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Chapter

A Comprehensive Review of 4(1*H*)-Quinolones and 4(1*H*)-Pyridones for the Development of an Effective Antimalarial

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Abstract

Malaria is a global public health issue. Despite the efforts in malaria prevention, nearly half the world's population is at risk of infection. Until present-day, researchers are struggling to design and discover an efficacious antimalarial. In comparison to most common antimalarial chemotypes that eliminate erythrocytic stages of *P. falciparum*, 4(1*H*)-quinolones and 4(1*H*)-pyridones exhibit antimalarial activity against multiple stages of the parasite. They have potential to treat blood stages of multidrug resistant *P. falciparum* malaria, eradicate dormant exoerythro stages of relapsing malaria species (*P. vivax*), and prevent transmission of infectious gametocytes to mosquitoes. However, thus far, the advancement of these chemotypes towards pre-clinical and clinical development has been impeded due to poor physicochemical properties, poor oral bioavailability, and poor dose-proportionality limiting preclinical safety and toxicity studies. Despite all these challenges, 4(1*H*)-quinolones and 4(1*H*)-pyridones continue to be at the forefront for the development of the next-generation antimalarials as they would have tremendous global public health impact and could significantly enhance current malaria elimination efforts.

Keywords: 4(1*H*)-quinolones, 4(1*H*)-pyridones, malaria, resistance, plasmodium, antimalarials

1. Introduction

1.1 Malaria

Malaria is a global, mosquito-borne, parasitic disease that is serious and fatal, putting people of 87 countries at risk. The population with the highest risk of infection are young children under the age of five and pregnant women living in the sub-Saharan Africa. In 2020, the World Health Organization (WHO) reported an estimate of 229 million malaria cases, with approximately 409,000 deaths in 2019 alone [1]. This is a significant decrease from that of ten years ago, where the global number of malaria cases and deaths were 243 million and 863,000, respectively. The increased efforts in malaria prevention had led to these decreases in cases [2].

Malaria is a disease caused by a protozoan parasite of the genus *Plasmodium*. These species are *P. falciparum*, *P. vivax*, *P. knowlesi*, *P. malariae*, and *P. ovale*, of which *P. falciparum* is the most common species of transmission [3, 4].

To develop efficacious antimalarial drugs, it is important to understand the *Plasmodium* lifecycle via its route of infection. Initially, the disease is transmitted into the host via a pregnant, female *Anopheles* mosquito when it takes a bloodmeal to feed her eggs by simultaneously injecting sporozoites and an anticoagulant to prevent blood clotting. Those that penetrate the blood vessels enter the bloodstream and head to the hepatocytes. The parasites entry point to the hepatocytes requires the penetration of the liver sinusoidal barrier, consisting of sentinel Kupffer cells [4].

Once *Plasmodium* sporozoites invade the hepatocytes, they do not mature immediately within the first invaded hepatocyte. They migrate through several hepatocytes, causing necrosis. When the parasite settles, these develop into the liver schizont form. Liver schizont is a multinucleate state of the cell during asexual reproduction called schizogony. One infected liver cell can develop into thousands of merozoites. These merozoites are released into the bloodstream when the hepatocytes bursts. Once these merozoites are released into the bloodstream, they will invade the erythrocytes through specific ligand-receptor interactions mediated by the proteins on the surfaces of the parasite and the erythrocyte [3, 5, 6].

Once inside the erythrocytes, the parasites can hide from the hosts' immune response. These merozoites begins to enlarge and become a uninucleate cell termed trophozoite. The nucleus of the trophozoites divides asexually to produce a schizont. The schizont then divides and produces merozoites. These merozoites can invade other erythrocytes and continue replicating. The clinical symptoms of malaria appear when these erythrocytes rupture and releases merozoites [3].

After many rounds of schizogony in the erythrocyte, some merozoites, rather than replicating, enter a sexual phase, where they develop into male and female gametocytes. Erythrocytes containing gametocytes do not rupture. Gametocytes are incapable of forming gametes within their hosts and form only when they are taken up by a mosquito. The importance of sexual differentiation is that it is responsible for the transmission from host to the *Anopheles* mosquito. The male and female gametocytes fuse within the mosquito, which forms a diploid zygote that becomes an ookinete. These ookinetes migrate to the midgut of the mosquito, pass through the gut wall, and form oocysts. The meiotic division of the oocysts occur and form sporozoites, which migrate to the salivary glands of the mosquito. This mosquito then injects these sporozoites to the next host, completing the transmission cycle [3].

Opposed to the common *P. falciparum* infection, *P. vivax* can infect the liver cells and remain dormant for as long as several years by remaining in the hepatocytes as hypnozoites, rather than developing into liver schizonts. This is the cause of malaria relapse (**Figure 1**) [7].

Unfortunately, this infection follows a vicious, never-ending cycle between human and mosquito, if a cure is not discovered for all forms of the parasite. Hence, the life cycle dictates design consideration from the onset of the discovery, optimization, and development of a new antimalarial agent.

1.2 Past and present antimalarial drugs

Decline in malaria cases are being observed due to the increased efforts in preventing, controlling, and treating malaria [8]. Still, chemotherapy is the most common method of prevention and treatment utilized for this infection. Of course, given the complex nature of the parasite, these drugs act differently towards
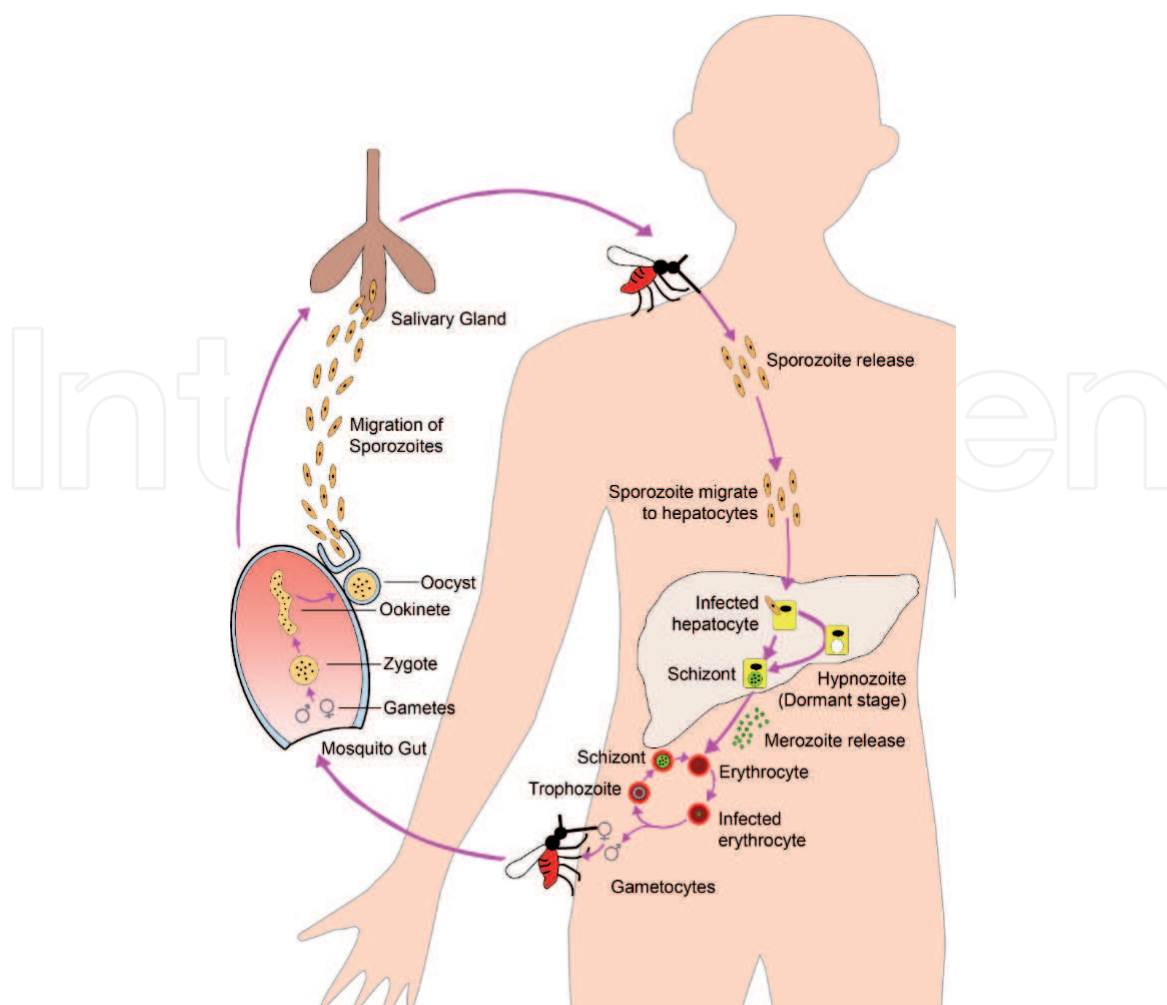
Figure 1.
*Schematic representation of the malaria life cycle.*

