Journal of Medicinal Chemistry

Article

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Discovery, Synthesis, and Optimization of Antimalarial 4(1*H*)-Quinolone-3-Diarylethers

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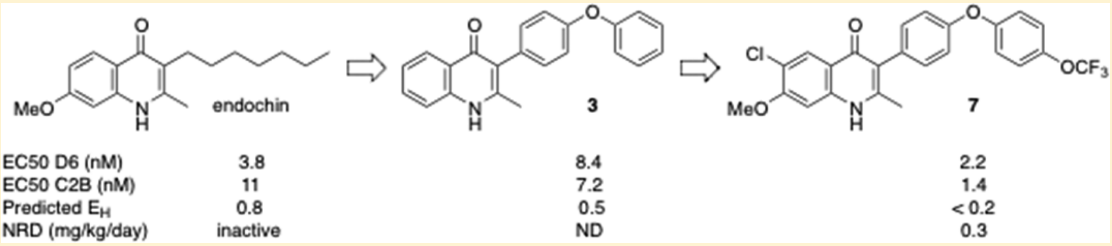
Supporting Information



	endochin	3	7
EC50 D6 (nM)	3.8	8.4	2.2
EC50 C2B (nM)	11	7.2	1.4
Predicted E_H	0.8	0.5	< 0.2
NRD (mg/kg/day)	inactive	ND	0.3

ABSTRACT: The historical antimalarial compound endochin served as a structural lead for optimization. Endochin-like quinolones (ELQ) were prepared by a novel chemical route and assessed for in vitro activity against multidrug resistant strains of *Plasmodium falciparum* and against malaria infections in mice. Here we describe the pathway to discovery of a potent class of orally active antimalarial 4(1*H*)-quinolone-3-diarylethers. The initial prototype, ELQ-233, exhibited low nanomolar IC_{50} values against all tested strains including clinical isolates harboring resistance to atovaquone. ELQ-271 represented the next critical step in the iterative optimization process, as it was stable to metabolism and highly effective in vivo. Continued analoging revealed that the substitution pattern on the benzenoid ring of the quinolone core significantly influenced reactivity with the host enzyme. This finding led to the rational design of highly selective ELQs with outstanding oral efficacy against murine malaria that is superior to established antimalarials chloroquine and atovaquone.

INTRODUCTION

Malaria is a deadly disease that has plagued human civilization down through recorded history and has been responsible for the deaths of millions, particularly infants, young children, and expectant mothers.[1] While progress has been made over the past decade to lessen the impact of the disease on global health, widespread multidrug resistance threatens to reverse recent gains brought about by the use of insecticide-treated bed nets and artemisinin-combination therapies. New drugs that are active against multiple stages of the *Plasmodium* parasite's life cycle, including transmissible forms in the host and mosquito vector, will be important for pressing forward with a worldwide effort to contain and eliminate malaria from the many countries where it remains endemic.[2]

Interest in the antimalarial properties of 3-alkyl-4(1*H*)-quinolones dates to the 1940s and the pioneering research led by Hans Andersag at Bayer IG Farbenindustrie in Elberfeld, Germany.[3] Their work on this particular class of molecules focused principally on endochin (see Figure 1) because it was highly active against blood and tissue stages of avian malaria (*Plasmodium cathemerium* in canaries), a preclinical model

Received: January 28, 2014
Published: April 10, 2014

   dx.doi.org/10.1021/jm500147k | J. Med. Chem. 2014, 57, 3818−3834

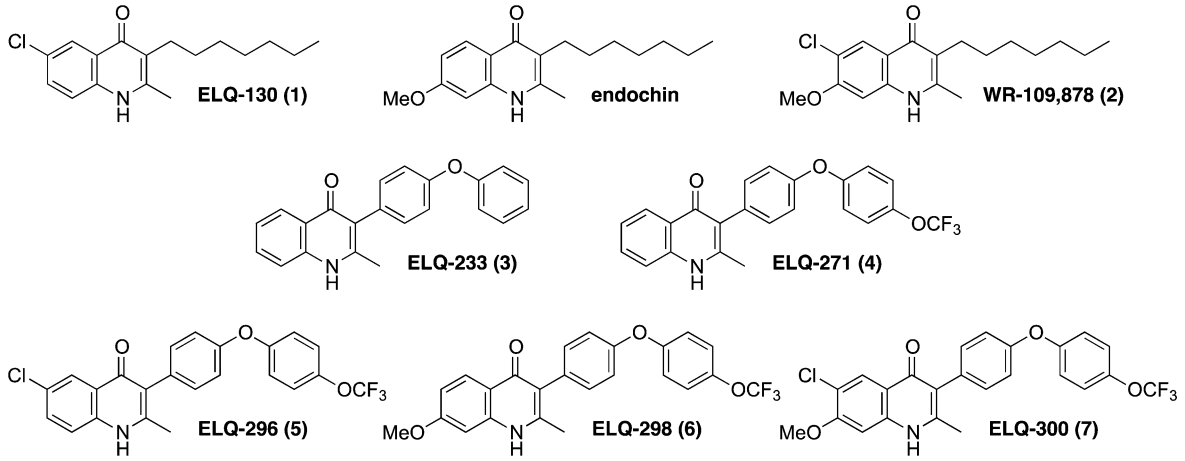


Figure 1. Lead optimization.

system in use at the time.[4] However, their attempts to demonstrate efficacy with endochin in humans failed. Recently we showed that endochin is extensively metabolized (including *O*-demethylation as well as hydroxylation of the ring nitrogen and heptyl side chain) to poorly active metabolites by human hepatic microsomal enzymes, thus providing one possible explanation for the patient treatment failures over six decades ago.[5]

With intent to develop an endochin-like quinolone (ELQ) that targets multiple stages of the malaria life cycle, we set out to design derivatives with enhanced metabolic stability and potent activity against drug resistant malaria. Our early work[5] showed that endochin analogues with a halogen at the 6-position, such as ELQ-130 (**1**) (Figure 1), were equipotent against *Plasmodium falciparum* strains resistant to chloroquine, quinine, mefloquine, and the folate antagonists, pyrimethamine and sulfadoxine, as well as the antirespiratory compound atovaquone (Figure 2).

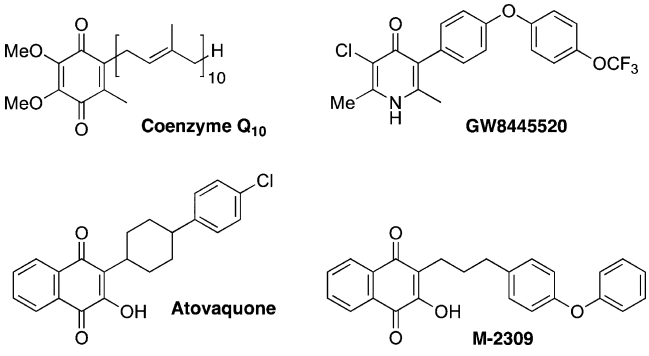


Figure 2. Chemical structures of compounds important to the design and optimization of endochin analogues (ELQs).

Our studies revealed that ELQs effectively shut down the parasite respiratory processes within minutes following drug addition.[6] A carbonate prodrug of a potent endochin analogue, 5,7-difluoro-3-heptyl-2-methylquinolin-4(1*H*)-one, was curative against patent malaria infections in mice, albeit at relatively high doses of 50−100 mg/kg/day.[5]

At this point, we joined a research consortium supported by the Medicines for Malaria Venture (Geneva, Switzerland) for chemical optimization of endochin. We proposed to replace the metabolically labile heptyl side chain at position 3 of endochin with a substituted diarylether, taking a cue for this from Yeates and co-workers,[7] who described a series of diphenylether containing pyridones that, like atovaquone, mimic the structure of coenzyme Q and target the *Plasmodium* cytochrome bc_1 complex.[7,8] A late lead from this series, GW844520, appears in Figure 2. Another clue to this modification came from earlier work by Fieser and colleagues, who described an antimalarial hydroxynaphthoquinone containing a 3-alkyl-diphenylether side chain (designated M-2309, Figure 2) with enhanced metabolic stability.[9] We report here the discovery of 4(1*H*)-quinolone-3-diarylethers and the in vitro and in vivo structure−activity profiles that significantly impacted the optimization path toward the design and selection of a preclinical candidate with the potential for prevention and treatment of malaria and for interfering with disease transmission.

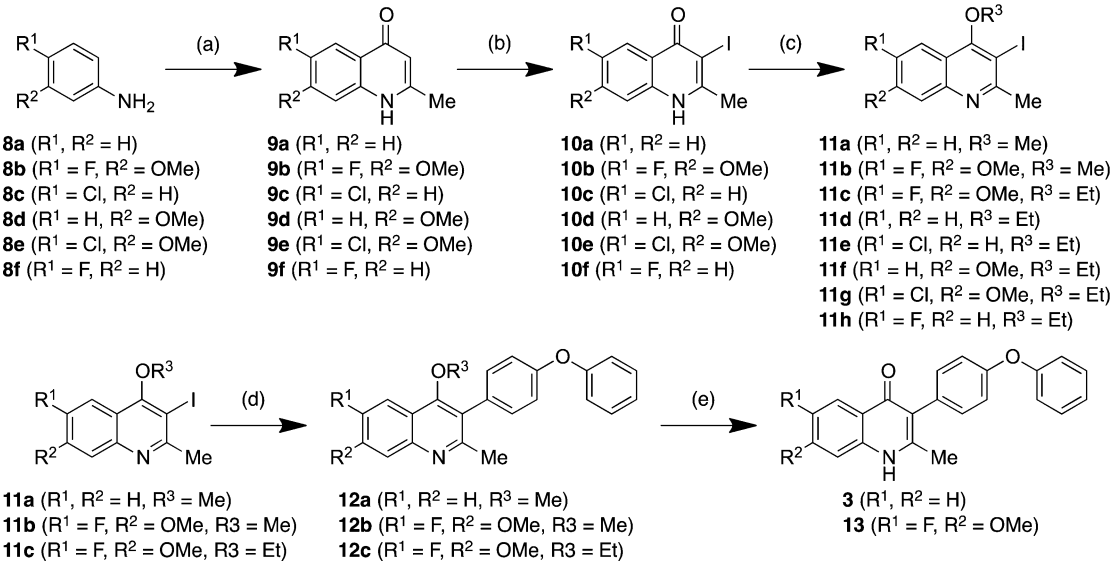
CHEMISTRY

Iodination of commercially available 4(1*H*)-quinolone **9a** with iodine in saturated aqueous potassium iodide solution and *n*-butylamine provided the 3-iodo-4(1*H*)-quinolone **10a** (Scheme 1). Direct Suzuki−Miyaura reaction of **10a** with 4-phenoxyphenyl boronic acid resulted in an inseparable mixture of products. 4(1*H*)-Quinolones suffer from low solubility in almost all solvents and are thus difficult to isolate chromatographically. Mphahlele and Mtshemla showed that 3-aryl-4(1*H*)-quinolones could be prepared via Suzuki−Miyaura reaction of 3-iodo-4-methoxyquinolones.[10] Conversion of **10a** to the corresponding 4-chloro-3-iodoquinoline in neat phosphorus oxychloride followed by nucleophilic displacement of the chloro substituent with sodium methoxide provided 3-iodo-4-methoxyquinolone **11a**. Suzuki−Miyaura reaction of **11a** with phenoxyphenylboronic acid in the presence of palladium tetrakis triphenyphosphine (0) and aqueous potassium carbonate provided 4-methoxyquinolone-3-diarylether **12a**. Removal of the methyl ether-protecting group with 3 equiv of BBr_3 provided the desired 4(1*H*)-quinolone-3-diarylether ELQ-233 (**3**) (Figure 1) in excellent overall yield.

Because the lead compound, endochin, has a methoxy group at the 7-position, it was important to demonstrate that the quinolone 4-methoxy ether could be selectively cleaved in the presence of an aryl methoxy moiety. Thus, the synthesis of 4(1*H*)-quinolone-3-diarylether ELQ-262 (**13**) was undertaken. Condensation of commercially available 4-fluoro-3-methoxyaniline **8b** and ethyl acetoacetate followed by thermal cyclization provided 2-methyl-4(1*H*)-quinolone **9b** via Conrad−Limpach synthesis.[11] Iodination using the method described above followed by selective oxygen alkylation using methyl iodide

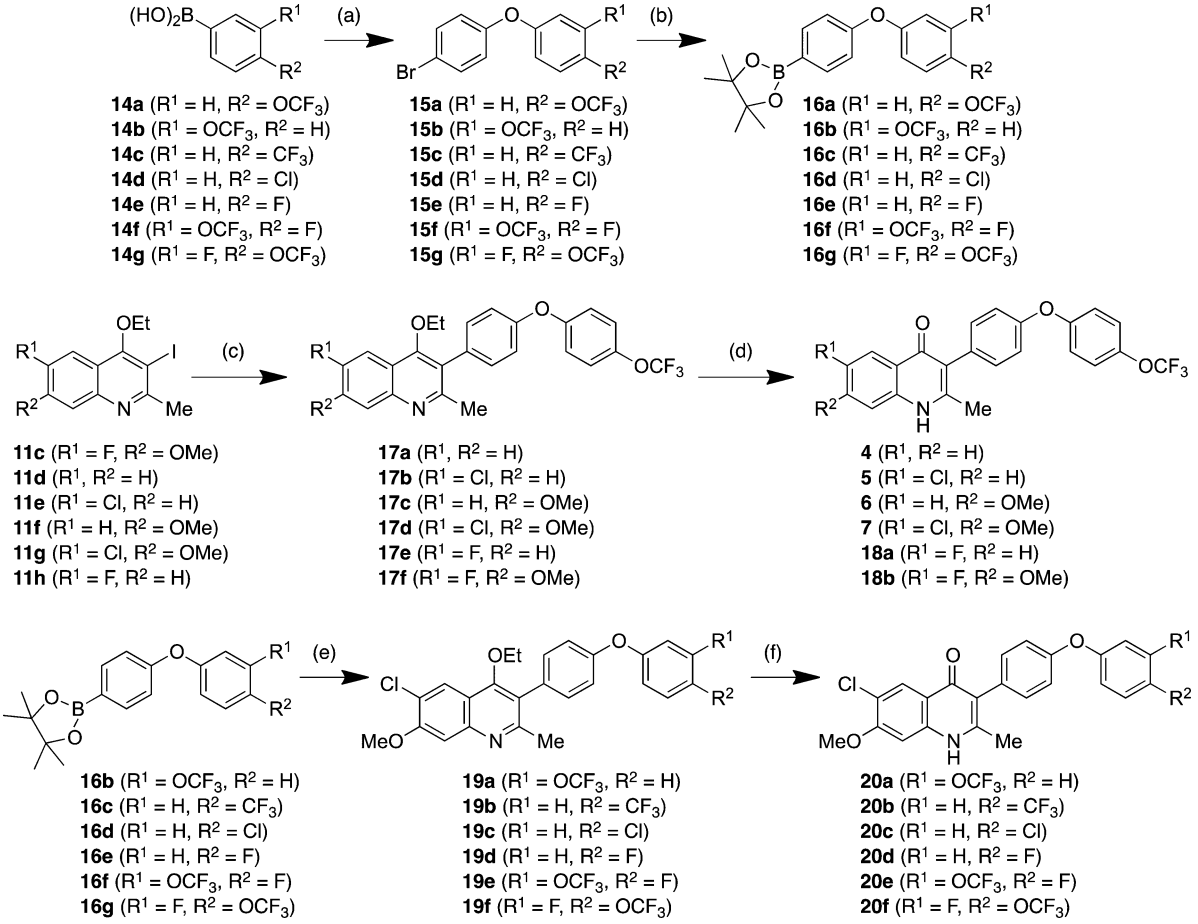
dx.doi.org/10.1021/jm500147k | J. Med. Chem. 2014, 57, 3818−3834

Scheme 1[a]



[a](a) (1) Ethyl acetoacetate, *p*-TsOH, benzene, reflux, (2) DOWTHERM A, 250 °C; (b) iodine, aq KI, *n*-butylamine, DMF; (c) MeI or EtI, K_2CO_3, DMF; (d) 4-phenoxyphenyl boronic acid, $Pd(PPh_3)_4$, aq K_2CO_3, DMF, 85 °C; (e) BBr_3, CH_2Cl_2 or 48% aq HBr, AcOH, 90 °C.

