# 4-AMINOQUINOLINES AS ANTIMALARIAL DRUGS

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*Malaria is a widespread parasitic infection with high mortality rates and a distribution which happens to correlate with the distribution of poverty. The class of drugs known as the 4-aminoquinolines has shown promise in treating malaria, especially infection by the Plasmodium falciparum parasite. While drugs such as chloroquine heralded the start of 4-aminoquinoline prophylaxis, resistance soon developed and became widespread. By investigating the structure-activity relationships of chloroquine, it was possible for medicinal chemists to devise novel compounds such as amodiaquine and isoquine for treatment of chloroquine-resistant strains of the parasite. These structural alterations ranged from compounds which could undergo bioactivation into toxic metabolites, to fluorinated alternatives and structural isomers. The present review aims to discuss the chemical investigation into chloroquine and its alternatives, and to examine the prospects of future antimalarial drugs of the 4-aminoquinoline class.*

## Introduction

Malaria is a blood-borne parasitic infection transmitted through the bite of a female mosquito of the genus *Anopheles*. There are around 30 species in this genus which act as malarial vectors, and around 3.2 billion people are at risk of this life-threatening disease (WHO, 2015). According to the WHO Malaria Fact Sheet (2015), 97 countries had ongoing malaria transmission in 2015 and between January and September 2015 there were an estimated 214 million clinical cases of malaria and over 400,000 deaths attributed to the disease. Malaria is caused by

protozoan (unicellular eukaryotic organisms) parasites of the genus *Plasmodium*, of which there are 5 species which infect humans (WHO, 2015) – *P. vivax, P. ovale, P. malariae, P. falciparum* and *P. knowlesi* (however *P. knowlesi* rarely causes disease in humans and only zoonotic transmission from monkey to human is known (Singh *et al.*, 2016). *P. falciparum*-induced malaria is widely regarded as one of the most dangerous forms of malaria, as it is most prevalent and cause the most deaths globally (WHO, 2015).
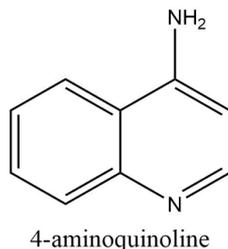


4-aminoquinoline

Figure 1. *The structure of 4-aminoquinoline, of which 4-aminoquinoline antimalarial are based.*

In 1934 the drug chloroquine was synthesised for the first time (Cook & Zumla, 2009), which became first in class of the quinine-based drugs termed the 4-aminoquinolines. The structure of 4-aminoquinoline (Figure 1) is based on the natural crystalline alkaloid quinine, found in Cinchona bark. While chloroquine was not the first synthetic antimalarial, it surpassed early compounds created by the company Bayer – such as mepacrine – and became a cost effective solution to falciparum malaria (that is, caused by *P. falciparum*). Clinical mepacrine use was discontinued due to reports of patients developing severe aplastic anaemia, psychotic reactions and exfoliative dermatitis. There were also minor adverse effects recorded, including yellow skin pigmentation (Peto, 1989). As mepacrine was declining in usage, chloroquine became available clinically, and this may have contributed to its widespread use. Resistance to chloroquine arose in four separate locations where falciparum malaria is endemic including the Thai-Cambodian border in the late 1950s (Harinasuta *et al.*, 1965), in Venezuela and Colombia strains of resistant *P. falciparum* were identified in 1960 (Moore & Lanier, 1961) and clinical cases of chloroquine resistance were detected in Papua New Guinea in the late 1970s (Grimmond *et al.*, 1976). Between 1978 and 1988, resistance to chloroquine was reported in all African countries, yet it remained as the first-choice treatment in many African countries into the early 2000s (Trape ,2001). As no effective malarial vaccines are yet available, health services rely on drugs for treatment and prophylaxis, however efficacy of antimalarial compounds has plummeted due to emerging *Plasmodium* resistance towards many different drug classes including first-line treatments such as chloroquine (Severini & Menegon, 2015). Drugs such as amodiaquine were developed, however they displayed adverse side effects and were redesigned as fluorinated derivatives.

Later, other 4-aminoquinolines such as isoquine were also synthesised. These compounds and their design are discussed in the main body of this review.

