School of Pharmacy

The Synthesis of the Antimalarial Compound Hydroxypiperaquine (HPQ)

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Declaration

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgment has been made.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.
Signature:

Date:

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Table of Abbreviations

1,3-DCP	1,3-dichloropropanol
4,7-DCQ	4,7-dichloroquinoline
ACT	Artemisinin-based combination therapy
CQ	Chloroquine
$CDCl_3$	Deuterated chloroform
CD_3OD	Deuterated methanol
DCM	Dichloromethane
DMF	N,N-dimethylformamide
DIPEA	Diisopropylethylamine
DMSO	Dimethylsulfoxide
D_2O	Deuterated water
EtOH	Ethanol
EtOAC	Ethyl acetate
Et_3N	Triethylamine
HPQ	Hydroxypiperaquine
iPrOH	Isopropanol
IR	Infrared
KI	Potassium iodide
LP	Light Petroleum
NMP	*N*-methyl pyrrolidinone
NMR	Nuclear magnetic resonance
PQ	Piperaquine
ppm	Parts per million
P. falciparum	*Plasmodium falciparum*
P. malariae	*Plasmodium malariae*
P. ovale	*Plasmodium ovale*
P. vivax	*Plasmodium vivax*
tBOC	Tertiarybutoxycarbonyl group
TLC	Thin layer chromatography
UV	Ultraviolet spectroscopy
WHO	World Health Organization

Abstract

Malaria remains one of the most common causes of illness and death in developing countries.[1] The development of new drugs to combat the disease is becoming one of the fastest growing research areas. Hydroxypiperaquine (HPQ) is an antimalarial bisquinoline compound related to the emerging antimalarial drug Piperaquine (PQ).[2] Various research programs are being conducted internationally in efforts to prepare PQ for possible clinical use in combination with artemisinin derivatives. The hydroxy compound (HPQ) has been described in the Chinese literature but no data exists for this compound within the Western literature.[3] The primary aim of this research project was to synthesise Hydroxypiperaquine *via* alternative synthetic pathways to that briefly described by Xu *et al*[3] by exploring various synthetic strategies based on literature synthetic procedures involving similar compounds. HPQ was synthesised through a three step synthetic process. In the first step, tertiary butoxy carbonyl (tBOC) piperazine was coupled with 4,7-dichloroquine (4,7-DCQ) to produce the intermediate 7-chloro-4-(tBOC piperazin-1-yl)quinoline. The second synthetic step involved the deprotection of 7-chloro-4-(tBOCpiperazinyl)quinoline to remove the tertiary butoxy carbonyl (tBOC) protecting group. The deprotected intermediate, 7-chloro-4-(piperazin-1-yl)quinoline, was subsequently reacted with 1,3-dichloropropanol in 1-pentanol to yield HPQ in the third step. This three step synthetic approach provides an alternative and efficient process to synthesise HPQ. The research provides important and specific details for the synthetic methodology involved in the synthesis of HPQ for future synthetic and biological research.

"*Malaria is a disease that is both preventable and curable, yet a child dies of malaria every 30 seconds*". [4]

1 INTRODUCTION

1.1 Malaria

Malaria is a major global health issue leading to the death of up to 3 million people annually.[5] Almost 40% of the world lives under the constant threat of contracting the disease. In 2003, according to the World Health Organization 350–500 million people worldwide became ill with malaria.[6] Malaria is an infection caused by the parasitic protozoan of genera *Plasmodium* and transmitted into humans *via* the mosquito.

1.1.1 Cause of Malaria

Malaria is caused by the protozoan Plasmodium (*P*), four species of which infect humans. These are *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae*. These parasites differ in morphology and have different life cycles. Almost all malaria related deaths and disease are caused by *P. falciparum*.[7] *P. falciparum* is the most pathogenic species causing malaria in humans; the febrile attacks caused by its infection lasting 48 hours. The pathogenicity of *P. falciparum* combined with its ability to acquire resistance to multiple drugs makes it the most dangerous species to humans. Most deaths occur due to a complication of the infection by *P. falciparum* in which infected erythrocytes adhere to the vascular endothelium of the post-capillary venules in the brain.[8, 9] *P. vivax* is the most globally widespread form of malaria prevailing in Central America, the Middle East, India and various countries in South East Asia.[10] This infection is rarely fatal but has the ability to form dormant hypnozoites in the liver, which can cause relapse (*P. ovale* can form dormant hypnozoites though relapse is very rare [10]). Similar to *P. falciparum*, both *P. vivax* and *P. ovale* exhibit a 48-hour cycle of fever. *P. malariae* causes benign quartan malaria with a febrile cycle of 72 hours.[9, 10] Its distribution is similar to *P. falciparum* but it only causes a very mild form of infection.

1.1.2 Transmission of Malaria

The vector responsible for malarial transmission is the female Anophelene mosquito (Figure 1).[9] The mosquito serves as a co-host for the disease and part of the parasitic life cycle is completed within its gut. As the mosquito injects its proboscis into a human blood capillary it salivates to dilute the blood and injects both anticoagulants and the parasitic sporozoites into the bloodstream.

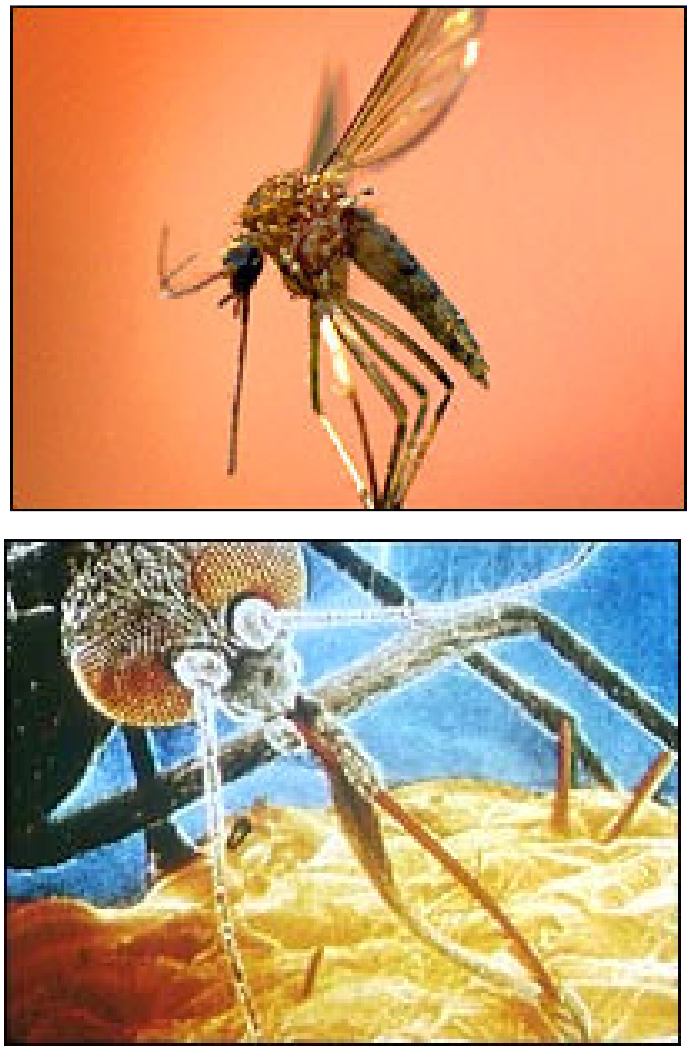

Figure 1 Female Anopheles mosquito

Malaria can also be transmitted by human blood transfusions and through the use of infected hypodermic needles though this is very rare.[7, 9]

1.1.3 Life Cycle of the Malarial Parasite

Figure 2 illustrates the lifecycle of the malarial parasite which begins in humans when an infected female mosquito bites, withdrawing blood and simultaneously injecting sporozoite-containing saliva directly into the bloodstream.[7, 9]

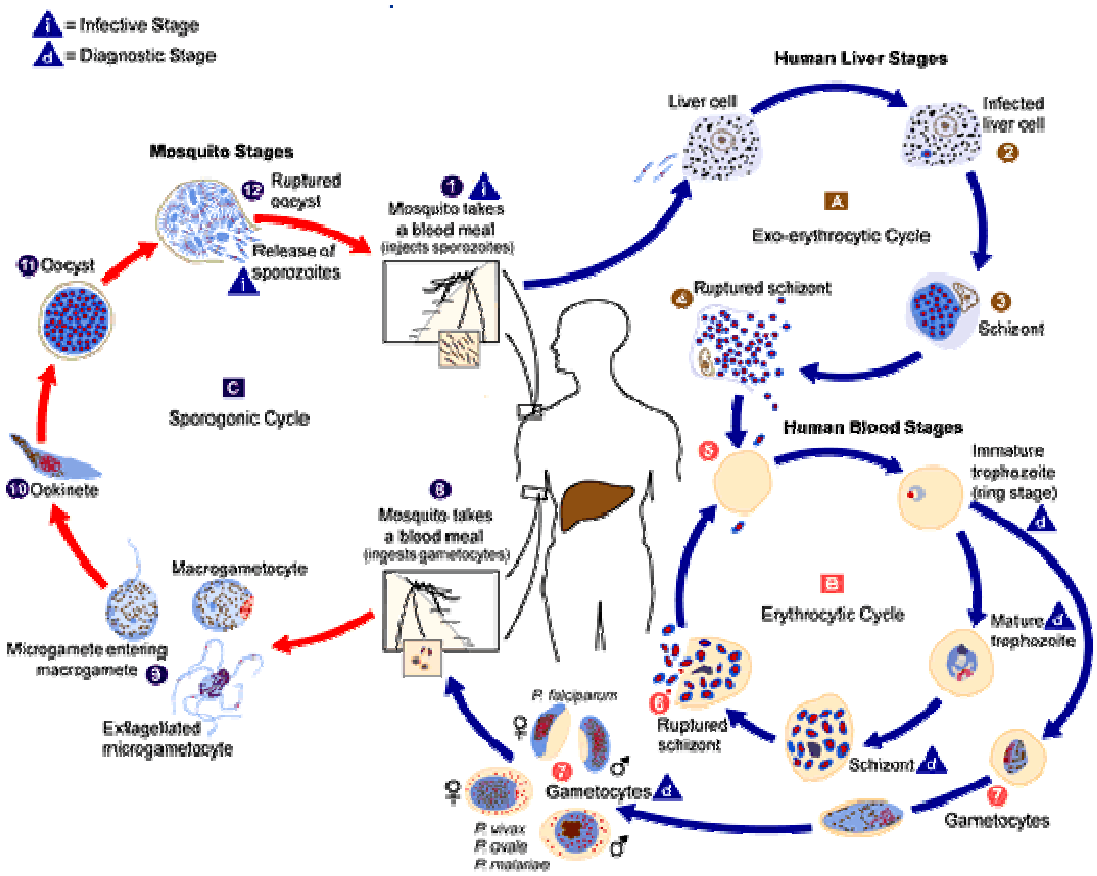

Figure 2 Life cycle of the malarial parasite [11]

With each bite the mosquito injects about 20 to 30 sporozoites into the circulatory system. The sporozoites multiply in the liver to form numerous merozoites.[7, 9] Within a week the merozoites re-enter the bloodstream where within the red blood cells (erythrocytes) they develop through the ring, trophozoite, and schizont stages (as shown on the right hand side of Figure 2). During these stages the infected erythrocyte does not come under attack from the immune system thereby shielding the parasite. Parasite growth is supported by the ingestion of the host's haemoglobin. The cycle of asexual division into daughter merozoites, their maturation to schizonts

and the release of toxins takes up to 48 hours and is accompanied by fever, which is typically cyclic. At this stage merozoites can invade new erythrocytes and the erythrocytic cycle recommences.[7,9] After several cycles, the intraerythrocytic parasites can develop into sexual stage gametocytes. These gametocytes are ingested when a mosquito bites an infected individual and the sexual life cycle commences in the mosquito gut where the gametocytes form fused oocytes. These oocytes can later form sporozoites that travel to the mosquito's salivary glands ready to reinfect another human host.[7-9]

1.2 Drugs employed in Malarial Therapy

The antimalarials drugs used globally are divided into the following five classes.[5]

- Quinolines (includes quinoline arylaminoalcohols)
- Antifolates
- Artemisinin derivatives
- Hydroxynaphthaquinones
- Antibacterial Agents

1.2.1 Quinolines

One of the first agents used for the treatment of malaria was the bark of cinchona tree, which was introduced to Europe from South America in the 17th century.[9] The active component contained in the bark is a quinoline based compound, Quinine (Figure 3). Quinine acts primarily as a blood schizonticide and it has little effect on sporozoites or pre-erythrocytic forms of malarial parasites.

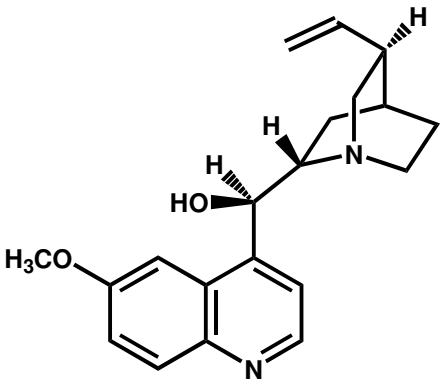

Figure 3 Chemical structure of Quinine

Quinolines which include for the most part the quinoline-based arylaminoalcohols can act on the malarial parasite *via* various pharmacological mechanisms. They are able to act on different stages of the parasitic life cycle. Chloroquine (Figure 4A) and other 4-aminoquinoline derivatives have been shown to inhibit DNA replication and RNA synthesis in the nucleus of the protozoal parasite.[8]

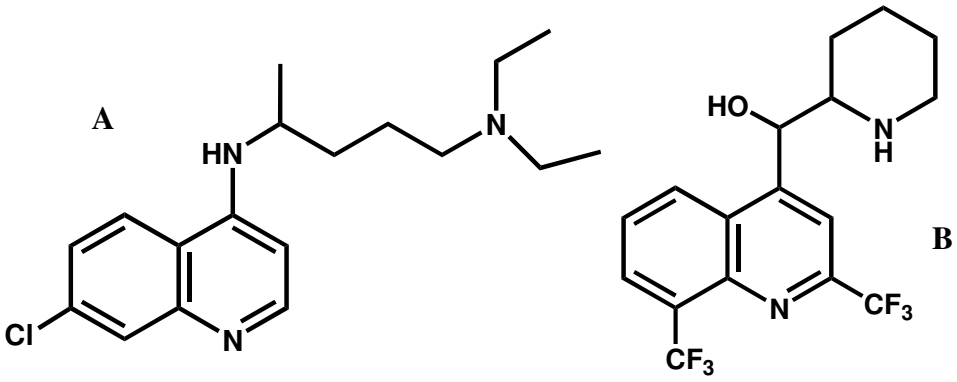

Figure 4 Chemical structures of Chloroquine (A) and Mefloquine (B)

Chloroquine is active on those stages of the parasitic life cycle in which the parasite actively degrades haemoglobin. Studies have shown swelling of the parasitic food vacuole and undigested haemoglobin in the parasitic endocytic vesicles suggesting that the food vacuole is the site of action of the quinoline antimalarials.[8, 12] The parasitic food vacuole is acidic in nature and the accumulation of basic compounds such as chloroquine and other aminoquinolines increases vacuole pH forcing unfavourable conditions for parasitic development and growth. The malarial parasite resides in the erythrocyte which contains high quantities of haemoglobin. The parasite feeds on the proteinicious globulin but the heme cofactor is very toxic to the parasite. To decrease the toxicity of heme, the parasite polymerises the heme to non-toxic haemozoin which is also known as malarial pigment. Chloroquine and most other quinoline antimalarials inhibit the heme polymerisation process thereby killing the parasite with an accumulation of toxic heme.[8, 13, 14] Mefloquine (Figure 4B) and Quinine are classified as quinolinementhols and have the same mechanism of action as chloroquine. Although mefloquine's mechanism of action is similar to chloroquine, studies suggest that the exact step in the parasite feeding process which it interferes with may be different.[8] The quinoline-based drug Chloroquine (CQ) will be discussed in detail in Section 1.3.

1.2.2 Antifolates

Dihydrofolate reductase (DHFR) and dihydropteroate synthase (DHPS) are key enzymes in parasitic *de novo* folate biosynthesis and their inhibition inhibits biosynthesis of pyrimidines and purines in the parasite.[1] Antifolate drugs inhibit either dihydrofolate reductase (DHFR) e.g. Pyrimethamine (Figure 5C) and Proguanil (Fig 5B), or dihydropteroate synthase (DHPS) e.g. Sulphodoxine. In *P. falciparum*, DHFR is a key enzyme in the redox cycle for the production of tetrahydrofolate which is required for essential parasitic thymidine and metheonine biosynthesis. The drugs Pyrimethamine and Proguanil act by inhibiting DHFR. Antifolate drugs are usually used in combination with other antimalarial agents e.g. 4-Aminoquinolines and Atavaquone. When DHFR or DHPS inhibitors are used alone resistance in parasites becomes rapidly evident due to single point mutations in the genes encoding for these enzymes.[15]

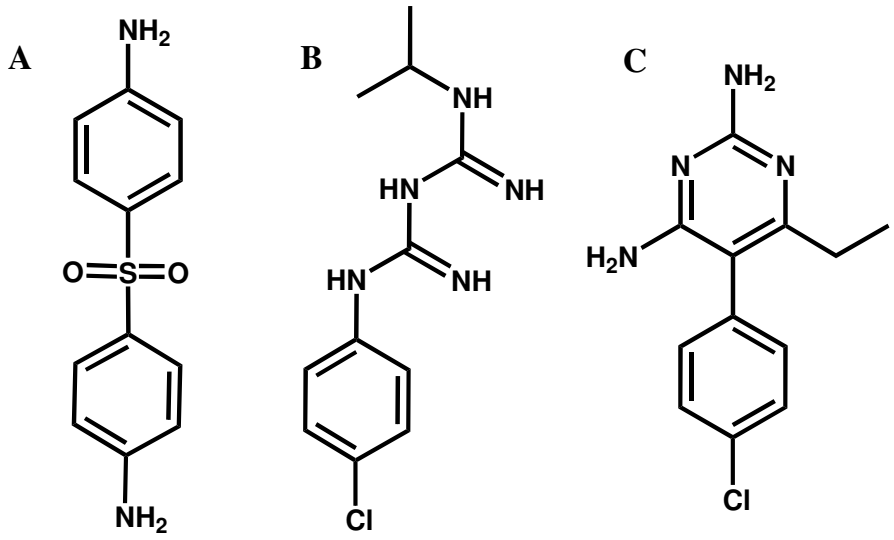

Figure 5 Chemical structures of Dapsone (A), Proguanil (B) and Pyrimethamine (C)

Pyrimethamine acts by inhibiting plasmodial dihydrofolate reductase (DHFR) whilst the sulpha drugs, with which it is combined e.g. Sulphodoxine, inhibit dihydropteroate synthase (DHPS). The concomitant blockage of dihydro folate synthesis and the inhibition of DHFR make the combination of DHPS and DHFR inhibitors synergistic – one inhibitor potentiates the activity of the other.[16] The new combination of Chlorproguanil and Dapsone (Figure 5A) known as the antimalarial formulation Lap Dap®[17] was developed to counterbalance the resistance to

Sulphodoxine-Pyrimethamine. These drugs were combined in order to obtain an antifolate combination with shorter *in vivo* residence time than Sulphodoxine - Pyrimethamine hence lowering the probability of selecting resistant parasites.[18] Clinical studies have shown good activity against strains resistant to the previous combination of Sulphodoxine–Pyrimethamine.[19] Resistance to antifolate drugs is due to an effective efflux mechanism that the parasite develops. In order to overcome this, new strategies such as the addition of Probenecid to the combination has been employed. This drug in particular serves to inhibit the drug efflux mechanism within the parasite.[19]

1.2.3 Artemisinin and its derivatives

The natural product Artemisinin (Figure 6) also known as Quinghaosu in China, (its country of origin) is derived from *Artemisia annua*, the sweet wormwood plant. A cold water extract of this plant has been used in traditional Chinese medicine for many years as a treatment for malarial fever.[1, 5, 20]

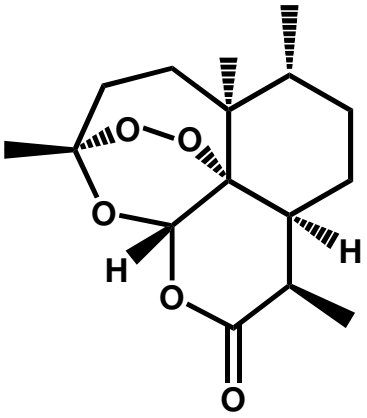

Figure 6 Chemical structure of Artemisinin

The active ingredient Artemisinin and its synthetic derivatives (artesunate, artemether, and arteether) have been used for the treatment of malaria since 1970 (when Artemisinin was first isolated).[1] Artemisinin reduces overall malaria transmission and decrease mortality rate due to rapid parasite clearance and targeting of gametocytes in the early developmental stage.[21] Artemisinin is biotransformed rapidly but does not

form any active metabolites. Artesunate and artemether are biotransformed to the active metabolite dihydroartemisinin (DHA) *in vivo* which has an elimination half life of approximately 1 hour. This ultra short elimination half life makes artemisinin derivatives unsuitable for sole therapy and enhances the opportunity of parasitic resistance. Artemisinin-based combination therapies (ACTs) were developed to overcome this issue and are currently the best anti-malarial drugs available.[20-22] Artemisinins have become very popular in combination therapies and as of 2005 have been adopted in national malaria campaigns by 43 countries.[4]

1.2.4 Hydroxynaphthaquinones

Hydroxynaphthaquinones are potent inhibitors of mitochondrial electron transport processes, which compete with the biological electron carrier ubiquinone.[23] In mammalian cells, ubiquinone-linked dehydrogenases are involved in energy generation *via* the synthesis of Adenosine triphosphate (ATP).[65] Atovaquone (Figure 7), a ubiquinone analogue binds to the cytochrome *bc1* complex of the parasite mitochondrial electron transport chain and inhibits cytochrome *c* reductase activity in *P. falciparum.* [1, 23]

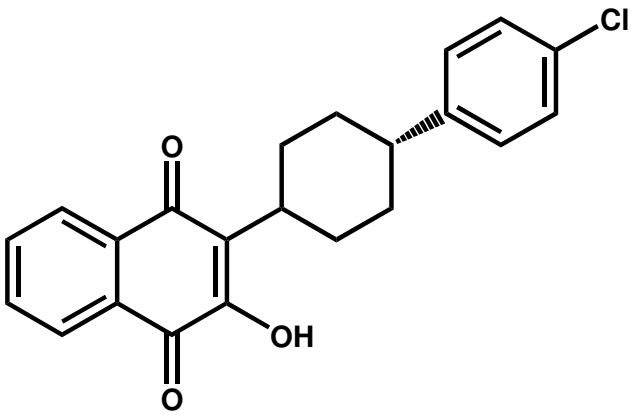

Figure 7 Chemical structure of Atovaquone

Atovaquone acts synergistically with Proguanil to collapse the parasite mitochondrial membrane potential.[23] This drug combination has been used very successfully in both malarial treatment and prophylaxis.[15] The site of action of hydroxynaphthaquinones is very different from other antimalarial classes. In spite of the above, resistance has emerged primarily due to point mutations in the cytochrome b gene of the parasite

which are much more rapid than the parasite mutations which confer chloroquine resistance.[23, 24]

1.2.5 Antibacterial Agents

Tetracycline (Figure 8) and Doxycycline are the most commonly used antibiotic antimalarials.[25] They have been used in combination mostly with quinine and chloroquine.[25-27] Antimalarial combinations with tetracycline have been shown to have good activity against both chloroquine resistant and chloroquine sensitive *P. falciparum* malaria.[25-27] However, combinations of tetracycline have not been proven safe in pregnancy therefore their use has been limited.[15] Various antibacterial drugs such as sulphonamides, clindamycin, rifampin and desferrioxamine have been used alone or in combination with quinolines but their activity is not exemplary when compared to the first line drugs e.g. Artemisinins.[25] Recent studies have shown that macrolide antibiotics such as erythromycin and azithromycin can also be used in combination with quinine and chloroquine.[25] The rationale for these combinations is the safety of macrolides in pregnancy.[28] Attempts to combine macrolides with artemisinin derivatives have not yielded satisfactory clinical results because of the marked antagonistic activity of these drugs.[29]

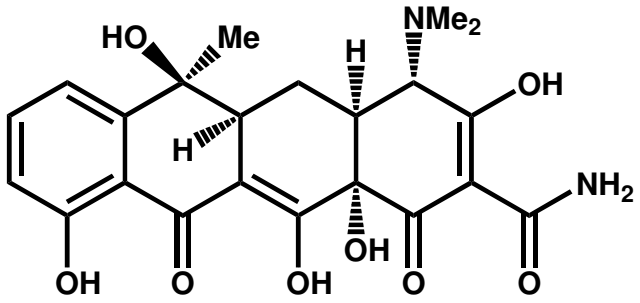

Figure 8 Chemical structure of Tetracycline

1.3 Chloroquine

Chloroquine (CQ, Figure 4A, Page 6) was introduced during the Second World War in 1943 by US researchers.[13] It was originally synthesized in 1934 by the Bayer Company (Germany) and was called Resochin.[30] Resochin was initially thought too toxic for clinical use and was ignored by the pharmaceutical establishment for a decade. However, during World War II allied troops captured a supply of the related drug Sontaquine, and began a re-evaluation of the two drugs. Resochin was found to be safe at therapeutic concentrations and was renamed chloroquine and underwent clinical trials in 1943. Since that time it has proved to be a highly effective, safe and a well tolerated drug for both the treatment and prophylaxis of malaria.[1, 13]

1.3.1 Chemical Properties of Chloroquine

CQ or 7-Chloro-4-(4-diethylamino-1-methylbutylamino)quinoline is a aminoquinoline alkaloid with potent antimalarial activity.[9] It is a weak base containing amine groups with pK_a (8.1, 9.9) values in the physiological range.[31] It exists in 3 different isoforms, the dextrorotatory *d*, levorotatory *l* and a racemic mixture of both *dl* all of which have equal potency.[9] CQ is slightly soluble in water, soluble in chloroform, ether and dilute acids. CQ is clinically available as chloroquine diphosphate, phosphate, hydrochloride and dihydrochloride.[32] The most common form, CQ diphosphate is readily soluble in water but practically insoluble in alcohol, methanol, ether and chloroform. Aqueous solutions for injection formulations of CQ often employ chloroquine diphosphate.[31]

