Synthesis and Evaluation of 3-Aryl-4(1H)-Quinolones as Orally Active Antimalarials: Overcoming Challenges in Solubility, Metabolism, and Bioavailability

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Synthesis and Evaluation of 3-Aryl-4(1H)-Quinolones as Orally Active Antimalarials: Overcoming Challenges in Solubility, Metabolism, and Bioavailability

by

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DEDICATION

To Katerynka and Mother, it all would not be possible without you.

“There are no beautiful surfaces without a terrible depth” – Friedrich Nietzsche
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ABSTRACT

Infectious diseases are the second leading cause of deaths in the world with malaria being responsible for approximately the same amount of deaths as cancer in 2012. Despite the success in malaria prevention and control measures decreasing the disease mortality rate by 45% since 2000, the development of single-dose therapeutics with radical cure potential is required to completely eradicate this deadly disease. Targeting multiple stages of the malaria parasite is becoming a primary requirement for new candidates in antimalarial drug discovery and development. Recently, 4(1H)-pyridone, 4(1H)-quinolone, 1,2,3,4-tetrahydroacridone, and phenoxyethoxy-4(1H)-quinolone chemotypes have been shown to be antimalarials with blood stage activity, liver stage activity, and transmission blocking activity. Advancements in structure-activity relationship and structure-property relationship studies, biological evaluation in vitro and in vivo, as well as pharmacokinetics of the 4(1H)-pyridone and 4(1H)-quinolone chemotypes is discussed in the first chapter of the dissertation.

Convenient synthetic approaches to 3-aryl-4(1H)-quinolones via metal-catalyzed and metal-free arylation of β-keto carbonyl compounds is addressed in Chapter 2. A clean arylation protocol of ethyl acetoacetate was developed by using hypervalent diaryl iodonium salts under mild and metal-free conditions. The scope of the reaction, using symmetric and unsymmetric iodonium salts varying in stercics and electronics was examined. This method has been applied for the synthesis of antimalarial compound ELQ-300, which is currently in preclinical development. Additionally, a first gram scale synthesis of ELQ-300 and its structurally related
4(1H)-quinolone P4Q-391 using operationally simple and highly yielding metal-catalyzed conditions have been shown.

Despite of 3-aryl-4(1H)-quinolone chemotypes displaying potent antimalarial activities against *Plasmodium* species *in vitro* and *in vivo*, their development is also associated with risks. 4(1H)-quinolones are known to be poorly soluble and thus represent challenging drug candidates for pharmacokinetic and bioavailability reasons. Disrupting of molecular crystal packing and prodrug approaches were employed to overcome solubility and bioavailability issues in current series. Quantum mechanics torsion profile calculations, $^{13}$C $T_1$ spin-lattice relaxation experiments as well as X-ray studies were conducted with the objective to determine possible effects improving key physicochemical properties such as solubility and stability.

As a backup strategy, a prodrug approach was developed enabling the 4(1H)-quinolone scaffold to be functionalized at the quinolone’s oxygen. In order to avoid any enzymatic dependences, an approach was developed in which the prodrug moiety was removed via a pH-triggered decay. Additionally, phosphate prodrugs regenerating the active compound via extrahepatic enzymes such as the ubiquitous alkaline phosphatase were investigated. The development of orally bioavailable prodrugs enabled an advance overcoming *in vivo* efficacy limitations and has been confirmed by pharmacokinetic profiling studies. The herein presented approaches present viable options for any pyridone quinolone antimalarial chemotype which are currently studied.
CHAPTER ONE: 4(1H)-PYRIDONE AND 4(1H)-QUINOLONE DERIVATIVES AS
ANTIMALARIALS WITH ERYTHROCYTIC, EXOERYTHROCYTIC, AND
TRANSMISSION BLOCKING ACTIVITIES\textsuperscript{1}

1.1 Overview

1.1.1 Main biologically active derivatives

Malaria continues its devastating impact on the health of human populations in tropical regions, with 207 million cases and approximately 0.7 million deaths in 2012.\textsuperscript{1} Half of the world’s population is at risk for malaria with the majority of deaths occurring on the African continent. Resistance to all known antimalarials and the limited availability of a large number of efficacious chemotypes are accountable for the wide spread of the disease. Even artemisinin and artemisinin combination therapies (ACT), currently considered the standard treatments, are showing signs of emerging resistance.\textsuperscript{2-5}

Over the last decade the number of deaths has decreased by approximately three times due to renewed efforts to control and kill mosquito populations, as well as improved accessibility to effective medicines.\textsuperscript{6} However, to eliminate and eradicate malaria, the discovery of novel therapeutics which have potential to be used as single-dose agent targeting \textit{P. falciparum} and as a radical cure of \textit{P. vivax} malaria is required. Ideally, such a candidate possesses potent efficacy against the blood stages of multidrug resistant malaria, blocks transmission of infectious gametocytes to mosquitoes, and eradicates the liver stages infections, in particular the dormant

\textsuperscript{1} The entire work presented in this chapter has been accepted with minor revisions for publication in Curr. Top. Med. Chem. 2014
forms (hypnozoites) of the relapsing malaria species (*P. vivax* and *P. ovale*). In the past, antimalarial drug discovery focused primarily on the erythrocytic stages of malaria, however in recent years, targeting the hypnozoites as well as the infective stages for mosquitoes has been considered to be equally important.

For a long period of time, 8-aminoquinolines such as primaquine were the only known antimalarials demonstrating liver stage activity, weak erythrocytic stage activity and transmission blocking activity simultaneously (Figure 1.1). However, toxicity in glucose-6-phosphate dehydrogenase deficient patients after administration of primaquine limits its use. Therefore, malaria elimination efforts continue to focus on the development of a compound that is safe and effective at killing malaria parasites at all stages of development. Fortunately, the investment into global antimalarial research over the last decade was expanded by non-profit organizations such as the Bill and Melinda Gates Foundation (USA), the Medicines for Malaria Venture (MMV, Switzerland), as well as by other for-profit organizations. In particular, the increased participation of academic drug discovery units in public-private partnerships with non-profit and pharmaceutical partners led to about 10 new antimalarial candidates in the early clinical phased of drug development. Unfortunately, only a few of these are capable of targeting multiple stages of the malaria parasite life cycle. One of these promising

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**Figure 1.1:** Structures of *(R,S)*-primaquine, atovaquone, as well as 4(1H)-quinolone and 4-pyridone-based chemotypes with antimalarial activity.
preclinical candidates targeting multiple stages of the malaria parasite is ELQ-300, which is structurally related to the 4(1H)-quinolones and the 4(1H)-pyridones.\textsuperscript{11}

Historically, 3-alkyl- and 3-phenyl-substituted 4(1H)-quinolones (P4Qs) and 1,2,3,4-tetrahydroacridones (THAs) were reported to possess causal prophylactic activity (kill growing exoerythrocytic stage parasites) and potent erythrocytic stage inhibition in avian malaria models, but not against malaria parasites in mammals.\textsuperscript{12-14} Similarly, compound ICI56,780, a phenoxyethoxy-substituted quinolone ester (PEQ) produced a radical cure (eradication of dormant exoerythrocytic stage parasites known as hypnozoites) in \textit{P. cynomolgi} infected rhesus monkeys.\textsuperscript{15, 16} Finally, the 4(1H)-pyridone clopidol (Figure 1.3) was an \textit{in vivo} efficacious antimalarial against blood stages and it was also considered to be curative against the exoerythrocytic stages (\textit{P. gallinaceum} and \textit{P. cynomolgi}) provided it was administered at high doses over a period of seven consecutive days.\textsuperscript{17} As these studies were conducted over 20-30 years ago without an adequate evaluation in current preclinical efficacy models and without assessing physicochemical properties of the compounds, a large number of research groups recently revisited the development of these chemotypes as antimalarials targeting multiple stages of the parasite.\textsuperscript{18-24} Herein, we will discuss these 4(1H)-pyridone and 4(1H)-quinolone chemotypes, which have potential to be unique antimalarials with blood stage activity, exoerythrocytic stage activity and transmission blocking activity.

The 4(1H)-pyridone and 4(1H)-quinolone pharmacophores possess a common structural moiety, which is probably also responsible for their unique antimalarial activity. It has been shown that the majority of these chemotype analogues are inhibitors of the plasmodial electron transport chain targeting the parasite’s \textit{bc}\textsubscript{1} complex.\textsuperscript{11} Remarkably to this date, atovaquone (Figure 1.1) is the only clinically relevant antimalarial drug inhibiting the same target.\textsuperscript{25}
1.1.2 Physicochemical properties of 4(1H)-pyridones and 4(1H)-quinolones

Pyridones are the carbonyl tautomeric forms of hydroxypyridines containing one oxygen at either the 2-, 3- or 4-position, while quinolones, often also called quinolinones, are pyridones to which an aromatic ring has been fused (Figure 1.2). Structural studies of 4(1H)-pyridones using X-ray crystallography, IR and liquid NMR suggest that the keto-form is favored in both solid and liquid states.\textsuperscript{26, 27} Ion binding, self-association, and strong solvent effects are responsible for the domination of the keto tautomer in the liquid state, in the solid state and in solutions. In particular, a crystal structure of 4(1H)-quinolone exists as a dimer linked by intermolecular hydrogen bonding (NH1..O4 distance 1.795 Å while the carbon oxygen bond length of 1.246 Å is in the range of a C=O double bond. At the same time, the presence of a carbon C4 chemical shift at 176.8 ppm in \textsuperscript{13}C NMR as well as the splitting of the C2 proton to a triplet in \textsuperscript{1}H NMR in DMSO indicate the carbonyl nature of the 4(1H)-quinolone.\textsuperscript{28} Both \textsuperscript{1}H NMR and \textsuperscript{13}C NMR data also confirm that 4(1H)-pyridones and 4(1H)-quinolones also exist as π-electron delocalized systems with aromatic character. 4(1H)-pyridones \textsuperscript{1.1} are aromatic as long as the lone pair of the nitrogen electrons can be delocalized into the ring. However, the degree of the aromaticity depends on the contribution of the pyridinium \textsuperscript{1.2} to the overall structure. 4(1H)-Pyridones act like classical aromatic compounds with electrophilic aromatic substitution (halogenation, nitration, etc.) occurring selectively at position C3, while the C4 carbonyl undergoes nucleophilic substitution by halogens when reacting with POCl\textsubscript{3} or PCl\textsubscript{5}. Finally, the powder FT-IR of 4(1H)-quinolones displays only a stretching of the C=O bond at 1505 cm\textsuperscript{-1} without a hydroxyl peak.\textsuperscript{28} Despite the
predominance of the keto-form in solid and liquid states, structural studies of gas phase pyridones reveal an enol character which strongly suggests the existence of a tautomeric equilibrium.27

1.1.3 Synthesis of 4(1H)-pyridones and 4(1H)-quinolones

The synthesis of 4(1H)-pyridones proceeds by a cyclocondensation of 1,3,5-tricarbonyl compounds with ammonia.29 The formation of asymmetrical 2,6-substituted 4(1H)-pyridones is possible via a cyclocondensation of dianions of β-dicarbonyl compounds with nitriles, although the best results are obtained by the procedure described by Letsinger through pyrone intermediates.30 Finally, 4(1H)-pyridones are also accessed via [5+1] hererocyclization of 2-azadienes with 1,1-carbonyldiimidazole.31 At the same time, synthesis of 4(1H)-quinolones is traditionally accomplished via Conrad-Limpach,32 Niementowski,33 Gould-Jacobs34 or Camps35 cyclizations. Among common disadvantages of these transformations are elevated reaction temperatures, low reproducibility and poor-to-moderate reaction yields. Recently, several novel synthetic approaches involving transition metal catalysis with improved yields and regioselectivity has been reported.36-38

1.2 Atovaquone and Other Naphthoquinones

Plasmodium cytochrome bc₁ complex is a validated antimalarial target with atovaquone (Figure 1.1) being the only drug targeting bc₁, which is currently in clinical use. The mitochondria play a pivotal role in the parasite life cycle and as a part of the energy household, bc₁ complex is actively involved in the electron transport chain. Fortunately, structural differences between the organelle’s components of the human cell and parasite’s one impelled the development of selective inhibitors targeting the Plasmodium bc₁ complex. There are two
specific ubiquinone binding sites (ubiquinol oxidation site $Q_0$ and the ubiquinone binding site $Q_i$) within the $bc_1$ complex and studies have shown that atovaquone competitively inhibits the $Q_0$ site.\textsuperscript{25}

Atovaquone belongs to the class of naphthoquinone and it was discovered in the early 1990s. Shortage of quinine during World War II led to the development of new antimalarial programs with a variety of chemotypes being tested in a bird assays. Opportunely, naphthoquinone compounds had emerged as an attractive class of antimalarials with suppressive \textit{in vivo} activities against \textit{P. lophurae} in ducks.\textsuperscript{39} SAR studies of more than three hundred naphthoquinones resulted in few 2-alkyl substituted compounds, which in 1943 were also tested in clinical trials. Unfortunately, the best results of the series showed only temporary suppression of fever extending for an of average of 10 days in eight cases.\textsuperscript{40} The clue to 2-alkyl naphthoquinones inactivity in human was revealed in the same report with poor PK parameters being the reasons for the failure.

Development of a reliable and adequate \textit{in vitro} assay against \textit{P. falciparum} allowed researchers at Welcome Research Laboratories to revisit the naphthoquinone chemotype in 1980s.\textsuperscript{41} Low metabolic stability was identified as the main reason for the poor performance in the efficacy models and main efforts were directed on improvement of the abovementioned property. A variety of 3-cycloalkyl-2-hydroxy naphthoquinones was tested \textit{in vitro} with some of them showing excellent subnanomalor activities. However, only one compound, atovaquone, was relatively stable to human liver microsomes showing promising antimalarial efficacy \textit{in vivo}.\textsuperscript{42} The clinical studies involving 1,395 patients were conducted between 1990 and 1996 and evaluated atovaquone alone or in combination with other drugs for treatment of malaria. The overall cure rate was more than 98\% in about 500 patients infected with \textit{P. falciparum}.\textsuperscript{43}
Atovaquone is used since then as a fixed-dose combination therapy with dihydrofolate reductase inhibitor proguanil (Malarone, GSK).

