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**SYNTHESIS AND INVESTIGATION OF QUINOLINE BASED β -
HAEMATIN FORMATION INHIBITORS AS POTENTIAL
ANTIMALARIALS**

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A thesis submitted in fulfillment of the
requirements for the degree of

Doctor of Philosophy

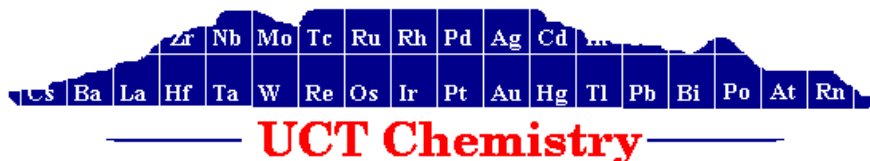
To the



UNIVERSITY OF CAPE TOWN
IYUNIVESITHI YASEKAPA • UNIVERSITEIT VAN KAAPSTAD

September 2010

Supervisors: Professor Timothy J. Egan and Professor Kelly Chibale



ABSTRACT

The question of whether or not replacing the 7-chloro group in the 4-aminoquinoline moiety with an electron withdrawing and hydrophilic substituent results in retention of antiplasmodial activity in the 4-aminoquinoline class of compounds was investigated. The effect of the lateral chain and the group at the 7-position of the 4-aminoquinoline ring on haem binding and inhibition of β -haematin formation were also evaluated. Accordingly, a series of 4-amino-7-X-, 4-methyl amino-7-X and 4-ethylene diamine-7-X-substituted quinoline molecules were successfully synthesised and evaluated. The group X (-CH₃, -OCH₃, -NH₂, -OH, -H, -CONH₂, -CN, -Cl, -NO₂ and -CF₃) was chosen from all four quadrants of a Hammett constant (σ) versus lipophilicity constant (π) Craig plot. In general, compounds with electron withdrawing groups were found to be good haem binders with the exception of -CONH₂. Electron releasing groups (-CH₃, -NH₂, OCH₃ and OH) were found to be weaker haem binders. An increase in binding constant (Log K) values was observed as the lateral chain was increased in length. Differences in log K values between 4-amino-7-X-quinoline and 4-ethylene diamine-7-X-quinoline compounds ranged from 0.05 to 0.85 log units. Furthermore, a significant correlation ($r^2 = 0.50$ and $P = 0.02$) between the lipophilicity constant and Log K values in the 4-amino-7-X-quinoline series was observed. 4-Aminoquinolines with electron releasing groups were either inactive or poor inhibitors of β -haematin formation; in contrast, compounds with electron withdrawing groups were efficient inhibitors of β -haematin formation. These observations suggest that the nature of the electron withdrawing substituents rather than the inherent lipophilicity of the substituent is the key feature governing the ability of each analogue to inhibit β -haematin formation. A series of 4-amino-7-X-quinoline compounds, where X is a subset of the substituents above, were

synthesised bearing the *N*¹,*N*¹-diethylpentane-1,4-diamine, 2-(dimethylaminomethyl) benzylamine and *N*-(4-(aminomethyl)benzyl)-*N*-methyl-1-(pyridine-2-yl)methanamine lateral chains. It was found that the compounds with electron withdrawing substituents at the 7-position of the quinoline ring retained activity against chloroquine sensitive (NF54) and chloroquine resistant (K1) strain of the malaria parasite. The -CN derivatives, which were the least lipophilic and electron withdrawing, had IC₅₀ values ranging from 8.88 to 93.52 nM and 14.22 to 456.29 nM for the NF54 and K1 strains respectively. In silico predictions indicate that the -CN substituted compounds may have overall better solubility and metabolic stability profiles relative to the chloro substituted compounds.

DECLARATION

I declare that this thesis is my own original research and all sources that I have used or quoted have been indicated and acknowledged by means of complete references.

Samkele Nsumiwa

ACKNOWLEDGMENTS

I would like to express sincere appreciation to Professor Timothy J. Egan and Professor Kelly Chibale for their unwavering support and guidance during the course of the project.

Special thank you to all my colleagues in the Department of Chemistry particularly those in the Organic Synthesis Lab and the Bio-Inorganic group.

Thank you to Dr. Frederic Douelle and Dr. Margaret Blackie for their efforts in my chemical education at UCT.

Thanks to my wife Saziso, the boys Nathan Bokani and Rileigh Dumoluhle for their patience.

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LIST OF ABBREVIATIONS

ACT	Artemisinin-based combination therapy
ADME	Absorption, Distribution, Metabolism and Excretion
AQ	Amodiaquine
BHIA ₅₀	50% inhibitory concentration (molar equivalents of drug relative to haem)
br	Broad singlet
CDCl ₃	Deuteriochloroform
CoMFA	Comparative molecular field analysis
CoMSiA	Comparative similarity analysis
CQ	Chloroquine
CQR	Chloroquine resistant
CQS	Chloroquine sensitive

d	Doublet
DCM	Dichloromethane
dd	Doublet of a doublets
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide
ECG	Electrocardiogram
Et ₃ N	Triethylamine
Fe(III)PPIX	Ferriprotoporphyrin IX
FQ	Ferroquine
GSK	GlaxoSmithKline
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
hERG	Human <i>ether-go-go</i> Related Gene

HPLC	High performance liquid chromatography
IC ₅₀	50% Inhibitory concentration
IR	Infrared spectroscopy
m	Multiplet
mp	Melting point
MAOS	Microwave assisted organic synthesis
MQ	Mefloquine
NMe	Methylamino
PDB	Pyridodibemequine
PfCRT	<i>Plasmodium falciparum</i> chloroquine resistant transporter protein
PfMDR1	<i>Plasmodium falciparum</i> multi drug resistant

PQ	Phenylequine
QN	Quinine
RBM	Roll Back Malaria
s	Singlet
SAR	Structure activity relationship
SEM	Standard error of the mean
t	Triplet
<i>t</i> -BuLi	Tertiary Butyllithium
TdP	Torsades de pointes
TLC	Thin layer chromatography
TMS	Trimethylsilane
WHO	World Health Organisation

σ_m *meta*-Hammett constant

σ_p *para*-Hammett constant

v/v Volume for volume

1. INTRODUCTION

1.1 Malaria

It is estimated that a third of the people in the world live in malaria endemic areas. According to estimates, a billion people carry the malaria parasite at any one time, making it the most significant parasitic infection in the world. [1.1] The World Malaria Report 2010 reports that there were 225 million clinical cases of malaria resulting in the death of 781, 000 people in 2009. [1.2] Malaria is prevalent in the tropical and subtropical areas of the world (Fig. 1). The majority of the world's poorer countries are in this region and thus the disease has the greatest economic impact on those countries that can least afford it. [1.3] In temperate areas such as Western Europe and the United States owing to, among other things, economic development and improved public health systems, the disease has been eradicated. [1.4]

Efforts such as the Roll Back Malaria (RBM) Partnership, launched in 1998 to provide coordinated response to the disease [1.5] and the Bill and Melinda Gates Foundation are among numerous organisations in the fight to control, eliminate and eventually eradicate malaria. Of these organisations, the Gates Foundation declared the most ambitious goal of eradication of the disease. [1.6] Statistics from the World Malaria Report 2010 indicate that the combined efforts of the various organisations are paying off with some countries recording more than 50% decrease in confirmed malaria cases. [1.2]

Control measures, as recommended by the World Health Organisation (WHO), include the use of insecticide-treated bed nets, indoor-residual spraying and accessibility of effective first line anti-malarial drugs. Obviously, the efficacy of the bed nets is limited in

that their use during the day and early evening is minimal. Insecticide resistance and economic challenges may be obstacles to realise the potential of indoor-residual spraying. [1.7] Vaccines could be an effective control however, despite years of research; a widely marketable vaccine is still not yet available.