different stages of the parasite. For this reason, antimalarials are categorized by their activity – blood schizonticidal, tissue schizonticidal, gametocytocidal, and sporontocidal drugs. Blood schizonticidal drugs are antimalarials that target the asexual erythrocytic stages of the parasite. Tissue schizonticidal drugs are antimalarials that target hypnozoites. Gametocytocidal drug are antimalarials that targets the sexual erythrocytic forms of the parasite in the blood. Lastly, sporontocidal drugs are antimalarials that prevent formation of malarial oocytes and sporozoites in infected mosquito (**Table 1**) [9, 10].

Quinine (**1**), the first medicine to treat malaria, is an alkaloid isolated from the bark of *Cinchona* trees, which targets the asexual and sexual blood stage (**Figure 2**). This initial discovery in the 17th century of a natural product with antimalarial activity was revolutionary, as it was the first successful use of a chemical compound to treat an infectious disease. Unfortunately, due to its toxic nature and its rise of resistant *P. falciparum* strain, **1** was abandoned [11, 12]. However, its structure became the inspiration for the development of current antimalarials [13].

Currently, the majority of drugs target the asexual blood stages of the parasite. The most utilized drugs are chloroquine (**2**), artemisinin (**3**), and mefloquine (**4**) (**Figure 2**). Mefloquine (**4**), discovered during World War II, is a highly effective against blood stages of all *Plasmodium spp.* that affects humans [14, 15]. Chloroquine (**2**), initially discovered during the 1930s, was deemed toxic. However, with re-evaluation in the 1940s, chloroquine (**2**) became the standard medication for the treatment of malaria. Given its many advantages, such as excellent bioavailability, low cost, low toxicity, and effectiveness, chloroquine (**2**) was predominately

	Classification via Activity			
	Blood Schizonticidal	Tissue Schizonticidal	Gametocytocidal	Sporontocidal
Chloroquine	(+)	(−)	(−)	(−)
Quinine	(+)	(−)	(+)	(−)
Primaquine	(−)	(+)	(+)	(+)
Artemisinin	(+)	(−)	(+)	(−)
Atovaquone	(+)	(+)	(−)	(−)
Mefloquine	(+)	(−)	(−)	(−)

Table 1.
Antimalarials targeting different forms of parasite.
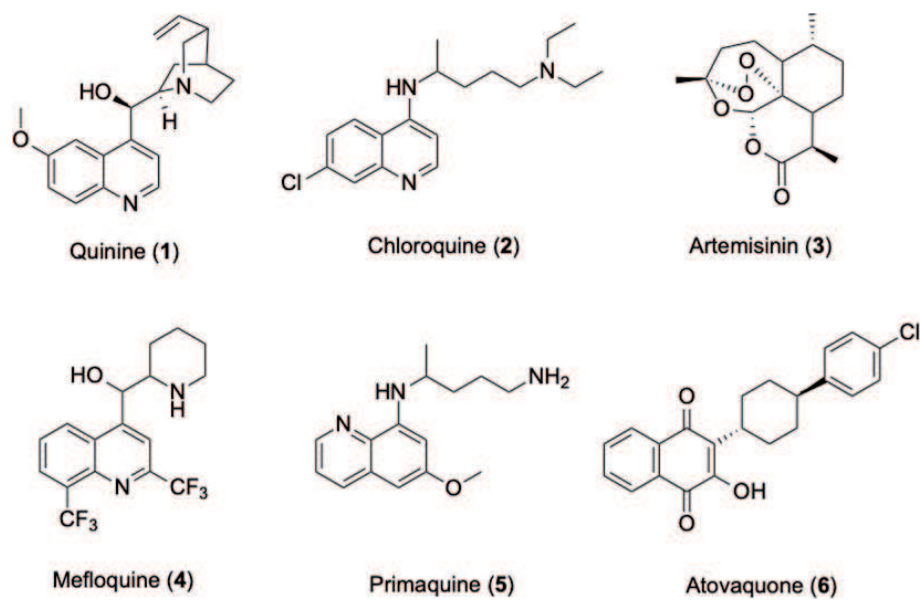
Figure 2.
Past and present antimalarial drugs.

used for at least two decades [15, 16]. Artemisinin (**3**), discovered in 1972, is a natural product isolated from *Artemisia annua* (sweet wormwood) and has been used as an antimalarial drug in China [15]. However, our supply of varying antimalarial is very limited, where we do not have many that target beyond the blood stage. Primaquine (**5**) and atovaquone (**6**) are the commonly utilized drug against liver stage parasites (**Figure 2**). Primaquine (**5**) has restricted use because it poses a major health concern, as it causes haemolysis for those with glucose-6-phosphate dehydrogenase (G6PD) deficiency. G6PD deficiency is an X-linked abnormality that is very common in tropical areas, where malaria is most prevalent [17].

Not only is the inadequate number of antimalarials that are active against the different life stages of parasite an issue, the development of resistance towards currently available drugs is a major concern. Resistance towards current antimalarial drugs are usually a result of a point mutation that can decrease drug accumulation through altered influx/efflux mechanism or change the affinity of the drug to its' validated targets [18, 19]. Actions have been taken by the WHO to act against resistance. Originally, artemisinin (**3**) was used as a monotherapy; however, given the relatively high recrudescence rate of approximately ten percent and the need for a seven-day course, this drug is now recommended by the WHO to be used in combination with another antimalarial. This is known as artemisinin combination

therapy (ACT). With the ACT, the treatment length is three days, which prevents the emergence of resistant strains [20, 21]. ACTs are a combination of drugs that have separate mechanism of action against the same stage of the parasite. In other words, ACTs would combine fast-acting artemisinin derivative antimalarial with a slow-acting, structurally different antimalarial. This enables the fast-acting anti-malarial to quickly reduce the parasite burden as the slow-acting antimalarial will completely eliminate the remaining parasite population [22].

Despite these efforts, increased resistance from mutant strains of *Plasmodium spp.* and inadequate number of antimalarials have urged the need of novel antimalarials that are active against multi-drug resistant strains for different forms of the parasite.