Unfortunately, mutations within the catalytic domain of the cytochrome $bc_1$ complex led to emergence of resistance to atovaquone.\(^4^4\) Additionally, resistance appeared to occur primarily through a single point mutation in position 268 in cytochrome $bc_1$.\(^4^5\) Despite the rapid development of resistance to atovaquone, the cross-resistance between this quinone and other antimalarials has not been observed.\(^4^3\) These results suggested the uniqueness of atovaquone’s mechanism of action in comparison to other marketed antimalarials. Although a series of mutations is required to achieve complete resistance to proguanil, the evolution of drug-resistant strains of $P. falciparum$ to Malarone is a developing threat and could pose an increasingly serious problem to public health worldwide.\(^4^6\) Antimalarial activity against atovaquone-resistant strains is mandatory requirements in preclinical development of novel $bc_1$ inhibitors.

Notwithstanding growing parasite resistance to atovaquone, the compound shows antimalarial activity against liver, blood and asexual stages of $Plasmodium$ life cycle. In order to eliminate malaria, compounds are required which not only target blood stages of the disease but also the liver stages as well as the infectious stages for mosquitoes. Ultimately, the centrality of malarial $bc_1$ complex makes it an attractive target for the development of antimalarials with blood, liver and transmission blocking activity.

1.3 (1H)-Pyridones

1.3.1 Historical overview

The antimalarial activities of clopidol 1.3, 3,5-dichloro-2,6-dimethyl-4(1H)-pyridone (Figure 1.3), were described by the Walter Reed Army Institute of Research (WRAIR) in the late 1960s.\(^1^7\) In a concise article, clopidol was reported to have $in vivo$ activity against $P. falciparum$
(chloroquine resistant), *P. berghei*, *P. cynomolgi* and *P. gallinaceum*. Unfortunately, clinical trials in humans at that time failed most likely because of pharmacokinetic issues (high clearance and low solubility) and simple acylations or sulfonations did not yield clopidol derivatives with improved physicochemical properties. Early investigations on the mechanism of action of clopidol indicated that the mitochondria electron chain was inhibited. This outcome was later confirmed by the Burroughs Welcome (BW) Laboratories. Importantly, compound 1.3 maintained potent antimalarial activity against atovaquone resistant strains of malaria suggesting a different site of action for 1.3 than the ubiquinol oxidation site Q\textsubscript{o} of the bc\textsubscript{1} complex for atovaquone.

1.3.2 Structure-activity relationship (SAR) studies of 4(1H)-pyridones

A few decades later, these results motivated scientists at GSK to renew lead optimization studies of a pyridine lead identified during the atovaquone development at BW. The C2, C3, C5, and C6 positions of 1.3 were optimized in a detailed structure-activity relationship study on the 4(1H)-pyridone scaffold. First, the halogenation of the C3 position significantly improved the activity *in vitro* and *in vivo* over unsubstituted analogues. However, no significant differences between the 3-Br or the 3-Cl analogues were observed (EC\textsubscript{50} (3-Cl) = 0.005 µM, EC\textsubscript{50} (3-Br) = 0.008 µM, and EC\textsubscript{50} (3-H) = 0.16 µM). Relative to the 3-halo-4(1H)-pyridones, other electron
withdrawing groups (3-NO₂ or 3-CF₃) did not display any improvements, while compounds with an electron donating substituent (3-OCH₃) lost all antimalarial activity.

For the C2 and C6 positions, a substantial loss in activity was observed when the 2-CH₃ and 6-CH₃ (EC₅₀ 3D7A = 0.2 µM) groups were replaced by CF₃ groups (EC₅₀ (3D7A) > 1 µM). This observation is in alignment with a previous report, in which electron-withdrawing groups (2-CF₃) have been shown to shift the tautomeric equilibrium towards the enol isomer.²⁶ These results strongly suggest the importance of the keto-tautomer to maintaining antimalarial activities.

Finally, substitution at the C5 position proved to play the most important role in terms of efficacy as well as physicochemical properties. Initial improvements of clopidol were achieved by changing one of the chloro substituents to an alkyl chain, a phenyl, the atovaquone-like side chain 4-(4-chlorophenyl)cyclohexyl, or a diaryl ether moiety (Figure 1.4).¹⁸ Introduction of a phenyl group at the C5 position modestly improved the in vitro activity against T9-96, a chloroquine sensitive strain of P. falciparum, by a factor of two over 1.3, while the installation of an n-octyl chain at C5 further enhanced the potency yielding an EC₅₀ of 4 µM. Nevertheless,
both compounds lost their in vivo activity in a 4-day suppressive mouse P. yoelii assay (ED$_{50}$ = 20 mg/kg and ED$_{50}$ > 60 mg/kg respectively) presumably due to metabolic issues. The use of a diaryl group mimicking the side chain of atovaquone dramatically decreased the EC$_{50}$ value to 0.4 µM, while the in vivo activity improved from an ED$_{50}$ value of 40 mg/kg for 1.3 to an ED$_{50}$ value of 0.6 mg/kg for the 5-diaryl-substituted 4(1H)-pyridone. Additional optimization of the distal aromatic ring and introduction of an oxygen as a spacer between the two aromatic rings of the 5-diaryl moiety provided compound 1.4 with promisingly low nanomolar in vitro activity and excellent in vivo activity with an ED$_{50}$ value of 0.2 mg/kg in the murine P. yoelii model. This optimization leading to GW844520 corresponds to an approximate 200-fold improvement in potency over the starting point clopidol. 18

In addition to excellent activity against erythrocytic stages of P. falciparum parasites, 1.4 also showed remarkable activity towards liver stages of P. falciparum (EC$_{50}$ = 48.8 µM) and blood stages of P. vivax (EC$_{50}$ < 4.88 µM), while no cytotoxicity against human cell lines was observed (EC$_{50}$ > 6.1 mM). Furthermore, a selectivity index was confirmed in inhibition studies, in which 1.4 was shown to selectively inhibit plasmodial cytochrome bc$_1$ complex over mammalian cytochrome bc$_1$. The IC$_{50}$ for plasmodial cytochrome bc$_1$ complex III (IC$_{50}$ = 0.002 µM) was shown to be approximately 250-fold lower than the corresponding human protein (IC$_{50}$ = 0.51 µM). 49 The inhibition of P. falciparum complex II and complex IV was weak (IC$_{50}$ > 3 µM).

Compound 1.4 was also tested against multidrug resistant strains (3D7A, FCR3, K1, Dd2, Hb3 and W2) of P. falciparum and showed no cross-resistance with any of the tested strains including the atovaquone resistant strain FCR3, suggesting a slightly different binding mode to cytochrome bc$_1$ despite its similar mechanism of action. 49 Importantly, the FCR3
ataquone resistant strain does not possess the clinically relevant amino acid 268 mutation in cytochrome b.

1.3.3 Preclinical studies of 4(1H)-pyridones

Based on the promising antimalarial profile, compound 1.4 entered preclinical trials in 2006; however, it was discontinued because of histopathological complication in skeletal and cardiac muscles. These results were probably caused by the high lipophilicity (logD pH7.4 = 2.79) and the long half-life ($t_{1/2} = 143$ h in a dog) of 1.4. Consequently, oral bioavailability of lead GW844520 was also extremely low at higher dosage (% F = 4 at a 2mg/kg dose in dogs and % F = 20 at a 10mg/kg dose in mice) mainly because of poor aqueous solubility (pH 2-12 < 0.1 µg/ml). The lack of dose linearity of 1.4 in the preclinical studies triggered the initiation of a back-up program at GSK with the goal to design 4(1H)-pyridones with higher solubilities and increased bioavailabilities. Introduction of a hydroxymethyl moiety at C6 yielded compound GSK932121 (1.5) (Figure 1.3), whose solubility at lower pHs (simulated gastric fluid (SGF) pH 1.2 = 372 mg/ml, fed state simulated intestinal fluid (FeSSIF) pH 5.0 = 2.34 mg/ml) was significantly increased in comparison to its solubility at pH 7.4 (PBS pH 7.4 < 0.1 mg/ml). As such, the oral bioavailability of 1.5 was improved to 16 % in a dog model at 2 mg/kg doses. Importantly, incorporation of the hydroxymethyl group at C6 retained the in vitro ($EC_{50}$ 3D7A = 0.002 µM) and in vivo efficacy ($ED_{50}$ = 0.3 mg/kg in the murine P. yoelii model). The formulation approach was used to enhance the oral bioavailability and allowed 1.5 to progress to first-time-in-human (FTIH) studies. Despite promising early results, these studies were terminated due to unexpected acute toxicity with the water-soluble phosphate prodrug 1.6 (Figure 1.3) in rats. Prodrug approach with compound 1.6 progressed into preclinical trials in parallel with FTIH study as a back-up. The toxicity was attributed to the inhibition of
mammalian bc₁ cells by parent compound 1.5. Despite the difficulties associated with the toxicity of GSK compounds in humans, 4(1H)-pyridones remain an attractive chemotype with potential to be efficacious not only against the blood forms of parasite, but also against other stages such as liver and mosquito forms acting as prophylactic and transmission blocking agent.

1.4 4(1H)-Quinolones

1.4.1 Historical overview

Before the beginning of World War II, German chemists from Bayer discovered prophylactic antimalarial activity with endochin (1.7), a 3-heptyl-7-methoxy-2-methyl-4(1H)-quinolone (Figure 1.5). The prefix “endo-“ was chosen as this new antimalarial appeared to inhibit sporozoites and infected endothelial cells of liver stages, while the suffix “-chin” was common to many antimalarials related or unrelated to the alkaloid quinine (German: chinin). It was not until the end of the war when the first results on the antiplasmodial activity of 1.7 were published. Remarkably, endochin showed activity in an avian malaria model against all three stages of the parasite life cycle: host liver, blood, and mosquito stages. Despite demonstrated efficacy of endochin towards P. gallinaceum in chicken and P. praecox in canaries, initial trials in humans failed, likely because of unfavorable pharmacokinetics. When better in vivo malaria models became available, 1.7 was tested by Walter Reed Army Institute of Research in 1974 in the gold standard P. cynomolgi infected Rhesus radical cure assay and found to be inactive.

Around the same time, Casey at the University of Bridgeport prepared 15 endochin analogues focusing on 3-alkenyl- and 3-alkyl-substituted 2-methyl-4(1H)-quinolones. Only two
of the tested compounds showed antimalarial activity in *P. gallinaceum* and *P. berghei* assays. The 4(1H)-quinolone with an (E)-(dodec-1-en-1-yl) side chain at the C3 position cured two out of five mice at a dose of 640 mg/kg in the Rane single dose malaria model. The *N*-oxide of endochin was the second compound displaying *in vivo* activity in the same malaria model confirming the initial assumption that the poor bioavailability of endochin limited its use in mammals. It is also noteworthy that in 1968 Lemke and co-workers reported a series of 3-unsubstituted 4(1H)-quinolone-2-carboxylates which were inactive against *P. berghei* in a murine model.

1.4.2 SAR studies of 4(1H)-quinolones: 3-alkyl derivatives

4(1H)-quinolones regained attention by several research teams at the beginning of this century. First, Kyle and co-workers at the Walter Reed Army Institute of Research reevaluated approximately 30 structurally related 4(1H)-quinolones for *in vitro* erythrocytic activity against D6 (chloroquine and atovaquone susceptible) and TM90-C2B (chloroquine, mefloquine, and atovaquone resistant) strains of *P. falciparum*. Notably, several 4(1H)-quinolones demonstrated exceptional *in vitro* activity with potencies in the low nanomolar ranges while the cross-resistance with atovaquone was not complete across the 4(1H)-quinolone series. Moreover, the efficacy of WR193211 (3-geranyl-substituted 2-methyl-4(1H)-quinolone, one of Casey’s compound) was reevaluated and proven to display modest *in vivo* activity in a modified Thompson Test with *P. berghei* (compound given daily for three days) with an ED$_{90}$ of 97 mg/kg. Subsequently in 2007, Riscoe and Winter reported similar results with endochin and 8 analogues thereof.
1.4.3 SAR studies of 4(1H)-quinolones: optimization of the C5-C8 benzenoid ring

For the optimization of endochin, Manetsch and Kyle combined an extensive structure-activity relationship study with a structure-property study as endochin appeared to be limited by its physicochemical properties. While the n-alkyl chain at the C3 position was identified to play an important role for the antimalarial activity, it was also found to be a major liability in terms of aqueous solubility and microsomal stability (Figure 1.6). Nevertheless, initial optimization efforts focusing primarily on antimalarial activity yielded 3-alkenyl-substituted 4(1H)-quinolones which were approximately 2-fold more potent in comparison to 3-alkyl-4(1H)-quinolones. In contrast, 3-alkynyl-substituted compounds or 4(1H)-quinolones unsubstituted at the C3 position completely lacked in antimalarial activity. Modest results were obtained with 3-aryl-substituted 4(1H)-quinolones displaying EC$_{50}$s in the submicromolar range.

A systematic approach following the Topliss operational scheme for aromatic substituents was applied to probe the benzenoid ring for steric and electronic effects. The C5, C6, C7, and C8 positions of 3-phenyl- or 3-benzyl-2-methyl-4(1H)-quinolones were altered with a methoxy-, a chloro-, or a dichloro-substitution. Best potencies were observed with compounds

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**Figure 1.6:** Structure-activity relationship (SAR) studies for the optimization of endochin
substituted at the benzenoid ring with a chloro group at the C6 or with a methoxy group at the C7 position. Combination of a 6-chloro and a 7-methoxy group within the same 4(1H)-quinolone greatly synergized the substituent effects and improved the *in vitro* potency up to 20-fold. In addition, the 6-chloro-7-methoxy substitution combination gave rise to an array of 4(1H)-quinolones displaying equal antimalarial potency against the TM90-C2B (atovaquone resistant) and the W2 (chloroquine resistant) strains of *P. falciparum* which consequently lowered the atovaquone cross-resistance index (RI: EC$_{50}$ ratio of TM90-C2B to W2) to an optimal value close to 1. Atovaquone resistance of TM90-C2B is caused by a specific mutation altering the wild type from Tyr$^{268}$ to Ser$^{268}$ in the parasite’s cytochrome b gene of cytochrome bc$_1$. Compounds with RI = 0.3 – 3.0 are considered acceptable in terms of risk to cross-resistance with atovaquone, whereas compounds with RI > 10 and RI < 0.1 are likely to have clinically relevant levels of cross-resistance with atovaquone.$^{43,59}$

The authors also attempted to lock a particular 4-quinolone in its tautomeric forms via *N*- or *O*-alkylation of the 4-quinolone core. Predominantly, the keto form of a 4(1H)-quinolone is favored over enol form in both solid and solution states.$^{28}$ Several *N*-methylated 3-benzyl-substituted 4-quinolones and their *O*-methylated analogues have been shown to completely lack in antimalarial activity in comparison to their corresponding 4(1H)-quinolones (Figure 1.6). These results suggest that the hydrogen bond donors and hydrogen bond acceptors typical of a 4(1H)-quinolone scaffold are likely required for a tight interaction with the parasite target.