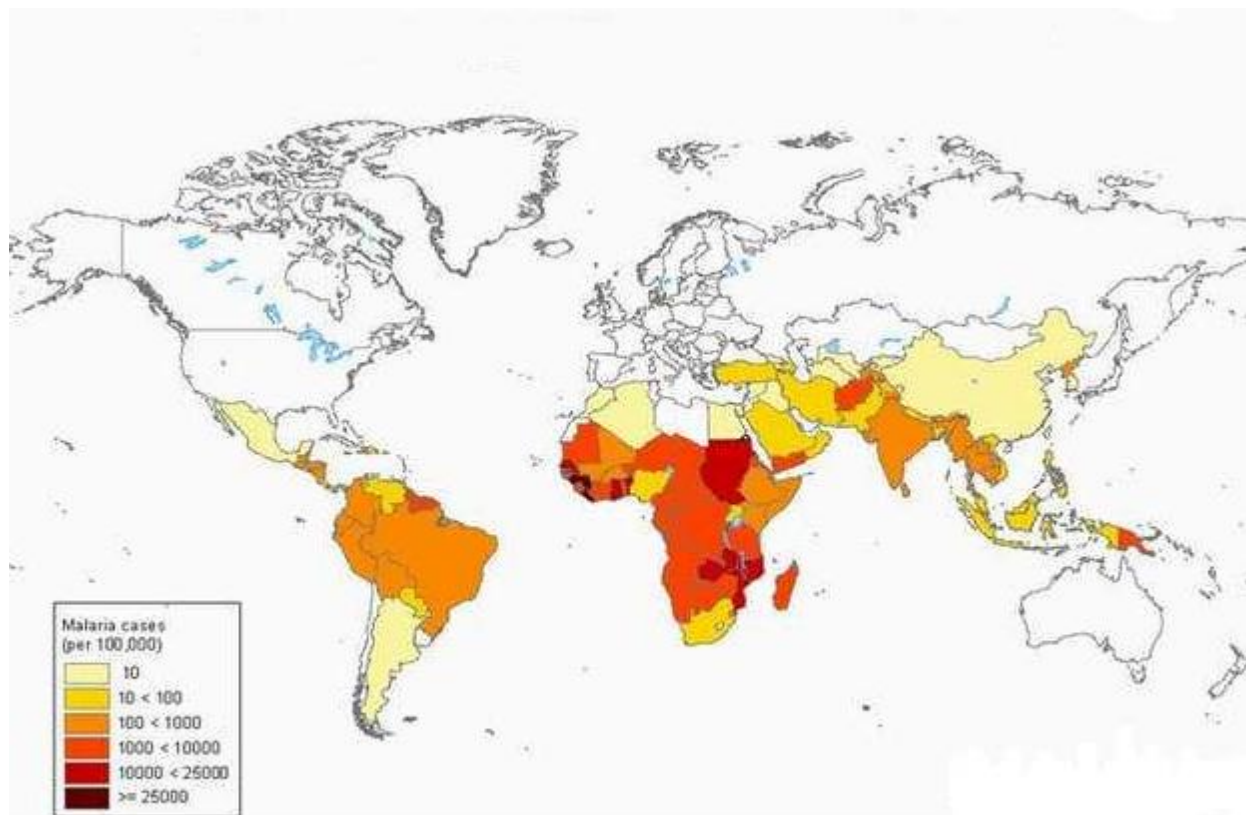


Figure 1: The distribution of malaria [1.8]

A protozoan infection that primarily affects red blood cells causes the disease. It is transmitted to humans by anopheles mosquitoes infected with *Plasmodium* parasites, four of which are known to use humans as their natural hosts. [1.9] Of the four major species of the genus *Plasmodia*, that infect human beings, the majority of malaria-related deaths can be attributed to *Plasmodium falciparum*. [1.10] This species is also known to be the most

resistant to the currently available medications. After *P. falciparum*, *P. vivax* is the most widespread human malaria. [1.10] The other two species, *P. ovale* and *P. malariae* are rarely fatal. [1.9] In recent years, particularly in south East Asia, increasing human infections by the parasite *P. knowlesi* have been reported. [1.11] It is increasingly being recognised as the fifth human malaria parasite. [1.12, 1.13]

The majority of malaria related deaths (70%) occur in Africa where infants and pregnant women are most at risk. [1.14] An increase in immigrants from endemic areas looking for jobs, tourism and the appropriate latitudes and benign climate are some factors likely to foster the resurgence of malaria, [1.15] most notably, in Southern Europe.

The development of the malaria parasite occurs in two unique hosts. A phase of sexual reproduction occurs in the mosquito vector and asexual reproduction, where male and female gametes are formed, occur in vertebrates. [1.16] Infection occurs when infected female *Anopheles* mosquito injects sporozoites (Fig. 2), the infective stage of the malaria parasite, into the skin while it takes its blood meal. [1.4, 1.17] This forms the basis of disease transmission and the parasite 'perceives' this stage as merely a way to reaching the mosquito where sexual reproduction takes place. [1.8] The sporozoites eventually reach their target cell, the hepatocyte, after penetrating skin and travelling through the circulatory system. [1.17]

In the liver, the sporozoites develop into a schizont form containing thousands of merozoites. [1.18] The schizonts form drug targets where blood and tissue schizonticides (Fig. 2) [1.8] would target asexual stages in the liver and disrupt asexual development of the parasite in red cells respectively.

Between 2 to 16 days from infection, depending on the invading *Plasmodium* species, merozoites are released when the host cell lyses into the bloodstream, where they infect erythrocytes initiating a cycle that ends with the release of more merozoites. This is the blood cycle that begins after an asymptomatic period of growth and multiplication in the liver. Immediately after invading the erythrocytes, merozoites differentiate into a ring stage that ingests erythrocyte cytoplasm, grows and gradually occupies the host cell, developing into trophozoites. [1.19, 1.20] Growth ceases in the subsequent blood schizont stage and the nucleus undergoes multiple divisions giving rise to daughter merozoites that escape and invade new erythrocytes, [1.20] restarting the cycle.

Concerted cell lysis triggers the clinical symptoms of malaria such as fever, chills and headache at this stage. The cycle is complete when some parasites develop into gametocytes and taken up by the mosquito when it has its blood meal. Once in the vector sexual reproduction takes place restarting the cycle.

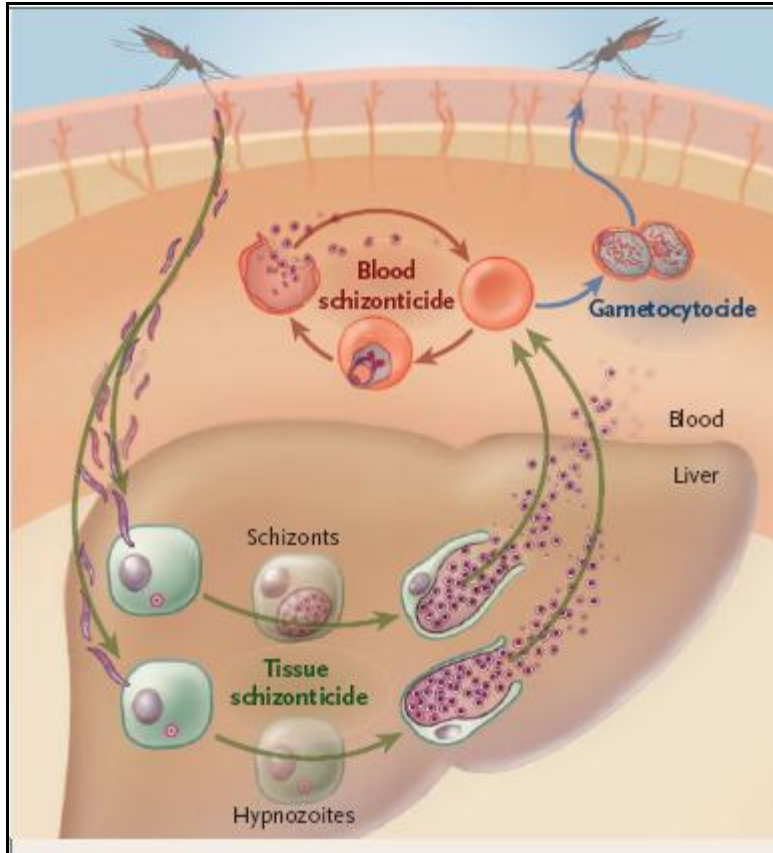


Figure 2: The life cycle of malaria parasite highlighting the blood and liver stages [1.8]

1.2 Current treatment/prevention methods

The incidence of the disease is now higher than it was a generation ago as a result of parasite resistance to available antimalarial drugs and insecticides. Given the complex life cycle (Fig. 2) of the malaria parasite this trend can be reversed by targeting the different stages of the parasite's development using drugs [1.21] or insecticides. Infection prevention and disease prevention are some of the current malaria control strategies [1.22] in use to arrest the incidence of the disease. In addition to the aforementioned preventive methods of consistent reduction of the mosquito vector by insecticides and avoiding nocturnal exposure using bed nets more needs to be done to control the burden of malaria.

A malaria vaccine would be a welcome addition to preventative measures against the disease. There has been significant investment in the search for vaccines and though there are several in development there are few front runners [1.61]. RTS, S/S02A, [1.23, 1.24] developed by GlaxoSmithKline (GSK), is in Phase 3 clinical trials. [1.25] This is evidence that the development of a malaria vaccine may be realistic. Chemoprevention, where high-risk groups, for example, pregnant women, the elderly and young children are regularly given antimalarials for prophylaxis is a useful way of reducing mortality and morbidity. [1.26] Chemoprophylaxis is being sidelined in countries with successful control programmes because of the escalating costs involved. Improving health care systems in malaria endemic countries ensures most people in need of care have access to effective treatment.