1.3 Recommended candidate profiles for the next generation of antimalarials

We are currently facing issues with current antimalarials due to resistant strains and lack of antimalarials to combat this problem. To develop the next generation of antimalarials, a description of the desired drug profile has been summarized in a Target Product Profile (TPP). TPPs are divided into different target candidate profiles (TCPs) since malaria chemotherapies will exist as combination therapy, containing more than one active ingredient. The main considerations across all TCPs are that these drugs are safe, affordable, and efficacious against multi-drug resistant *Plasmodium spp*. [23, 24].

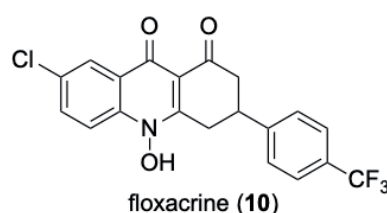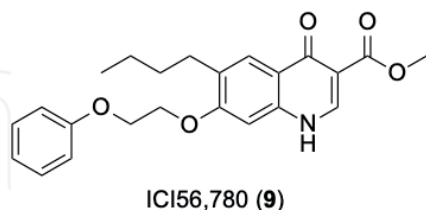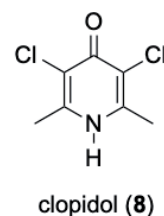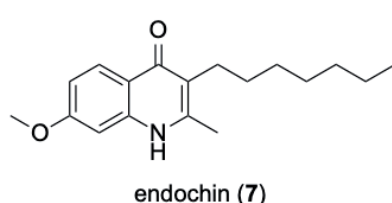## 2. Development of 4(1H)-quinolones and 4(1H)-pyridones

Abiding to the TCPs to develop a novel class of antimalarials is the current measure taken to assist in the eradication of malaria. 4(1H)-quinolones and 4(1H)-pyridones are a promising class of antimalarials, exhibiting potent activity [25, 26]. Structurally, 4(1H)-quinolones and 4(1H)-pyridones are synthetic compounds that contain the common ring, making them distinctive (**Figure 3**).

Previously discovered 4(1H)-quinolones and 4(1H)-pyridones with antimalarial activity in either avian, rodent, or primate were quickly abandoned without adequate evaluation. Recently, various research groups re-evaluated these older antimalarial 4(1H)-quinolones, 4(1H)-pyridones, and its' derivatives – endochin (**7**), clopidol (**8**), ICI56,780 (**9**), and floxacrine (**10**) – which shed light to the possibility that they can be viable leads if improvements to their physicochemical properties are successfully accomplished (**Figure 4**).

To improve physicochemical properties, it is necessary to scrutinize the properties, such as molecular weight, polar surface area, rotatable bonds, hydrogen bond acceptors and hydrogen bond donors as introduced in Lipinski's paper introducing Rule of Five and other subsequent papers [27, 28]. Another key feature to consider is the complexity of the molecule via Fsp^3, which measures saturation of the
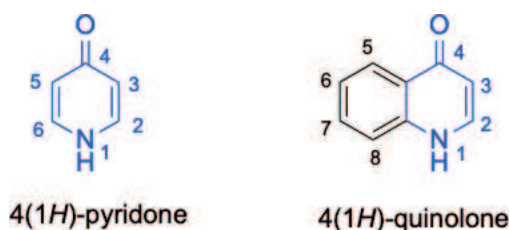
Figure 3.
4(1H)-quinolone and 4(1H)-pyridone scaffold.

Figure 4.
*Original 4(1H)-pyridones, 4(1H)-quinolones, and its' derivatives with antimalarial activity.*

compound. It has been observed that increase saturated carbons leads to higher aqueous solubility – a major downfall in 4(1*H*)-quinolone and 4(1*H*)-pyridone type molecules [29].

2.1 4(1*H*)-pyridones

2.1.1 Optimization of clopidol

An anticoccidial drug clopidal (**8**) was discovered to possess antimalarial activity in the 1960s by the Walter Reed Army Institute of Research. Assays presented activity towards four strains of *Plasmodium spp.* – *P. berghei*, *P. gallinaceum*, *P. cynomologi*, and *P. falciparum* [25, 30].

After several decades, scientists at GlaxoSmithKline (GSK) exerts efforts to optimize clopidal (**8**) due to emerging evidence that **8** may employ similar cytochrome bc_1 inhibition, like atovaquone (**6**) [31]. They hypothesize that clopidal (**8**) acts as a ubiquinone-mimic; therefore, replacing C-3 chlorine with a lipophilic sidechain. Installing an *n*-octyl chain improved *in vitro* activity (IC_{50} = 4 µM) but diminished *in vivo* activity ($ED_{50} \geq 60$ mg/kg). They hypothesized that the difference in activity may be due to metabolic degradation of the alkyl chain. To resolve this issue regarding the chemically labile sidechain, they installed the *trans*-(4-chlorophenyl) cyclohexyl sidechain of atovaquone (**6**), known to be resistant to metabolism, which significantly increased both *in vitro* and *in vivo* activity (IC_{50} = 0.05 µM; ED_{50} = 0.6 mg/kg). This led to the study of biaryl analogues, where 4-phenoxyphenyl sidechain also exhibited similar *in vitro* and *in vivo* activity. More in-depth SAR study was performed on 5-(phenoxyphenyl)-4(1*H*)-pyridones (**Figure 5**) [32].
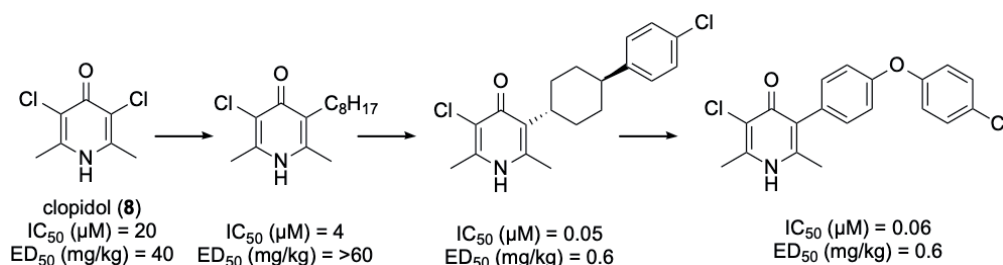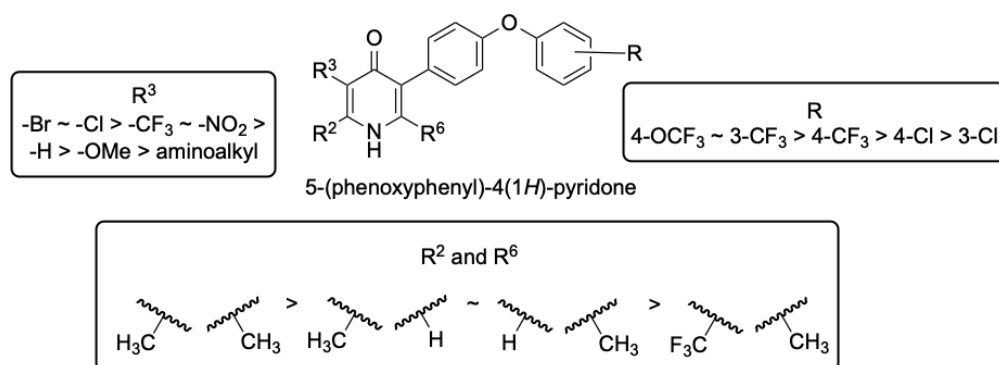

