



Synthesis and structure–activity relationships of antimalarial 4-oxo-3-carboxyl quinolones

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ABSTRACT

Malaria is endemic in tropical and subtropical regions of Africa, Asia, and the Americas. The increasing prevalence of multi-drug-resistant *Plasmodium falciparum* drives the ongoing need for the development of new antimalarial drugs. In this light, novel scaffolds to which the parasite has not been exposed are of particular interest. Recently, workers at the Swiss Tropical Institute discovered two novel 4-oxo-3-carboxyl quinolones active against the intra-erythrocytic stages of *P. falciparum* while carrying out rationally directed low-throughput screening of potential antimalarial agents as part of an effort directed by the World Health Organization. Here we report the design, synthesis, and preliminary pharmacologic characterization of a series of analogues of 4-oxo-3-carboxyl quinolones. These studies indicate that the series has good potential for preclinical development.

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1. Introduction

Malaria, caused by the protozoal species *Plasmodium*, is one of the most devastating infectious diseases in the world. More than 500 million cases are reported annually, with 1–3 million deaths.^{1,2} The most lethal form of malaria is caused by *Plasmodium falciparum* and the rapid spread of chloroquine-resistant (CQ-R) and multi-drug-resistant (MDR) *P. falciparum* contributes to the deteriorating malaria situation.^{3–5} Thus, the development of new antimalarial drugs that are active against CQ-R and MDR strains is urgently needed.^{6–8}

Among the potential classes of antimalarials, the acridone derivatives have been known since 1940s. Both 7-methoxy tetrahydroacridone (**1**) and endochin (**2**) (Fig. 1) have prophylactic and therapeutic activity in canaries infected with *Plasmodium praecox* or *Plasmodium gallinaceum*.^{9,10} However, endochin is not effective against human malaria.¹¹ Endochin-like 4(1H)-quinolones bearing extended alkenyl or alkyl side chains at the 3 position have potent activity against both chloroquine-sensitive (CQ-S) and MDR *P. falciparum* strains.^{11,12} The primary target of these compounds is thought to be the parasite cytochrome *bc*₁ complex.¹² Additionally,

Abbreviations: TDR, special programme for research and training in tropical diseases; CQ-R, chloroquine-resistant; CQ-S, chloroquine-sensitive; MDR, multi-drug-resistant; PAMPA, parallel artificial membrane permeability assay; TI, therapeutic index; NOE, nuclear Overhauser effect.

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the 4(1H)-quinolone esters [WR197236 (**3**) and WR194905 (**4**)] have potent anti-relapse activity in rhesus monkeys infected with *Plasmodium cynomolgi* B.¹³ It has been reported that *Plasmodium Berghei* quickly developed resistance to WR197236 (**3**) when it was used alone.¹⁴ Acridinedione derivatives WR 243251 (**5**) and WR249685 (**6**) also have significant potency against CQ-R and CQ-S strains in vitro.^{15–17} WR 249685 (**6**) targets the quinol oxidation site of the *bc*₁ complex.¹⁸

The World Health Organization's Special Programme for Research and Training in Tropical Diseases (TDR) recently initiated a rationally directed screening campaign to explore the antimalarial potential of commercially available and proprietary compounds that resemble known antimalarials or compounds with known anti-parasitic effects. As part of this campaign, workers at the Swiss Tropical Institute recently identified two 4(1H)-quinolone derivatives, TDR42098 (**7**) and TDR17516 (**8**), with potent antimalarial activity against both the CQ-R K1 and CQ-S NF54 strains of *P. falciparum* (Fig. 1). Although these compounds are structurally similar to the quinolones described above, their simpler structures, lacking the long fatty side chains, present some potential for improved physicochemical properties.¹⁹ We report here the development of synthetic routes that allow the rational perturbation of all potentially important functional groups on the 4(1H)-quinolone scaffold and the use of these routes to probe the fundamental structure–activity relationships of the series. These studies led to the discovery of analogues with potency equal to or greater than that of mefloquine in drug-resistant strains.

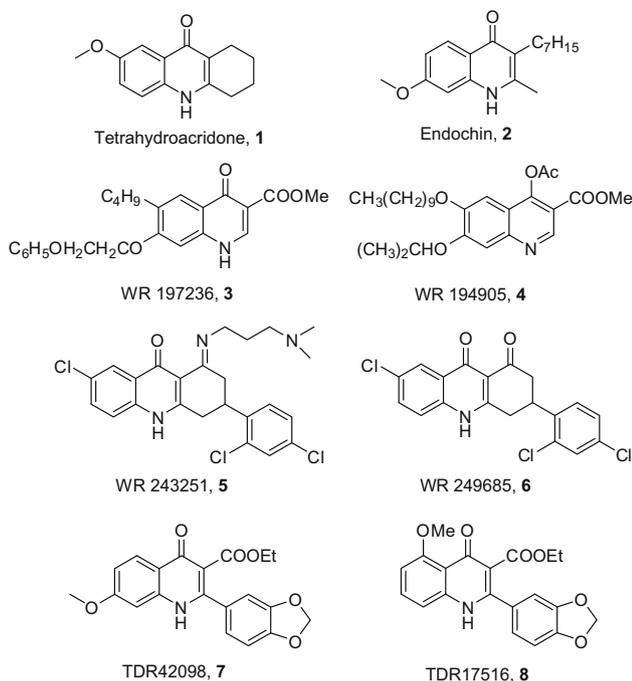


Figure 1. Reference compounds.

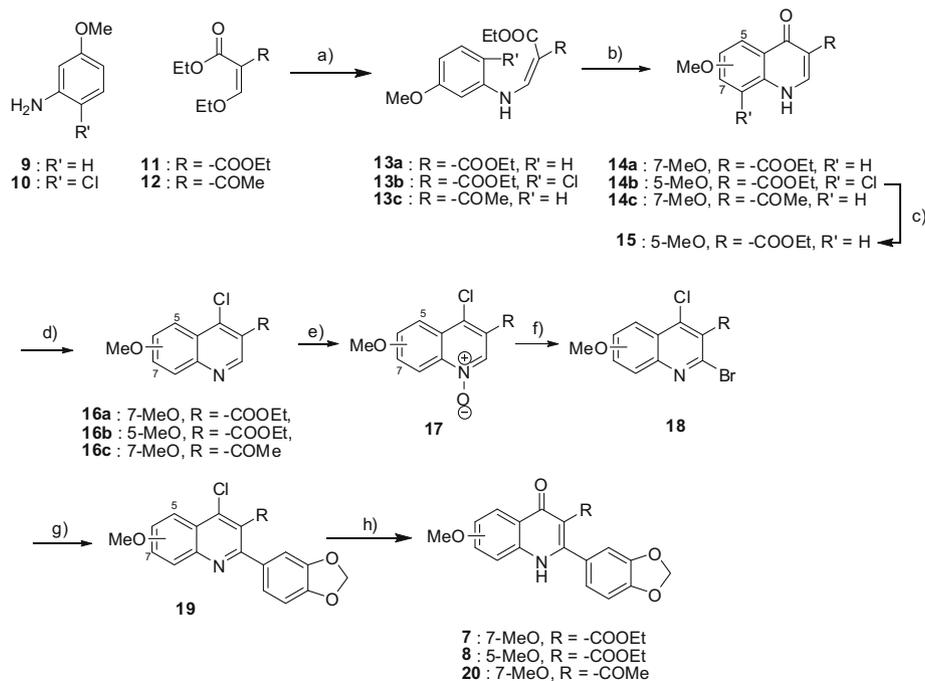
2. Synthesis of targeted analogues of 7 and 8

There were several issues to be addressed in preliminary hit validation on this series. First, the prior work discussed above had indicated that absorption limited pharmacokinetics were a major potential worry with poor solubility and cellular permeability being the major physiochemical drivers. Second, this particular compound series shares significant pharmacophore overlap with

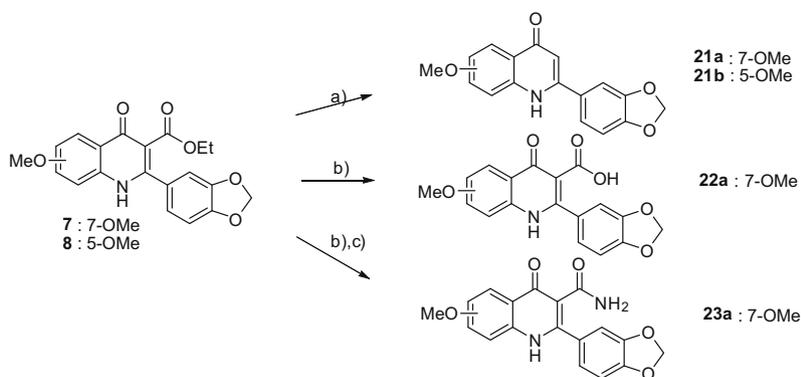
the quinolone antibiotics, which may raise an issue of target mediated cyto- and geno-toxicity. Finally, the methylenedioxy and methoxy groups pose potential metabolic clearance challenges. The preliminary survey of structure–activity relationships was designed to assess liabilities related to all of these issues.

On the basis of their chemical structures, the 4-oxo-3-carboxyl quinolone analogues were divided into four groups. Quinolones containing 3-carboxylic acid constitute a class of antibacterial agents that target bacterial type II topoisomerase.²⁰ Therefore, Series I consisted of derivatives with 3-carboxylic acid, 3-carboxylic amide, or a 3-acetyl group designed to explore whether this moiety was required and if any potential interactions with topoisomerases could be avoided while retaining activity. Series II included derivatives with reduced functionalities at the 3 and 4 positions, with the same overall goal. Series III and IV focused on exploring substituted 2-aryl groups of 7-methoxy quinolones and 5-methoxy quinolones, respectively, with the intention of identifying any alternate ring functionalities that would have lower metabolic liabilities with equivalent potency.