Among the most promising candidates in the initial library of approximately 70 4(1H)-quinolones was P4Q-95 (1.8, Figure 1.7) with EC$_{50}$s comparable to endochin (P4Q-95: EC$_{50}$ (W2) = 26 nM, EC$_{50}$ (TM90-C2B) = 15 nM; endochin: EC$_{50}$ (W2) = 8.6 nM; EC$_{50}$ (TM90-C2B) = 47 nM). Importantly, 1.8 also displayed better microsomal stability and slightly better aqueous
solubility in comparison to endochin. Based on these promising results, 3-phenyl substituted P4Q-95 was assessed *in vivo* in mice (Thompson test, *P. berghei*), but unfortunately 8 lacked in *in vivo* antimalarial activity most likely due to low aqueous solubility.  

A first successful *in vivo* efficacy study with an optimized endochin analogue was reported by Riscoe and co-workers in 2011. For the orally administered prodrug ELQ-125, a polyethylene glycol carbonate derivative of 3-heptyl-5,7-diflouro-2-methyl-4-quinolone, an ED$_{50}$ value of 11 mg/kg was obtained against *P. yoelii* infections in CF1 mice. ELQ-125 emerged from an optimization study comprising approximately thirty 3-alkyl-substituted 4(1H)-quinolones differing primarily at their benzenoid ring substituents. The most potent compounds had EC$_{50}$ values in the picomolar range against the chloroquine resistant strain Dd2, although these compounds were not as potent against the atovaquone resistant strain TM90-C2B. For example, the resistance index for compound ELQ-125 was approximately 365 (ELQ-125: EC$_{50}$ (Dd2) = 0.4 nM, EC$_{50}$ (TM90-C2B) = 146 nM) suggesting strong potential for reduced efficacy against clinically relevant atovaquone resistant strains of malaria.

1.4.4 SAR studies of 3-aryl-4(1H)-quinolones and advancement of P4Q-391 and ELQ-300 into preclinical development

The experimental evidence that the orally administered prodrug ELQ-125 displayed good *in vivo* efficacy underscored the great promise of endochin derivatives as antimalarials. In addition to this, the potent *in vitro* antimalarial activity, the advantageous resistance index, the moderately improved physicochemical properties, as well as the synthetic tractability of P4Q-95 motivated the various scientists (University of South Florida, Oregon Health and Sciences University, Drexel University, Monash University, and MMV) to combine efforts in a multidisciplinary lead optimization program. Concerted optimization efforts led to the discovery
of 3-diarylethers-4(1H)-quinolones ELQ-271 (1.9), ELQ-300 (1.10) and P4Q-391 (1.11), of which ELQ-300 was nominated as a preclinical candidate (Figure 1.7).\(^\text{11}\) The C3 substituent of ELQ-271 and ELQ-300 correspond exactly to the side chain of GSK’s analogue GW8444520, while the 6-chloro-7-methoxy benzenoid substituent in ELQ-300 and P4Q-391 is identical to the benzenoid substitution pattern of 4(1H)-quinolone P4Q-95 (Figure 1.7). The combination of these substitutions at C3 improved physiochemical properties whilst 6-chloro-7-methoxy proved critical for remarkable selectivity for parasite versus mammalian cytochrome b.

ELQ-300 was potent in the \textit{in vitro} erythrocytic assay against a chloroquine-sensitive strain (EC\(_{50}\) (Dd6) = 2.2 nM) and multidrug-resistant strains of \textit{P. falciparum} (EC\(_{50}\) (W2) = 1.8 nM) including the atovaquone-resistant clinical clones (EC\(_{50}\) (TM90-C2B) = 1.7 nM). Furthermore, both ELQ-300 and P4Q-391 showed excellent \textit{in vivo} efficacy against the blood stages (Thompson Test, \textit{P. berghei}: ELQ-300 ED\(_{50}\) = 0.016 mg/kg; P4Q-391 ED\(_{50}\) = 0.27 mg/kg) and the liver stages (\textit{P. berghei} sporozoite infection of mice followed by bioluminescence imaging: ELQ-300 blocking dose > 0.03 mg/kg; P4Q-391 blocking dose > 0.1 mg/kg). Moreover, both compounds were extremely effective in blocking transmission to the mosquito vector. Gametocytes treated with as low as 0.1 µM of ELQ-300 did not develop past stage III while a minimum concentration of 1 µM was needed for P4Q-391 to inhibit gametocyte growth past
stage IV. ELQ-300 and P4Q-391 were also shown to inhibit the formation of zygote, ookinete, and oocyst forms of the mosquito life cycle. The mechanism of action for both compounds was also found to be associated to the cytochrome $bc_1$ complex. Fortunately, although the $Q_0$ site of plasmodial mitochondrial cytochrome $bc_1$ complex is a common target for both drugs, the selectivity index for ELQ-300 was greatly improved (>18000) for *Plasmodium* $bc_1$ versus human $bc_1$ due to the 6-chloro-7-methoxy substitution pattern.

One limitation of ELQ-300 is its poor aqueous solubility (fasted state simulated intestinal fluid (FaSSIF) pH 6.5 = 0.3 µM) which has direct impact on the pharmacokinetic profile of the compound.\textsuperscript{11} The oral bioavailability of ELQ-300 in mice was excellent (~100%) at a dose of 0.3 mg/kg with PEG-400 vehicle in a *P. yoelii* efficacy model. However, increasing the dose concentration resulted in a decrease of bioavailability. The absence of dose-proportionality impedes the determination of the therapeautic index and *in vivo* toxicity. Alternative approaches such as prodrugs or formulation are required to overcome this solubility constraint. Nevertheless, as of today, ELQ-300 remains one of the five preclinical candidates in the MMV pipeline.\textsuperscript{10}

1.4.5 SAR studies of 4(1H)-quinolones: 2-substituted derivatives

O’Neil, Ward and coworkers developed a series of 2-substituted 4(1H)-quinolones with a dual mechanism of action against two enzymes of the *Plasmodium* mitochondrial electron transport chain: the NADH:ubiquinone oxidoreductase (*P. falciparum* NDH2) and cytochrome $bc_1$.\textsuperscript{22} Hydroxy-2-dodecyl-4(1H)-quinolone (HDQ), a known *Pf*NDH2 inhibitor was utilized as a starting point for the selection of approximately 17,000 compounds, which were tested in a high-through-put
screening against PfNDH2. Follow-up hit-to-lead optimization yielded frontrunner 4(1H)-quinolone CK-2-68 (1.13), in which the 2-dodecyl side chain of HDQ was replaced by a bis-aryl side chain (Figure 1.8). Generally, the overall trends of this structure-activity relationship study (Figure 1.9) closely resemble the trends of the 3-substituted quinolones reported by Manetsch, Kyle and Riscoe (Figure 1.6).

![Figure 1.9: SAR studies of 2-aryl-4(1H)-quinolones](image)

The benzenoid ring of the 4(1H)-quinolone core tolerated substitutions at the C6 and C7 positions, while any modification at C5 or C8 significantly decreased the antiplasmodial activities (Figure 1.9). Secondary in vitro activities of C3 methyl substituted 4Qs versus unsubstituted analogs are overtaken by better physiochemical properties of the formers due to increased rotational barrier along quinolone-aryl σ bond. More importantly, the nature of the C2 and C3 substituents determined the selectivity of this 4(1H)-quinolone series with 2-diaryl substituted compounds being selective towards PfNDH2, while 3-substituted analogues preferentially target the parasite’s cytochrome bc1 complex. Compound 1.13 has good activity against the blood with an EC$_{50}$ value of 36 nM against 3D7 and a strong inhibition of PfNDH2
(IC\textsubscript{50} = 16 nM). The compound also showed 100% suppressive activity in mice infected with \textit{P. berghei} (Peter’s 4 day \textit{in vivo} test) administrated either as a prodrug or in conjunction with a solubilizing formulation.\textsuperscript{22} Selective 4(1H)-quinolones including CK-2-68 were also tested against multidrug resistant strains of \textit{P. falciparum} and while the \textit{in vitro} activity against the chloroquine resistant clone W2 were similar to 3D7 (resistance index of approximately 1), compounds were not equipotent towards the atovaquone resistant strain TM90-C2B (EC\textsubscript{50} = 178 nM).

The low aqueous solubility and the necessity of approaches utilizing a prodrug moiety or formulations led to a design of compounds containing a heterocyclic sidechain.\textsuperscript{62} In comparison to \textbf{1.13}, compound SL-2-25 (\textbf{1.14}, Figure 1.10) conserved the promising antimalarial activity against the 3D7 strain of \textit{P. falciparum} (EC\textsubscript{50} = 54 nM) and the target \textit{PfNDH2} (IC\textsubscript{50} = 14 nM), while an oral administration showed activity with an ED\textsubscript{50} value of 1.9 mg/kg in a \textit{P. berghei} model of malaria when given as a phosphate salt. At the same time \textbf{1.14} is also equipotent against the parasite \textit{bc\textsubscript{1}} complex (IC\textsubscript{50} = 15 nM). However, it shows reduced activity towards the atovaquone-resistant strain TM90-C2B (EC\textsubscript{50} = 156 nM), yielding a resistance index of approximately 3. Generally, 2-pyridyl-4(1H)-quinolones are considered to be attractive templates for further lead optimization of 4(1H)-quinolones with dual mechanisms of action.
1.5 3-Ester-4(1H)-Quinolones

1.5.1 Historical overview

At Imperial Chemical Industries (ICI), Ryley and Peters reported a study on the development of an anti-coccidial agent as a potent antimalarial.\textsuperscript{15} Compound ICI56,780, a 7-(2-phenoxyethoxy)-4(1H)-quinolone (PEQ) (1.15, Figure 1.11), was shown to possess causal prophylactic (single dose of 30 mg/kg subcutaneous) and blood schizonticidal activity (ED\textsubscript{50} = 0.05 mg/kg) in rodent malaria models. Unfortunately a high degree of resistance to 1.15 was obtained after one passage in \textit{P. berghei} infected mice and the lack of oral bioavailability led to abandonment of this series of compounds. Approximately twenty years later, Puri and Dutta retested 1.15 and produced radical cures (eradicate dormant hypnozoites) in \textit{P. cynomolgi} infected Rhesus monkeys.\textsuperscript{16} Antimalarials which have proven \textit{in vivo} activity at eradicating hypnozoites of \textit{P. cynomolgi} have potential to be used as radical cures representing targets of highest priority for the worldwide malaria eradication initiative.

1.5.2 SAR studies of phenoxyethoxy-substituted quinolone esters (PEQs)

To overcome the resistance issue related to the PEQs, the Kyle and Manetsch laboratories conducted a structure-activity relationship study on a series of 29 novel PEQs in 2011.\textsuperscript{23} The most promising compound of this PEQ series was 6-butyl-3-(2-fluoro-4-(trifluoromethyl)phenyl)-7-(2-phenoxyethoxy)-4(1H)-quinolone which was reported to have a more than 200 fold improvement in RI (EC\textsubscript{50} (TM90-C2B) = 31 nM, EC\textsubscript{50} (W2) = 28 nM; RI = 1.1) over the parent compound 1.15 (EC\textsubscript{50} (TM90-C2B) = 0.05 nM, EC\textsubscript{50} (W2) = 11.2 nM; RI = 224). Superior blood stage activity and improved RI values were obtained with the 7-(2-
phenoxyethoxy) substituted 4(1H)-quinolone, while the presence of the hydrophobic butyl group at the C6 position was identified to be important in maintaining potent antimalarial activities. In contrast, the majority of the reported PEQs suffered from poor metabolic stability and limited aqueous solubility [unpublished data].

Similarly, an antimalarial series of twenty 4(1H)-quinolone esters that were mono-substituted at the C6 or C7 positions with one aryloxy or benzyloxy moiety was reported by Cowley et al in 2012. Each of the compounds was tested only in vitro against the chloroquine-sensitive strain 3D7 of *P. falciparum*. The structure-activity relationship data suggests that a C7 aryloxy or benzyloxy substituent was preferred over the same analogues substituted at C6 by an approximate factor of 300. Furthermore, the importance of the ester group at C3 was also established. The most promising quinolone ester from this series was confirmed to be active against the plasmodial bc1 complex (IC50 = 1.3 nM). Docking studies with the best compound binding to the Qo site of yeast bc1 complex suggested the existence of strong hydrogen bonding between the quinolone’s nitrogen and oxygen with the His182 and Glu272 residues.63

1.5.3 SAR studies of 2-aryl-3-ester-4(1H)-quinolones

An extensive work on lead optimization of 2-aryl-3-ester-4(1H)-quinolones was reported by Guy and co-workers. Approximately 100 derivatives of 4(1H)-quinolones were synthesized and tested against an array of *P. falciparum* strains (chloroquine and pyrimethamine resistant strain K1; chloroquine, mefloquine, pyrimethamine, and atovaquone resistant strain TM90-C2B; chloroquine sensitive strain D10; and DHODH inhibitor resistant strain D10_yDHOD). A systematic approach including a methodical variation of steric, electrostatics and hydrophobics following Topliss and Hansch analysis was applied to conduct the optimization. A meta-substituted aryl group at the C2 position was established to be extremely important for
antimalarial activity. In order to investigate this trend more precisely, more than 20 additional analogues were synthesized and tested in vitro (Figure 1.12). Hydrophobic and electron-donating groups at the meta-position were determined to be the preferred C2 aryl substituents. Interestingly, a linear correlation between the pEC₅₀ (K1) values and the hydrophobicity ($R^2 = 0.74$) was identified while performing Hansch analysis. In contrast, no correlation between antimalarial activity and electronic effects were observed, which highlights a direct relationship between hydrophobicity and potency for these 2-aryl-3-ester-4(1H)-quinolones. For these 4(1H)-quinolone esters, it was therefore concluded that during the optimization process the antimalarial activity and key physicochemical properties needed to be carefully counterbalanced.