Figure 5.
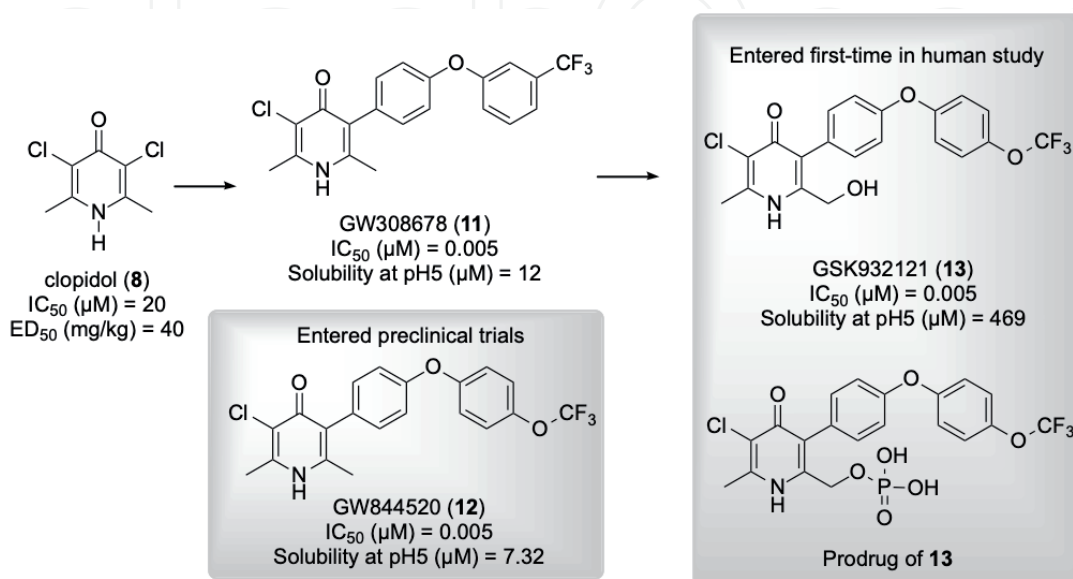*Initial optimization of clopidol.*

Variations to the C-2, C-3, and C-6 position were made and further improvements could not be observed (**Figure 6**). Variations on the aryl group made a major impact on the *in vitro* activity. This led to GW308678 (**11**) and GW844520 (**12**), where **12** entered preclinical trials [32]. However, after observing mild and reversible histopathological findings in skeletal and cardiac muscles, this study was abandoned. They hypothesized that these findings were due to its high lipophilic nature and long half-life. Therefore, they sought to improve the physicochemical properties [25].

To improve the physicochemical properties of GW844520 (**12**), the scientists at GSK introduced a hydroxymethyl group on C-2 position that significantly diminished the activity (IC_{50} = 0.13 μM). Interestingly, its isomer with the hydroxymethyl group on the C-6 position maintained high level of antimalarial activity (IC_{50} = 0.005 μM) with improved solubility. Simultaneously, a prodrug approach was introduced to optimize solubility. The newly introduced hydroxy group offered more chemically stable prodrugs than previous attempts utilizing the C4-OH. This led to GSK932121 (**13**) and a phosphate ester prodrug of **13**, where it was selected to enter human trials. Regrettably, this study was terminated due to toxicological findings from lack of species-specific target selectivity of the parent drug (**Figure 7**) [25, 33].

Recently, various linkers and heteroaromatic rings have been investigated. Compounds with rigid linker (alkyne) are still active; however, relative to their

Figure 6.
SAR of 5-(phenoxyphenyl)-4(1H)-pyridones.

Figure 7.
Overall optimization scheme of clopidol.

flexible linker (ether and alkane) counterparts, their potency decreases by a ten-fold. They anticipate that the flexibility allows the compound to mold into the active site with correct hydrophobic interactions. Another observation was that replacing the proximal phenyl ring with a pyridine ring maintains the activity and improves pharmacokinetic profiles. This demonstrates the potential of analogues of **13** in hopes of improving pharmacokinetic and toxicology profiles of 4(1*H*)-pyridones [34].

2.2 4(1*H*)-quinolones

With renewed efforts to optimize 4(1*H*)-quinolones, three main factors are considered –aqueous solubility, resistance index (RI), and potency. Aqueous solubility is essential in developing a dose-proportional pharmacokinetic profile for orally administered drugs. The RI is the ratio of the effective concentration to kill 50% of the parasite population of two clinically relevant malaria strains, where one is the parent or sensitive strain. For instance, W2 and TM90-C2B are frequently used, where W2 is the multi-drug resistant strain that is atovaquone-sensitive and TM90-C2B is atovaquone-resistant due to a point mutation in cytochrome *b*. Given this, RI would be the EC_{50} of TM90-C2B divided by the EC_{50} of W2 [35]. Ideally, this value is 1, which demonstrates that the drug is equally potent against both malaria strains; values between 0.3 and 3.0 are acceptable. Finally, low nanomolar potency of both strains is preferred.

The balance amongst these three qualities are necessary for the development of a potent, orally bioavailable antimalarial quinolones.

2.2.1 Optimized of floxacrine

In 1974, an evaluation of floxacrine (**10**) for antimalarial activity was performed. It was discovered that **10** exhibited casual prophylactic activity. However, it had several liabilities – cardiovascular toxicity, parasite drug resistance, and poor aqueous solubility [36, 37]. Efforts have been made to synthesize analogous compounds devoid of these drawbacks. In the 1990s, WR243251 (**14**), a 1,2,3,4-tetrahydroacridin-9(10*H*)-ones (THAs), was discovered and did not display any cardiovascular toxicity (**Figure 8**) [37]. However, it still displayed resistance and had poor aqueous solubility [37, 38].

Due to limited exploration of THAs since the discovery of WR243251, Manetsch and Kyle initiated a structure–activity relationship (SAR) and a structure–property relationship (SPR) studies to better understand THAs and its ability to exert activity towards both the blood and liver stage of the parasite.

Altering the number of carbons on the saturated ring system significantly reduced the activity and the aqueous solubility. Here on out, the scientists modified the 5-, 6-, 7-, and 8- position of the benzenoid ring with the 6-membered saturated


WR234251 (**14**)
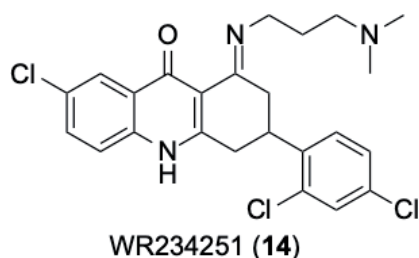
Figure 8.
Structure of WR243251.