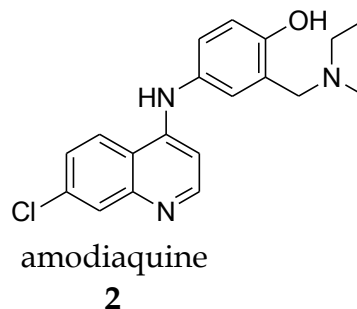
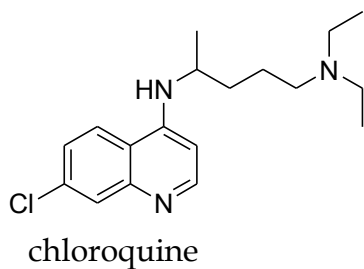
According to the World Malaria Report 2010, countries with considerable reduction in malaria cases have succeeded in employing preventive measures such as distributing bed nets and spraying households. [1.2] When prevention fails, cost-effective, fast acting antimalarial drugs with few side effects need to be available to the exposed.

Current antimalarial drugs (Fig. 3) can be grouped into seven groups viz: antibiotics, arylaminoalcohols, antifolates, artemisinins, 8-aminoquinolines, 4-aminoquinolines and inhibitors of the respiratory chain. [1.4]

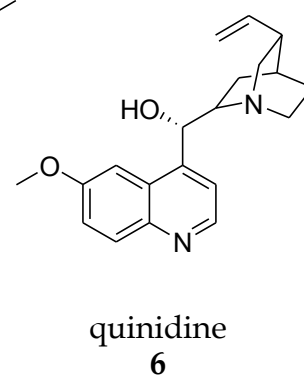
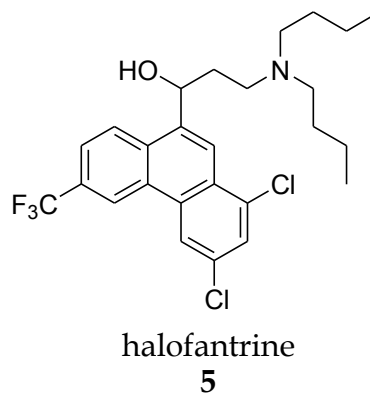
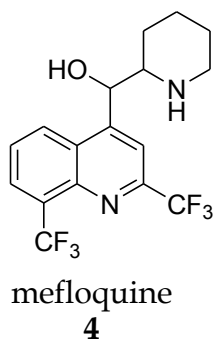
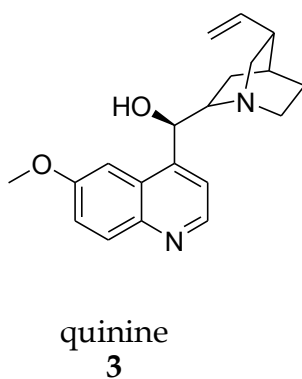
Doxycycline is an example of an antibiotic drug with antimalarial activity. These drugs are usually combined with other faster acting antimalarials such as artemisinins because they are slow acting. [1.27] Antifolates include sulfadoxine and pyrimethamine and they act by inhibiting dihydropteroate synthase and dihydrofolate reductase respectively. Atovaquone is the best known among the inhibitors of the respiratory chain. This class of antimalarial drugs is used for chemoprevention and the treatment of uncomplicated *P.*

falciparum malaria. The atovaquone-proguanil combination is known as Malarone® [1.28] and offers limited protection against the disease. Halofantrine, an arlyaminoalcohol, is not widely used because it has severe cardiac side effects. Mefloquine is generally used in combination with artesunate. When used alone it is associated with neurotoxicity. [1.29]

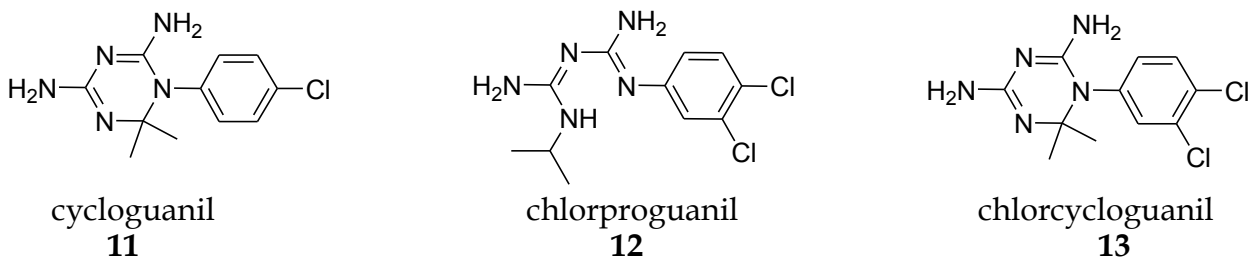
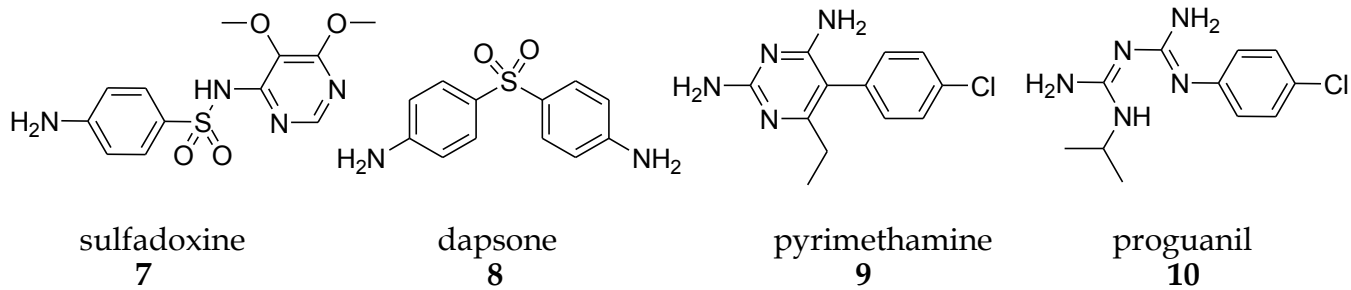
4-Aminoquinolines: inhibitors of haemozoin formation



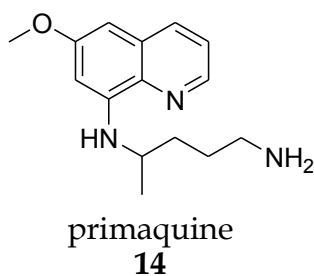
Arylaminoalcohols: inhibition of haemoglobin access into the food vacuole



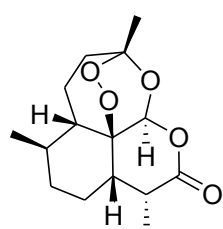
Antifolates: inhibition of tetrahydrofolate biosynthesis



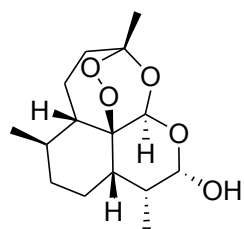
8-Aminoquinolines: inhibition of pentos phosphate cycle in liver stage parasites



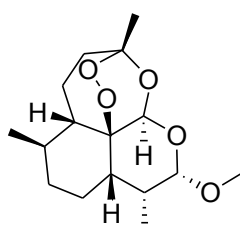
Artemisinin: inhibition of PfATP6 calcium pump



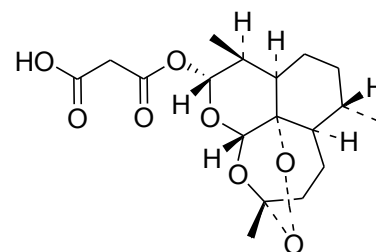
artemisinin
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dihydroartemisinin
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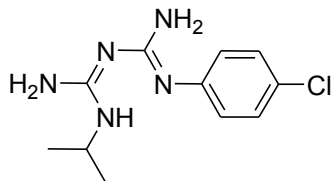


artemether
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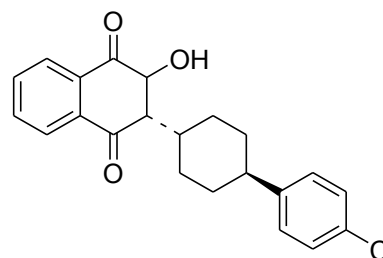


artesunate
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Inhibitors of the respiratory chain: inhibition of mitochondrial electron transport chain