Scheme 2[a]

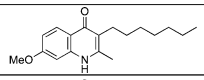
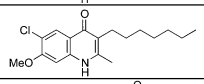
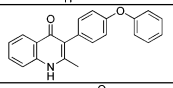
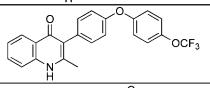
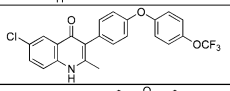
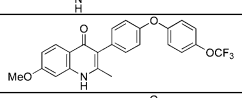
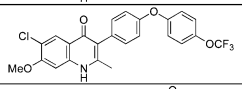
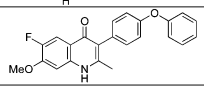
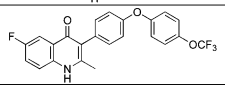
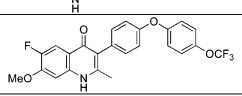
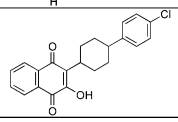


[a](a) 4-Bromophenol, $Cu(OAc)_2$, DIPEA, pyridine, DCM; (b) $Pd(dppf)_2Cl_2$, bis(pinacolato)diboron, KOAc, DMF, 80 °C; (c) **16a**, $Pd(PPh_3)_4$, aq K_2CO_3, DMF, 85 °C; (d); 48% aq HBr, AcOH, 90 °C; (e) **11g**, $Pd(PPh_3)_4$, aq K_2CO_3, DMF, 85 °C; (f) 48% aq HBr, AcOH, 90 °C.

and potassium carbonate provided the 3-iodo-4-methoxyquinolone **11b**. 4-Methoxyquinolone-3-diarylether **12b** was obtained from **11b** using the Suzuki reaction described above. However, deprotection of **12b** with 3 equiv of BBr_3 resulted in the cleavage

dx.doi.org/10.1021/jm500147k | J. Med. Chem. 2014, 57, 3818−3834

Table 1. Optimization of Benzenoid Ring Substituents[a]

Compound	Structure	cLogP	EC_{50} D6 (nM)	EC_{50} Dd2 (nM)	EC_{50} Tm90-C2B (nM)	IC_{50} Cytotox. (μM)	IC_{50} Human Cyt. bc_1 (μM)
endochin		3.35	3.8	3.1	11	> 10	> 10
2		3.91	1.4	2.3	0.80	ND	> 10
3		3.70	8.4	12	7.2	> 10	ND
4		5.22	4.1	5.9	7.5	> 10	1.9
5		5.78	3.2	5.6	2.3	> 10	> 10
6		5.10	1.7	3.4	2.3	> 10	> 10
7		5.66	2.2	2.5	1.4	> 10	> 10
13		3.73	40	43	8.5	> 10	ND
18a		5.38	1.5	2.4	1.7	> 10	> 10
18b		5.26	1.4	1.4	0.50	> 10	> 10
atovaquone		3.68	0.10	0.10	7,700	> 10	0.41

[a]clogP values were calculated using ChemDraw Ultra software (version 12). EC_{50} values are the average of at least three determinations, each carried out in triplicate. Cytotoxicity assays (IC_{50} values) were carried out with human hepatoma derived HepG2 cells and performed in triplicate across an initial concentration range of 2.5 nM to 10 μM. Full details of each of these biological and biochemical assays can be found in the Experimental Section. ND = not determined.

of both methyl ethers. Various selective deprotection methods were evaluated by HPLC including less BBr_3 (1.5 equiv), refluxing 48% aqueous HBr, and refluxing 1:1 48% aqueous HBr and acetic acid. However, it was not possible to selectively deprotect the 4-methoxy position in the presence of the 7-methoxy moiety. Considering that an ethyl group would form a more stable carbocation intermediate under acidic cleavage conditions compared to the corresponding methyl ether, we prepared 4-ethoxyquinolone-3-diarylether **12c**. Deprotection of **12c** in 1:2 48% aqueous HBr and acetic acid at 90 °C gave complete and selective conversion to 4(1*H*)-quinolone-3-diarylether **13**.

The boronic ester of the trifluoromethoxy diarylether side chain **16a** was then prepared (Scheme 2). Copper-mediated coupling of commercially available 4-bromophenol and 4-trifluoromethoxyphenyl boronic acid **14a** with Hunig's base and pyridine afforded the bromo diarylether **15a**.[12] Palladium-mediated coupling of **15a** and bis(pinacolato) diboron with potassium acetate gave the desired boronic ester **16a**. A series of benzenoid ring substitituted 4(1*H*)-quinolone-3-diarylethers (Table 1) was prepared from the appropriate 3-iodo-4-ethoxy-quinolones **11c–h** and boronic ester **16a** using the above method. A series of 4(1*H*)-quinolone-3-diarylethers varying in the side chain portion of the molecule (Table 3) was prepared from 3-iodo-4-ethoxy-quinolone **11g** and the appropriate boronic esters **16b–g** also using the above method.

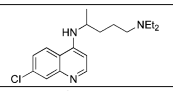
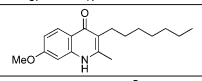
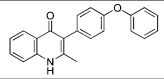
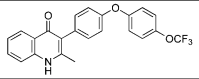
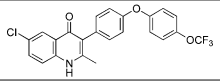
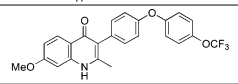
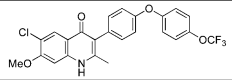
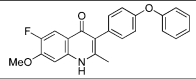
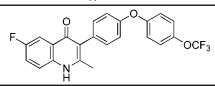
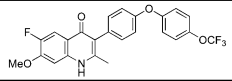
Preparation of heterocyclic diarylethers using the copper (II) acetate coupling described above proved problematic. However, a series of heterocyclic diarylether compounds (**22a–c** and **28**) was prepared by coupling of commercially available aryl iodides **21a–c** with 4-trifluoromethoxy phenol (Scheme 3) or by coupling of 4-bromophenol with 1-iodo-4-trifluoromethylpyridine (Scheme 4) in the presence of copper (I) iodide, picolinic acid, and tribasic potassium phosphate.[13] A series of 4(1*H*)-quinolone-3-diarylethers with heterocyclic side chains (Table 4) was prepared using the above method.

RESULTS AND DISCUSSION

Here we describe some of the key compounds and assays that helped to guide us through the discovery phase and to accelerate the optimization process leading to highly active molecules of the 4(1*H*)-quinolone-3-diarylether class of antimalarials. At the outset of research activities with the Medicines for Malaria Venture, the primary challenges to endochin optimization were

dx.doi.org/10.1021/jm500147k | J. Med. Chem. 2014, 57, 3818–3834

Table 2. Optimization of Benzenoid Ring Substituents: Metabolic Stability and in Vivo Efficacy[a]

Compound	Structure	Predicted E_H (human microsomes)	ED_{50} (mg/kg/day)	NRD (mg/kg/day)
chloroquine		ND	2.2	> 64
endochin		0.8	inactive	inactive
3		0.5	ND	ND
4		< 0.2	0.1	3.0
5		< 0.2	0.2	> 3.0
6		0.5	ND	ND
7		< 0.2	0.02	0.3
13		0.6	ND	ND
18a		< 0.2	0.1	3.0
18b		< 0.2	0.05	ND

[a]Detailed procedures for assay of microsomal stability and for in vivo assessment of antimalarial activity against *P. yoelii* infected mice can be found in the Experimental Section. ND = not determined.

clear: (1) enhance metabolic stability, (2) enhance aqueous solubility, (3) eliminate cross-resistance in clinical strains of *P. falciparum*, and (4) diminish the propensity for resistance, i.e., relative to atovaquone for which resistance arises quickly.

Early in the analoging work we obtained X-ray diffraction analysis of the crystal structure for the endochin derivative 5,7-difluoro-3-heptyl-2-methylquinolin-4(1*H*)-one.[5] The crystal structure revealed extensive $\pi-\pi$ stacking in the Z plane and an extensive network of intermolecular H-bonds in the $X-Y$ plane (see Supporting Information Figure S1). A decision was made to place an aryl group at position 3 because the adjacent 2-position CH_3 would force an out-of-plane movement of the bulky aromatic ring, thereby potentially disrupting $\pi-\pi$ interactions and reducing the crystal lattice energy. We chose the lipophilic diphenylether moiety because it was a key structural element for the highly active antimalarial GSK pyridones[7,14] and earlier 2-hydroxy-naphthoquinones from Fieser's research program.[9,15]

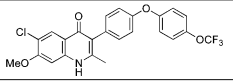
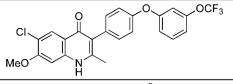
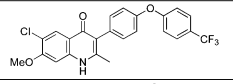
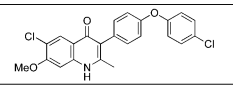
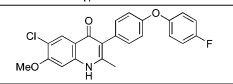
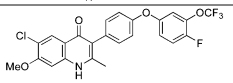
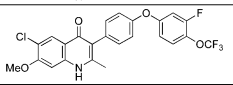
All of the compounds were evaluated for parasite growth inhibitory activity in vitro by a microplate-based assay in which SyBr Green 1 fluorescent dye was utilized to quantitate parasite double stranded DNA following a 72 h incubation period.[16] In vivo efficacy was determined in a murine *Plasmodium yoelii* model in which animals were randomly placed in groups of four and administered test drugs by oral gavage on four sequential days following the day of inoculation. The in vivo data are expressed as ED_{50} values and reflect the dose (estimated from dose–response curves) for suppression of parasitemia by 50% relative to vehicle-only controls as assessed on day 5 of each study. Drug treated animals that were parasite free on day 30 of the experiment are defined as "cures", and the amount of drug that was needed to achieve a cure is referred to as the "non-recrudescence dose" (NRD).

Compounds **3** and **13** were prepared as described above from commercially available phenoxyphenylboronic acid. Unsubstituted in the benzenoid ring of the quinolone core, compound **3**, the first compound in this subseries, showed equipotency (EC_{50} values 10 nM) against all three tested strains including multidrug resistant strains and an atovaquone-resistant clinical isolate Tm90-C2B (Table 1). Compound **13**, with a fluorine atom at position 6 and a methoxy group at position 7, also exhibited impressive low nanomolar EC_{50} values against all three strains. Both of these compounds were metabolically unstable in the presence of rat and human liver microsomes (Table 2) with in vitro degradation half-lives of 10.6–92.7 min and microsome-predicted hepatic extraction ratios (E_H) in the intermediate to high range of 0.5–0.8. In contrast, there was no detectable degradation in the microsomal matrix with ELQ-271 (**4**) (Figure 1), and the intrinsic antiplasmodial activity was enhanced about 2-fold over its progenitors. Clearly, the introduction of an OCF_3 group at the *para* position of the outermost ring of the diarylether side chain stabilized the drug against liver derived microsomal enzymes.

The effect of substituents on the benzenoid ring of the quinolone core was further explored to optimize the scaffold for potency and lack of cross-resistance, focusing especially on the

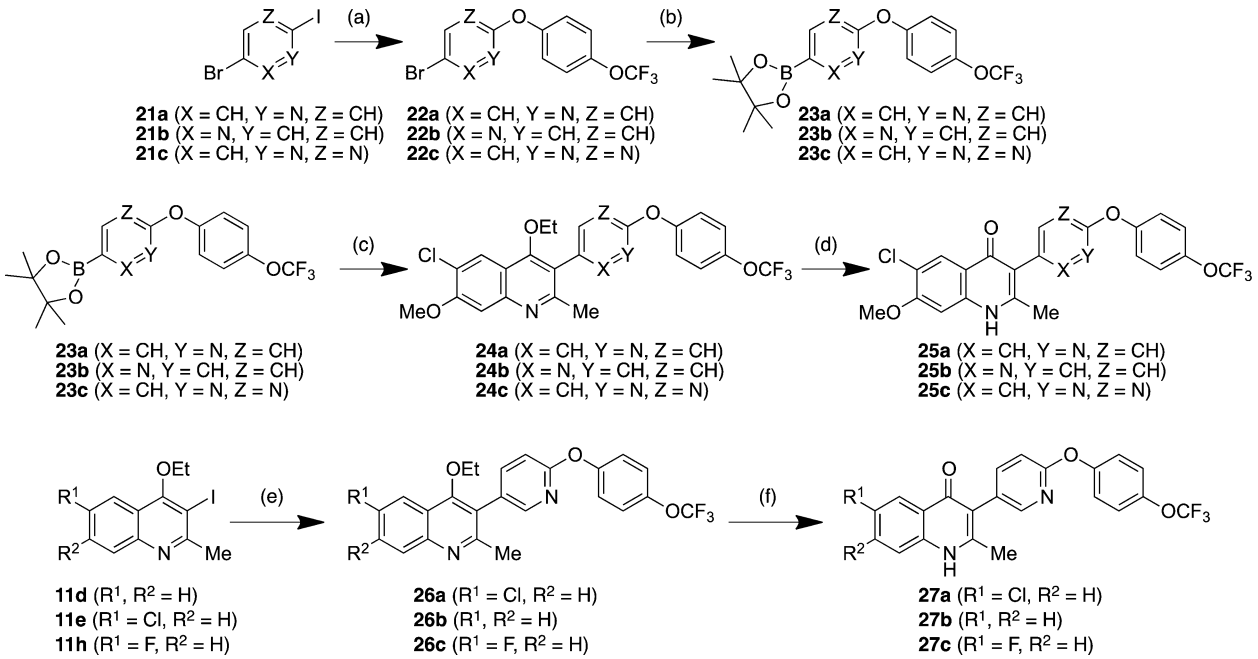
dx.doi.org/10.1021/jm500147k | J. Med. Chem. 2014, 57, 3818–3834

Table 3. Optimization of Diarylether Side Chain: Substituents[a]

Compound	Structure	cLogP	EC_{50} D6 (nM)	EC_{50} Dd2 (nM)	EC_{50} Tm90-C2B (nM)	IC_{50} Cytotox. (μM)
7		5.66	2.2	2.5	1.4	> 10
20a		5.66	0.03	0.03	< 0.03	> 10
20b		5.05	0.3	0.3	0.05	> 10
20c		4.69	1.9	1.8	1.2	> 10
20d		4.29	0.8	3.8	ND	> 10
20e		5.81	0.4	0.5	0.3	> 10
20f		5.81	12	12	5.8	> 10

[a]clogP values were calculated using ChemDraw Ultra software (version 12). EC_{50} values are the average of at least three determinations, each carried out in triplicate. Cytotoxicity assays (IC_{50} values) were carried out with human hepatoma derived HepG2 cells and performed in triplicate across an initial concentration range of 2.5 nM to 10 μM. Full details of each of these biological and biochemical assays can be found in the Experimental Section. ND = not determined.