The synthetic route for the derivatives in Series I is illustrated in Schemes 1 and 2. The intermediate 4(1*H*)-quinolones (**14**) were prepared from anilines and diethyl (2-ethoxymethylene)malonate (**11**) or ethyl 2-(ethoxymethylene)acetoacetate (**12**), yielding enamines (**13**). Gould–Jacobs cyclization of **13**, following the published procedure, gave the desired quinolones (**14**).^{21–24} The precursor 4(1*H*)-quinolone (**15**) was obtained by the reduction of **14b** with H₂ in the presence of a catalytic amount of 5% Pd/C.²⁴ The 4(1*H*)-quinolones (**14a**, **14c**, and **15**) were converted to 4-chloro-quinolines (**16**) and then oxidized to yield *N*-oxide derivatives (**17**). Bromide **18** was produced from *N*-oxide **17** through bromination with POBr₃. An *m,p*-methylenedioxyphenyl group was introduced at the 2 position by a Suzuki coupling reaction of **19** and the corresponding boronic acid at 75 °C. Control of the reaction temperature was important in this step to avoid cross-coupling at both the 2 and 4 positions. The desired 4(1*H*)-quinolones (**7**, **8**, and **20**) were prepared by the hydrolysis of 4-chloroquinolines (**19**) in AcOH/H₂O.



Scheme 1. Synthesis of 4(1*H*)-quinolones. Reagents and conditions: (a) EtOH, 130 °C, 3 h; (b) Ph₂O, reflux, 4–6 h; (c) 5% Pd/C, H₂, MeOH, rt, overnight; (d) POCl₃, 1,4-dioxane, 120 °C, 1 h; (e) *m*-chloroperbenzoic acid, CHCl₃, rt, 4 h; (f) POBr₃, CHCl₃, rt, 1 h; (g) 3,4-methylenedioxyphenyl boronic acid, Pd(PPh₃)₄, CsCO₃, 1,4-dioxane/H₂O, 75 °C, 3 h; (h) AcOH/H₂O (4:1), 120 °C, 1 h.



Scheme 2. Synthesis of 3-carboxylic acid and 3-carboxylic amide quinolones. Reagents and conditions: (a) NaOH (1 N, aqueous), 130 °C, overnight; (b) KOH (1 N, aqueous), 75 °C, overnight; (c) *N,N'*-carbonyldiimidazole, DMF, 65 °C, 2.5 h, NH_4OH (aqueous), rt, overnight.

Further derivatives in Series I were synthesized as depicted in Scheme 2. Decarboxylation of **7** and **8** yielded **21a** and **21b**. Hydrolysis of **7** gave 3-carboxylic acid quinolone (**22a**), which was easily converted to 3-carboxylic amide (**23a**).

Compounds in Series II were synthesized as shown in Schemes 3 and 4. The 4-methoxy quinoline (**24**) and 4-methylamino quinoline derivatives (**25**) were prepared by the treatment of **19** with NaOMe and MeNH₂, respectively (Scheme 3). Compounds **26a** and **26b** were prepared from the reduction of **19** with H₂ in the presence of 5% Pd/C (Scheme 4). The treatment of **19a** with diisobutylaluminum hydride (DIBAL) selectively reduced the carboxylate group of **19a** and gave the 4-chloro-hydroxymethyl quinoline (**27a**). However, DIBAL reduced both 4-chloro and 3-carboxylate groups of **19b**. The synthesis of 3-hydroxymethyl quinoline (**29a** and **29b**) was performed by the reduction of **19a** and **19b** in the presence of LiAlH₄ as the reducing agent. Alkylation of **27a** and **29b** was performed to give the corresponding ethyl ethers (**28a** and **30b**).

The synthesis of Series III (**31**) and Series IV (**32**), with various substituted aromatic rings at the C-2 position, is shown in Scheme 5. 2-Aryl quinolone derivatives (**31–32**) were prepared from the cross-coupling of 2-bromo quinoline (**19a** and **19b**) with appropriate boronic acids, followed by refluxing in AcOH/H₂O.

Extensive prior work in the antibacterial field has indicated that it is important to be aware of the quinolone/quinoline tautomerism, which determines where the hydrogen bond donor is located in the inhibitor and influences the interaction between inhibitors and both targets and membranes. The relative thermodynamic stabilities of the quinolone and quinoline forms are predicted to be similar.^{25–27} The more polar quinolone tautomer is predicted to be predominantly adopted in the solid state and in aqueous solu-

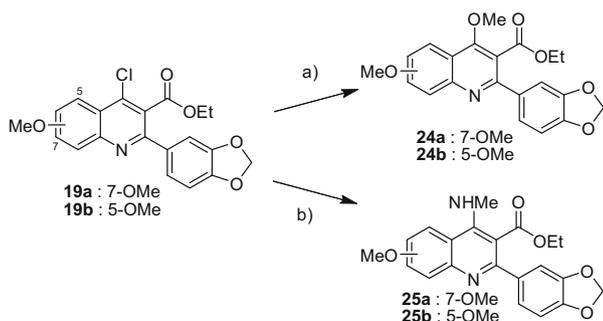
tion.^{25,26,28} The presence of a carboxylate group at the 3 position could act as a hydrogen bond acceptor for the 4-OH group and thus might stabilize the enol quinoline tautomer.²⁵ To explore this tautomerism, we studied the Fourier transform infrared (FTIR) spectrum (KBr pellet) and nuclear Overhauser effects (NOE; in DMSO) of compound **7**. We observed carbonyl vibrations at 1722.47 cm⁻¹, 1632.53 cm⁻¹, and 1627.52 cm⁻¹ by FTIR, indicating the presence of the quinolone tautomer of compound **7** in the solid state. The quinolone tautomer was also supported by the NOE studies of compound **7**, as shown in Figure 2. A characteristic NOE was observed between the *N*-proton and the quinolone proton at the 8 position, as well as between the *N*-proton and the *ortho*-protons of the *m,p*-methylenedioxyphenyl group.

3. Biological activities and physical properties of analogue series

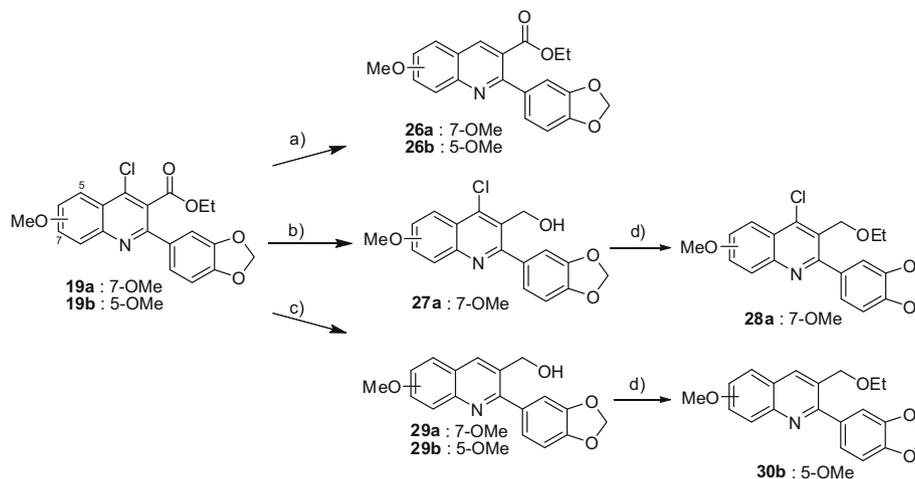
All compounds described in this work were evaluated for anti-malarial activity against the CQ-S *P. falciparum* strain 3D7 and the CQ-R strain K1 using a previously described assay.²⁹ For all compounds, concentration response curves were defined using 10-point, twofold dilution schemes. Each experiment was performed in triplicate, and all experiments were independently replicated at least twice. Data are reported as average values with standard deviations based upon all replications of the experiments. Additionally, for the reasons discussed above, the aqueous solubility and passive membrane permeability of the compounds were measured in neutral physiologically isotonic buffers. Each experiment was performed in triplicate, and all experiments were independently replicated at least twice. Data are reported as average values with standard deviations based upon all replications of the experiments.

3.1. Series I: Quinolones with various substituents at the 3-position

The antimalarial activity of 7-methoxy quinolones varied within a large range of EC₅₀ values, as shown in Table 1. The carboxyl ester **7** had the best antimalarial potency (EC₅₀ ~0.25 μM against both the K1 and 3D7 strains). The replacement of the 3-carboxyl ester group by 3-carboxylic acid or 3-carboxylic amide abolished its activity against both strains (**7** vs **22a**, and **7** vs **23a**, Table 1). The introduction of an acetyl group at the 3 position caused a nearly 90% drop in potency (**7** vs **20**, Table 1). We also observed that the absence of a carboxylic ester group in compound **21a** resulted in a lack of activity against both strains. The 5-methoxy quinolone was <10% as active as 7-methoxy quinolone (**7** vs **8**, Table 1). The solubility of these compounds was dependent on



Scheme 3. Synthesis of 4-methoxy and 4-methylamino derivatives. Reagents and conditions: (a) NaOMe, MeOH/THF, 85 °C, 30 min; (b) MeNH₂ (2 N in MeOH), THF, 85 °C, 30 min.



Scheme 4. Reduction of 4-chloroquinolones **19**. Reagents and conditions: (a) 5% Pd/C, H₂, Et₃N, MeOH/THF, rt, overnight; (b) DIBAL, THF, 0 °C to rt, 7 h; (c) LiAlH₄, THF, –42 °C, 1 h, rt, overnight; (d) NaH (60% dispersion on mineral oil), EtI, THF, overnight.