1.3.2 Mechanism of Action of Chloroquine

CQ is a potent schizonticide, active against the erythrocytic forms of all strains of plasmodia that cause malaria in humans.[7, 9, 13] It has some gametocytocidal activity in *P.vivax*, *P. ovale* and *P. malariae*, but does not have an effect on sporozoites or hypnozoites within the liver.[13] Despite extensive research, the definitive mechanism of action of CQ is still unknown, although a number of facts related to its action are now generally accepted.[8, 14] It is known that CQ accumulates in high concentrations in the plasmodium food vacuole *via* an ion trapping mechanism and inhibits

detoxification of heme.[33] It is therefore believed that this acidic compartment is where the compound exerts its antimalarial effect. A number of hypotheses have been raised to explain the mechanism of action of quinoline and related compounds. It was demonstrated that the 4-aminoquinoline antimalarials were able to interact with both mammalian and malarial parasite DNA *in vitro*.[34,35] It is thought that binding to the parasitic DNA prevents both RNA synthesis and DNA replication which leads to cell death. However, this does not explain the ability of CQ to kill parasites at concentrations much lower than the concentration at which it is toxic to mammalian cells.[8]

Another hypothesis relating to its pharmacological activity is the inhibition of parasite feeding. As the parasite matures within the host erythrocyte, it ingests small packets of haemoglobin by endocytosis.[13, 36] These haemoglobin containing vesicles are transported to the parasite's acidic food vacuole where the outer membrane of the double membrane is digested by a series of proteases. Ferriprotoporphyrin IX (FP) is freed during the breakdown of the haemoglobin. CQ inhibits the biocrystallization of FP to haemozoin and also inhibits the normal degradation of accumulated FP by reduced glutathione.[9, 37] Thus the changes seen shortly after the treatment of malarial parasites with pharmacological concentrations of the drug include swelling of the parasitic food vacuole and accumulation of undigested haemoglobin in the endocytic vesicles.[12] CQ is thought to act by selectively targeting the parasite and inhibiting the parasite specific process of haemoglobin degradation.[8] CQ forms complexes with free FPIX which cannot be detoxified in the parasite by polymerization.[13]

1.3.3 Pharmacokinetics of Chloroquine

CQ is well absorbed orally, intramuscularly and subcutaneously in both healthy and diseased adults and children.[38] It has a bioavailability of 70 to 80% when administered orally.[39] Half time of absorption is 0.56 hours and peak plasma concentration is reached 1.5 to 3 hours after ingestion. It is well distributed throughout the tissues and becomes concentrated in the infected erythrocytes. CQ has also been shown to exist extensively in kidney, liver, spleen and lung tissue and is strongly bound to melanin containing cells.[30] It has a very high apparent volume of distribution (between 100 and 1000 L/kg) and is 50 to 65% protein bound. CQ is

extensively metabolised by cytochrome P450 isoenzymes 2C8, 3A4 and 2D6 in the liver.[38] The major metabolite desethylchloroquine retains the antimalarial activity of chloroquine.[38, 40, 41] The major route of CQ excretion is by a relatively slow hepatic metabolism combined with renal excretion. It is excreted very slowly; about 55% is excreted in the urine and 19% in the faeces.[30] CQ has a long, highly variable half life between 8 and 58 days. The long *in vivo* residual time of CQ is believed to lead to drug resistance.[7, 9, 41]

1.3.4 Therapeutic Uses of Chloroquine

CQ is taken both prophylactically and as a curative treatment for malaria. For the treatment of an adult, 600 mg base is given followed by 300 mg base 6 hours later. The regimen is then 300 mg base per day at 24 and 48 hr. In the treatment of a child, 10 mg/kg base is given (maximum 600 mg). This is followed by 5 mg/kg base 6 hours later (maximum 300 mg) and 5mg/kg per day base at 24 and 48 hours. For malarial prophylaxis in adults 300 mg base is given orally per week beginning one week before and continuing for 4 weeks after exposure.[1]

1.3.5 Resistance to Chloroquine

The most significant problem relating to CQ is parasitic drug resistance. Its long half life and natural selection pressure on the parasite population has contributed significantly to CQ resistance.[42, 43] CQ resistance is caused by increased drug efflux from the parasitic food vacuole or altered uptake rate.[8] There are several potential explanations for the altered uptake rate, reflecting changes in the transmembrane pH in resistant parasites, altered membrane permeability characteristics, and, paradoxically, an efflux pump operating prior to cytosolic appearance of drug. Two mdr like genes (multi drug resistant), pfmdr1 and pfmdr2 have been found in *P. falciparum*.[8] Some evidence has suggested that mutations in pfmdr1 might be associated with resistance.[8] CQ resistance can also be caused by reduced affinity of CQ for heme thereby reducing CQ uptake. Initially CQ resistance was thought to be genetically attributed to cg2, a gene encoding for the polymorphic protein located at the periphery of the parasite.[13] This mechanism may result in an increased drug efflux or decreased drug influx leading to a decrease in the accumulation of CQ in drug

resistant Plasmodium.[43] However, this hypothesis has been ruled out by the transformation studies performed recently by Fidock *et al*.[44]

1.4 Piperaquine

Piperaquine (PQ, Figure 9) is a bisquinoline antimalarial drug that was first synthesised independently by both the Shanghai Pharmaceutical Industry Research Institute in (China) and the Rhone Poulenc Company (France) in the late 1960s.[45] It is effective against *P. vivax* and *P. falciparum*, including strains of *P. falciparum* resistant to chloroquine. It is available as a free base and a tetraphosphate salt. Bisquinolines have been extensively studied in recent years because of their potent antimalarial activity against chloroquine resistant strains.[2]

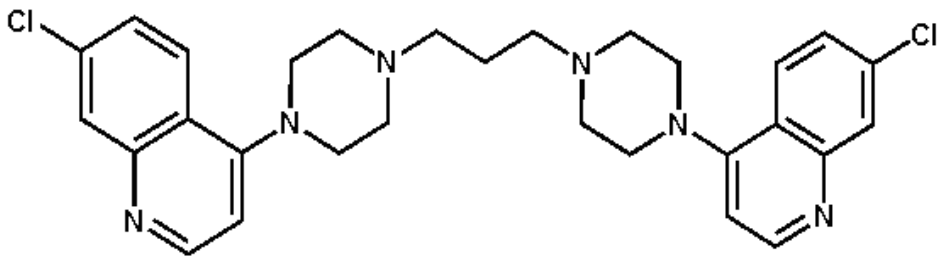

Figure 9 Chemical structure of Piperaquine

1.4.1 Chemical Properties of Piperaquine

Piperaquine (PQ) or 1, 3-bis [1-(7-chloro-4 quinolyl)-4-piperazinyl] propane (MW 535.51) is a member of the 4-aminoquinoline group that includes CQ. PQ in the free base form is a pale white to yellow crystalline powder with a melting point of 212–213°C.[46,47] It is a hexabasic compound (pK_a = 8.92) that is only sparingly soluble in water at neutral and alkaline pH, but has high lipid solubility (log P = 6.16).[48] PQ is usually formulated as its water soluble tetraphosphate salt (MW 927.48)-a white to pale yellow crystalline power, freely soluble in water. It has a slightly bitter taste and a melting point of 246–252°C.[2] The compound is known to be photosensitive in aqueous solution turning from yellow to deep orange on prolonged light exposure.[2]

1.4.2 Mechanism of Action of Piperaquine

Electron microscopy studies undertaken to study the effect of PQ on *P. berghei* ANKA strain have shown that the first morphological changes are swelling of the

food vacuole, vesiculation and accumulation of undigested haemoglobin.[49] This suggests that the action of PQ involves blockage of the function of the food vacuole. CQ and bisquinolines such as PQ are weakly basic drugs and readily accumulate in the acidic parasitic food vacuole by several different mechanisms e.g. active transport and diffusion along a pH gradient. When present in the food vacuole, CQ and the bisquinolines compete for the heme substrate. CQ has been shown to interact tightly with free heme and is known to induce premature termination of haemozoin polymers. Bisquinolines which are essentially two quinoline moieties linked together may chelate heme more efficiently and inhibit the catalase activity of heme to an even greater extent.[50] The greater efficacy of the bisquinolines against CQ resistant parasites has been explained by the greater number of protonation sites compared with CQ which may allow these derivatives to be accumulated in higher concentrations within the CQ resistant parasites.[13, 51]

1.4.3 Pharmacokinetics of Piperaquine

The pharmacokinetic properties of PQ were only studied very recently despite its availability as a first line antimalarial agent in China for over three decades. The first pharmacokinetic data for PQ in humans was published by Hung *et al* in 2004.[52] They reported that the absorption of PQ was slow, with mean absorption half times of 9.1 and 9.3 hours in adults and children, respectively. Slow absorption can likely be attributed to the high lipophilicity of PQ. A more recent study by Davies and Illet at the University of Western Australia has shown the effects of food on absorption of PQ.[52] They concluded that piperaquine absorption is increased in the presence of a high fat diet.[53] They also established that the mean terminal elimination half-life was long in both adults (543 hours) and children (324 hours), whilst the mean volume of distribution at steady state/bioavailability (Vss/F) was very large in adults (574 L/kg) and children (614 L/kg). Plasma protein binding of PQ has not been measured but is estimated to be around 97%.[49] The metabolism of PQ in humans has not been studied in detail. However, since PQ has no primary functional groups that could generate Phase 2 polar metabolites, it seems likely that a Phase I oxidative process occurring on either the quinoline or PQ ring structures may occur during its metabolism.[2]

1.4.4 Therapeutic Uses Of Piperaquine

PQ phosphate was first used for human antimalarial prophylaxis in China in 1978.[2] The standard clinical regimen dose employed was; 1.5–3.0 g PQ base given in divided doses over 2 or 3 days. PQ was highly active against both chloroquine sensitive and chloroquine resistant species of *P. falciparum*.[54] Due to fears related to the development of resistance and reports concerning cross-resistance between PQ and CQ, combination therapies with artemisinin derivatives were developed. PQ based artemisinin combination therapy was developed in the form of CV8 in Vietnam, a formulation containing PQ, dihidroartemisinin, trimethoprim and primaquine.[55] This combination has certain advantages over artesunate- mefloquine but has limited use because of lack of safety data in children and pregnancy. A new combination of piperaquine 'Artekin' which contained dihidroartemisinin (DHA) and PQ phosphate was developed to reduce cost and toxicity.[15, 56] Studies in children and adults in Cambodia showed very good activity and safety for this drug combination.[56]

1.4.5 Resistance to Piperaquine

Clinical studies have shown some degree of cross resistance between CQ and PQ.[13, 57] Resistance to PQ follows the same routes as CQ and is thought to have developed as a consequence of decreased drug accumulation.[14] Processes changing the rate of influx of the drug such as changes in pH or a specific transport mechanism may decrease drug accumulation.[58] Another mechanism is the active efflux of the drug from the food vacuole. Initially when bisquinolines such as PQ and hydroxypiperaquine (HPQ) were developed it was thought that their bulky structure would inhibit the efflux mechanisms developed by the parasite.[14, 58] It has been suggested by Vennerstrom *et al* that the bulky structure may not be recognized by proteins involved in conferring quinoline resistance.[50]

1.5 Hydroxypiperaquine

Hydroxypiperaquine (HPQ, Fig 10) is a bisquinoline antimalarial drug first synthesized by the Chinese group led by Xu Dingqiu in 1971.[3] HPQ displays very similar antimalarial activity to its parent compound PQ, both drugs show rapid and effective antimalarial activity.[3]

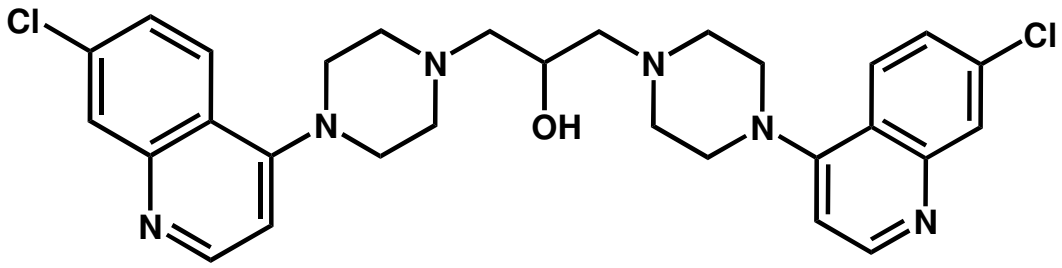

Figure 10 Chemical structure of Hydroxypiperaquine

1.5.1 Chemical Properties of Hydroxypiperaquine

HPQ base is a white or yellowish white crystalline powder, which is both odourless and tasteless. It has a melting point of 177-178.5°C. Its water soluble phosphate salt, HPQ phosphate is a pale yellow crystalline powder. It is also odourless, but with a slightly bitter taste and a melting point 237-238°C.[3]

1.5.2 Mechanism of Action of Hydroxypiperaquine

Chinese studies have shown HPQ to be highly effective schizonticide with rapid action against asexual stages of sensitive strains of P. *falciparum* and *P. vivax*.[54] It has also been demonstrated that, HPQ displays very good activity against CQ resistant *P. falciparum*.[54] The mechanism of action is believed to be similar to that of CQ and other bisquinolines although no formal studies have been undertaken.[3, 8, 58, 59] *In vitro* studies have reported that HPQ displays more rapid action than PQ on *P. berghei.* However, its physiological effect on the parasitic fine structure was found to be similar to that of PQ.[59]

1.5.3 Pharmacokinetics of Hydroxypiperaquine

Pharmacokinetic and pharmacodynamic studies of HPQ have not been published in the non-Chinese scientific literature. All scientific and clinical data available on the drug has been published in Chinese scientific literature (mostly written in Chinese). Pharmacokinetic data is not available, but it is believed that HPQ unlike PQ would possess a shorter biological half life due to its additional hydroxyl group. By virtue of this polar group, HPQ appears to be a prime candidate for Phase 2 metabolic conjugation processes e.g. glucuronidation and sulfation, which may significantly increase its biological clearance rate thus reducing its biological half life (Figure 11).

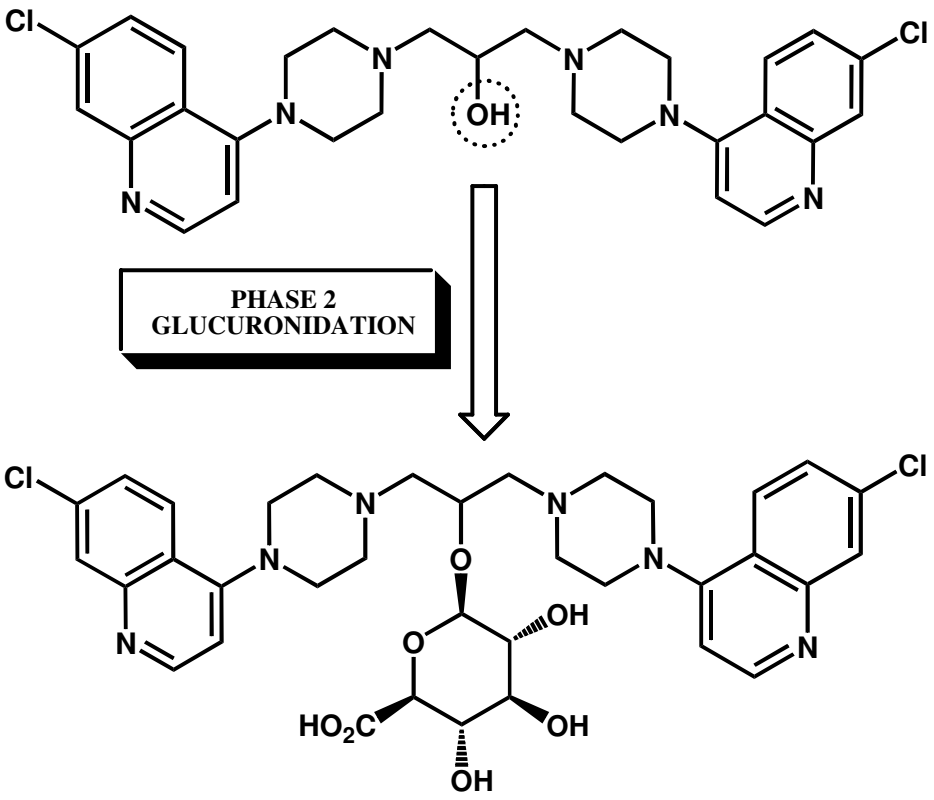

Figure 11 Phase 2 glucuronidation of HPQ

1.5.4 Therapeutic Uses of Hydroxypiperaquine

Initial reports on the clinical application of HPQ were reported in 1973, involving field studies undertaken in the South Western Yunnan province of China.[3,60] Between

June and October 1973, 93 patients infected with acute *P. falciparum* malaria were treated with HPQ at a total dose of 1.5 g over three days. CQ phosphate at the same dose was given as a reference drug to some patients. The study reported that the cure rate for hydroxypiperaquine was 98.33%.[60] HPQ was more effective than CQ with a difference in fever and parasite clearance time which was statistically significant.[60] Its side-effects were few, and included somnolence, nausea and dizziness. When compared to CQ the intensity of the side-effects experienced were milder.[60] Following this study, trials were performed with the drug against P. *vivax* malaria from 1974-1977 in the malaria endemic villages of Central China.[57] Approximately 700 patients infected with P. *vivax* malaria patients were treated with 1.2 g HPQ orally, (divided in two doses) and 350 reference patients were given oral chloroquine. However, results suggested no difference in mean parasite clearance time for both drugs.[3,57] The toxicity observed with CQ, e.g. retinal toxicity, occurs largely because of its long-half life and high plasma concentrations. It is known that the hydroxylated derivative of chloroquine, hydroxychloroquine is 2-3 times less toxic than the parent CQ.[61] The introduction of a hydroxyl group into one of the *N*-ethyl groups of chloroquine gives hydroxychloroquine reduced toxicity by introducing a readily available functionality that could undergo glucuronidation leading to detoxification and more rapid excretion.[61] A similar increase in the safety profile would be expected in HPQ due to its additional hydroxyl group.

1.5.5 Resistance to Hydroxypiperaquine

Initial *in vitro* studies on *P. falciparum* performed in China and Vietnam reported that HPQ showed cross resistance to CQ only at a very low level.[3, 13] Clinical trials undertaken in Yunnan province (1975) showed HPQ cured the CQ resistant cases of *P. falciparum* malaria in the control wing.[60] A similar trial was performed in Hainan Island were CQ resistant *P. falciparum* malaria is known to be common. Confirmed CQ resistant cases were selected for treatment with HPQ and the study showed a cure rate of 90.62%.[3] This is in contrast with several studies which have shown marked cross resistance between CQ and bisquinolines.[50,58,62,63] Experimental resistance models have been developed in China for resistance studies on chloroquine, piperaquine and HPQ.[59] All three resistant lines developed in ANKA strains of *P.*

berghei exhibited a clear cross resistance to artesunate.[59] Cross resistance between PQ/CQ and HPQ/CQ was not significant.[59]

1.5.6 Synthesis of Hydroxypiperaquine

The synthesis and chemical properties of HPQ have only been very briefly described in a review published by Xu *et al* in 1988.[3] This review was published in an obscure medical research journal (Journal of Medical College of the People's Liberation Army) and represents the only published synthetic work undertaken on the drug HPQ. The review article briefly describes a three step scheme for the synthesis of HPQ (Figure 12, reproduced from review published by Xu *et al*[3]). Essential details such as molar ratios of the reactants, reaction conditions and solvents were not specified for any of these steps. Brief details regarding the chromatographic (TLC and HPLC) and spectral (UV, IR, ^{1}HNMR) analysis of HPQ are provided in a brief section but with virtually no information relating to the analytical procedures. There have been no published reports on the reproducibility of this synthesis or the validity of the analytical data.

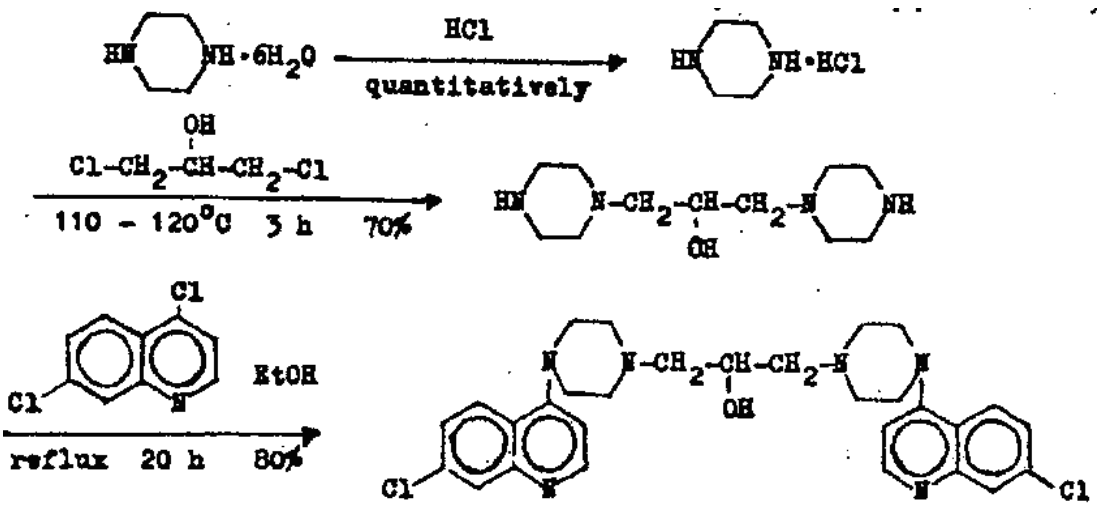

Figure 12 Synthetic scheme for Hydroxypiperaquine (reproduced)[3]

HPQ was synthesised by what appears to be at first glance a simple three step reaction process (Figure 12). In the first step, piperazine was converted to its hydrochloride salt using hydrochloric acid. In the second step, 1,3 dichloropropanol (1,3-DCP, the alkyl linker unit) was reacted with the piperazine salt to generate the bispiperazinyl-2-hydroxypropane intermediate. For the third and final step, 4,7- dichloroquinoline

(4,7-DCQ) was reacted with the bispiperazinyl intermediate in refluxing ethanol for 20 hours. Whilst the synthetic process appears to be uncomplicated and straightforward, previous attempts at reproducing this approach have failed in our laboratories.[64] The most common problems encountered were the formation of the monosubstituted piperazinyl chloropropanol and the presence of inseparable starting materials-especially piperazine (in large quantities after the 2nd step). Both by-products and starting materials were virtually impossible to remove from the reaction mixture in some instances, and in various steps resulted in low yields of unacceptable impure compounds.[64] In addition, the method is difficult to monitor from a standard chromatographic standpoint e.g. TLC with UV detection, due to a lack of a chromophore in reactants and products in the first two synthetic steps.

1.6 Aim of the Research Project

The primary aim of this project was to synthesise the antimalarial drug HPQ *via* alternative pathways to those described previously. The major objectives of the research program were to explore various synthetic strategies based on curent literature synthetic procedures involving similar compounds. The research was to explore various synthetic routes to generate the antimalarial compound HPQ – initially *via* methodology utilising *N*-monoprotected derivatives of piperazine. To accomplish this, a range of *N*-monoprotected derivatives of piperazine was to be synthesised. The *N*-monoprotected derivatives of piperazine were employed in turn to synthesize a range of *N*-monoprotected piperazine bis-adducts containing the 2-hydroxypropane linker unit. These bis-adducts should be easy to deprotect (i.e. remove the protecting group) using established literature methods. This deprotection step would be followed by a coupling reaction (catalysed by base) with 4,7-dichloroquinoline (4,7-DCQ) to generate HPQ (Figure 13).

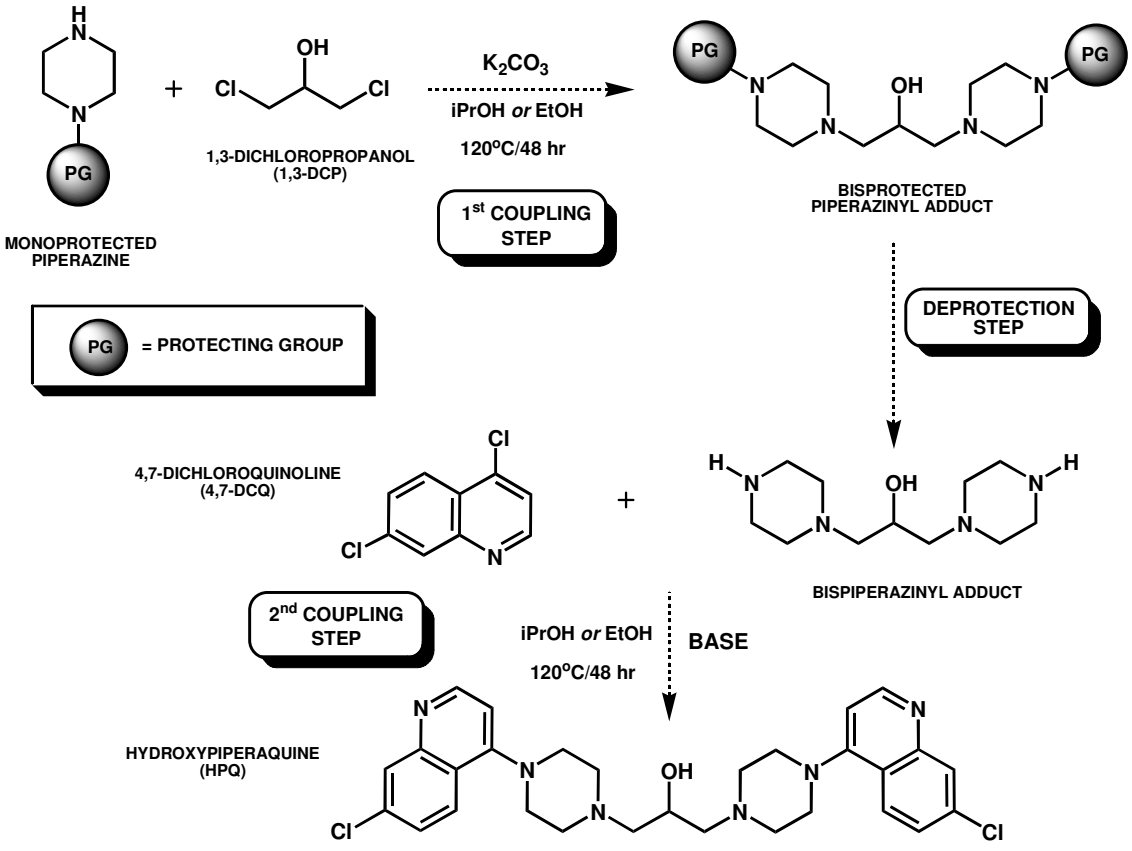

Figure 13 Proposed synthesis of HPQ using protected piperazine and 1,3-DCP

As an alternative approach, the synthesis of HPQ *via* the coupling of 4,7-DCQ and piperazine in the first step was to be considered (Figure 14). Piperazine, upon reaction with 4,7-DCQ has two possible amino reactive sites, which can lead to the formation of an array of compounds e.g. unreacted piperazine, mono and disubstituted piperazinyl adducts. To overcome this potential problem, suitably mono *N*-protected derivatives of piperazine could be utilised e.g. tertiarybutoxycarbonyl piperazine (tBOC-piperazine), benzylpiperazine and ethoxycarbonylpiperazine. These reagents, which already have one end of the piperazine molecule effectively blocked rendering it unreactive thus ensuring the reaction with 4,7-DCQ generates a single product namely a mono-*N*-protected piperazinyl quinoline. This product should then be easily deprotected and reacted with a half molar equivalent of 1,3-DCP to yield HPQ.