Scheme 3[a]

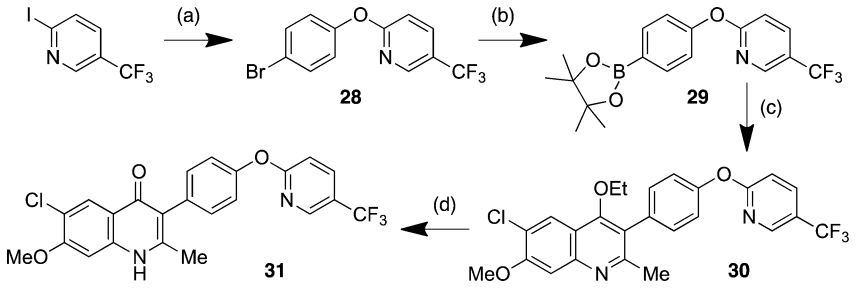


[a](a) 4-Trifluoromethoxy phenol, CuI, picolinic acid, K_3PO_4, DMSO, 80 °C; (b) $Pd(dppf)_2Cl_2$, bis(pinacolato)diboron, KOAc, DMF, 80 °C; (c) **11g**, $Pd(PPh_3)_4$, aq K_2CO_3, DMF, 85 °C; (d) 48% aq HBr, AcOH, 90 °C; (e) **19a**, $Pd(PPh_3)_4$, aq K_2CO_3, DMF, 85 °C; (f) 48% aq HBr, AcOH, 90 °C.

atovaquone resistant isolate. On the basis of earlier work by Andersag relating to endochin (which exhibits modest cross resistance vs Tm90-C2B), our earlier discovery of 6-position halogenated variants **1** and 6-fluoro-3-heptyl-2-methylquinolin-4(1*H*)-one (i.e., lack of atovaquone cross resistance),[5] as well as the 6-Cl/7-OCH_3 combination provided by WR-109,878 (**2**) as published previously by Cross et al.[17] (and also lacking atovaquone cross resistance), we completed the synthesis of ELQ-296 (**5**), ELQ-298 (**6**), ELQ-300 (**7**), ELQ-314 (**18a**), and ELQ-316 (**18b**) (Scheme 2). In vitro microsomal assays showed

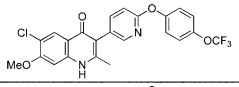
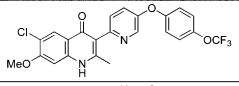
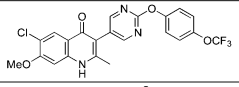
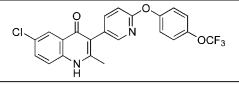
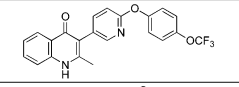
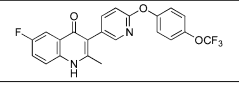
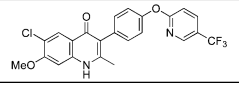
dx.doi.org/10.1021/jm500147k | J. Med. Chem. 2014, 57, 3818−3834

Scheme 4[a]



[a](a) CuI, picolinic acid, K_3PO_4, DMSO, 80 °C; (b) $Pd(dppf)_2Cl_2$, bis(pinacolato)diboron, KOAc, DMF, 80 °C; (c) **11g**, $Pd(PPh_3)_4$, aq K_2CO_3, DMF, 85 °C; (d) 48% aq HBr, AcOH, 90 °C.

Table 4. Optimization of Diarylether Side Chain: *N*-Heterocycles[a]

Compound	Structure	cLogP	EC_{50} D6 (nM)	EC_{50} Dd2 (nM)	EC_{50} Tm90-C2B (nM)	IC_{50} Cytotox. (μM)
25a		5.04	0.3	0.2	0.4	> 10
25b		4.74	0.4	0.5	0.2	> 10
25c		4.23	> 2,500	> 2,500	> 2,500	> 10
27a		5.16	3.6	6.1	1.5	> 10
27b		4.60	3.0	5.8	4.3	> 10
27c		4.76	13	13	11	> 10
31		4.43	8.1	6.8	4.1	> 10

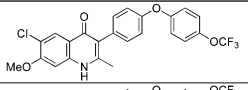
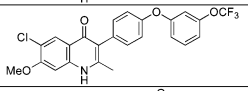
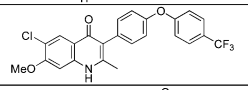
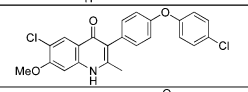
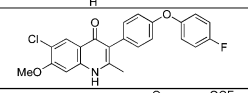
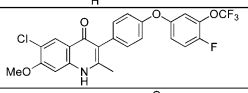
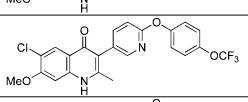
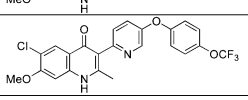
[a]clogP values were calculated using ChemDraw Ultra software (version 12). EC_{50} values are the average of at least three determinations, each carried out in triplicate. Cytotoxicity assays (IC_{50} values) were carried out with human hepatoma derived HepG2 cells and performed in triplicate across an initial concentration range of 2.5 nM to 10 μM. Full details of each of these biological and biochemical assays can be found in the Experimental Section. ND = not determined.

that **6** was metabolized at a moderate to intermediate rate in both rat and human hepatic microsomal mixtures, while the other compounds in this set were highly stable to metabolism. All of these molecules exhibited low nanomolar EC_{50} values against *P. falciparum* strains D6, Dd2, and Tm90-C2B. It is noteworthy that compound **7** was also highly active against *P. falciparum* strain Tm93-C1088 with an EC_{50} value of 1.0 nM (see Supporting Information Table S2). Like Tm90-C2B, this parasite[18] was isolated from a Thai patient who experienced a recrudescence of parasitemia after treatment with atovaquone. The inhibitory effect of this subseries of molecules was highly specific, as evidenced by the lack of cytotoxicity exhibited by any member of the set against proliferating HepG2 cells (a human hepatoma cell line) at concentrations as high as 10 μM.

The in vivo antimalarial efficacy of this initial series of ELQ derivatives is summarized in Table 2 and compared to endochin, the original lead, as well as chloroquine, a 4-aminoquinoline antimalarial drug. Consistent with earlier reports and our observation that it is metabolically unstable, endochin was inactive in vivo while the estimated ED_{50} value for chloroquine was 2.2 mg/kg/day (CQ was not curative in this model even at doses as high as 64 mg/kg/day). With the minimum structural elements needed for potency and metabolic stability, **4** showed a dramatic enhancement of in vivo efficacy over endochin with an ED_{50} value of 0.1 mg/kg/day and parasite-free cures, established on day 30, at 3 mg/kg/day. The 6-chloro analogue (**5**) was much less effective than **4**, which may relate to low aqueous solubility and poor oral bioavailability. Compound **7** (delivery vehicle: PEG400), with the 6-Cl/7-OCH_3 substitution pattern, provided excellent in vivo values against *P. yoelii* in mice with ED_{50} and ED_{90} values of 0.02 and 0.06 mg/kg/day, respectively, and a nonrecrudescence cure dose of 0.3 mg/kg/day. It is noteworthy that **7** was shown previously to be 30-fold more effective than atovaquone against murine malaria in side-by-side tests.[19] The 6-F and 6-F/7-OCH_3 analogues (i.e., **18a** and **18b**) within this series, with reduced cLogP values relative to **7**, were also highly

dx.doi.org/10.1021/jm500147k | J. Med. Chem. 2014, 57, 3818−3834

Table 5. In Vivo Activity of Selected ELQs[a]

Compound	Structure	Predicted E_H (human microsomes)	ED_{50} (mg/kg/day)	NRD (mg/kg/day)
7		< 0.2	0.02	0.3
20a		0.5	> 3	> 3
20b		0.3	0.03	1
20c		< 0.28	0.05	> 0.3
20d		< 0.28	< 1	> 3
20e		ND	0.06	> 3
25a		< 0.28	0.3	> 3
25b		< 0.28	< 1	> 3

[a]ND = not determined.

active in vitro and stable to metabolism. However, they were somewhat less efficacious against malaria in mice, which may relate to differences in pharmacokinetics.

It is important to note that a prodrug of the GSK pyridone progressed until toxicology results revealed unexpected acute toxicity in rats that was putatively linked to inhibition of mammalian cytochrome bc_1 complex.[14] Because of the close structural similarity between the pyridone and 4(1*H*)-quinolone-diarylethers and out of concern for potential host toxicity, each of the 4(1*H*)-quinolone-3-diarylethers in the original set (**4**–**7** and **18a**–**b**) was screened for inhibition of human cytochrome bc_1 complex (derived from HEK-293 cells) activity (Figure 3). An interesting SAR trend emerged from the in vitro inhibition profile. For **4**, in which each position of the core benzenoid ring is occupied by an H atom, the IC_{50} level against host cytochrome bc_1 was 1.85 μM. ELQs with a 6-position halogen atom (e.g., compound **5** and **18a**) or a $-OCH_3$ group in position 7 (e.g., compound **6**), or both (e.g., compounds **7** and **18b**), were not inhibitory toward the human enzyme at concentrations as high as 10 μM. Evaluation of **7** for inhibition of *P. falciparum* cytochrome bc_1 complex provided an EC_{50} value of 0.56 nM, thereby highlighting the remarkable parasite selectivity (≥20000-fold) of this analogue as well as its superiority over **4** (IC_{50} = 8.9 nM) and atovaquone (IC_{50} = 2 nM) as inhibitory molecules in this assay.[19] Overall, we observed that the degree of host vs parasite selectivity increased as the size of the core increased from the pyridone to the quinolone ring system. We assume that this is due to steric hindrance in binding of the larger quinolone core to the more restrictive Q_i site of the human cytochrome bc_1. The evidence further shows that addition of bulky substituents to the 6- or 7-position widens the selective advantage and minimizes the potential developmental risk associated with the inhibition of host cytochrome bc_1 complex.

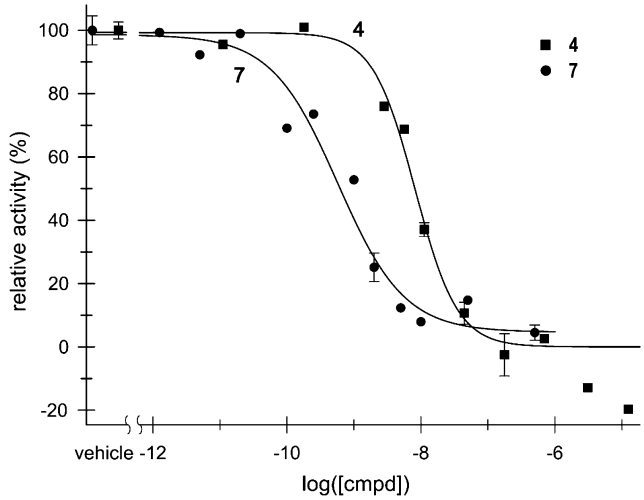


Figure 3. Inhibition of *P. falciparum* cytochrome bc_1 complex by **4** (squares) and **7** (circles).

Other structural variations were explored including the *meta*-OCF_3 congener of **7**. This compound, ELQ-307 (**20a**), exhibited spectacular activity in vitro against *P. falciparum*; however, it was metabolically unstable in the presence of hepatic microsomes and ineffective in vivo. In an attempt to capture the high intrinsic activity of **20a** in a metabolically stable form, we prepared ELQ-352 (**20e**), which added a fluoro substituent *ortho* to the trifluoromethoxy group in **20a**. While **20e** had an excellent antiplasmodial profile with subnanomolar EC_{50} values against all three reference strains, it was both less potent than **20a** in vitro and far less effective than **7** in vivo. This result indicated that there is sensitivity to the SAR in this region, and the fluoro substituent diminished exposure and efficacy as a consequence.

dx.doi.org/10.1021/jm500147k | J. Med. Chem. 2014, 57, 3818–3834

To demonstrate this, ELQ-353 (**20f**) was prepared, which added a *meta*-fluoro substituent to **7**. With decreased in vitro potency and in vivo efficacy relative to **7**, compound **20f** confirmed the findings that even a small modification to **7** results in a decrease in activity.

Finally, the 4(1*H*)-quinolone-3-diarylether series described here includes molecules with a high selectivity index against the parasite mitochondrial electron transport chain. Potent against all life cycle stages of *P. falciparum* including infections in the liver and bloodstream and with particular sensitivity exhibited by developing forms within the mosquito vector, **7** has been selected as a preclinical candidate.[20] In preclinical animal studies, **7** demonstrated nonlinear pharmacokinetics, i.e., at low doses required for a therapeutic effect oral bioavailability was good to excellent from the PEG400 solution formulation, however, it fell off rapidly at higher doses, presumably due to solubility limited absorption.[19] A focused medicinal chemistry plan was developed to address the poor aqueous solubility and oral bioavailability issues that limit exposures of **7** at higher doses that are needed to establish a therapeutic safety window. Side chain analogues with reduced cLogP values in which the terminal OCF_3 moiety was replaced by CF_3 (ELQ-309, **20b**), Cl (ELQ-323, **20c**), and F (ELQ-333, **20d**) exhibited impressive antiplasmodial activity with EC_{50} values in the low to subnanomolar range. In vitro tests revealed that they were metabolically stable in the presence of human and rat microsomes. The introduction of nitrogen atoms in the form of pyridyl or pyrimidyl rings at either the inner or outermost rings of the diarylether yielded ELQs with excellent, e.g., ELQs 310 (**25a**) and 313 (**25b**), to poor ELQ-329 (**25c**), in vitro activity against *P. falciparum*. Unfortunately, none of the compounds that were selected for further study from this series (**25a** and **20c**) provided a significant enhancement over the blood levels achieved with **7** at oral doses of 1 and 10 mg/kg, and they were at least 3−10 times less effective than **7** against murine malaria.

