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Progress in the Optimization of 4(1*H*)-Quinolone Derivatives as Antimalarials Targeting the Erythrocytic, the Exoerythrocytic and the Transmitting Stages of the Malaria Parasite

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Abstract: Malaria is one of the leading infectious diseases occurring mainly in tropical and subtropical areas. Although available antimalarial tools have reduced the number of fatalities, there is still an urgent need for the development of new and more efficacious treatments to cure and eradicate malaria especially due to emerging resistance to all antimalarial drugs. Research was initiated to revisit antimalarial compounds which were deemed unsuitable as a result of poor understanding of physicochemical properties and the optimization thereof. The 4(1*H*)-quinolones are a class of compounds with demonstrated activity against malaria parasites. Recent optimization of the long-known core led to two highly promising compounds, *i.e.* P4Q-391 and ELQ-300, with great selective activity against all stages of the parasite's life cycle and good physicochemical properties. In this paper, we discuss the key steps on the way to these compounds, which fuel hope to find a suitable treatment for the prevention, cure and eradication of malaria.

Keywords: Antimalarials · 4(1*H*)-Quinolone · Structure–Activity Relationship (SAR) · Structure–Property Relationship

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1. Introduction

1.1 *Malaria*

Malaria continues to be one of the most significant public health problems in the world. With 40% of the world's population living in malaria endemic areas, malaria is one of the most devastating parasitic diseases. More than 200 million infections and over 0.4 million deaths were reported in 2015.[1] Malaria in humans arises from the infection with parasites of one of five species of the genus *Plasmodium* (*P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae*, and *P. knowlesi*). With 96% of all infections, *P. falciparum* causes the majority of severe malaria cases. *P. vivax* is responsible for the other severe cases, whereby 58% of the infections by *P. vivax* occur in South-East Asia. Several other *Plasmodium* species cause malaria only in animals and some of these have been shown to be very valuable for *in vivo* studies. For example, *P. berghei* infects rodents, *P. cynomolgi* primates and *P. yoelii* a variety of non-human mammals such as rodents.[2]

As part of a complex life cycle[3] involving a mosquito vector and a vertebrate host, the malaria parasite is transmitted to humans following a bite of an infected female *Anopheles* mosquito.

First, the mosquito injects malaria parasites, called sporozoites, which undergo one round of asexual reproduction (exoerythrocytic schizogony) in the liver. The resulting merozoites are released into the circulatory system and invade erythrocytes, red blood cells, in which the parasite ingests the host cell cytoplasm and undergoes asexual replication (erythrocytic schizogony). At the end of this replication cycle, the host erythrocyte ruptures and releases merozoites along with numerous antigens and waste products, which lead to typical malaria symptoms such as headache, fever and rigors, nausea and vomiting, anorexia, immunosuppression and apoptosis.[4] The released merozoites further invade new erythrocytes and continue the parasite's asexual multiplication in red blood cells. As an alternative to erythrocytic schizogony, a small proportion of asexual parasites form gametocytes, a sexual stage of the malaria parasite. Gametocytes

do not cause pathology in the human host and disappear from systemic circulation if not taken up by a mosquito during a bite to continue the parasite's life cycle (transmission). After a mosquito is infected during ingestion, the gametocytes transform into either micro- or macrogametes (gametogenesis) and initiate the sporogonic cycle within the mosquito host. In the sporogonic cycle, parasites form new sporozoites *via* meiotic division (sporogony), which accumulate in the mosquito's salivary gland and remain ready until they are inoculated into a new host. Therefore, the presence of gametocytes in systemic circulation of infected individuals is imperative for malaria to remain endemic.[5] It is worth mentioning, that *P. vivax* has the ability to defer exoerythrocytic replication and stay dormant (hypnozoites) in liver cells before continuing the further life cycle. They can be reactivated after months or even years to cause relapse of the clinical infection.[6]