The current accepted mechanism of 4-aminoquinolines involves haemoglobin degradation by the *P. falciparum* parasite. During the red blood cell stage of *P. falciparum* infection (between the trophozoite and mature trophozoite stages), the parasite digests the haemoglobin of the human host as a source of amino acids through specialized processes (Elliott *et al.*, 2008). This degradation of haemoglobin releases the iron protoporphyrin "heme" as a by-product, which is toxic to the parasite – it can inhibit enzymes such as proteases and DNA polymerases and also damage the protozoan's membranes via lipid peroxidation (Aft & Mueller, 1983; Schmitt *et al.*, 1993; Vincent 1989; Orjih AU et. al, 1981). The *P. falciparum* parasite utilises a detoxification technique in which the monomeric heme ($\alpha$-hematin) is converted into the crystalline polymer hemazoin (otherwise known as $\beta$-hematin), which is non-toxic to the parasite (Slater & Cerami, 1992). It is thought that this is where 4-aminoquinolines exert their activity, and this will be examined in the case of chloroquine, amodiaquine and isoquine.

## Synthetic 4-aminoquinolines

### Chloroquine



*Chloroquine*
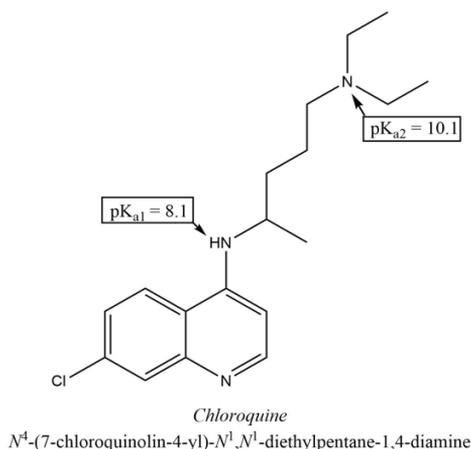$N^4$-(7-chloroquinolin-4-yl)-$N^1$,$N^1$-diethylpentane-1,4-diamine

Figure 2. *The structure of Chloroquine. Adapted from Vandekerckhove & D'hooghe (2015).*

Chloroquine was the first synthetic antimalarial drug and the first-in-class of the 4-aminquinolines, and it was synthesised in 1934 by chemists working for the German company Bayer (Cook & Zumla, 2009). The current predicted mechanism

of chloroquine is that the slightly basic chloroquine (pKa1 = 8.1, pKa2 = 10.1) (Olliaro 2001) accumulates down the pH gradient into the acidic digestive vacuole of the *P. falciparum* parasite, where the aforementioned haemoglobin digestion occurs. While the environment outside of the digestive vacuole is at physiological pH (pH = 7.4), the inside of the digestive vacuole has pH = 5.5 (O'Neill *et al.*, 2012). In this acidic environment the chloroquine structure become chloroquine$^{2+}$, and cannot readily diffuse back out of the digestive vacuole and instead begins to accumulate (Mushtaque & Shahjahan, 2015). In the digestive vacuole, chloroquine$^{2+}$ inhibits heme crystallisation to hemazoin by intercalating between the polymeric crystal packing and forms a dimeric hematin complex through π-π stacking interactions with the heme protoporphyrin ring (Shelnutt, 1983; White, 1978). This form of heme-drug complexation therapy results in the steady increase in heme concentration within the acidic digestive vacuole, which is toxic to the parasite and will eventually lead to death of the *P. falciparum* (Hempelmann, 2007).