SUMMARY AND CONCLUDING REMARKS

Our interest in endochin derived from earlier work on the larger acridone scaffold.[21] The hydrolysis of quinacrine produced an acridone (3-chloro-7-methoxyacridone) with impressive antiplasmodial and antirespiratory properties, and this same molecule also served as the catalyst for Hans Andersag's optimization program that spotlighted endochin for its pronounced effectiveness against multiple stages of malaria infection.[3] Our efforts to chemically alter the structure of endochin to enhance its clinical utility have shown that additions to the benzenoid ring of the quinolone core have the greatest influence on intrinsic antiplasmodial activity, cross-resistance, and selective inhibition of host and parasite cytochrome bc_1 complexes. The 6-Cl/7-OCH_3 substitution pattern yielded analogues that were not cross resistant to atovaquone, a highly desirable attribute given that atovaquone is a key component of a clinical formulation with proguanil that is used in treatment of malaria. Replacement of endochin's extended alkyl group with a diphenylether at position 3 resulted in >1000-fold greater efficacy in vivo compared to the original lead molecule. Presumably, the overall boost in antimalarial efficacy in going from endochin to **7** is due to the presence of the 3-position diphenylether side chain that imparts enhanced metabolic stability while maintaining acceptable, albeit low, aqueous solubility.[19]

In summary, we discovered a novel route to synthesis of 3-diarylether substituted 4(1*H*)-quinolones. Unable to find reaction conditions that would allow for the selective deprotection of 4-*O*-methylether quinoline intermediates in the presence of an essential core methoxy group, we devised a successful route through the corresponding 4-*O*-ethylethers. The parallel convergent approach that is described is straightforward, utilizes inexpensive and readily available reagents, yields the desired quinolone in high purity (>99%), and is amenable to large-scale production. All of these factors are important given that antimalarial drugs need to be inexpensive so that they can be made available to populations in need in the developing world where the disease is endemic and resources are limited.

One of the greatest challenges to the clinical development of **7** for human use against malaria is the identification of a clinical formulation that improves exposures at higher doses needed to establish safety and tolerability in preclinical species and humans. Higher doses are also needed to establish whether and if it would be feasible to use **7** to achieve one-dose cures of falciparum malaria. Conventional formulation technologies such as particle size reduction,[22] use of solubilizing excipients or complexation agents,[23] as well as the development of amorphous spray-dried dispersions[24] and prodrugs[25] are all part of an ambitious program of research that is currently underway to resolve this issue so that the clinical potential for this new drug can be fully explored.

EXPERIMENTAL SECTION

General Chemistry. Anhydrous solvents and reagents were purchased from various fine chemical suppliers and were used without further purification. Inert atmosphere operations were conducted under argon in flame-dried glassware. ^{1}H NMR spectra were taken on a Bruker 400 MHz instrument. Data reported were calibrated to internal TMS (0.0 ppm) for all solvents and are reported as follows: chemical shift, multiplicity (bs, broad singlet; s, singlet; d, doublet; t, triplet; q, quartet; and m, multiplet), coupling constant, and integration. High-resolution mass spectrometry (HRMS) using electrospray ionization was performed by the Portland State University BioAnalytical Mass Spectrometry Facility. Final compounds were judged to be >95% pure by HPLC analysis using an HP1100 HPLC at 254 nm with Phenomenex Luna C8(2) reverse phase column (5 mm, 50 mm × 2 mm i.d) at 40 °C and eluted with methanol/water with 0.5% TFA and acetonitrile/water with 0.5% TFA at 0.4 mL/min. Further information is provided in the Supporting Information accompanying this report in which we have employed this separation system to characterize the relative hydrophobicity for each of the synthesized 4(1*H*)-quinolone-3-diarylethers (i.e., retention time) and correlated these results to cLogP values calculated with ChemDraw Ultra software (Cambridgesoft, version 12).

General Procedure A. A solution of aniline (63.5 mmol), ethyl acetoacetate (63.5 mmol), and catalytic *para*-toluene sulfonic acid (1.59 mmol) in 65 mL of benzene was stirred 6 h at reflux with a Dean−Stark trap. The reaction mixture was then concentrated in vacuo and the resulting Schiff base added to 65 mL of boiling (250 °C) DOWTHERM A and stirred 20 min at 250 °C. The reaction mixture was cooled to room temperature. After trituration with ethyl acetate, the product 4(1*H*)-quinolone was collected by filtration.

General Procedure B. To a stirred solution of 4(1*H*)-quinolone (62.2 mmol) and *n*-butylamine (622 mmol) in dimethylformamide (125 mL) cooled by a room temperature water bath was added iodine (62.2 mmol) in a saturated solution of aqueous potassium iodide (62 mL). The reaction mixture was stirred 12 h at room temperature. Residual iodine was quenched with excess 0.1 M aqueous sodium thiosulfate, and the resulting solution was concentrated in vacuo. The residue was resuspended in water and filtered to give the product 3-iodo-4(1*H*)-quinolone.

General Procedure C. To a stirred solution of 3-iodo-4(1*H*)-quinolone (5.72 mmol) in dimethylformamide (57 mL) was added potassium carbonate (11.4 mmol) at room temperature. The resulting suspension was stirred 0.5 h at 50 °C. Ethyl iodide (8.58 mmol) was added dropwise at room temperature, and the reaction mixture was

stirred 8 h at 50 °C. The solvent was removed in vacuo, and the resulting residue was resuspended in ethyl acetate and water and filtered. The organic layer was extracted with brine, dried over magnesium sulfate, and concentrated in vacuo to give the product 3-iodo-4(1*H*)-quinolone O-ethyl ether.

General Procedure D. Using a method adapted from Hart et al.,[12] to a solution of boronic acid (36.5 mmol) and 4-bromophenol (24.3 mmol) in dichloromethane (250 mL) over heat-activated 3 Å molecular sieves was added copper (II) acetate (24.3 mmol), diisopropylethylamine (121 mmol), and pyridine (121 mmol). The reaction mixture was stirred 12 h at room temperature under positive pressure of dry air and concentrated in vacuo. The resulting residue was resuspended in ethyl acetate and water. The organic layer was extracted with 0.5 M HCl and brine, dried over magnesium sulfate, and concentrated in vacuo. Purification by silica gel chromatography (ethyl acetate/hexanes) provided product bromo diaryl ether.

General Procedure E. Using a method adapted from Maiti and Buchwald,[13] to a flask containing aryl iodide (5.00 mmol), phenol (6.00 mmol), copper (I) iodide (0.500 mmol), picolinic acid (1.00 mmol), and potassium phosphate (10.0 mmol) was added dry DMSO (10 mL). The reaction mixture was heated to 80 °C, stirred for 18 h, and allowed to cool. The reaction was then diluted with ethyl acetate (50 mL) and water (5 mL) and separated. The aqueous phase was extracted with ethyl acetate (2 × 50 mL). The combined organic phases were treated with brine, dried over magnesium sulfate, filtered, and concentrated in vacuo. The resulting residue was purified by flash chromatography (ethyl acetate/hexanes).

General Procedure F. To a solution of bromo diaryl ether (33.0 mmol) in DMF (130 mL) over heat-activated 3 Å molecular sieves (13 g) was added 1,1′-bis(diphenylphosphino)ferrocene dichloropalladium (II) (1.65 mmol), bis(pinacolato) diboron (36.3 mmol), and potassium acetate (99.0 mmol). The reaction mixture was heated to 80 °C, stirred for 18 h, cooled, and filtered. The filtrate was concentrated in vacuo, resuspended in ethyl acetate and water, and separated. The organic layer was extracted with brine and dried over magnesium sulfate. Purification by silica gel chromatography (ethyl acetate/hexanes) provided the product boronic ester.

General Procedure G. To a solution of 3-iodo-4(1*H*)-quinolone *O*-ethyl ether (3.35 mmol), boronic acid/ester (5.02 mmol), and palladium (0) tetrakis triphenylphosphine (0.168 mmol) in degassed dimethylformamide (17 mL) was added 6.7 mL of a 2 N aqueous potassium carbonate solution. The reaction mixture was stirred 18 h at 85 °C, filtered through celite, and concentrated in vacuo. The resulting residue was resuspended in ethyl acetate and water and separated. The organic layer was extracted with brine, dried over magnesium sulfate, and concentrated in vacuo. Purification by silica gel chromatography (ethyl acetate/dichloromethane) provided the product 4(1*H*)-quinolone *O*-ethyl ether.

General Procedure H. To a solution of 4(1*H*)-quinolone *O*-ethyl ether (2.30 mmol) in acetic acid (10 mL) was added a 50% aqueous hydrobromic acid solution (5 mL). The reaction mixture was stirred 24 h at 90 °C, cooled, and neutralized with a saturated potassium hydroxide solution. The product 4(1*H*)-quinolone-3-diaryl ether was triturated in ethyl acetate and recrystallized from DMF/methanol.

2-Methyl-3-(4-phenoxyphenyl)quinolin-4(1*H*)-one (3). To a solution of 4-methoxy-2-methyl-3-(4-phenoxyphenyl)quinoline **12a** (1.60 mmol) in anhydrous dichloromethane (12 mL) was added boron tribromide (0.23 mL) at room temperature. The reaction mixture was stirred 18 h at room temperature, quenched with 10 mL of water, and filtered. The filter cake was triturated in ethyl acetate and recrystallized from methanol to give the title compound as a flaky off-white solid (314 mg, 60% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.87 (s, 1H), 8.20−8.25 (m, 1H), 7.74−7.83 (m, 2H), 7.39−7.52 (m, 3H), 7.33 (d, J = 8.3 Hz, 2H), 7.18 (t, J = 7.2 Hz, 1H), 7.04−7.12 (m, 4H), 2.37 (s, 3H). HRMS (ESI) m/z for [$C_{22}H_{18}NO_2^+$]: calculated 328.1332, found 328.1329.

2-Methyl-3-(4-(4-(trifluoromethoxy)phenoxy)phenyl)-quinolin-4(1*H*)-one (4). The title compound was prepared from 4-ethoxy-2-methyl-3-(4-(4-(trifluoromethoxy)phenoxy)phenyl)-quinoline **17a** according to general procedure H. Yield: 84%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.65 (s, 1H), 8.09 (d, J = 7.2 Hz, 1H), 7.64 (td, J = 7.6, 1.4 Hz, 1H), 7.54 (d, J = 8.1 Hz, 1H), 7.42 (d, J = 8.5 Hz, 2H), 7.27−7.31 (m, 3H), 7.15−7.18 (m, 2H), 7.07−7.09 (m, 2H), 2.27 (s, 3H). HRMS (ESI) m/z for [$C_{23}H_{17}F_3NO_3^+$]: calculated 412.1155, found 412.1158.

6-Chloro-2-methyl-3-(4-(4-(trifluoromethoxy)phenoxy)-phenyl)quinolin-4(1*H*)-one (5). The title compound was prepared from 6-chloro-4-ethoxy-2-methyl-3-(4-(4-(trifluoromethoxy)-phenoxy)phenyl)quinoline **17b** according to general procedure H. Yield: 73%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.85 (s, 1H), 8.00−8.03 (m, 1H), 7.66−7.70 (m, 1H), 7.57−7.61 (m, 1H), 7.39−7.45 (m, 2H), 7.28−7.32 (m, 2H), 7.15−7.20 (m, 2H), 7.06−7.11 (m, 2H), 2.27 (s, 3H). HRMS (ESI) m/z for [$C_{23}H_{16}ClF_3NO_3^+$]: calculated 446.0765, found 446.0766.

7-Methoxy-2-methyl-3-(4-(4-(trifluoromethoxy)phenoxy)-phenyl)quinolin-4(1*H*)-one (6). The title compound was prepared from 4-ethoxy-7-methoxy-2-methyl-3-(4-(4-(trifluoromethoxy)-phenoxy)phenyl)quinoline **17c** according to general procedure H. Yield: 40%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.65 (s, 1H), 8.09 (d, J = 7.2 Hz, 1H), 7.64 (td, J = 7.6, 1.4 Hz, 1H), 7.54 (d, J = 8.1 Hz, 1H), 7.42 (d, J = 8.5 Hz, 2H), 7.27−7.31 (m, 3H), 7.15−7.18 (m, 2H), 7.07−7.09 (m, 2H), 2.27 (s, 3H). HRMS (ESI) m/z for [$C_{24}H_{19}F_3NO_4^+$]: calculated 442.1261, found 442.1263.

6-Chloro-7-methoxy-2-methyl-3-(4-(4-(trifluoromethoxy)-phenoxy)phenyl) quinolin-4(1*H*)-one (7). The title compound was prepared from 6-chloro-4-ethoxy-7-methoxy-2-methyl-3-(4-(4-(trifluoromethoxy)phenoxy)phenyl) quinoline **17d** according to general procedure H. Yield: 84%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.97 (s, 1H), 8.05 (s, 1H), 7.42 (d, J = 8.7 Hz, 2H), 7.29 (d, J = 8.3 Hz, 2H), 7.17 (d, J = 8.3 Hz, 2H), 7.12 (s, 1H), 7.08 (d, J = 8.7 Hz, 2H), 3.97 (s, 3H), 2.26 (s, 3H). HRMS (ESI) m/z for [$C_{24}H_{17}ClF_3NO_4$]: calculated 475.0798, found 475.0801.

6-Fluoro-7-methoxy-2-methylquinolin-4(1*H*)-one (9b). The title compound was prepared from commercially available 4-fluoro-3-methoxyaniline **8b** according to general procedure A. Yield: 51%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.21 (bs, 1H), 7.62 (d, J = 11.8 Hz, 1H), 7.02 (d, J = 7.4 Hz, 2H), 5.81 (s, 1H), 3.89 (s, 3H), 2.28 (s, 3H).

6-Chloro-2-methylquinolin-4(1*H*)-one (9c). The title compound was prepared from commercially available 4-chloroaniline **8c** according to general procedure A. Yield: 34%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.72 (bs, 1H), 7.67 (dd, J = 8.9, 2.5 Hz, 2H), 7.48 (d, J = 8.7 Hz, 1H), 7.35 (d, J = 8.9 Hz, 1H), 6.37 (s, 1H), 2.06 (s, 3H), 1.86 (s, 1H).

7-Methoxy-2-methylquinolin-4(1*H*)-one (9d). The title compound was prepared from commercially available 3-methoxyaniline **8d** according to general procedure A. Yield: 36%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.78 (s, 1H), 7.89 (d, J = 9.5 Hz, 1H), 7.35−7.49 (m, 1H), 6.85 (dq, J = 4.6, 2.4 Hz, 1H), 5.79 (s, 1H), 3.81 (s, 3H), 2.34 (s, 1H), 2.27 (s, 3H).

6-Chloro-7-methoxy-2-methylquinolin-4(1*H*)-one (9e). The title compound was prepared from commercially available 4-chloro-3-methoxyaniline **8e** according to general procedure A. Yield: 60%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.54 (bs, 1H), 7.94 (s, 1H), 7.02 (s, 1H), 5.86 (s, 1H), 3.94 (s, 3H), 2.31 (s, 3H).

6-Fluoro-2-methylquinolin-4(1*H*)-one (9f). The title compound was prepared from 4-fluoroaniline **8f** according to general procedure A. Yield: 20%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.73 (s, 1H), 7.68 (dd, J = 9.3, 2.8 Hz, 1H), 7.46−7.62 (m, 2H), 5.93 (s, 1H), 2.35 (s, 3H).

4-Hydroxy-3-iodo-2-methylquinolone (10a). The title compound was prepared from commercially available 4-hydroxy-2-methylquinolone **9a** according to general procedure B. Yield: 99%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.13 (1H, bs), 8.08 (1H, d, J = 8.3 Hz), 7.68 (1H, m), 7.55 (1H, d, J = 8.3 Hz), 7.35 (1H, m), 2.65 (3H, s).