Scheme 5. Exploring the 2-aryl group. Reagents and conditions: (a) ArB(OH)₂, Pd(PPh₃)₄, CsCO₃, 1,4-dioxane/H₂O, 75 °C, 3 h; (b) AcOH/H₂O (4:1), 120 °C, 1 h.

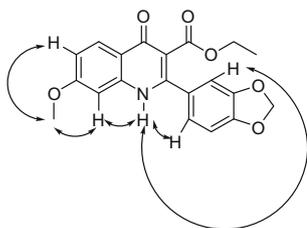
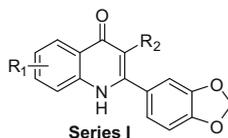


Figure 2. Determination of quinolone/quinoline tautomerism of compound **7** in DMSO by NOE.

Table 1

Summary of Series I compounds: antimalarial activity, solubility, and permeability across an artificial membrane (PAMPA)



	R1	R2	EC ₅₀ ^a (μM) K1 strain	EC ₅₀ ^a (μM) 3D7 strain	Solubility ^b (μM)	Permeability ^c (10 ^{–6} cm/s)
7	7-OMe	COOEt	0.25 ± 0.01	0.24 ± 0.02	>100	100 ± 13
22a	7-OMe	COOH	>10	>10	>100	19 ± 3
23a	7-OMe	CONH ₂	>10	>10	>100	18 ± 2
20	7-OMe	COCH ₃	2.32 ± 0.02	2.00 ± 0.30	36 ± 1	88 ± 17
21a	7-OMe	H	>10	>10	3 ± 0	146 ± 15
8	5-OMe	COOEt	3.01 ± 0.08	2.81 ± 0.11	>100	47 ± 7
21b	5-OMe	H	>10	>10	1 ± 0	173 ± 24

^a EC₅₀ values are reported as the mean ± SD of at least two independent experiments. Positive controls were mefloquine (EC₅₀ in K1: 0.04 ± 0.02 μM; EC₅₀ in 3D7: 0.16 ± 0.02 μM) and chloroquine (EC₅₀ in K1: 1.61 ± 0.30 μM; EC₅₀ in 3D7: 0.09 ± 0.02 μM).

^b Solubility in PBS buffer, pH 7.4, containing 5% DMSO. The detection limit of the solubility assay was 100 μM. Two standards were tested in the same assay: albendazole (3 ± 0.1 μM, poorly soluble) and carbamazepine (>100 μM, highly soluble).

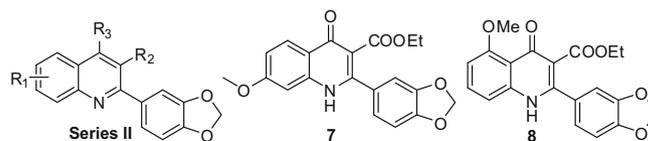
^c Permeability at pH 7.4. Three standards were tested in the same assay: ranitidine HCl (1.2 ± 0.7 × 10^{–6} cm/s, poorly permeable), carbamazepine (145 ± 13 × 10^{–6} cm/s, moderately permeable), and verapamil HCl (1960 ± 290 × 10^{–6} cm/s, highly permeable).

3-substituents. Compounds with 3-carboxylate, 3-acid, and 3-amide groups, such as **7**, **22a**, **23a**, and **8**, were highly soluble (>100 μM in phosphate-buffered saline [PBS] with DMSO at pH 7.4). Compounds **21a** and **21b**, which lacked 3-substituents, were less soluble (3 and 1 μM, respectively). In addition, we observed tolerable permeability (18–173 × 10^{–6} cm/s) for all compounds in Series I. Compounds with hydrophilic groups, such as **22a** and **23a**, did not penetrate the lipid layer as well as compound **7**. Because of the limitations of 7-methoxy and 5-methoxy quinolones containing carboxylate at the 3-position, we focused our attention on compounds containing the 7-methoxy and 3-carboxyl ester substituents.

3.2. Series II: Quinoline analogues

The changes in properties arising from reduced functionalities at the 3 and 4 positions are shown in Table 2. Changing the functional keto at the 4 position to a 4-methoxy group led to a 2–3-fold decrease in potency (**7** vs **24a**, and **8** vs **24b**, Table 2), suggesting that the 4-keto moieties in **7** and **8** makes them more active than 4-methoxy quinolones. This finding motivated us to evaluate the

Table 2
Summary of Series II compounds: antimalarial activity, solubility, and permeability across an artificial membrane (PAMPA)



	R1	R2	R3	EC ₅₀ ^a (μM) K1 strain	EC ₅₀ ^a (μM) 3D7 strain	Solubility ^b (μM)	Permeability ^c (10 ⁻⁶ cm/s)
7	–	–	–	0.25 ± 0.01	0.24 ± 0.02	>100	100 ± 13
8	–	–	–	3.01 ± 0.08	2.81 ± 0.11	>100	47 ± 7
24a	7-Ome	COOEt	OMe	0.71 ± 0.09	0.61 ± 0.01	24 ± 3	783 ± 139
25a	7-Ome	COOEt	NHMe	>10	>10	59 ± 11	2013 ± 179
26a	7-Ome	COOEt	H	>10	>10	11 ± 2	649 ± 44
19a	7-Ome	COOEt	Cl	>10	>10	0.4 ± 0.1	735 ± 0
29a	7-Ome	CH ₂ OH	H	>10	>10	65 ± 6	356 ± 22
28a	7-Ome	CH ₂ OEt	Cl	>10	>10	2 ± 0	802 ± 370
24b	5-Ome	COOEt	OMe	6.00 ± 0.20	6.10 ± 0.90	51 ± 4	193 ± 14
25b	5-Ome	COOEt	NHMe	>10	>10	67 ± 0	1240 ± 154
26b	5-Ome	COOEt	H	>10	>10	10 ± 2	918 ± 171
29b	5-Ome	CH ₂ OH	H	>10	>10	69 ± 0	644 ± 20
30b	5-Ome	CH ₂ OEt	H	>10	>10	16 ± 3	2796 ± 1746

^a EC₅₀ values are reported as the mean ± SD of at least two independent experiments. Positive controls were mefloquine (EC₅₀ in K1: 0.04 ± 0.02 μM; EC₅₀ in 3D7: 0.16 ± 0.02 μM) and chloroquine (EC₅₀ in K1: 1.61 ± 0.30 μM; EC₅₀ in 3D7: 0.09 ± 0.02 μM).

^b Solubility in PBS buffer, pH 7.4, containing 5% DMSO. The detection limit of the solubility assay was 100 μM. Two standards were tested in the same assay: albendazole (3 ± 0.1 μM, poorly soluble) and carbamazepine (>100 μM, highly soluble).

^c Permeability at pH 7.4. Three standards were tested in the same assay: ranitidine HCl (1.2 ± 0.7 × 10⁻⁶ cm/s, poorly permeable), carbamazepine (145 ± 13 × 10⁻⁶ cm/s, moderately permeable), and verapamil HCl (1960 ± 290 × 10⁻⁶ cm/s, highly permeable).

activity of *N*-alkyl derivatives. However, we found that *N*-ethylated **7³⁰** was inactive against both K1 and 3D7 strains (EC₅₀ >10 μM). In Series II, other reduced functionalities at the 4 position or at both the 3 and 4 positions all caused a complete loss of activity (**25a–b**, **26a–b**, **28a**, **29b**, and **30b**, Table 2). The solubility of this series ranged widely (from 0.4 to >100 μM). Compounds containing 4-methoxy, 4-methylamino, or 3-hydroxymethyl (**24a–b**, **25a–b**, **29a–b**) were acceptably soluble (24–69 μM). All the tested quinolones were moderately or highly permeable (193–2796 × 10⁻⁶ cm/s). The results of these two sets of experiments prompted us to strongly focus our subsequent work on compounds with the 4(1*H*)-quinolone moiety.

3.3. Series III: 7-Methoxy quinolones with various 2-aryl groups

In the Series III compounds, the influence of the aryl group at the C-2 position of 7-methoxy quinolones was explored (Table 3). Compared with reference compound **7** (EC₅₀: 0.25 μM), the unsubstituted phenyl group (**31a**) and the 2'-thienyl ring (**31b**) at the C-2 position led to 3-fold and 12-fold decreases in antimalarial potency, respectively. *meta* Substitutions generally gave increased potency, depending on the nature of the substituent. The *meta*-methoxy phenyl compound (**31c**) was the most potent analogue (EC₅₀ in K1: 0.13 μM; EC₅₀ in 3D7: 0.10). *meta*-Methyl phenyl (**31d**), *meta*-trifluoromethyl phenyl (**31e**), and *meta*-chloro phenyl (**31f**) analogues all had potencies approximately equivalent to that of the lead compound (**7**). However, the *meta*-fluoro phenyl (**31g**) and *meta*-nitro phenyl (**31h**) analogues were slightly less potent. The presence of *para*-substituents on the phenyl ring, such *para*-methoxy (**31i**), *para*-*t*-butyl (**31j**), *para*-trifluoromethyl (**31k**), and *para*-chloro (**31l**), led to an overall decrease in antimalarial potency, with EC₅₀ values between 1 μM and >10 μM. On the other hand, *ortho* substitution on the phenyl at the C-2 position caused the complete loss of potency against both strains (**31n** and **31o**). The solubility of Series III compounds varied between 2 and >100 μM. Interestingly, *para*-substituents on the 2-aryl group caused poor solubility, as shown in compounds **31i–l** with 2–8 μM of solubility. All 7-methoxy quinolones in Series III had moderate to high permeability. These studies

made it clear that *meta*-substituted 2-phenyl rings gave the best overall average potency. They also confirmed that the potency of the original lead compounds could be retained while replacing the potentially metabolically labile and genotoxic methylenedioxy substitution pattern with more stable and less toxicologically troublesome substituents.