Regarding the structure-activity relationships (SAR), the terminal amino group was identified as essential for accumulation, as this is one of the sites where protonation in the digestive vacuole occurs. The two aromatic rings of the quinoline moiety also proved useful for the π-π stacking interactions and presence of a chlorine atom at the 7-position of the quinoline also correlates with higher antimalarial activity (O'Neill *et al.*, 2012; Mushtaque & Shahjahan, 2015). Based on these functionalities, Koh *et al.*, (1994) proposed a structure for the chloroquine binding sites on heme, which is shown in Figure 3. Heme is also known as *α*-hematin or ferriprotoporphyrin IX. The carbon chain between the tertiary amine and secondary amine is referred to as the 'linker'.
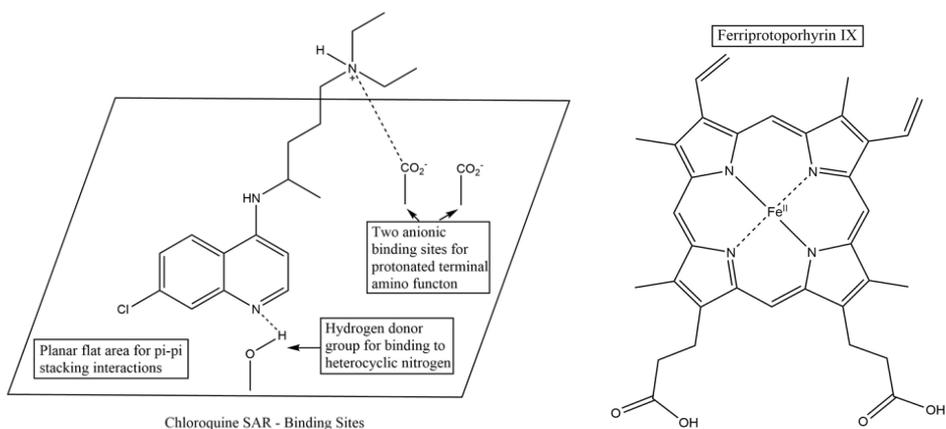


Figure 3. *Binding of chloroquine to the key features of the proposed receptor sites (top) in ferriprotoporphyrin IX (bottom). Adapted from O'Neill et al., (1997) and Koh et al., (1994)*

In the paper published by Koh *et al.* (1994), it was initially proposed that the central iron atom of ferriprotoporphyrin IX can bind to the aromatic quinoline nitrogen, and that the carboxylates could bind to the proton of the protonated nitrogen atom belonging to the 4-aminquinoline side chain. It should be noted however that Koh only accounted for the protonation of the terminal amino group and not the quinoline nitrogen, and so while the original binding model is reasonably accurate otherwise, it fails to account for any interactions the protonated heterocyclic nitrogen may have. This was highlighted in a publication by O'Neill *et al.* (1997). In this publication, O'Neill *et al.* (1997) proposed that the receptor proposed by Koh *et al.* was in fact ferriprotoporphyrin IX since it contains a planar flat region of 30 – 40 Å to interact with the aromatic quinoline moiety, two negatively charged carboxylates to bind to the protonated nitrogen of the terminal amino group of the alkyl side chain, and a central iron atom which can act as an acceptor site for the quinoline nitrogen. The distance between the quinoline nitrogen and the alkyl chain nitrogen was calculated as 8.30 Å (monoprotonated), which is quite similar to distances between the central iron atoms and the carboxylate oxygens in heme (calculated as 8.20 Å, analysed via X-ray crystallography) (O'Neill *et al.*, 1997).

Unfortunately, resistance to chloroquine gradually arose and has now become a major health concern for the developing world. As mentioned in the introductory section of this review, chloroquine resistance is now widespread everywhere that malaria is endemic. As the mechanism of action of parasite inhibition has still not been definitively proved, the mechanism of resistance is also debated. Chloroquine-sensitive parasites appear to accumulate much more of the drug in the digestive vacuole than resistant strains (Yayon *et al.*, 1984; Saliba *et al.*, 1998; Fitch, 1970). Research has identified point mutations in the gene encoding for *P. falciparum* chloroquine-resistance transporter (PfCRT) protein as a cause for the reduced drug accumulation (Bray *et al.*, 2005). A recent report (Chinappi *et al.*, 2010) stated that the PfCRT is found in the digestive vacuole and contains 10 predicted membrane-spanning domains (Cooper *et al.*, 2007). Furthermore, isolates exhibiting the chloroquine-resistant prototype not only carry the PfCRT point mutation but a charge-loss mutation K76T, often presented as two single mutations (K76N and K76I), which overall will affect the accumulation in the parasite. (Huaman *et al.*, 2004; Cooper *et al.*, 2005; Fidock *et al.*, 2000; Cooper *et al.*, 2002) The debate as to whether the point mutation in the gene encoding for PfCRT is the mechanism of chloroquine resistance in P. falciparum is ongoing, and the empirical data can be interpreted in numerous different ways. Several research groups are still devising novel approaches to pinpointing the mechanism of chloroquine resistance, yet there is no solid undisputed answer yet (Chinappi *et al.*, 2010).