6-Fluoro-3-iodo-7-methoxy-2-methylquinolin-4(1*H*)-one (10b). The title compound was prepared from 6-fluoro-7-methoxy-2-methylquinolin-4(1*H*)-one **9b** according to general procedure B. Yield: 42%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.11 (bs, 1H), 7.70 (d, J = 11.7 Hz, 1H), 7.09 (d, J = 7.4 Hz, 1H), 3.95 (s, 3H), 2.61 (s, 3H).

6-Chloro-3-iodo-2-methylquinolin-4(1*H*)-one (10c). The title compound was prepared from 6-chloro-2-methylquinolin-4(1*H*)-one **9c** according to general procedure B. Yield: 48%. ^{1}H NMR (400 MHz,

DMSO) δ 8.02 (dd, J = 7.9, 2.3 Hz, 1H), 7.71 (dd, J = 8.8, 2.5 Hz, 1H), 7.60 (d, J = 8.8 Hz, 1H), 2.64 (s, 3H).

3-Iodo-7-methoxy-2-methylquinolin-4(1*H*)-one (10d). The title compound was prepared from 7-methoxy-2-methylquinolin-4(1*H*)-one **9d** according to general procedure B. Yield: 88%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.96 (bs, 1H), 7.97 (d, J = 8.91 Hz, 1H), 6.91−6.96 (m, 2H), 3.86 (s, 3H), 2.60 (s, 3H).

6-Chloro-3-iodo-7-methoxy-2-methylquinolin-4(1*H*)-one (10e). The title compound was prepared from 6-chloro-7-methoxy-2-methylquinolin-4(1*H*)-one **9e** according to general procedure B. Yield: 89%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.65 (bs, 1H), 7.59 (s, 1H), 6.41 (s, 1H), 3.91 (s, 3H), 2.18 (s, 3H).

6-Fluoro-3-iodo-2-methylquinolin-4(1*H*)-one (10f). The title compound was prepared from 6-fluoro-2-methylquinolin-4(1*H*)-one **9f** according to general procedure B. Yield: 97%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.25 (bs, 1H), 7.73 (dd, J = 9.3, 2.4 Hz, 1H), 7.56−7.67 (m, 2H), 2.64 (s, 3H).

3-Iodo-4-methoxy-2-methylquinoline (11a). A solution of 4-hydroxy-3-iodo-2-methylquinolone **10a** in phosphorus oxychloride (80 mL) was refluxed 3 h. The reaction mixture was cooled to 0 °C, quenched with a cold, saturated sodium hydroxide solution, and extracted with ethyl acetate. The organic layer was extracted with brine, dried over magnesium sulfate, filtered, and concentrated in vacuo to give 4-chloro-3-iodo-2-methylquinoline (6.68 g, 88% yield) as an off-white solid. 4-Chloro-3-iodo-2-methylquinoline (1.55 g, 5.11 mmol) was added to a solution of sodium metal (1.00 g, 43.5 mmol) in dry methanol (40 mL).[10] The reaction mixture stirred 18 h at reflux and concentrated in vacuo. The resulting residue was resuspended in ethyl acetate and water. The organic layer was extracted with water and brine, dried over magnesium sulfate, filtered, and concentrated in vacuo. The resulting off-white solid was recrystallized from hexanes and ethyl acetate to give white crystals (0.866 g, 56% yield). ^{1}H NMR (400 MHz, DMSO) δ 7.94−8.12 (m, 2H), 7.58−7.83 (m, 2H), 3.99 (s, 3H), 2.86 (s, 3H).

6-Fluoro-3-iodo-4,7-dimethoxy-2-methylquinoline (11b). To a stirred solution of **10b** (5.72 mmol) in dimethylformamide (57 mL) was added potassium carbonate (11.4 mmol) at room temperature. The resulting suspension was stirred 0.5 h at 50 °C. Methyl iodide (8.58 mmol) was added dropwise at room temperature, and the reaction mixture was stirred 8 h at 50 °C. The solvent was removed in vacuo, and the resulting residue was resuspended in ethyl acetate and water and filtered. The organic layer was extracted with brine, dried over magnesium sulfate, and concentrated in vacuo to give the title compound (30% yield) as an off-white solid. ^{1}H NMR (400 MHz, DMSO) δ 7.78 (d, J = 11.8 Hz, 1H), 7.56 (d, J = 8.2 Hz, 1H), 3.99 (s, 3H), 3.95 (s, 3H), 2.82 (s, 3H).

4-Ethoxy-6-fluoro-3-iodo-7-methoxy-2-methylquinoline (11c). The title compound was prepared from 6-fluoro-3-iodo-7-methoxy-2-methylquinolin-4(1*H*)-one **10b** according to general procedure C. Yield: 77%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (d, J = 11.5 Hz, 1H), 7.42 (d, J = 8.0, 1H), 4.18 (q, J = 7.0 Hz, 2H), 2.92 (s, 3H), 1.60 (t, J = 7.0 Hz, 3H).

4-Ethoxy-3-iodo-2-methylquinoline (11d). The title compound was prepared from 4-hydroxy-3-iodo-2-methylquinolone **10a** according to general procedure C. Yield: 99%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00−8.03 (m, 2H), 7.68−7.72 (m, 1H), 7.48−7.52 (m, 1H), 4.21 (q, J = 7.0 Hz, 2H), 2.96 (s, 3H), 1.62 (t, J = 7.0 Hz, 3H).

6-Chloro-4-ethoxy-3-iodo-2-methylquinoline (11e). The title compound was prepared from 6-chloro-3-iodo-2-methylquinolin-4(1*H*)-one **10c** according to general procedure C. Yield: 96%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (d, J = 2.4 Hz, 1H), 7.94 (d, J = 9.0 Hz, 1H), 7.62 (dd, J = 9.0, 2.4 Hz, 1H), 4.20 (q, J = 7.0 Hz, 2H), 2.95 (s, 3H), 1.63 (t, J = 7.0 Hz, 3H).

4-Ethoxy-3-iodo-7-methoxy-2-methylquinoline (11f). The title compound was prepared from 3-iodo-7-methoxy-2-methylquinolin-4(1*H*)-one **10d** according to general procedure C. Yield: 99%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89 (d, J = 9.1 Hz, 1H), 7.33 (d, J = 2.5 Hz, 1H), 7.13 (dd, J = 9.1, 2.5 Hz, 1H), 4.19 (q, J = 7.0 Hz, 2H), 3.93 (s, 3H), 2.93 (s, 3H), 1.60 (t, J = 7.0 Hz, 3H).

6-Chloro-4-ethoxy-3-iodo-7-methoxy-2-methylquinoline (11g). The title compound was prepared from 6-chloro-3-iodo-7-methoxy-2-methylquinolin-4(1*H*)-one **10e** according to general procedure C. Yield: 99%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (s, 1H), 7.40 (s, 1H), 4.19 (q, J = 7.1 Hz, 2H), 4.02 (s, 3H), 2.92 (s, 3H), 1.61 (t, J = 7.1 Hz, 3H).

4-Ethoxy-6-fluoro-3-iodo-2-methylquinoline (11h). The title compound was prepared from 6-fluoro-3-iodo-2-methylquinolin-4(1*H*)-one **10f** according to general procedure C. Yield: 69%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (dd, J = 9.2, 5.2 Hz, 1H), 7.60 (dd, J = 9.2, 2.8, 1H), 7.45 (ddd, J = 9.1, 8.3, 2.8 Hz, 1H), 4.20 (q, J = 7.0 Hz, 2H), 2.94 (s, 3H), 1.62 (t, J = 7.0 Hz, 3H).

4-Methoxy-2-methyl-3-(4-phenoxyphenyl)quinoline (12a). The title compound was prepared from 3-iodo-4-methoxy-2-methylquinoline **11a** according to general procedure G. Yield: 80%. ^{1}H NMR (400 MHz, DMSO) δ 8.09 (dd, J = 8.3, 1.0 Hz, 1H), 7.96 (d, J = 8.3 Hz, 1H), 7.74 (ddd, J = 8.4, 6.9, 1.4 Hz, 1H), 7.58 (ddd, J = 8.1, 6.9, 1.1 Hz, 1H), 7.37−7.49 (m, 4H), 7.16−7.23 (m, 1H), 7.09−7.15 (m, 4H), 3.58 (s, 3H), 2.42 (s, 3H).

6-Fluoro-4,7-dimethoxy-2-methyl-3-(4-phenoxyphenyl)-quinoline (12b). The title compound was prepared from 6-fluoro-3-iodo-4,7-dimethoxy-2-methylquinoline **11b** according to general procedure G. Yield: 84%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (d, J = 11.8 Hz, 1H), 7.49 (d, J = 8.0 Hz, 1H), 7.35−7.44 (m, 2H), 7.29−7.35 (m, 2H), 7.20−7.08 (m, 5H), 4.03 (s, 3H), 3.57 (s, 3H), 2.50 (s, 3H).

4-Ethoxy-6-fluoro-7-methoxy-2-methyl-3-(4-phenoxyphenyl)-quinoline (12c). The title compound was prepared from 4-ethoxy-6-fluoro-3-iodo-7-methoxy-2-methylquinoline **11c** according to general procedure G. Yield: 91%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (d, J = 11.8 Hz, 1H), 7.48 (d, J = 8.0 Hz, 1H), 7.39 (t, J = 8.4, 7.5 Hz, 2H), 7.27−7.33 (m, 3H), 7.07−7.13 (m, 4H), 4.03 (s, 3H), 3.70 (q, J = 7.0 Hz, 2H), 2.50 (s, 3H), 1.18 (t, J = 7.0 Hz, 3H).

6-Fluoro-7-methoxy-2-methyl-3-(4-phenoxyphenyl)-quinolin-4(1*H*)-one (13). The title compound was prepared from 4-ethoxy-6-fluoro-7-methoxy-2-methyl-3-(4-phenoxyphenyl)quinoline **12c** according to general procedure H. Yield: 89%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.69 (s, 1H), 7.70 (d, J = 11.8 Hz, 1H), 7.42 (t, J = 8.0 Hz, 2H), 7.25 (d, J = 8.6 Hz, 2H), 6.96−7.20 (m, 6H), 3.95 (s, 3H), 2.24 (s, 3H). HRMS (ESI) m/z for $[C_{23}H_{18}FNO_3]$: calculated 375.1271, found 375.1270.

1-Bromo-4-(4-(trifluoromethoxy)phenoxy)benzene (15a). The title compound was prepared from commercially available 4-(trifluoromethoxy)phenylboronic acid **14a** according to general procedure D. Yield: 63%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 (d, J = 9.3 Hz, 1H), 7.19 (d, J = 8.9 Hz, 1H), 6.99 (d, J = 9.3 Hz, 1H), 6.89 (d, J = 8.9 Hz, 1H).

1-(4-Bromophenoxy)-3-(trifluoromethoxy)benzene (15b). The title compound was prepared from commercially available 3-(trifluoromethoxy)phenylboronic acid **14b** according to general procedure D. Yield: 69%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.16−7.69 (m, 2H), 6.54−7.14 (m, 2H), 1.54 (s, 2H), 1.26 (s, 2H).

1-Bromo-4-(4-(trifluoromethyl)phenoxy)benzene (15c). The title compound was prepared from commercially available 4-(trifluoromethyl)-phenylboronic acid **14c** according to general procedure D. Yield: 62%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37−8.00 (m, 2H), 6.68−7.15 (m, 2H), 1.55 (s, 2H), 1.26 (s, 2H).

1-Bromo-4-(4-chlorophenoxy)benzene (15d). The title compound was prepared from commercially available 4-chlorophenylboronic acid **14d** according to general procedure D. Yield: 59%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41−7.46 (m, 2H), 7.27−7.32 (m, 2H), 6.91−6.95 (m, 2H), 6.85−6.89 (m, 2H).

1-Bromo-4-(4-fluorophenoxy)benzene (15e). The title compound was prepared from commercially available 4-fluorophenylboronic acid **14e** according to general procedure D. Yield: 46%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39−7.43 (m, 2H), 7.01−7.07 (m, 2H), 6.94−6.99 (m, 2H), 6.82−6.86 (m, 2H).

4-(4-Bromophenoxy)-1-fluoro-2-(trifluoromethoxy)benzene (15f). The title compound was prepared from commercially available 4-fluoro-3-(trifluoromethoxy)phenylboronic acid **14f** according to general procedure D. Yield: 66%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42−7.49 (m, 2H), 7.17 (t, 1H, J = 9.26), 6.96−6.98 (m, 1H), 6.86−6.92 (m, 3H).

4-(4-Bromophenoxy)-2-fluoro-1-(trifluoromethoxy)benzene (15g). The title compound was prepared from commercially available

dx.doi.org/10.1021/jm500147k | J. Med. Chem. 2014, 57, 3818−3834

3-fluoro-4-(trifluoromethoxy)phenylboronic acid **14g** according to general procedure D. Yield: 45%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47−7.51 (m, 2H), 7.36−7.40 (m, 1H), 6.91−6.95 (m, 2H), 6.81 (dd, 1H, J = 10.92, 2.75), 6.75 (ddd, 1H, J = 9.00, 2.87, 1.63).

4,4,5,5-Tetramethyl-2-(4-(4-(trifluoromethoxy)phenoxy)-phenyl)-1,3,2-dioxaborolane (16a). The title compound was prepared from 1-bromo-4-(4-(trifluoromethoxy)phenoxy)-benzene **15a** according to general procedure F. Yield: 92%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (d, J = 8.9 Hz, 2H), 7.19 (d, J = 8.9 Hz, 2H), 6.97−7.04 (m, 2H), 1.35 (s, 12H).

4,4,5,5-Tetramethyl-2-(4-(3-(trifluoromethoxy)phenoxy)-phenyl)-1,3,2-dioxaborolane (16b). The title compound was prepared from 1-(4-bromophenoxy)-3-(trifluoromethoxy)benzene **15b** according to general procedure F. Yield: 90%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 (d, J = 9.0 Hz, 2H), 7.36 (t, J = 8.3 Hz, 1H), 6.99 (dm, J = 8.2, 1.1 Hz, 1H), 6.94 (d, J =9.0 Hz, 2H), 6.88 (s, 1H), 6.69 (d, J = 8.9 Hz, 1H).

4,4,5,5-Tetramethyl-2-(4-(4-(trifluoromethyl)phenoxy)-phenyl)-1,3,2-dioxaborolane (16c). The title compound was prepared from 1-bromo-4-(4-(trifluoromethyl)phenoxy)benzene **15c** according to general procedure F. Yield: 85%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (dt, J = 8.57 Hz, 2H), 7.58 (dt, J = 8.47 Hz, 2H), 7.06 (d, J = 8.38 Hz, 2H), 7.03 (dt, J = 8.59 Hz, 2H), 1.35 (s, 12H).

2-(4-(4-Chlorophenoxy)phenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (16d). The title compound was prepared from 1-bromo-4-(4-chlorophenoxy)benzene **15d** according to general procedure F. Yield: 97%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77−7.80 (m, 2H), 7.27−7.32 (m, 2H), 6.93−6.98 (m, 4H), 1.34 (s, 12H).

2-(4-(4-Fluorophenoxy)phenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (16e). The title compound was prepared from 1-bromo-4-(4-fluorophenoxy)benzene **15e** according to general procedure F. Yield: 72%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75−7.79 (m, 2H), 6.97−7.06 (m, 4H), 6.92−6.95 (m, 2H), 1.34 (s, 12H).

