** = 38.

compound, **8b** following oral administration (20 mg/kg) were $C_{max}$ 3.7 μg/mL, $T_{max}$ 7.0 h, $T^{1/2}$ of 9.9 h, $V_d$ 3970.8 mL/kg, AUC0-t 69.3 μg.h/mL and a ClT of 276.3 mL/h/kg (see Supporting Information). These pharmacokinetic parameters are consistent with a once-daily oral dosing target product profile.

## CONCLUSIONS

To conclude, a 4−6 step synthesis of a range of heterocylic quinolones with potent antimalarial activity both in vitro and in vivo have been reported. Several compounds within this series have been proven to be potent against the novel PfNDH2 enzymatic target. Representative analogue SL-2-25 (**8b**) demonstrates outstanding antimalarial activity, reduced ClogP, and improved solubility. **8b** has antimalarial activity against the 3D7 strain of *P. falciparum* of 54 nM, PfNDH2 activity of 15 nM and an $ED_{50}/ED_{90}$ of 1.87/4.72 mg/kg when formulated as the phosphoric acid salt.

## EXPERIMENTAL SECTION

All reactions that employed moisture sensitive reagents were performed in dry solvent under an atmosphere of nitrogen in oven-dried glassware. All reagents were purchased from Sigma Aldrich or Alfa Aesar chemical companies, and were used without purification. Thin layer chromatography (TLC) was carried out on Merck silica gel 60 F-254 plates and U.V. inactive compounds were visualized using iodine or anisaldehyde solution. Flash column chromatography was performed on ICN Ecochrom 60 (32-63 mesh) silica gel eluting with various solvent mixtures and using an air line to apply pressure. NMR spectra were recorded on a Bruker AMX 400 ($^1$H, 400 MHz; $^{13}$C, 100 MHz) spectrometer. Chemical shifts are described in parts per million ($\delta$) downfield from an internal standard of trimethylsilane. Mass spectra were recorded on a VG analytical 7070E machine and Fisons TRIO spectrometer using electron ionization (EI) and chemical ionization (CI). All compounds were found to be >95% pure by HPLC unless specified below. See Supporting Information for experimental and data on all intermediates.

**General Procedure for the Synthesis of Quinolones 8.** The appropriately substituted oxazoline **7** (4 mmol, 1.0 equiv) was added to a solution of ketone **5** (4 mmol, 1.0 equiv) and *para*-toluenesulfonic acid (20 mol %) in *n*-butanol (10 mL). The reaction mixture was heated to 130 °C under nitrogen and stirred for 24 h. The solvent was removed under a vacuum and water (20 mL) was added. The aqueous solution was extracted with EtOAc (3 × 20 mL), dried over $MgSO_4$, and concentrated under a vacuum. The product was purified by column chromatography (eluting with 20% −80% EtOAc in *n*-hexane) to give quinolone **8**.

**8b**: White solid (Yield 69%); mp 277−278 °C; $^1$H NMR (400 MHz, DMSO) $\delta$ 11.76 (s, 1H), 8.90 (d, $J$ = 2.1 Hz, 1H), 8.37 − 8.31 (m, 2H), 8.25 (d, $J$ = 8.2 Hz, 1H), 8.17 (dd, $J$ = 8.2, 2.2 Hz, 1H), 8.15 (dd, $J$ = 7.0, 1.5 Hz, 1H), 7.65 (ddd, $J$ = 8.2, 6.8, 1.4 Hz, 1H), 7.60 (d, $J$ = 7.7 Hz, 1H), 7.55 (d, $J$ = 8.2 Hz, 2H), 7.33 (ddd, $J$ = 8.0, 6.8, 1.2 Hz, 1H), 1.96 (s, 3H); $^{13}$C NMR (101 MHz, DMSO) $\delta$ 176.98, 155.52, 149.75, 144.79, 139.95, 138.49, 137.46, 131.83, 130.30, 129.34, 129.17 (C-8′), 125.37, 123.50, 123.22, 121.70, 120.35, 119.18, 118.51, 115.59, 12.39; HRMS (ESI) $C_{22}H_{16}N_2O_2F_3$ $[M + H]^+$ requires $m/z$ 397.1164, found 397.1173. Anal. $C_{22}H_{15}N_2O_2F_3$ requires C 66.67%, H 3.81%, N 7.07%, found C 66.77%, H 3.73%, N 6.98%.