Interestingly, long before PfCRT had been identified, it was observed that in some cases increasing or decreasing the length of the alkyl side chain could overcome the unknown resistance mechanism (Bray & Ward, 1998). This led some research groups to begin altering the properties of the N-alkyl amino side chain and later, the aromatic rings of the quinoline structure, which spurred on the discovery of new 4-amioquinolines.

## Amodiaquine

The spread of chloroquine resistance acted as a catalyst for new research into the pharmacology and development of novel 4-aminoquinoline antimalarials. The structure of one of these compounds, amodiaquine, is shown as Figure 4. Amodiaquine contains many of the same structural features as chloroquine with the primary structural difference between them being the presence of a phenol group in the alkyl side chain.
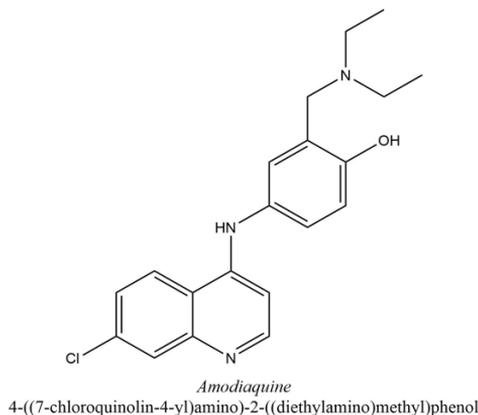


*Amodiaquine*
4-((7-chloroquinolin-4-yl)amino)-2-((diethylamino)methyl)phenol

Figure 4. *The structure of amodiaquine. Adapted from O'Neill et al., (2003).*

Amodiaquine was predicted to exhibit many of the same structural interactions as those detailed for chloroquine, with the planar quinoline system engaging in π-π stacking interactions with the heme protoporphyrin ring (Shelnutt, 1983; White, 1978), and preventing heme crystallisation. Working from the receptor model suggested by Koh *et al.* (1994), work published by O'Neill *et al.* (1997) showed the possible interactions of amodiaquine with the receptor, which are obviously quite similar to that of chloroquine.
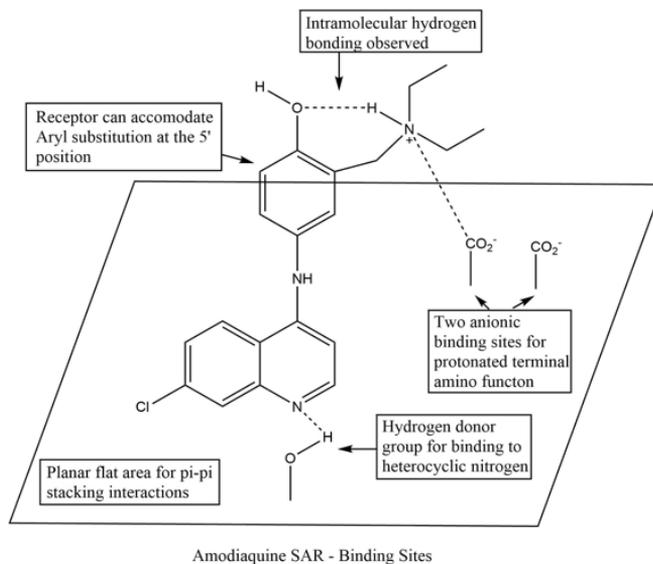
Amodiaquine SAR - Binding Sites

Figure 5. *Binding of amodiaquine to the key features of the proposed receptor sites in heme. Adapted from (O'Neill et al., 1997)*

Amodiaquine displayed very high efficacy in treating the chloroquine resistant strains of *P. falciparum*. In vitro assays against the chloroquine-sensitive strain HB3 and chloroquine-resistant strain K1 (Table 1) provided evidence that amodiaquine was a lead compound for treatment of chloroquine resistant *P. falciparum* malaria.