2-(4-(4-Fluoro-3-(trifluoromethoxy)phenoxy)phenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (16f). The title compound was prepared from 4-(4-bromophenoxy)-1-fluoro-2-(trifluoromethoxy)-benzene **15f** according to general procedure F. Yield: 75%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77−7.83 (m, 2H), 7.14−7.18 (m, 1H), 6.88−7.02 (m, 4H), 1.35 (s, 12H).

2-(4-(3-Fluoro-4-(trifluoromethoxy)phenoxy)phenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (16g). The title compound was prepared from 4-(4-bromophenoxy)-2-fluoro-1-(trifluoromethoxy)-benzene **15g** according to general procedure F. Yield: 81%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80−7.85 (m, 2H), 7.21−7.27 (m, 1H), 7.00−7.04 (m, 2H), 6.80−6.86 (m, 1H), 6.75−6.79 (m, 1H), 1.35 (s, 12H).

4-Ethoxy-2-methyl-3-(4-(4-(trifluoromethoxy)phenoxy)-phenyl)quinoline (17a). The title compound was prepared from 4-ethoxy-3-iodo-2-methylquinoline **11d** and 4,4,5,5-tetramethyl-2-(4-(4-(trifluoromethoxy)phenoxy)-phenyl)-1,3,2-dioxaborolane **16a** according to general procedure G. Yield: 84%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12−8.14 (m, 1H), 8.01−8.04 (m, 1H), 7.70 (ddd, J = 8.5, 6.9, 1.5 Hz, 1H), 7.51 (ddd, J = 8.2, 6.9, 1.2 Hz, 1H), 7.34−7.37 (m, 2H), 7.22−7.25 (m, 2H), 7.07−7.15 (m, 4H), 3.76 (q, J = 7.0 Hz, 2H), 2.54 (s, 3H), 1.19 (t, J = 7.0 Hz, 3H).

6-Chloro-4-ethoxy-2-methyl-3-(4-(4-(trifluoromethoxy)-phenoxy)phenyl)quinoline (17b). The title compound was prepared from 6-chloro-4-ethoxy-3-iodo-2-methylquinoline **11e** and 4,4,5,5-tetramethyl-2-(4-(4-(trifluoromethoxy)phenoxy)phenyl)-1,3,2-dioxaborolane **16a** according to general procedure G. Yield: 76%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (dd, J = 2.4, 0.4 Hz, 1H), 7.95 (dd, J = 9.0, 0.3 Hz, 1H), 7.61 (dd, J = 9.0, 2.4 Hz, 1H), 7.32−7.36 (m, 2H), 7.22−7.25 (m, 2H), 7.12−7.14 (m, 2H), 7.09−7.11 (m, 2H), 3.73 (q, J = 7.0 Hz, 2H), 2.52 (s, 3H), 1.19 (t, J = 7.0 Hz, 3H).

4-Ethoxy-7-methoxy-2-methyl-3-(4-(4-(trifluoromethoxy)-phenoxy)phenyl)quinoline (17c). The title compound was prepared from 4-ethoxy-3-iodo-7-methoxy-2-methylquinoline **11f** and 4,4,5,5-tetramethyl-2-(4-(4-(trifluoromethoxy)phenoxy)phenyl)-1,3,2-dioxaborolane **16a** according to general procedure G. Yield: 72%. ^{1}H NMR (400 MHz, DMSO) δ 8.00 (d, J = 9.1 Hz, 1H), 7.35−7.48 (m, J = 14.3, 11.1, 1.6 Hz, 5H), 7.12−7.24 (m, 5H), 3.92 (s, 3H), 3.71 (q, J = 7.0 Hz, 2H), 2.41 (s, 3H), 1.09 (t, J = 7.0 Hz, 3H).

6-Chloro-4-ethoxy-7-methoxy-2-methyl-3-(4-(4-(trifluoromethoxy)phenoxy)phenyl) quinoline (17d). The title compound was prepared from 6-chloro-4-ethoxy-3-iodo-7-methoxy-2-methylquinoline **11g** and 4,4,5,5-tetramethyl-2-(4-(4-(trifluoromethoxy)phenoxy)-phenyl)-1,3,2-dioxaborolane **16a** according to general procedure G. Yield: 85%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 (s, 1H), 7.43 (s, 1H), 7.31−7.36 (m, 2H), 7.22−7.26 (m, 2H), 7.07−7.14 (m, 4H), 4.04 (s, 3H), 3.71 (q, J = 7.1 Hz, 2H), 2.49 (s, 3H), 1.18 (t, J = 7.1 Hz, 3H).

4-Ethoxy-6-fluoro-2-methyl-3-(4-(4-(trifluoromethoxy)-phenoxy)phenyl)quinoline (17e). The title compound was prepared from 4-ethoxy-6-fluoro-3-iodo-2-methylquinoline **11h** and 4,4,5,5-tetramethyl-2-(4-(4-(trifluoromethoxy)phenoxy)phenyl)-1,3,2-dioxaborolane **16a** according to general procedure G. Yield: 84%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (dd, J = 9.2, 5.2 Hz, 1H), 7.72 (dd, J = 9.5, 2.9 Hz, 1H), 7.45 (td, J = 8.7, 2.9 Hz, 1H), 7.33−7.36 (m, 2H), 7.23−7.26 (m, 2H), 7.08−7.15 (m, 4H), 3.72 (q, J = 7.0 Hz, 2H), 2.51 (s, 3H), 1.19 (t, J = 7.0 Hz, 3H).

4-Ethoxy-6-fluoro-7-methoxy-2-methyl-3-(4-(4-(trifluoromethoxy)-phenoxy)-phenyl)quinoline (17f). The title compound was prepared from 4-ethoxy-6-fluoro-3-iodo-7-methoxy-2-methylquinoline **11c** and 4,4,5,5-tetramethyl-2-(4-(4-(trifluoromethoxy)-phenoxy)phenyl)-1,3,2-dioxaborolane **16a** according to general procedure G. Yield: 87%. ^{1}H NMR (400 MHz, DMSO) δ 8.06 (s, 1H), 7.87 (d, J = 8.6 Hz, 1H), 7.75 (d, J = 11.9 Hz, 1H), 7.56 (d, J = 8.3 Hz, 1H), 7.42 (ddd, J = 16.3, 7.1, 1.6 Hz, 2H), 7.17−7.27 (m, 2H), 7.14 (d, J = 9.1 Hz, 1H), 7.01 (d, J = 8.6 Hz, 1H), 4.01 (s, 3H), 3.70 (q, J = 7.0 Hz, 2H), 2.41 (s, 3H), 1.09 (t, J = 7.0 Hz, 3H).

6-Fluoro-2-methyl-3-(4-(4-(trifluoromethoxy)phenoxy)-phenyl)quinolin-4(1*H*)-one (18a). The title compound was prepared from 4-ethoxy-6-fluoro-2-methyl-3-(4-(4-(trifluoromethoxy)phenoxy)-phenyl)quinoline **17e** according to general procedure H. Yield: 67%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.83 (s, 1H), 7.71−7.74 (m, 1H), 7.62 (dd, J = 9.1, 4.7 Hz, 1H), 7.56 (td, J = 8.6, 3.0 Hz, 1H), 7.42 (d, J = 8.5 Hz, 2H), 7.28−7.31 (m, 2H), 7.15−7.19 (m, 2H), 7.06−7.10 (m, 2H), 2.27 (s, 3H). HRMS (ESI) m/z for $[C_{23}H_{16}F_4NO_3^+]$: calculated 430.1061, found 430.1059.

6-Fluoro-7-methoxy-2-methyl-3-(4-(4-(trifluoromethoxy)-phenoxy)phenyl)quinolin-4(1*H*)-one (18b). The title compound was prepared from 4-ethoxy-6-fluoro-7-methoxy-2-methyl-3-(4-(4-(trifluoromethoxy)phenoxy)phenyl)quinoline **17f** according to general procedure H. Yield: 68%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.78 (s, 1H), 7.69−7.74 (m, 1H), 7.40−7.43 (m, 2H), 7.29 (d, J = 8.5 Hz, 2H), 7.15−7.19 (m, 2H), 7.10−7.15 (m, 1H), 7.06−7.09 (m, 2H), 3.96 (s, 3H), 2.25 (s, 3H). HRMS (ESI) m/z for $[C_{24}H_{17}F_4NO_4]$: calculated 459.1094, found 459.1093.

6-Chloro-4-ethoxy-7-methoxy-2-methyl-3-(4-(3-(trifluoromethoxy)phenoxy)-phenyl)quinoline (19a). The title compound was prepared from 6-chloro-4-ethoxy-3-iodo-7-methoxy-2-methylquinoline **11g** and 4,4,5,5-tetramethyl-2-(4-(3-(trifluoromethoxy)-phenoxy)phenyl)-1,3,2-dioxaborolane **16b** according to general procedure G. Yield: 84%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 (s, 1H), 7.34−7.43 (m, 4H), 7.14−7.17 (m, 2H), 6.97−7.02 (m, 2H), 6.90−6.93 (m, 2H), 4.04 (s, 3H), 3.71 (q, J = 7.02 Hz, 2H), 2.50 (s, 3H), 1.18 (t, J = 7.02 Hz, 3H).

6-Chloro-4-ethoxy-7-methoxy-2-methyl-3-(4-(4(trifluoromethyl)-phenoxy)phenyl)-quinoline (19b). The title compound was prepared from 6-chloro-4-ethoxy-3-iodo-7-methoxy-2-methylquinoline **11g** and 4,4,5,5-tetramethyl-2-(4-(4-(trifluoromethyl)-phenoxy)phenyl)-1,3,2-dioxaborolane **16c** according to general procedure G. Yield: 70%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 (s, 1H), 7.63 (d, J = 8.5, 2H), 7.43 (s, 1H), 7.36−7.39 (m, 2H), 7.15−7.18 (m, 2H), 7.13 (d, J = 8.6 Hz, 2H), 4.05 (s, 3H), 3.72 (q, J = 7.0 Hz, 2H), 2.50 (s, 3H), 1.19 (t, J = 7.0 Hz, 3H).

6-Chloro-3-(4-(4-chlorophenoxy)phenyl)-4-ethoxy-7-methoxy-2-methylquinoline (19c). The title compound was prepared from 6-chloro-4-ethoxy-3-iodo-7-methoxy-2-methylquinoline **11g** and 2-(4-(4-chlorophenoxy)phenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane **16d** according to general procedure G. Yield: 78%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06−8.19 (m, 1H), 7.21−7.63 (m, 5H), 6.92−7.18 (m, 4H), 4.04 (s, 3H), 3.70 (q, 2H), 2.49 (s, 3H), 1.17 (t, J = 6.9, 2.1 Hz, 3H).

dx.doi.org/10.1021/jm500147k | J. Med. Chem. 2014, 57, 3818−3834

6-Chloro-4-ethoxy-3-(4-(4-fluorophenoxy)phenyl)-7-methoxy-2-methylquinoline (19d). The title compound was prepared from 6-chloro-4-ethoxy-3-iodo-7-methoxy-2-methylquinoline **11g** and 2-(4-(4-fluorophenoxy)-phenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane **16e** according to general procedure G. Yield: 95%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (s, 1H), 7.42 (s, 1H), 7.25−7.34 (m, 3H), 7.07 (m, 5H), 4.04 (s, 3H), 3.70 (q, J = 7.0 Hz, 2H), 2.49 (s, 3H), 1.18 (t, J = 7.0 Hz, 3H).

6-Chloro-4-ethoxy-3-(4-(4-fluoro-3-(trifluoromethoxy)-phenoxy)phenyl)-7-methoxy-2-methylquinoline (19e). The title compound was prepared from 6-chloro-4-ethoxy-3-iodo-7-methoxy-2-methylquinoline **11g** and 2-(4-(4-fluoro-3-trifluoromethoxy)phenoxy)-phenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane **16f** according to general procedure G. Yield: 82%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (s, 1H), 7.59 (ddd, J = 9.5, 6.9, 5.7 Hz, 1H), 7.29−7.49 (m, 4H), 6.93−7.28 (m, 3H), 4.04 (s, 3H), 3.71 (q, J = 7.1 Hz, 2H), 2.49 (s, 3H), 1.17 (t, J = 7.0 Hz, 3H).

6-Chloro-4-ethoxy-3-(4-(3-fluoro-4-(trifluoromethoxy)-phenoxy)phenyl)-7-methoxy-2-methylquinoline (19f). The title compound was prepared from 6-chloro-4-ethoxy-3-iodo-7-methoxy-2-methylquinoline **11g** and 2-(4-(3-fluoro-4-(trifluoromethoxy)phenoxy)-phenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane **16g** according to general procedure G. Yield: 70%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (s, 1H), 7.34−7.46 (m, 4H), 7.30 (td, J = 8.7, 1.0 Hz, 1H), 7.12−7.19 (m, 2H), 6.89 (dd, J = 14.4, 3.6 Hz, 1H), 4.05 (s, 3H), 3.71 (q, J = 7.0 Hz, 2H), 2.50 (s, 3H), 1.18 (t, J = 7.0 Hz, 3H).

6-Chloro-7-methoxy-2-methyl-3-(4-(3-(trifluoromethoxy)-phenoxy)phenyl)quinolin-4(1*H*)-one (20a). The title compound was prepared from 6-chloro-4-ethoxy-7-methoxy-2-methyl-3-(4-(3-(trifluoromethoxy)phenoxy)-phenyl)quinoline **19a** according to general procedure H. Yield: 88%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.41 (s, 1H), 8.03−8.17 (m, 1H), 7.51−7.60 (m, 1H), 7.28−7.38 (m, 2H), 7.04−7.25 (m, 6H), 3.99 (s, 3H), 2.28 (s, 3H). HRMS (ESI) m/z for $[C_{24}H_{17}ClF_3NO_4]$: calculated 475.0798, found 475.0796.

6-Chloro-7-methoxy-2-methyl-3-(4-(4-(trifluoromethyl)-phenoxy)phenyl)quinolin-4(1*H*)-one (20b). The title compound was prepared from 6-chloro-4-ethoxy-7-methoxy-2-methyl-3-(4-(4-(trifluoromethyl)phenoxy)-phenyl)quinoline **19b** according to general procedure H. Yield: 88%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.68 (s, 1H), 8.01 (s, 1H), 7.77 (d, J = 8.7 Hz, 2H), 7.31−7.35 (m, 2H), 7.20 (d, J = 8.6 Hz, 2H), 7.13−7.17 (m, 2H), 7.08 (s, 1H), 3.95 (s, 3H), 2.24 (s, 3H). HRMS (ESI) m/z for $[C_{24}H_{17}ClF_3NO_3]$: calculated 459.0849, found 459.0850.

6-Chloro-3-(4-(4-chlorophenoxy)phenyl)-7-methoxy-2-methylquinolin-4(1*H*)-one (20c). The title compound was prepared from 6-chloro-3-(4-(4-chlorophenoxy)phenyl)-4-ethoxy-7-methoxy-2-methylquinoline **19c** according to general procedure H. Yield: 89%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.65 (s, 1H), 8.00 (s, 1H), 7.44−7.48 (m, 2H), 7.25−7.28 (m, 2H), 7.03−7.11 (m, 5H), 3.96 (s, 3H), 2.24 (s, 3H). HRMS (ESI) m/z for $[C_{23}H_{17}Cl_2NO_3]$: calculated 425.0585, found 425.0582.