3.4. Series IV: 5-Methoxy quinolones with various 2-aryl groups

In this series, 12 compounds with various aryl groups at the 2-position had no measurable antimalarial activity against either the K1 or 3D7 strains (Table 4). In general, the compounds in Series IV that did have activity were significantly less potent than those in Series III. We observed moderate (22 μM) to high solubility (>100 μM) for compounds in Series IV, except for **32j** (1 μM), which included a hydrophobic *t*-butyl group. The permeability of Series IV compounds varied from 37 × 10⁻⁶ cm/s to 1143 × 10⁻⁶ cm/s. The results of this study focused our work on 7-methoxy substituted compounds.

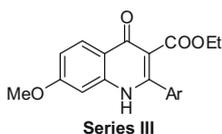
3.5. Toxicity studies

To provide preliminary assessment of inherent toxicity, all compounds were screened using a panel of mammalian cell lines, including an embryonic kidney cell line (HEK 293), a lymphoblastoid cell line (Raji), a hepatocellular carcinoma cell line (HepG2), and a fibroblast cell line (BJ). The cytotoxicity of each compound was first evaluated by screening for effects on cell viability at a fixed concentration of 27.5 μM. No compound was more than 60% cytotoxic at this concentration in any cell line. Compounds that were 30–60% cytotoxic at 27.5 μM were further tested to determine their potencies. Each experiment was performed in triplicate, and all experiments were independently replicated at least twice. Data are reported as average values with standard deviations based upon all replications of the experiments.

Since HEK 293 cells were the most sensitive of the four cell lines, a therapeutic index (TI) was defined as the ratio between the EC₅₀ in the cytotoxicity assay on HEK 293 cells and the EC₅₀

Table 3

Summary of Series III compounds: antimalarial activity on CQ-resistant strain K1 and CQ-sensitive strain 3D7, solubility, and permeability across an artificial membrane (PAMPA)



	Aryl group	EC ₅₀ ^a (μM) K1 strain	EC ₅₀ ^a (μM) 3D7 strain	Solubility ^b (μM)	Permeability ^c (10 ⁻⁶ cm/s)
7	<i>m,p</i> -Methylenedioxy-phenyl	0.25 ± 0.01	0.24 ± 0.02	>100	100 ± 13
31a	Phenyl	0.82 ± 0.07	0.79 ± 0.04	>100	194 ± 41
31b	2-Thienyl	3.10 ± 0.60	2.87 ± 0.01	>100	105 ± 10
31c	<i>m</i> -MeO-phenyl	0.13 ± 0.01	0.10 ± 0.01	>100	172 ± 19
31d	<i>m</i> -Me-phenyl	0.30 ± 0.01	0.29 ± 0.01	48 ± 8	227 ± 7
31e	<i>m</i> -CF ₃ -phenyl	0.22 ± 0.02	0.21 ± 0.02	19 ± 1	569 ± 80
31f	<i>m</i> -Cl-phenyl	0.18 ± 0.02	0.20 ± 0.01	58 ± 8	948 ± 39
31g	<i>m</i> -F-phenyl	0.55 ± 0.02	0.59 ± 0.05	>100	188 ± 14
31h	<i>m</i> -NO ₂ -phenyl	0.87 ± 0.01	0.84 ± 0.02	13 ± 1	397 ± 19
31i	<i>p</i> -MeO-phenyl	1.15 ± 0.22	1.35 ± 0.12	8 ± 2	196 ± 20
31j	<i>p</i> - <i>t</i> Bu-phenyl	1.02 ± 0.03	1.93 ± 1.69	2 ± 0	876 ± 54
31k	<i>p</i> -CF ₃ -phenyl	>10	3.60 ± 0.07	8 ± 0	312 ± 15
31l	<i>p</i> -Cl-phenyl	>10	2.09 ± 0.27	3 ± 0	501 ± 71
31m	<i>m,p</i> -Cl, Cl-phenyl	2.90 ± 2.80	2.52 ± 0.90	17 ± 3	763 ± 19
31n	<i>o</i> -Me-phenyl	>10	>10	>100	100 ± 13
31o	<i>o</i> -Cl-phenyl	>10	>10	28 ± 1	674 ± 81

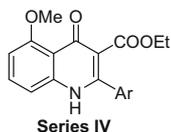
^a EC₅₀ values are reported as the mean ± SD of at least two independent experiments. Positive controls were mefloquine (EC₅₀ in K1: 0.04 ± 0.02 μM; EC₅₀ in 3D7: 0.16 ± 0.02 μM) and chloroquine (EC₅₀ in K1: 1.61 ± 0.30 μM; EC₅₀ in 3D7: 0.09 ± 0.02 μM).

^b Solubility in PBS buffer, pH 7.4, containing 5% DMSO. The detection limit of the solubility assay was 100 μM. Two standards were tested in the same assay: albendazole (3 ± 0.1 μM, poorly soluble) and carbamazepine (>100 μM, highly soluble).

^c Permeability at pH 7.4. Three standards were tested in the same assay: ranitidine HCl (1.2 ± 0.7 × 10⁻⁶ cm/s, poorly permeable), carbamazepine (145 ± 13 × 10⁻⁶ cm/s, moderately permeable), and verapamil HCl (1960 ± 290 × 10⁻⁶ cm/s, highly permeable).

Table 4

Summary of Series IV compounds: antimalarial activity on CQ-resistant strain K1 and CQ-sensitive strain 3D7, solubility, and permeability across an artificial membrane (PAMPA)



Compound	Aryl group	EC ₅₀ (μM) ^a K1 strain	EC ₅₀ (μM) ^a 3D7 strain	Solubility ^b (μM)	Permeability (10 ⁻⁶ cm/s) ^c
8	<i>m,p</i> -Methylenedioxy-phenyl	3.01 ± 0.08	2.81 ± 0.11	>100	47 ± 7
32a	Phenyl	>10	>10	>100	37 ± 1
32c	<i>m</i> -MeO-phenyl	7.98 ± 3.09	9.94 ± 0.13	>100	64 ± 13
32d	<i>m</i> -Me-phenyl	9.60 ± 2.00	>10	>100	126 ± 14
32e	<i>m</i> -CF ₃ -phenyl	>10	>10	>100	377 ± 33
32f	<i>m</i> -Cl-phenyl	>10	>10	>100	196 ± 38
32g	<i>m</i> -F-phenyl	>10	>10	>100	79 ± 12
32h	<i>m</i> -NO ₂ -phenyl	>10	>10	49 ± 2	110 ± 6 ^d
32i	<i>p</i> -MeO-phenyl	>10	8.80 ± 2.00	>100	78 ± 4
32j	<i>p</i> - <i>t</i> Bu-phenyl	>10	>10	1 ± 0	1143 ± 56
32k	<i>p</i> -CF ₃ -phenyl	>10	>10	22 ± 3	457 ± 72
32l	<i>p</i> -Cl-phenyl	>10	>10	>100	211 ± 15
32m	<i>m,p</i> -DiCl-phenyl	>10	>10	39 ± 5	781 ± 77
32n	<i>o</i> -Me-phenyl	>10	>10	>100	92 ± 13
32o	<i>o</i> -Cl-phenyl	>10	>10	>100	112 ± 28

^a EC₅₀ values are reported as the mean ± SD of at least two independent experiments. Positive controls were Mefloquine (EC₅₀ in K1: 0.04 ± 0.02 μM; EC₅₀ in 3D7: 0.16 ± 0.02 μM) and Chloroquine (EC₅₀ in K1: 1.61 ± 0.30 μM; EC₅₀ in 3D7: 0.09 ± 0.02 μM).

^b Solubility in PBS buffer, pH 7.4, containing 5% DMSO. The detection limit of the solubility assay was 100 μM. Two standards were tested in the same assay: albendazole (3 ± 0.1 μM, poorly soluble) and carbamazepine (>100 μM, highly soluble).

^c Permeability at pH 7.4. Three standards were tested in the same assay: ranitidine HCl (1.2 ± 0.7 × 10⁻⁶ cm/s, poorly permeable), carbamazepine (145 ± 13 × 10⁻⁶ cm/s, moderately permeable), and verapamil HCl (1960 ± 290 × 10⁻⁶ cm/s, highly permeable).

^d Permeability was measured at pH 4.0. Two standards were tested in the same assay: ranitidine HCl (0, poorly permeable) and carbamazepine (143 ± 7 × 10⁻⁶ cm/s, moderately permeable).

in the antimalarial assay on the K1 strain. For compounds <30% cytotoxic at 27.5 μM, the potency of these compounds as a cytotoxin was conservatively estimated at 27.5 μM. Eight compounds had a TI >10, with three having a TI >100 and two of those having a TI >200 (Fig. 3). This reflected the ability of compounds to inhibit

parasite growth at concentrations significantly lower than those producing mammalian cytotoxicity.