Table 1. *In vitro assay of antimalarial activity of chloroquine and amodiaquine, adapted from O'Neill et al., (2003). Amodiaquine tested as hydrochloride salt, whilst chloroquine tested as diphosphate. IC50 indicates concentration required for 50% inhibition in vitro.*

| Drug | $IC_{50}$ (nM) HB3 Strain | SD ± Mean | $IC_{50}$ (nM) K1 Strain | SD ± Mean |
|---|---|---|---|---|
| Chloroquine | 14.98 (6) | 3.98 | 183.82 (6) | 11.13 |
| Amodiaquine | 9.60 (9) | 3.73 | 15.08 (9) | 9.36 |

Whilst amodiaquine displayed exceptional activity against the chloroquine resistant K1 strain, the clinical use of the drug has been extremely restricted due to hepatotoxicity and agranulocytosis (Neftel *et al.*, 1986; Lind *et al.*, 1973; Bray & Ward, 1998). Although very seldom used due the adverse side effects, researchers began investigating how the toxicity of amodiaquine was exerted: the drug's efficacy and potential to become a first-line treatment for chloroquine-resistant malaria were high, and so if the mechanism of toxicity was understood it may be possible to prepare analogues without adverse side effects. Studies of amodiaquine metabolism in rats showed that the drug is excreted in bile as a 5′ thioether conjugate, with glutathione and/or cysteinyl forms (Harrison *et al.*, 1992). This excretion indicates that the compound must undergo some form of bioactivation to either an amodiaquine quinoneimine (AQQI) or an amodiaquine semiquinoneimine (AQSQI) with later conjugative addition (Maggs *et al.*, 1988). Work published recently has recently been ascribed the toxicity of amodiaquine to oxidation by cytochrome P450 enzymes (O'Neill *et al.*, 2012). The mechanism of this toxicity is shown in Figure 6. The adverse effects likely occur when glutathione stores have been depleted and the AQQI metabolite persists. Indeed this metabolism is quite similar to the metabolism observed in paracetamol (acetaminophen) overdose, in which conjugation of glutathione depletes stores until conjugation no longer occurs, and an electrophilic arylating compound which can covalently bind to macromolecules is formed, initiating the onset of hepatotoxicity (Beckett *et al.*, 1985).

Due to the side effects of amodiaquine, it is not often used in a clinical setting. Studies of the toxicity inspired the synthesis of *para*-fluorinated alternatives, where the hydroxyl group of the phenol is substituted by a fluorine have been prepared, as these compounds cannot undergo *in vivo* bioactivation to the toxic quinoneimines (Staines & Krishna, 2012). The current reasoning for why they do not undergo bioactivation is to do with the strength of the C-F bond and the high oxidation potential of these compounds (O'Neill *et al.*, 1994). The cost of the starting materials for fluorinated alternatives tends to be expensive compared to that of the parent compound, and when one is investigating drugs for a disease found primarily in the third world, cost must be considered. Hence in 2003, a reasonably cheap alternative to amodiaquine, with high efficacy against chloroquine resistant and sensitive strains was synthesised – isoquine (O'Neill *et al.*, 2003).
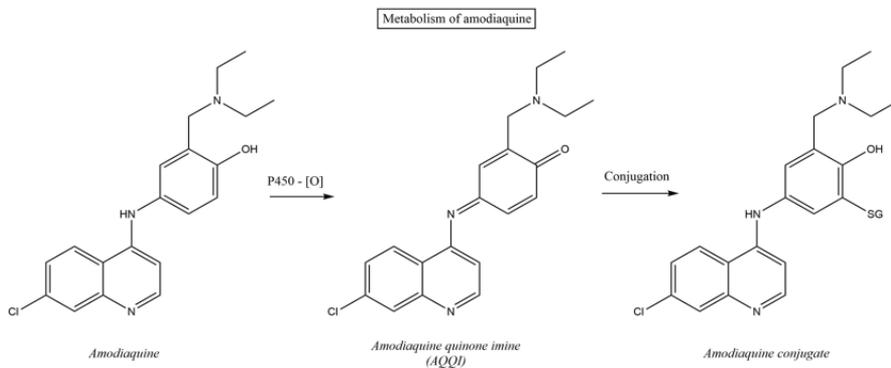
Metabolism of amodiaquine