Introduction of an electron-donating group at the C7 position of the 4(1H)-quinolone benzenoid ring greatly improved the antimalarial activity, while an electron-withdrawing group caused a significant drop in potency (Figure 1.12). At the same time, the addition of a halogen substituent at the C6 position improved the antimalarial activity primarily against the TM90-C2B strain and consequently the RI value to approximately 1. Lastly, incorporation of different...
solubilizing moieties at the C3 ester position substantially reduced the potency in comparison to the ethyl ester 4(1H)-quinolone starting point.

Parallel assessment of the in vitro activity and physicochemical property data led to the decision to test nine of these 4(1H)-quinolone esters in a Thompson test for in vivo activity. Of all the tested compounds, only two showed suppressive activities. For instance, ethyl 2-(3-chlorophenyl)-6-fluoro-7-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylate displayed low nanomolar EC50s against K1 (EC50 = 80 nM) and TM90-C2B (EC50 = 200 nM) strains of P. falciparum, but it was highly soluble at pH 7.4 even at concentrations of 20 µM and possessed good permeability (PAMPA pH 7.4 = 410 cm/s). Not surprisingly, it was efficacious in vivo, demonstrating a 57% suppression of parasitemia on day 6 postinfection at an oral dose of 30 mg/kg.

Despite the excellent activities in vitro for the best 2-aryl-3-ester-4(1H)-quinolones, they are approximately 50-fold less potent in vivo in comparison to the 4(1H)-quinolones such as ELQ-300 or P4Q-391 (Figure 1.7) developed by the Manetsch and Riscoe groups. These compounds also appear to target the plasmodial cytochrome bc1 complex as increased toxicity was observed with repeated dosing suggesting partial inhibition of mammalian bc1 complex. Nonetheless, 2-aryl-3-ester-4(1H)-quinolones possess great potential for future development as there is high potential for additional bioavailable improvements.

Recent screening of 1037 existing drugs targeted against P. berghei liver stages identified decoquinate 1.16 as the most potent inhibitor (EC50 = 2.6 nM) (Figure 1.13). This result is not unexpected considering that antimalarial activity of decoquinate against P. berghei was first
reported by Ryley and Peters in their anti-coccidial study. In addition, Puri and Dutta also showed that compound \textbf{1.16} possessed anti-hypnozoite activity in a \textit{P. cynomolgi} infected Rhesus model when administrated as the ester prodrug WR 194905. Further investigation of this quinolone ester confirmed selective inhibition of plasmodial cytochrome \textit{bc} \textsubscript{1} complex.

1.6 1,2,3,4-Tetrahydroacridones

1.6.1 Historical overview

Antimalarial activity of 1,2,3,4-tetrahydroacridin-9(10H)-ones (THAs) was reported initially by Stephen and co-workers in 1947. More than a dozen THA analogues were tested using an \textit{in vivo} assay against \textit{P. gallinaceum} infections in chicks with a few compounds having minimum effective doses as low as 12.5 mg per 100 g (Figure 1.14, compound \textbf{1.17}). At approximately the same time, endochin (Figure 1.5) was discovered to have prophylactic antiplasmodial activity, which surpassed the prophylactic activity of most of the THAs. Because of this, the next 50 years of optimization efforts focused primarily on 4(1\textit{H})-quinolones and 4(1\textit{H})-quinolone esters instead of the THAs. Nevertheless, interesting reports on the antimalarial properties of THAs were published in the past decades.
1.6.2 Floxacrine and other dihydroacridinediones

The dihydroacridinediones, which are structurally related to the THAs (Figure 1.14, compound 1.18), were studied in great detail over the course of last 30 years. Floxacrine (Figure 1.15, compound 1.19), first reported by Durckheimer in 1975, was discovered to have promising antimalarial activity including causal prophylactic activity.\(^{66}\) Extensive optimization of floxacrine was conducted by several research groups with the hope to address the poor aqueous solubility, atovaquone cross resistance, and the dose-dependent chronic periarteritis caused by floxacrine.\(^{67}\) Compound WR243251 (1.20), a prodrug of WR243246 (1.21), was developed and shown to generate cures in Aotus monkeys infected with the Smith strain (chloroquine, quinine, and pyrimethamine resistant) of \(P. falciparum\) at a dose of 16 mg/kg. Unfortunately, prodrug 1.20 was abandoned in the late preclinical development stage because of cross-resistance to atovaquone\(^{68}\) and compound instability related to a spontaneous decomposition of the prodrug 1.20 into 1.21 [unpublished data]. Noteworthy, despite a substantial similarity between floxacrine and 1.21, both compounds appear to have different mechanisms of action with floxacrine inhibiting parasites via heme-mediated processes whereas the single (\(S\))-enantiomer of 1.21 was shown to be a highly selective inhibitor of the ubiquinol oxidation site \(Q_o\) of the \(P. falciparum bc_1\) complex.\(^{69}\)

Recently, Riscoe and co-workers developed acridone T3.5 (1.22), a fully aromatized THA analogue, which possesses excellent blood stage antimalarial activity via a dual mode of action (Figure 1.16).\(^{70}\) Compound 1.22 was shown to be equipotent (RI ~ 1) in \textit{in vitro} erythrocytic assays against the chloroquine-sensitive D6 (EC\(_{50}\) = 45 nM) and the multidrug-
resistant *P. falciparum* strains Dd2 (EC\(_{50} = 77\) nM) and also the atovaquone-resistant clinical clone TM90-C2B (EC\(_{50} = 71\) nM). 1.22 also showed suppressive *in vivo* efficacy in a *P. berghei* murine model decreasing parasitemia by 95% at a dose of 100 mg/kg per day. The curative effect was recorded at higher doses (256 mg/kg per day). The dual mode of action of compound 20 was attributed to the ability of disrupting hemozoin formation (tricyclic aromatic acridone core) as well as chemosensitizing via acid trapping. While no *in vivo* pharmacokinetic data has been reported so far, the T3.5 acridone chemotype is a promising template for further development of novel antimalarials.

1.6.3 SAR studies of 1,2,3,4-tetrahydroacridones

Finally, an extensive structure-activity relationship and structure-property relationship study on more than 100 THAs was reported by Kyle and Manetsch.\(^{20}\) A systematic approach following the Topliss operational scheme for aromatic substituents was applied to investigate the
benzenoid ring while also looking at the saturated ring using the findings of Stephen and Kesten.\textsuperscript{67} In general, the results (Figure 1.17) were similar to the previously observed structure-activity relationship data for the 4(1\textit{H})-quinolones (Figure 1.6). Despite the initial report from Kesten on the importance of the mono-chloro substitution of the THA core, best antimalarial activity was obtained when the C6 position was substituted with an electron-donating substituent and C7 was substituted with a weakly electron-withdrawing group such as a halogen. Substitutions at C5 and C8 were not well tolerated perhaps because of the disruption of intermolecular hydrogen bonding between amine and carbonyl oxygen to the biological target. The THA core containing a six member unsubstituted aliphatic ring was reported to be most active. 7-Chloro-6-methoxy-1,2,3,4-tetrahydroacridin-9(10\textit{H})-one was reported with EC\textsubscript{50} values lower than 30 nM for the W2 and TM90-C2B strains. Although the THAs are less potent than the 4(1\textit{H})-quinolones, the best THAs had an excellent \textit{in vitro} therapeutic index and were equipotent for the atovaquone resistant and susceptible strains. Unfortunately, the THAs suffered from poor solubility and unfavorable microsomal stability yielding poor performance of the THA analogues \textit{in vivo} [rodent models, unpublished data]. Additional functionalization of acridin-9(10\textit{H})-ones via alternative approaches such as prodrug or formulations are required to obtain good oral efficacy.

1.7 Conclusions

4(1\textit{H})-pyridone and 4(1\textit{H})-quinolone chemotypes display potent antimalarial activities against \textit{Plasmodium} species \textit{in vitro} and \textit{in vivo}. Some of these chemotypes inhibit the electron-transport chain in \textit{P. falciparum} and hence possess a mechanism of action similar to the one of atovaquone. The progression of 4(1\textit{H})-pyridone GSK932121 into first-time-in-human studies
featured the full potential that these chemotypes have, although the program had to be stopped because of potential toxicity issues. Not surprisingly, several research groups focus their resources in developing and optimizing 4(1H)-quinolone-based antimalarials. Recently, ELQ-300 and structurally related 4(1H)-quinolone analogues have been shown to be highly active against *P. falciparum* and *P. vivax* at all life cycle stages. Due to this antimalarial activity profile in conjunction with favorable pharmacokinetics, ELQ-300 has been selected as a preclinical candidate in the MMV drug discovery program. At the same time, *in vitro* and *in vivo* efficacious 2-diheteroaryl 4(1H)-quinolones with dual mechanism of action have been developed, which inhibit the NADH:ubiquinone oxidoreductase *P. falciparum* NDH2 and the cytochrome *bc*1 complex simultaneously. These 2-substituted 4(1H)-quinolones are currently undergoing further development in the MMV pipeline.

Nevertheless, the development of 4(1H)-pyridone and 4(1H)-quinolone antimalarials are also associated with risks. 4(1H)-Pyridones and 4(1H)-quinolones are known to be poorly soluble and thus represent challenging drug candidates for pharmacokinetic and bioavailability reasons. In addition, as the parasite electron-transport chain is quite often the biological target, 4(1H)-pyridone or 4(1H)-quinolone inhibitor design requires structural features inheriting a high selectivity over the corresponding mammalian targets. Furthermore, the potential to induce cross-resistance with atovaquone has to be carefully monitored during the optimization process. In particular compounds with greatly reduced potential for inducing resistance (e.g., ELQ-300) are required due to the high frequency of resistance selection with atovaquone. Also an ideal antimalarial compound should have a rapid onset of action, which is a property none of the compounds of this series has yet overcome. Despite these hurdles, the potent blood stage activity and the demonstrated potential to kill hypnozoites in the gold standard *P. cynomolgi* infected
Rhesus model makes the herein discussed 4(1H)-pyridones, the 4(1H)-quinolones, the THAs and the PEQs attractive chemotypes for the development of novel drugs to treat multidrug resistant malaria and to aid the malaria elimination campaign.

1.8 References Cited


55. Casey, A. C. *Synthesis of some 4-quinolones and related structures for evaluation as potential antimalarial agents*; University of Bridgeport: Bridgeport, CT, **1974**.


66. Durckheimer, W.; Raether, W.; Seliger, H. G. Tetrahydroacridones having chemotherapeutic action and process for preparing them. US3947449, **1976**.


CHAPTER 2: SYNTHETIC APPROACHES FOR THE CONVENIENT ACCESS TO 3-ARYL-4(1H)-QUINOLONES

2.1 Introduction

Ethyl acetoacetate (EAA) is a versatile and well-established reagent in organic synthesis. EAA's combined electrophilic and nucleophilic nature makes it a convenient reagent for the preparation of a variety of products of different structural complexity. In medicinal chemistry, 2-aryl substituted EAA s provide access to diverse classes of biologically active scaffolds such as important heterocyclics. Various 2-aryl EAA derived compounds were well documented in the literature as antifungal, antibacterial, antitubercular and antitumoral agents. These also served as TNF-α inhibitors, α2C-adrenoreceptor antagonists, DMT1 blockers and HCV NS5B polymerase inhibitors. In addition, a series of 3-aryl-4(1H)-quinolone compounds, synthesized from corresponding aniline and 2-phenyl EAA, were reported to have excellent low nanomolar activity against malaria. Notably, extensive development of this 3-aryl-4(1H)-quinolone chemotype against *P. falciparum* and *P. vivax* malaria at all parasite life cycle stages resulted in ELQ-300 (Figure 2.1), which recently entered preclinical studies. Based on the advantage and importance of 2-aryl EAA s as starting materials, suitable and straightforward access to these compounds is required.

Historically, 2-aryl EAA s are prepared using various metal-mediated and metal-free reaction conditions. In a classical approach, 2-aryl-2-acylacetonitriles are converted to 2-aryl

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1 The first part of the work presented has been submitted to Chem.Com. for review
EAAs in two steps under harsh acidic conditions via an imidate intermediate in low to moderate yields. Ethyl 2-arylacetate could also be acylated under basic conditions with acetyl chloride or acetic anhydride to obtain the target compounds. These transformations, however, are low-yielding and produce the deacylated by-product (starting material), which in most cases is inseparable from the EAA product. Finally, under Pd-mediated or Cu-mediated conditions with the appropriate metal ligands, EAA could be treated with aryl halides and base to obtain the target compounds at elevated temperatures. In turn, metal-catalyzed reactions suffer from accompanying ligand arylation and product deacylation that is heavily dependent on the nature and quantity of the base used. Moreover, minimal or no usage of expensive metal catalysts is highly advisable in drug discovery because of malfunctions at the cellular level. All of these facts prompted us to develop a protocol where the 2-aryl EAAs can be obtained easily under mild metal-free reaction conditions.