Quinolones are a well known class of bactericidal drugs that inhibit bacterial topoisomerase II or topoisomerase IV, thereby blocking bacterial DNA replication.^{20,31–33} Quinolone antibacterial drugs have

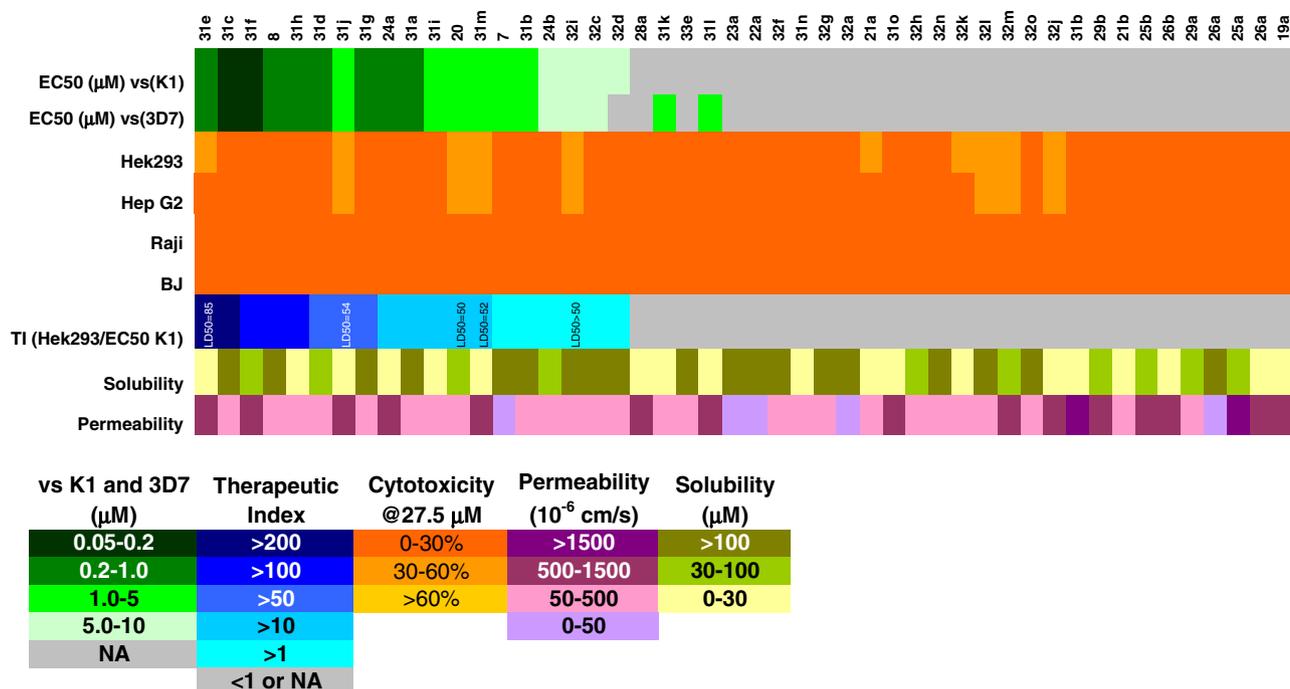


Figure 3. Activity and physicochemical property summary sorted by therapeutic index. Antimalarial activity is represented as the EC₅₀ value in green, with darker squares indicating higher potency. Cytotoxicity data is represented as the percentage cell death relative to untreated control at a fixed 27.5 µM screening concentration in orange, with lighter squares indicating more cell death. The therapeutic index is represented as a ratio of the cytotoxicity value in HEK 293 cells to the EC₅₀ in the K1 strain in blue, with darker squares indicating higher potency (LD₅₀ in HEK 293 cells applied to estimate the therapeutic index.). Permeability (PAMPA) at pH 7.4 is represented in purple, with darker squares indicating higher permeability. Solubility is represented in yellow, with darker squares indicating higher solubility.

been reported to have weak antimalarial activity against *P. falciparum* parasites, suggesting that the malarial topoisomerase shares some overlapping sensitivity with the bacterial.^{34,35} Because of the similarity of analogues of **7** to antibacterial quinolones, we evaluated the antibacterial activity of five compounds, **7**, **21a**, **24a**, **31a**, and N-ethylated **7** on the *Escherichia coli* BL21 strain. None of the tested compounds inhibited bacterial growth at concentrations up to 200 µM, whereas the control fluoroquinolone was highly active. Moreover, our antimalarial testing showed that the introduction of the 3-carboxylic acid or an N-ethyl group on the quinolone nitrogen, functional features of quinolone antibacterial agents, abolished antimalarial potency. These limited findings suggest that topoisomerase II of *P. falciparum* is not a target for the quinolone ester derivatives reported herein.

Because the MMV/WHO product profile specifies orally available drugs, drug-like antimalarial candidates should possess a balance between antimalarial potency, solubility, permeability, and toxicity. Among the subseries studied, only analogues (**31c–h**) in Series III containing a *meta*-substituted phenyl at the 2-position had such properties. Compound **31c**, equipotent with mefloquine against the 3D7 strain, was identified as the most active analogue with high solubility (>100 µM) and reasonable permeability (172×10^{-6} cm/s). Exploring *meta*-substituents of the 2 aromatic ring should provide the possibility to improve both potency and physicochemical properties. Moreover, isosteres of 3-carboxylate would likely be valuable in future investigations.

4. Conclusion

Our structure–activity relationship studies revealed that several factors influence the antimalarial potency of quinolone analogues: (1) an ethyl carboxylate at the 3 position was superior to a carboxylic acid, carboxylic amide, or other replacements; (2) removal of the 4-oxo moiety led to a strong decrease in antimalarial potency;

(3) a 7-methoxy group was favored over a 5-methoxy group; and (4) an aryl ring at the C-2 position caused large variations in antimalarial potency. With respect to the C-2 aryl ring, *meta*-substituents were superior to *para*-substituents and *ortho*-substituents. Compounds **31c–h** had reasonably drug-like properties. In particular, compound **31c** was the most promising analogue.

With respect to structure–property relationships, there were no clear structural associations that governed either solubility or hydrophobicity other than a loose correlation between hydrophobicity of introduced groups and decreased solubility. There were no clear associations between potency and either solubility or permeability.

Overall, this series shows promise for further development with a particular focus on understanding absorption, distribution, metabolism, excretion, and toxicology (ADMET). Clearly two early goals are improving potency and aqueous solubility. Additionally, isosteres of the carboxyl ester, isosteres of the 4(1*H*)-quinolone, and exploration of solubility-enhancing substituents at the *meta* position of the 2 aromatic ring are all appropriate venues for future work.

5. Experimental section

5.1. General methods

3,4-Methylenedioxyphenyl boronic acid was purchased from Boron Molecular, and all other chemical reagents were from Acros, Aldrich, or Combi-Blocks. All materials were obtained from commercial suppliers and used without further purification. Thin layer chromatography was performed using a Silica Gel 60 F254 plate from EMD. Purification of compounds was done by normal phase column chromatography (Biotage SP1). ¹H NMR, ¹³C NMR, and 2D NOE spectra were recorded on a Bruker 400 MHz. Chemical shifts were expressed in ppm relative to tetramethylsilane, which was used as an internal standard. Purity was estimated using high-per-

formance liquid chromatography/mass spectrometry (Alliance HT, Micromass ZQ 4000, and RP-C18 Xterra column, 5 μm , 6 \times 50 mm [Waters]) or ultra-performance liquid chromatography/mass spectrometry (Acquity PDA detector, Acquity SQ detector, and Acquity UPLC BEH-C18 column, 1.7 μm , 2.1 \times 50 mm [Waters]). FTIR spectrums were recorded on a Thermo Nicolet IR 100 FTIR spectrometer. Compounds prepared in our laboratory were generally 90–98% pure. Efficacy data were obtained only on compounds that were at least 95% pure.

5.2. Ethyl 7-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylate (14a) and 3-acetyl-7-methoxyquinolin-4(1H)-one (14c)

Ethyl 7-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylate (**14a**) was prepared from *m*-aminoanisole (**9**) (15 mmol) and diethyl 2-(ethoxymethylene)malonate (**11**) (3.309 mL, 16.5 mmol), followed by Gould–Jacobs cyclization according to the published procedure.²² 3-Acetyl-7-methoxyquinolin-4(1H)-one (**14c**) was prepared in a similar manner from *m*-aminoanisole and ethyl 2-(ethoxymethylene)acetoacetate.²³

5.3. Ethyl 5-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylate (15)

The intermediate compound ethyl 8-chloro-5-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylate (**14b**) was prepared in a manner similar to that of **14a** from 2-chloro-5-methoxyanilin (**10**) and diethyl 2-(ethoxymethylene)malonate (**11**). Following the treatment of **14b** with H_2 in the presence of a catalytic amount of 5 wt % Pd/C at room temperature (rt) overnight, the catalyst was removed by filtration through Celite, and the filtrate was concentrated under vacuum to produce compound **15** at a 97% yield.

5.4. Ethyl 2-bromo-4-chloro-7-methoxyquinoline-3-carboxylate (18a)

Phosphorus oxychloride (0.72 mL, 7.8 mmol) was added to a suspended solution of 3-carboxylate quinolone (**14a**) (1.6 g, 6.5 mmol) in 6 mL of 1,4-dioxane. The mixture was sealed in a reaction tube and stirred at 120 °C for 1 h. After being cooled to rt, the reaction mixture was poured into ice water and then neutralized by adding K_2CO_3 until the pH was approximately 7. 4-Chloro quinoline intermediate **16a** was extracted by CH_2Cl_2 (30 mL \times 2). Organic layers were combined, dried over Na_2SO_4 , filtered, and concentrated to produce **16a** at an 86% yield. No purification was performed on **16a**.

4-Chloro quinoline intermediate **16a** was treated with *m*-chloroperbenzoic acid (1.74 g, 7.8 mmol) in 30 mL of CHCl_3 at rt for 4 h. The reaction was quenched by adding NaHCO_3 aqueous solution. The aqueous layer was extracted by CH_2Cl_2 (30 mL \times 2). The combined organic layers were dried over Na_2SO_4 , filtered, and concentrated to produce *N*-oxide intermediate **17a** at a 92% yield, which was used for the next step without the further purification.