**8h**: Pale yellow solid (yield 30%); mp 317−319 °C; $^1$H NMR (400 MHz, DMSO) $\delta$ 11.83 (s, 1H), 8.90 (d, $J$ = 1.8 Hz, 1H), 8.39−8.31 (m, 2H), 8.24 (t, $J$ = 8.7 Hz, 1H), 8.22 − 8.11 (m, 2H), 7.55 (d, $J$ = 8.2 Hz, 2H), 7.30 (dd, $J$ = 10.1, 2.4 Hz, 1H), 7.20 (td, $J$ = 8.8, 2.5 Hz, 1H), 1.95 (s, 3H); $^{13}$C; HRMS (ESI) $C_{22}H_{14}N_2O_2F_4{}^{23}Na$ $[M + Na]^+$ requires $m/z$ 437.0889, found 437.0905.

**Procedure for the Synthesis of Pro-Drug 55.** Sodium hydride (0.57 mmol, 2.5 equiv) was added at 0 °C to a stirred solution of


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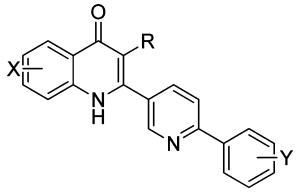
Table 6. In Vitro Antimalarial Activities of Bicyclic Pyridine Quinolones versus 3D7 *Plasmodium falciparum*[a]



| compound | R | X | Y | $IC_{50}$ (nM) 3D7 ± SD/ ($IC_{50}$ (nM) $PfNDH_2$) |
|---|---|---|---|---|
| **8b** | Me | H | 4-$OCF_3$ | 54 ± 6/(15) |
| **8c** | Me | 5-OMe | 4-$OCF_3$ | >1000 |
| **8d** | Me | 6-Cl | 4-$OCF_3$ | 298 ± 47 |
| **8e** | Me | 6-OMe | 4-$OCF_3$ | >1000 |
| **8f** | Me | 6-$OCF_3$ | 4-$OCF_3$ | >1000 |
| **8g** | Me | 6-$CF_3$ | 4-$OCF_3$ | >1000 |
| **8h** | Me | 7-F | 4-$OCF_3$ | 75 ± 9/(4.2) |
| **8j** | Me | 7-Cl | 4-$OCF_3$ | 373 ± 74 |
| **8k** | Me | 7-$CF_3$ | 4-$OCF_3$ | >1000 |
| **8l** | Me | 7-$SO_2$Me | 4-$OCF_3$ | >1000 |
| **8m** | Me | 8-OMe | 4-$OCF_3$ | >1000 |
| **8n** | Me | 5-F, 7-F | 4-$OCF_3$ | 286 ± 28/(2.6) |
| **8o** | Me | 6-F, 7-F | 4-$OCF_3$ | 344 ± 68 |
| **8p** | Me | 6-Cl, 7-F | 4-$OCF_3$ | 390 ± 20 |
| **8q** | Me | 6-F, 7-Cl | 4-$OCF_3$ | 390 ± 80 |
| **8r** | Me | H | 4-$CF_3$ | 102 ± 19/(13.9) |
| **8s** | Me | H | 2-F, 4-F | 219 ± 62/(88.6) |
| **8t** | Me | H | 3-OMe | 556 ± 41 |
| **10a** | Me | 6-OH | 4-$OCF_3$ | 280 ± 50 |
| **10b** | Me | 7-OH | 4-$OCF_3$ | 202 ± 79 |
| **10c** | Me | 8-OH | 4-$OCF_3$ | >1000 |
| **11a** | Me | H | 3-OH | >1000 |
| **11b** | Me | H | 4-OH | >1000 |
| **12e** | Me | H | 2-$CF_3$ | 109 ± 13/(352) |
| **12f** | Me | H | 2-F | 151 ± 22/(131) |
| **21** | $CO_2$Et | H | 4-$OCF_3$ | 987 ± 17 |
| **22** | $CH_2$OH | H | 4-$OCF_3$ | 103 ± 18/(407) |
| **27** | OMe | H | 4-$OCF_3$ | 93 ± 2 |