Figure 6. *Metabolism of amodiaquine by the enzyme cytochrome P450, showing the highly toxic metabolite AQQI which leads to agranulocytosis and hepatotoxicity. Adapted from O'Neill et al., (2003).GS indicates glutathione, an antioxidant.*
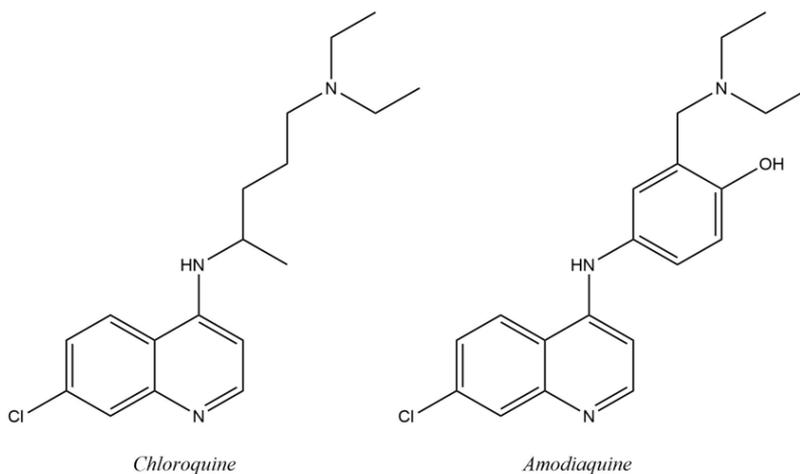


Figure 7. *Comparison of the structures of chloroquine and amodiaquine.*

## Isoquine

Isoquine is a structural analogue of amodiaquine with high antimalarial activity and no possibility of toxic quinoneimine metabolite formation, as the *in vivo* bioactivation is prevented (O'Neill *et al.*, 2003). Isoquine has been formulated for oral administration and displays high $ED_{50}$ (median effective dose) activity of 1.6 and 3.7 mg/kg against the *P. yoelli* NS strain (a strain used for laboratory infection of mice) compared to 7.9 and 7.4 mg/kg for amodiaquine. Furthermore, isoquine provides a cost effective and potentially safer alternative to amodiaquine, as its metabolism is quite different; isoquine and its Phase I metabolites appear to undergo clearance by Phase II glucuronidation, evidenced by the lack of glutathione metabolites in the bile (O'Neill *et al.*, 2003). Isoquine is now being investigated further and is considered a lead compound for the treatment of chloroquine-sensitive and chloroquine-resistant *P. falciparum* malaria. It is currently reaching the end of Phase I clinical trials, conducted by GlaxoSmithKline in the UK (GlaxoSmithKline 2010-Present).

## Future analogues

A huge field of currently active research deals with synthesising hybrid compounds of 4-aminoquinolines with other classes of antimalarial drugs. As it stands, conjugation of some of the 4-aminoquinolines to natural product antimalarials has proved useful – for example, the synthesis of many different artemisinin-quinoline hybrid dimers was recently published by the Lombard group, displaying reasonably high potency with low resistance occurrence (Lombard *et al.*, 2011). In terms of other forms of modification, side chain modification with oxalimide linkers, $\alpha$-ketoamide linkers coupled in indole and hybrid aminoquinoline-triazine derivatives have been reported, with some of the these compounds reporting quite high antiplasmodial activity (Sunduru *et al.*, 2009).

Novel motifs for conjugation to quinoline backbones are constantly emerging from different research groups. One example are the 7-Chloroquinoline-chalcone hybrids which so far have shown somewhat low antimalarial activity (Sharma *et al.*, 2009) but are being researched further because they are cost effective and further modification could potentially boost their efficacy (Vandekerckhove & D'hooghe, 2015). Ultimately, the field of 4-aminoquinoline and 7-chloroquinoline modification could provide cost effective and potent antimalarial drugs, whether that is through conjugation with other natural antimalarials like artemisinin or Cinnamic acid (Wiesner *et al.*, 2001) or through carbon linker modification, or some of the other many forms of modification published in recent years (Vandekerckhove & D'hooghe, 2015). These modifications show varying degrees of toxicity and potency, however what is quite interesting is that Wiesner et. al reported in 2001 that Cinnamic acid derivatives show markedly low toxicity (Wiesner *et al.*, 2001). As Cinnamic acid is a water-soluble natural product obtained

from oil of cinnamon (and also shea butter), perhaps natural product conjugation to the quinoline moiety should be focused on for the future of cheap antimalarial conjugates with low toxicity.