1.2 *Antimalarials*

Recently, a decline in malaria morbidity and mortality has been observed as a result of combined efforts in prevention, control and treatment of malaria worldwide.[7] Strategies for malaria prevention involve different approaches to control or kill the mosquito population and reduce human–mosquito contact. Chemotherapy is considered of highest importance as part of the general prevention strategy as well as the treatment of infected individuals. At present, several antimalarial drugs are available that act differently on the various life cycle stages with most drugs targeting primarily the blood stages of the parasite. Chloroquine[8] (**1**), mefloquine[9] (**2**) and artemisinin[10] (**3**) are the most common and widely used representatives of this class of antimalarials and are therefore suitable for the treatment of acute malaria (Fig. 1). Additionally, **1** and **2** are used for malaria prophylaxis. Only a few antimalarial drugs are known to show activity extending beyond the blood stages. Primaquine (**4**), for example, is the only clinically proven drug that effectively kills hypnozoites and is active against gametocytes.[11] However, the use of primaquine is significantly limited as it causes fatal hemolysis in glucose-6-phosphate dehydrogenase deficient patients.[12] Another drug targeting liver stage parasites is atovaquone (**5**), which selectively inhibits the parasite's mitochondrial electron transport chain at the cytochrome bc_1 complex.[13] Besides the limited number of antimalarial drugs attacking exoerythrocytic parasites, the efficacy of currently available antimalarials is further diminished by widespread resistance. In some countries in South-East Asia resistant parasites against the currently most widely used artemisinin and its derivatives have already been found,[14] even though they are utilized in Artemisinin-based Combination Therapies (ACTs) to improve treatment efficacy and to protect the artemisinin derivatives against the development of resistance.[10] Due to the lack of antimalarials leading to radical cures (eradicate dormant exoerythrocytic stages of the parasite) and the constant emergence of multidrug resistance against all common antimalarials in many regions, a new rise in the malaria burden and mortality will inevitably occur without new and effective next-generation medicines at hand. Therefore, the most significant need for future malaria elimination efforts is to identify a new compound that is safe and effective at killing parasites at all stages of development in the patient without susceptibility to resistance.[14b,c]

Whereas in the past, antimalarial drug discovery was mainly focused on the erythrocytic stages of malaria, the ability to target multiple stages of the parasite's life cycle has recently become a primary requirement for new candidates. The ideal drug candidates should be potent against the blood stages, block the transmission and/or the development of infectious gametocytes, and eradicate the liver stage parasites, in particular the dormant forms (hypnozoites). Additionally, the Malaria Eradication Research Agenda (malERA) defined Target Product Profiles (TPPs) recommending the development of antimalarial drugs, which are a safe, orally bioavailable and economical ($1.00 per administration for adults and $0.24 for infants).[15] The recommendations focus on the development of a Single Exposure Radical Cure and Prophylaxis (SERCaP) medicine that is suitable for the use in mass drug administration programs to eliminate and eradicate malaria.

Recent understanding of the mechanism of action and resistance to current drugs combined with improvements in physicochemical property assessment, synthetic methodologies and *in vivo* efficacy protocols suggest that some old leads could become viable drug candidates if renewed efforts can overcome chemotype specific hurdles (Fig. 2). One example of a known promising lead is the 4(1*H*)-quinolone series, which has been recognized to have tremendous potential for prevention, eradication, and treatment of malaria. In the 1940s, endochin (**6**), a representative of that class, was identified to be a causal prophylactic (kill growing liver stage parasites) and potent erythrocytic stage agent in avian malaria models.[16]

Despite its promise as an antimalarial agent targeting the mitochondrial bc_1 complex of the parasite,[17] the further development of endochin-like antimalarials languished primarily because of its ineffectiveness in *in vivo* efficacy studies. The fact that endochin is poorly absorbed and easily metabolized to inactive metabolites is the main reason for the disappointing results obtained in the *in vivo* efficacy studies.

In 1970, potent antimalarial activity of ICI56,780 (**7**, Fig. 2), a compound belonging to a class of 3-ester quinolones featuring a characteristic 2-phenoxyethoxy substituent, was reported.[18] Early on, prophylactic and blood schizontocidal activity in rodent malaria models was proven, but later on, **7** was also shown to produce radical cures, *i.e.* eradication of hypnozoites in *P. cynomolgi* infected rhesus monkeys.[18,19] Unfortunately, a high degree of resistance emerged after one passage in *P. berghei* infected mice and the lack of oral bioavailability led to the abandonment of further development of this compound series.[18]