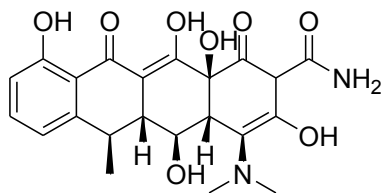


proguanil
19

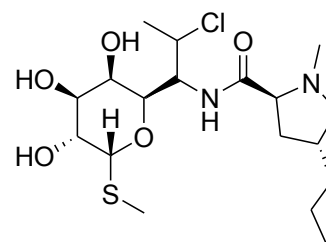


atovaquone
20

Antibiotics: inhibition of prokaryote-like protein biosynthesis



doxycycline
21



clindamycin
22

Figure 3: The current antimalarial drugs classified according to their chemical structure [1.4]

Currently, artemisinin-based combination therapy (ACT) is the recommended first line therapy for the treatment of malaria by the World Health Organisation (WHO). Artemisinins are fast acting, efficacious, safe and relatively cheap. They are derived from artemisinin lactone the active ingredient of the *Artemisia annua* plant. [1.30] Artemether and artesunate are common examples and they act on both the early and late stages of the malaria parasite life cycle in the human host. To delay the onset of resistance, artemisinins are recommended for combination with other classes of antimalarial drugs, a standard that has been adopted by many countries. [1.31] Reports have emerged of the failure of artemisinin monotherapy in South East Asia and the potential imminent emergence of resistance. [1.32]

4-aminoquinolines and 8-aminoquinolines, chloroquine (**1**) and primaquine (**14**) respectively, are discussed below.

1.3 Chloroquine (CQ) and related compounds

Primaquine (**14**) (Fig. 5) is the only 8-aminoquinoline drug available and is the official drug used against *P. vivax*. It is also however used for chemoprevention against *P. falciparum*. [1.33] Primaquine is related to CQ but unlike CQ it is used to treat and eradicate species that cause relapsing malaria such as *P. vivax*. Tafenoquine, an analogue of primaquine, has been found to be more potent and less toxic than primaquine, [1.34] additionally it is long acting and therefore does not need to be taken frequently like primaquine. These drugs act against the sexual stage and pre-erythrocytic stage of the *Plasmodium* parasites. This class of drugs forms part of the quinoline based drugs the best known of which is chloroquine (**1**) (Fig. 4), a 4-aminoquinoline.

CQ was the drug of choice for the Global Eradication Program when it was launched in 1955. [1.4] Amodiaquine (AQ) (**2**) can be used as an alternative against low chloroquine-

resistant parasites but has severe hepatic side effects after prolonged use, whereas CQ causes gastrointestinal disturbances because of its rapid absorption. [1.80]

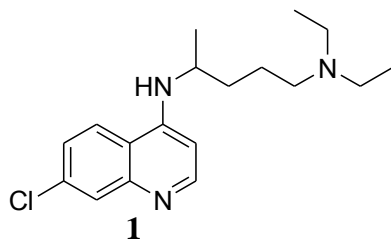


Figure 4: The structure of chloroquine (CQ)

Synthetic antimalarials first made an appearance as far back as the 1930s and intensive research has been invested in the field since then. Despite this, the repertoire of clinically available drugs remains limited. [1.35] Thus there is an increasing need for the rational development of effective and cheap antimalarial drugs. Chloroquine (CQ) (**1**) (Fig. 4) was once considered to be one of the most important remedies for the treatment of malaria. [1.36] Though it still remains the most widely prescribed drug in the developing world, chloroquine has lost most of its efficacy due to the emergence of resistance.

Quinoline antimalarials such as CQ have had a long and successful history. The development of chloroquine was precipitated by a need for a synthetic alternative to quinine. [36] Since then many quinoline based drugs (Fig. 5) have been important antimalarials. In fact, the quinoline nucleus is an important motif in heterocyclic compounds found in many natural and synthetic products with pharmacological activities such as anti-inflammatory, anticancer, antibacterial and antiviral agents. [1.37] Quinine (**3**) was isolated from the Cinchona tree in 1820 and was used as an antimalarial agent. Due to its severe undesirable side effects it has found limited use.

Systematic development of quinine (**3**) led to the discovery of CQ a potent and cheap antimalarial. [1.38] CQ has several pharmacokinetic and pharmacological advantages over all other antimalarial drugs. [1.39] The main advantages of CQ are its fast action, low toxicity, good bioavailability in oral dosage form, water solubility and high volume of distribution in the body. Emergence of chloroquine resistance led to the subsequent discovery of mefloquine (**4**), amodiaquine (**2**). Piperaquine (**23**), a bis-4-aminoquinoline, compounds that have two 4-aminoquinoline moieties attached by a linker of various length and nature have been found to be effective against chloroquine resistant strains. Their steric bulk, preventing them from being transported by *pfcr* is thought to explain their activity. [1.40] Most recently, ferroquine (**24**) has followed suite and all the above mentioned drugs are quinoline based. [1.41] FQ is currently in Phase IIB clinical trials, [1.42] has exhibited low toxicity and has shown no cross-resistance with CQ. [1.43]

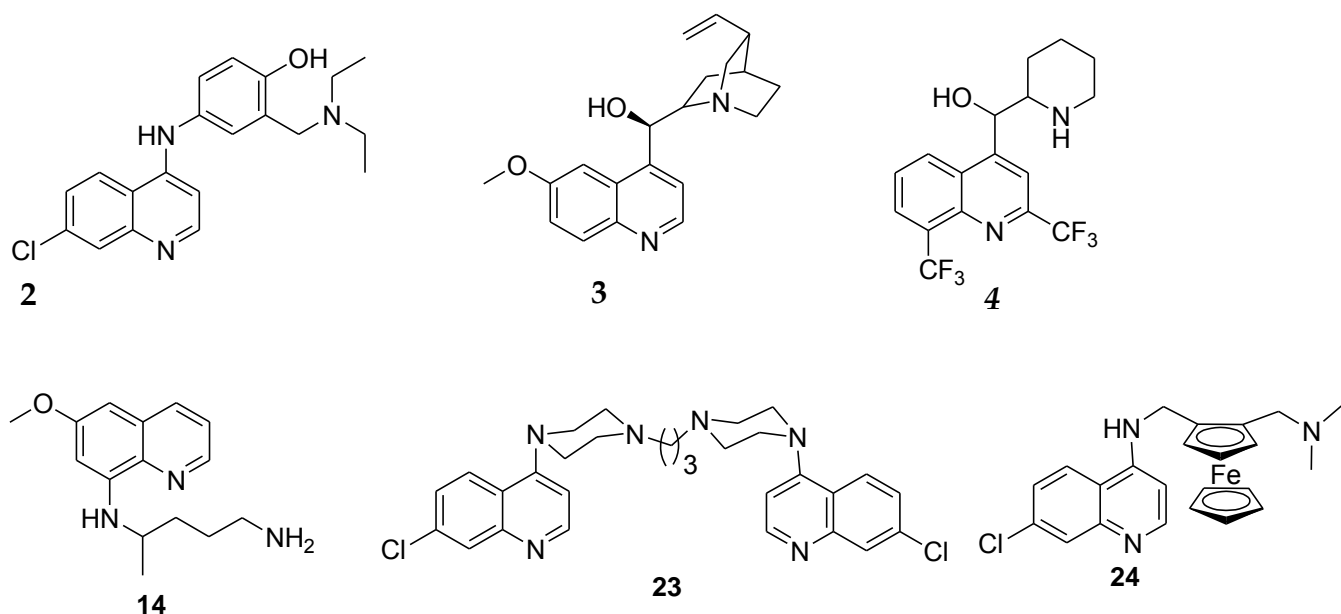


Figure 5: Quinoline containing antimalarials; amodiaquine (AQ) (**2**), quinine (QN) (**3**), mefloquine (MQ) (**4**), primaquine (**14**), piperaquine (**23**) and ferroquine (FQ) (**24**).

1.4 Mechanism of action of 4-aminoquinolines

The antimalarial activity of CQ and 4-aminoquinolines is thought to occur in the digestive vacuole of the malaria parasite. During the blood stage (Fig. 2) of the *P. falciparum* life cycle, the parasite digests host erythrocyte haemoglobin by a process which releases the toxic waste product, iron(II)protoporphyrin IX ($\text{Fe}^{\text{II}}\text{PPIX}$) which undergoes auto oxidation to form iron(III)protoporphyrin IX ($\text{Fe}^{\text{III}}\text{PPIX}$) or haematin [1.44, 1.45] also toxic. The desirable product comprises globin which is further broken down to amino acids.