Phosphorus oxybromide (2.06 g, 7.2 mmol) was added to the solution of *N*-oxide intermediate **17a** in 30 mL of CHCl_3 . After being stirred at rt for 1 h, the reaction was quenched by adding ice water and then neutralized by adding K_2CO_3 until the pH was approximately 7. The aqueous layer was extracted by CH_2Cl_2 (30 mL \times 2). The combined organic layers were dried over Na_2SO_4 , filtered, and concentrated. Purification was done by flash column chromatography to give the corresponding compounds **18a** at a 71% yield over three steps. (SP1, 40S, SiO_2 , flow rate: 40 mL/min, gradient: 1–20% ethyl acetate in hexanes over 7 CV; 20% ethyl acetate in hexanes over 10 CV). ^1H NMR (400 MHz, CDCl_3) δ 8.01 (d, $J = 9.2$, 1H), 7.25 (dd, $J = 6.3$, 15.4, 2H), 4.45 (q, $J = 7.2$, 2H), 3.88 (s, 3H), 1.39 (t, $J = 7.2$, 3H). ^{13}C NMR (401 MHz, CDCl_3) δ 164.63, 162.83, 150.13,

140.35, 136.96, 127.43, 125.79, 121.86, 119.65, 107.16, 62.82, 55.89, 14.05. Electrospray ionization mass spectrometry (MS [ESI]) calculated for $\text{C}_{13}\text{H}_{11}\text{BrClNO}_3$ [M+H]⁺, 343.97; found, 344.65.

5.5. Ethyl 2-bromo-4-chloro-5-methoxyquinoline-3-carboxylate (18b)

The compound was prepared in a manner similar to that of **18a** from **15** for a 48% yield over three steps. ^1H NMR (400 MHz, CDCl_3) δ 7.59–7.68 (m, 3H), 6.96 (d, $J = 7.5$, 1H), 4.49 (q, $J = 7.1$, 2H), 3.95 (s, 3H), 1.43 (t, 3H), ^{13}C NMR (400 MHz, CDCl_3) δ 164.82, 156.82, 150.09, 139.31, 136.71, 132.02, 130.79, 121.60, 116.74, 108.10, 62.73, 56.17, 13.99. MS (ESI) calculated for $\text{C}_{13}\text{H}_{11}\text{BrClNO}_3$ [M+H]⁺, 343.97; found, 344.65.

5.6. 1-(2-Bromo-4-chloro-7-methoxyquinolin-3-yl)ethanone (18c)

The compound was prepared in a manner similar to that of **18a** from **14c** for a 23% yield over three steps. ^1H NMR (400 MHz, CDCl_3) δ 7.84 (d, $J = 9.2$, 1H), 7.14–7.02 (m, 2H), 3.72 (s, 3H), 2.45 (s, 3H), ^{13}C NMR (401 MHz, CDCl_3) δ 199.08, 162.73, 150.11, 138.54, 135.50, 133.07, 125.52, 121.94, 119.88, 107.12, 55.91, 31.28. MS (ESI) calculated for $\text{C}_{12}\text{H}_9\text{BrClNO}_2$ [M+H]⁺, 313.96; found, 314.09.

5.7. General procedure for 7, 8, 20, 31a–o, 32a–o

The flask was charged with 2-bromo-4-chloro quinoline intermediate **18** (0.3 mmol), proper boronic acid (0.31 mmol), $\text{Pd}(\text{PPh}_3)_4$ (8.6 mg, 0.0075 mmol) in 2 mL of 1,4-dioxane. The flask was degassed three times. To the mixture was added the solution of CsCO_3 (0.195 g, 0.6 mmol) in 0.6 mL of H_2O . The flask was degassed again three times. The reaction mixture was stirred at 75 °C for 3 h. After being cooled to rt, the bottom aqueous layer was removed, and the organic layer was concentrated and purified by flash column chromatography to produce the intermediate compound ethyl 2-aryl-4-chloroquinoline-3-carboxylate. (SP1, 12S, SiO_2 , flow rate: 6 mL/min, gradient: 1–20% ethyl acetate in hexanes over 7 CV; 20% ethyl acetate in hexanes over 10 CV).

The solution of ethyl 2-aryl-4-chloroquinoline-3-carboxylate intermediate (0.2 mmol) in 1 mL of $\text{AcOH}/\text{H}_2\text{O}$ (4:1) was refluxed at 120 °C for 1 h. The reaction mixture was concentrated and neutralized by adding five drops of NH_4OH . Purification was performed by flash column chromatography to produce the desired compounds. (SP1, 12S, SiO_2 , flow rate: 12 mL/min, gradient: 40% ethyl acetate in hexanes over 5 CV; 100% ethyl acetate over 10 CV). Compound **7** is given as an example. For characterization of **8**, **20**, **31a–o**, and **32a–o**, see [Supplementary data](#).

5.8. Ethyl 2-(benzo[d][1,3]dioxol-5-yl)-7-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylate (7)

A 59% yield was produced over two steps. ^1H NMR (400 MHz, DMSO) δ 11.77 (s, 1H), 8.01 (d, $J = 9.0$, 1H), 7.07–6.98 (m, 6H), 6.14 (d, $J = 4.3$, 2H), 4.03 (d, $J = 7.1$, 3H), 3.86 (s, 3H), 1.03 (t, $J = 7.1$, 3H). ^{13}C NMR (400 MHz, DMSO) δ 173.12, 166.46, 162.29, 148.82, 148.32, 147.36, 141.29, 127.29, 126.74, 122.55, 118.70, 115.22, 113.83, 108.45, 101.76, 99.74, 60.17, 55.48, 13.80. MS (ESI) calculated for $\text{C}_{20}\text{H}_{17}\text{NO}_6$ [M+H]⁺, 368.11; found, 368.02.

5.9. 2-(Benzo[d][1,3]dioxol-5-yl)-7-methoxyquinolin-4(1H)-one (21a)

The suspended solution of quinolone **7** (0.73 g, 2 mmol) in 10 mL of 1 N NaOH was stirred at 130 °C overnight. The cooled solution was neutralized by adding 1 N HCl solution until the pH

was approximately 7. The white precipitate was formed, collected by filtration, and dried under a vacuum for a 75% yield. ^1H NMR (400 MHz, DMSO) δ 11.40 (s, 1H), 7.96 (d, $J = 8.9$, 1H), 7.42–7.35 (m, 2H), 7.13 (dd, $J = 5.3$, 27.9, 2H), 6.89 (dd, $J = 2.4$, 8.9, 1H), 6.23 (s, 1H), 6.13 (s, 2H), 3.85 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 161.50, 148.77, 148.58, 147.79, 126.28, 121.36, 119.42, 112.82, 108.52, 107.32, 101.63, 55.30. MS (ESI) calculated for $\text{C}_{17}\text{H}_{13}\text{NO}_4$ $[\text{M}+\text{H}]^+$, 296.09; found, 296.07.

5.10. 2-(Benzo[d][1,3]dioxol-5-yl)-5-methoxyquinolin-4(1H)-one (21b)

The compound was prepared in a manner similar to that of **21a** from **8** for an 80% yield. ^1H NMR (400 MHz, DMSO) δ 11.17 (br, 1H), 7.43 (dd, $J = 25.9$, 54.7, 4H), 7.09 (d, $J = 8.2$, 1H), 6.77 (s, 1H), 6.13 (s, 3H), 3.33 (s, 9H). ^{13}C NMR (101 MHz, DMSO) δ 177.95, 148.91, 147.81, 131.74, 121.50, 114.93, 108.54, 107.35, 104.28, 101.66, 55.73. MS (ESI) calculated for $\text{C}_{17}\text{H}_{13}\text{NO}_4$ $[\text{M}+\text{H}]^+$, 296.09; found, 295.97.

5.11. 2-(Benzo[d][1,3]dioxol-5-yl)-7-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (22a)

Ethyl ester **7** (0.110 g, 0.3 mmol) underwent hydrolysis in 5 mL of 1 N KOH at 75 °C overnight. The mixture was neutralized by adding 1 N HCl (aqueous), followed by filtration to produce the compound at a 55% yield. ^1H NMR (400 MHz, DMSO) δ 12.75 (s, 1H), 8.20 (d, $J = 9.0$, 1H), 7.24–6.99 (m, 5H), 6.13 (s, 2H), 3.89 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 178.08, 165.30, 163.43, 156.61, 148.32, 146.59, 140.52, 128.70, 126.98, 122.51, 117.52, 116.15, 109.44, 107.79, 106.68, 101.44, 100.12, 55.78. MS (ESI) calculated for $\text{C}_{18}\text{H}_{13}\text{NO}_6$ $[\text{M}+\text{H}]^+$, 340.08; found, 339.86.

5.12. 2-(Benzo[d][1,3]dioxol-5-yl)-7-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxamide (23a)

N,N' -Carbonyldiimidazole (49 mg, 0.3 mmol) was added to the corresponding carboxylic acid **22a** (50 mg, 0.15 mmol) in 1 mL of DMF. After being stirred at 65 °C for 2.5 h, the mixture was cooled to rt and then poured into 5 mL of iced NH_4OH . The solvent was evaporated under a vacuum. To the residue was added 5 mL of iced water, and precipitation was observed. The desired product was collected by filtration at a 56% yield. ^1H NMR (400 MHz, DMSO) δ 11.63 (s, 1H), 8.06 (d, $J = 8.9$, 1H), 7.12–6.89 (m, 6H), 6.12 (s, 2H), 3.85 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 174.58, 167.18, 162.15, 149.85, 148.19, 146.80, 140.86, 128.99, 126.99, 122.45, 119.07, 116.10, 113.77, 108.92, 108.01, 101.46, 99.50, 55.44. MS (ESI) calculated for $\text{C}_{18}\text{H}_{14}\text{N}_2\text{O}_5$ $[\text{M}+\text{H}]^+$, 339.10; found, 338.94.