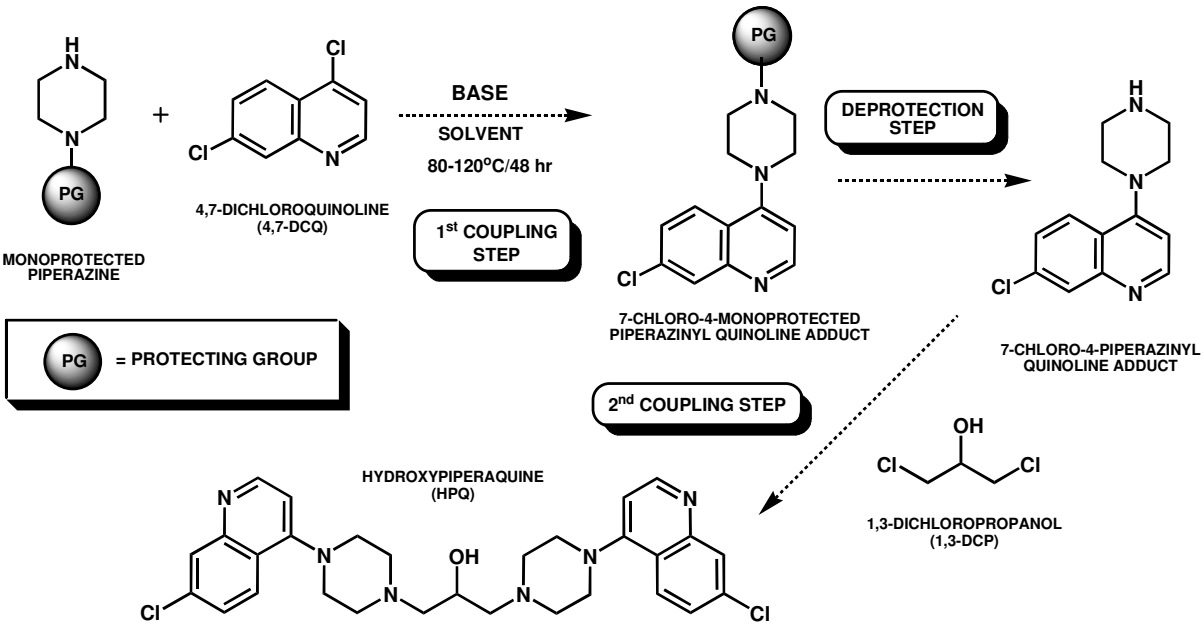

Figure 14 Proposed synthesis of HPQ using protected piperazine and 4,7-DCQ

For a protecting group to find wide application in organic synthesis, it must fulfil several criteria. In particular it must be selectively introduced into the molecule to be protected under mild conditions and in high yield; functional groups other than that to be protected must not be attacked. It should be stable under all the conditions used

during subsequent synthetic steps, including purification steps, until its deprotection is necessary. [65] It should be introduced and removed with the help of readily available reagents and should be easily purified. Only a few protecting groups meet all of these demands, and in most cases a compromise must be found, in which the most important criteria are addressed.[66] Benzyl groups can be commonly used for the protection of alcohols, carboxylic acids, amines, and diols.[65] They can be removed under fairly mild conditions e.g. hydrogenolysis. Ethoxy carbonyl group and tertiary butoxy carbonyl (tBOC) group are very commonly used in synthetic reactions to protect amines. However, they are acid labile protecting groups and readily removed by acid-mediated hydrolysis using strong acids.[65, 66] Among a number of protecting groups, tBOC is considered to be one of the most useful protecting groups. Its popularity is largely due to its ease of addition and removal.[65] It has several advantages such as its stability to hydrolysis in basic conditions and inertness to many nucleophilic reagents.[67] As stated above, reagents required for its removal must be acidic. A variety of reagents have been employed which include hydrochloric acid in ethyl acetate, sulphuric acid in dioxane, anhydrous hydrogen fluoride, boron trifluoride and trifluoroacetic acid.[65]

1.6.1 Significance of the Research

The research proposed, if successful, will be significant in many respects. Upon future publication this research will represent the only detailed synthetic procedure for the antimalarial HPQ. In addition HPQ generated during this program will be biologically tested within the School of Pharmacy's antimalarial research program (under the supervision of Dr Kevin Batty) to generate significant biological data regarding the compound. This data will supplement research data acquired from the School of Pharmacy's ongoing PQ research program. HPQ as an antimalarial has potentially numerous advantages over piperaquine. The additional hydroxyl group in HPQ appears to make it a prime candidate for Phase 2 metabolic processes which should increase its clearance rate thus reducing its biological half life. Theoretically the long half life of PQ is a major issue with regard to possible future malarial resistance thus diminishing the drug's therapeutic use. In addition to this, hydroxyl group present in HPQ should in theory be readily derivatized to afford a range of simple ester prodrugs.[68, 69] Phosphate and succinate prodrugs of HPQ should

significantly increase the water solubility of the drug. The issue of low water solubility is a recognised problem with both HPQ and PQ.

2 MATERIALS & METHODS

2.1 General Details

All reagents, starting materials and solvents were purchased from the Sigma-Aldrich Chemical Company, unless otherwise stated. These materials were used without further purification. Solvents used were of laboratory grade and reactions were performed using Quickfit^{TM} glassware. Fume hoods were used when necessary, and stirrer heating mantles were employed for heating methods. Propylene glycol oil baths were employed for the most part. ^{1}H NMR (Nuclear Magnetic Resonance) spectroscopy was performed on a Varian Gemini 200 MHz spectrometer (School of Applied Chemistry, Curtin University of Technology). Deuterated solvents including methanol (CD_3OD), chloroform ($CDCl_3$), dimethyl sulfoxide (DMSO-d6) and deuterium oxide (D_2O) were employed as NMR solvents. All chemical shifts (δ) are quoted in parts per million (ppm) and referenced against solvent peaks in the sample e.g. $CHCl_3$ at δ7.27 for $CDCl_3$. Thin layer chromatography (TLC) analysis was performed on 20 × 20 cm precoated silica gel, aluminium backed plates, impregnated with a fluorescent indicator (254 nm). A Spectroline model CM 10 florescence analysis cabin fitted with short and long wavelength UV lamps was used to view the TLC plates. Flash column chromatography was performed using silica as an adsorbent. Vacuum solvent evaporation was performed using a Buchi R-200 rotary evaporater.

2.1.1 Synthetic Approaches to the 1,3-(Bispiperazinyl)-propan-2-ol Intermediate using Mono-*N*-Protected Piperazine Derivatives

The synthetic methods employed in attempts to prepare the intermediate 1,3-(bis piperazinyl)-propan-2-ol are described below. The methodology is similar to that outlined in the initial stages of the synthetic process described by Xu *et al.* [3] However, our attempts would utilise mono-*N*-protected piperazine derivatives not piperazine. For the first step of our alternate approach, efforts were made to synthesize a range of *N*-mono protected piperazine derivatives. These derivatives were to be used, in the second synthetic coupling step with 1,3-dichloropropanol (1,3-DCP) to generate 1,3-(bis (*N*-protected)piperazinyl)-propan-2-ol. Subsequent deprotection of the protected adduct using a suitable acidic medium should yield the desired intermediate 1,3-(bispiperazinyl)-propan-2-ol. It was initially anticipated that the above approach would yield materials relatively free from impurities which would only require minor purification by recrystallisation.

2.1.1.1 Synthesis of 1-benzylpiperazine dihydrochloride [70]

1-Benzylpiperazine dihydrochloride was synthesised in a two step reaction based on the method as described by Cymerman *et al.* [70]

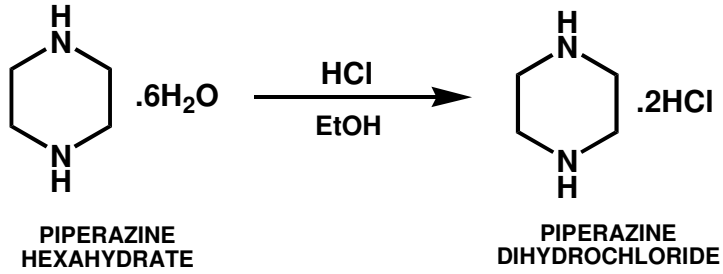

Figure 15 Synthesis of Piperazine Dihydrochloride salt

To a solution of 12.521 g (2.50 mmol) of piperazine hexahydrate in 50 mL of absolute ethanol, 16.25 mL (161.2 mmol) of hydrochloric acid (10M) was added dropwise using a Pasteur pipette (10M HCl: piperazine hexahydrate in the molar ratio of 1 : 2.5). The reaction flask was cooled in an ice bath to maintain a temperature of 25°C. After stirring for 20-30 minutes, the contents of the flask were cooled to about 0°C, and the crystalline white solid yielded was collected by vacuum filtration and washed

with two 25 mL portions of ice-cold absolute ethanol. The identity of the solid was confirmed as piperazine dihydrochloride salt by ^{1}H NMR spectroscopy (D_2O).[70] An ethanolic solution (50 mL) of piperazine hexahydrate (3.285 g, 0.65 mmol), was warmed to 65°C using a hot water bath. To this solution, 3 g (0.51 mmol) of piperazine dihydrochloride was added with stirring. The solution was vigorously stirred for 5 min during which time benzyl chloride (2.136 g, 0.25 mmol) was added *via* a Pasteur pipette.

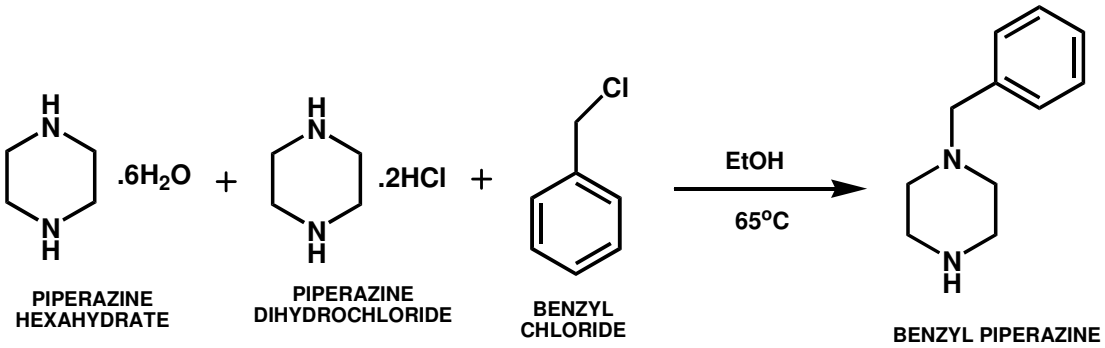

Figure 16 Synthesis of 1-Benzylpiperazine

The separation of white needles started almost immediately. The solution was stirred for an additional 25 minutes at 65° C, and then cooled to room temperature. The solution was allowed to stand in an ice bath for about 30 minutes. Crystalline piperazine dihydrochloride monohydrate was collected by vacuum filtration. The unreacted salt was washed with three 10ml portions of ice-cold absolute ethanol and dried. The combined filtrate and washings from the piperazine dihydrochloride were cooled in an ice bath to 0°C and treated with 25 mL of absolute ethanol saturated with concentrated hydrochloric acid. After the solution was mixed, it was allowed to stand for 10-15 minutes in the ice bath. The precipitated white plates formed were collected *via* suction filtration, washed with diethyl ether, and dried by rotary evaporation. TLC analysis and a ^{1}H NMR spectrum (D_2O) confirmed that the crystalline plates formed were the product required, 1-benzylpiperazine dihydrochloride.

2.1.1.2 Attempted Synthesis of 1-ethoxycarbonylpiperazine[71-73]

1-Ethoxycarbonylpiperazine was attempted according to the method described by Krys'ko *et al.* [72]

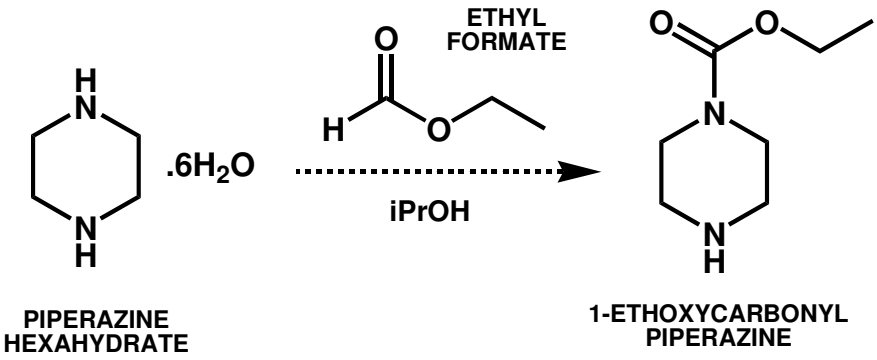

Figure 17 Synthesis of 1-ethoxycarbonylpiperazine [72]

An aqueous solution (50 mL) of 5 g piperazine hexahydrate (1 mmol) was acidified with acetic acid to pH 4–5 and cooled to 0°C. To this solution was slowly added a solution of 15 mL (185 mmol) ethyl formate in 50 mL of isopropanol. The mixture was stirred for 2 h and diluted with water to 150 mL. The excess of ethyl formate was extracted with two portions of 100 mL hexane. The remaining aqueous solution was neutralized with aqueous $NaHCO_3$ to pH 7, and an organic extraction was performed with chloroform. The chloroform extracts were combined, washed sequentially twice with water (75 mL) and saturated NaCl (75 mL), then dried over anhydrous sodium sulphate (Na_2SO_4). The drying agent was separated by filtration, and DCM evaporated to give a residue that was further dried in a vacuum oven to yield a golden brown oil. However, TLC analysis of the oil showed multiple spots and comparison of ^1H NMR spectral data from the literature source clearly showed that the reaction had failed to generate the desired product (mostly starting materials present).

2.1.1.3 Attempted coupling reaction of 1-benzylpiperazine and 1,3-dichloropropanol

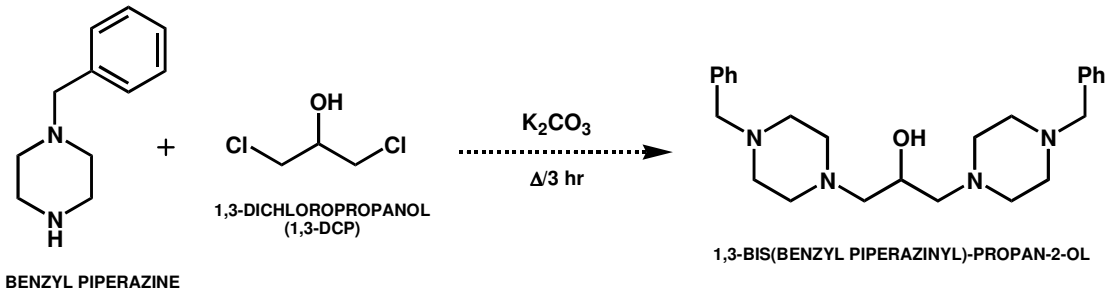

Figure 18 Synthesis of 1,3–bis(benzyl piperazinyl)propan–2-ol

The product of the procedure 2.1.1.1, 1-benzylpiperazine dihydrochloride 498.366 mg (2 mmol) was dissolved in 20 mL of isopropanol.[74] The solution was stirred with 691 mg of K_2CO_3 (5 mmol) and 100 µL of 1,3-dichloropropanol (1 mmol). The mixture was refluxed for 3 hr. TLC was performed at regular intervals but after 3 hrs the TLC analysis did not show any new spots for the product. Therefore, the reaction was allowed to run for an additional 24 hrs. TLC analysis after 24 hr showed a new compound spot. The reaction mixture was mixed with DCM (50 mL) and undissolved inorganic salts (e.g. K_2CO_3) were removed *via* filtration. The filtrate obtained was evaporated to dryness using a rotary evaporater to remove all solvent (isopropanol and DCM). However, ^{1}H NMR spectrum (D_2O) of the white solid obtained did not show the presence of the desired bispiperazinyl product required.

2.1.1.4 Attempted coupling reaction of tertiary butoxycarbonyl piperazine(t-BOC-piperazine) and 1,3-dichloropropanol (1,3-DCP) in ethanol

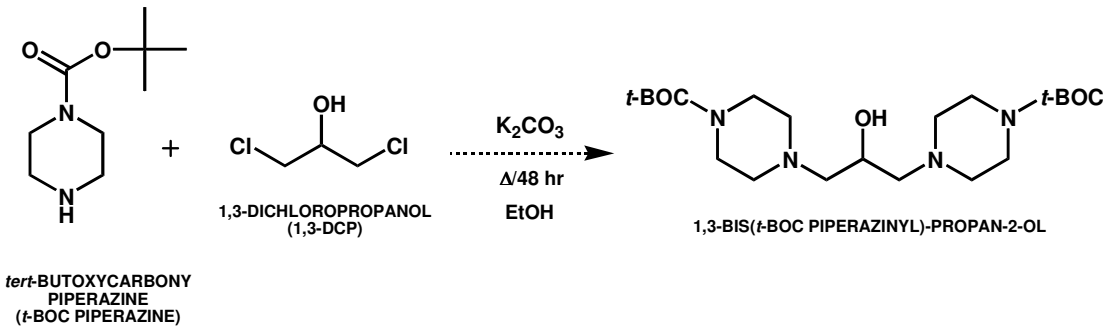

Figure 19 Synthesis of 1,3–Bis(tBOC-piperazinyl)propan 2-ol

The method below describes an attempt to couple 1,3-dichloropropanol with commercially acquired tertiary butoxycarbonyl piperazine (tBOC piperazine).

To a solution of 500 mg of tBOC piperazine (2.6 mmol) in 5 mL of absolute ethanol, 371 mg of K_2CO_3 (2.6 mmol) and 128 µl of 1,3-dichloropropanol (1.3 mmol) were added with stirring. The reaction mixture was stirred at reflux temperature for 24-48 hr.[74] TLC was performed at regular intervals using tBOC piperazine (in DCM) as a reference standard. TLC plates from 24 hour onwards showed multiple spots which were very close to each other and various mobile phase mixtures failed to separate the mixture further.[74,75] The reaction mixture was suspended in DCM and filtered to remove K_2CO_3. The filtrate obtained was evaporated to dryness to remove all solvents (ethanol and DCM) yielding a dark brown sticky solid. ^{1}H NMR spectrum ($CDCl_3$) showed peaks pertaining to the tBOC group and the formation of the product. However, coupled with the TLC analysis (showing multiple spots), it was evident that a complex mixture of compounds was present.

2.1.1.5 Attempted coupling reaction of tBOC piperazine and 1,3-dichloropropanol in isopropanol.

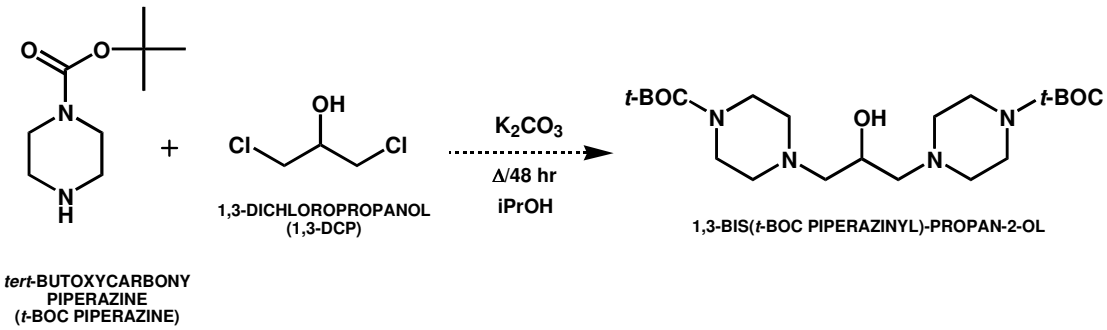

Figure 20 Synthesis of 1,3-Bis(tBOC piperazinyl)propan-2-ol

To a solution of, 500 mg of tBOC piperazine (2.6 mmol) in 10 mL of isopropanol, 371 mg of K_2CO_3 (2.6 mmol) and 128 µl of 1,3-dichloropropanol (1.3 mmol) were added with stirring. The reaction mixture was refluxed for 24-48 hr.[74] As in the previous attempt, the progress of the reaction was monitored by TLC. The same workup procedure was employed as for the previous attempt to yield a similar dark brown sticky solid. ^{1}H NMR spectral analysis ($CDCl_3$) of this solid yielded a similar spectrum to the previous attempt.

2.1.2 Approaches to the Synthesis of the 7-Chloro-4-(piperazine-1-yl) quinoline Intermediate using 4,7-Dichloroquinoline (4,7-DCQ)

The approaches described in the previous section to synthesise 1,3-(bis piperazinyl)-propan-2-ol *via* *N*-monoprotected derivatives of piperazine did not produce intermediates of satisfactory purity. Hence an alternative approach for the synthesis of HPQ was embarked upon. The following approach involved the synthesis of a 7-chloro-4-(piperazine-1-yl)quinoline intermediate as described below (Figure 21). This intermediate does not possess a conventional protecting group but instead contains an essential building block for HPQ synthesis i.e. the 7-chloroquinoline unit. This unit also serves to block or protect one of the amino groups present in piperazine, thereby only permitting a coupling reaction at the remaining amino group. The below coupling product could then be reacted with 1,3-DCP to give HPQ.[63, 74, 76-81] The below intermediate, 7-chloro-4-(piperazine-1-yl)quinoline, can be synthesised *via* two different synthetic methods. Piperazine could be coupled with 4,7-DCQ in the presence of organic bases (e.g. triethylamine, diisopropylethylamine) or inorganic bases (e.g. K_2CO_3) to generate 7-chloro-4-(piperazine-1-yl)quinoline as described in Figure 21.

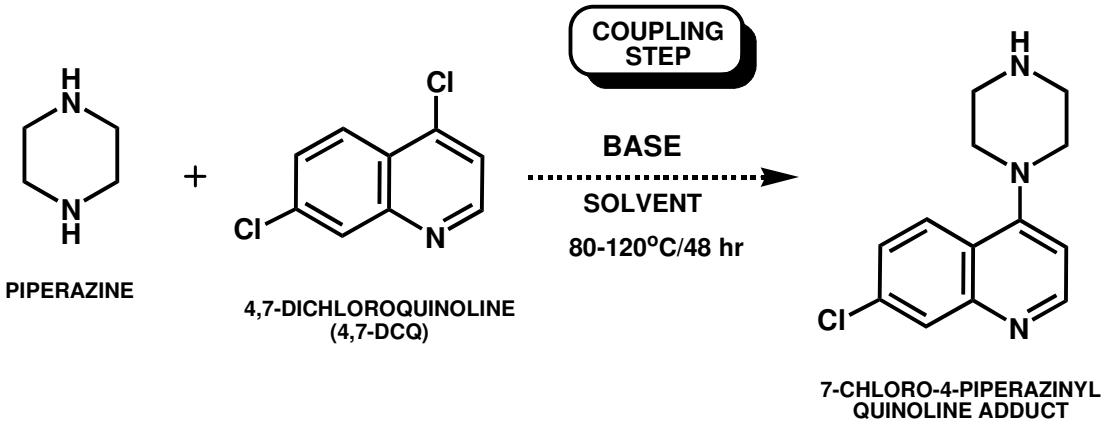

Figure 21 Coupling reaction of Piperazine and 4,7-DCQ

Alternatively tBOC piperazine could be coupled with 4,7-DCQ in the presence of the above bases to generate 7-chloro-4-(tBOC-piperazine-1-yl) quinoline as described in Figure 22. This compound on subsequent deprotection in a suitable acidic medium would yield 7-chloro-4-(piperazine-1-yl) quinoline.

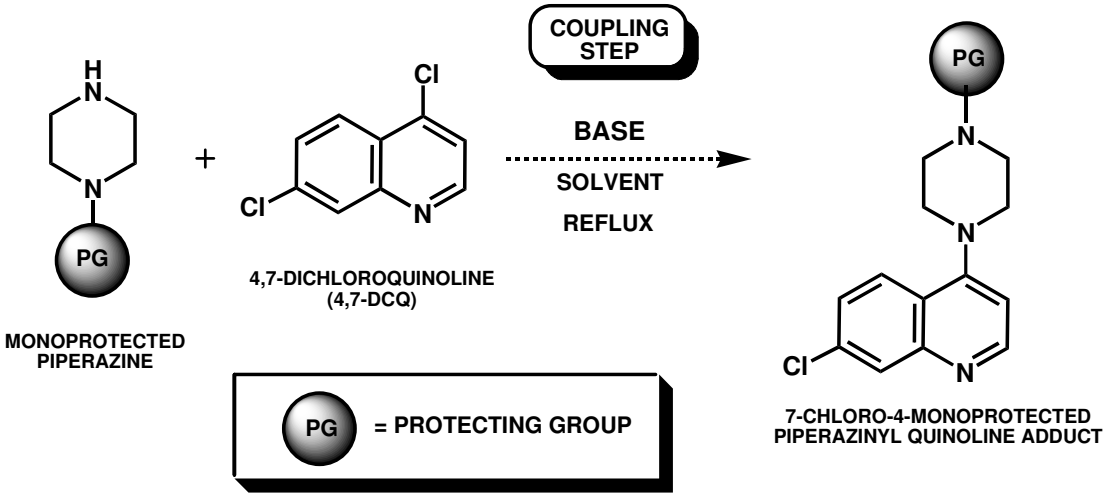

Figure 22 Coupling reaction of Protected Piperazine and 4,7-DCQ

2.1.2.1 Attempted coupling reaction of 4,7- DCQ and piperazine in triethylamine base[77, 82]

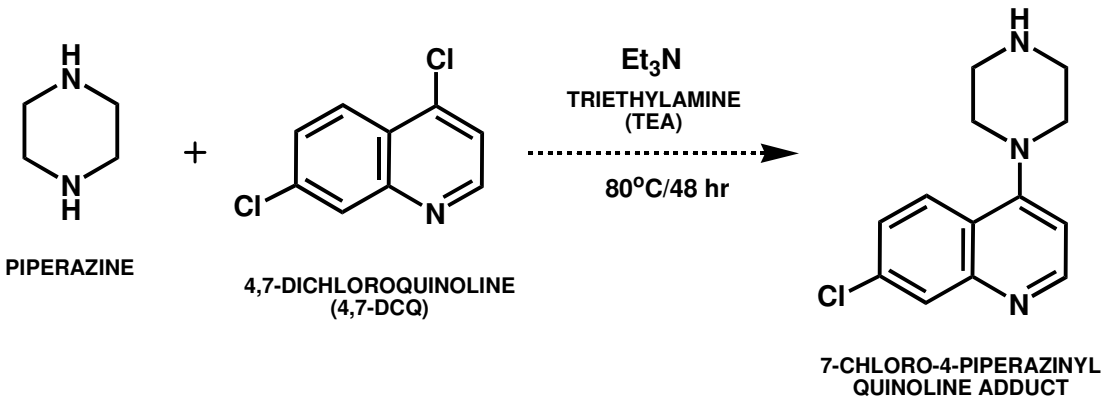

Figure 23 Coupling reaction of 4,7-DCQ and Piperazine in triethylamine

A solution of 500 mg of 4,7-dichloroquinoline (4,7-DCQ) (2.5 mmol) and 1.076 g of piperazine (12.5 mmol) in 10 mL of triethylamine was heated for 48 hr at 80°C. TLC analysis was performed at regular intervals (DCM: MeOH: Et_3N 8.9:1:0.1 and DCM: Ethanol: Ammonia 100:8:1). The reaction mixture was purified *via* flash column chromatography using silica and DCM: MeOH: Et_3N (8.9:1:0.1) as the mobile phase.[83-85] The product fractions obtained were concentrated using a rotary evaporater and analysed *via* ^{1}H NMR spectroscopy ($CDCl_3$). The ^{1}H NMR spectrum of the dark brown resinous solid obtained showed peaks for the presence of trace amounts of 4,7-DCQ (starting material) and impurities such as ethanol, water and silicone grease. There were no peaks for the formation of the product.