The amino acids are essential to the parasite for protein synthesis, metabolism and parasite growth. [1.46] It is estimated that up to 75% of the haemoglobin in an infected host erythrocyte is degraded. [1.44] This creates a huge toxic waste problem for the parasite. A unique adaptation of the malaria parasite is its ability to detoxify haematin to form dimerised non toxic haemozoin (β -haematin is its synthetic equivalent) (Fig. 6). Formation of haemozoin occurs when one haematin moiety associates via the carboxylate group with the Fe^{3+} centre of an adjacent haematin forming the insoluble material. This mechanism allows the parasite to degrade haemoglobin with impunity. [1.47]

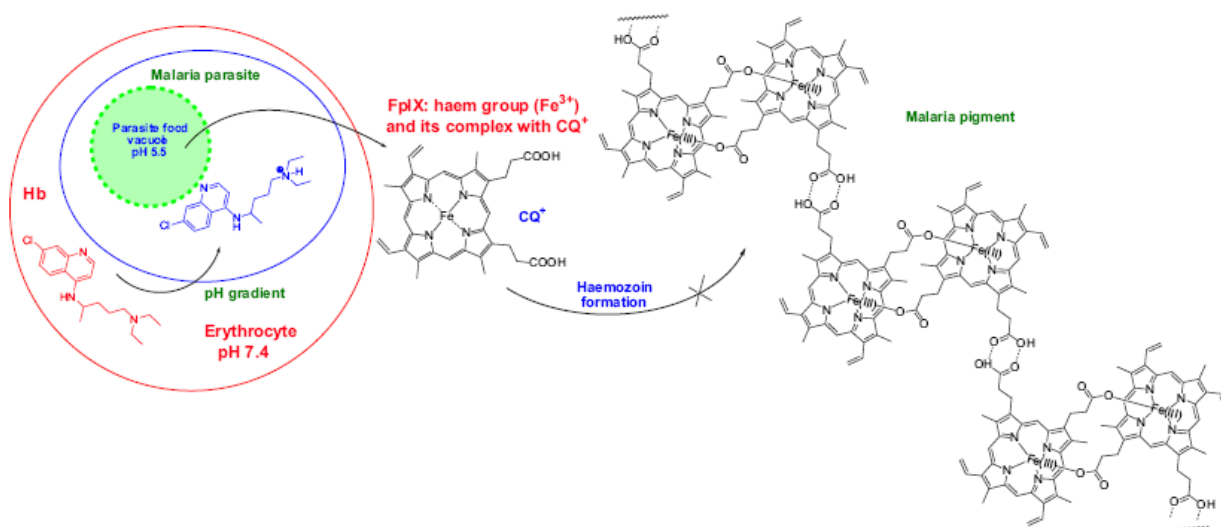


Figure 6: Mechanism of action of CQ and related 4-amioquinoline drugs [1.48]

CQ and 4-aminoquinoline compounds are understood to enter the digestive vacuole of the malaria parasite and interfere with the dimerisation and detoxification of haematin, inhibiting the formation of haemozoin. [1.49] This inhibits parasite growth and leads to its death. The exact killing mechanism is not known though the peroxidation of parasite lipid membranes, damage of DNA and oxidation of protein are implicated in the death of the parasite. [1.50] There are several important elements in the 4-aminoquinolines which are necessary for antiplasmodial efficacy.

1.4.1 Structure activity relationships of 4-aminoquinolines

The interaction of CQ and related compounds with haematin, as described above, is an important event for antiplasmodial activity. Studies [1.51, 1.52] suggest that CQ binds to haematin in the form of two μ -oxo dimers predominantly in a π - π stacking orientation. CQ is a diprotic weak base, pK_a 8.1 & 10.2, and in its unprotonated form will pass through membranes of the erythrocytes and the parasite membranes and eventually accumulate in the parasite digestive vacuole (Fig. 6) [1.48] where it interacts with haematin.

The 4-aminoquinoline pharmacophore is generally accepted to play a crucial role in this process of the interaction with Fe(III)PP1X which results in the inhibition of haemozoin formation [1.53] and parasite growth. The presence of a basic amino group in the lateral chain is known to play an important role in the accumulation of the drug in the acidic vacuole, where the drug becomes protonated, membrane impermeable and subsequently trapped in the acidic compartment, a process known as pH trapping. [1.53, 1.54, 1.55] Iwaniuk *et al* and Natarajan *et al* replaced the 4-quinolinyl amino moiety with an ether and sulphur or oxygen group respectively. This reduced the basicity of the quinolinyl nitrogen and lowered the antimalarial activity of the compounds. [1.55, 1.56] The resistance index (RI), defined as the ratio of chloroquine resistance (CQR) strain IC_{50} /chloroquine sensitive (CQS) strain IC_{50} , of the compounds though was improved.

Other studies have focused on the modification of the basic amine side chain as well as different substituents at the 7-position of the quinoline ring. Changes on the ring system and removal of chlorine at the 7-position alters the physicochemical properties of the compound but is not significantly correlated to activity against CQR strains. [1.38, 1.53] On the other hand, the variation of the CQ side chain, for example short (2-3 carbon) and long (10-12 carbon) chains have shown promising results [1.57] against both CQR and CQS strains, while structural modifications of the 7-chloroquinoline ring reduces antiplasmodial activity. However, recent work on substitutions on the quinoline ring is almost exclusively focused on the 7-position thus limiting room for comprehensive conclusions. The studies above lay the foundation to investigate and map the structure activity relationship (SAR) of CQ and 4-aminoquinolines.

In light of these studies [1.51, 1.53, 1.57] relating to accumulation, formation of drug-haem complex and exertion of toxic effects by the complex, the individual moieties responsible for activity have been identified (Fig. 7). Individually, these elements are not sufficient to cause parasite death. The inhibition of β -haematin formation has been demonstrated in 4-aminoquinoline analogues with Cl at the 7-position and is important for antiplasmodial activity. Other substituents, such as -Br, -NO₂, -NH₂, -F, -I, -CF₃ and -OCH₃ have been investigated to find the role of -Cl at that position by determining the effect of replacing it. [1.53] It has been found that the antiplasmodial activity ranges from none in none substituted (H) derivatives to moderate in -F and -CF₃, IC_{50s} 60 nM and 41 nM respectively for short (2 carbon) chain diaminoalkane side chains. [1.57]

The group at the 7-position (Fig. 7) has been identified as the critical feature required for inhibition of β -haematin formation. Studies, [1.53, 1.54, 1.57] have demonstrated that 4-aminoquinolines with a -Cl atom at this position show the most potent inhibition of β -haematin formation. However, whilst the presence of the Cl has been established as being

crucial, it is not clear why this is so. Physicochemical properties of the derivatives are greatly influenced by the group at the 7-position, where electron releasing substituents such as $-NH_2$ and $-CH_3$ would tend to raise the pK_a of the compound. Electron withdrawing groups such as $-I$ and $-NO_2$ would tend to reduce the pK_a . There is enough evidence supporting pH trapping for the accumulation of the drug in the digestive vacuole of the parasite, and it is instructive to note therefore that the substituent at the 7-position will have an influence in this process.

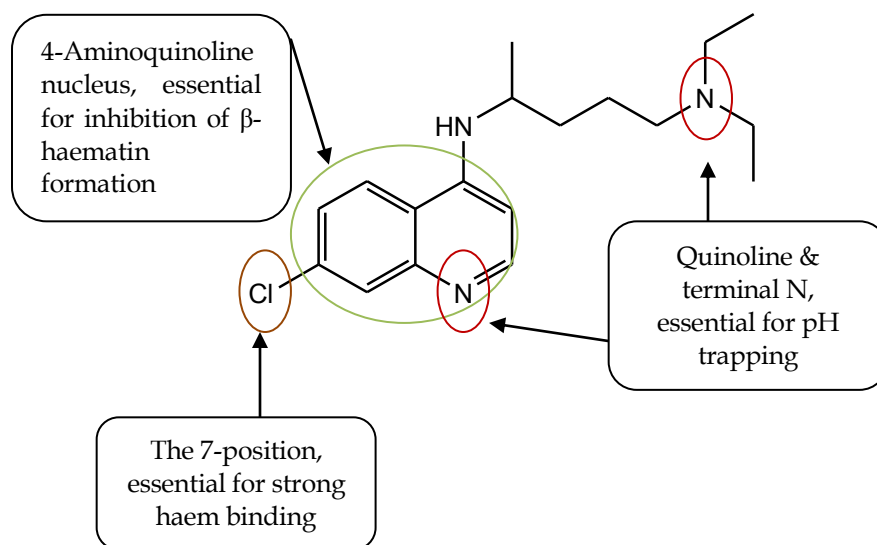


Figure 7: The structure activity relationships of CQ [1.54]

The group at the 7-position is ideally proposed to be (i) moderately electron withdrawing which will cause only a moderate decrease in pK_a of the molecule and thus enable the compound to accumulate at the site of action while ensuring inhibition of β -haematin formation and (ii) be strongly lipophilic. A strongly lipophilic group will enable the compound to associate more strongly with haematin and it was proposed that the more strongly it associates the more strongly it would inhibit the formation of β -haematin.