5.13. Ethyl 2-(benzo[d][1,3]dioxol-5-yl)-4,7-dimethoxyquinoline-3-carboxylate (24a)

To fresh sodium methoxide solution, which was prepared from Na (9 mg, 0.4 mmol) in 1 mL of MeOH, was added the corresponding 4-chloro quinoline **19a** (77 mg, 0.2 mmol) in 0.5 mL of dried tetrahydrofuran (THF). After the mixture was refluxed at 85 °C for 30 min, the solvent was evaporated under a vacuum. The obtained residue was purified by flash column chromatography to produce the compound at a 44% yield (SP1, 12S, SiO_2 , flow rate: 6 mL/min, gradient: 1–20% ethyl acetate in hexanes over 7 CV; 20% ethyl acetate in hexanes over 10 CV). ^1H NMR (400 MHz, CDCl_3) δ 8.06 (d, $J = 9.2$, 1H), 7.49 (s, 1H), 7.20 (ddd, $J = 1.7$, 8.2, 23.1, 3H), 6.91 (d, $J = 8.0$, 1H), 6.04 (s, 2H), 4.27 (q, $J = 7.2$, 2H), 4.16 (s, 3H), 3.98 (s, 3H), 1.20 (t, $J = 7.2$, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ 167.60, 162.08, 161.98, 157.82,

151.17, 148.32, 147.78, 134.11, 123.65, 122.56, 119.81, 116.79, 116.28, 109.14, 108.19, 107.51, 101.28, 62.83, 61.77, 55.67, 13.88. MS (ESI) calculated for $\text{C}_{21}\text{H}_{19}\text{NO}_6$ $[\text{M}+\text{H}]^+$, 382.13; found, 382.03.

5.14. Ethyl 2-(benzo[d][1,3]dioxol-5-yl)-4,5-dimethoxyquinoline-3-carboxylate (24b)

The compound was prepared in a similar manner from **19b** for a 77% yield. ^1H NMR (400 MHz, CDCl_3) δ 7.70–7.57 (m, 2H), 7.18 (d, $J = 8.0$, 1H), 6.84 (dd, $J = 7.9$, 16.0, 2H), 5.96 (s, 2H), 4.24 (q, $J = 7.0$, 2H), 3.98 (s, 3H), 3.96 (s, 3H), 1.17 (t, $J = 7.1$, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ 167.27, 162.79, 156.76, 155.77, 151.62, 148.56, 147.89, 133.46, 130.78, 122.76, 122.36, 121.82, 114.04, 109.17, 108.24, 106.17, 101.31, 64.15, 61.68, 56.33, 14.02. MS (ESI) calculated for $\text{C}_{21}\text{H}_{19}\text{NO}_6$ $[\text{M}+\text{H}]^+$, 382.13; found, 382.03.

5.15. Ethyl 2-(benzo[d][1,3]dioxol-5-yl)-7-methoxy-4-(methylamino)quinoline-3-carboxylate (25a)

The 4-chloro quinoline **19a** (77 mg, 0.2 mmol) and 2 mL of MeNH_2 (2 M in THF) were refluxed at 85 °C for 30 min. After the solution was cooled to rt, the solvent was evaporated. Then, to the residue was added 2 mL of H_2O . The precipitate was collected by filtration. The compound was produced at a 63% yield. ^1H NMR (400 MHz, MeOD) δ 8.09 (d, $J = 9.2$, 1H), 7.22 (s, 1H), 7.10 (d, $J = 9.2$, 1H), 6.91 (dd, $J = 10.2$, 20.3, 2H), 6.01 (s, 2H), 4.02 (q, $J = 7.1$, 2H), 3.92 (s, 3H), 3.07 (s, 3H), 1.01 (t, $J = 7.1$, 3H). ^{13}C NMR (101 MHz, MeOD) δ 170.17, 161.59, 158.46, 150.74, 148.81, 147.82, 147.30, 134.96, 123.06, 121.76, 116.64, 112.40, 108.57, 107.27, 106.28, 105.58, 101.18, 60.97, 54.48, 30.94, 12.45. MS (ESI) calculated for $\text{C}_{21}\text{H}_{20}\text{N}_2\text{O}_5$ $[\text{M}+\text{H}]^+$, 381.15; found, 381.32.

5.16. Ethyl 2-(benzo[d][1,3]dioxol-5-yl)-5-methoxy-4-(methylamino)quinoline-3-carboxylate (25b)

The compound was prepared in a manner similar to that of **25a** from **19b** for a 56% yield. ^1H NMR (400 MHz, MeOD) δ 7.55 (t, $J = 8.2$, 1H), 7.42 (d, $J = 8.5$, 1H), 6.94 (dd, $J = 10.9$, 28.2, 4H), 6.00 (d, $J = 3.0$, 2H), 4.07 (s, 3H), 4.01 (q, $J = 7.2$, 2H), 2.96 (s, 3H), 1.03 (t, $J = 7.2$, 2H). ^{13}C NMR (101 MHz, MeOD) δ 170.13, 158.15, 157.54, 152.34, 148.75, 147.78, 147.25, 134.45, 129.94, 121.61, 120.37, 109.65, 108.51, 107.24, 106.57, 104.97, 101.17, 61.12, 55.53, 30.48, 12.44. MS (ESI) calculated for $\text{C}_{21}\text{H}_{20}\text{N}_2\text{O}_5$ $[\text{M}+\text{H}]^+$, 381.15; found, 380.98.

5.17. Ethyl 2-(benzo[d][1,3]dioxol-5-yl)-7-methoxyquinoline-3-carboxylate (26a)

The corresponding 4-chloro quinoline **19a** (93 mg, 0.24 mmol) in 5 mL of MeOH and 2 mL of THF was treated with H_2 in the presence of Et_3N (34 μL , 0.24 mmol) and 5 mol % of 5 wt % Pd/C. After the mixture was stirred at rt overnight, the Pd/C was filtered out though Celite. The filtrate was concentrated and then purified by flash column chromatography to produce the compound at a 65% yield. (SP1, 12S, SiO_2 , flow rate: 6 mL/min, gradient: 1–20% ethyl acetate in hexanes over 7 CV; 20% ethyl acetate in hexanes over 10 CV). ^1H NMR (400 MHz, CDCl_3) δ 8.55 (s, 1H), 7.76 (d, $J = 9.0$, 1H), 7.23–7.20 (m, 1H), 7.14 (d, $J = 1.8$, 1H), 7.06 (d, $J = 8.0$, 1H), 6.88 (d, $J = 8.0$, 1H), 6.00 (s, 2H), 4.23 (q, $J = 7.2$, 2H), 3.95 (s, 3H), 1.17 (t, $J = 7.1$, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ 167.85, 162.77, 157.87, 148.25, 147.61, 139.21, 129.31, 123.01, 121.08, 120.81, 109.37, 108.15, 107.00, 106.71, 101.27, 61.48, 55.80, 13.96. MS (ESI) calculated for $\text{C}_{20}\text{H}_{17}\text{NO}_5$ $[\text{M}+\text{H}]^+$, 352.12; found, 352.02.

5.18. Ethyl 2-(benzo[d][1,3]dioxol-5-yl)-5-methoxyquinoline-3-carboxylate (26b)

The compound was prepared in a manner similar to that of **26b** from **19b** for a 41% yield. ¹H NMR (400 MHz, CDCl₃) δ 8.98 (s, 1H), 7.68–6.85 (dd, *J* = 6.7, 13.9, 2H), 7.17 (d, *J* = 1.8, 1H), 7.07 (dd, *J* = 1.8, 8.0, 1H), 6.87 (m, 2H), 5.99 (s, 2H), 4.24 (q, *J* = 7.2, 2H), 4.02 (s, 3H), 1.19 (t, *J* = 7.1, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 167.12, 156.62, 154.66, 147.90, 147.22, 146.63, 133.28, 130.84, 123.43, 121.98, 120.24, 117.34, 108.35, 107.11, 103.78, 100.23, 60.51, 54.89, 12.96, 0.02. MS (ESI) calculated for C₂₀H₁₇NO₅ [M+H]⁺, 352.12; found, 352.02.

5.19. (2-(Benzo[d][1,3]dioxol-5-yl)-4-chloro-7-methoxyquinolin-3-yl)methanol (27a)

To the 4-chloro quinoline **19a** (108 mg, 0.28 mmol) in 3 mL of THF was added DIBAL (239 mg, 1.68 mmol) at 0 °C. The mixture was stirred at 0 °C for 10 min, and then at rt overnight. The reaction was quenched by adding saturated Na₂SO₄·10H₂O solution at 0 °C. The mixture was stirred at 0 °C for 20 min, and then at rt for 30 min. The suspended solid was filtered out through Celite. The filtrate was concentrated and purified by flash column chromatography (SP1, 25S, SiO₂, flow rate: 12 mL/min, gradient: 1–20% ethyl acetate in hexanes over 7 CV; 20% ethyl acetate in hexanes over 10 CV; 20–80% ethyl acetate in hexanes over 2 CV; 80% ethyl acetate in hexanes over 3 CV). The compound was obtained at an 82% yield. ¹H NMR (400 MHz, CDCl₃) δ 8.14 (d, *J* = 9.3, 1H), 7.49 (br, 1H), 7.29–7.25 (m, 3H), 6.93 (d, *J* = 8.4, 1H), 6.02 (s, 2H), 4.89 (d, *J* = 6.4, 2H), 3.94 (s, 3H).