2.1.2.2 Attempted coupling reaction of 4,7-DCQ and piperazine in triethylamine and N-methyl pyrrolidinone bases [77, 82]

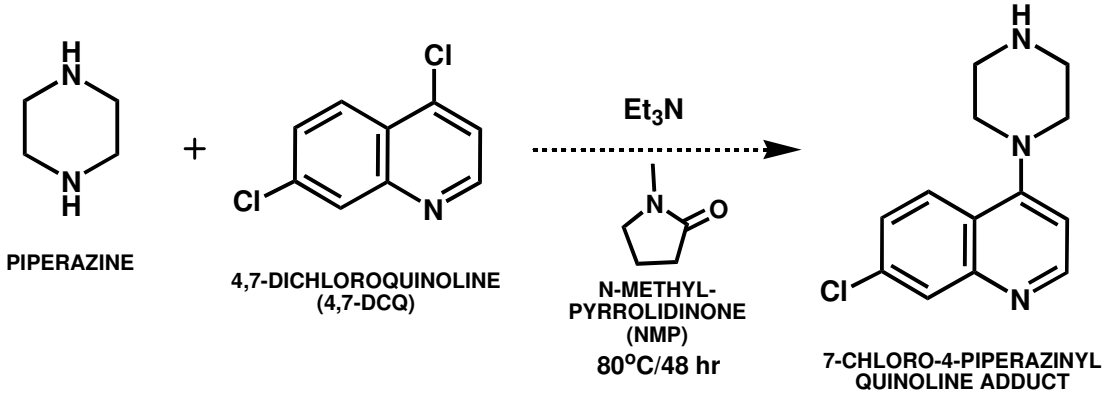

Figure 24 Coupling reaction of 4,7-DCQ and piperazine in triethylamine and *N*-methyl pyrrolidinone

In a mixture of Et_3N (3 mL) and *N*-methyl pyrrolidinone (NMP,7 mL), 500 mg of 4,7-DCQ (2.5 mmol) and 1.076 g of piperazine (12.5 mmol) was dissolved and heated for 48 hr at 80°C. TLC was performed at regular intervals (DCM: Ethanol: Ammonia in the ratio 100:8:1). A solution of 4,7-DCQ in DCM was used as the reference spot to assess the progress of the reaction. TLC analysis after 48 hours showed the presence of new spots and the reaction mixture was extracted and purified *via* a basic work up. The work up procedure included extraction with three 50 mL portions of 10% NaOH solution and subsequently with three 50 mL portions of saturated NaCl solution. The organic phase was dried using 10 g of sodium sulphate, filtered and concentrated using a rotary evaporator. A shiny chocolate coloured solid free from solvent was obtained. Spectral analysis of the solid was performed by ^1H NMR ($CDCl_3$), but did not show peaks for the desired product (only NMP was present).

2.1.2.3 Attempted coupling of 4,7-DCQ and piperazine with catalytic amounts of KI [78, 82]

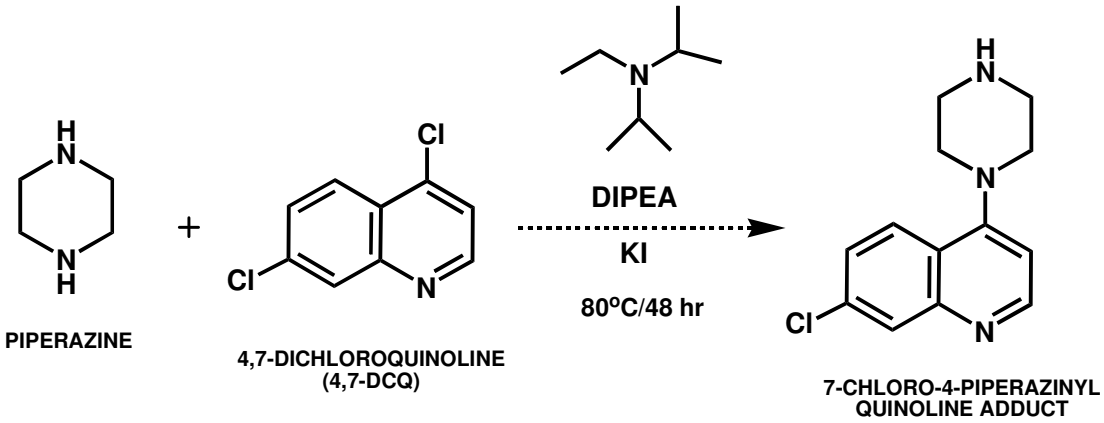

Figure 25 Attempted coupling of 4,7-DCQ and piperazine with catalytic amounts of KI

A solution of 1.980 g of 4,7-DCQ (10 mmol), 4.307 g of piperazine (50 mmol) and 100 mg of KI in 20 mL of diisopropyl ethylamine (DIPEA, Hunig's base) was heated for 48 hr at 80°C. TLC was performed at regular intervals (DCM: Ethanol: Ammonia 100:8:1). After 48 hr the reaction mixture was extracted *via* a basic work up.[83, 85] A shiny chocolate coloured solid free from solvent was obtained. However, the ^{1}H NMR spectrum ($CDCl_3$) of the solid showed peaks for 4,7-DCQ only.

2.1.2.4 Attempted coupling reaction of 4,7-DCQ and piperazine

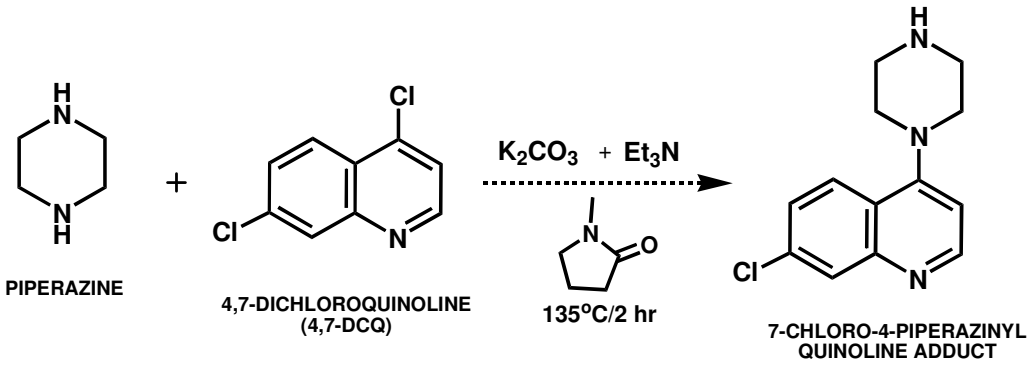

Figure 26 Coupling reaction of 4,7-DCQ and piperazine

A mixture of 10.8 g of piperazine (125.37 mmol), 1.047 g of K_2CO_3(7.58 mmol), 5.28 mL of triethylamine (37.98 mmol) and 5.00 g of 4,7-DCQ (25.25 mmol) was stirred in 17.7 mL of NMP at 135°C for 2h. TLC analysis was performed at 1 hour interval (DCM: Ethanol: Ammonia 100:8:1). After cooling to room temperature the reaction mixture was diluted with 200 mL DCM. The reaction mixture was washed twice with 50 mL brine, dried with $MgSO_4$ and concentrated using a rotary evaporater. [83, 85] The resulting yellow oil was purified by column chromatography on silica using CH_2Cl_2/ MeOH (4:1) as the eluent. The fractions containing the product were concentrated using rotary evaporater to give a pale yellow oil which was analysed by ^{1}H NMR spectroscopy ($CDCl_3$). However, the ^{1}H NMR spectrum given showed no product peaks and the presence of piperazine only.

2.1.2.5 Attempted coupling reaction of 4,7-DCQ and tBOC-piperazine in diisopropyl ethylamine base[78, 82]

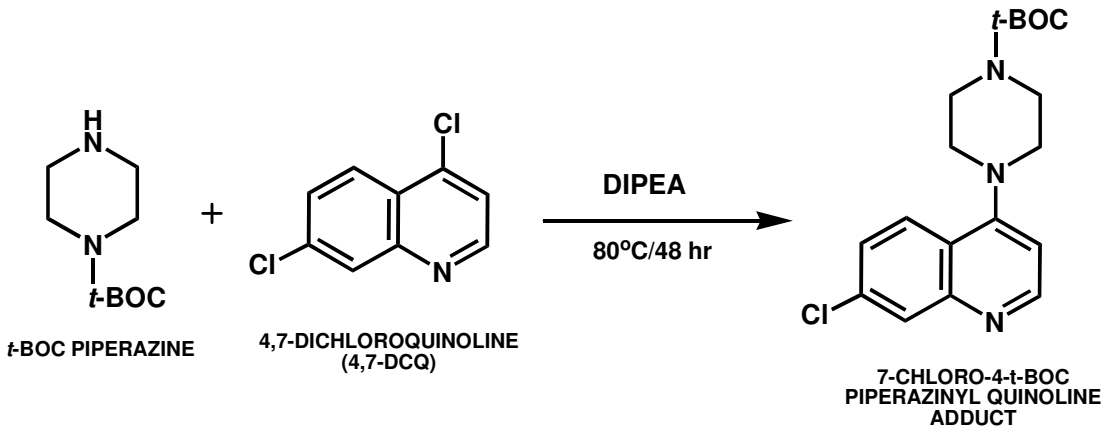

Figure 27 Coupling reaction of 4,7-DCQ and tBOC-piperazine in diisopropyl ethylamine (DIPEA)

A solution of 1 g of 4,7-DCQ (5 mmol) and 1 g of tBOC-piperazine (5 mmol) in 10 mL of DIPEA was heated for 48 hr at 80^0 C. The TLC (DCM: Ethanol: Ammonia 100:8:1) performed on the reaction mixture showed the formation of new compounds. The reaction mixture was worked up with 10% NaOH and saturated NaCl.[83, 85] The organic phase obtained was dried and concentrated as in the previous attempt (see page 40). A shiny chocolate coloured solid free from solvent was obtained. Flash column chromatography purification was performed with DCM: Ethanol: Ammonia 200:8:1 as the eluent solvent mixture. Combined fractions with expected spots pertaining to the product were dried using a rotary evaporator. The ^{1}H NMR spectrum ($CDCl_3$) of the shiny chocolate coloured solid obtained showed peaks for the formation of the product but also displayed peaks pertaining to starting materials i.e. 4,7-DCQ, tBOC-piperazine and DIPEA (minute quantities of DCM solvent were also evident).

2.1.2.6 Coupling reaction of 4,7-DCQ and tBOC-piperazine in DIPEA with catalytic amounts of KI[78, 82]

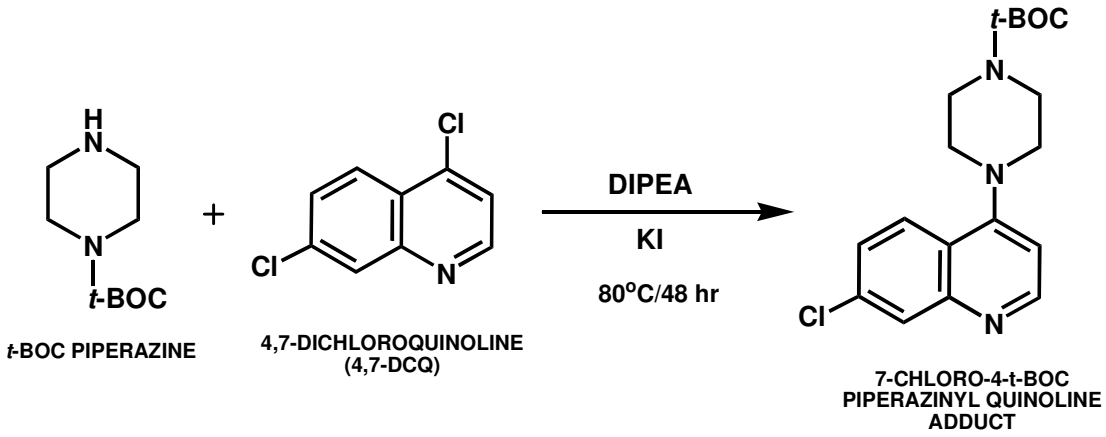

Figure 28 Coupling reaction of 4,7-DCQ and tBOC-piperazine in diisopropyl ethylamine with catalytic amounts of KI

A solution of 1.980 g of 4,7-DCQ (10 mmol), 1.86 g of tBOC-piperazine (10 mmol) and 100 mg of KI in 20 mL of DIPEA was heated for 48 hr at 80°C. TLC analysis was performed at regular intervals (DCM: Ethanol: Ammonia 100:8:1). The mixture was purified *via* basic work up as in the previous reactions. A shiny chocolate coloured solid (free from solvent) was obtained. Flash column chromatography purification was performed with DCM: Ethanol: Ammonia 200:8:1 as the eluent solvent mixture. The ^{1}H NMR spectrum ($CDCl_3$) of the chocolate coloured solid given showed peaks for the intermediate 7-chloro-4-(tBOC-piperazine-1-yl)quinoline. The amount of product obtained was 1.610 g. Based on 10 mmol of 4,7-DCQ and tBOC piperazine utilised the reaction yield was 46%. The product was relatively free from impurities such as solvent and starting material apart from minute quantities of tBOC piperazine, which could be seen from the integration of the 9H singlet (tBOC) at δ 1.50 ppm; this is probably due to spinning side bonds. There also where two small multiplets at δ 3.00 and δ 3.60 ppm which could be attributed to the tBOC piperazine starting material. ^{1}H NMR (200MHz, $CDCl_3$) δ ppm 1.50 (singlet, 9H, tBOC), 3.43 (multiplet, 4H, 2 H-2^1& 6^1) , 3.72 (doublet, 4H, 2 H-5^1& 3^1), 6.95 (doublet, 1H, H-3), 7.50 (doublet of doublets, 1H, H-6), 7.94 (doublet, 1H, H-5), 8.40 (doublet, 1H, H-8), 8.61 (doublet, 1H, H-2).

2.1.2.7 Deprotection of the tBOC coupling product, 7-chloro-4-(tBOC piperazine-1-yl) quinoline.[87, 88]

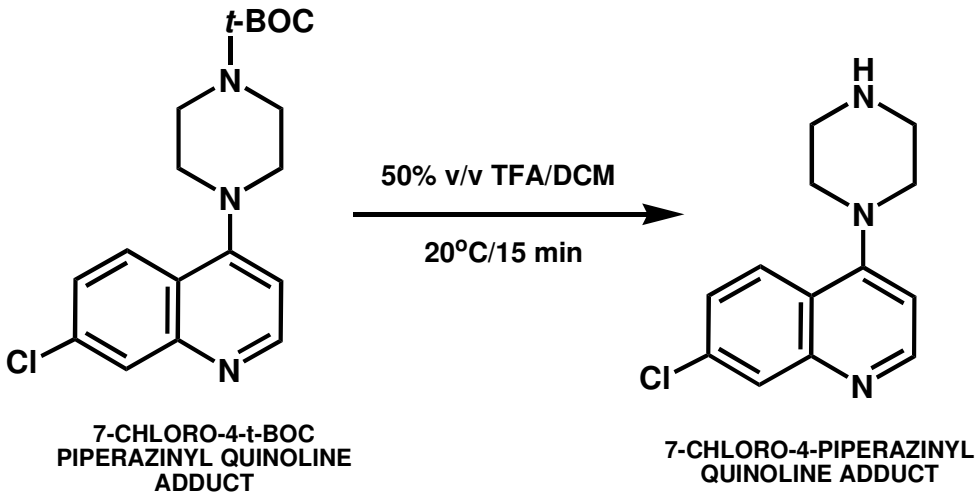

Figure 29 Synthesis of 7-chloro-4-(piperazine-1-yl)quinoline

Deprotection of the coupling product from the last procedure was performed to remove the tertiary butoxycarbonyl (tBOC) group and render the secondary amino site free for reaction with 1,3- dichloropropanol for the final synthesis of HPQ.

The products from the reactions described in Sections 2.1.2.5 (730 mg) and 2.1.2.6 (3.0 g) were placed into two separate 100 mL round bottom flasks. To each flask, 50% TFA: DCM (10 mL of TFA and 10 mL of DCM) were added and stirred for 15 mins at room temperature. By-products and excess TFA were removed *via* the following basic workup procedure. Contents of both the flasks were combined and the product mixture was made alkaline using sodium bicarbonate and then washed with 50 mL 10% NaOH and 50 mL saturated brine.[83, 85] The organic phase was separated and dried using $MgSO_4$. Upon concentration in a rotary evaporator, a pale brown coloured solid (1.720g) was obtained. The reaction yield was 64.7% based on the combined mass of the starting materials. ^{1}H NMR spectra ($CDCl_3$) of the solids were obtained and compared to the literature data.[76, 77] The spectrum correlated with the peaks specified in the literature and showed the presence of the desired deprotected product. ^{1}H NMR (200MHz, $CDCl_3$) δ ppm 2.18 (singlet, 1H, H-4^1), 3.43 (singlet, 8H, 2 H-5^1& 3^1 and 2 H-2^1& 6^1) , 6.95 (doublet, 1H, H-3), 7.50 (doublet of doublets, 1H, H-6), 7.94 (doublet, 1H, H-5), 8.40 (doublet, 1H, H-8), 8.61 (doublet, 1H, H-2).

2.1.3 Synthesis of Hydroxypiperaquine (HPQ)

2.1.3.1 Synthesis of HPQ using an organic base [77, 82]

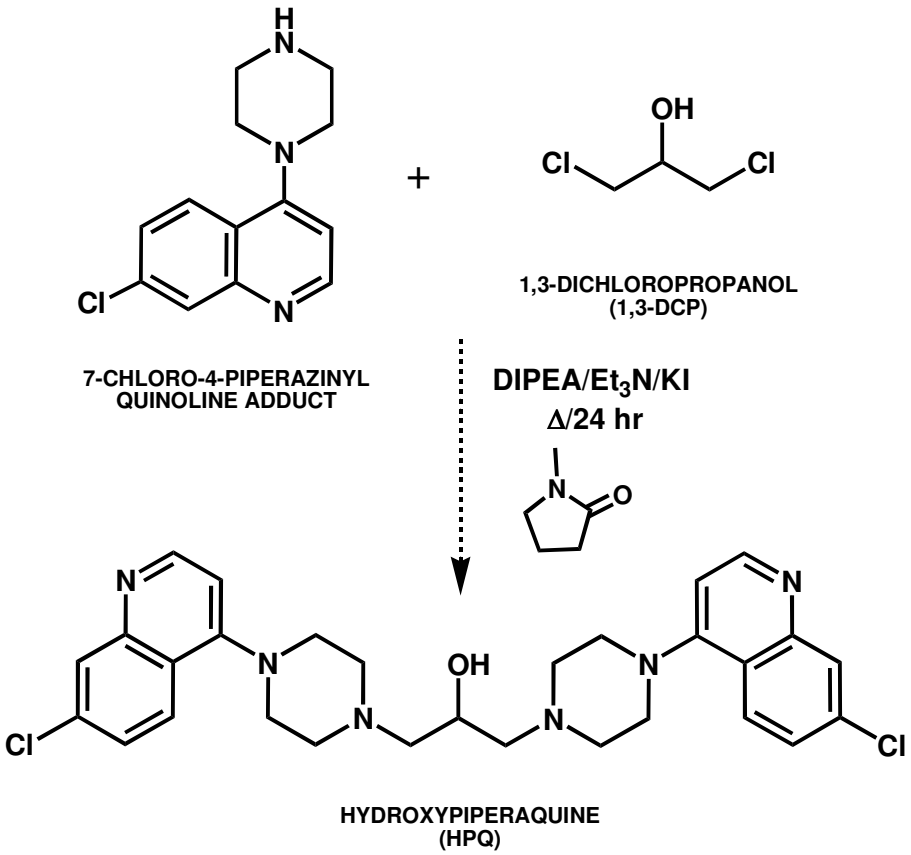

Figure 30 Synthesis of HPQ using an organic base

In a solvent and base mixture of 2 mL of DIPEA, 5 mL of TEA and 2 mL of NMP, 248 mg of 7-Chloro-4-(piperazin-1-yl)quinoline (1 mmol) and 50 µl of 1,3-dichloropropanol were dissolved. 10 mg of KI was added (as a catalyst) and the reaction mixture was continuously refluxed for 24 hr. TLC was performed in DCM: Ethanol: Ammonia (100:8:1) and a DCM solution of 7-chloro-4-(piperazin-1-yl)quinoline was used as a reference to assess the reaction progress. TLC after 24 hr showed a new spot therefore the reaction mixture was purified *via* a basic work up as described in previous sections (see Page 40). The pale chocolate coloured solid obtained was analysed by ^{1}H NMR spectroscopy ($CDCl_3$). However, the spectrum clearly showed that the desired product (HPQ) was not present. Impurities like solvents (NMP and DCM) and starting material (1,3-DCP) were present.

2.1.3.2 Synthesis of HPQ using an inorganic base in isopropanol [63, 82]

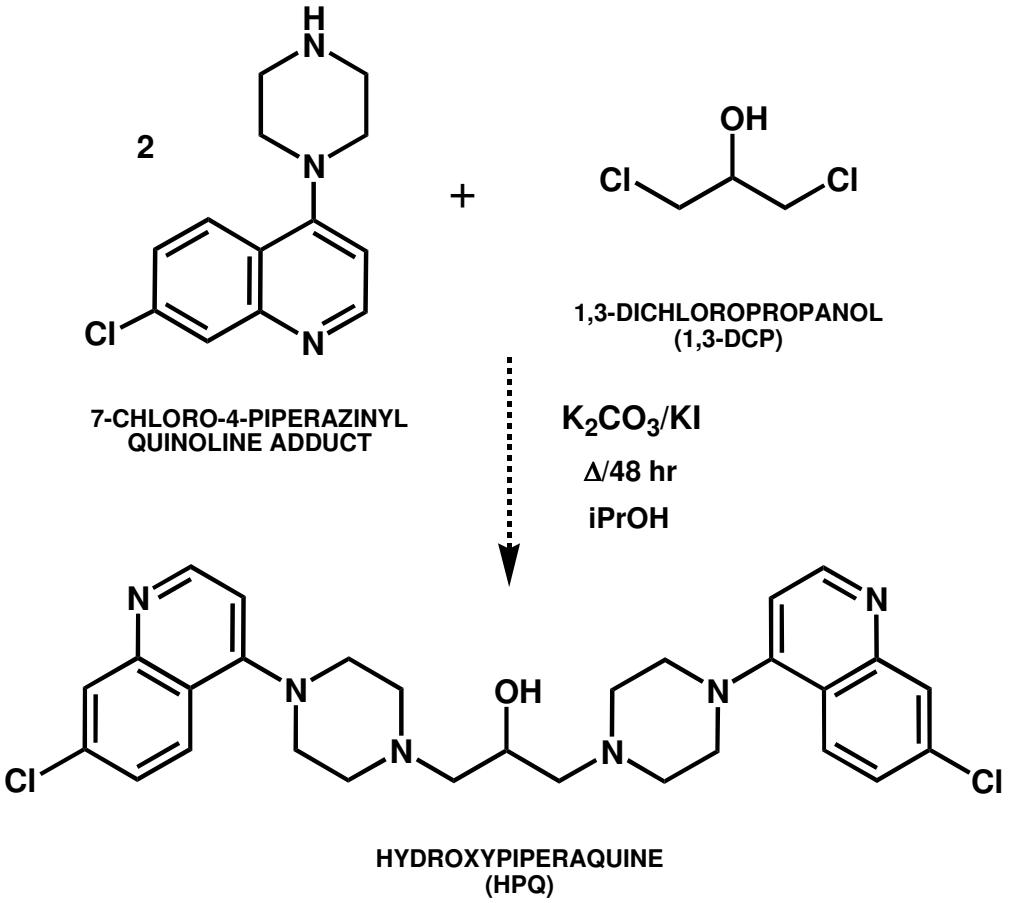

Figure 31 Synthesis of HPQ using an inorganic base

To a solution of 500 mg of 7-chloro-4-(piperazin-1-yl)quinoline (2.5 mmol) and 128 µl of 1,3-dichloropropanol (1.3 mmol) in 10 mL of isopropanol, 370 mg of K_2CO_3 (2.7 mmol) and 100 mg of KI were added. The mixture was stirred at reflux for 48 h. TLC was performed in DCM: Ethanol: Ammonia 100:8:1. The reaction mixture was purified *via* a basic workup as described in the previous reactions (see Page 40) to yield a pale chocolate coloured solid. As for the latter attempt, (from the ^{1}H NMR spectrum) there appeared to be no HPQ present in the solid. (Peaks observed for chloroform and water only).