Approximately at the same time, the 4(1*H*)-pyridone clopidol (**8**, Fig. 2) was found to be an efficacious antimalarial against blood stages.[20] It also displayed curative activity against exoerythrocytic stages of the parasite. Despite potent *in vivo* activity in mice, clinical trials in humans failed, primarily because of low aqueous

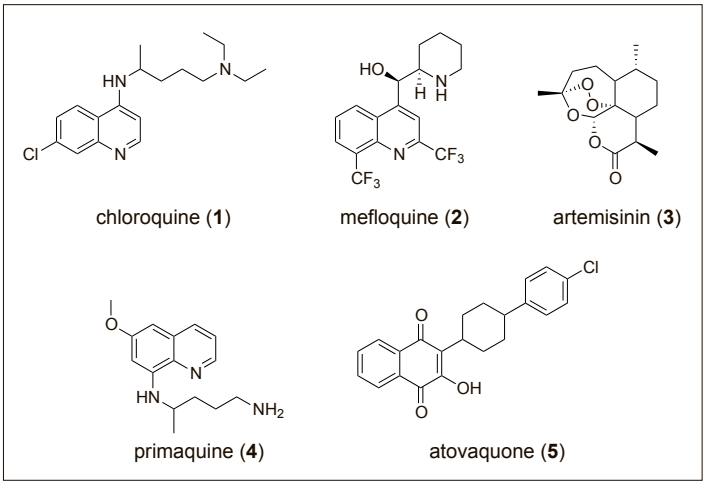


Fig. 1. Structures of most common and widely used antimalarials **1**–**5**.

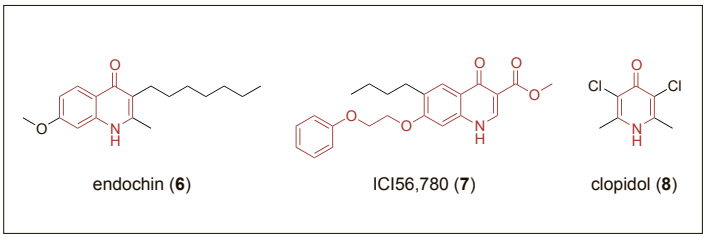


Fig. 2. Structures of old leads **6–8** that displayed antimalarial potency but did not make it to market. The core of the structure and key features are marked in red.

solubility and high clearance. Clopidol (**8**), like atovaquone (**5**) and many antimalarial 4(1*H*)-quinolones, disrupts the parasite's mitochondrial electron chain. Remarkably, clopidol (**8**) maintains potent antimalarial activity against atovaquone resistant strains suggesting a different site of action than the ubiquinol oxidation site Q_o of the bc_1 complex targeted by atovaquone.[21]

2. Development of Antimalarials Targeting the Parasite's Respiration

2.1 *4(1H)-Pyridones*

Decades later, scientists at GSK (GlaxoSmithKline) renewed lead optimization studies founded on clopidol. In a detailed structure–activity relationship (SAR) study, they optimized the substituents in 2-, 3-, 5- and 6-position of the 4(1*H*)-pyridone scaffold.[22]

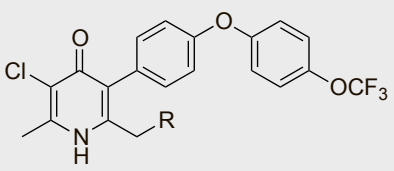
They found that the substituent in 5-position has the biggest impact on activity and physicochemical properties. Changing one of the chloro substituents of clopidol to an alkyl chain, a phenyl group, the atovaquone-typical sidechain 4-(4-chlorophenyl)cyclohexyl or a diarylether moiety decreased the EC_{50} values. Nevertheless, only compounds with diarylether sidechains significantly improved *in vivo* activity.[22] Installing a 4-(4-(trifluoromethoxy) phenoxy)phenyl moiety led to GW844520 (**9**, Table 1) with low nanomolar *in vitro* activity and excellent *in vivo* activity with an ED_{50} value of 0.2 mg/kg in a murine *P. yoelii* model. In 2006, compound **9** entered preclinical trials, but it was discontinued because of histopathological complications in skeletal and cardiac muscles.[23] Attempts to improve the physicochemical properties led to a pyridone with a hydroxymethyl moiety in 6-position (GSK932121, **10**), which showed acute cardiotoxicity attributed to the inhibition of mammalian mitochondrial cytochrome bc_1 cells and was thus withdrawn from human trials resulting in the abandonment of the entire 4(1*H*)-pyridone series.[24]