[1.53] However, it should be noted that the $-\text{NO}_2$ substituent is a strong β -haematin formation inhibitor, without being very lipophilic.

The 4-aminoquinoline nucleus (Fig. 7) was found to be the haematin associating nucleus as the introduction of an alkyl side chain to the amino group of the 4-aminoquinoline had no significant effect on the association strength. [1.53, 1.54, 1.58] Cheruku *et al* also found that the CQ-haematin μ -oxo dimer is largely a function of the 4-aminoquinoline substructure and the lateral chain contributes minimally to the binding. [1.59] The inclusion of $-\text{Cl}$ at the 7-position had only a slight effect on the association constant and it was concluded that the 4-aminoquinoline nucleus was necessary but not sufficient for antiplasmodial effect. [1.53] In the same study the quinoline and terminal nitrogen were identified as essential for pH trapping of the molecule once it enters the digestive vacuole. Egan and co-workers concluded in these studies that the 4-aminoquinoline nucleus of CQ, basic nitrogen attached to the aminoalkyl side chain and the chloro ($-\text{Cl}$) group at the 7-position as being essential for antiplasmodial activity. [1.53] This was confirmation of earlier work done by the group who had proposed the structure activity relationship of CQ and related 4-aminoquinoline antimalarial drugs. [1.54]

1.5 Resistance of *P. falciparum* to CQ

1.5.1 Emergence and geography

Despite all the advances in science, the incidence of malaria is now higher than it was 30 years ago. Parasite resistance to commonly used antimalarial drugs and, in some parts of the world, the free flow of counterfeit and substandard drugs [1.60] have exacerbated the problem. In addition to the spread of resistance, the World Bank report (2001) implicates the deterioration of healthcare systems, population displacement caused mainly by civil unrest, human migration and periodic changes in weather patterns as combining to aggravate the malaria problem.

One of the first confirmed cases of chloroquine resistance occurred in the same area of western Cambodia in the late 1950s where there have been recent reports of artemisinin resistance. [1.32] In addition to the Cambodia location, resistance has originated and spread from a number of locations worldwide. From Southern Asia CQ resistance rapidly spread to East Africa in the late 1970s (Fig. 8) and by mid-1980s the majority of sub-Saharan Africa was affected. [1.61] Resistance also appeared independently in South America and the regions of Papua New Guinea and Melanesia. [1.62]

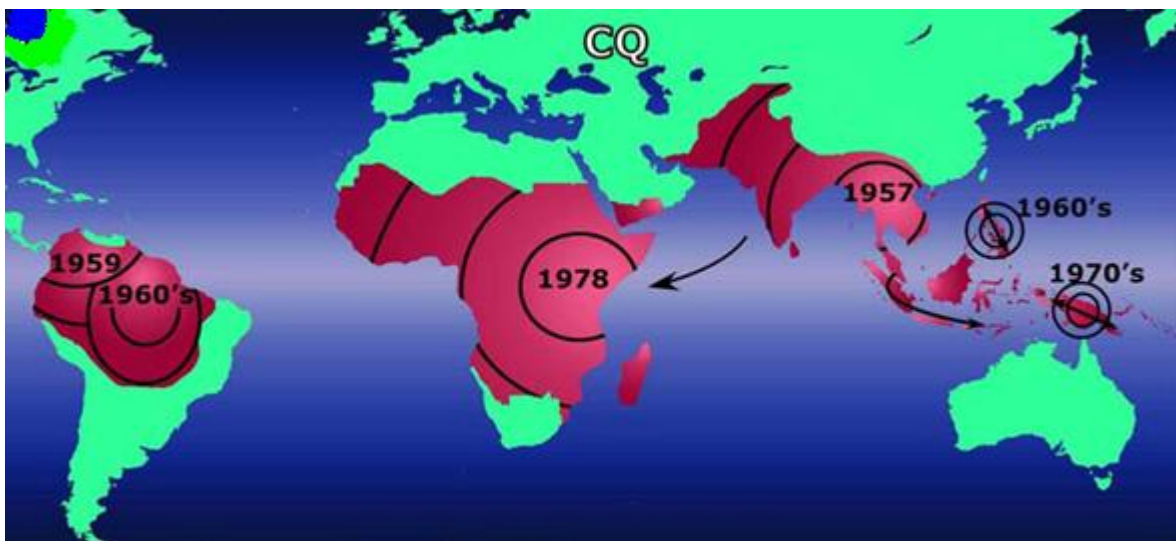


Figure 8: Spread of chloroquine resistance from Southern Asia to Africa [1.62]
Areas in red indicate presence of CQR *P. falciparum* while black circles indicate spread of CQR

1.5.2 Resistance mechanisms

Though resistance to CQ has been apparent for about 50 years the understanding of the underlying mechanism(s) has been slow. The molecular basis for the resistance of the parasite to common malaria drugs is now understood and the key genetic determinative mechanisms have been characterised for drugs such as chloroquine. [1.63] The major mutation implicated in resistance to CQ is the putative transporter, the *P. falciparum* chloroquine resistance transporter (*pfcr*) protein, [1.64] particularly the substitution at

amino acid K76T, [1.62] where lysine (K) is replaced by threonine (T) at position 76. This mutation always acts in concert with a series of other mutations thought to be compensatory mutations in the protein. [1.65] Indeed, since resistance took some time to emerge it was evident that multiple gene mutations are required to produce the resistant phenotype. [1.66]

Evidence also shows that there are other genes that play an additive role such as the *P. falciparum* multi drug resistance analogue (*pfmdr1*) that create important digestive vacuole physiological changes necessary for CQ resistance. [1.64] The observations made suggest the appearance of resistance to CQ after many years of widespread use reinforces the aforementioned multigenic mutations hypothesis. This is thought to be brought about by extensive and prolonged drug pressure on parasite populations. [1.67]

The resistance to CQ is usually attributed to the accumulation of substantially lower amounts of the drug in erythrocytes infected with CQR parasites relative to those with CQS parasites. Although this may not be the only factor contributing to the resistance of the parasite, the concentration of the drug is thought to be reduced to levels below those required to inhibit the formation of β -haematin. [1.68] There is experimental evidence that attempts to explain this scenario. The experiment uses *Xenopus laevis* oocytes to express wild type and resistant forms of *pfCRT* to determine its function, thought to involve the efflux of CQ from the target site. [1.65] In the experiment the authors compared the function of the mutant *pfCRT* from the CQ resistant *P. falciparum* strain Dd2 with that of the wild-type from the CQ sensitive strain D10. They found that the oocytes expressing the CQR *pfCRT* showed a 5-fold increase in the uptake of protonated CQ relative to the controls and oocytes expressing CQS *pfCRT*. The measurements were done in an acidic medium to mimic the digestive vacuole (Fig. 6) where the majority of the drug is protonated. The results were found to be consistent with the CQR *pfCRT* being central to

the transport of the protonated drug from the digestive vacuole. The authors went further to demonstrate the importance of the K76T mutation in *pfcr*. The introduction of this and other mutations to the CQR *pfcr* resulted in the loss of CQ transport activity. [1.65] The introduction of K76T to the CQS *pfcr* did not result in any significant increase in the uptake of protonated CQ. This, the authors allude, shows K76T mutation is necessary but not sufficient for the efflux of CQ from the target site.

1.5.3 Resistance and structure activity relationships

The 7-chloroquinoline nucleus with varying side chains has been a feature of recently synthesised antimalarials. [1.38] Among these, the drug FQ (**23**) [1.69] is a promising lead, and in combination with artesunate is currently in advanced stages of clinical trials. FQ is a CQ derivative with a ferrocenyl moiety attached at the 4-position. Previous studies have shown that aminoquinolines with a lateral chain with a metallocene are active against CQR parasites. The primary mechanism of action of FQ in addition to the redox properties of the ferrocene/ferrocinium system is thought to be similar to that of CQ, [1.70] in as far as haematin association is concerned.