2.2 Hypervalent Iodine Compounds

Hypervalent iodine compounds and diaryliodonium salts in particular have recently captured the attention of synthetic chemists as mild and selective reagents. One of the biggest advantages of the above mentioned salts is metal-free reaction conditions to overcome cost and toxicity of the organometallic chemistry in medicinally interesting compounds. In recent literature, arylation of carbonyl compounds and nucleophiles like O, N, S, Se, etc. under various conditions was reported with excellent yields using highly electrophilic hypervalent aryliodonium salts. Asymmetric synthesis via chiral iodonium salts is also being investigated, although it is in the initial stages of research. With the inspiration of the above mentioned successes, we were encouraged to extend the scope to a metal-free arylation of EAA.
Noteworthy, the reaction between EAA and diphenyliodonium trifluoroborate to prepare 2-phenyl EAA has been initially reported by a Soviet group in 1984.\textsuperscript{25} To our knowledge, the arylation of EAA with diaryliodonium salts has not been explored by any research group since this original report. Interestingly, a failed attempt of EAA reaction with diphenyliodonium salt was reported in 1999.\textsuperscript{26} By the virtue of having one-pot synthetic access to various biaryl iodonium salts and with some of them being commercially available nowadays, it was envisioned that a general and simple arylation protocol can be established under mild conditions.\textsuperscript{17}

### 2.3 Arylation of Ethyl Acetoacetate (EAA) with Symmetrical Diphenyliodonium Salts

Diphenyliodonium tetrafluoroborate \textbf{2.1a} was chosen as a test substrate in the optimization of arylation reaction conditions resulting in 2-phenyl EAA in DMF with \textit{t}-BuOK. To avoid any possible solubility issues with iodonium salts, DMF was the solvent of preference, though most of the salts with BF\textsubscript{4} and OTf anions are soluble in nonpolar solvents. When 1:1 ratio of iodonium salt and EAA was used, the reaction was low yielding because of the formation of double arylated products evident from LC-MS. However, improved yields were obtained when 1:2.5 ratio of iodonium salt and EAA was used. On top, the formation of side products was suppressed according to \textit{1}H-NMR analysis of the crude product. It was found that after reaction completion, the addition of HCl solution in one portion is mandatory to avoid the formation of deacylated product via a retro-Claisen reaction. Among the different bases screened, Cs\textsubscript{2}CO\textsubscript{3} and \textit{t}-BuOK resulted in the best and most reproducible yields. Of the two bases, \textit{t}-BuOK was preferred due to its low cost. Later, having these conditions set, the influence of various diphenyliodonium anions on the course of the arylation reaction was examined. Arylations with a
diphenyliodonium triflate provided similar yields to the tetrafluoroborate, however, the hexafluorophosphate resulted in a higher 60% yield. The reaction with an iodonium salt with a bromide anion resulted in poor yields possibly due to a combination of competing nucleophilicity of the bromide anion and the low solubility of the bromide salt in DMF. Although the yield with the PF₆ salt is slightly better than the arylations with BF₄ and OTf anions, it was preferred to proceed with the latter two due to their easy accessibility. Despite initial moderate yields with the unsubstituted diphenyliodonium salt, we wanted to explore the reaction further by probing diverse electron-rich, electron-deficient and sterically hindered electrophiles with the reasonable assumption of getting improved results particularly when using iodonium salts substituted at the aryl rings with electron withdrawing groups.

**Table 2.1: Optimization of the arylation reaction conditions**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Base</th>
<th>Time (h)</th>
<th>X⁻</th>
<th>Yield (2.2a) (%)&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>t-BuOK</td>
<td>18</td>
<td>BF₄ (2.1a)</td>
<td>53</td>
</tr>
<tr>
<td>2</td>
<td>Cs₂CO₃</td>
<td>20</td>
<td>BF₄ (2.1a)</td>
<td>55</td>
</tr>
<tr>
<td>3</td>
<td>KOH</td>
<td>24</td>
<td>BF₄ (2.1a)</td>
<td>35</td>
</tr>
<tr>
<td>4</td>
<td>K₂CO₃</td>
<td>28</td>
<td>BF₄ (2.1a)</td>
<td>25</td>
</tr>
<tr>
<td>5</td>
<td>NaH</td>
<td>20</td>
<td>BF₄ (2.1a)</td>
<td>40</td>
</tr>
<tr>
<td>6</td>
<td>t-BuOK</td>
<td>24</td>
<td>OTf (2.1b)</td>
<td>45</td>
</tr>
<tr>
<td>7</td>
<td>t-BuOK</td>
<td>24</td>
<td>Br (2.1c)</td>
<td>10</td>
</tr>
<tr>
<td>8</td>
<td>t-BuOK</td>
<td>24</td>
<td>PF₆ (2.1d)</td>
<td>60</td>
</tr>
</tbody>
</table>

<sup>a</sup>Reaction conditions: salt 2.1 was added to the enolate solution of EAA and the reaction was run for the tabulated time.<br><sup>b</sup>Isolated yields combined in keto and enol form.

Firstly, various symmetrical iodonium salts with tetrafluoroborate or triflate anions were prepared and subsequently treated with EAA under optimized conditions. The resultant 2-aryl EAAs were then converted to the corresponding 3-aryl-4(1H)-quinolones under modified conditions.
Conrad-Limpach conditions using 4-chloro-3-methoxyaniline.\textsuperscript{27} All the results are summarized in Table 2.1.

Generally, arylations of numerous ortho- substituted substrates are problematic in metal-mediated conditions and result in poor yields due to steric bulkiness.\textsuperscript{14} Remarkably, the ortho-substitution was tolerated well in our case with salts 2.1b and 2.1e getting converted to greater than 70\% of product signifying an advantage of the selected methodology. In addition, the arylation with 2,4-dimethyl substituted salt 2.1h delivered 60\% yield, whereas dimesityliodonium example 2.1c resulted only in 10\% yield. Noteworthy, compound 2.1c was obtained in the enol form exclusively, impeding the formation of the enamine during the Conrad-Limpach cyclization. Unsurprisingly, increasing the substitution of the aromatic ring resulted in slightly lower yields in the cyclization step due to steric factors (Table 2.2).

The arylation of EAA using electron-rich electrophiles like 4-\textit{tert}-butyl took relatively longer times and revealed marginally lower yields. Moreover, any attempts with 4-methoxy substituted salt 2.1m did not deliver the required product even after prolonged reaction times. The unreactivity of salt 2.1m is probably due to reduced electrophilicity of the iodine center. 4-Chloro substituted salt 2.1f produced the corresponding product in respectable yields under these reaction conditions. Noticeably, salts 2.1g and 2.1l, which contain deactivating groups, behaved excellently, reacting to the corresponding arylated products in less time with yields over 90\%. Likely, these results can be linked to an enhanced electrophilicity of the iodine center.

Traditionally, the Conrad-Limpach reaction, which was initially reported in 1887\textsuperscript{28}, is low-yielding and involves harsh conditions requiring high-boiling solvents like diphenyl ether or polyphosphoric acid. In this report, we were able to improve the overall yields in average by 10\% by using microwave assisted conditions in toluene as a solvent and cutting the cyclization
time to 3 minutes (Table 2.2). Under classical high temperature thermal conditions in Ph₂O the formation of quinolone is usually accompanied with multiple side products which interfere with isolation.

**Table 2.2: Arylation of ethyl acetoacetate (EAA) with symmetrical salts**

<table>
<thead>
<tr>
<th>Entry</th>
<th>R</th>
<th>Yield 2.2ᵃ (%)</th>
<th>Yield 2.3ᵇ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>H (2.1a)</td>
<td>53 (2.2a)</td>
<td>60 (2.3a)</td>
</tr>
<tr>
<td>2</td>
<td>2-CH₃ (2.1e)</td>
<td>73 (2.2b)</td>
<td>42 (2.3b)</td>
</tr>
<tr>
<td>3</td>
<td>2,4,6-tri CH₃ (2.1f)</td>
<td>10 (2.2c)</td>
<td>No Reaction (2.3c)</td>
</tr>
<tr>
<td>4</td>
<td>4-CF₃ (2.1g)</td>
<td>93 (2.2d)</td>
<td>62 (2.3d)</td>
</tr>
<tr>
<td>5</td>
<td>2-F (2.1h)</td>
<td>75 (2.2e)</td>
<td>54 (2.3e)</td>
</tr>
<tr>
<td>6</td>
<td>4-Cl (2.1i)</td>
<td>70 (2.2f)</td>
<td>68 (2.3f)</td>
</tr>
<tr>
<td>7</td>
<td>4-tBu (2.1j)</td>
<td>65 (2.2g)</td>
<td>67 (2.3g)</td>
</tr>
<tr>
<td>8</td>
<td>2,4-di CH₃ (2.1k)</td>
<td>60 (2.2h)</td>
<td>38 (2.3h)</td>
</tr>
<tr>
<td>9</td>
<td>4-COOCH₃ (2.1l)</td>
<td>90 (2.2i)</td>
<td>67 (2.3i)</td>
</tr>
<tr>
<td>10</td>
<td>4-OCH₃ (2.1m)</td>
<td>No Reaction</td>
<td>N/A</td>
</tr>
</tbody>
</table>

⁴Reaction conditions: a) t-BuOK, DMF, EAA, 5 to 21 h, r.t.; b) 4-chloro-3methoxyaniline, AcOH, benzene, Dean-Stark, 24 to 48 h, then MW, toluene, 3 min, 255 °C. ⁵Isolated yields.

Switching the reaction solvent to toluene in a microwave allowed the isolation of analytically pure samples by precipitation with no further need for recrystallization.

### 2.4 Arylation of EAA with Unsymmetrical Diphenyliodonium Salts

Next, the chemoselectivity trends were examined with unsymmetrical salts. A few examples with varying sterics and electronics were prepared for this purpose followed by treatment under optimized arylation conditions. The results are summarized in the Table 3.
Table 2.3: Arylation of EAA with unsymmetrical salts

\[
\begin{align*}
\text{BF}_4^- \text{ or } OTf & \quad \text{R} \quad \text{a} \quad \text{COOEt} \quad \text{COOEt} \quad \text{b} \quad \text{with 2.2} \\
\begin{array}{c|c|c|c}
\text{Entry} & \text{R} & \text{Yield 2.2/2.2a (\%)} & \text{Yield 2.3 (\%)} \\
1 & \text{mesityl (2.1n)} & 2.2c/2.2a 36/0 & \text{No reaction (2.3c)} \\
2 & 4-NO_2 (2.1o) & 2.2j/2.2a 83/0 & 39 (2.3j) \\
3 & 4-\text{CF}_3 (2.1p) & 2.2d/2.2a 89/0 & 62 (2.3d) \\
4 & 4-\text{Cl}-3-\text{pyridyl (2.1q)} & 2.2k/2.2a 50/12 & \text{Trace (2.3k)} \\
\end{array}
\end{align*}
\]

\(^a\)Reaction conditions: a) t-BuOK, DMF, EAA, 5 to 21 h, r.t.; b) 4-chloro-3-methoxyaniline, AcOH, benzene, Dean-Stark, 24 to 48 h, then MW, toluene, 3 min, 255 °C.

Excellent chemoselectivity was observed in the case of phenylmesityl substituted salt 2.1n, resulting in EAA 2.2c exclusively in 36\% yield. The mesityl group was readily transferred compared to the phenyl group. Compared to the symmetrical salt 2.1f, the improved yield for this arylation may be due to the steric differences of both rings attached to the iodine center. During the arylation of salts 2.1o and 2.1p, the electron-deficient rings were selectively and exclusively transferred to produce the products 2.3j and 2.3d in excellent yields. Arylation with salt 2.1q resulted in a 4:1 ratio of the pyridyl ring and phenyl ring products in 62\% overall yield. However the Conrad-Limpach cyclization of 2.2k did not perform well and produced only trace amount of corresponding quinolone after several attempts of purification. Arylation pursuits using the 4-anisylphenyl iodine compound to deliver the expected unsubstituted product 2.2a were not successful. The observed chemoselectivity trends are consistent with selectivities reported in the literature for metal-free reactions. The electron-poor rings are preferentially transferred over the electron-rich rings and bulky rings readily transferred over rings lacking in sterics.
2.5 Synthesis of ELQ-300 via Diphenyliodonium Salts

After having the arylation conditions set, this strategy was applied for the synthesis of antimalarial compound **ELQ-300** (Figure 2.1). The iodonium salt **2.6** was prepared according to the reported procedure and subsequently treated with 4-iodophenol to obtain the biaryl ether **2.7** in excellent yield. Next, boronic acid **2.8** was prepared from aryl iodide **2.7** and converted cleanly to the appropriate iodonium salt **2.9**. The salt **2.9** was then treated with EAA under standard arylation conditions providing the corresponding substituted EAA **2.10** in 52% yield in its pure form of the keto-enol tautomers. This result is better than the previously reported arylation obtained in the Cu-catalyzed reaction, where only 30% yield was obtained with the contamination of inseparable deacylated product. Additionally, one equivalent of the intermediate **7** is formed during the reaction, which could be reused to make the salt **2.9**. Finally, EAA **2.10** was treated under Conrad-Limpach conditions to obtain **ELQ-300**.

![Figure 2.1: Synthesis of ELQ-300 via the hypervalent iodonium salt route](image-url)
2.6 Gram Scale Syntheses of Antimalarial 4(1H)-Quinolones ELQ-300 and P4Q-391

4(1H)-Quinolones belong to the well-established class of heterocyclic compounds being approved by FDA for use in human primarily as antibacterials.\textsuperscript{30-32} 4(1H)-quinolones have also shown to be potent anticancer, antimicrobial and antiviral agents, with some natural products containing a 4(1H)-quinolone scaffold inhibiting HIV integrase and interfering with postintegrational processes.\textsuperscript{33-37} Recently, 3-alkyl and 3-aryl-4(1H)-quinolones have been reported to possess excellent antimalarial activity in low nanomolar range.\textsuperscript{38, 39} Not surprisingly, several research groups including our group focused their resources in developing and optimizing 4(1H)-quinolone-based antimalarials.\textsuperscript{9, 40-42} Currently, ELQ-300 and its structurally related 4(1H)-quinolone P4Q-391 (Figure 2.2) have been shown be highly active against \textit{P. falciparum} and \textit{P. vivax} at all life cycle stages.\textsuperscript{10} Due to the antimalarial activity profile and pharmacokinetics, ELQ-300 has been selected by Medicines of Malaria Venture as a preclinical candidate. In order to determine the safety profile required to nominate the candidate entering preclinical development, large amounts of 4(1H)-quinolones ELQ-300 and P4Q-391 were needed. Herein, we report a practical and operationally simple gram scale syntheses of ELQ-300 and P4Q-391.

For supplies during the initial lead optimization stages, we devised and executed an 8-step synthetic route according to the reported procedures described in Figure 2.3.\textsuperscript{10, 40, 43} However, the utilization of expensive and harsh reagents made the method disadvantageous for large scale preparations. In addition, the reported route is operationally tedious particularly for
the purification steps. In contemplation of increasing the overall synthetic yields, decreasing the number of synthetic steps, and reducing the expenses, alternative synthetic routes were explored. In order to improve the preparation, the following issues were considered to be addressable:

1) The observed low-to-moderate yield was a result of the Ullmann couplings of compounds 2.18 and 2.19. In particular, for the synthesis of the ortho-fluoro substituted biaryl ether 2.19, which was used for the synthesis of P4Q-391, was only achieved in 39% yield. Because the Ullmann-type coupling was the first step in the synthetic sequence, it contributed profoundly for the low overall yields. An alternative to this reaction would ideally enhance the final yield and avoid the usage of stoichiometric amounts of heavy metals.

2) The isolation and purification of the intermediates 2.13, 2.14, 2.18 and 2.19 were commonly complicated requiring both flash chromatography purification and recrystallization. Introduction of an aryl substitution in C2 position of EAA prior to the Conrad-Limpach cyclization would not only simplify the purification step but also elude the use of the harsh acid deprotection step.