6-Chloro-3-(4-(4-fluorophenoxy)phenyl)-7-methoxy-2-methylquinolin-4(1*H*)-one (20d). The title compound was prepared from 6-chloro-4-ethoxy-3-(4-(4-fluorophenoxy)phenyl)-7-methoxy-2-methylquinoline **19d** according to general procedure H. Yield: 92%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.14 (bs, 1H), 8.08 (s, 1H), 7.24−7.29 (m, 4H), 7.12−7.17 (m, 3H), 6.99−7.03 (m, 2H), 3.98 (s, 3H), 2.27 (s, 3H). HRMS (ESI) m/z for $[C_{23}H_{17}ClFNO_3]$: calculated 409.0881, found 409.0879.

6-Chloro-3-(4-(4-fluoro-3-(trifluoromethoxy)phenoxy)-phenyl)-7-methoxy-2-methylquinolin-4(1*H*)-one (20e). The title compound was prepared from 6-chloro-4-ethoxy-3-(4-(4-fluoro-3-(trifluoromethoxy)phenoxy)-phenyl)-7-methoxy-2-methylquinoline **19e** according to general procedure H. Yield: 84%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.68 (s, 1H), 8.00 (s, 1H), 7.56 (t, J = 9.6 Hz, 1H), 7.31−7.35 (m, 2H), 7.26−7.30 (m, 2H), 7.15 (dt, J = 9.1, 3.4 Hz, 1H), 7.06−7.08 (m, 3H), 3.97 (s, 3H), 2.23 (s, 3H). HRMS (ESI) m/z for $[C_{24}H_{16}ClF_4NO_4]$: calculated 493.0704, found 493.0706.

6-Chloro-3-(4-(3-fluoro-4-(trifluoromethoxy)phenoxy)-phenyl)-7-methoxy-2-methylquinolin-4(1*H*)-one (20f). The title compound was prepared from 6-chloro-4-ethoxy-3-(4-(3-fluoro-4-(trifluoromethoxy)phenoxy)-phenyl)-7-methoxy-2-methylquinoline **19f** according to general procedure H. Yield: 84%. ^{1}H NMR (400 MHz, DMSO) δ 11.67 (s, 1H), 8.00 (s, 1H), 7.60 (s, 1H), 7.19−7.39 (m, J = 22.3, 9.4 Hz, 3H), 7.00−7.19 (m, 3H), 6.94 (d, J = 5.4 Hz, 1H), 3.97 (s, 3H), 2.25 (s, 3H). HRMS (ESI) m/z for $[C_{24}H_{16}ClF_4NO_4]$: calculated 493.0704, found 493.0707.

5-Bromo-2-(4-(trifluoromethoxy)phenoxy)pyridine (22a). The title compound was prepared from commercially available 5-bromo-2-iodopyridine **21a** according to general procedure E. Yield: 91%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (dd, J = 2.55, 0.62 Hz, 1H), 7.78−7.81 (m, 1H), 7.22−7.27 (m, 2H), 7.13−7.18 (m, 2H), 6.87 (dd, J = 8.70, 0.64 Hz, 1H).

5-Bromo-2-(4-(trifluoromethoxy)phenoxy)pyrimidine (22c). The title compound was prepared from commercially available 5-bromo-2-iodopyrimidine **21c** according to general procedure E. Yield: 79%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.58 (s, 2H), 7.25−7.30 (m, 2H), 7.19−7.23 (m, 2H).

5-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)-2-(4-(trifluoromethoxy)phenoxy)pyridine (23a). The title compound was prepared from 5-bromo-2-(4-(trifluoromethoxy)phenoxy)pyridine **22a** according to general procedure F. Yield: 63%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.54−8.56 (m, 1H), 8.05−8.08 (m, 1H), 7.20−7.26 (m, 2H), 7.12−7.18 (m, 2H), 6.88−6.91 (m, 1H), 1.34 (s, 12H).

2-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)-5-(4-(trifluoromethoxy)phenoxy)pyridine (23b). 2-Bromo-5-(4-(trifluoromethoxy)-phenoxy)pyridine **22b** was prepared from commercially available 2-bromo-5-iodopyridine **21b** according to general procedure E and used without further purification. The title compound was prepared from compound **22b** according to general procedure F. Yield (over two steps): 38%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17−8.22 (m, 1H), 7.69−7.74 (m, 1H), 7.20−7.28 (m, 2H), 7.12−7.19 (m, 2H), 7.00−7.04 (m, 1H), 1.34 (s, 12H).

5-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)-2-(4-(trifluoromethoxy)phenoxy)pyrimidine (23c). The title compound was prepared from 5-bromo-2-(4-(trifluoromethoxy)phenoxy)pyrimidine **22c** according to general procedure F. Yield: 38%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.84 (s, 2H), 7.21−7.30 (m, 4H), 1.35 (s, 12H).

6-Chloro-4-ethoxy-7-methoxy-2-methyl-3-(6-(4-(trifluoromethoxy)phenoxy)pyridin-3-yl)quinoline (24a). The title compound was prepared from 6-chloro-4-ethoxy-3-iodo-7-methoxy-2-methylquinoline **11g** and 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-2-(4-(trifluoromethoxy)phenoxy)pyridine **23a** according to general procedure G. Yield: 47%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (dd, J = 2.4, 0.6 Hz, 1H), 8.08 (s, 1H), 7.74 (dd, J = 8.4, 2.4 Hz, 1H), 7.43 (s, 1H), 7.25−7.30 (m, 4H), 7.09 (dd, J = 8.4, 0.6 Hz, 1H), 4.04 (s, 3H), 3.74 (q, J = 7.0 Hz, 2H), 2.50 (s, 3H), 1.20 (t, J = 7.0 Hz, 3H).

6-Chloro-4-ethoxy-7-methoxy-2-methyl-3-(5-(4-(trifluoromethoxy)phenoxy)pyridin-2-yl)quinoline (24b). The title compound was prepared from 6-chloro-4-ethoxy-3-iodo-7-methoxy-2-methylquinoline **11g** and 2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-5-(4-(trifluoromethoxy)phenoxy)pyridine **23b** according to general procedure G. Yield: 22%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16−8.21 (m, 1H), 8.08 (s, 1H), 7.74 (dd, J = 8.4, 2.4 Hz, 1H), 7.40−7.45 (m, 1H), 7.08 (dd, J = 8.4, 0.4 Hz, 1H), 4.04 (d, J = 2.8 Hz, 3H), 3.74 (q, J = 7.0 Hz, 2H), 2.50 (s, 3H), 1.20 (t, J = 7.0 Hz, 3H).

6-Chloro-4-ethoxy-7-methoxy-2-methyl-3-(2-(4-(trifluoromethoxy)phenoxy)pyrimidin-5-yl)quinoline (24c). The title compound was prepared from 6-chloro-4-ethoxy-3-iodo-7-methoxy-2-methylquinoline **11g** and 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-2-(4-(trifluoromethoxy)phenoxy)pyrimidine **23c** according to general procedure G. Yield: 51%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.61 (s, 2H), 8.06 (s, 2H), 7.44 (s, 1H), 7.23−7.39 (m, 4H), 4.05 (s, 3H), 3.79 (q, J = 7.0 Hz, 2H), 2.54 (s, 3H), 1.62 (s, 3H), 1.24 (t, J = 7.0 Hz, 3H).

6-Chloro-7-methoxy-2-methyl-3-(6-(4-(trifluoromethoxy)-phenoxy)pyridin-3-yl)quinolin-4(1*H*)-one (25a). The title compound was prepared from 6-chloro-4-ethoxy-7-methoxy-2-methyl-3-(6-(4-(trifluoromethoxy)-phenoxy)pyridin-3-yl)quinoline **24a** according to general procedure H. Yield: 76%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.78 (s, 1H), 8.03 (d, J = 2.1 Hz, 1H), 8.01 (s, 1H), 7.78 (dd, J = 8.4, 2.4 Hz, 1H), 7.44 (d, J = 8.5 Hz, 2H), 7.30−7.34 (m, 2H), 7.14

dx.doi.org/10.1021/jm500147k | J. Med. Chem. 2014, 57, 3818−3834

(d, J = 7.0 Hz, 1H), 7.08 (s, 1H), 3.97 (s, 3H), 2.26 (s, 3H). HRMS (ESI) m/z for $[C_{23}H_{16}ClF_3N_2O_4]$: calculated 476.0751, found 476.0749.

6-Chloro-7-methoxy-2-methyl-3-(5-(4-(trifluoromethoxy)-phenoxy)pyridin-2-yl)quinolin-4(1*H*)-one (25b). The title compound was prepared from 6-chloro-4-ethoxy-7-methoxy-2-methyl-3-(5-(4-(trifluoromethoxy)phenoxy)pyridin-2-yl)quinoline **24b** according to general procedure H. Yield: 98%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.87 (s, 1H), 8.02−8.03 (m, 2H), 7.77−7.80 (m, 1H), 7.42−7.47 (m, 2H), 7.30−7.34 (m, 2H), 7.10−7.14 (m, 2H), 3.97 (s, 3H), 2.27 (s, 3H). HRMS (ESI) m/z for $[C_{23}H_{16}ClF_3N_2O_4]$: calculated 476.0751, found 476.0755.

6-Chloro-7-methoxy-2-methyl-3-(2-(4-(trifluoromethoxy)-phenoxy)pyrimidin-5-yl)quinolin-4(1*H*)-one (25c). The title compound was prepared from 6-chloro-4-ethoxy-7-methoxy-2-methyl-3-(2-(4-(trifluoromethoxy)phenoxy)pyrimidin-5-yl)quinoline **24c** according to general procedure H. Yield: 51%. ^{1}H NMR (400 MHz, DMSO) δ 8.07 (s, 4H), 7.96 (s, 2H), 7.13 (s, 2H), 3.92 (s, 3H), 2.29 (s, 3H). HRMS (ESI) m/z for $[C_{22}H_{15}ClF_3N_3O_4]$: calculated 477.0703, found 477.0705.

6-Chloro-4-ethoxy-2-methyl-3-(6-(4-(trifluoromethoxy)-phenoxy)pyridin-3-yl)quinoline (26a). The title compound was prepared from 6-chloro-4-ethoxy-3-iodo-2-methylquinoline **11e** and 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-2-(4-(trifluoromethoxy)-phenoxy)pyridine **23a** according to general procedure G. Yield: 31%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (dd, J = 2.4, 0.6 Hz, 1H), 8.06 (d, J = 2.3 Hz, 1H), 7.96 (d, J = 9.0 Hz, 1H), 7.74 (dd, J = 8.4, 2.4 Hz, 1H), 7.64 (dd, J = 9.0, 2.4 Hz, 1H), 7.24−7.29 (m, 4H), 7.10 (dd, J = 8.4, 0.6 Hz, 1H), 3.75 (q, J = 7.0 Hz, 2H), 2.53 (d, J = 3.3 Hz, 3H), 1.22 (t, J = 7.0 Hz, 3H).

4-Ethoxy-2-methyl-3-(6-(4-(trifluoromethoxy)phenoxy)-pyridin-3-yl)quinoline (26b). The title compound was prepared from 4-ethoxy-3-iodo-2-methylquinoline **11d** and 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-2-(4-(trifluoromethoxy)phenoxy)pyridine **23a** according to general procedure G. Yield: 32%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20−8.21 (m, 1H), 8.11 (dd, J = 8.3, 0.8, 1H), 8.04 (d, J = 8.4, 1H), 7.69−7.77 (m, 2H), 7.50−7.55 (m, 1H), 7.25−7.33 (m, 4H), 7.07−7.10 (m, 1H), 3.78 (q, J = 7.0 Hz, 2H), 2.54 (s, 3H), 1.22 (t, J = 7.0 Hz, 3H).

4-Ethoxy-6-fluoro-2-methyl-3-(6-(4-(trifluoromethoxy)-phenoxy)pyridin-3-yl)quinoline (26c). The title compound was prepared from 4-ethoxy-6-fluoro-3-iodo-2-methylquinoline **11h** and 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-2-(4-(trifluoromethoxy)-phenoxy)pyridine **23a** according to general procedure G. Yield: 38%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (dd, J = 2.4, 0.6 Hz, 1H), 8.02 (dd, J = 9.2, 5.2 Hz, 1H), 7.72 (ddd, J = 12.2, 8.9, 2.6 Hz, 2H), 7.47 (ddd, J = 9.2, 8.3, 2.9 Hz, 1H), 7.25−7.30 (m, 4H), 7.10 (dd, J = 8.4, 0.6 Hz, 1H), 3.75 (q, J = 7.0 Hz, 2H), 2.52 (s, 3H), 1.21 (t, J = 7.0 Hz, 3H).

6-Chloro-2-methyl-3-(6-(4-(trifluoromethoxy)phenoxy)-pyridin-3-yl)quinolin-4(1*H*)-one (27a). The title compound was prepared from 6-chloro-4-ethoxy-2-methyl-3-(6-(4-(trifluoromethoxy)-phenoxy)pyridin-3-yl)quinoline **26a** according to general procedure H. Yield: 71%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.95 (s, 1H), 8.03 (dd, J = 8.9, 2.4 Hz, 2H), 7.80 (dd, J = 8.3, 4.1 Hz, 1H), 7.69−7.72 (m, 1H), 7.61 (d, J = 8.9 Hz, 1H), 7.42−7.47 (m, 2H), 7.30−7.35 (m, 2H), 7.12−7.16 (m, 1H), 2.29 (s, 3H). HRMS (ESI) m/z for $[C_{22}H_{15}ClF_3N_2O_3^+]$: calculated 447.0718, found 447.0718.

2-Methyl-3-(6-(4-(trifluoromethoxy)phenoxy)pyridin-3-yl)-quinolin-4(1*H*)-one (27b). The title compound was prepared from 4-ethoxy-2-methyl-3-(6-(4-(trifluoromethoxy)phenoxy)pyridin-3-yl)-quinoline **26b** according to general procedure H. Yield: 80%. ^{1}H NMR (400 MHz, DMSO) δ 8.06 (d, J = 7.9 Hz, 1H), 8.02 (d, J = 1.8 Hz, 1H), 7.78 (dd, J = 8.4, 2.4 Hz, 1H), 7.52 (t, J = 8.5 Hz, 2H), 7.43 (d, J = 8.3 Hz, 2H), 7.26−7.33 (m, 2H), 7.21 (t, J = 7.3 Hz, 1H), 7.10 (d, J = 8.9 Hz, 1H), 2.26 (s, 3H). HRMS (ESI) m/z for $[C_{22}H_{16}F_3N_2O_3^+]$: calculated 413.1108, found 413.1110.