2.2 *4(1H)-Quinolones*

Recently, the Manetsch and Kyle research groups renewed efforts to optimize the previously reported ICI56,780 (**7**) compound series. With a focus on overcoming the cross-resistance issues inherent to this compound series, several analogs of 7-(2-phenoxyethoxy)-4(1*H*)-quinolone (**7**), called PEQs, were prepared and tested against the clinically relevant multidrug resistant malarial strains W2 (chloroquine and pyrimethamine resistant) and TM90-C2B (chloroquine, mefloquine, pyrimethamine and atovaquone resistant).[25] Removal of the ester functionality in the 3-position severely affected the potency increasing the EC_{50} values against W2 by a factor of more than 8000. Despite this significant potency reduction, it was noted that the resistance index, which is the ratio of the effective concentrations needed to kill 50% of the population of parasites (EC_{50}) for TM90-C2B and W2 strains (RI = (EC_{50} TM90-C2B)/(EC_{50} W2)), approached the ideal value of 1. This stands in stark contrast to the large RI values for the origin **7** and analogues thereof, in which the 6- or 7-substituent were removed. Follow-up SAR studies proved the original *n*-butyl and the 2-phenoxyethoxy substituents in 6- and 7-position to be optimal for potency, whereby especially changes of the 7-substituent greatly affected the antimalarial activity. The introduction of an alkyl group or an amide in 3-position led to compounds with an improved RI but noticeably decreased *in vitro* potency. 3-Aryl and 3-halogen substituted PEQs, however, displayed good (but not as good as the original ICI56,780) potencies against both strains and acceptable RIs. The best compromise between a reasonable antimalarial potency and low cross-resistance was achieved by the addition of a methyl group in 2-position in combination with a substitution of the 3-ester substituent by a bromo (compound **11**, Table 2; 50-fold improved RI) or *o*-fluoro-*p*-(trifluoromethyl) phenyl moiety (compound **12**, Table 2; 200-fold improved RI).[25] Several *in vitro* tests suggested the modified compounds continued to be active against blood and liver stages.[25b] However, one of the biggest limitations of this compound series remains the poor aqueous solubility affecting the oral bioavailability unfavorably. In a modified Thompson test in mice, compound **11** showed 61% inhibition on day 6 post-infection (PI) after oral administration of 10 mg/kg of **11**, formulated in HEC/Tween, on day 3, 4 and 5 PI, whereas the original ICI56,780 did not show any inhibition on day 6 PI. Nevertheless, parasitemia rapidly rebounded so that mice were sacrificed on day 13 PI.[25b]

Analogously to the optimization of 4(1*H*)-quinolone ester **7**, the Manetsch laboratory and others focused on studies on endochin derivatives termed P4Qs and ELQs.[26] Manetsch and co-workers verified that the substituent in 3-position is of highest importance for *in vitro* antimalarial activity, but that the *n*-alkyl chain is one of the major liabilities due to poor aqueous solubility and unacceptable microsomal stability. Accordingly, a first set of compounds without any substituents at the benzenoid ring led to moderately active

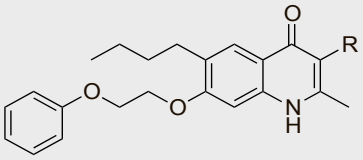
Table 1. Frontrunner compounds of GSK's 4(1*H*)-pyridone series and their key properties.



Compound	GW844520 (**9**)[22,24]	GSK932121 (**10**)[24]
R	H	OH
EC_{50}(3D7A)[a] [nM]	5.0	2.0
log$D_{7.4}$[b]	2.8	2.4
$t_{½}$(dog)[c] [h]	143	42
solubility $_{FeSSIF(5.0)}$[d] [μM]	1.7	5.5
solubility $_{PBS(7.4)}$[d] [μM]	<0.3	<0.3

[a]Effective concentrations needed to kill 50% of the population of parasites; [b]Logarithm of the distribution-coefficient at pH 7.4; [c]Half-life in a living dog; [d]Solubility in given medium: FeSSIF, Fed State Simulated Intestinal Fluid (pH 5.0); PBS, Phosphate-buffered saline (pH 7.4).