3) The conditions for the Conrad-Limpach cyclization were sub-optimal lowering the yields. In many instances, the cyclization of the crude enamine intermediate in high boiling solvent results in a dark crude product, which was arduous to purify. Optimization of the Conrad-Limpach cyclization step would definitely add to the overall synthetic outcome.
Main optimization efforts first focused on alternatives to the low yielding metal-catalyzed Ullmann coupling reaction. Hypervalent iodine compounds and diaryliodonium salts in particular captured our attention as mild and selective reagents during the initial exploration.\(^{24}\) One of the biggest advantages using hypervalent iodine compounds were the metal-free reaction conditions overcoming cost and toxicity issues related to the use of the organometallic reagents in the initial approach. The correspondent diaryl iodonium salt was prepared following the literature procedure as shown in the Figure 2.4.\(^{44}\) The corresponding iodobenzene 2.17 and boronic acid 2.22 were treated with anhydrous mCPBA and BF\(_3\)-OEt\(_2\) in dichloromethane provided the corresponding clean iodonium salt 2.23 in 65% yield as a white solid which required no further purification. This reaction was repeated several times at a 20 gram scale without any hurdles. Subsequently, the arylation of phenols 2.15 and 2.16 with the iodonium salt
2.23 using $t$-BuOK furnished biarylethers 2.18 and 2.19 in excellent yields after simple flash chromatography with mere hexane.\textsuperscript{18}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.4.png}
\caption{Synthesis of biarylethers via diaryliodonium salts}
\end{figure}

With the biarylether compounds in hand, the arylation of EAA was targeted (Figure 2.5). The arylation of either diethylmalonate or EAA has been reported in the literature with different halobenzenes.\textsuperscript{14, 45} The copper-mediated reactions using the ligands such as L-proline or 2-phenylphenol were also documented.\textsuperscript{14} The arylation attempts of biarylether 2.18 with EAA with L-proline and CuI yielded trace amount of product along with an unwanted ligand arylation. Surprisingly, the coupling reaction between EAA and aryl iodide 2.18 with CuI, Cs\textsubscript{2}CO\textsubscript{3} and 2-phenylphenol conditions provided the required product in 30\% yield however with the inseparable mixture of deacylated product, which was evident from NMR and MS analysis.\textsuperscript{45} The mixture of product was subjected to the traditional Conrad-Limpach cyclization conditions producing ELQ-300 with 30\% yield.\textsuperscript{28} Though the synthesis was done in four steps compared to the route described in the scheme-I, the overall yield was 5.2\%. The formation of product as inseparable mixture with deacylated product was also an added disadvantage. At the same time, the arylation reaction between EAA and the biarylether 2.19 for the synthesis of P4Q-391 was unsuccessful. Alteration of catalysts, ligands, bases and solvents under various conditions did not produce product presumably because of the ortho-fluoro substitution impact.
 Unsatisfied yields for the copper-catalyzed arylation in case of ELQ-300 intermediate and the lack of formation of product with biaryliodide 2.19 for the synthesis of P4Q-391 led to the development of an alternative strategy described in the Figure 2.6. Principally, the versatile nature of 1,3-dioxin-4-ones of generating diversified building blocks in organic synthesis has been well known and documented.\textsuperscript{46,47} They act as protected enol forms of acetyl acetic acid and thermal or photochemical ring opening with a choice of nucleophiles lead to variety of applicable products. In the present scenario, 1,3-dioxin-4-one 2.26 was first iodinated at C5 position to obtain 5-iodo-1,3-dioxin-4-one 2.27 and subsequently, the coupling of diarylether part was performed.\textsuperscript{48} In this context, boronic acid 2.21 was treated with iodo compound 2.27 under Pd-mediated conditions achieved 5-arylated 1,3-dioxin-4-one 2.31 product in 32% yield. To circumvent the low yield, boronic acid 2.21 was converted to its air stable and more reactive trifluoroborate 2.29 by treating with inexpensive KHF\textsubscript{2}.\textsuperscript{49} Then the coupling between trifluoroborate 2.29 with iodo compound 2.27 under reported conditions resulted in much better
yield of 70%. To our delight, the similar coupling reaction between 2.27 and borate salt 2.28 resulted in 91% yield and in both cases products were easily isolated with high purity. Next, thermal ring opening of aryl substituted dioxinones 2.30 and 2.31 with ethanol in xylenes

revealed aryl substituted EAAs 2.24 and 2.25 in excellent yields (~90%).

Once the clean aryl substituted EAAs were obtained, the modification of the Conrad-Limpach conditions took place. As mentioned earlier, this reaction often resulted in low yields for some substrates with isolation issues via a precipitation in high boiling diphenylether. First, EAAs 2.24 and 2.25 were refluxed with 1.5 equivalent of 4-chloro-3-methoxy aniline under acidic medium in dry benzene to produce the enamine intermediate. As a part of the optimization, aniline related baseline impurities were removed by quick chromatography

Figure 2.6: Synthesis of ELQ-300 and P4Q-391 via a Cu-mediated coupling with 1,3-dioxin-4-one

a) NIS, AcOH, 70%; b) KHF₂, MeOH, H₂O, 70 °C, 24 h, 81% with 2.21 and 70% with 2.20; c) Pd(dba)_2, 2.27, K₂CO₃, 2:1 dioxane:water, 80 °C, (75 min and 91% with 2.28) and (4 h and 70% with 2.29) d) ethanol, xylenes, 120 °C, 5 h, 90%; e) 1) 4-chloro-3-methoxy aniline, AcOH, benzene, Dean-Stark trap, reflux, 24 h; 2) toluene, 3 min, MV, 230 °C, 65% for ELQ-300 and 81% for P4Q-391
assuming that these contaminations were causing problems for the precipitation of the final 4(1H)-quinolone after the cyclization step. Reasonably clean enamine was also taken in toluene instead of diphenyl ether, and subjected to microwave heating conditions at 265 °C for three minutes. The 3-aryl-4(1H)-quinolone precipitated immediately and the reaction vials were placed in -20 °C freezer overnight to allow the crystallization to complete. Much satisfactory yields of 65% and 81% were obtained for ELQ-300 and P4Q-391 respectively. The entire sequence for the generation of both 3-diarylether-4(1H)-quinolones was checked and validated in multi-gram scale gratifyingly without major issues.

2.7 Conclusions

In conclusion, to the best of our knowledge, a metal-free arylation on EAA using diaryliodonium salts was broadly studied for the first time. Commercial availability or straightforward accessibility of iodonium salts makes this method convenient and operationally simple. The arylation with symmetrical salts with electron-rich rings delivers good yields with the exception of the reaction with 4-methoxy substituted aryliodonium salt. Furthermore, excellent results are obtained with symmetrical salts containing electron-deficient rings. Importantly, the moderate yields of arylations using ortho-substituted iodonium salts stands in stark contrast to metal-mediated synthetic approaches with similar substrates, which usually do not perform well. Finally, impressive chemoselectivities were also obtained in the case of unsymmetrical salts. Overall this method demonstrated enhanced selectivity and versatility giving straightforward access to medicinally and pharmaceutically interesting EAA derived complex molecules.

Also a first multi-gram scale synthesis for antimalarial compounds ELQ-300 and P4Q-391 has been accomplished in good overall yield. All the reactions in the sequence are
operationally simple and having high value of reproducibility. This route is enriched with high yielding reactions particularly arylation of phenol with iodonium salt, modified Conrad-Limpach conditions and clean aromatic and aliphatic Suzuki-Miyuara coupling reactions.

2.8 References Cited


27. See electronic supplementary information (ESI) for details

29. unpublished data


CHAPTER 3: STRATEGIES TO IMPROVE AQUEOUS SOLUBILITY AND BIOAVAILABILITY OF ANTIMALARIAL 3-ARYL-4(1H)-QUINOLONES

3.1 Introduction

Despite recent decrease in global morbidity and mortality caused by malaria, it remains a dangerous parasitic disease with over 200 million infection cases reported and an estimated 0.6 million of deaths in 2012. Children under age of five are accounted for almost 80% of documented mortality cases with the majority of deaths occurring in sub-Saharan Africa. Five species of the genus *Plasmodium* (*P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae*, and *P. knowlesi*) are responsible for malaria in humans and *P. falciparum* is the most dangerous one often being lethal. Importantly, the dormant liver stages that exist in *P. vivax* and *P. ovale* are usually underestimated, however those are capable of relapsing blood stage malaria at a later time without a mosquito bite. As of 2012, parasite resistance to one of the most widely used antimalarial chloroquine has been confirmed in ten countries making artemisinin combination therapies (ACTs) the last resort medicine to combat *P. vivax* malaria. Unfortunately, resistance to artemisinin has already emerged at least in four countries of South-East Asia and there is an urgent need for new antimalarial drugs to overcome documented resistance and at the same time be readily bioavailable due to economic reasons. Commonly, antimalarial drug discovery focuses on the erythrocytic stages of malaria that cause disease. Yet to eliminate malaria, compounds are required to target exoerythrocytic stages, namely the liver stages as well as the infectious stages for mosquitoes. Recent reevaluation and optimization studies of 4(1H)-
quinolones, 4(1H)-pyridones, 1,2,3,4-tetrahydroacridones, 4(1H)-quinolone esters, 2-aryl-4(1H)-quinolones, and 3-carboxyl-4(1H)-quinolones led to new probes with promising in vitro erythrocytic stage activity and improved physicochemical properties. Particularly, 3-phenyl-substituted 6-chloro-7-methoxy-4(1H)-quinolones (P4Qs) identified by Riscoe and our group have been shown to be potent in vitro against the clinically relevant isolates of P. falciparum W2 (chloroquine and pyrimethamine resistant) and TM90-C2B (chloroquine, mefloquine, pyrimethamine and atovaquone resistant) with EC50 constants in the single digit nanomolar range. Moreover, these compounds have also been tested against J774 mammalian cells demonstrating to inhibit parasite growth over mammalian cells with a selectivity of 50 or greater. Further optimization lead to 4(1H)-quinolones P4Q-391 and ELQ-300, which have been shown to not be only potent against blood stages and liver stages of the parasite, but also efficient at blocking transmission.

During the optimization, a variety of P4Qs with acceptable microsomal stability and good membrane permeability have been identified, which motivated us to test frontrunner compounds P4Q-95, P4Q-146 and P4Q-158 (Figure 3.1) first for in vivo efficacy against the blood stages in a modified Thompson test and against the liver stages using a GFP expressing P. berghei strain. As a result, compounds P4Q-158 exhibited respectable suppressive in vivo activity against the liver and the blood stages whereas P4Q-146 was slightly active and P4Q-95 appeared to be completely inactive (Table 3.1).

Both compounds P4Q-146 and P4Q-158 are slightly more in vitro active than P4Q-95, nevertheless they are either better soluble (increasing aqueous solubility by “out-of-plane” aryl
substituent due to α-methyl group in P4Q-146) or metabolically more stable (blocking of metabolically labile site by CF₃-group in P4Q-158). In addition, 3-aryl-4(1H)-quinolone leads exhibited gametocidal activity as shown by significant reduction or complete inhibition of oocyst development in mosquitoes (average number for untreated gamocyte 15.3, Table 3.1) and reduction of the average number of exflagellation per field (data not shown). Compounds were also tested against EE stages of malaria, with P4Q-158 was highly potent against liver stage parasites, while P4Q-146 was unable to effectively prevent the development of liver stage parasites in vivo.

Table 3.1: Biological and physicochemical properties of frontrunners compounds P4Q-95, P4Q-146 and P4Q-158

<table>
<thead>
<tr>
<th>Compound</th>
<th>P4Q-95</th>
<th>P4Q-146</th>
<th>P4Q-158</th>
</tr>
</thead>
<tbody>
<tr>
<td>W2 EC₅₀ (nM)</td>
<td>26.2</td>
<td>5.83</td>
<td>12.0</td>
</tr>
<tr>
<td>TM90-C2B EC₅₀ (nM)</td>
<td>15.3</td>
<td>4.03</td>
<td>6.6</td>
</tr>
<tr>
<td>RI = EC₅₀(C2B)/EC₅₀(W2)</td>
<td>0.58</td>
<td>0.69</td>
<td>0.5</td>
</tr>
<tr>
<td>Cytotoxicity J774 EC₅₀ (mM)</td>
<td>&gt;33.4</td>
<td>&gt;31.9</td>
<td>&gt;27.2</td>
</tr>
<tr>
<td>CI = EC₅₀(J774)/EC₅₀(C2B)</td>
<td>&gt;2.184</td>
<td>&gt;7.908</td>
<td>&gt;4.102</td>
</tr>
<tr>
<td>Log D₇.₄</td>
<td>2.8</td>
<td>2.5</td>
<td>2.3</td>
</tr>
<tr>
<td>Permeability Pe pH=7.4 (10⁻⁶ cm/s)</td>
<td>48.3</td>
<td>191</td>
<td>3.0</td>
</tr>
<tr>
<td>Solubility pH=7.4 (µM)</td>
<td>5.7</td>
<td>8.7</td>
<td>0.3</td>
</tr>
<tr>
<td>MW (g/mol)</td>
<td>299.7</td>
<td>313</td>
<td>367</td>
</tr>
<tr>
<td>Microsomes human T½ (min)</td>
<td>99.5</td>
<td>70.8</td>
<td>min degrad.</td>
</tr>
<tr>
<td>Microsomes mouse T½ (min)</td>
<td>45.8</td>
<td>24.0</td>
<td>min degrad.</td>
</tr>
<tr>
<td>Microsomes rat T½ (min)</td>
<td>135.3</td>
<td>59.1</td>
<td>219</td>
</tr>
<tr>
<td>Bioavailability, F%</td>
<td>&lt;2</td>
<td>&lt;2</td>
<td>~3</td>
</tr>
<tr>
<td>In vivo: parasitemia suppression on day 6PE (%)</td>
<td>not active</td>
<td>17</td>
<td>92</td>
</tr>
<tr>
<td>Transmission blocking potential (average number of oocysts)</td>
<td>0.1</td>
<td>0.0</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

In order to support our hit-to-lead optimization efforts of this important class of antimalarials, we decided to investigate in details why these frontrunner compounds displayed such significant differences in the various in vivo efficacy assays. Subsequent pharmacokinetic studies with lead quinolone compounds suggest the aqueous solubility is the major reason for poor bioavailability and moderate in vivo activity. Interestingly, a few advanced 4(1H)-quinolones of the series, ELQ-300 and structurally related analogue P4Q-391 (Figure 3.2), have been recently shown be highly active against P. falciparum and P. vivax at all life cycle stages.
ELQ-300 has been selected as a preclinical candidate in the MMV drug discovery program and currently placed in preclinical development. Despite of excellent antimalarial profile ELQ-300 also lacks in aqueous solubility.\(^\text{14}\) Moreover, the absence of dose-proportionality caused by low solubility impedes the determination of the therapeutic index and \textit{in vivo} toxicity.