6-Fluoro-2-methyl-3-(6-(4-(trifluoromethoxy)phenoxy)-pyridin-3-yl)quinolin-4(1*H*)-one (27c). The title compound was prepared from 4-ethoxy-6-fluoro-2-methyl-3-(6-(4-(trifluoromethoxy)-phenoxy)pyridin-3-yl)quinoline **26c** according to general procedure H. Yield: 82%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.15 (s, 1H), 8.05 (d, J = 2.2 Hz, 1H), 7.79−7.84 (m, 1H), 7.75 (dd, J = 9.3, 2.9 Hz, 1H), 7.69 (dd, J = 9.1, 4.7 Hz, 1H), 7.61 (dd, J = 8.6, 2.9 Hz, 1H), 7.44 (d, J = 8.6 Hz, 2H), 7.30−7.36 (m, 2H), 7.15 (d, J = 8.4 Hz, 1H), 2.31 (s, 3H). HRMS (ESI) m/z for $[C_{22}H_{15}F_4N_2O_3^+]$: calculated 431.1013, found 431.1015.

2-(4-Bromophenoxy)-5-(trifluoromethyl)pyridine (28). The title compound was prepared from commercially available 2-iodo-5-trifluoromethylpyridine according to general procedure E. Yield: 99%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.43 (td, J = 1.69, 0.84 Hz, 1H), 7.92 (ddd, J = 8.67, 2.55, 0.55, 1H), 7.52−7.55 (m, 2H), 7.01−7.06 (m, 3H).

2-(4-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)phenoxy)-5-(trifluoromethyl)pyridine (29). The title compound was prepared from 2-(4-bromophenoxy)-5-(trifluoromethyl)pyridine **28** according to general procedure F. Yield: 99%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.41−8.45 (m, 1H), 7.87−7.91 (m, 2H), 7.12−7.16 (m, 2H), 6.99−7.06 (m, 2H), 1.35 (s, 12H).

6-Chloro-4-ethoxy-7-methoxy-2-methyl-3-(4-((5-(trifluoromethyl)pyridin-2-yl)oxy)phenyl)quinoline (30). The title compound was prepared from 2-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenoxy)-5-(trifluoromethyl)pyridine **29** according to general procedure G. Yield: 82%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.49 (s, 1H), 8.12 (s, 1H), 7.95 (dd, J = 8.7, 2.4 Hz, 2H), 7.38−7.47 (m, 1H), 7.24−7.32 (m, 2H), 7.08 (d, J = 8.6 Hz, 2H), 4.05 (s, 3H), 3.72 (q, J = 7.0 Hz, 2H), 2.53 (s, 3H), 1.19 (t, J = 7.0 Hz, 3H).

6-Chloro-7-methoxy-2-methyl-3-(4-((5-(trifluoromethyl)-pyridin-2-yl)oxy)phenyl)quinolin-4(1*H*)-one (31). The title compound was prepared from 6-chloro-4-ethoxy-7-methoxy-2-methyl-3-(4-((5-(trifluoromethyl)-pyridin-2-yl)oxy)phenyl)quinoline **30** according to general procedure H. Yield: 66%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.69 (s, 1H), 8.61−8.64 (m, 1H), 8.25 (dd, J = 8.7, 2.5, 1H), 8.01 (s, 1H), 7.30−7.33 (m, 2H), 7.28 (d, J = 8.8 Hz, 1H), 7.20−7.25 (m, 2H), 7.08 (s, 1H), 3.97 (s, 3H), 2.26 (s, 3H). HRMS (ESI) m/z for $[C_{23}H_{16}ClF_3N_2O_3]$: calculated 460.0802, found 460.0802.

Biology. *Parasite Culture and Drug Sensitivity.* *P. falciparum* parasite lines D6 (chloroquine sensitive, MRA-285) and Dd2 (multidrug resistant, MRA-156) were obtained from MR4, ATCC Collection, Manassass, Virginia, and deposited by D. E. Kyle and T. E. Wellems, respectively. Atovaquone resistant clinical isolates, Tm90-C2B and Tm93-C1088, each containing a $Y_{(TAT)}268 \Rightarrow S_{(TCT)}268$ transformation in the cytochrome *b* gene, were originally collected from Thai patients with recrudescent parasites following atovaquone therapy.[18,26] These two isolates were obtained from the frozen parasite repository of WRAIR, Division of Experimental Therapeutics (Silver Spring, Maryland) and were kindly provided by Victor Melendez.

Laboratory strains of *P. falciparum* were cultured in human erythrocytes by standard methods under a low oxygen atmosphere (5% O_2, 5% CO_2, 90% N_2) in an environmental chamber.[27] The culture medium was 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). The parasites were maintained in fresh human erythrocytes suspended at a 2% hematocrit in complete medium at 37 °C. Stock cultures were subpassaged every 3−4 days by transfer of infected red cells to a flask containing complete medium and uninfected erythrocytes.

EC_{50} Determination by the Fluorescence-Based SyBr Green Assay. In vitro antimalarial activity of the 4(1*H*)-quinolone-3-diarylether derivatives was assessed by the SYBR Green I fluorescence-based method described previously by us[16] with minor modifications.[21] Briefly, experiments were set up in triplicate in 96-well plates (Costar, Corning) with 2-fold dilutions of each drug across the plate in a total volume of 100 μL and at a final red blood cell concentration of 2% (v/v). The dilution series was initiated at a concentration of 1 μM, and the experiment was repeated beginning with a lower initial concentration for those compounds in which the EC_{50} value was below 10 nM. Automated pipetting and dilution was carried out with the aid of a programmable Precision 2000 robotic station (BioTek, Winooski, VT). An initial parasitemia of 0.2% was attained by addition of normal uninfected red cells to a stock culture of asynchronous parasite infected red cells (PRBC). The plates were incubated for 72 h at 37 °C in an atmosphere of 5% CO_2, 5% O_2, and 90% N_2. After this period, the SYBR Green I dye−detergent mixture (100 μL) was added and the plates were incubated at room temperature for an hour in the dark and then placed in a 96-well fluorescence plate reader (Spectramax Gemini-EM,

dx.doi.org/10.1021/jm500147k | J. Med. Chem. 2014, 57, 3818−3834

Molecular Diagnostics) for analysis, with excitation and emission wavelength bands centered at 497 and 520 nm, respectively. The fluorescence readings were plotted against the logarithm of the drug concentration, and curve fitting by nonlinear regression analysis (GraphPad Prism software) yielded the drug concentration that produced 50% of the observed decline relative to the maximum readings in drug-free control wells (EC_{50}). Chloroquine and atovaquone were used as internal controls to establish zero percent viability and cross-resistance.

HepG2 Cytotoxicity Assay. Drugs were dissolved in DMSO to make 10 mM stock solutions. Human hepatocarcinoma cells (HepG2) were maintained on RPMI-1640 medium supplemented with 10% fetal bovine serum at 37 °C in a humidified 5% CO_2 atmosphere. Cells were seeded at a density of 2×10^4 per well in 96-well flat-bottom tissue culture plates containing complete medium in a total volume of 160 μL/well. The cells were allowed to attach at 37 °C overnight. On the following day, drug solutions (40 μL/well) were serially diluted with complete culture medium across the plate to achieve a concentration range of 2.5 nM to 10 μM. The plates were then incubated at 37 °C and 5% CO_2 for another 24−36 h. Afterward, the medium was aspirated and replaced with complete RPMI medium (200 μL/well), and the plates were incubated for an additional 24 h at 37 °C and 5% CO_2. An aliquot of a stock solution of resazurin (Alamar Blue, prepared in 1× PBS) was then added at 20 μL per well (final concentration 10 μM), and the plates were returned to the incubator for 3 h. After this period, fluorescence in each well, indicative of cellular redox activity, was measured in a Gemini EM plate reader with excitation wavelength at 560 nm and emission wavelength at 590 nm.[28] IC_{50} values were determined by nonlinear regression analysis of logistic concentration−fluorescence intensity curves (GraphPad Prism software).

In Vivo Efficacy against Murine Malaria. The in vivo activity of selected ELQ derivatives was assessed against the blood stages using a modified 4-day test.[29] Mice (female, CF1, Charles River Laboratories) were infected intravenously with $2.5-5.0 \times 10^5$ *P. yoelii* (Kenya strain, MR4 MRA-428) parasitized erythrocytes from a donor animal. Drug administration commenced the day after the animals were inoculated (day 1). The test compounds were dissolved in PEG-400 and administered by oral gavage once daily for four successive days; chloroquine phosphate was used as a positive control. On the fifth day, blood films were prepared and the extent of parasitemia was determined by microscopic examination of Giemsa stained smears. Initially, ELQ analogues were administered at 0.1, 0.3, 1.0, 3.0, and 10 mg/kg/day. ED_{50} values (mg/kg/day) were derived graphically from the dose required to reduce parasite burden by 50% relative to drug-free controls. If necessary, the initial dose range was adjusted to include higher or lower dosages for accurate assessment of the ED_{50}. Animals remaining parasite free 30 days after the last drug dose were considered cured of their infection. The malaria infection in this model system was rapidly fulminated, producing average parasitemias of ≈30% in untreated control animals by day 5. 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.

Enzymology (Assay for Inhibition of Human Cytochrome bc_1). *Isolation of HEK-293 Derived Mitochondria.* HEK-293 cells were grown in DMEM containing 10% FCS using standard methods. When cell monolayers were confluent, flasks were treated with trypsin and the detached cells were pelleted and washed twice in ice-cold PBS. The washed cells were resuspended in PBS containing 1 mM PMSF and processed three times with an ice-cold Dounce homogenizer. Large or insoluble matter was removed from the broken cells by centrifugation at 800*g* for 10 min. The pellet was discarded, and the supernatant was centrifuged at 20000*g* for 40 min. The pellet containing mitochondria was resuspended in a minimum of PBS and made 30% (v/v) glycerol for storage at −80 °C until needed for enzyme assays.

Measurement of Cytochrome bc_1 Complex Inhibition (Derived from Human HEK-293 Cells). Mitochondrial fraction was diluted to a concentration that yielded suitable activity levels (usually $10^{-4}-10^{-3}$ absorbance units per second; see below) and dispersed in 2 mg/mL *n*-dodecyl β-D-maltoside. The mixture was allowed to incubate for 45 min on ice and then clarified by microcentrifugation at 10000*g*. Enzymatic activity was measured in the following reaction buffer: 50 mM Tricine, 100 mM KCl, 4 mM KCN, 50 μM ferric cytochrome *c* (horse heart, Sigma), 0.1 mg/mL *n*-dodecyl β-D-maltoside, and 50 μM decylubiquinol (prepared freshly before each experiment by reduction of decylubiquinone with sodium borohydride followed by HCl quenching), pH = 8.0. Cytochrome *c* reductase measurements were made at 550−542 nm at 30 °C. Measurements were initiated by the addition of decylubiquinol, and a baseline was collected for approximately 20 s to account for the nonenzymatic reduction of cytochrome *c* by decylubiquinol. Once the baseline collection was complete, enzyme was added to the mixture and the reaction was allowed to proceed. Once the kinetic trace had been collected, the baseline was subtracted from the initial rate of enzymatic activity. The activity of each kinetic trace is reported as the fraction of activity with respect to control uninhibited enzyme activity under identical conditions.

Isolation of P. falciparum Mitochondria. Mitochondria from the trophozoite stage of *P. falciparum* were prepared according to the protocol published by Mather et al.[30] A particular care was taken to minimize contamination with hemozoin in mitochondrial preparations from parasites.

Ubiquinol−Cytochrome c Oxidoreductase (Cytochrome bc_1) Activity: P. falciparum. Cytochrome *c* reductase activity was assayed by a modification of the method of Trumpower and Edwards.[31] The assay was performed at 35 °C in a stirred cuvette with a final volume of 1 mL containing various amounts of mitochondrial preparation (generally ∼6−12 μL), 100 μM 2,3-dimethoxy-5-methyl-6-decyl-1,4 benzohydroquinone (Q_DH_2) (5 μL 20 mM), 100 μM horse heart cytochrome *c* (Sigma Aldrich), 0.1 mg/mL *n*-dodecyl-β-D-maltoside, 60 mM HEPES (pH 7.4), 10 mM sodium malonate, 1.0 mM EDTA, and 2.0 mM KCN. The reduction of cytochrome *c* was recorded with a modified SLM-AMINCO DW2C dual wavelength spectrophotometer (Online Instrument Systems, Inc., Bogart, GA, USA) in dual mode (550−541 nm). The short chain ubiquinol analogue Q_DH_2 was prepared by reducing Q_D in dimethyl sulfoxide with sodium borohydride and acidifying the mixture with concentrated HCl and stored under argon in aliquots at −80 °C.

Methods to Assess in Vitro Microsomal Stability. Compounds were incubated at 37 °C and 1 μM concentration in human liver microsomes (BD Gentest, Discovery Labware Inc., Woburn, MA) suspended in 0.1 M phosphate buffer (pH 7.4) at a final protein concentration of 0.4 mg per mL. Metabolic reactions were initiated by the addition of an NADPH-regenerating system (1 mg/mL NADP, 1 mg/mL glucose-6-phosphate, 1 U/mL glucose-6-phosphate dehydrogenase) and $MgCl_2$ (0.67 mg/mL) and were quenched at various time points up to 60 min by the addition of ice-cold acetonitrile. Quenched samples were centrifuged, and the relative loss of parent compound over the course of the incubation was monitored by LC-MS using either a Micromass single quadrupole, triple quadrupole, or TOF mass spectrometer (Waters Corporation, Milford, MA). 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, an in vitro intrinsic clearance value, and a predicted in vivo intrinsic clearance (CL_{int}) value according to the methods of Obach.[32] In vivo CL_{int} values were converted to a predicted in vivo hepatic extraction ratio (E_H) using the following equation: $E_H = CL_{int}/Q + CL_{int}$, where Q is liver blood flow which was assumed to be 20.7, 55.2, and 90 mL/min/kg for humans, rats, and mice, respectively.[33]

■ ASSOCIATED CONTENT

Supporting Information

Additional details describing the crystal structure of 5,7-difluoro-3-heptyl-2-methylquinolin-4(1*H*)-one and relative log *P* values of selected ELQs. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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Co-authors M.K.R., A.N., J.X.K., Y.L., D.J.H., J.N.B., and R.W.W. are listed as coinventors on a U.S. patent that is relevant to this work (US Patent 2014/0045888 A1 published February 13, 2014). The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript.

■ ACKNOWLEDGMENTS

This project was supported by the Medicines for Malaria Venture (0095/08), and by the National Institutes of Health AI079182 and AI100569 (M.K.R.) and AI028398 (M.W.M., A.M.P., and A.B.V.). M.K.R. also receives funding from the United States Department of Veterans Affairs and the Stanley Medical Research Institute. All of the authors would like to recognize the outstanding leadership provided by Ian Bathurst (deceased, 26 June 2011) who served as a codirector for this project. This project was established as a collaboration by the MMV with the University of South Florida, and all of the authors would like to recognize the significant contributions to the overall 4(1*H*)-Quinolone Optimization Project provided by Roman Manetsch and Dennis Kyle and members of their respective laboratories.

■ ABBREVIATIONS USED

ACT, artemisinin combined therapies; CQ, chloroquine; EC50, 50% effective concentrations (relative to drug-free controls); E_H, predicted human hepatic extraction ratio based on in vitro clearance values; ELQ, endochin-like quinolone; HFF, human foreskin fibroblasts; MMV, Medicines for Malaria Venture; NRD, nonrecrudescence dose; OHSU, Oregon Health & Science University; SAR, structure−activity relationship; WRAIR, Walter Reed Army Institute of Research

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