Evidence seems to suggest the 4-aminoquinoline nucleus will circumvent resistance since the side chain is the primary recognition site for resistance. [1.35] De *et al* and other researchers have demonstrated that resistance is compound specific rather than class specific. [1.47, 1.49, 1.57, 1.58] CQ analogues with altered side chains have been found to be active against CQ resistant strains of *P. falciparum*. To this end, rational design and synthesis of CQ analogues bearing the 4-aminoquinoline sub-structure should still be able to lead to improved activity particularly against the resistant strain. In resistant parasites, the observations are that the drug accumulates to a lesser extent and this would be in some way influenced by the reduced association strength of the drug to its target. Studies have indicated that this is as a result of changes in the uptake of the drug. [1.61]

As with any other drug it is essential to elucidate its mode of action so as to understand how the parasite becomes resistant. Fortunately with regards to CQ and related drugs there is a general consensus that the mechanism of action and the mechanism by which the parasite becomes resistant to the drugs are mutually independent processes.

Indeed, in spite of the negatives there still lies a silver lining in the dark clouds viz:

- (i) Drug resistance to quinoline antimalarial drugs does not appear to result from any change in the structure of the drug target.
- (ii) Under experimental conditions, close analogues of CQ have been shown to retain full activity against CQ resistant strains and,
- (iii) Advances in the understanding of the mechanism of action of CQ and quinoline antimalarial drugs together with their structure activity relationships have improved prospects of developing novel quinoline antimalarial drugs [1.54]

1.6 Countering resistance

1.6.1 Resistance reversal agents

A typical feature of CQR parasites is that certain compounds can re-sensitise them to CQS, this is known as 'reversibility.' [1.72] As already alluded to, the molecular basis of resistance stems from the fact that the drug does not accumulate to toxic levels within the parasite as a result of active efflux of the drug from site of action. As such, if the active efflux is inhibited by a reversal agent, the drug can accumulate and exert its toxic effects. Verapamil (**25**) (Fig. 9), a calcium channel blocker, was one of the earliest identified reversal agents and is known inhibit the active efflux of drugs from resistant cells. [1.73] Martin *et al* found a synergistic effect of CQ and verapamil suggesting resistance could indeed emanate from active efflux of the drug from the resistant cells. [1.73]

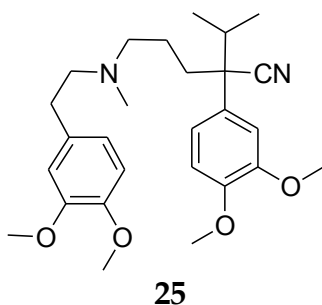


Figure 9: Resistance reversal agent verapamil

It is known that reversal agents are themselves toxic to parasites, but it is not known what methods they use to exert their toxic effects. It is also not known whether their antiplasmodial effect can predict the extent of reversibility of CQR strains as, for example, South American 7G8 strain is less responsive to verapamil reversal effects. [1.72] In addition to verapamil, other structurally diverse compounds have been shown to be resistance reversal agents. Dibenzyl-suberanylpiperazine derivatives, plant derived compounds, the antipsychotic phenothiazine and primaquine, an antimalarial used for treating liver stage of *P. vivax* have been shown to be resistance reversers. [1.74]

Burgess *et al* have described a series of compounds as “reversed chloroquines.” [1.75] The compounds are a hybrid of the 4-amino-7-chloroquinoline substructure and a reversal agent. The pharmacophore of these compounds consists of a nitrogen atom linking two aromatic rings separated by a short aliphatic chain (Fig. 10). [1.75] As shown in Fig. 10 the molecule would contain elements which will facilitate haem binding, inhibition of β -haematin formation, drug accumulation by pH trapping and the resistant reversal agent. Compound **26** (Fig. 10) was the first prototype synthesised by the group. The drug has nanomolar range IC_{50} against both chloroquine sensitive and chloroquine resistant strains of *P. falciparum*. A major drawback for the compound is that it is quite lipophilic ($ClogP \approx 8.9$), though encouragingly is able to clear parasitemia to less than 1% in mouse model via oral dose. [1.76]

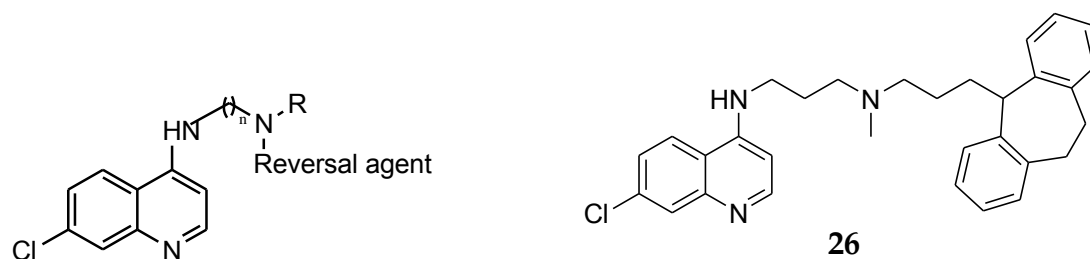


Figure 10: General structure of “reversed chloroquine” agent and the first prototype (**26**) compound

1.7 Medicinal chemistry perspective of CQ and related compounds

One of the most important physicochemical properties of a chemical compound is its aqueous solubility and this information is relevant throughout the drug discovery and development process. [1.77] Absorption-related physicochemical properties are important in drug discovery because they influence the rate and extent of absorption through biological membranes, a critical determinant of bioavailability of the compound. As already described above, the terminal nitrogen in the lateral chain and the quinoline nitrogen are important in the pH trapping process because of their basicity. The extent of association with Fe(III)PP1X, pK_a , lipophilicity, strength of inhibition of β -haematin formation of the drug are some of the physicochemical properties thought to play an important role in the antiparasitic activity of CQ and other 4-aminoquinoline drugs. [1.78] Due to their lipophilic and basic nature quinoline antimalarial drugs are thought to possess hERG liability. The human *Ether- α -go-go* Related Gene (hERG) is a K^+ channel that represents a key part of the cardiac action potential. It encodes potassium (K^+) channel that creates the rapidly activating delayed rectifier K^+ current called I_{Kr} , an important component that regulates the duration of the cardiac action potential. [1.79] CQ toxicity is thought to be related to its interaction with cardiac conduction and myocardium. [1.80]

Inhibition of the hERG K^+ channel by pharmaceutical agents or as a result of inherited dysfunction results in the obstruction of the flow of K^+ thus lengthening the time required

to repolarise the cell, [1.81] which is manifested as an elongation of the QT interval (LQT) in the electrocardiogram (ECG) (Fig. 11). A life threatening ventricular arrhythmia, torsades de pointes (TdP) is implicated in the blockade of hERG K⁺ channel, [1.64, 1.82] as a result a number of commercial drugs (Fig. 12) have been withdrawn from the market or have major labeling restrictions. [1.81] These drugs include terfenadine (antihistamine), cisapride a gastrointestinal drug and grepafloxacin (**27**) (trade name Raxar) which was used to treat bacterial infections. Quinoline based drugs such as quinine (**3**), quinidine (**6**) and halofantrine (**28**) are also known to have hERG liability. The chemical structures of CQ, QN and other 4-aminoquinoline substructures exert toxic effects by interacting with what is now known to be the hERG K⁺ channel causing the elongation of the QT interval (Fig. 11) resulting in potentially fatal conditions. [1.83] A study, [1.84] demonstrated that certain quinoline derivatives such as mefloquine (**4**) have hERG liability. The drug is lipophilic (log P 3.9) and extensively distributed in the tissues ensuring levels in the heart are sufficient to interact with the K⁺ channels. [1.84]

Considering the implications of interactions with pharmaceutical products, hERG liability has emerged as an important safety consideration in drug discovery. [1.81] A drug is considered to be within the safe margin for hERG when the ratio of its hERG IC₅₀ to the maximum free drug plasma concentration is greater than 30. For a drug to satisfy this criterion it will typically have a hERG IC₅₀ greater than 10 μM. [1.82]