Table 2. Frontrunner compounds of the 7-(2-phenoxyethoxy)-4(1*H*)-quinolone series and their key properties.

		
Compound	PEQ-1020 (**11**)[26b]	PEQ-437 (**12**)[25a]
R	Br	2-F-4-CF_3-Ph
EC_{50}(W2)[a] [nM]	2.6	28
EC_{50}(TM90-C2B)[a] [nM]	12	31
RI[b]	4.69	1.11
log$D_{7.4}$[c]	4.2	–
solubility$_{6.5}$[d] [µM]	1–1.9	–
Thompson test[e]:		
dose [mg/kg]	10	–
inhibition on day 6 PI [%]	61	–
survival days	13	–

[a]Effective concentrations needed to kill 50% of the population of parasites; [b]RI = (EC_{50} TM90-C2B)/(EC_{50} W2); [c]Logarithm of the distribution-coefficient at pH 7.4; [d]Solubility at pH 6.5; [e]Mice were infected with 1×10^6 *P. berghei-GFP* parasites and then orally treated once a day on days 3–5 PI with test compound in a HEC/Tween solution. Inhibition and number of days animals survived is given compared to control, untreated animals.

compounds by introduction of an alkenyl, bromo, benzyl or phenyl moiety in 3-position. Further, the Manetsch laboratories investigated the influence of the substituent on the benzenoid ring (5-, 6-, 7- and 8-position). It was found that substituents in 5- and 8-position are not tolerated, while substituents in 6- and 7-position influence the compounds' antimalarial activity as well as the RI. A remarkable synergistic effect, improving the antimalarial activity by a factor of 30 and eliminating the cross-resistance, was identified for compounds with a 6-chloro and 7-methoxy substitution pattern. The influence of the substituents on the 4(1*H*)-quinolone core's electronics possibly affect the hydrogen-bond donor and acceptor ability of these analogues. Specifically, the 6-chloro substituent increases the acidity of the quinolone NH and possibly strengthens the binding between the 4(1*H*)-quinolone and the macromolecular target. In contrast, the electron-donating nature of the methoxy group in 7-position, improves the ability of the carbonyl group to accept hydrogen bonds in the binding pocket. Of a subseries comprising of 6-chloro-7-methoxy-substituted 4(1*H*)-quinolones, P4Q-105 (**13**) with an ethyl group in 3-position provided the best compromise between potent antimalarial activity, acceptable RI value, improved aqueous solubility and prolonged half-life (Table 3). In contrast, compounds with a longer alkyl chain displayed better potency but suffered from a bad RI and unacceptable physicochemical properties. Another promising compound was P4Q-95 (**14**) with a phenyl group in 3-position. In comparison to endochin, compound **14** displayed a better microsomal stability and slightly improved aqueous solubility whereas the antimalarial potency was unaffected.

Generally, the measured aqueous solubility of the vast majority of 4(1*H*)-quinolones was worse than predicted from log$D_{7.4}$. Experimental data suggests that strong lattice energy and intermolecular hydrogen bonding are the main reasons for the unusually poor solubility.[26] Analogues within a set of specific 3-aryl-substituted 4(1*H*)-quinolones which display a better aqueous solubility supported this hypothesis.[27] This improvement was ascribed to a weaker interaction between the molecules and less dense molecular crystal packing induced by structural features perturbing the crystal packing and/or hydrogen bonds. In comparison to the *in vivo* inactive P4Q-95 (**14**), the additional methyl group in the 3-phenyl ring of P4Q-146 (**15**, Table 3) not only increased the 4(1*H*)-quinolone's solubility, but also rendered P4Q-146 (**15**) weak suppressive antimalarial activity *in vivo*. The observed activity was attributed to the slightly better solubility of **15**, while the low metabolic stability has a limiting effect on the *in vivo* efficacy. The findings regarding the aqueous solubility were also supported by quantum mechanical (QM) calculations and X-ray diffraction (XRD) analysis.