ring. It became evident that the 6- and 7-position are key positions for antimalarial activity, which also affected the RI. The electronics on the 6-position did not significantly alter the activity. However, the 7-position was more sensitive, as it displayed preference for electron donating groups to retain potency and maintain the RI between 0.3 and 3. Furthermore, an inverse relationship was observed between potency and aqueous solubility. Substituting either or both the 5-position and 8-position, decreased activity and increased solubility. With this, THA-114 (**15**) and THA-115 (**16**) were found to exhibit nanomolar antimalarial activity with an acceptable RI that lacks toxicity. However, its solubility did not fall within the acceptable range of 40 μM or greater (**Figure 9**). Developing a candidate that is both active and has optimal physicochemical properties is difficult; nonetheless, it is necessary. The inability to develop such molecules leads to issues with *in vivo* efficacy studies and hinders with safety assessment studies [39].
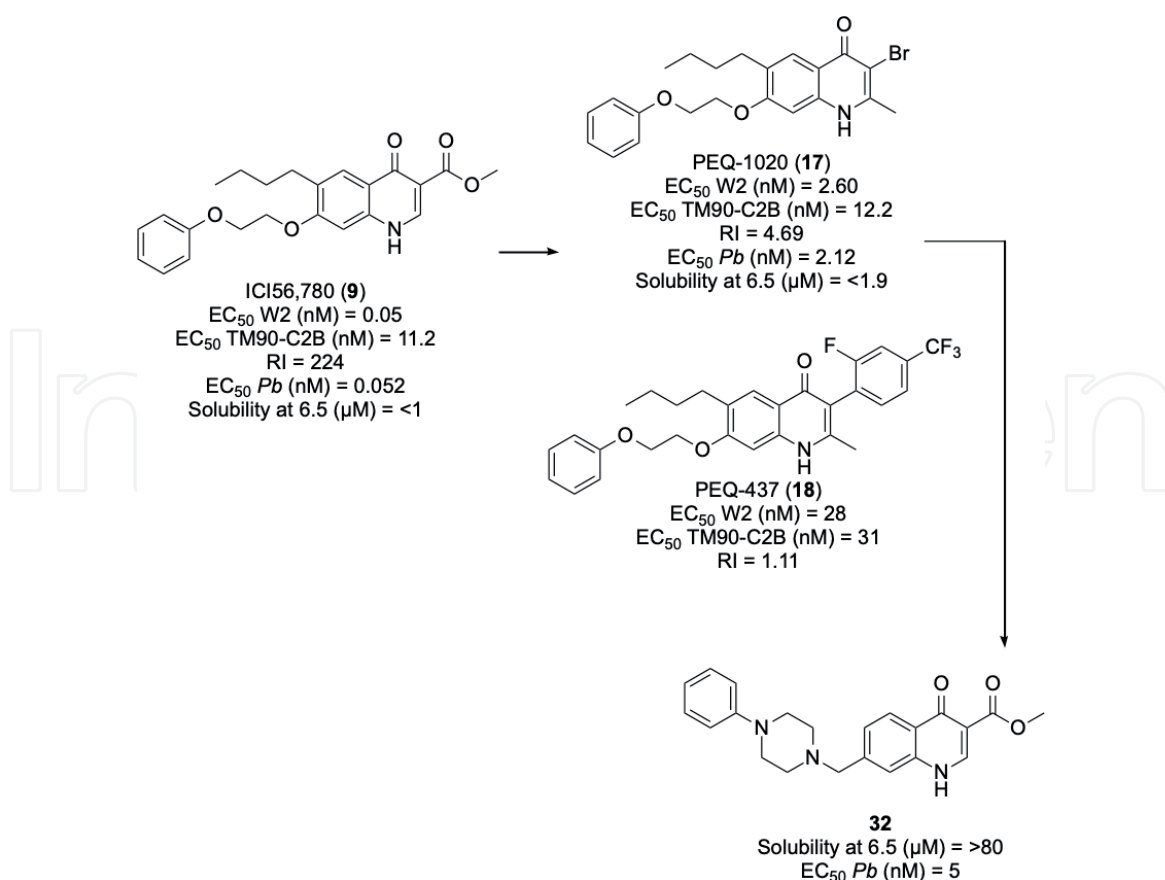
2.2.2 Optimization of ICI56,780

Scientists at Imperial Chemical Industries (ICI) developed ICI56,780 (**9**) as an antimalarial agent. What was striking about this compound series was its activity against blood stage and liver stage parasites in *P. cynomolgi* in rhesus monkeys and *P. berghei* in mice. However, disappointingly, resistance was developed in *P. berghei* after one passage [40, 41].

Manetsch and Kyle initiated work on SAR to optimize **9**. ICI56,780 (**9**) was utilized as the reference molecule, where they observed excellent potency for both W2 and TM90-C2B and *in vitro* liver stage activity; however, the potential to observe cross–resistance with atovaquone was high. Examination of the 2-, 3-, 6-, and 7-position concluded that the original 6- and 7- substituents are optimal. 3-position was rather interesting. Alteration significantly decreased the activity; however, remained within acceptable range. Interestingly, the RI fell into an appropriate range. Finally, the 2-position was examined with and without a methyl group, where the analogue with the methyl group displayed higher potency. This led to PEQ-1020 (**17**) an PEQ-437 (**18**). With promising *in vitro* blood stage data, compound **17** were tested *in vivo*. The insoluble nature of the compound **17** hindered *in vivo* testing and displayed poor activity (61% inhibition at day 6) (**Figure 10**) [42, 43].
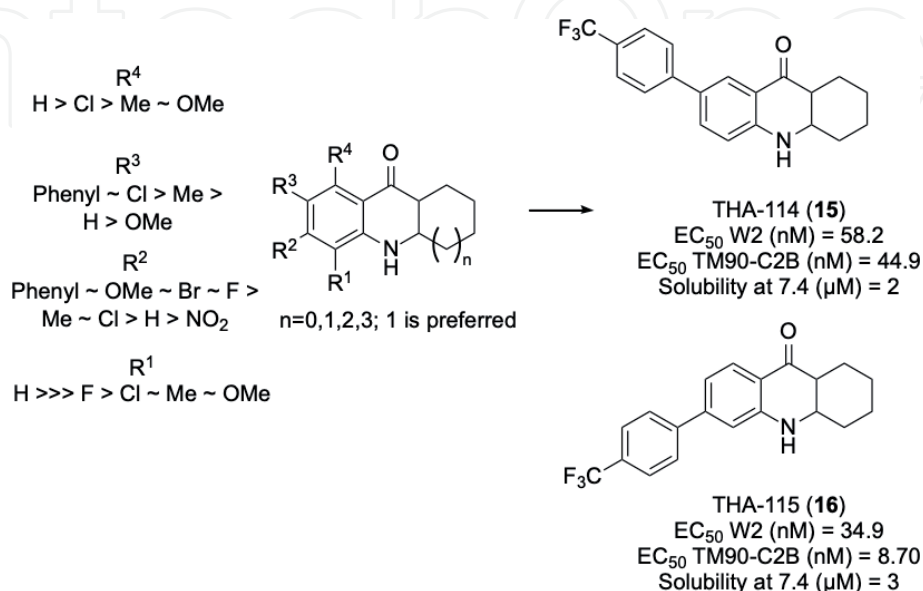
Figure 9.
Optimization of floxacrine.
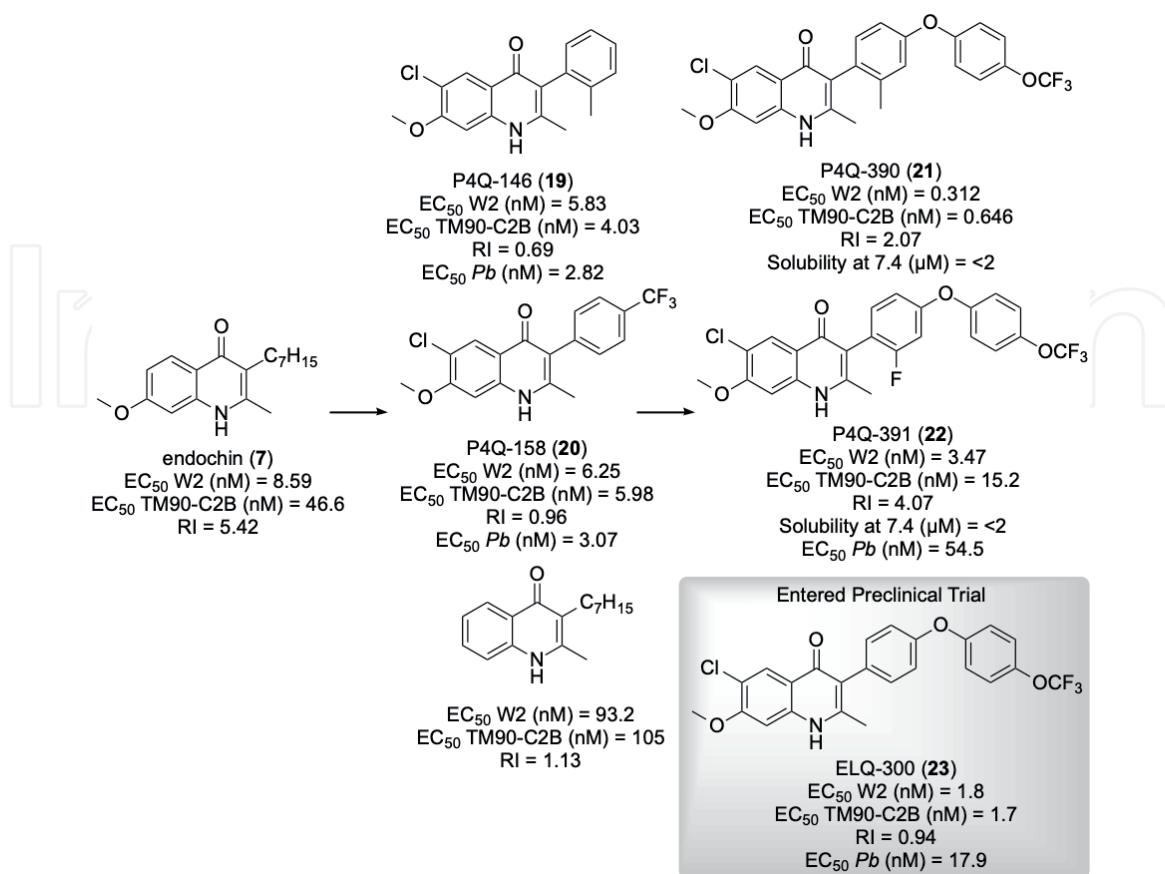
Figure 10.
Optimization of ICI56,780.