2.1.3.3 Synthesis of HPQ from 7- chloro-4-(piperazin-1-yl) quinoline using inorganic base in ethanol [63]

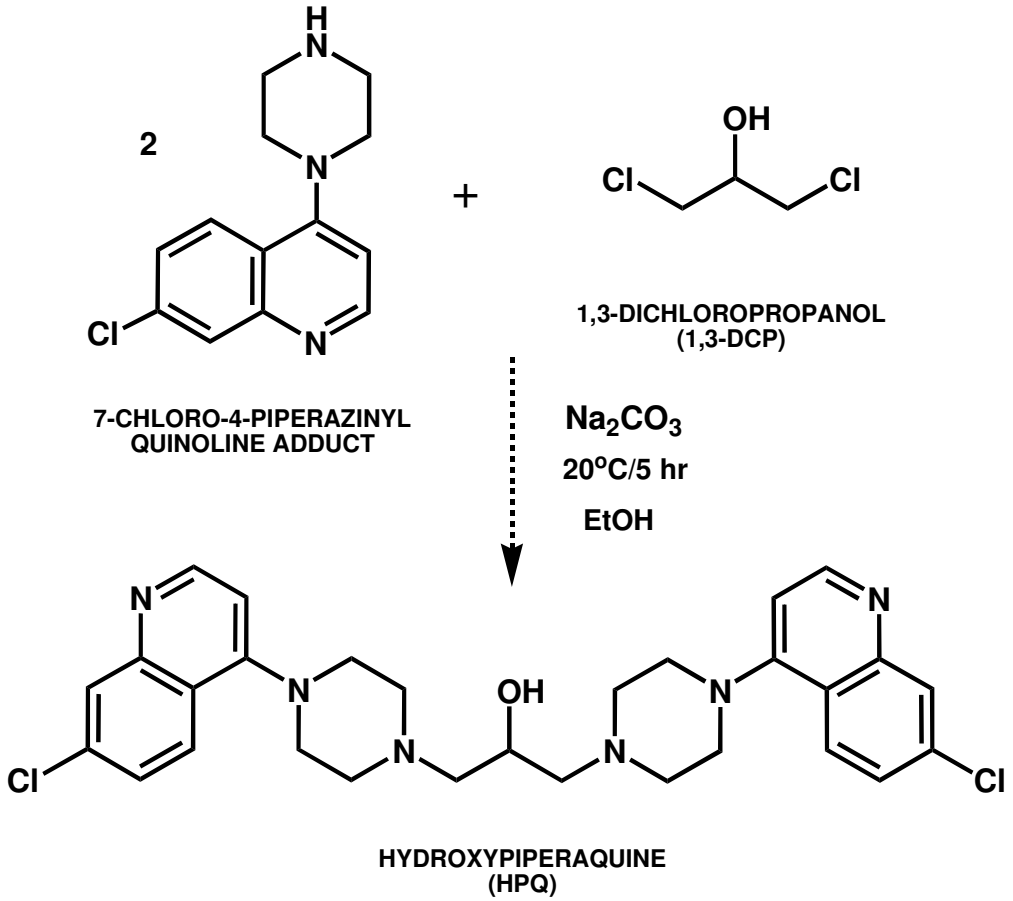

Figure 32 Synthesis of HPQ from 7-chloro-4-(piperazin-1-yl)quinoline using Na_2CO_3

A mixture of 248 mg of 7-chloro-4-(piperazin-1-yl)quinoline (1 mmol) and 50 µL of 1,3-dichloropropanol (0.5 mmol) were stirred in 1 mL of ethanol. To this was added with stirring 52.5 mg (0.5 mmol) of Na_2CO_3. The mixture was stirred at room temperature for 5 hr. To workup the solution, 15 mL of DCM was added and the solution was subsequently dried over Na_2SO_4. After filtration the solvent was removed by rotary evaporation yielding a dark brown oily liquid. The oil present was analysed by ^1H NMR spectroscopy ($CDCl_3$). As for both previous attempts, the spectrum obtained showed no peaks pertaining to HPQ (only 1,3-DCP was present).

2.1.3.4 Synthesis of HPQ using an inorganic base in amyl alcohol

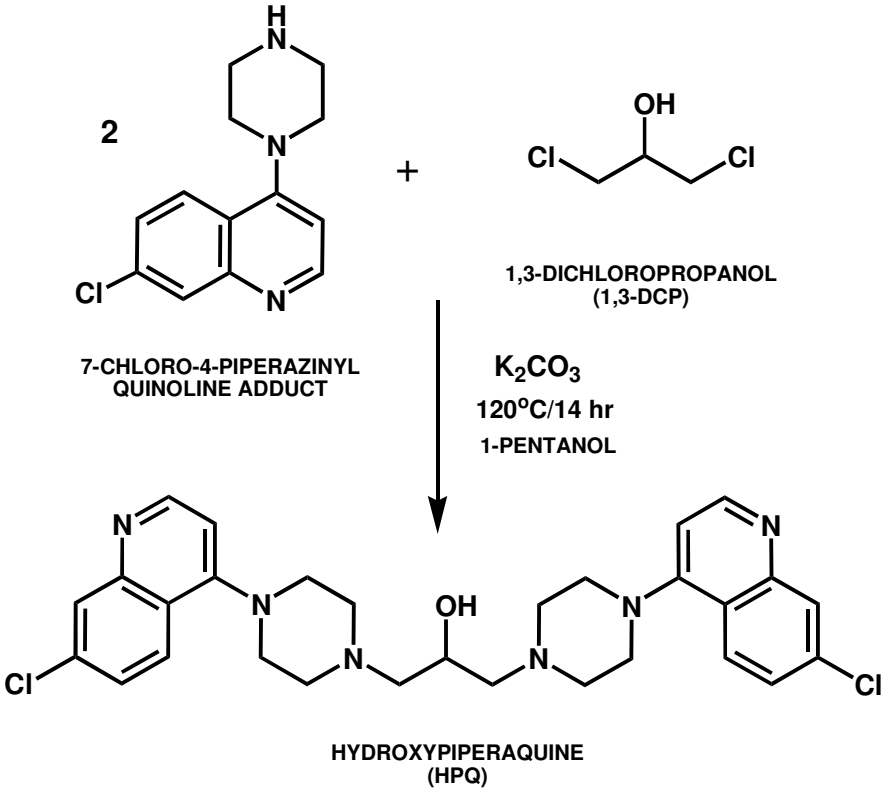

Figure 33 Synthesis of HPQ using an inorganic base in amyl alcohol

To a stirring solution of 0.992g of 7-chloro-4-(piperazin-1-yl)quinoline (4 mmol) in 10 mL of amyl alcohol (1-pentanol), 0.660 g of K_2CO_3 was added. To this solution, 200 µL of 1,3-dichloropropan-2-ol (2.1 mmol) was added using a Pasteur pipette. The solution was heated at a temperature of 120°C overnight (14 hours). After 14 hours, a dark solid material suspended in a clear pale yellow solution was produced. The material was extracted and purified *via* a basic work up as described in previous sections (see Page 40). A shiny chocolate coloured solid free from any solvent was obtained after the workup. TLC analysis on this compound showed considerable amounts of impurities and the starting material. Flash column chromatography was undertaken to purify the solid using an eluent of gradually increasing polarity starting with 200:8:1 DCM: Ethanol: Ammonia. The fractions containing the suspected product were concentrated using rotary evaporater to give a brownish yellow coloured solid (683mg). Based on the ratio, 2 mmol of 7-chloro-4-(piperazin-1-yl)quinoline and 1mmol of 1,3-DCP produce 1mmol of HPQ the reaction yield for 2 mmol of HPQ was 59.0% (with solvent impurities). The ^{1}H NMR spectrum of the solid obtained showed peaks for the aromatic part of HPQ consistent with the reference spectral data

for HPQ as described by Xu *et al* [3]. A few trace impurities such as amyl alcohol seen in the non-aromatic region and DCM were present. ^{1}H NMR (200MHz, $CDCl_3$) δ ppm 2.30 (Doublet, 4H, CH_2-propanol × 2), 2.80 (Singlet, 8H, 2H-5[1] and 2H-3[1]piperazine× 2), 3.40 (Broad singlet, 8H, 2H-2[1] and 2H-6[1]piperazine × 2), 3.60 (Broad singlet, 1H, OH), 4.10 (Pentet, 1H, CHOH), 6.88 (Doublet, 2H, H-3 × 2), 7.49 (Doublet of doublets, 2H, H-6 × 2), 7.97 (Doublet, 2H, H-5 × 2), 8.10 (Doublet, 2H, H-8 × 2), 8.71 (Doublet, 2H, H-2 × 2).

3 RESULTS & DISCUSSION

3.1 Outline of the Synthetic Approaches to HPQ

3.1.1 HPQ synthesis described by Xu *et al* [3]

Whilst numerous journal articles and reports have addressed its antimalarial efficacy and clinical uses of hydroxypiperaquine (HPQ), the literature regarding its synthesis is virtually non-existent. The sole exception to this is the review published nearly 20 years ago by Xu *et al* in the Journal of the Medical College of the People's Liberation Army (JMCPLA).[3] The review contains the only published synthetic work relating to HPQ but there have been no subsequent reports on its efficiency, reliability and reproducibility.

The synthesis and chemical properties of HPQ were first described very briefly in the above review by Xu *et al*.[3] They synthesised HPQ *via* a simple four step reaction procedure. However, the review does not include any specific experimental details pertaining to the synthetic procedure and the only information relating to the synthesis is in the form of a diagrammatic reaction scheme shown below (Figure 34).

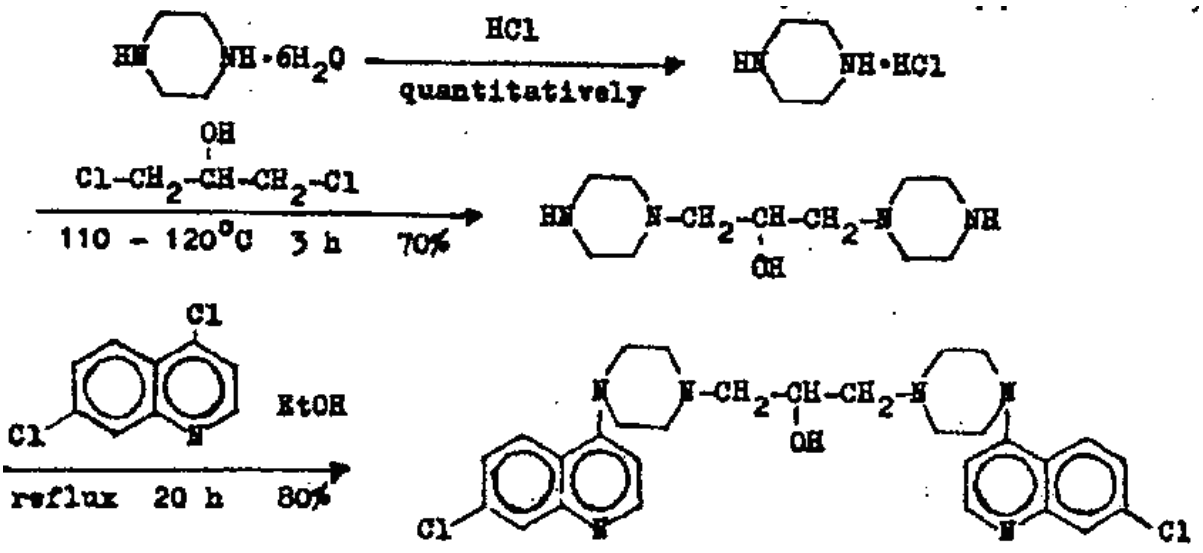

Figure 34 Reproduced scheme for the synthesis of HPQ described by Xu *et al* [3]

In the first step of the synthetic procedure, piperazine was converted to its hydrochloride salt using hydrochloric acid (HCl) but no solvent was specified. For the second step, 1,3-dichloropropanol (1,3-DCP) was reacted with the above piperazine salt. A reaction temperature of 110-120°C was specified and the reaction

was carried out for 3 hours yielding 1,3-bis-(piperazinyl)-propan-2-ol (70% yield). Oddly, no solvent is specified for this second step. In the third step, 4,7-dichloroquinoline (4,7-DCQ) was coupled with the above bispiperazinyl product by refluxing in ethanol for 20 hours to give HPQ (80% yield). Whilst this approach appeared straightforward, previous attempts at reproducing the synthesis in our laboratory were unsuccessful.[64] The lack of experimental details and conditions for all the steps significantly hampered its reproduction. In particular, important information relating to the amounts of reagents and reaction solvents for each step were not provided. In earlier efforts to reproduce the synthesis, the reaction conditions were varied (in particular relative amounts of reactants) and numerous problems were encountered.[64] The most common problems encountered were the formation of monosubstituted piperazinyl chloropropanol and the presence of starting materials (especially piperazine) in very large quantities. Both by-products and unreacted starting materials proved very difficult to remove from the reaction mixture *via* conventional means resulting in low and unacceptable yields of products at best (at worst crude and pharmaceutically unacceptable product mixtures). In addition, rapid analytical monitoring of the first two steps of the process was particularly difficult as both piperazine and 1,3-DCP lack an effective chromophore, making TLC plates difficult to visualise using conventional UV detection. TLC analysis previously performed using various stains for plate visualisation and compound detection (e.g. anisaldehyde, iodine and potassium permanganate) met with very little success.

3.1.2 Summary of synthetic approaches

The present research explored various synthetic strategies to synthesize HPQ in an efficient manner. The starting compound, piperazine is dibasic and contains two secondary amino groups both of which have the same susceptibility to nucleophilic substitution reactions using alkyldichlorides. Reaction of piperazine *via* this process can lead to the formation of an array of alkylated compounds - namely monosubstituted and disubstituted products (and depending on the conditions possibly unreacted piperazine). To overcome this issue, previous attempts at the synthesis utilised an excess of piperazine where in fact piperazine acts as both reactant and base catalyst for the substitution reaction. It has been demonstrated by many studies that base catalysts increase nucleophilicity of amino compounds *via* amino group

deprotonation.[89] An alternative approach which may limit the amount of bis by–products is to utilise mono-*N*-protected derivatives of piperazine. It was intended in our initial proposal to synthesize or possibly commercially acquire a selection of these protected derivatives namely tertiary butoxy carbonyl piperazine (tBOC piperazine), 1-benzylpiperazine and 1-ethoxycarbonylpiperazine. Most of these protecting groups have a UV chromophore which would assist in the monitoring of the reactions by TLC with UV detection. Also, a protected piperazine should in theory react more cleanly with the alkyl dichloride (1,3-DCP), at the unprotected amino group as it would be the only site available for nucleophilic attack. The protected product generated on subsequent removal of the protecting group should provide the desired intermediate, 1,3-bis-(piperazinyl)-propan-2-ol, which could then be coupled with two molar equivalents of 4,7-DCQ to provide HPQ (Figure 35).

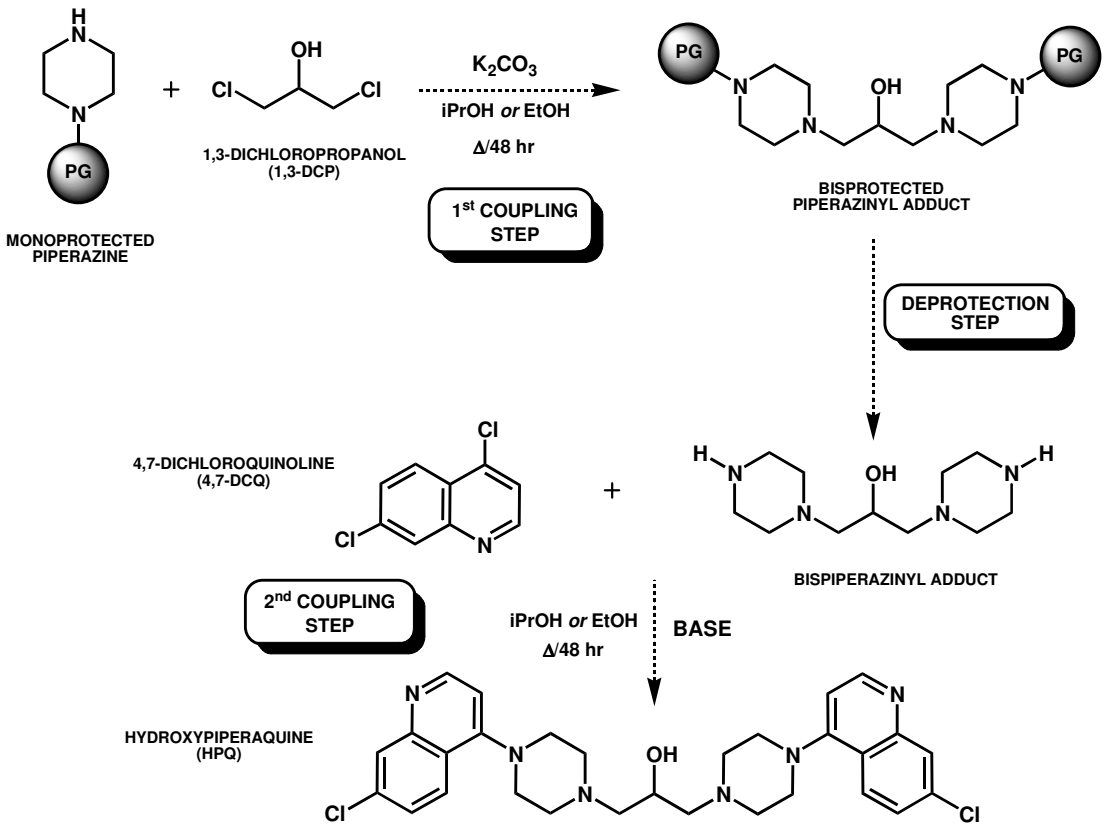

Figure 35 Proposed synthesis of Bis-(protected piperazinyl)propan-2-ol

In addition to the above, a second alternative synthetic approach to HPQ is possible, involving the direct coupling reaction of 4,7-DCQ and either piperazine or an *N*-monoprotected piperazine derivative. This would generate one of the two

intermediates, 7-chloro-4-(piperazin-1-yl)quinoline (Figure 36) or 7-chloro-4-(*N*-monoprotected piperazin-1-yl)quinoline (Figure 37). Both intermediates could subsequently be reacted with 1,3-DCP to afford HPQ.

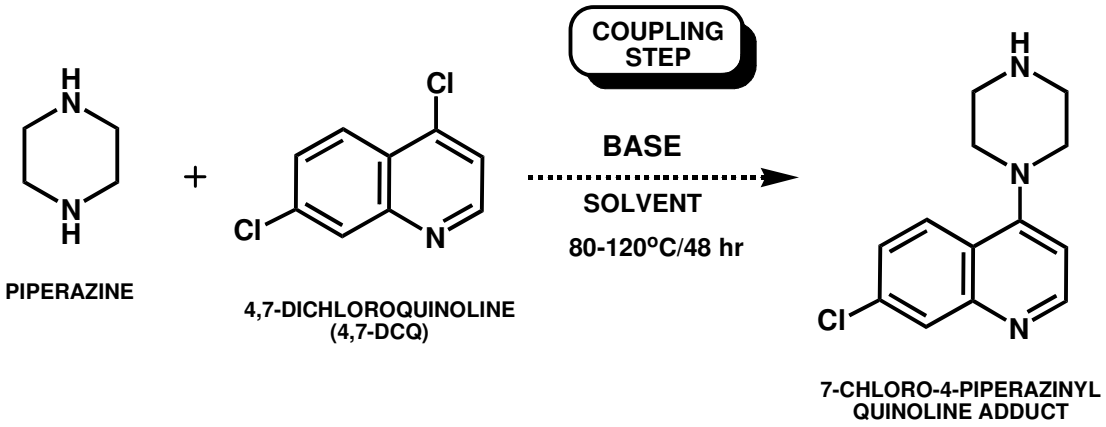

Figure 36 Proposed synthesis of 7-Chloro-4-(piperazin-1-yl)quinoline

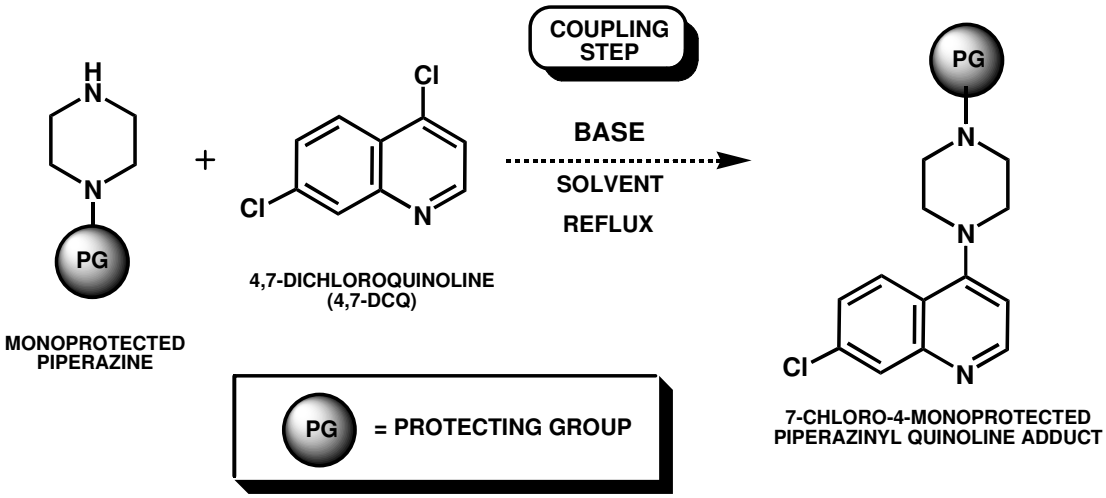

Figure 37 Proposed synthesis of 7-Chloro-4-(protected piperazin-1-yl)quinoline

All starting materials, intermediates and products generated *via* this approach would posses a chromophore and hence the above reactions should be easily monitored *via* conventional TLC with UV detection. A literature review revealed that three different methods have been previously published to synthesise 7-chloro-4-[piperazin-1-yl]quinoline. The first literature method by Vennerstrom described the synthesis of novel bisquinolines and synthesized 7-chloro-4-[piperazin-1-yl]quinoline by reaction of 4,7-DCQ and piperazine in a molar ratio of 1:10.[63] The reactants were refluxed under argon atmosphere for 24 hours in 2-ethoxyethanol solvent. They showed that by simply cooling and reheating the reaction mixture the excess unreacted piperazine

could be distilled off. The second approach by Chiyanzu *et al* described the synthesis of 4-aminoquinoline isatin derivatives.[76] 7-chloro-4-[piperazin-1-yl]quinoline was synthesized as an intermediate by reacting 4,7-DCQ and piperazine in a 1:5 molar ratio. They used both inorganic (K_2CO_3) and organic bases (triethylamine) as catalysts in *N*-methyl-2-pyrrolidinone solvent. The preparation of 7-chloro-4-[piperazin-1-yl]quinoline was carried out at 135°C for 4 hours under a nitrogen atmosphere. The third method described by Clarkson *et al* coupled 4, 7-DCQ with piperazine in a 1:5 molar ratio in the presence of both K_2CO_3 and triethylamine under nitrogen at 135ºC for 2 hours in *N*-methyl-2-pyrrolidinone.[77] It was our intention to repeat the latter method (see section 3.3.1) as all the reagents used in this method were available in our laboratory and the reaction conditions were easily reproducible. However, the reported product yield of 87.5 % was the major reason why this method was attempted.

3.2 Synthetic Approaches to HPQ using *N*-Mono Protected Piperazine Derivatives

Three methods using *N*-mono protected derivatives of piperazine were to be attempted.

- Coupling of 1-benzylpiperazine with 1,3-dichloropropanol to synthesise bis (benzyl)piperazinyl-propan-2-ol.
- Coupling of tBOC-piperazine with 1,3-dichloropropanol to synthesise bis (tBOC)piperazinyl-propan-2-ol.
- Coupling of 1-ethoxycarbonylpiperazine with 1,3-dichloropropanol to synthesise bis(ethoxycarbonyl)piperazinyl-propan-2-ol.

Each bis-adduct should be easily deprotected and subsequently coupled with two molar equivalents of 4,7-DCQ to provide HPQ. Whilst, tBOC piperazine is a commercially available compound from the Sigma-Aldrich Chemical Company, 1-benzylpiperazine and 1-ethoxycarbonylpiperazine were synthesized in our laboratory according to recent literature methods.[70, 72]

3.2.1 Synthesis of 1-Benzylpiperazine Dihydrochloride

1-Benzylpiperazine dihydrochloride was synthesised according to the literature method of Cymerman *et al* (see page 29).[70] This method involved the synthesis of 1-benzylpiperazine *via* a two step process. The first step involved the synthesis of piperazine dihydrochloride monohydrate. This in turn was reacted in the second step with benzyl chloride in the presence of piperazine hexahydrate (organic base catalyst and assists in reagent solvation) to afford 1-benzylpiperazine dihydrochloride. The methods described were facile, in comparison to an alternative method described by Bergbreiter *et al* using 1-ethoxycarbonylpiperazine.[90] By following Cymerman method, 1-benzylpiperazine dihydrochloride was successfully quantitatively synthesised.[70]

3.2.2 Synthesis of 1-Ethoxycarbonylpiperazine

The method used to synthesise 1-ethoxycarbonylpiperazine was based on a synthetic method published by Krys'ko *et al*, (see page 31).[72] They describe the synthesis of 1-ethoxycarbonylpiperazine by the reaction of an aqueous solution of piperazine with ethyl formate in isopropanol.[72] Alternative literature methods included the reaction of piperazine with ethyl chloroformate in xylene described by Kushner *et al*.[73] After careful scrutiny of the methods available the Krys'ko method appeared to be simpler, because of the ready availability of all reagents involved.

However, attempts to reproduce the above synthesis were not successful. Upon closer examination of the research article a typographical error was noted in the experimental section relating to the reactants employed. Ethyl formate was specified in this section but the accompanying reaction scheme featured ethyl chloroformate. Clearly the latter reagent should have been employed in the synthesis of the 1-ethoxycarbonyl piperazine. Further attempts to synthesize ethoxycarbonylpiperazine were not pursued because two alternative protected piperazines were now available to us - namely 1-benzylpiperazine and commercially available tBOC piperazine. Time constraints and reagent unavailability did not permit us to reattempt the above synthesis with ethyl chloroformate. In addition, other errors in the paper relating to

the use of incorrect molecular weights and molar amounts of reagents were also noted upon further examination.