Herein, we report quantum mechanics (QM) torsion profile calculations, \(^\text{13}\)C \(T_1\) spin-lattice relaxation experiments, as well as X-ray studies conducted with the objective to determine structural modifications improving P4Q's physicochemical properties such as solubility resulting in an increased bioavailability. We also describe the development of prodrug approaches to circumvent the observed bioavailability limitations. Prepared prodrugs have been tested for chemical stability and aqueous solubility at different pHs. In a \textit{P. berghei} infected mouse model, the three most promising prodrugs have subsequently been shown to be more \textit{in vivo} efficacious than their corresponding parent compounds. Finally, the increased antimalarial activity of these prodrugs was demonstrated to be derived from the significantly improved oral bioavailability. Most importantly, the herein presented prodrug approaches are easily applicable in any antimalarial 4(1\(H\))-quinolone or 4(1\(H\))-pyridone scaffolds with limited oral bioavailability.\(^\text{9,15,16}\)

\textbf{3.2 Disruption of 3-Aryl-4(1\(H\))-Quinolones’s Molecular Planarity and Symmetry}

\textbf{3.2.1 Rotational barrier calculations}

As the main structural difference between \textbf{P4Q-95} and \textbf{P4Q-146} is the methyl group in \textit{ortho}-position of the 3-phenyl ring, the rotational barriers along the C3-C12 bond of both 3-aryl-
4(1H)-quinolones were first established using quantum mechanics (QM) calculations by technique previously described (Figure 3.3).\textsuperscript{13} Relaxed dihedral angle scans (starting with 0° when both methyl group of \textbf{P4Q-146} quinolone and \textit{o}-methyl-3-phenyl are directed towards each other in the same plane) with a torsion angle increment of 15° were carried out employing a HF/6-31G** method using Jaguar application in Maestro suite (version 9.2, Schrödinger, Inc). The structures were optimized prior to the torsion scan using the same level of theory. The torsion profile was then obtained by plotting 24 energy points versus dihedral angles.

Previously, the calculated rotational barrier $\Delta E_{\text{rot}}$ of 20 kcal/mol was reported as a suitable threshold between atropisomers and non-atropisomers.\textsuperscript{17} The prediction accuracy of QM calculations is 86% over experimental values and makes it therefore a practical tool to predict atropisomerism along the drug discovery process. Based on the QM energy profile obtained for \textbf{P4Q-95}, the lowest energy barrier $\Delta E_{\text{rot}}$ that allowed the interconversion between geometrical
isomers was 11.9 kcal/mol. This result suggested that P4Q-95’s torsional rotation half-time at room temperature was in the order of milliseconds with a free rotation along C3-C12 bond completely lacking of any axial chirality. In contrast, the minimal energy barrier $\Delta E_{\text{rot}}$ of P4Q-146 has been calculated to be 23.2 kcal/mol implying that the half-time for the same bond rotation was in the range of hours. Compounds with torsion angle rotational energy barriers $\leq 30$ $\Delta E_{\text{rot}} \geq 20$ kcal/mol have the potential to form atropisomers with corresponding interconversion half-lives ($t_{1/2}$) in the order of minutes to months. According to the classification proposed by LaPlante and co-workers,\textsuperscript{13} P4Q-95 is a symmetrical compound with a rapid free rotation along C3-C12 bond and belongs to the Class 1 of the atropisomers classification. On the other hand, P4Q-146 is experiencing delayed axial interconversions and corresponds to the Class 2 of compounds, however, should be developed as a stereoisomer mixture as long as the racemization is faster relative to the in vivo elimination rates.

Leaving the atropisomer discussion aside, the significantly increased rotational energy barrier of P4Q-146 suggested also a more orthogonal orientation of the 3-aryl substituent relative to the 4(1H)-quinolone plane. QM calculations evoked that in comparison to P4Q-95, the out-of-plane 3-aryl ring of P4Q-146 likely alters the molecular crystal packing increasing the aqueous solubility. In fact, the aqueous solubility determined by an HPLC protocol in our group, revealed a 1.5-fold increase in solubility for 2-methylphenyl substituted 4(1H)-quinolone P4Q-146 over P4Q-95 (solubility P4Q-95 $\sim 5.7$ $\mu$M; P4Q-146 $\sim 8.7$ $\mu$M). Introduction of the ortho-substituent into the 3-phenyl ring of the 4(1H)-quinolone is indeed changing the overall energy profile of the molecule and could be used as one of the medicinal chemistry’s tools to enhance bioavailability.
3.2.2 Spin-lattice relaxation experiment

To further substantiate our out-of-plane findings, NMR-based experiments measuring the relaxation times were conducted. $^{13}$C spin-lattice relaxation $T_1$ values are excellent parameters for monitoring relatively slower segmental motion or flexibility of C-H vectors.\(^1\) We used the inversion recovery technique using inverse-gated proton decoupling to inspect the rotation of the C3-C12 bond by determining all $^{13}$C $T_1$ values of the compounds P4Q-95 and P4Q-146 (see the Supporting Information for details). The moiety motion of 3-aryl-4(1H)-quinolone compounds were compared using the determined $T_1$ values (Figure 3.4). Shorter times are characteristic of a relatively slower fragment motion and decreased flexibility.\(^1\) Since the $T_1$ values of quinolone P4Q-95 were ~0.2 seconds shorter than the ones of P4Q-146 (the average values are 0.5 seconds versus 0.7 seconds), it can be concluded that the 3-aryl ring of P4Q-146 is more rigid. These results support our QM energy calculations indicating that the C3-C12 bond rotation of P4Q-146 is slower.

3.2.3 X-ray studies

Finally, X-ray studies were conducted to confirm different crystal packings depending on whether a P4Q possesses atropisomeric character or not (Figure 3.5). Based on the fact of elevated melting points of P4Q class (m.p. > 230 °C), the poor solubility could be attributed to the high crystallinity of the molecule.\(^1\) Although the hydrogen bonding distances between the quinolone’s oxygens and nitrogens are similar for P4Q-95 (2.800 Å) and P4Q-146 (2.687 and
2.808 Å), linear molecule chains have been observed in the crystal of P4Q-146, whereas for P4Q-95 a zigzag-like packing was determined. Moreover, for P4Q-95, the density was 6.7% higher than the one of P4Q-146 suggesting that the additional methyl group of P4Q-146 prevents an efficient packing and hence disrupt the intermolecular interactions led to the high crystallinity. It has been shown before, the disruption of molecular planarity is effective method of increasing aqueous solubility to up to 350-fold improvement.\textsuperscript{20} In order to free a molecule from its crystal in case of high packing, an elevated level of free energy is required. The difference in energy must be indemnified by the release of solvation energy during the solvation step. Basically, performed X-rays studies are in accordance with the QM calculations and the spin-lattice NMR experiment confirming the disturbance of molecular crystal packing for the 3-phenyl-4(1H)-quinolone chemotype with the installation of the ortho-substitution.

3.3 Prodrug Approaches

3.3.1 Introduction and synthesis

There are no big surprises of 3-aryl-4(1H)-quinolones being poorly soluble as more than 30% of all drug discovery compounds have been reported with a reduced solubility of 10 µM
and lower. Decreased aqueous solubility has been identified as one of the most critical parameters limiting the therapeutic use of a variety of drug candidates. The formulation techniques such as the use of solubilizing agents, salt formation and particle size modification are commonly utilized to overcome solubility barriers, however often these cannot provide sufficient improvements. It has been also shown by other groups that an enhanced water solubility and an improved oral bioavailability may be achieved by impinging the crystal packing or by decreasing the melting point of the lead compound. The development of prodrugs – deliberately modified forms of the active ingredient that can undergo an enzymatic and/or chemical transformation in vivo – is an alternative and well-documented approach to increase aqueous solubility and bioavailability. Interestingly, approximately 10% of world marketed drugs are classified as prodrugs with about a third of new molecular entities (NME) approvals folded into this category in 2008. Prodrugs typically developed via chemical modification of activated functional groups into esters, ethers, carbonated, carbamates, oximes, amides and phosphates.

In the case of 4(1H)-quinolones, the keto form is favored over the enol form in both solid and solution states. It was shown by our group, that methylation of P4Qs occur almost equally at both N1 and O4 site of quinolone. However, the alkylation using alkyl halides with two and more carbons yielded the O-substituted products exclusively. These results enabled us to use the
hydroxyl group of the 4-quinolinol as a development point for a prodrug approach (Figure 3.6). We first decided to derivatize the 4-hydroxy group of the P4Qs via a simple esterification/acylation with the assumption of altering the intermolecular hydrogen bonds of 4(1H)-quinolones disrupting the high crystallinity of the compounds. The synthesis of ester, carbamate and carbonate 4(1H)-quinolones was straightforward using cesium carbonate as a base in DMF yielding the desired products in moderate to good yields (Figure 3.7A). 3-Aryl-4(1H)-quinolones were prepared as previously described via a Conrad-Limpach cyclization of 2-aryl substituted ethyl acetoacetate with 3-chloro-4-methoxy aniline.

**Figure 3.7:** Synthesis of 4(1H)-quinolones and prodrugs via O-alkylation/acylation
3.3.2 Prodrug’s antimalarial activities and structure-property relationship (SPR) studies

The synthesized compounds were tested against the clinically relevant multi-drug resistant malarial strains W2 (chloroquine and pyrimethamine resistant) and TM90-C2B (chloroquine, mefloquine, pyrimethamine and atovaquone resistant). The human malaria parasite *P. falciparum* was grown *in vitro* in dilute human erythrocytes in RPMI 1640 media containing 10% heat inactivated plasma and the potency for each 4(1H)-quinolone prodrug has been calculated against the individual strains as the 50% effective concentration (EC$_{50}$).

In parallel to the *in vitro* antimalarial activity testing, standard physicochemical properties were determined to identify potential compound liabilities. All compounds were assessed for aqueous solubility and partition coefficient LogD using HPLC-based protocols, which were previously described. Passive transcellular permeability was determined using the standard parallel artificial membrane permeability assay (PAMPA).

The summary of antimalarial *in vitro* activities and physicochemical properties are shown in Table 3.2. Generally, introduction of ester (P4Q-464) and carbonate (P4Q-463) groups into O4 position of P4Q-146 has not affected the biological activities but at the same time increased the lipophilicity of the molecule. More importantly, implemented promoieties did not improve the aqueous solubility which was the primary objective of our prodrug approach. Installation of a carbamate into 4(1H)-quinolone (P4Q-413 and P4Q-465) did not affect the solubility either, while permeability was increased by more than 10 times. Surprisingly, carbamates derivatives were also completely inactive against *P. falciparum* using *in vitro* cellular assay.
### Table 3.2: *In vitro* antimalarial activities and SPR data for *P4Q-146* prodrugs

<table>
<thead>
<tr>
<th>Name</th>
<th>MW</th>
<th>EC&lt;sub&gt;50&lt;/sub&gt; [nM]</th>
<th>Solubility [µM]</th>
<th>PAMPA Pe 10⁻⁶</th>
<th>LogD</th>
</tr>
</thead>
<tbody>
<tr>
<td>P4Q-146</td>
<td>313.8</td>
<td>5.8</td>
<td>8.7</td>
<td>191</td>
<td>2.5</td>
</tr>
<tr>
<td>P4Q-464</td>
<td>369.8</td>
<td>3.3</td>
<td>2.9</td>
<td>143</td>
<td>4.9</td>
</tr>
<tr>
<td>P4Q-463</td>
<td>385.8</td>
<td>2.4</td>
<td>0.5</td>
<td>504</td>
<td>4.2</td>
</tr>
<tr>
<td>P4Q-413</td>
<td>412.9</td>
<td>&gt;6054</td>
<td>0.03</td>
<td>1237</td>
<td>2.6</td>
</tr>
<tr>
<td>P4Q-465</td>
<td>410.9</td>
<td>2185</td>
<td>2.9</td>
<td>1555</td>
<td>2.8</td>
</tr>
<tr>
<td>P4Q-366</td>
<td>401.8</td>
<td>8.8</td>
<td>0.1</td>
<td>46</td>
<td>4.6</td>
</tr>
<tr>
<td>P4Q-351</td>
<td>429.9</td>
<td>7.3</td>
<td>1.6</td>
<td>100</td>
<td>5.3</td>
</tr>
<tr>
<td>P4Q-369</td>
<td>445.9</td>
<td>16.3</td>
<td>2.9</td>
<td>430</td>
<td>2.5</td>
</tr>
<tr>
<td>P4Q-414</td>
<td>495.4</td>
<td>19.4</td>
<td>35</td>
<td>194</td>
<td>3.2</td>
</tr>
<tr>
<td>P4Q-427</td>
<td>494.4</td>
<td>not stable</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>P4Q-428</td>
<td>508.4</td>
<td>not stable</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

3.3.3 *pH-Triggered prodrugs*

In the classical prodrug approach, covalent linkage between the parent drug and prodrug moiety is cleaved through an enzymatic hydrolysis. For example, almost half of all marketed prodrugs are activated this way with the majority been esters. Various esterases found in the blood, liver and other tissues are responsible for the cleavage of the ester bond. Significant
differences in specific animal enzyme activities, however, challenge the accurate prediction of parent compound release in vivo. The phosphate is the only prodrug that typically hydrolyzed at similar rates in different species by alkaline phosphatase. On the other side, despite the satisfactory chemical stability, phosphates could lead to the precipitation of parent compound in the intestinal lumen.\textsuperscript{22}

An alternative approach that can regenerate the parent compound via chemical methods has been identified as a superior way to avoid inter- and intraspecies variability involved with enzymatic activities. For example, regeneration of the active drug can be accomplished via a pH

![Figure 3.8: pH-Activated prodrugs](image1)

![Figure 3.9: Aqueous stability of P4Q-414 at different pHs](image2)
mediated process of cyclization-activated prodrugs.\textsuperscript{24} Ideally, such a prodrug moiety would consist of a latent nucleophile which is not activated at a pH range 1-5 (GIT pH) and undergo a controlled intramolecular cyclization releasing the parent drug once the pH is elevated (Figure 3.8).