Given the necessity to improve aqueous solubility, ionizable piperazinyl-substituted analogues were developed. The linker length between the piperazine moiety and the benzenoid ring was investigated, along with the various piperazinyl-substituent. Ethylene linker analogues were found to diminish blood stage activity, while methylene linker analogues were most active. Amongst the *N*-phenylpiperazinyl, *N*-benzylpiperazinyl, and *p*-methoxybenzylpiperazinyl substituents, *N*-phenylpiperazinyl was most potent. When the piperazinyl-substituent was placed on the 6-position rather than the 7-position, the analogues were void of activity. Based on previously developed analogues, 3-position was investigated. Similarly, 3-halo substituted analogs improved RI; however, diminished activity compared to that of the methyl ester at the 3-position. This led to the discovery of **32** and due to its high potency against *in vitro* liver stage activity, **32** was further evaluated in an *in vivo* assay against liver stage. Compound **32** was able to generate cures at oral doses of 25 mg/kg or higher [44].

2.2.3 Optimization of endochin

Endochin (**7**) was discovered during World War II by Hans Andersag, a German chemist from Bayer [45, 46]. This compound had been identified to be a causal prophylactic and potent erythrocytic stage agent in avian models [47]. However, it was deemed ineffective against human malaria due to its physicochemical properties and its inactive metabolite that formed in the presence of cytochrome P450 (CYP450) enzymes [45]. This had inspired research teams, like the one of Manetsch and Kyle or Riscoe, to diversify such molecule.

The two groups worked together to synthesize a series of compounds, termed ELQ for endochin-like quinolones and P4Q for phenyl/aryl substituted in the 4-position of 4(1*H*)-quinolones, to undertake SAR and SPR studies. By removing either

substituent on the 3- or 7-position, it was determined that the 3-position is essential to the activity of endochin. Because the alkyl chain on the 3-position was metabolically labile, they modified the 3-position. By diversifying the 3-position, it suggested that the active site was hydrophobic with a reasonably sized pocket. The substituents on the benzenoid ring was also investigated, where installing a chlorine at the 6-position and a methoxy- group on the 7-position increased potency through the nature of electronics. The chlorine increases the binding affinity by increasing the acidity of N-H. The methoxy-group increases the potential of the carbonyl to accept hydrogen bonds with the active site. Substitutions at the 5- and 8-positions were not tolerated well. To optimize variably in the SAR study, further studies utilized 3-alkylphenyl-4(1*H*)-quinolones. It was revealed that *para* position observed higher activity, *para* and *ortho* position had acceptable RI, and *ortho* position exhibited the higher aqueous solubility. Of this series, P4Q-146 (**19**) and P4Q-158 (**20**) were the compounds with the best characteristics – single-digit nanomolar activity with acceptable RI and lack of toxicity. To further optimize the aryl-substituent, moieties with two aromatic rings were investigated, where some were inspired by the GSK pyridones. This led to P4Q-390 (**21**). P4Q-391 (**22**), and ELQ-300 (**23**), where they exhibited low single-digit nanomolar activity with acceptable RI (**Figure 11**). P4Q–391 (**22**) and ELQ-300 (**23**) are especially unique because of its activity against exo-erythrocytic stage, which includes liver schizonts, gametocytes, and ookinetes and oocytes. Antimalarials that are capable of preventing the transmission of malaria is extremely important. For this reason, ELQ-300 (**23**) was selected by Medicines for Malaria Venture (MMV) to undergo preclinical development, where it resulted in causal prophylaxis in mice malarial models and complete inhibition of oocyst formation. Unlike the structurally similar GSK 4(1*H*)-pyridone, **23** was a species–specific inhibitor. However, due to the poor solubility of this compound, a proper safety margin could not be established and was, therefore, abandoned for future tests [48–54].

Figure 11.
*Optimization of endochin to ELQ-300.*
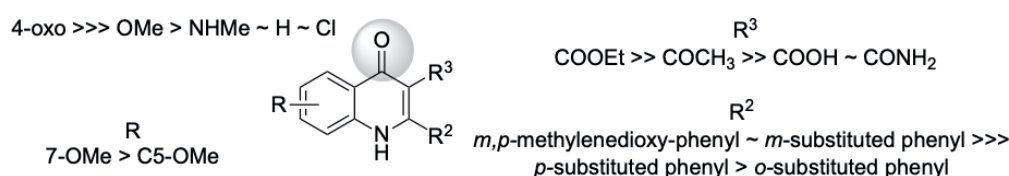
2.2.4 Optimization of TDR molecules

The Guy laboratory is also one of the various groups that are working to optimize quinolones to develop a potent antimalarial; however, their approach was slightly different. Rather than utilizing the older antimalarials, this group utilized two compounds that had confirmed antimalarial activity through the WHO's Special Programme for Tropical Disease Research (TDR). TDR42098 (**24**) and TDR17516 (**25**), differing in the position of the methoxy group on the benzenoid ring, was identified and confirmed to have antimalarial activity from a screening campaign by TDR at Tibotec using 17,472 non-proprietary compounds sourced from SPECS (**Figure 12**) [55].