To further optimize the antimalarial activity, the aqueous solubility and the microsomal stability, 4(1*H*)-quinolones with a 3-phenyl moiety substituted with various heteroatom-containing functional groups were prepared. One promising compound was the 3-(*p*-(trifluoromethyl)phenyl) substituted P4Q-158 (**16**) possessing excellent EC_{50} values and a nearly perfect RI of 0.96 in addition to high human liver microsomal stability (Table 3).

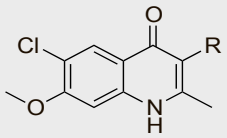
In comparison to **15**, 4(1*H*)-quinolone **16** not only exhibited a better *in vivo* potency against blood stages,[27] but *via* bioluminescent imaging of mice infected with a luciferase expressing *P. berghei* parasite, Kyle and Manetsch also demonstrated that **16** is *in vivo* active against liver stage parasites.[28] Furthermore, assessment of the transmission-blocking activity with *P. falciparum* or *P. berghei* infected mice, also demonstrated that P4Q-146 and other 4(1*H*)-quinolones reduced or prevented the exflagellation of male gametocytes and, more importantly, prevented parasite transmission to the mosquito vector.[29]

Further optimization also revealed that 3-biphenyl- and 3-diarylether-substituted 4(1*H*)-quinolones display low single-digit nanomolar EC_{50} values and acceptable RI values.[27]

For example, in comparison with the phenyl-substituted 4(1*H*)-quinolone **14**, analogue **17** with a 4-phenoxyphenyl moiety in 3-position not only displayed a better *in vitro* potency, but with suppressive activity (87% on day 6 PI) at a 10 mg/kg dose on day 3, 4 and 5 PI, it was also more efficacious in the modified Thompson test with *P. berghei* infected mice. Even though the *in vivo* efficacy of **17** was increased (primarily by an improved microsomal stability), **17** did not cure the mice so that they had to be sacrificed 3 days after the control mice.

In accordance with the discussed observations, further improvements of the *in vitro* antimalarial activity, microsomal stability and/or aqueous solubility were achieved with 3-diarylether-4(1*H*)-quinolones **18**–**20** (Table 4), whose 3-diarylether-moiety is substituted with a trifluoromethoxy substituent in *para*-position of the distal aryl ring (ELQ-300, **20**) and an additional methyl group (compound **18**) or a fluorine (P4Q-391, **19**) in *ortho*-position of the proximal aryl ring.[27,30] Analogues **18**–**20** showed excellent *in vitro* activity with subnanomolar or single-digit nano-

Table 3. Key steps in the SAR of the 4(1*H*)-quinolone scaffold based on endochin and their key properties.



Compound	P4Q-105 (**13**)[26]	P4Q-95 (**14**)[26,27]	P4Q-146 (**15**)[27]	P4Q-158 (**16**)[27]	P4Q-341 **17**[27]
R	Et	Ph	2-CH_3-Ph	4-CF_3-Ph	4-(OPh)-Ph
EC_{50}(W2)[a] [nM]	48	26	5.8	6.3	2.4
EC_{50}(TM90-C2B)[a] [nM]	28	15	4.0	6.0	1.3
RI[b]	0.57	0.59	0.69	0.96	0.55
log$D_{7.4}$[c]	2.4	1.7	2.5	2.4	3.7
$t_{½}$(human liver microsomes)[d] [min]	34	128	–	>250	–
solubility $_{PBS7.4}$[e] [μM]	10–20	2–6	6–12	<2	<2
Thompson test[f]:					
dose [mg/kg]	–	10	10	10	10
inhibition on day 6 PI [%]	–	<1	17	92	87
survival days	–	0	0	3	4

[a]Effective concentrations needed to kill 50% of the population of parasites; [b]RI = (EC_{50} TM90-C2B)/(EC_{50} W2); [c]Logarithm of the distribution-coefficient at pH 7.4; [d]Half-life in human liver microsomes; [e]Solubility in given medium: PBS, Phosphate-buffered saline (pH 7.4); [f]Mice were infected with 1×10^6 *P. berghei-GFP* parasites and then orally treated once a day on days 3–5 PI with test compound in a PEG400 solution. Inhibition and number of days animals survived is given compared to control, untreated animals.