[a] P*fbc*$_1$ $IC_{50}$ data(nM): **8b** = 15, **8h** = 26.8.

quinolone (0.23 mmol, 1.0 equiv) in dry THF (10 mL). After 1 h, tetrabenzyl pyrophosphate (0.19 mmol, 0.8 equiv) was added and the stirring continued for 20 min. The mixture was filtered and the filtrate concentrated under a vacuum at a temperature below 35 °C. The residue was dissolved in DCM, washed with $NaHCO_3$ aq, dried over $MgSO_4$, and concentrated under vacuum to give phosphonate **55**. Where necessary the product was purified by flash column chromatography (eluting with 10% ethyl acetate in *n*-hexane).

**55**: Pale yellow solid (yield 70%); MP 257−258 °C; $^1$H NMR (400 MHz, DMSO) $\delta$ 11.77 (s, 2H), 8.91 (s, 1H), 8.37−8.27 (m, 3H), 8.17 (dd, $J$ = 6.9, 1.7 Hz, 2H), 8.02 (d, $J$ = 8.4 Hz, 1H), 7.76 (t, $J$ = 7.6 Hz, 1H), 7.68−7.59 (m, 1H), 7.54 (m, 2H), 2.46 (s, 3H); $^{31}$P NMR (162 MHz, DMSO) $\delta$ −1.17, −5.89.

**Procedure for the Synthesis of Pro-Drug 56.** **8b** (124 mg, 0.31 mmol) in anhydrous THF was added $^t$BuOK (52.7 mg, 0.47 mmol) at room temperature. The mixture was stirred for 1/2 h. 4-Morpholinecarbonyl chloride (0.05 mL, 0.41 mmol) was added. The mixture was stirred for a further 2 h (followed by TLC). The reaction was quenched with brine and was extracted with ethyl acetate, dried over $Na_2SO_4$, filtered, and concentrated to an oil. The crude product was purified by column chromatography using 20% ethyl acetate in hexane to give the title compound.

**56**: White solid (125 mg, Yield 78%); mp 150−151 °C; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.96 (dd, $J$ = 2.2, 0.8 Hz, 1H), 8.15 (d, $J$ = 8.4 Hz, 1H), 8.13−8.10 (m, 2H), 8.08 (dd, $J$ = 8.1, 2.3 Hz, 1H), 7.91−7.81 (m, 2H), 7.73 (ddd, $J$ = 8.4, 6.9, 1.4 Hz, 1H), 7.60 (ddd, $J$ = 8.2, 6.9, 1.1 Hz, 1H), 7.36 (d, $J$ = 8.0 Hz, 2H), 3.91 (m, 2H), 3.89 (m, 2H), 3.85 (m, 2H), 3.67 (m, 2H), 2.40 (s, 3H); HRMS (ESI) $C_{27}H_{23}N_3O_4F_3$ $[M + H]^+$ requires $m/z$ 510.1641, found 510.1637.

**Biology.** *Parasite Culture.* *Plasmodium* blood stage cultures[15] and drug sensitivity[16] were determined by established methods. $IC_{50}$s (50% inhibitory concentrations) were calculated by using the four-parameter logistic method (Grafit program; Erithacus Software, United Kingdom)

*High-Throughput Screening (HTS).* PfNDH2 activity was measured using an end-point assay in a 384-well plate format. Final assay concentrations used were 200 $\mu$M NADH, 10 mM KCN, 1 $\mu$g/mL F571 membrane,[6] and 20 $\mu$M decylubiquinone (dQ). A pre-read at 340 nm was obtained prior to the addition of dQ to initiate the reaction followed by a post-read at 1 min. HDQ was used as positive control at 5 $\mu$M. The agreed QC pass criteria was Z′ > 0.6 and signal/background >10. Compounds were selected by the described chemoinformatics algorithms from the Biofocus DPI compound library (Galapagos Company).