Utilizing **24** and **25** as the reference compound, the Guy laboratory modified the 2-, 3-, 5-, and 7-position to see its effect on the activity, solubility, and permeability. In the first series of compounds, the 3-position was investigated. Removal of the carboxyl ester resulted in the total loss of activity and solubility for both analogues of **24** and **25**. Replacing the carboxylate with either an acid or amide also resulted in the total loss of activity and due to its hydrophilic nature, loss of permeability was observed. In the next series, quinoline analogues were investigated, where any alterations to the 4-oxo observed total loss of activity. It is possible that the ability for the quinolones to undergo tautomerization is a necessary characteristic to have antimalarial activity. Finally, they investigated the 2-position by installing varying aryl groups. Alterations of analogue **25** at the 2-position diminished antimalarial activity; however, analogues of **24** observed different results. Installing an unsubstituted phenyl group decreased activity by approximately two-fold, while maintaining solubility and increasing permeability. *Ortho* substituted phenyl rings diminished any activity. *Para* substituted phenyl rings either decreased activity by four-fold or completely, in addition to significant decrease in aqueous solubility. *Meta* substituted rings observed the best potency with acceptable aqueous solubility and increased permeability (**Figure 13**) [56].

Findings from the initial SAR study prompted the Guy laboratory to further investigate 3-carboxy-4(1*H*)-quinolones. Various *meta* substituted phenyl rings were installed to the 2-position. Introduction of hydrophobic groups, such as methyl, vinyl, and phenyl, retained similar potency to **24**. Installation of alkoxy groups on the *meta* position displayed great improvement to the potency. Strong electron withdrawing groups, such as nitro, acetyl, and methyl sulfonyl, and H-bond donors
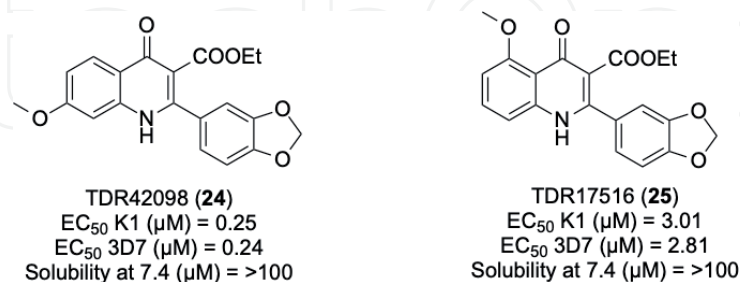
Figure 12.
TDR molecules identified as compounds with antimalarial activity.

Figure 13.
Initial optimization of TDR molecules.

resulted in loss of activity. This suggests that hydrophobic electron-donating group on the *meta*-position of the phenyl group is most ideal; however, like other quinolones, increasing activity with hydrophobic substituents tends to decrease aqueous solubility and permeability – essential characteristics for oral bioavailability [57].

7-position was also investigated, where findings also displayed that small hydrophobic electron-donating group improves potency, while an electron withdrawing group diminishes the potency [57].

Multi-substituted benzenoid ring was investigated to observe if there are any synergistic effect of varying substituents. 5,7- and 6,7- dihalogenated compounds were inactive towards multidrug-resistant (MDR) strains. Similar results were observed from 6,7-dimethoxy analogues. Simultaneous incorporation of a methoxy and halo group was investigated, where it exhibited sub-micromolar to nanomolar activity when the halogen was on the 6 position and the methoxy was on the 7 position. When installing a methoxy group on the 6 position and the halogen on the 7 position, antimalarial activity against all strains were lost [57].

Finally, the carbonyl substituent on the 3-position was investigated. Varying chain lengths and incorporation of morpholinyl, pyrrolidinyl, and *N,N*-dimethyl amino functionalities were installed; however, these changes reduced potency without any increase in solubility of the compounds (**Figure 14**) [57].

13 compounds that had an appropriate balance amongst activity, solubility and permeability were selected by the Guy laboratory to test for microsomal activity. Compounds **26** and **27** displayed the most promising liver microsomal activity ($CL_{int,in vitro}$ < 4 uL/min/mg in human and mouse microsomes). These two compounds also displayed highest C_{max} and AUC, which is indicative of extensive systemic exposure after oral administration. This was observed in *in vivo* antimalarial activity assay, where these two compounds were the only compounds that suppressed parasite growth (**Figure 15**) [57].

2.2.5 Optimization of HDQ

Similar to the Guy laboratory, the O'Neill and Ward utilized a unique approach that led them to study quinolones to treat malaria. Originally, hydroxy-2-dodecyl-4(1*H*)-quinolone (HDQ, **28**) was known to be active against alternative NADH
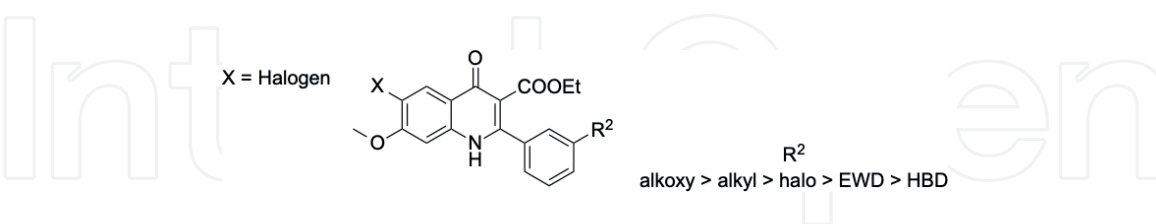
Figure 14.
Recent optimization of TDR molecule by the guy lab.
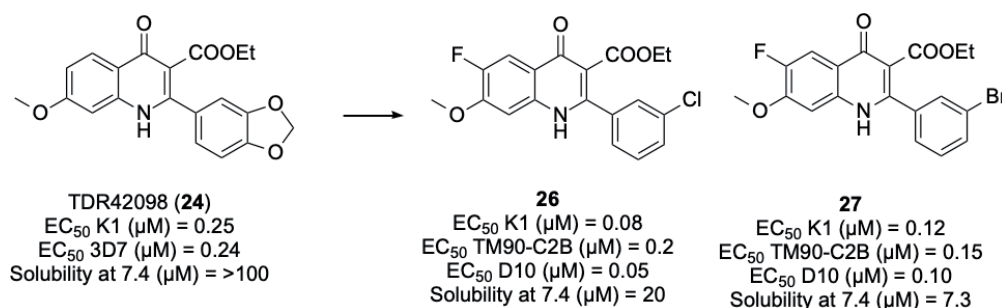
Figure 15.
Overall optimization of TDR molecule to frontrunner compounds.
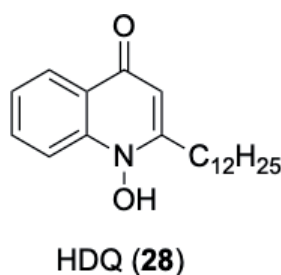
Figure 16.
*Hydroxy-2-dodecyl-4(1H)-quinolone (HDQ).*

dehydrogenase from the fungus *Yarrowia lipolytica* (**Figure 16**). In 2007, HDQ (**28**) exhibited nanomolar activity against *P. falciparum* and *T. gondii* [58]. This led O'Neill and Ward to perform various chemoinformatic methods (molecular fingerprinting, turbo similarity, principal component analysis, Bayesian modeling, and bioisosteric replacements) to select compounds for high-throughput screening. 17,000 compounds were selected from a commercial library of approximately 750,000 compounds from Biofocus DPI. Afterwards, these molecules were subjected to a sequential high-throughput screening method utilizing an *in vitro* assay against recombinant PfNDH2. This led to the selection of quinolone core as the main target chemotype for their SAR study [59–61].

Quinolones with 2-substituted monoaryl were selected as the template, as it contained a lipophilic side chain that was not metabolically labile like the aliphatic chain on HDQ (**28**) [59–61].