3.2.3 Coupling reaction of Protected piperazine and 1,3-Dichloropropanol

Three methods for the coupling of 1,3-dichloropropanol with *N*-protected piperazine were attempted. The first involved the coupling of 1-benzylpiperazine with 1,3-dichloropropanol (see page 32). The second and the third methods involved the attempted coupling of tBOC-piperazine with 1,3-dichloropropanol in two different solvents (see pages 33 and 34). The coupling of 1-benzylpiperazine with 1,3-dichloropropanol was attempted using isopropanol as a solvent. Isopropanol was selected because of results from a solubility assessment performed by Fong. [64] This earlier work performed in our laboratory showed that both starting materials (1-benzylpiperazine and 1,3-dichloropropanol) were very soluble in low molecular weight alcohols such as methanol, isopropanol and ethanol. High molecular weight alcohols e.g. amyl alcohol were not used inspite of their higher boiling points as they showed poor solubility for the reactants.[64] It was speculated that the use of a higher boiling point solvent (isopropanol), allowing a higher reflux temperature, would improve the reaction yield. Potassium carbonate (K_2CO_3) was used as a catalytic base in all of the three coupling methods attempted. The use of K_2CO_3 as an inorganic base in nucleophilic substitution reactions has been described by various research groups.[74,76,77,91] This inorganic base can be used in a variety of organic solvents. Both Clarkson and Chiyanzu *et al* described the use of K_2CO_3 in *N*-methyl-2-pyrrolidinone as a solvent.[76, 77] They have also used an organic base, triethylamine, in combination with K_2CO_3. It has also been used in dimethyl formamide (DMF) by Ryckebusch *et al*.[92] Kumar *et al* have described the use of K_2CO_3 for the synthesis of hydroxychloroquine in the absence of any solvent.[82] We decided to attempt the coupling of 1-benzylpiperazine with 1,3-dichloropropanol under continuous reflux conditions for 3 hr using K_2CO_3 in iPrOH. (see Figure 18, Page 32).

However, the TLC analysis of the reaction mixture after 3 hours did not show any new compound spots for a product, therefore the reaction was continued for 24 hours. Further TLC analysis showed multiple spots after 24 hours. However, ^{1}H NMR

spectral analysis of the reaction mixture following workup did not indicate the presence of the desired product.

The second method attempted the coupling reaction of tBOC-piperazine with 1,3-dichloropropanol (see Figure 19, Page 33). The conditions employed for this reaction were similar to the previous coupling reaction except for the use of ethanol as the solvent. Again the selection of solvent was primarily based on its boiling point and its ability to dissolve tBOC piperazine. However, a mixture of compounds was found to be present on ^{1}H NMR spectral analysis of the brown solid obtained.

The final method attempted was similar to the latter approach but involved the coupling of 1,3-dichloropropanol with tBOC-piperazine in isopropanol (see Figure 20, Page 35). The reaction conditions were the same as the previous two reactions i.e. the reaction was carried out at reflux for 48 hours. However, TLC analysis of the reaction showed various spots which could not be separated even with various combinations of mobile phase mixtures e.g. DCM: ethanol: ammonia, DCM: methanol: triethylamine and petroleum ether: ethyl acetate.

The failure of all the above methods suggested that aprotic solvents such as dimethylformamide, acetone or acetonitrile would have possibly been more suitable for these reactions. The above reactions are probably operating *via* a substitution nucleophilic bimolecular (S_N2) mechanism. It has been observed that polar aprotic solvents can increase the rate of such reactions by up to 10^9 fold (by increasing the reactivity of the nucleophile). The nucleophile under aprotic conditions is not surrounded by a shell of solvent molecule (a nucleophilic solvation envelope) unlike in the presence of protic solvents (e.g. methanol) where its nucleophilicity is diminished due to hydrogen bonding with the solvent.[93] Previous attempts in our laboratory involving the use of aprotic solvents have been problematic. The use of dimethyl formamide (DMF) has met with some success but difficulties have arisen in relation to the removal of this high boiling point solvent *via* conventional rotary evaporation.[64] Due to time constraints, this solvent and other aprotic solvents such as acetone, tetrahydrofuran, dimethyl sulfoxide and dioxane were not trialled

3.3 Synthetic Approaches to 7-Chloro-4-(piperazin-1-yl) quinoline Using 4,7-Dichloroquinoline

Two synthetic approaches were attempted to synthesise the above intermediate 7-chloro-4-(piperazin-1-yl)quinoline. The first approach was a direct method which involved the coupling of 4,7-DCQ with piperazine in the presence of either inorganic base or organic bases. The second approach involved the coupling of 4,7-DCQ with tBOC protected piperazine to give 7-chloro-4-(tBOC piperazin-1-yl)quinoline. On subsequent deprotection with trifluoroacetic acid (TFA) this product would provide 7-chloro-4-(piperazin-1-yl)quinoline. Numerous methods have been published relating to the synthesis of similar compounds by the coupling of 4,7-DCQ with a variety of primary, secondary or diamino compounds. The solvents, bases and reaction conditions employed in these published reactions will be discussed in the sections below.

3.3.1 Attempted Coupling of 4,7-DCQ and Piperazine

Four different methods for the coupling reaction of 4,7-DCQ and piperazine utilizing a variety of solvents and bases were attempted (see pages 35-40). The first method involved the coupling of 4,7-DCQ with piperazine using triethylamine as an organic base (see page 37). The reaction was carried out by heating the reactants at a temperature of 80ºC for 48 hours. This approach was based on similar synthetic procedures.[76-78, 81, 82, 91] Triethylamine has been employed as a base by Kumar *et al* in the synthesis of hydroxychloroquine.[82] The use of this base has been further cited in methods described by Clarkson *et al*[77], Beagley *et al*[80] and Chiyanzu *et al*.[76] Kumar emphasized the use of organic bases such as triethylamine, diisopropylethylamine in the absence of any solvents i.e. the base functions as both catalyst and solvent.[82] Other published methods employed organic bases in solvents such as *N*-methyl-2-pyrrolidinone, isopropanol and ethanol.[50, 63, 76, 77, 91] The role of base in nucleophilic aromatic substitution reactions can be critical to the success of these reactions. The above reaction proceeds *via* a substitution nucleophilic aromatic (S_NAr) mechanism (see Fig 38 on the next page). This involves a two step process in which the first step

involves the attack of the nucleophilic amine (in this case the 2° cyclic amine piperazine) on the heteroaryl halide (in this case 4,7-DCQ) to give a resonance stabilised carbanion intermediate. The formation of this intermediate is often the rate limiting step. This is followed by the loss of the halide leaving group (chloride) in the second step.

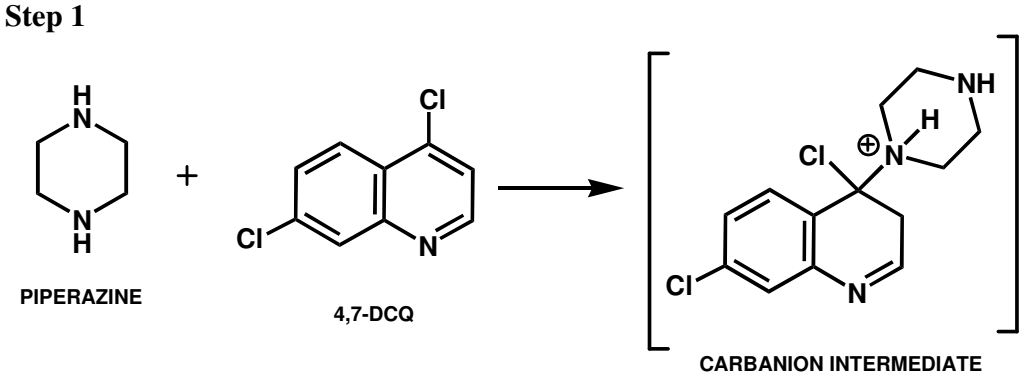

Figure 38 Proposed S_NAr mechanism for the reaction of 4,7-DCQ & piperazine

Bases catalyse the above process by increasing the rate of the second step .[89] Under protic solvent conditions, it is generally believed that base catalysts function by deprotonating the quaternary amino group of the intermediate followed by the rapid loss of the chloride. The base may also assist the loss of chloride directly *via* a protonated form of the base catalyst (conjugate acid).[89]

Most published methods for the synthesis of similar compounds use an excess of piperazine[76,77] or other diamine when coupling with 4,7-DCQ and therefore the unreacted piperazine functions as an organic base. A 4,7-DCQ : piperazine molar ratio of 1:5 was employed in our first attempt (Figure 23) as presented for the method published by Clarkson *et al* (synthesis of Totarol amino alcohol derivatives).[77]

However, the quantities of both reactants were relatively high in comparison to the second base/solvent (Et_3N) used and therefore complete dissolution of the reactants in the basic solvent was not achieved. The material obtained after work up was a hard dark brown resinous solid soluble in DCM. TLC analysis on the substance showed multiple compound spots. Flash column chromatography was performed but, this procedure was not wholly successful. Most of the piperazine originally present in the reaction mixture was removed by the chromatographic purification step but 4,7-DCQ still remained. The presence of TLC spots of very close proximity meant that the amounts of fractions containing the suspect product were also contaminated with impurities. However, the ^1H NMR spectrum ($CDCl_3$) of these fractions showed mostly presence of 4,7-DCQ (trace quantities of water, ethanol and silicone grease were also present). Reference spectra from the literature were compared with the spectrum obtained to clarify and confirm the above conclusions.[63, 76,77]. The lack of reactant solvation might be one possible reason why this reaction failed. The lower reaction temperature of 80°C was selected (compared to the higher temperatures used in other literature methods) to avoid charring and degradation of the reactants because they were not in solution. This lower temperature obviously avoided compound degradation but meant the reaction had to be carried out over a longer period (48 hours). The lower temperature may also have played a significant role in the reaction's failure.

The second attempt at the coupling of 4,7-DCQ with piperazine was similar to the above method except for the use of triethylamine base in *N*-methyl-2-pyrrolidinone solvent (see Figure 24, Page 38). This attempt was based on the method by Beagley *et al* in which triethylamine and *N*-methyl-2-pyrrolidinone were used in the molar ratio 3:7 for a similar synthetic reaction (synthesis of ruthenocene-chloroquine analogues).[91] The same reaction conditions were further cited by Chiyanzu *et al* using triethylamine, K_2CO_3 and *N*-methyl-2-pyrrolidinone (for 4 hours at 135ºC).[76]

After 48 hours at 80ºC, TLC analysis performed on the reaction mixture showed the formation of a new product which was subsequently purified using a basic work up. A basic work up with NaOH was employed as it was believed that the product formed was an HCl salt (the reaction produces two molar equivalents of HCl as by-product). To generate the free piperazinyl base, a stronger base (NaOH) is added which

displaces the weak base product from its salt. The free amino base can then be extracted into an organic solvent i.e. DCM. Saturated brine was used in the work up for two reasons. Brine is used to dry the organic phase and restricts the dissolution of the free base into the aqueous phase. After work up, the shiny chocolate coloured compound afforded was analysed by ^{1}H NMR spectroscopy. However, the spectrum showed peaks pertaining to *N*-methyl-2-pyrrolidinone only. Neither the starting materials (4,7-DCQ and piperazine) nor the desired product were present.

The third attempt at the coupling of 4,7-DCQ with piperazine was based (partly) on the previously described attempts and the success of a similar reaction with tBOC protected piperazine which will be discussed in the later section (see Figure 25, Page 39).[82] The use of diisopropyl ethylamine (DIPEA) as a solvent-base was described in two recent literature methods describing the coupling of 4,7-DCQ with aminoheptanes and aminopiperidine.[78, 96] These methods describe the coupling of 4,7-DCQ with 4-aminopiperidine (Madrid *et al* [78]) using DIPEA as a solvent-base (100°C for 20 hours) and the synthesis of antiprion polyquinoline derivatives (Klingenstein *et al*).[96] In this latter method, 4,7-DCQ was coupled with diaminoheptane in the presence of DIPEA using 1-pentanol as a solvent.[96] Piperazine reacts with 4,7-DCQ in a similar fashion to the compounds mentioned above, though the molar ratios of the reactants might not be similar. After careful review of the methods it was decided that a ratio of 1:5 (4,7-DCQ: piperazine) would be used for our reaction.[76,77] This ratio was selected to primarily minimise the formation of bis- substituted by-product. As has been mentioned earlier, most previous research methods describing similar coupling reactions of 4,7-DCQ and secondary and tertiary amines have utilized the ratio of 1:3 or more.[76, 77, 82] In addition, the use of catalytic amounts of potassium iodide (KI) as described by Kumar *et al* is known to increase the efficiency of nucleophilic substitution reactions.[82] They have claimed that the molar ratio of the reactants can be decreased from 1:5 to 1:1 when KI is employed as a catalyst. Potassium iodide catalyses the reaction by substitution of the chorine atom on the quinoline ring (4-chloro site) with an iodine atom, to form a stable iodide intermediate. Iodide is subsequently displaced more readily than chloride due to the weak carbon-halogen bond thus making the nuclear carbon (C-4) more prone to nucleophilic substitution by the amine.[93]

Therefore, in our third attempt at the coupling reaction of piperazine and 4,7-DCQ the solvent-base used was DIPEA and catalytic amounts of KI were added. The reaction produced a dark brown substance which was purified *via* a basic work up. However, as in previous attempts the procedure generated a shiny chocolate coloured solid with an ^{1}H NMR spectrum ($CDCl_3$) that showed the presence of 4,7-DCQ only(minute quantities of solvent impurities were also observed).

The fourth and final attempt at the coupling of 4,7-DCQ with piperazine was based directly on a published method by Clarkson *et al* (See Figure 26, Page 40).[77] As mentioned at the beginning of this chapter, three different research groups had synthesized 7-chloro-4-[piperazin-1-yl]quinoline previously using fairly similar approaches. The method described by Clarkson *et al* which coupled 4,7-DCQ with piperazine in a 1:5 ratio in presence of K_2CO_3 and triethylamine was selected for the present research.[77] All the steps in the procedure including the work up with 4:1 DCM: MeOH and column chromatography were repeated as described in the literature method. However, the ^{1}H NMR spectrum ($CDCl_3$) of the pale yellow oil that was formed did not show any peaks for the desired product, but indicated the presence of piperazine only. The reason for the reaction's failure could not be determined, bearing in mind that the procedure was reproduced exactly. Due to time constraints, no further investigation or repeat of this reaction was attempted.

3.3.2 Coupling Reaction of 4,7-DCQ and tBOC-Piperazine

The problems of direct coupling of piperazine with 4,7-DCQ have been discussed in the sections above (Section 3.1.1 and 3.1.2). One of the strategies to overcome these issues, as mentioned earlier is to protect piperazine with a suitable protecting group. In this section, the attempted coupling reactions of tBOC-piperazine with 4,7-DCQ are discussed. As mentioned earlier, tBOC-piperazine is a commercially available substance therefore no attempts were made to synthesise this compound in our laboratory.

Two methods were made to couple 4,7-DCQ with tBOC-piperazine (Pages 41 and 42). The first method was partly based on a literature method described by Madrid *et al.*[78] They coupled 4,7-DCQ with 4-aminopiperidine using DIPEA as a solvent-base at 100^0C for 20 hours.[78] In our first attempt, DIPEA was used as the solvent and the reaction conditions were virtually unchanged except a milder reaction temperature of 80°C (for 48 hours) was employed(see Figure 27, Page 41). DIPEA was used for its better solubility as reported by Madrid and Klingenstein *et al.*[78,96] However, the use of tBOC-piperazine in the present research meant that there was very little scope for the formation of a bis substituted product unless deprotection occurred in the reaction flask. This later scenario is highly unlikely since strongly acidic conditions are required for this to occur. 4,7-DCQ and tBOC-piperazine were mixed in the molar ratio of 1:1. This was based on the assumption that with only one available site on tBOC-piperazine for nucleophilic aromatic substitution it would react quantitatively with 4,7-DCQ. This is in contrast to previous literature methods which have reacted protected amines in a higher molar ratio while coupling them with 4,7-DCQ. These include a method by Solomon *et al* in which mono-BOC-protected diaminoalkanes and 4,7-DCQ were reacted in the molar ratio 1:2.[94] After completion of the reaction, a shiny chocolate coloured solid was obtained. The ^{1}H NMR spectrum ($CDCl_3$) of this solid showed peaks for the desired product. But 4,7-DCQ and tBOC-piperazine were also evident. The 9H singlet for tBOC at δ 1.45 ppm was larger than expected and the 12H isopropyl doublet normally observed for DIPEA (observed at δ 1-2 ppm) appeared to be overlapping with the product tBOC singlet. Another possible reason for the extension of this singlet could be the presence of unreacted tBOC-piperazine. Flash column chromatography was performed on the crude solid and ^{1}H NMR spectral

analysis performed on the solid obtained after the purification showed the product was present; but that impurities such as 4,7-DCQ, tBOC-piperazine and DIPEA were also evident.

The second attempt was similar to the above reaction except for the use of a catalytic amount of KI (see Page 42). After refluxing for 48 hours at 80°C in DIPEA, TLC analysis showed almost no traces of starting material. A work up procedure based on earlier reactions was used to purify the crude product (Section 3.3.1). The ^{1}H NMR spectrum ($CDCl_3$) on the solid obtained showed that the desired product, 7-chloro-4-(tBOC piperazin-1-yl)quinoline had been successfully synthesized with only minor impurities present. The reaction yield was approximately 46 % (including minor impurities). The following table lists the product peaks that were observed for 7-chloro-4-(tBOC piperazin-1-yl)quinoline.

Table 1 Peak interpretation results for 7-chloro-4-(tBOC piperazin-1-yl)quinoline

Chemical shift (δ)	Peak type	Proton	Assignment
8.61	Doublet	1H	H-2
8.40	Doublet	1H	H-8
7.94	Doublet	1H	H-5
7.50	Doublet of doublet	1H	H-6
6.95	Doublet	1H	H-3
3.72	Multiplet	4H	2×H-5^{1}&3^{1}
3.43	Multiplet	4H	2×H-2^{1}&6^{1}
1.50	Singlet	9H	tBOC (3×CH_3)

3.3.2.1 Deprotection of 7-chloro-4-(tBOC piperazin-1-yl) quinoline

The next step required the deprotection of 7-chloro-4-(tBOC piperazin-1-yl)quinoline afforded in the previous coupling reaction, to remove the tertiary butoxy carbonyl group (See Page 43). The deprotected product 7-chloro-4-(piperazin-1-yl)quinoline would subsequently be coupled by reacting with 1,3-dichloropropanol (1,3-DCP) for the final synthesis of HPQ. Various methods for the deprotection of the tBOC group have been described in the literature.[65,66] The procedure should be achievable under relatively mild conditions using strong acids such as HCl and trifluoroacetic acid (TFA) in the presence or absence of DCM as solvent.[67,87] Fray *et al* describe a

method for the deprotection of *N*-Boc-piperazine using 50% TFA: DCM at 20ºC for 30 mins.[67, 87, 88] The use of HCl and ethyl acetate was described by Stahl *et al*.[97] Solomon *et al* deprotected the *N*-Boc-protected derivatives of 4-aminoquinolines using 20% HCl in dioxane.[94] In the present work, a 50% (v/v) TFA: DCM mixture was used to deprotect 7-chloro-4-(tBOC piperazin-1-yl)quinoline. The end products from the successful coupling reactions described above (section 3.3.2) were stirred separately with 50% (v/v) TFA: DCM at room temperature for 15 mins. The method appeared to be very efficient because ^{1}H NMR spectral analysis of the solid after work up showed only deprotected product. The reaction yield for the deprotection step was 65 %. Trace quantities of DCM were present along with two broad peaks at δ 4.00 ppm and δ 3.90 ppm and a triplet at δ 3.35 ppm. These impurity peaks were also common to other spectra obtained in our laboratory using the same source of NMR solvent ($CDCl_3$) and represent an inert contaminant in the solvent bottle. The following table lists the product peaks that were observed for 7-chloro-4-(piperazin-1-yl)quinoline.

Table 2 Peak interpretation results for 7-chloro-4-(piperazin-1-yl)quinoline

Chemical shift (δ)	Peak type	Proton	Assignment
8.61	Doublet	1H	H-2
8.40	Doublet	1H	H-8
7.94	Doublet	1H	H-5
7.50	Doublet of doublet	1H	H-6
6.95	Doublet	1H	H-3
4.00	Multiplet	8H	2H at 2^1, 6^1, 5^1& 3^1
3.2	Multiplet	1H	H-4^1

3.4 Synthesis of Hydroxypiperaquine (HPQ)

Following on from the successful synthesis of the intermediate 7-chloro-4-(piperazin-1-yl) quinoline, described in the previous section the next synthetic step planned was to couple this compound with 1,3-dichloropropanol to generate HPQ (see Page 24). Four separate attempts were made to synthesize HPQ (see Pages 44-48). Two different approaches based on literature methods were initially attempted.[63, 82] The first approach using an organic base; was based on a similar nucleophilic substitution reaction discussed in the above section (Section 3.3). The second approach utilised inorganic bases such as potassium carbonate and sodium carbonate in various solvents.

3.4.1 Synthesis of HPQ in organic base

The first attempted synthesis of HPQ in DIPEA was based on literature methods as described by Madrid *et al* and Klingenstein *et al*.[78, 96] The method described by Madrid *et al* did not employ solvent, but instead used large quantities of DIPEA as base and solvent.[78] In the present work, the coupling of 7-chloro-4-(piperazin-1-yl)quinoline with 1,3-dichloropropanol was performed in *N*-methylpyrrolidinone (NMP) with DIPEA and Et_3N as the bases (see Page 44). Catalytic amounts of KI were used according to Kumar's method.[82] The reaction was carried out at reflux for 24 h. The ^1H NMR spectrum ($CDCl_3$) of the pale chocolate coloured solid obtained was compared to reference data for HPQ from the review published by Xu *et al*.[3] However, the spectrum obtained indicated that the above reaction was not successful. The spectrum showed peaks pertaining to NMP, 1,3-DCP and trace quantities of DCM only.

3.4.2 Synthesis of HPQ in an inorganic base

The first attempt using an inorganic base (see Page 45) was based on the synthesis of hydroxychloroquine as described by Kumar *et al*.[82] The reactants (7-chloro-4-(piperazin-1-yl)quinoline and 1,3-dichloropropanol) were refluxed in the presence of K_2CO_3 and KI for 48 hours in isopropanol.[76,77,91] However, the ^1H NMR spectrum for the crude compound given showed only peaks for solvent and water. The sample was reanalysed after a basic work up procedure but, the spectrum for the obtained residue only showed peaks for chloroform and water.

The second attempt using an inorganic base (see Page 46) was based on the synthesis of bis (4-(7-chloroquinolin-4-yl) piperazin-1-yl)methane described by Vennerstrom *et al* (Figure 38). This compound is very similar in structure to HPQ and was synthesised by reacting 7-chloro-4-(piperazin-1-yl)quinoline with formaldehyde in methanol at room temperature.[63]

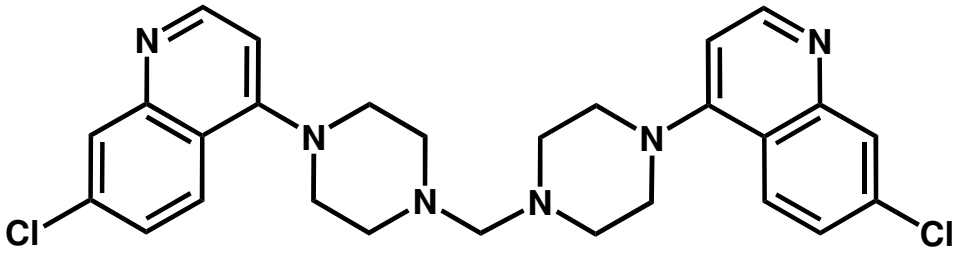

Figure 39 Structure of Bis(4-(7-chloroquinolin-4-yl) piperazin-1-yl)methane

In our attempt to synthesize HPQ from 7-chloro-4-(piperazin-1-yl)quinoline similar reaction conditions were employed to that described for the synthesis of the above compound. However, the ^{1}HNMR spectrum for the dark brown oily liquid obtained did not show any peaks for HPQ and only 1,3-DCP was present.

The third attempt using K_2CO_3 (see Page 47) was based on a method described by Ryckebusch *et al*.[92] They synthesized a range of piperazine derivatives *via* nucleophilic substitution reactions of 4,7-DCQ with bis-(3-aminopropyl)piperazine in 1-pentanol (amyl alcohol). This solvent has been previously used by Fong in our laboratory for the attempted synthesis of HPQ.[64] The reactants were refluxed for 14 hours at 120ºC with K_2CO_3. Following purification of the crude solid obtained by column chromatography the reaction yielded a brownish yellow solid which contained minute impurities. The ^{1}H NMR spectrum ($CDCl_3$) for the brownish yellow solid was compared to the spectrum described by Xu *et al*[3] and this conformed that HPQ was present in a crude form. The partial success of this reaction can possibly be attributed to 1-pentanol. Pentanol is a high molecular weight alcohol and is more lipophilic/less polar resulting in lower solvation of the nucleophile (similar to aprotic solvents).[93] This would allow it to possibly react more efficiently than the previous alcohols used in the first two attempts. The following table lists the product peaks that were

observed for HPQ. Apart from these peaks, trace amounts of DCM and 1-pentanol were observed in the spectrum.

Table 3 Peak interpretation results for HPQ

Chemical shift (δ)	Peak type	Proton	Assignment
8.71	Doublet	2H	H-2 × 2
8.10	Doublet	2H	H-8 × 2
7.97	Doublet	2H	H-5 × 2
7.49	Doublet of doublet	2H	H-6 × 2
6.88	Doublet	2H	H-3 × 2
4.10	Pentat	1H	CH-OH
3.60	Broad singlet	1H	OH
3.40	Broad singlet	8H	2 × 2H at 2^1 and 6^1piperazine
2.80	Singlet	8H	2 × 2H at 5^1 and 3^1piperazine
2.30	Doublet	4H	CH_2-propanol × 2

Though impurities were present in the final product, future attempts at the synthesis of HPQ then using an improved chromatographic purification and recrystallisation should yield a product of relatively high purity. It was planned at the start of the research program that upon successful completion of each synthetic step, each step would be reproduced and possibly modified to further optimize the reactant proportions, solvent and base quantities. This would have resulted in a final synthetic route with improved product yields. The above optimization process could not be undertaken due to time restrictions. However, this research is a part of ongoing work at the School of Pharmacy (Curtin University of Technology) to explore the synthesis of HPQ and will endeavour to undertake this optimization process.

The final synthetic approach to HPQ synthesis in this research was achieved *via* a three step synthetic process (Figure 39). In the first step, commercially available tBOC piperazine was coupled with 4,7-DCQ in a molar ratio of 1:1 in presence of KI in DIPEA at reflux for 48 hours. The reaction produced the intermediate compound 7-chloro-4-(tBOC piperazin-1-yl)quinoline (46 % yield). The introduction of tBOC piperazine as the starting material was crucial and prevented the past major complication arising from contamination of the product with excess piperazine.