molar EC_{50} values. Furthermore, **18–20** also displayed excellent *in vivo* activity in the Thompson test (99% inhibition on day 6 PI or ED_{50} of 0.27 mg/kg/d for P4Q-391 and 0.016 mg/kg/d for ELQ-300) with a curative blood-stage dose of 1 mg/kg (**19**, **20**) and 10 mg/kg (**18**). In accordance to that, compounds **19** and **20** were also shown to possess good oral bioavailability in mice (~100% at efficacious doses) and the highest exposure in mice after oral administration observed within the 4(1*H*)-quinolone series. They exhibit long *in vivo* half-life (15–32 h), low plasma clearance (0.3–0.77 mL/(min×kg)), and a low volume of distribution (0.7–1.2 L/kg) following intravenous administration in mice and rats.[30] Both compounds are also characterized by selective inhibition of the parasite's coenzyme Q cycle, which is required for *de novo* pyrimidine biosynthesis, by inhibition of mitochondrial cytochrome bc_1 complex (selectivity indices over human >10,000).[31] Besides the proven potency against the blood stages, they are highly active against exoerythrocytic stages of *P. berghei* and *P. cynomolgi* liver schizonts.[30] Additionally, they target the sexual and vector stage parasites that are crucial for the transmission of malaria, which was shown *in vitro* and *in vivo*. Long-term experiments with Dd2 strains of *P. falciparum* exposed to the potential drug compound for several weeks suggested that there is no propensity for the quinolone-3-diarylethers to induce resistance in malaria parasites.

2.3 *Synthesis*

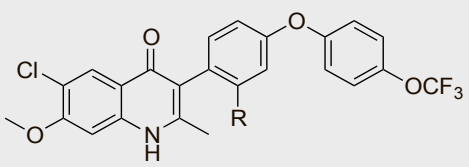
The Manetsch laboratory developed and implemented practical synthetic routes to provide structurally diverse 4(1*H*)-quinolones at appropriate amounts required for the optimization efforts and the studies to assess *in vivo* efficacy, pharmacokinetics, and safety profiles.[32] First, a divergent multistep synthesis was implemented by the synthesis of the in 3-position unsubstituted 4(1*H*)-quinolone *via* Conrad-Limpach reaction using easily accessible amines and ethyl acetoacetate followed by a regioselective iodination to obtain intermediate 3-iodo-4(1*H*)-quinolone (Scheme 1).[32a] Upon Suzuki-Miyaura cross-coupling of the iodo intermediate with the corresponding boronic acid using a Pd/SPHOS system different 3-aryl-4(1*H*)-quinolones are accessible. Using the unsubstituted ethyl acetoacetate as substrate combined with a subsequent derivatization secured easily accessible substrates and a high yielding Conrad-Limpach reaction, which was often observed to occur in low yield when 2-substituted β-ketoester were used.

However, to overcome low yields and harsh reaction conditions needed for preparation of some analogs, the Manetsch group also developed an unprecedented arylation protocol of ethyl acetoacetate providing clean access to substrates needed for the Conrad-Limpach reaction.[32b] The arylation of ethyl acetoacetate is achieved with diaryliodonium hexafluorophosphates and ^{t}BuOK as base. The resultant 2-aryl ethylacetoacetates can then be converted to the corresponding 3-aryl-4(1*H*)-quinolones under modified Conrad-Limpach conditions using microwave-assistance. Utilizing the new conditions the yield was increased and the isolation of the final quinolone was simplified due to less side products.