*Enzymology.* *P. falciparum* cell-free extracts were prepared from erythrocyte-freed parasites as described previously,[13] and recombinant PfNDH2 was prepared from the *Escherichia coli* heterologous expression strain F571.[6] PfNDH2 and $bc_1$ activities were measured as described previously.[6,13]

*Pharmacology.* In vivo efficacy studies were measured against *P. berghei* (NS-Strain) in the standard 4-day test.[14] All in vivo studies


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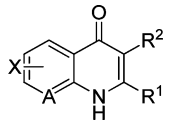
Table 7. In Vitro Antimalarial Activities of Other Bicyclic Quinolones versus 3D7 *Plasmodium falciparum*



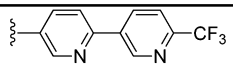
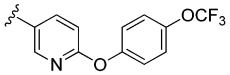
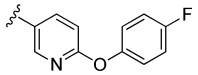
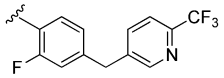
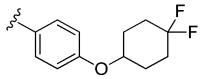
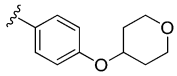
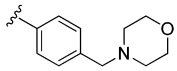
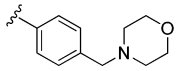
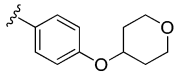
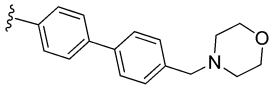
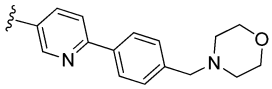
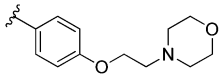
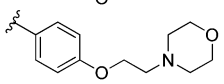
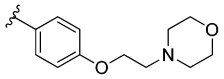
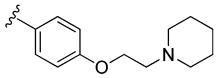
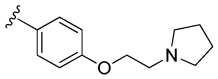
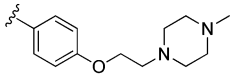
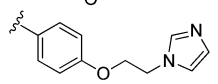
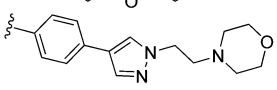
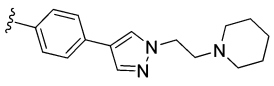
| Compound | $R^1$ | $R^2$ | X | A | $IC_{50}$ (nM) 3D7 ± SD/($IC_{50}$ (nM) $PfNDH_2$) |
|---|---|---|---|---|---|
| **8v** |  | Me | H | CH | 370 ± 80 |
| **8w** |  | Me | H | CH | 40 ± 10/(110) |
| **8x** |  | Me | H | CH | 279 ± 16/(588) |
| **8y** |  | Me | H | CH | 136 ± 12 |
| **8z** |  | Me | H | CH | 319 ± 100 |
| **9a** |  | Me | H | CH | >1000 |
| **31a** |  | Me | 7-Cl | CH | >1000 |
| **31b** |  | Me | H | CH | 32 ± 8 |
| **35** |  | Me | H | N | >1000 |
| **36a** |  | Me | H | CH | 496 ± 30 |
| **36b** |  | Me | H | CH | >1000 |
| **40a** |  | Me | H | CH | >1000 |
| **40b** |  | Me | 6-Cl | CH | >1000 |
| **40c** |  | Me | 7-Cl | CH | >1000 |
| **40d** |  | Me | H | CH | >1000 |
| **40e** |  | Me | H | CH | >1000 |
| **40f** |  | Me | H | CH | >1000 |
| **40g** |  | Me | H | CH | >1000 |
| **42a** |  | Me | H | CH | >1000 |
| **42b** |  | Me | H | CH | >1000 |



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Table 7. continued

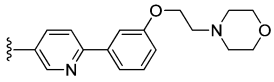
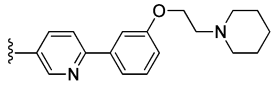
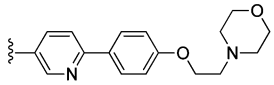
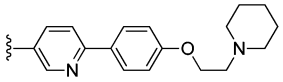
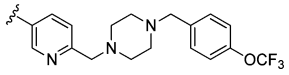
| Compound | $R^1$ | $R^2$ | X | A | $IC_{50}$ (nM) 3D7 ± SD/($IC_{50}$ (nM) $PfNDH_2$) |
|---|---|---|---|---|---|
| **46a** |  | Me | H | CH | >1000 |
| **46b** |  | Me | H | CH | >1000 |
| **46c** |  | Me | H | CH | >1000 |
| **46d** |  | Me | H | CH | 600 |
| **53** |  | H | H | CH | 679 ± 80 |