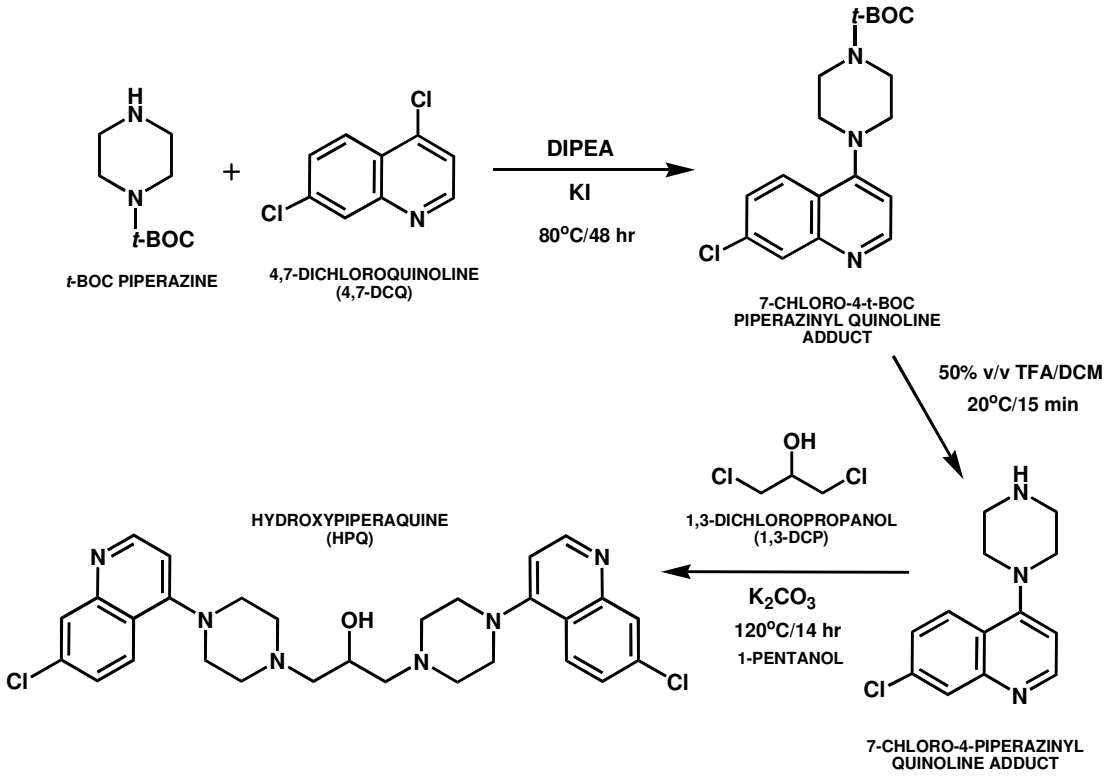

Figure 40 Three step synthetic route to HPQ

The second synthetic step involved the deprotection of 7-chloro-4-(tBOC piperazin-1-yl)quinoline to remove the tBOC protecting group and render the amine site on the piperazine molecule free for nucleophilic substitution reaction with 1,3-DCP. This was achieved cleanly and efficiently by deprotection with TFA: DCM mixture (50% (v/v)). (65 % yield)

The deprotected intermediate (7-chloro-4-(piperazin-1-yl)quinoline) was then reacted with 1,3-DCP using K_2CO_3 in 1-pentanol to generate HPQ. The reaction was performed with a 2:1 molar equivalent ratio of the reactants at 120ºC for 14 hours(59 % yield) 1-Pentanol proved an ideal solvent, dissolving both starting materials with ease and permitting the desired high reflux temperature for reaction to occur efficiently. However, even after chromatographic purification, the product (HPQ) synthesised was not completely free from solvent impurities (DCM and 1-pentanol). Unfortunately due to time constraints this step was not optimised and neither was any effort made to improve the purification procedure.

4 CONCLUSIONS

It is estimated that over 40% of the world's population is exposed to malaria and 270 million of those exposed are infected with malarial parasites. [13] The emergence and spread of drug-resistant malarial parasites has become a major global issue because chemotherapy remains the most important means of controlling malaria. [16,18] Therefore the development of novel drugs and drug combinations to replace older drugs is imperative.

Hydroxypiperaquine (HPQ) is a bisquinoline antimalarial drug which displays very similar antimalarial activity to its parent compound piperaquine (PQ).[3] HPQ as an antimalarial has potentially numerous advantages over piperaquine. The additional hydroxyl group in HPQ appears to make it a prime candidate for Phase 2 metabolic processes e.g. glucuronidation, which should increase its clearance rate thus reducing its biological half life (piperaquine has a very long biological half life). This may lower the probability of drug resistance. The additional hydroxyl group should also be readily derivatized to afford a range of simple ester prodrugs in order to address a range of pharmaceutical issues.[68,69]

The primary aim of this research project was to synthesise HPQ *via* alternative synthetic pathways to that briefly described in the literature.[3] The major objectives of the research program were to explore various synthetic strategies based on literature synthetic procedures involving similar compounds. In this study, various synthetic approaches, employing a variety of reaction conditions, reactants, catalyst and solvents were trailed in attempts to achieve the synthesis of HPQ. The success and efficacy of experiments performed were assessed *via* ^{1}H NMR spectroscopy and TLC exclusively.

Initial synthetic approaches attempted to synthesize the intermediate 1,3-(bis piperazinyl)-propan-2-ol from 1,3-DCP and protected piperazine. Protected piperazines such as 1-benzylpiperazine and tBOC piperazine were employed. However, this strategy was not successful. After the failure of this approach, the synthesis of the intermediate 7-chloro-4-(piperazin-1-yl)quinoline from 4,7-DCQ *via* a tBOC protected piperazine was achieved. The synthesis of HPQ was finally achieved by reaction of the deprotected intermediate with 1,3-dichloropropanol using K_2CO_3 in 1-pentanol.

The scheme below illustrates the final three step synthetic route for the synthesis of hydroxypiperaquine (HPQ).

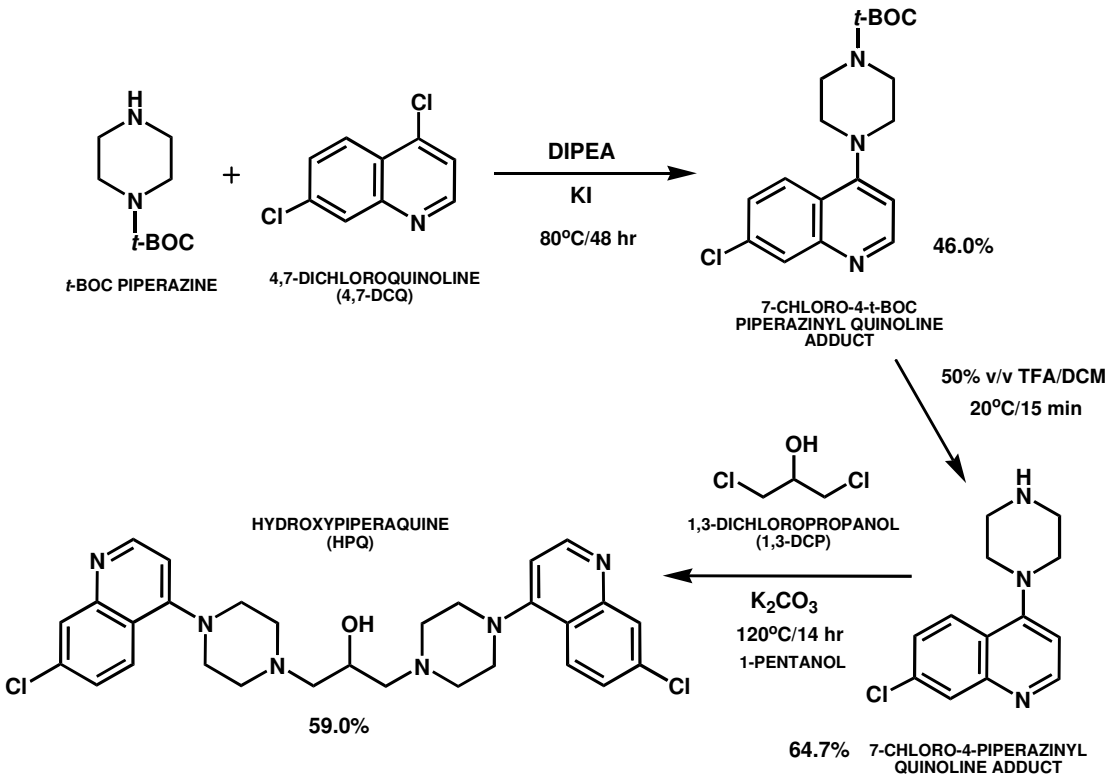

Figure 41 Summary of HPQ synthesis (Three step synthetic route to HPQ)

In conclusion, the above synthetic approach provides a relatively efficient process for the synthesis of HPQ from relatively accessible starting materials and reagents. However, further refinement and optimization of the reaction conditions and reactant proportionalities will be required. The use of time consuming column chromatography could possibly be replaced by recrystallisation to afford the compound in a very high level of purity. Unlike the previously reported synthesis of HPQ by Xu *et al*[3], this research provides specific details of the synthetic methodology involved for the synthesis of HPQ.

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6 APPENDICES

Figure 42 ^{1}H NMR spectrum ($CDCl_3$) for the reaction 2.1.2.1

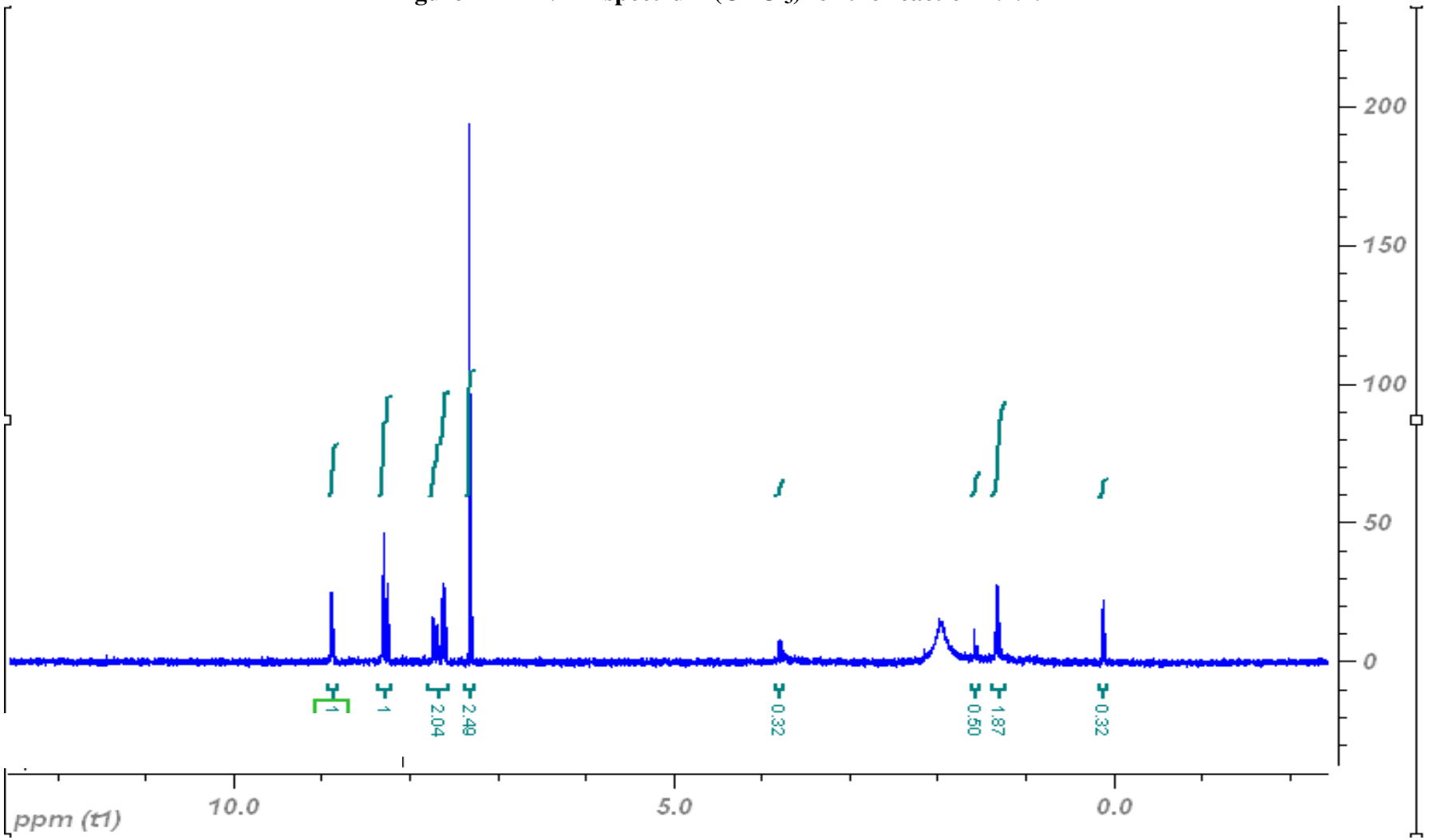


Figure 43 ^{1}H NMR spectrum ($CDCl_3$) for the reaction 2.1.2.2

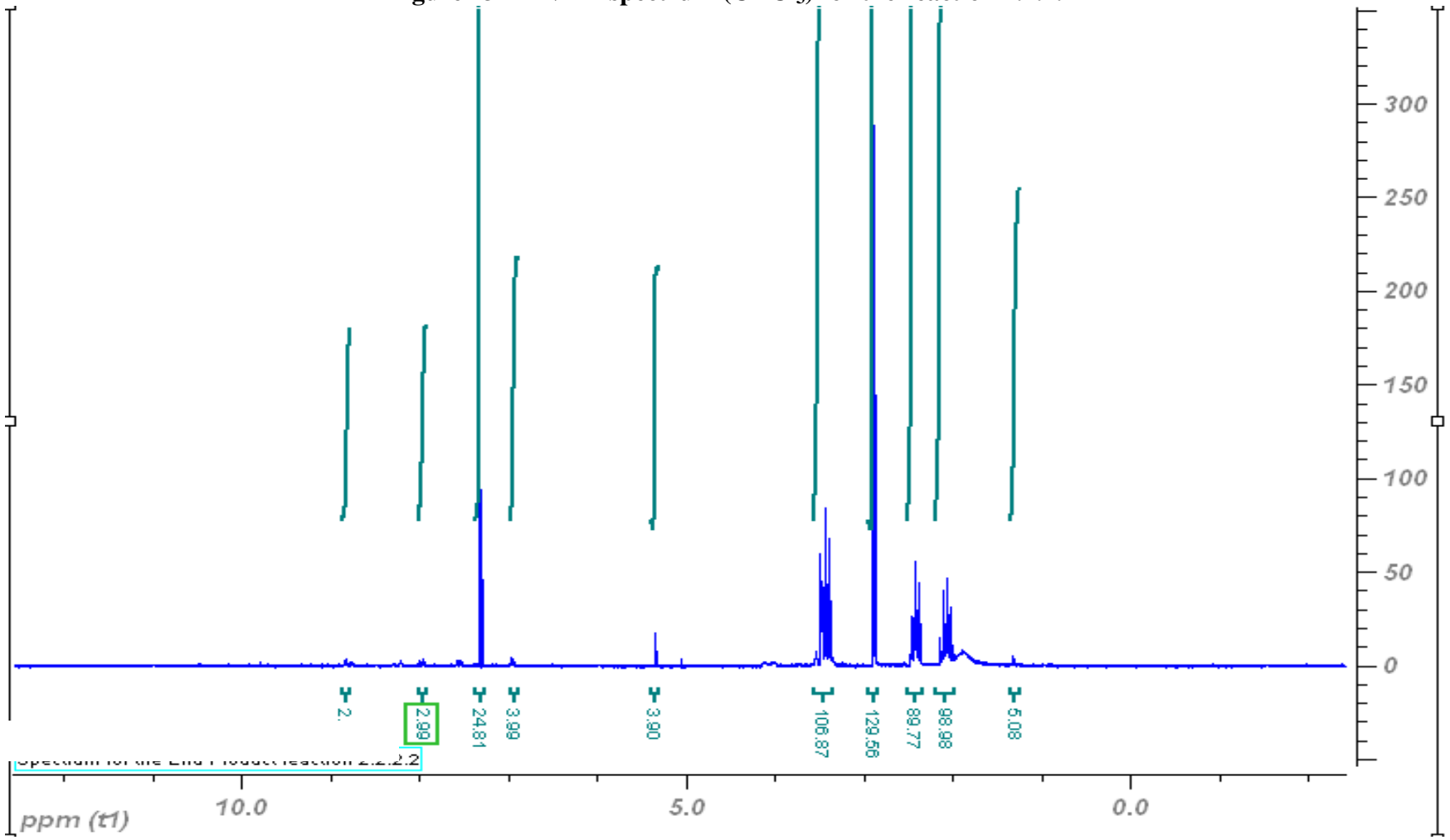


Figure 44 ^{1}H NMR spectrum ($CDCl_3$) for the reaction 2.1.2.3

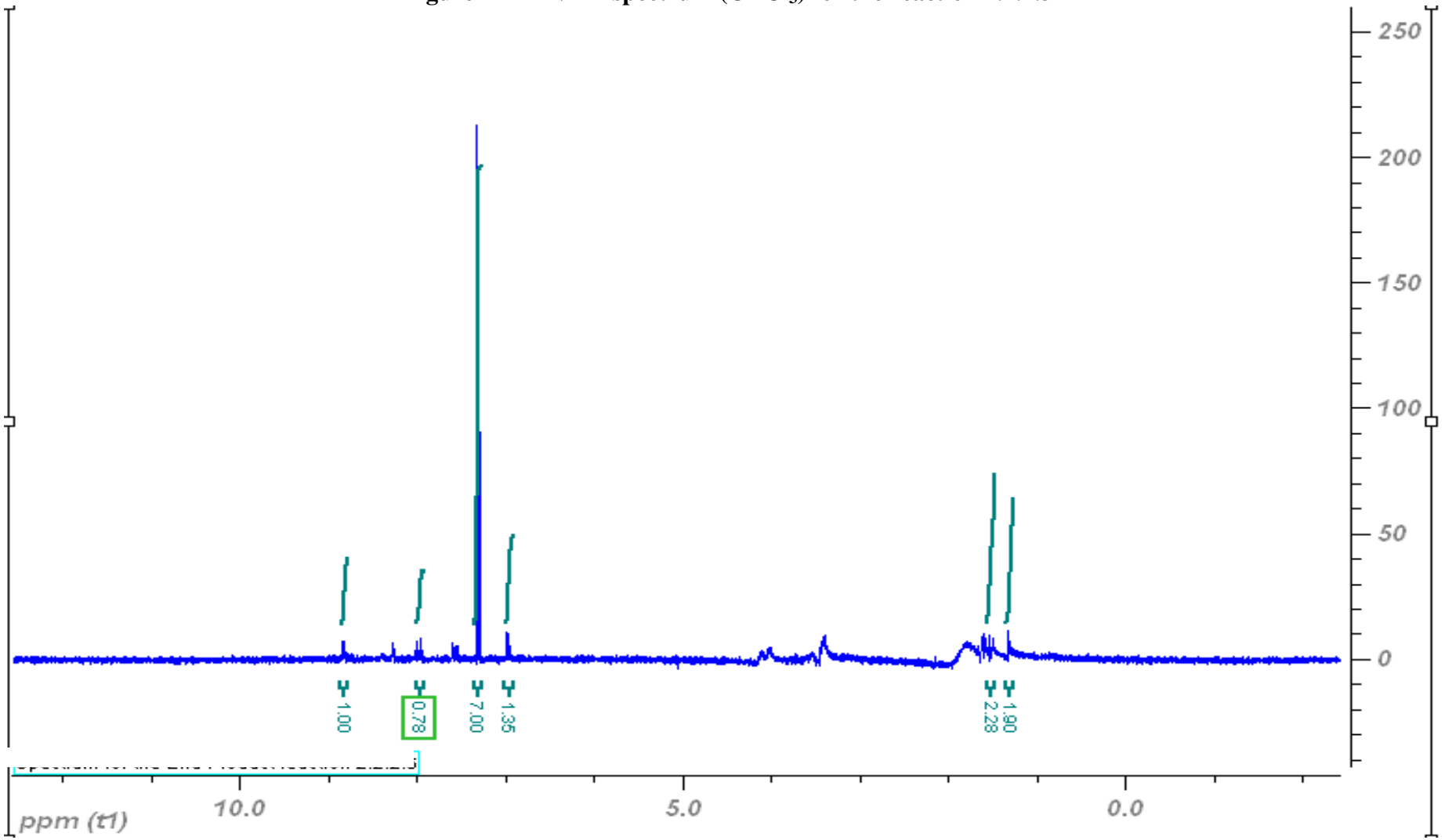


Figure 45 ^{1}H NMR spectrum ($CDCl_3$) for the reaction 2.1.2.5

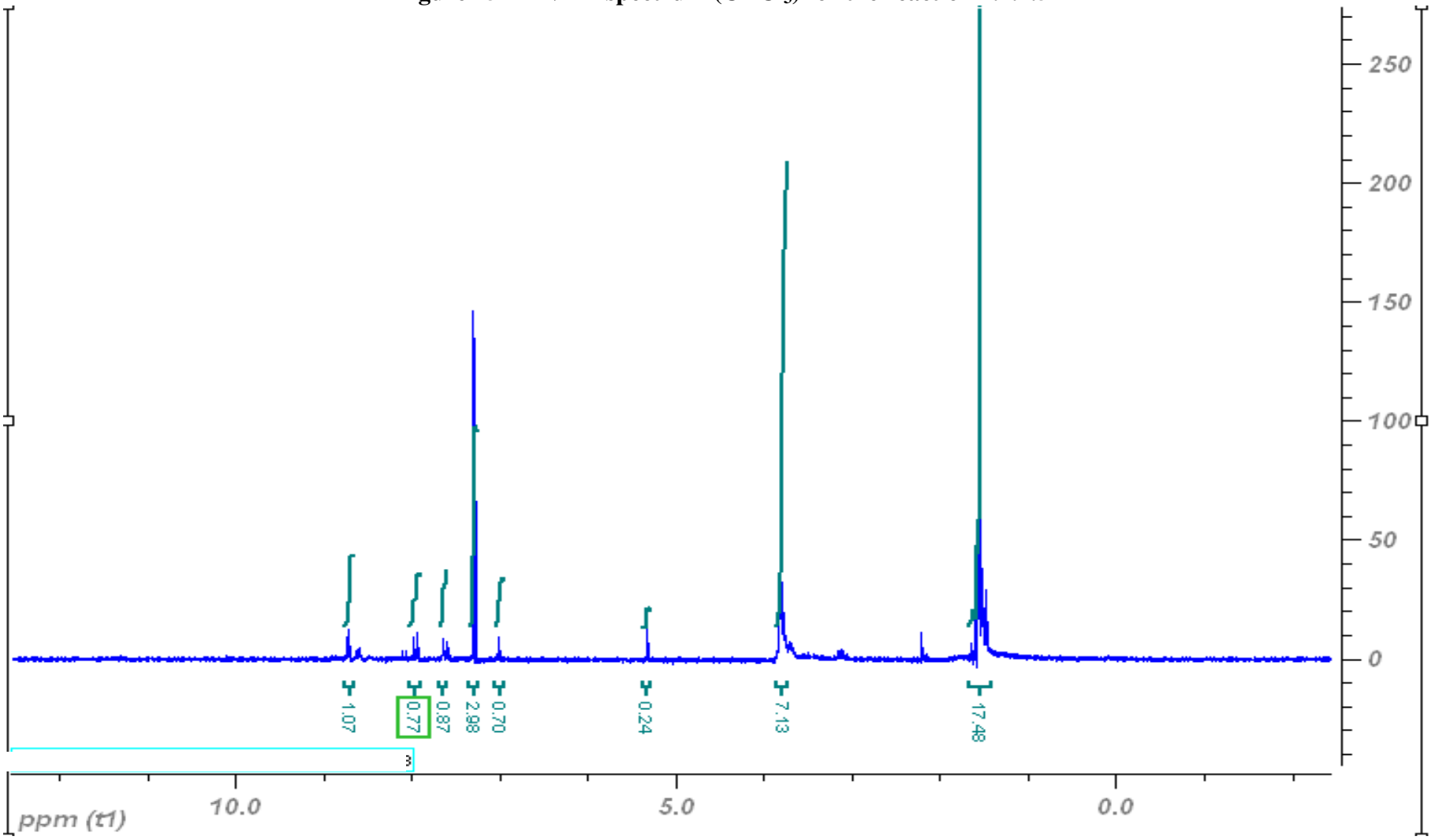
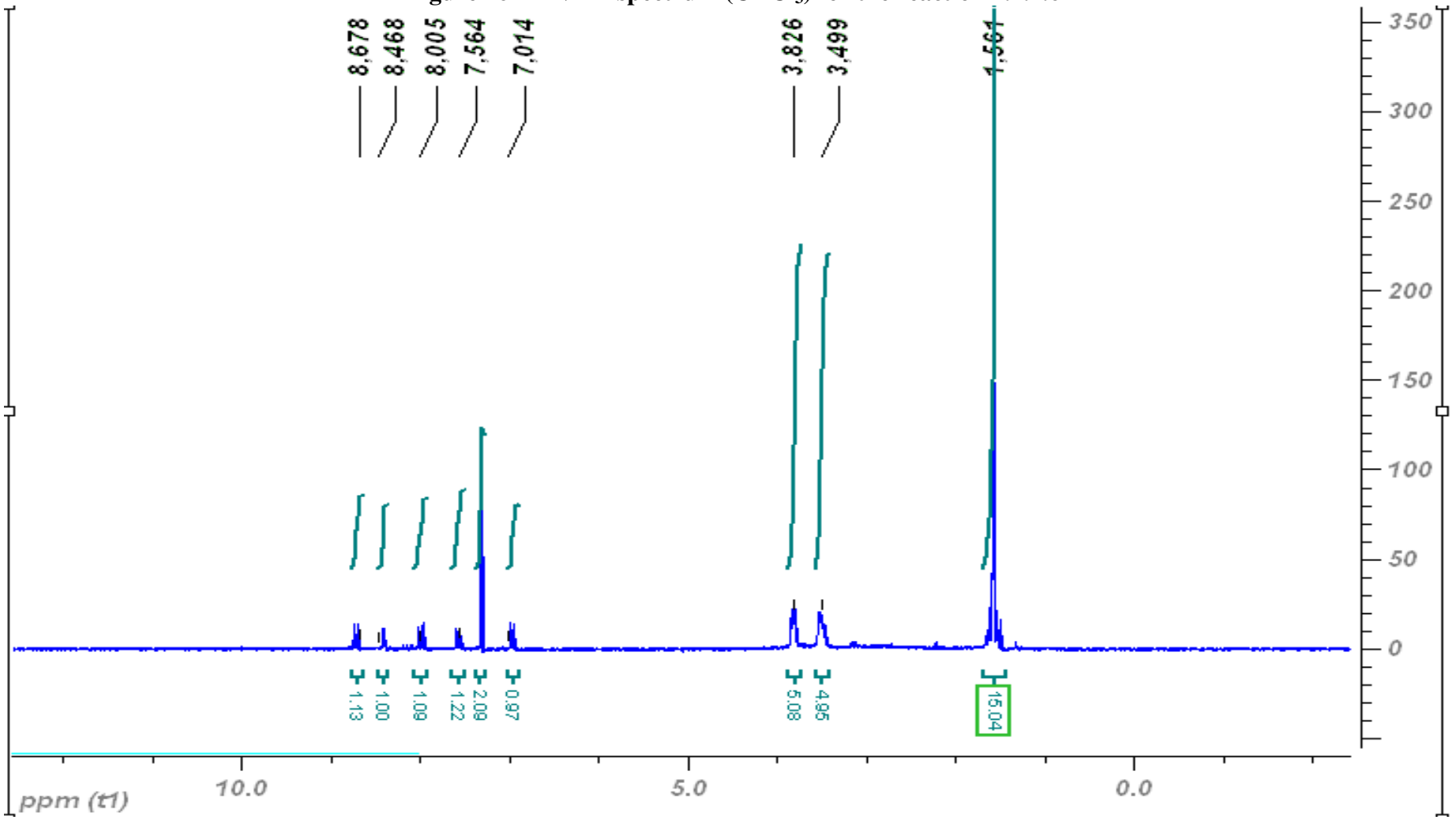