To avoid an animal-patient dependence associated with an enzymatic cleavage and the same time develop a prodrug which would be chemically stable at low pH and soluble in water, we designed carbonate prodrugs \textbf{P4Q-369}, \textbf{P4Q-414} and carbamates \textbf{P4Q-427} and \textbf{P4Q-428}. In theory, the nucleophilicity of the amine nitrogen is controlled by pH level of a solution and been fully protonated at acidic pHs. The same nitrogen would attack the electrophilic carbon on carbonate (carbamate) when the pH level increased, forming either a 5- (\textbf{P4Q-369}, \textbf{P4Q-427}) or 6- member (\textbf{P4Q-414}, \textbf{P4Q-428}) ring. The reaction is controlled by both acid-base equilibrium and the rates of new ring formation.

The synthesis of Boc-protected alkylxycarbonyloxymethyl (AOCOM) halides was accomplished as reported before in good yields (Scheme 1B).\textsuperscript{25} Corresponding alkyl halide was reacted with P4Q compound in presence of base to give AOCOM phenolic prodrug which upon deprotection yielded water-soluble HCl salts \textbf{P4Q-369}, \textbf{P4Q-414}, \textbf{P4Q-427} and \textbf{P4Q-428}. High decomposition rates to parent \textbf{P4Q-146} of carbamates \textbf{P4Q-427} and \textbf{P4Q-428} at low pHs was noticed upon deprotection of AOCOM prodrug and this pair was abandoned from further development. In contrast, carbonate AOCOM analogues (\textbf{P4Q-369}, \textbf{P4Q-414}) showed excellent aqueous stability-release profiles (Figure 3.9). The stability was assessed in buffers with different pHs (2, 5.5, 6.5 and 7.4) and simulated gastric fluid (SGF) and quantified by LCMS. According to the profile received, \textbf{P4Q-414} is stable at the pH level 2 - 5.5 and start decomposing at pH ~ 6.5 (35 \% decomposition in 2 hours). Fast release (100\% in 1 hour) of parent compound \textbf{P4Q-}}
146 is occurring when pH is raised to 7.4. Generally, the stability of P4Q-414 at physiological stomach pHs and its rapid release at pH > 7 is optimal for development of cyclization-activated prodrugs.

3.3.4 In vivo efficacy of selected prodrugs P4Q-146 and P4Q-158

Three 4(1H)-quinolones prodrugs of each P4Q-146 and P4Q-158 were selected to undergo screening for in vivo efficacy based on improved aqueous solubility. P4Q-158 was chosen as the parent compound possessed enhanced microsomal stability resulting from the para-substituted CF<sub>3</sub> group. Compound P4Q-146 seemed to be a good candidate derived from the out-of-plane substitution effect. The screen involved treating mice once per day with 10 mg/kg or 50 mg/kg of test compound delivered in 1% HEC on days 3 through 5 of post-exposure and then assessing parasitemia on days 6 post-exposure. Compounds with greater than 50% inhibition of parasitemia on that day were considered to be active.

Table 3.3: In vivo efficacy of P4Q-158 and P4Q-146 prodrugs

<table>
<thead>
<tr>
<th>Compound (Route)</th>
<th>10 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Suppression Day 6PE</td>
<td>Day of death (avg)</td>
</tr>
<tr>
<td>P4Q-146 (po)</td>
<td>29</td>
<td>13</td>
</tr>
<tr>
<td>P4Q-369 (po)</td>
<td>71</td>
<td>16</td>
</tr>
<tr>
<td>P4Q-414 (po)</td>
<td>52</td>
<td>13</td>
</tr>
<tr>
<td>P4Q-594 (po)</td>
<td>39</td>
<td>15</td>
</tr>
<tr>
<td>P4Q-158 (po)</td>
<td>11</td>
<td>14</td>
</tr>
<tr>
<td>P4Q-621 (po)</td>
<td>75</td>
<td>13</td>
</tr>
<tr>
<td>P4Q-435 (po)</td>
<td>82</td>
<td>13</td>
</tr>
<tr>
<td>P4Q-595 (po)</td>
<td>39</td>
<td>16</td>
</tr>
</tbody>
</table>

Suppression activity of six prodrugs and parent compounds P4Q-146 and P4Q-158 is summarized in Table 3.3. pH-triggered AOCOM prodrugs of P4Q-158 with both two and three methylene bridge were the most active compounds displaying over 95% inhibition at day 6 post-
infection at higher dose. Reduced in vivo efficacy averaging a 75% inhibition and 82% inhibition day 6 post-exposure was observed for P4Q-158’s prodrugs at lower dose 10 mg/kg. At the same time, compounds P4Q-414 and P4Q-369 derived from early lead P4Q-146 were not that active, however still possessed suppressive activities. Surprisingly, phosphate prodrugs of both P4Q-158 and P4Q-146 were not better than parents’ compounds suggesting delivery issues.

3.3.5 In vivo pharmacokinetics

In vivo PK experiments were performed in order to assess the systemic exposure in mice following oral administration of P4Q-158. Mouse plasma was collected via cardiac puncture at different time-points (0.5 hr, 1 hr, 2 hr, 4 hr, 24 hr) post-treatment with experimental compound
at a dose of 10 mg/kg and analyzed by LCMS (QqQ Agilent 7830) (Figure 3.10). **P4Q-158** was slowly absorbed after oral administration, with maximum plasma concentrations being observed 120 min post-dose, and concentrations remained above the lower limit of quantification (LLQ) up to ~24 h post-dose. Nevertheless, the maximum plasma concentration (C\text{max}) of **P4Q-158** has never passed 365 nM. Introducing of pH-sensitive moieties in prodrug **P4Q-621** and **P4Q-435** significantly improved maximum plasma concentration (12 µM and 21µM accordingly) and more importantly increased the AUC value in 35 and 50 folds respectively. The PK profile for phosphate prodrug revealed a maximum plasma concentration of ~1 µM with an AUC value even lower than the parent **P4Q-158**. The poor PK performance of the phosphate is in accordance with *in vivo* efficacy data and could be explained by stability issues.

### Table 3.4: PK parameters of **P4Q-158** and **P4Q-158** prodrugs

<table>
<thead>
<tr>
<th></th>
<th><strong>P4Q-158</strong></th>
<th><strong>P4Q-621</strong></th>
<th><strong>P4Q-435</strong></th>
<th><strong>P4Q-595</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>MW</td>
<td>367.75</td>
<td>535.34</td>
<td>549.37</td>
<td>521.72</td>
</tr>
<tr>
<td>Dose [mg/kg]</td>
<td>10.00</td>
<td>10.00</td>
<td>10.00</td>
<td>10.00</td>
</tr>
<tr>
<td>C\text{max} [µmol/L]</td>
<td>0.36</td>
<td>11.99</td>
<td>21.21</td>
<td>1.11</td>
</tr>
<tr>
<td>T\text{max} [min]</td>
<td>120</td>
<td>240</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>AUC (0\rightarrow\infty) [min*µmol/L]</td>
<td>223</td>
<td>8033</td>
<td>11665</td>
<td>121</td>
</tr>
<tr>
<td>Vd [L/kg]</td>
<td>75.53</td>
<td>1.56</td>
<td>0.86</td>
<td>17.27</td>
</tr>
<tr>
<td>Cl [mL/min/kg]</td>
<td>121.94</td>
<td>2.33</td>
<td>1.56</td>
<td>158.41</td>
</tr>
<tr>
<td>t\text{1/2} apparent [hrs]</td>
<td>7.15</td>
<td>7.74</td>
<td>6.35</td>
<td>1.26</td>
</tr>
</tbody>
</table>

Notably, all four compounds are cleared from the system in less than 24 hours which could be accounted by the accelerated metabolism of parent 4(1H)-quinolone. In order to be curative, however, antimalarial compound must stay in the blood for at least 36 hours.
3.4 Conclusions

The potent blood stage activity and demonstrated potential to kill hypnozoites in the gold standard *P. cynomolgi* infected Rhesus model make the P4Qs attractive chemotypes for the development of novel drugs to treat multidrug resistant malaria, to eradicate EE stages, to block transmission, and to aid the malaria elimination campaign. At the same time, the major liability of P4Qs class of compounds is low aqueous solubility and as a result unacceptable bioavailability. Both disrupting of crystal packing and prodrug approaches were employed to overcome solubility hurdles. QM rotational barrier calculations and NMR-based experiments confirmed rotational energy increase with implementation of methyl group into ortho- position of 3-phenyl ring. X-ray analysis of **P4Q-95** and **P4Q-146** also revealed that the dihedral angle for **P4Q-95** is approaching 180°, while the ortho-methyl-phenyl group in **P4Q-146** is completely orthogonal ($\Phi = 90^\circ$) to the quinolone plane. These results confirm that structural modifications altering the crystal packing improving the aqueous solubility. As an alternative, we have developed a prodrug approach, in which the prodrug is highly soluble and a parent compound is released by a pH-triggered mechanism. A selected set of prodrugs has been shown to possess improved *in vivo* antimalarial efficacy in a *P. berghei* infected mouse. The efficacy data was confirmed by pharmacokinetic profiling of pH-trigger prodrugs *in vivo*.

3.5 References Cited


SUMMARY

Among the priorities in antimalarial drug research is the development of a drug candidate, which has potential to be used for single exposure radical cures. The majority of antimalarial chemotypes being currently in development target the erythrocytic stages of the disease but lack potent activity against the liver stages and the mosquito stages of malaria. This challenge is aggravated by the lack of rapid and well-validated assays to assess liver stage activity as well as transmission blocking activity. Yet, to eliminate and completely eradicate malaria, antimalarial compounds are required to target these three stages of the parasite lifecycle. Moreover, new antimalarial drugs have to be readily orally bioavailable and at the same time have to overcome documented resistance. Currently, there are about a dozen of new antimalarial candidates in the early clinical phases of drug development. However, of these few promising compounds, only a few of these are potent at targeting multiple stages of the malaria parasite. One of these promising candidates is ELQ-300, which is structurally related to the 4(1H)-quinolone class of compounds.

Recent reevaluation and optimization studies of 4(1H)-quinolones lead to new probes with potent in vitro erythrocytic stage activity and improved physicochemical properties. Particularly, 3-phenyl-substituted 6-chloro-7-melthoxy-4(1H)-quinolones (P4Qs) have been identified by Manetsch and Kyle to be in vitro active against the clinically relevant isolates of P. falciparum including atovaquone resistant strain with EC50 constants in the single digit nanomolar range. These compounds have also been tested against J774 mammalian cells.
inhibiting parasite growth over mammalian cells with a spectacular selectivity factor of 50 or greater. More importantly, radical cures were achieved \textit{in vivo} while evaluating efficacy with selected 3-aryl-4(1H)-quinolones at doses as low as 3 mg/kg and 1 mg/kg. Additional optimization led to 4(1H)-quinolones \textbf{P4Q-391} and \textbf{ELQ-300}, which have been shown to be equally potent in all three stages of malaria lifecycle.

Nomination of a preclinical candidate requires its availability in large amounts in order to properly determine the safety profile. We have developed a practical and operationally simple gram-scale syntheses of both \textbf{ELQ-300} and \textbf{P4Q-391}, which was used for the synthesis of multiple grams of selected 4(1H)-quinolones. In parallel, a clean arylation protocol of ethyl acetoacetate was developed, which uses hypervalent diaryl iodonium salts under mild and metal-free conditions. The scope of this reaction using symmetric and unsymmetric iodonium salts varying in steric and electronics was examined. Furthermore, this synthetic approach has been applied for the synthesis of the preclinical candidate \textbf{ELQ-300}.

Notwithstanding, low aqueous solubility and unacceptable pharmacokinetic and bioavailability profiles are considered to be the major drawbacks of the P4Q class of compounds. The bioavailability is a function of the fraction absorbed and the clearance of the compound. At the same time, absorption is largely impacted by physicochemical properties such as permeability, solubility and dissolution rate. Poor solubility is not only limiting the absorption of the compound to the blood but also causing the absence of dose-proportionality required for the determination of the therapeutic index and \textit{in vivo} toxicity. Noteworthy, the deficient solubility of 3-aryl-4(1H)-quinolones is common for experimental candidates as about 40% of all drug candidates produced from high-throughput screenings are poorly soluble. A variety of techniques such as salt formation, particle size reduction and introduction of solubilizing agents have been
used to overcome these barriers. However, such an increase in solubility is rather temporary, causing the solute to reverse to its crystal form, which is thermodynamically more stable. The strategies of introducing hydrogen bond donating or accepting groups into the molecule are commonly used to improve the aqueous solubility. The disruption of molecular planarity or molecular symmetry is alternatives yet underutilized strategies to enhance solubility. Moreover it is more universal and could be more effective than a decrease in lipophilicity. For the selected P4Q frontrunners P4Q-146 and P4Q-158, quantum mechanics torsion profile calculations, $^{13}$C T$_1$ spin-lattice relaxation experiments as well as X-ray studies were conducted with the objective to determine possible effects improving key physicochemical properties such as solubility and stability. The data strongly suggests that P4Qs with atropisomeric character possess improved in vivo antimalarial efficacy in a P. berghei infected mouse model.

Solubility hurdles were also conquered with a prodrug strategy especially when such a limitation is caused by compounds in which first-pass metabolism is not the main route of the systemic drug delivery. At the same time, preference is given to prodrugs that can regenerate the parent compound via chemical methods rather than the ones depending on enzymatic reactions. We have implemented and examined a prodrug approach, in which the prodrug is highly soluble and stable at low pH, but decomposes to its parent compound by elevating the pH of the media. A selected set of pH-triggered prodrugs of 3-aryl-4(1H)-quinolones has been shown to possess improved in vivo antimalarial efficacy in a P. berghei infected mouse model. More importantly, the pH-regulated strategy of releasing the parent compound is easily applicable to the other classes of 4(1H)-quinolone compounds. For instance, several research groups including ours have focused their resources in developing and 4(1H)-quinolone antimalarials such as 4(1H)-pyridones, 1,2,3,4-tetrahydroacridones, 4(1H)-quinolone esters, 2-aryl-4(1H)-quinolones, and 3-
carboxyl-4(1H)-quinolones which all being limited by the poor solubility. We believe that our work in the area of alternative routes of enhancing solubility will provide insights and a flexible strategy to overcome this physicochemical barrier for all 4(1H)-quinolone-based antimalarial drug candidates.