Table 8. In Vitro Antimalarial Activities of Selected Quinolones versus TM90C2B

| compound | $IC_{50}$ (nM) TM90C2B ± SD | compound | $IC_{50}$ (nM) TM90C2B ± SD |
|---|---|---|---|
| **8b** | 344 ± 36 | **12e** | 381 ± 144 |
| **8d** | 410 ± 49 | **12f** | 324 ± 76 |
| **8r** | 314 ± 87 | **16b** | 1150 ± 95 |
| **12a** | 326 ± 42 | **17** | 361 ± 79 |

Table 9. In Vitro Antimalarial Activities of Selected Quinolones versus W2

| compound | $IC_{50}$ (nM) W2 ± SD/($IC_{50}$ (nM) 3D7 ± SD) | compound | $IC_{50}$ (nM) W2 ± SD ($IC_{50}$ (nM) 3D7 ± SD) |
|---|---|---|---|
| **8b** | 50 ± 12.7 (54 ± 6) | **8y** | 67 ± 3.9 (136 ± 12) |
| **8h** | 70 ± 9.3 (75 ± 9) | **12e** | 88 ± 22 (109 ± 13) |
| **8v** | 329 ± 41 (370 ± 80) | **12f** | 120 ± 6.2 (151 ± 22) |
| **8w** | 50 ± 2.5 (40 ± 10) | **31b** | 3073 ± 104 (32 ± 8) |

Table 10. In Vivo Peters' Standard 4-Day Test − Oral Administration[a]

| | | % parasite clearance on day 4 (20 mg/kg po) | | |
|---|---|---|---|---|
| | | vehicle | | |
| compound | ClogP | SSV | DET | $Na_2CO_3$ |
| atovaquone | 6.35 | 100 | 100 | ND |
| **8b** | 4.36 | 87.5 | 100 | ND |
| **8b·$H_3PO_4$** | | 100 | ND | ND |
| **55** | 4.94 | ND | ND | 100 |
| **56** | 5.61 | 100 | ND | ND |

[a]Four-day suppressive activity of key compounds in male CD-1 mice infected with *Plasmodium berghei* (NS Strain) Mice were exposed to the infection via intraperitoneal injection and then orally dosed with the relevant compound. Data were obtained from five mice per group.

were approved by the appropriate institutional animal care and use committee and conducted in accordance with the International Conference on Harmonization (ICH) Safety Guidelines.

Table 11. In Vivo (Oral) Antimalarial Activities of Quinolone 8b versus *Plasmodium berghei*

| compound | $ED_{50}$ (mg/kg) | $ED_{90}$ (mg/kg) |
|---|---|---|
| **8b** | 12.75 | 27.3 |
| **8b·$H_3PO_4$** | 1.87 | 4.72 |
| atovaquone | 0.07 | 0.11 |
| chloroquine | 3.3 | 4.6 |
| artemether | 3.1 | 5.8 |

## ■ ASSOCIATED CONTENT

### Ⓢ Supporting Information

Supporting Information includes the following: (1) Experimental details for all intermediates. (2) Further details on chemoinformatics (3) Measured solubility values.This material is available free of charge via the Internet at http://pubs.acs.org.

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## ■ ABBREVIATIONS

SAR, structure−activity relationship; NADH, nicotinamide adenine dinucleotide; $bc_1$, ubihydroquinone; ADMET, absorption, distribution, metabolism, excretion, toxicity; HTS, high-throughput screen; PTSA, *para*-toluene sulfonic acid; LAH,

lithium aluminum hydride; DMF, dimethylformamide; *m*-CPBA, *meta*-chloro per benzoic acid; KOH, potassium hydroxide; THF, tetrahydrofuran; DCM, dichloromethane; NADPH, nicotinamide adenine dinucleotide phosphate; NMP, *N*-methyl-2-pyrrolidone; SSV, standard suspension vehicle; DET, 5% DMSO and 5% EtOH in tetraglycol; PCC, Pyridinium chlorochromate

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