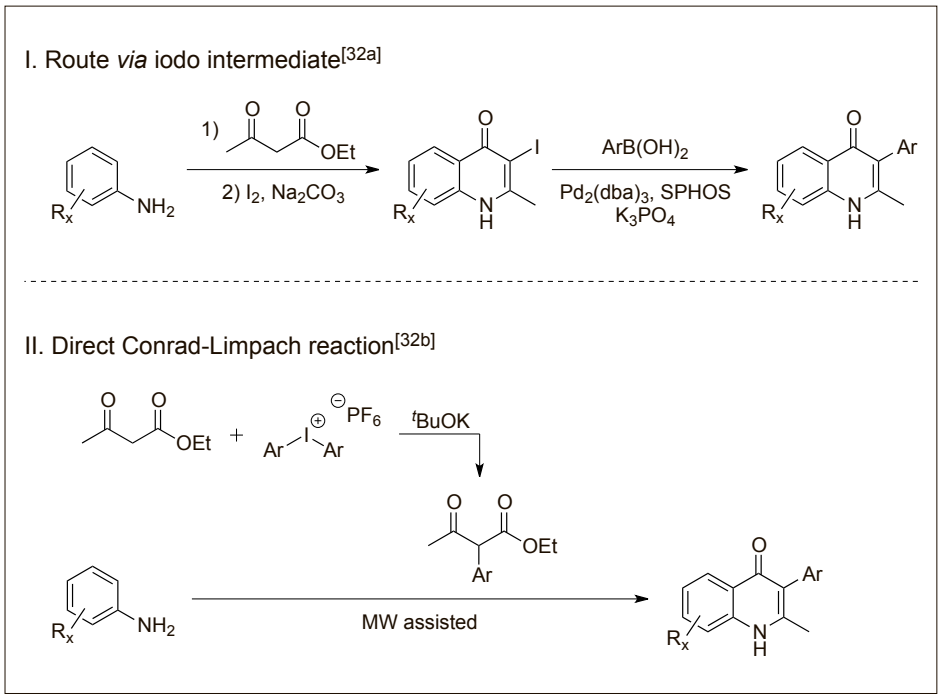
3. Conclusion

Quinolone-3-diarylethers represent a new antimalarial series active on a clinically validated pathway that overcomes the shortcomings of atovaquone, a clinical drug with the same enzyme target. Spearheaded by Medicines for Malaria Venture, in 2013 ELQ-300 was selected to undergo preclinical development. The advancement of it towards Phase I studies was deferred due to poor oral bioavail-

Table 4. Frontrunner compounds of the 4(1*H*)-quinolone series and their key properties.

			
Compound	P4Q-390 **18**[27]	P4Q-391 (**19**)[27,30]	ELQ-300 (**20**)[30]
R	CH_3	F	H
EC_{50}(W2)[a] [nM]	0.3	3.7	1.8
EC_{50}(TM90-C2B)[a] [nM]	0.6	15	1.7
RI[b]	2.07	4.07	0.94
log$D_{7.4}$[c]	2.1	3.4	4.2
$t_{½}$(mice)[d] [h]	–	32	15
solubility $_{PBS7.4}$[e] [µM]	<2	8	16
Thompson test[f]:			
dose [mg/kg]	10	1	1
inhibition on day 6 PI [%]	99	99	99
survival days	23 (curative)	23 (curative)	23 (curative)
ED_{50}[g] [mg/kg/d]	–	0.27	0.016

[a]Effective concentrations needed to kill 50% of the population of parasites; [b]RI = (EC_{50} TM90-C2B)/(EC_{50} W2); [c]Logarithm of the distribution-coefficient at pH 7.4; [d]Half-life in living mice; [e]Solubility in given medium: PBS, Phosphate-buffered saline (pH 7.4); [f]Mice were infected with 1×10^6 *P. berghei-GFP* parasites and then orally treated once a day on days 3–5 PI with test compound in a PEG400 solution; Inhibition and number of days animals survived is given compared to control, untreated animals; [g]Dose of drug that suppresses 50% of parasitemia on day 6 PI.



Scheme 1. Synthesis of 3-aryl-4(1*H*)-quinolones *via* a multi-step route including a 4(1*H*) -quinolone intermediate (I) and a direct route utilizing ethylacetoacetates which are arylated in advance (II).

ability limiting specific preclinical studies and blood exposure required to achieve single-dose cures. Moreover, the projected costs per treatment were considered to be uneconomical due to the need for advanced formulations to achieve an appropriate oral bioavailability. Thus, other ways are investigated to increase the aqueous solubility and the oral bioavailability of frontrunner ELQ-300 and P4Q-391 without interfering with the exceptional *in vitro* and *in vivo* potency against erythrocytic and exoerythrocytic stages of the parasite. One possible way to address the challenging physicochemical properties without using expensive formulation techniques is through the development of soluble prodrugs. In the Manetsch laboratories, prodrug approaches are currently under investigation and first promising results will be reported in due course.

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