Initially, the quinolone core was modified. Installing a 8-aza-4(1*H*)-quinolone core reduced antimalarial activity. Similar to the previous optimization of various quinolones, chlorine and fluorine is well-tolerated, methoxy on the 7-position is well-tolerated, and large substituents on the benzenoid ring is not well tolerated. Interestingly, unsubstituted 3-position provides a slight increase in activity; however, this small advantage is outweighed by the decrease in solubility. Given that there is a large hydrophobic pocket at the active site, these researchers investigated 2-substituted biaryl quinolones. It became clear to the researchers that a monoaryl group could not obtain an IC$_{50}$ of below 500 nM. Investigation of the biaryl began with modification of the linkers. Variations on linkers (*p*-CH$_2$, *m*-CH$_2$, and *p*-O) did not affect the activity of the compound, as they were all well-tolerated. The terminal substituent on the distal phenyl group is also dependent on the other substituents on the molecule. In general, OCF$_3$ is the optimal terminal group, while a large EWG was less tolerated. This led to the discovery of CK-2-68 (**29**). Due to poor solubility, the use of prodrug moiety and altering formulations provided a proof of concept that CK-2-68 (**29**) clears the parasites *in vivo* [59–62].

To improve solubility properties, heterocyclic substituents were introduced to the quinolone core. It was observed that the distal ring is most favorable as a phenyl ring; however, a pyridine ring for the replacement of the proximal aromatic ring demonstrates great potency, reduced ClogP, and improved solubility. However, even with the improved solubility, the *in vivo* testing had to utilize a prodrug moiety to establish a proof of concept that **30** does clear the parasites *in vivo* [60].

The most recent attempt to improve solubility was to utilize a bioisostere of benzene rings. Pyrazoles have been well-documented to improve solubility by reducing ClogP. The optimization of the other substituents follows their previous findings (**Figure 17**) [62].
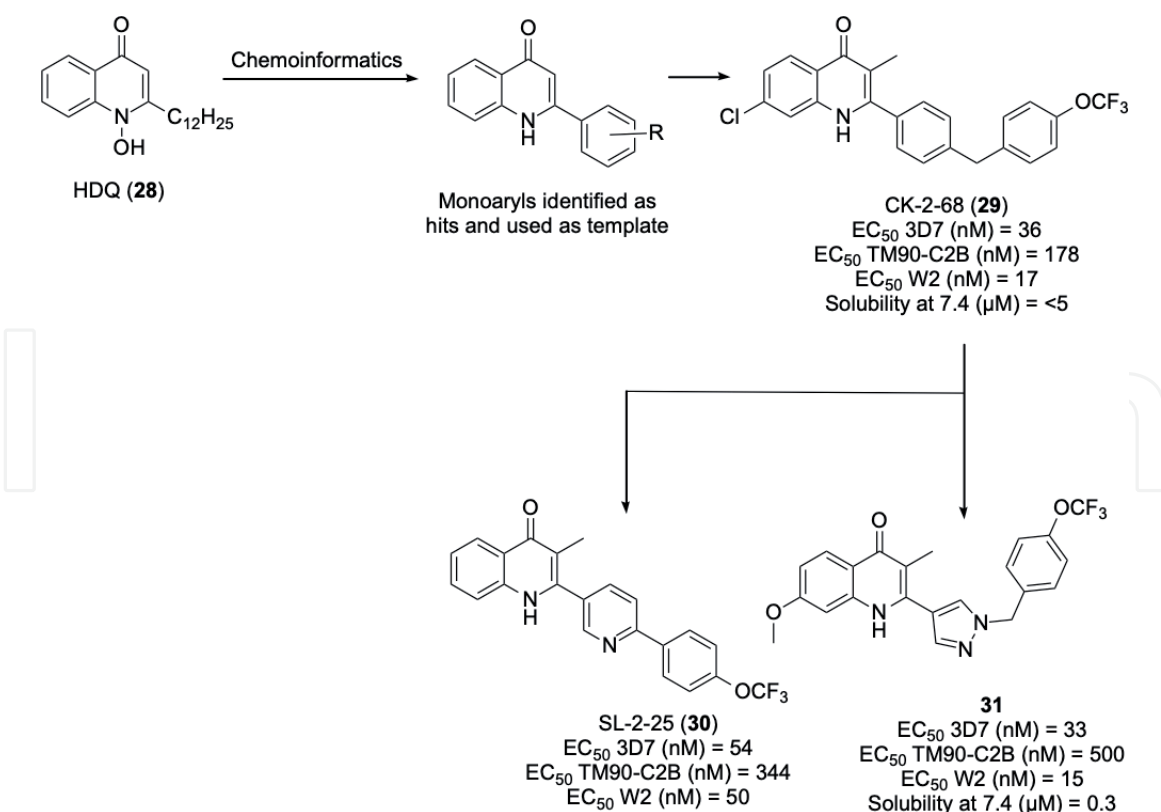
Figure 17.
Optimization of HDQ.

3. Conclusion

While malaria remains to be a global health threat, developing a novel class of drugs has become essential to treat and prevent the spread of this disease. 4(1*H*)-quinolones and 4(1*H*)-pyridones both display potential of developing into potent antimalarial agents due to recent re-evaluation of old 4(1*H*)-quinolones and 4(1*H*)-pyridones possessing antimalarial activity. These historical quinolones display promising activity against both erythrocytic and exo-erythrocytic stages of the parasite. Various research groups invested their efforts and resources to optimize these 4(1*H*)-pyridones or 4(1*H*)-quinolones since it is perfect for the malaria eradication initiative.

The frontrunner compound for 4(1*H*)-pyridone is **13**, which displayed single-digit nanomolar activity against the erythrocytic stage with excellent solubility. However, after entering first-time in human study, **13** displayed toxicity, which terminated the study.

Unlike 4(1*H*)-pyridones, 4(1*H*)-quinolones (P4Qs, ELQs, THAs, TDR analogues, and HDQ analogues) lack cytotoxicity. This is essential to develop a species-specific inhibitor. The frontrunner compounds for ELQ/P4Q are **22** and **23**, which displayed low nanomolar activity for both the erythrocytic and exo-erythrocytic stages. In addition to this, **22** and **23** displayed activity against the transmitting stages, making these molecules especially important. The frontrunner compounds for PEQ are **17**, **18**, and **32**, which displayed low nanomolar activity for both the erythrocytic and liver stages. Another molecule that is promising. The frontrunner compounds for THA are **15** and **16**, which displayed nanomolar activity for the erythrocytic stage. The frontrunner compound for TDR analogues are **26** and **27**, which displayed nanomolar activity for the erythrocytic stage. The frontrunner compound for HDQ analogues are **30** and **31**, which displayed nanomolar activity for the erythrocytic stage.

Since many research teams focus solely on the activity against the blood stage, compounds **22**, **23**, **17**, **18**, and **32** are especially promising, as these display exo-erythrocytic activity, along with the erythrocytic activity.

Nevertheless, one major downfall with the development of 4(1H)-quinolones and 4(1H)-pyridones is the poor aqueous solubility. This prevents proper development of pharmacokinetic profiles for drug candidacy; therefore, failed clinical development and development was halted in the early 80s.

Thankfully, by early 2000s, the field of medicinal chemistry advanced, where researchers could optimize historical quinolones to develop them into drug-like molecules. Even with the variety of chemotypes, the approach towards optimization is quite similar amongst the various research teams.

Despite these obstacles, 4(1H)-quinolones have a great potential of becoming the next class of antimalarials. These molecules lack toxicity and have acceptable physicochemical properties, aside from solubility. With increased understanding to improve aqueous solubility, it is recommended to continue research on anti-malarial quinolones, as they have great potential of becoming orally bioavailable antimalarials.

Acknowledgements

Conflict of interest

The authors declare no conflict of interest.

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