5.20. (2-(Benzo[d][1,3]dioxol-5-yl)-7-methoxyquinolin-3-yl)methanol (29a)

To LiAlH₄ (15 mg, 0.4 mmol) in 1 mL of dry THF was added the solution of the corresponding 4-chloro quinoline **19a** (50 mg, 0.13 mmol) in 1 mL of THF at –42 °C. The reaction mixture was stirred at –42 °C for 1 h, then at rt overnight. The reaction was quenched by adding 1 N NaOH aqueous solution. The suspended solid was filtered out through Celite. The filtrate was concentrated and purified by flash column chromatography (SP1, 12S, SiO₂, flow rate: 6 mL/min, gradient: 1–30% ethyl acetate in hexanes over 7 CV; 30% ethyl acetate in hexanes over 10 CV). The compound was obtained at a 36% yield. ¹H NMR (400 MHz, CDCl₃) δ 8.25 (s, 1H), 7.72 (d, *J* = 9.0, 1H), 7.50 (s, 1H), 7.19 (dd, *J* = 2.5, 8.9, 1H), 7.14–7.09 (m, 2H), 6.90 (d, *J* = 8.0, 1H), 6.01 (s, 2H), 4.78 (s, 2H), 3.93 (s, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 171.18, 161.21, 148.17, 147.76, 129.90, 128.48, 123.00, 122.52, 120.15, 109.59, 108.32, 106.63, 101.31, 60.41, 55.64. MS (ESI) calculated for C₁₈H₁₅NO₄ [M+H]⁺, 310.11; found, 309.98.

5.21. (2-(Benzo[d][1,3]dioxol-5-yl)-5-methoxyquinolin-3-yl)methanol (29b)

The compound was prepared in a manner similar to that of **29a** from **19b** for a 42% yield. ¹H NMR (400 MHz, MeOD) δ 8.83 (s, 1H), 7.68–7.59 (m, 2H), 7.12–6.96 (m, 5H), 6.05 (s, 2H), 4.70 (s, 2H), 4.06 (s, 3H). ¹³C NMR (101 MHz, MeOD) δ 160.28, 156.62, 149.17, 148.55, 134.56, 133.53, 132.22, 131.30, 124.13, 121.20, 120.81, 110.47, 109.11, 105.76, 102.84, 62.55, 56.47. MS (ESI) calculated for C₁₈H₁₅NO₄ [M+H]⁺, 310.11; found, 310.24.

5.22. 2-(Benzo[d][1,3]dioxol-5-yl)-4-chloro-3-(ethoxymethyl)-7-methoxyquinoline (28a)

The corresponding 3-hydroxymethyl quinoline **27a** (34 mg, 0.1 mmol) and NaH (60% dispersion in mineral oil, 8 mg, 0.2 mmol)

in 1 mL of dry THF were stirred at rt for 1 h. To the reaction mixture was added Et₃N (42 μL, 3 mmol). The mixture was stirred at 75 °C for 1 h, followed by quenching with H₂O. The aqueous layer was extracted by dichloromethane (3 × 3 mL). Organic layers were combined, dried over Na₂SO₄, filtered, and concentrated. The crude product was purified by flash column chromatography (SP1, 12S, SiO₂, flow rate: 6 mL/min, gradient: 1–20% ethyl acetate in hexanes over 7 CV; 20% ethyl acetate in hexanes over 10 CV). The compound was obtained at an 80% yield. ¹H NMR (400 MHz, CDCl₃) δ 8.14 (d, *J* = 9.3, 1H), 7.43 (s, 1H), 7.26 (dd, *J* = 7.2, 9.3, 3H), 6.91 (d, *J* = 7.8, 1H), 6.01 (s, 2H), 4.60 (s, 2H), 3.93 (s, 3H), 3.60 (q, *J* = 7.0, 2H), 1.27 (t, *J* = 7.0, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 176.98, 168.05, 155.07, 147.59, 125.84, 124.88, 123.57, 120.90, 120.72, 109.95, 108.15, 101.29, 67.61, 66.39, 55.78, 15.27. MS (ESI) calculated for C₂₀H₁₈ClNO₄ [M+H]⁺, 372.10; found, 371.99.

5.23. 2-(Benzo[d][1,3]dioxol-5-yl)-3-(ethoxymethyl)-5-methoxyquinoline (30b)

The compound was prepared in a manner similar to that of **28a** from **29b** for a 45% yield. ¹H NMR (400 MHz, CDCl₃) δ 8.70 (s, 1H), 7.72 (s, 1H), 7.59 (t, *J* = 8.2, 1H), 7.21–7.16 (m, 2H), 6.91 (d, *J* = 8.0, 1H), 6.84 (d, *J* = 7.8, 1H), 6.01 (s, 2H), 4.55 (s, 2H), 4.01 (s, 3H), 3.56 (q, *J* = 7.0, 2H), 1.25 (t, *J* = 7.0, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 155.13, 147.65, 128.76, 123.33, 119.71, 109.85, 108.20, 104.29, 101.24, 70.30, 66.15, 55.83, 15.27. MS (ESI) calculated for C₂₀H₁₉NO₄ [M+H]⁺, 338.14; found, 338.39.

5.24. Biological assay

Two *P. falciparum* strains, CQ-S 3D7 and CQ-R K1, were used in this study and were provided by the MR4 Unit of the American Type Culture Collection (Manassas, VA).

Asynchronous parasites were maintained in culture based on the method of Trager.³⁶ Parasites were grown in the presence of fresh group O-positive erythrocytes (Lifeblood, Memphis, TN) in Petri dishes at a hematocrit of 4–6% in RPMI-based medium. It consisted of RPMI 1640 supplemented with 0.5% AlbuMAX II, 25 mM HEPES, 25 mM NaHCO₃ (pH 7.3), 100 μg/mL hypoxanthine, and 5 μg/mL gentamicin. Cultures were incubated at 37 °C in a gas mixture of 90% N₂, 5% O₂, and 5% CO₂. For EC₅₀ determinations, 20 μL of RPMI 1640 with 5 μg/mL gentamicin were dispensed per well in an assay plate (384-well microplate, clear-bottom, tissue-treated). Next, 40 nL of compound, previously serially diluted in a separate 384-well white polypropylene plate, were dispensed in the assay plate, and then 20 μL of a synchronized culture suspension (1% rings, 10% hematocrit) were added per well to make a final hematocrit and parasitemia of 5% and 1%, respectively. Assay plates were incubated for 72 h, and the parasitemia was determined by a method previously described.³⁷ Briefly, 10 μL of 10X Sybr Green I, 0.5% v/v Triton, and 0.5 mg/mL saponin solution in RPMI were added per well. Assay plates were shaken for 30 s, incubated in the dark for 4 h, and then read with the Envision spectrofluorometer at Ex/Em 485 nm/535 nm. EC₅₀s were calculated with Graphpad PRISM software.

5.25. Solubility

Solubility assays were carried out on a Biomek FX lab automation workstation (Beckman Coulter, Inc., Fullerton, CA) using μSOL Evolution software (pION Inc., Woburn, MA) as follows: 10 μL of compound stock was added to 190 μL of 1-propanol to make a reference stock plate. Next, 5 μL of this reference stock plate was mixed with 70 μL of 1-propanol and 75 μL of PBS (pH 7.4 and 4, respectively) to make the reference plate, and the UV spectrum (250–500 nm) of the reference plate was read. Then, 6 μL of

10 mM test compound stock was added to 600 μ L of PBS in a 96-well storage plate and mixed. The storage plate was sealed and incubated at rt for 18 h. The suspension was then filtered through a 96-well filter plate (pION Inc., Woburn, MA). Next, 75 μ L of filtrate was mixed with 75 μ L of 1-propanol to make the sample plate, and the UV spectrum of the sample plate was read. Calculations were done using μ SOL Evolution software based on the area under the curve (AUC) of the UV spectrum of the sample plate and the reference plate. All compounds were tested in triplicate.

5.26. Permeability

A parallel artificial membrane permeability assay (PAMPA) was conducted on a Biomek FX lab automation workstation (Beckman Coulter, Inc., Fullerton, CA) with PAMPA evolution 96 command software (pION Inc., Woburn, MA) as follows: 3 μ L of 10 μ M test compound stock was mixed with 600 μ L of system solution buffer, pH 7.4 or 4 (pION Inc., Woburn, MA) to make diluted test compound. Then 150 μ L of diluted test compound was transferred to a UV plate (pION Inc., Woburn, MA), and the UV spectrum was read as the reference plate. The membrane on a preloaded PAMPA sandwich (pION Inc., Woburn, MA) was painted with 4 μ L of GIT lipid (pION Inc., Woburn, MA). The acceptor chamber was then filled with 200 μ L of acceptor solution buffer (pION Inc., Woburn, MA), and the donor chamber was filled with 180 μ L of diluted test compound. The PAMPA sandwich was assembled, placed on the Gut-Box controlled environment chamber and stirred for 30 min. The aqueous boundary layer was set to 40 μ m for stirring. The UV spectrum (250–500 nm) of the donor and the acceptor were read. The permeability coefficient was calculated using PAMPA Evolution 96 Command software (pION Inc., Woburn, MA) based on the AUC of the reference plate, the donor plate, and the acceptor plate. All compounds were tested in triplicate.

5.27. Cytotoxicity screens

Cytotoxicity was tested on BJ, Raji, HEK 293, and HepG2 cell lines using the Alamar blue assay as described previously.³⁸ The final concentration of the tested compounds was 27.5 μ M in 0.5% DMSO. The cell viability was normalized to a DMSO control.

5.28. Antibacterial screens

E. coli BL21(DE3) competent cells and 1 mL of pre-warmed SOB medium were shaken at 300 rpm at 37 °C for 1 h. Cells were plated on pre-warmed LB-agar selective plates and cultured at 37 °C overnight. Isolated colonies were diluted in LB broth and grown in broth for 4 h at 37 °C to an absorbance of \sim 0.6 at 600 OD. Cultures were further diluted by a 10-fold addition of broth. Then 196 μ L of cultures and 4 μ L of the test compound in DMSO were added into each well of a 96-well plate. Assay plates were incubated at 37 °C for 18 h. The positive control drug was ciprofloxacin.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.02.013.

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