Table 2. *In vitro assay of antimalarial activity of chloroquine, amodiaquine and isoquine, adapted from O'Neill et al., (2003). Amodiaquine tested as hydrochloride salt, whilst chloroquine tested as diphosphate. Isoquine tested as free-base. IC50 indicates concentration required for 50% inhibition in vitro.*

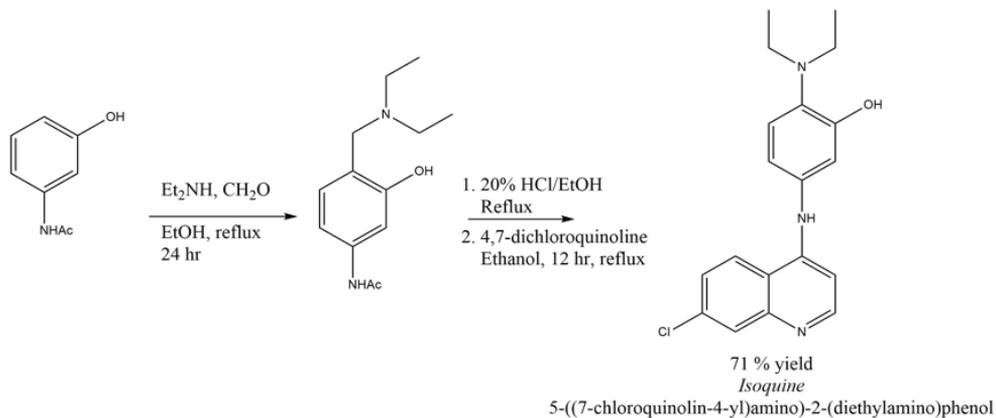| Drug | $IC_{50}$ (nM) HB3 Strain | SD ± Mean | $IC_{50}$ (nM) K1 Strain | SD ± Mean |
|---|---|---|---|---|
| Chloroquine | 14.98 (6) | 3.98 | 183.82 (6) | 11.13 |
| Amodiaquine | 9.60 (9) | 3.73 | 15.08 (9) | 9.36 |
| Isoquine | 12.65 (9) | 4.75 | 17.63 (9) | 7.00 |
| Isoquine diphosphate | 9.02 (3) | 4.06 | 6.01 (3) | 8.00 |



Figure 8. *Synthesis of isoquine. Adapted from O'Neill et al., (2003)*

## Discussion and Conclusions

Malaria is a widespread disease, with a distribution close to the distribution of poverty over the world. Reliable drugs for parasite prophylaxis are of immense value, and while the first of the 4-aminoquinolines, chloroquine, showed high efficacy and prophylaxis, resistance soon arose and became widespread. Drugs based off this early 4-aminoquinoline show similar degrees of activity and in some cases, structural analogues display higher efficacy than previously encountered. The scope of 4-aminoquinoline modification is huge – conjugation to natural product antimalarials which show reasonably low toxicity and promising potency. Modification of the length and nature of the carbon linker is shown in the case of amodiaquine and isoquine, however that appears to only be the beginning. In amodiaquine, the carbon linker transformation to a phenol proved useful in increasing potency, yet induced adverse side effects such as hepatotoxicity. The regioisomer isoquine was hence designed based on amodiaquine, and proved to be an effective antimalarial with low toxicity and $IC_{50}$ values similar to that of amodiaquine for the treatment of chloroquine-resistant malaria. In isoquine, rational drug design with respect to Phase I metabolism was implemented, and it is this kind of rationale which may lead to further advances in reducing the toxicity of quinoline-based antimalarials.

In terms of the future of synthetic 4-aminoquinolines and their derivatives, there are a wide range of conjugates and modified structures being researched, other than amodiaquine and isoquine. Functionalisation and/or interconversion of the tertiary and secondary amine component in chloroquine has proved beneficial, showing high potency. Furthermore, natural product derivatives, especially those from Cinnamic acid, have shown very low toxicity in studies published in the last six to seven years. In the future of antimalarial research, it is likely that cheap natural product conjugates and dimers based off the 4-aminoquinoline backbone structure or even the isoquine structural motif will find clinical use in plasmodia prophylaxis. Indeed, the area of antimalarial drug design requires careful navigation around toxicity and indeed with novel analogues such as isoquine and its potential for modification, we very well could be on the brink of discovering a safe and cheap alternative for treating resistant strains of *Plasmodium* parasites.

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