Figure 46 ^{1}H NMR spectrum ($CDCl_3$) for the reaction 2.1.2.6



Chemical shift (δ)	Peak type	Proton	Assignment
8.61	Doublet	1H	H-2
8.40	Doublet	1H	H-8
7.94	Doublet	1H	H-5
7.50	Doublet of doublet	1H	H-6
6.95	Doublet	1H	H-3
3.72	Multiplet	4H	H-5^1&3^1
3.43	Multiplet	4H	H-2^1&6^1
1.50	Singlet	9H	tBOC (3×CH_3)

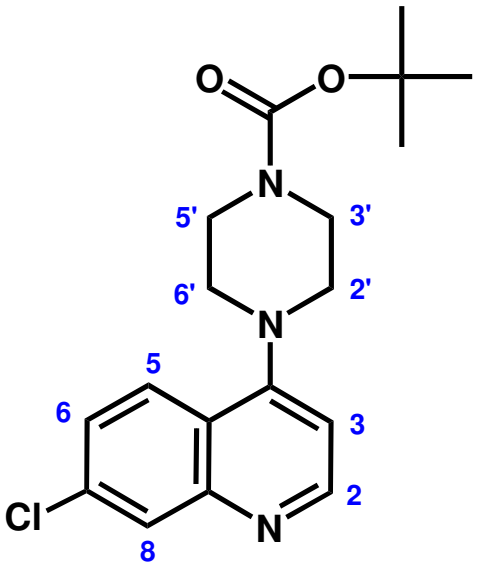
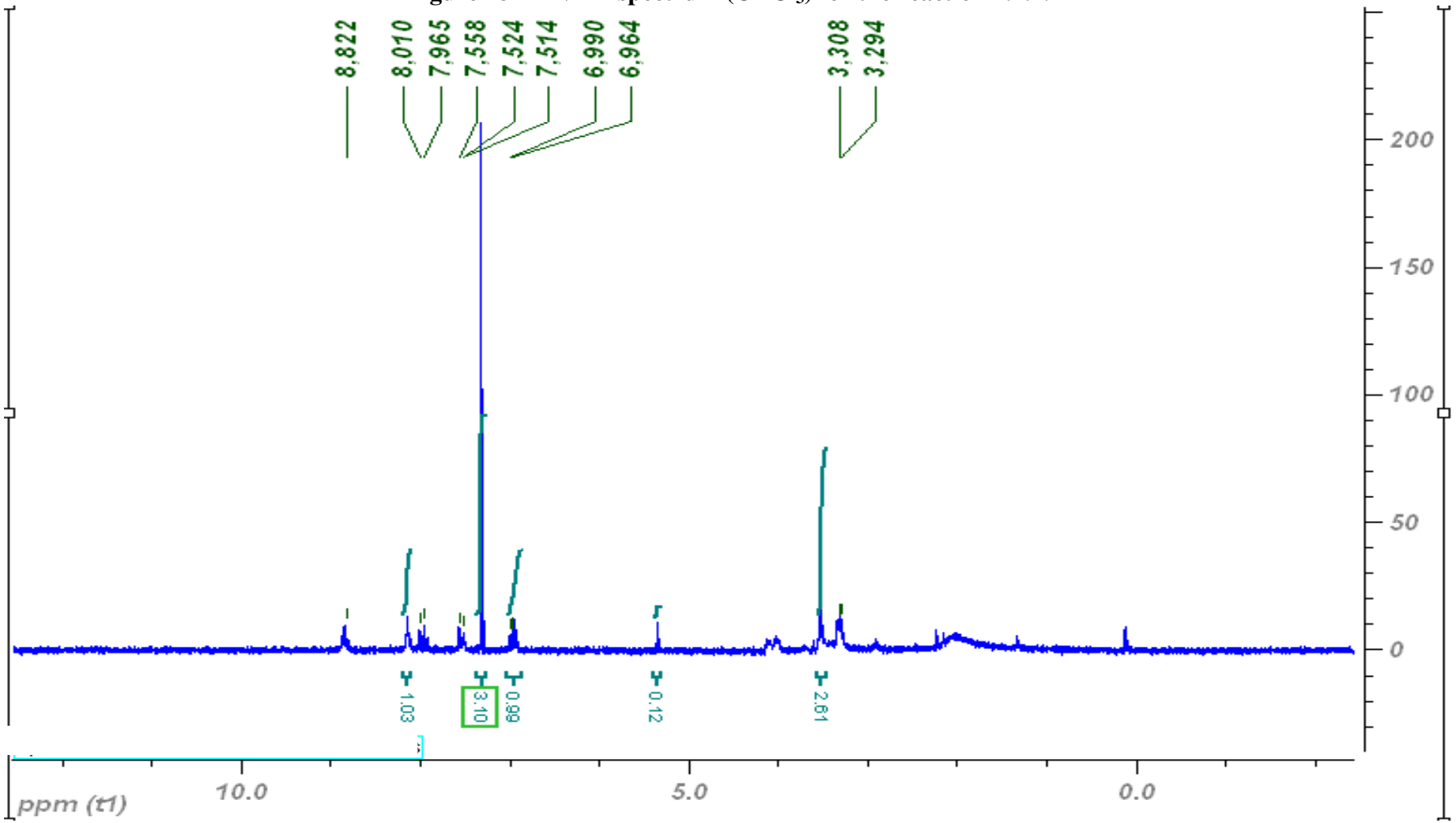


Figure 47 Peak interpretation results and numbered structure of 7-chloro-4-(tBOC piperazin-1-yl)quinoline

Figure 48 ^{1}H NMR spectrum ($CDCl_3$) for the reaction 2.1.2.7



Chemical shift (δ)	Peak type	Proton	Assignment
8.61	Doublet	1H	H-2
8.40	Doublet	1H	H-8
7.94	Doublet	1H	H-5
7.50	Doublet of doublet	1H	H-6
6.95	Doublet	1H	H-3
3.43	Multiplet	8H	2H ×(H-2^{1}& 3^{1}, 5^{1}&6^{1})
1.50	Singlet	1H	H-4^{1}

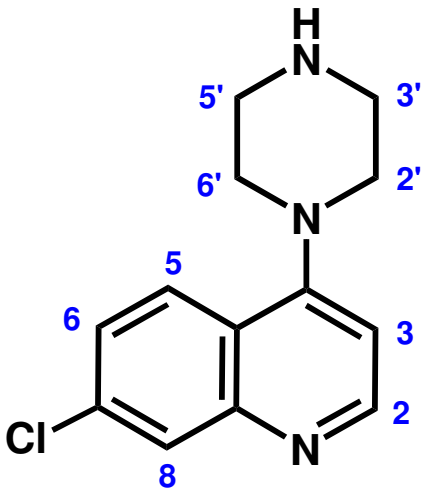


Figure 49 Peak interpretation results and numbered structure of 7-chloro-4-(piperazin-1-yl)quinoline

Figure 50 ^{1}H NMR spectrum ($CDCl_3$) for the reaction 2.1.3.1

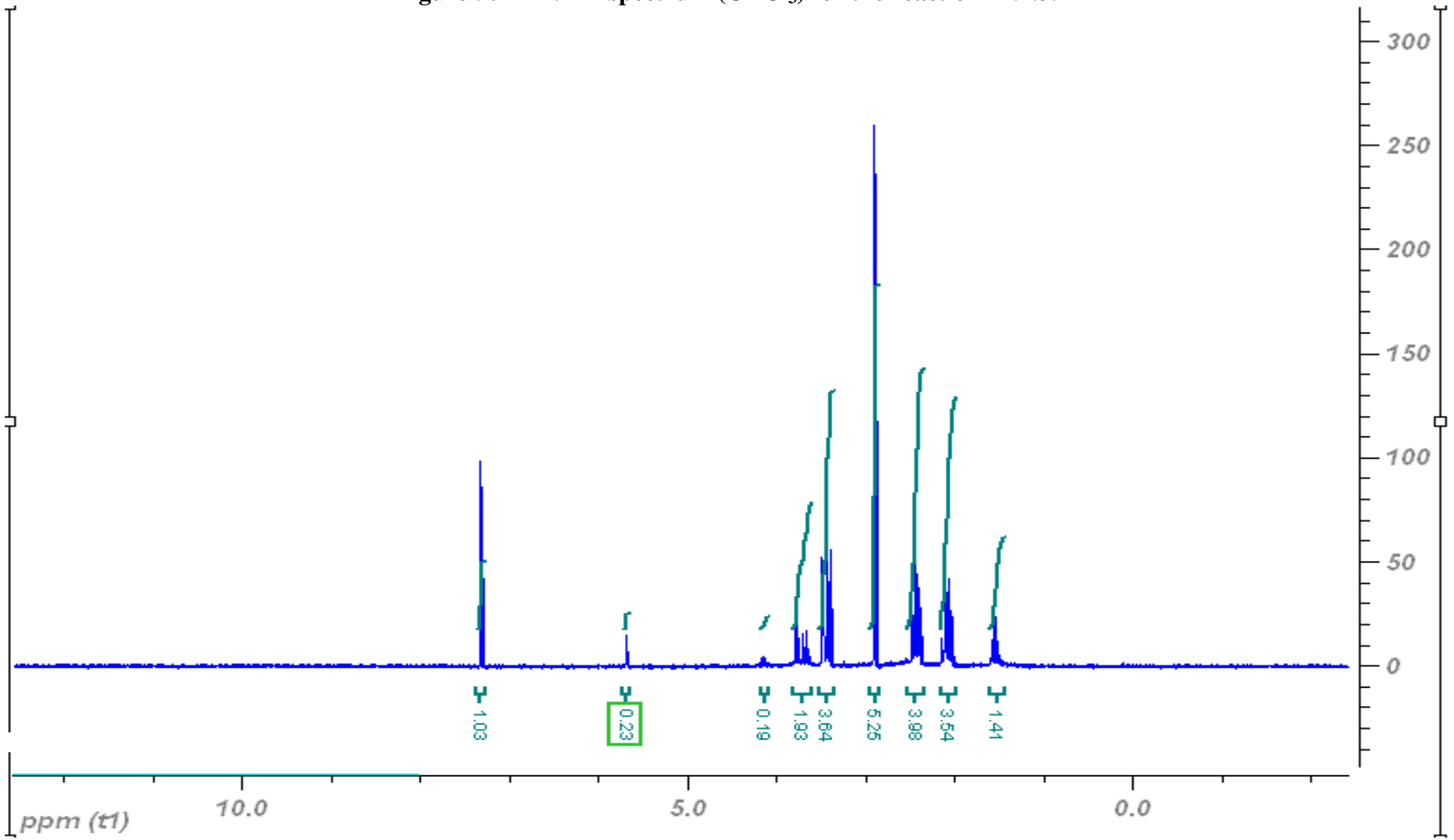


Figure 51 ^{1}H NMR spectrum ($CDCl_3$) for the reaction 2.1.3.2

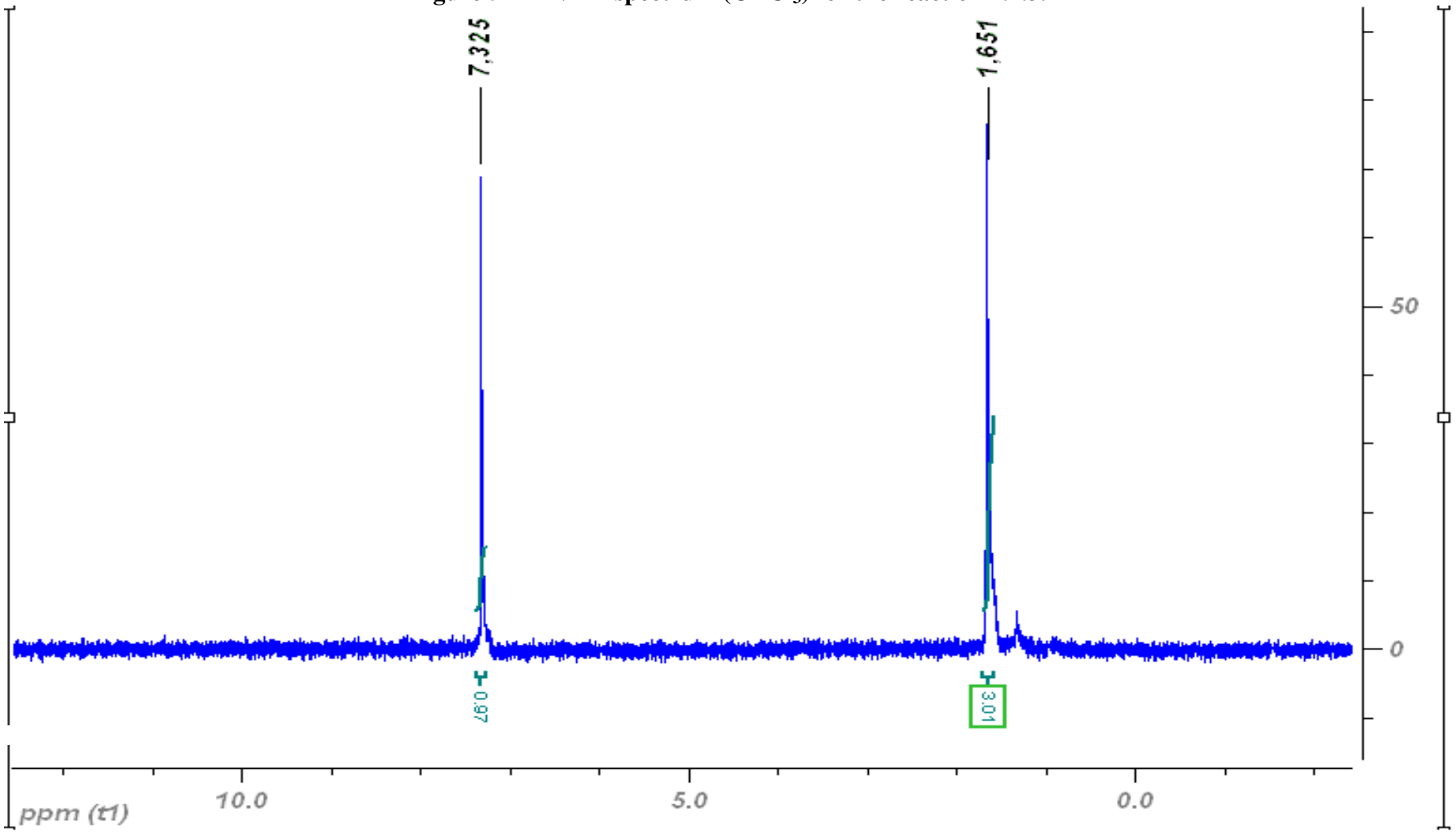


Figure 52 ^{1}H NMR spectrum ($CDCl_3$) for the reaction 2.1.3.3

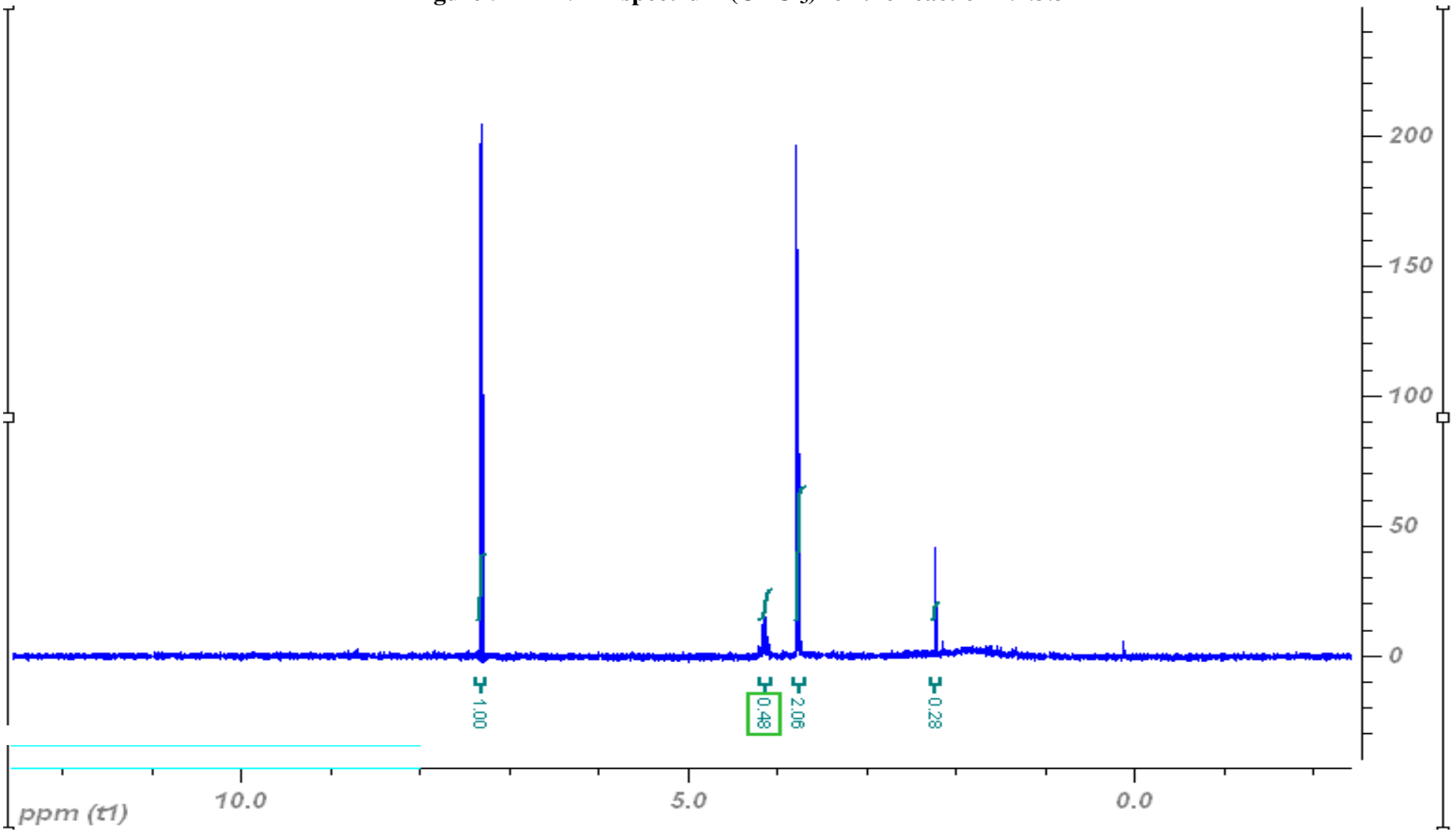
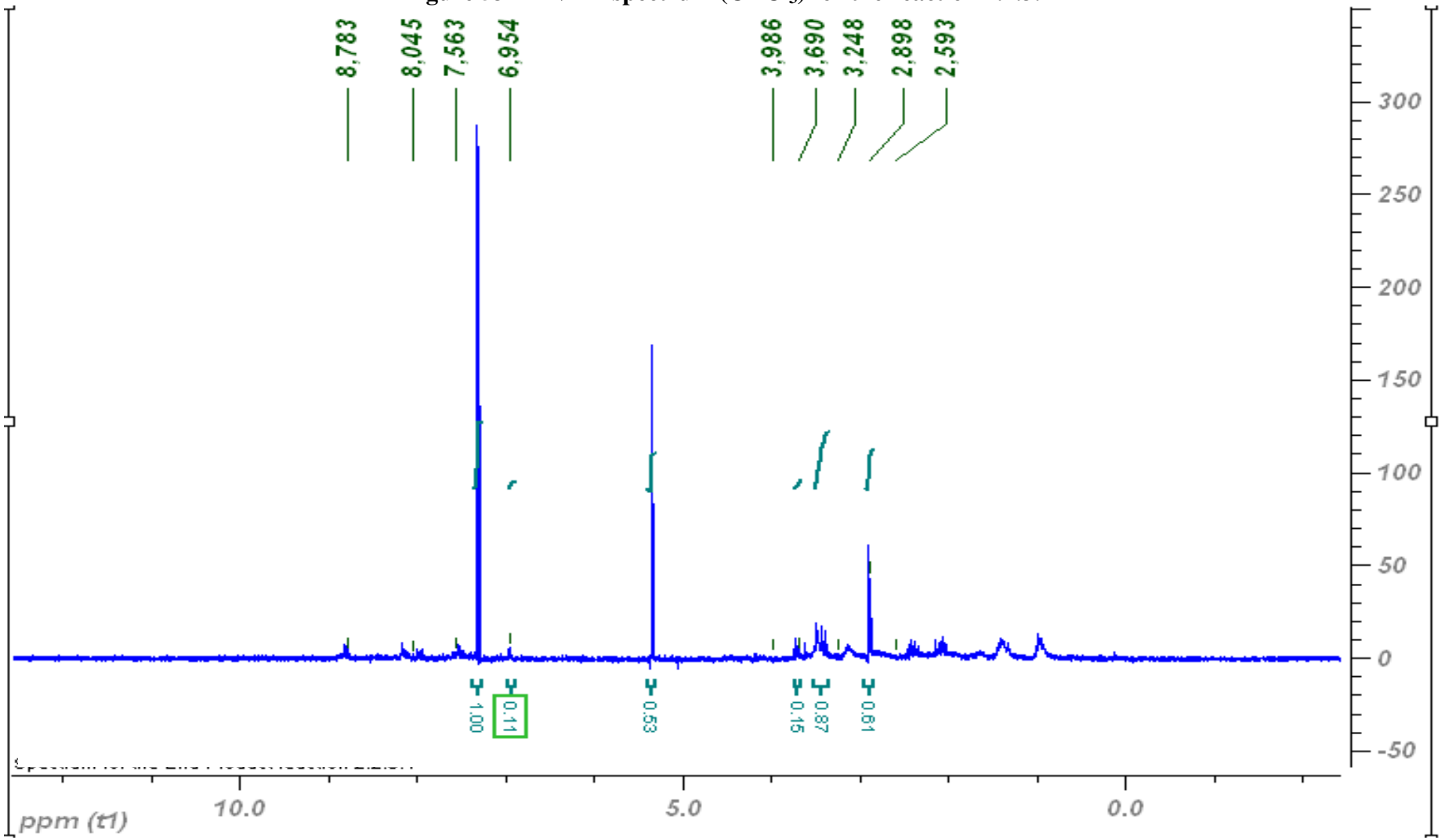


Figure 53 ^{1}H NMR spectrum ($CDCl_3$) for the reaction 2.1.3.4



Chemical shift (δ)	Peak type	Proton	Assignment
8.71	Doublet	2H	H-2 × 2
8.10	Doublet	2H	H-8 ×2
7.97	Doublet	2H	H-5 ×2
7.49	Doublet of doublet	2H	H-6 ×2
6.88	Doublet	2H	H-3 ×2
4.10	Pentet	1H	CHOH
3.60	Broad singlet	1H	OH
3.40	Broad singlet	8H	2H ×(2^{1} and 3^{1}piperazine)
2.80	Singlet	8H	2H ×(5^{1} and 6^{1}piperazine)
2.30	Doublet	4H	CH_2-propanol ×2

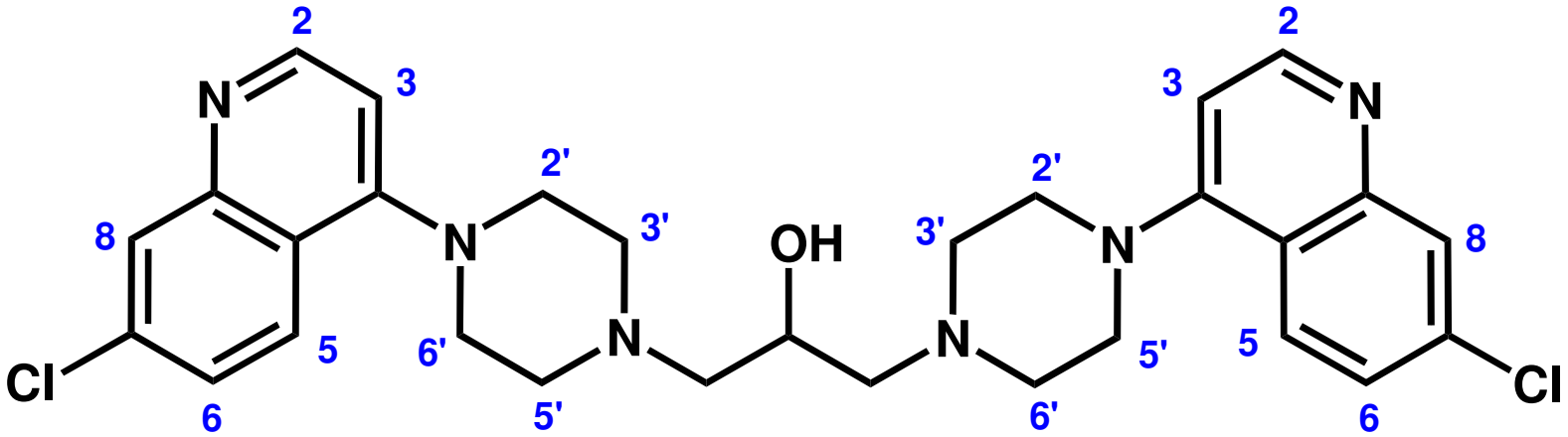


Figure 54 Peak interpretation results and numbered structure of HPQ