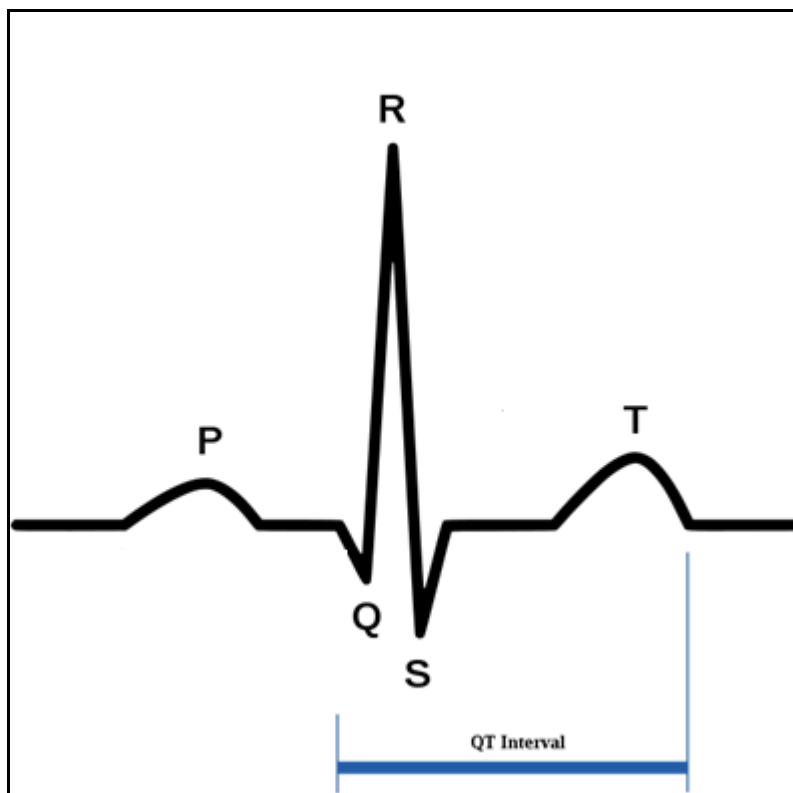


Figure 11: An ECG of the cardiac action potential

There are a number of computational methods to predict hERG liability. Though these methods differ slightly, they all agree that basic and/or lipophilic compounds are strongly implicated in hERG liability. [1.82] 4-Aminoquinoline drugs such as CQ possess the physicochemical characteristics described earlier that would potentially make them prone to hERG liability. Indeed, computational studies have been done using comparative field molecular analysis (CoMFA) and comparative field similarity analysis (CoMSiA) comprising 31 and 28 molecules respectively. [1.85, 1.86] The studies found aromatic (hydrophobic) and central nitrogen as well as ionizable centres were key features for binding to hERG.

The rational design of antimalarials bearing substituents with features to reduce lipophilicity, basicity and/or electron releasing properties is a strategy being pursued in

drug discovery and development to make drug candidates devoid of the physicochemical properties likely to make them prone undesirable side effects.

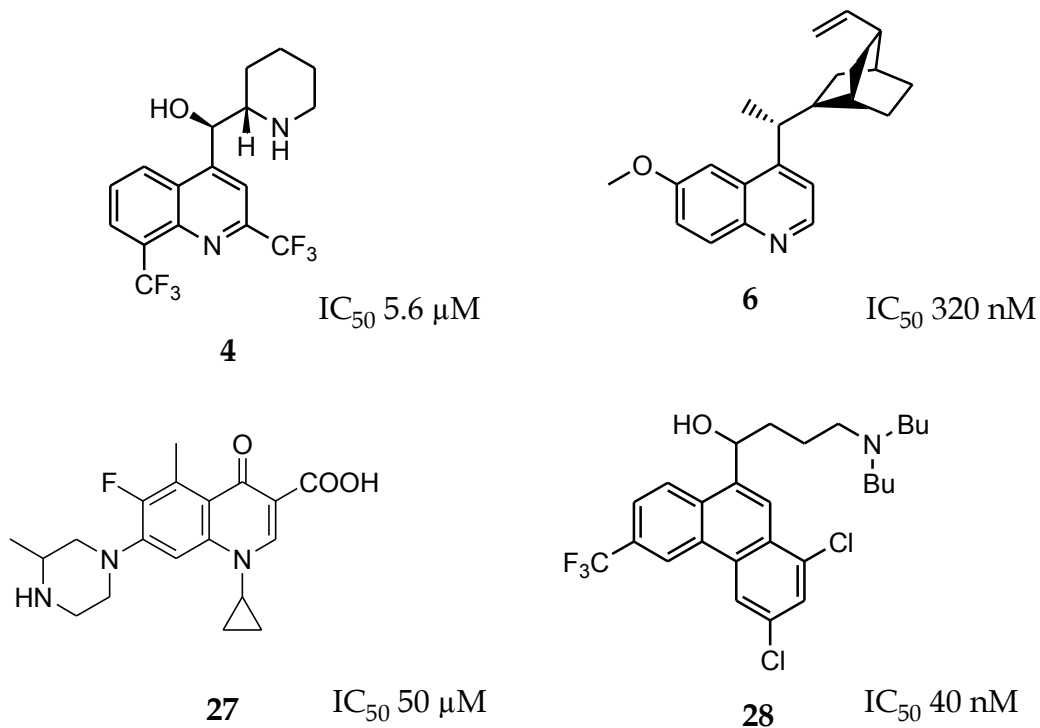


Figure 12: Commercially available quinoline or quinoline-related drugs that have been withdrawn or had major labeling restrictions due to hERG liability, viz; mefloquine (**4**), quinidine (**6**), grepafloxacin (**27**) and halofantrine (**28**). [1.87]

To minimise costs, preclinical drug candidates are screened very early in the drug discovery process. The promiscuous nature of the hERG K^+ channel interaction with pharmaceutical agents means that this is a significant factor in drug development.

In addition to hERG, drug candidates can run afoul by binding to and inhibiting proteins such as cytochrome P450s (CYP450). These are a class of enzymes metabolize xenobiotics and pharmaceutical agents and make them easier to be cleared from the body. [1.88]

CYP3A family is the most important in terms of human drug metabolism as it is involved in the clearance of 60% of all marketed drugs. [1.89] In particular, CYP3A4 is predominant in intestinal and hepatic drug metabolism and catalytically the most promiscuous. [1.88] Potent inhibition of this and other CYP450s can lead to accumulation of a co-administered drug that would ordinarily be metabolized, causing it to accumulate in the body resulting in adverse effects. Owing to their affinity for hydrophobic molecules CYP450s may be involved in many drug-drug interactions, known potent CYP450 inhibitors (Fig. 13) always carry warning labels advising of this potential. [1.90] Computational modeling for CYP3A has identified hydrogen bonding as well as hydrophobicity as important determinants for binding. CYP3A4 is also known to have a general affinity for lipophilic and basic compounds. [1.91]

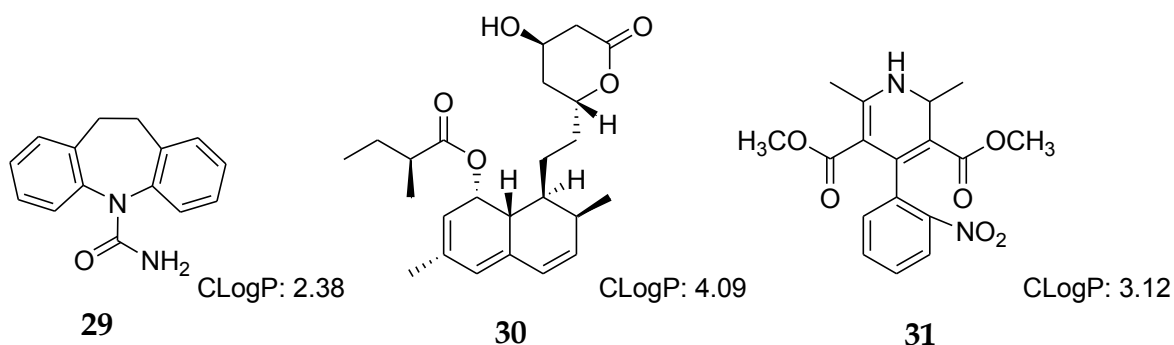


Figure 13: Carbamazepine (**29**), lovastatin (**30**) and nifedipine (**31**) examples of known CYP3A4 inhibitors. [1.91]

1.8 Drug design

Drug discovery tools have been rapidly developing over time and powerful computer modeling software programs are now used routinely. The conventional Craig plot (Fig. 14) was developed in the early 1970's for finding the most suitable substituents during a drug development programme. The plot is a two dimensional scheme of Hammett

constant (σ) versus lipophilicity constant (π). The plot was employed to select substituents such that their (substituents') electronic and the lipophilic contributions to activity could be quantitatively and separately evaluated.

The Hammett constant (σ) relates to the distribution of electrons in a molecule depending on the electron releasing or withdrawing groups attached to it. A negative σ value indicates the substituent is electron releasing whilst a positive value indicates electron withdrawing groups. The lipophilicity constant (π), also known as the hydrophobicity constant, represents the contribution a substituent has on the partition coefficient of a molecule. [1.91] A positive value indicates the substituent has higher lipophilicity while a negative value indicates lower lipophilicity than hydrogen.

Selection of substituents from all four quadrants of the plot would ensure availability of a wide range of properties. As a result of statistical independence between the sigma (σ) and pi (π) constants, [1.92] this would facilitate the correlation of structure and biological function with these parameters.

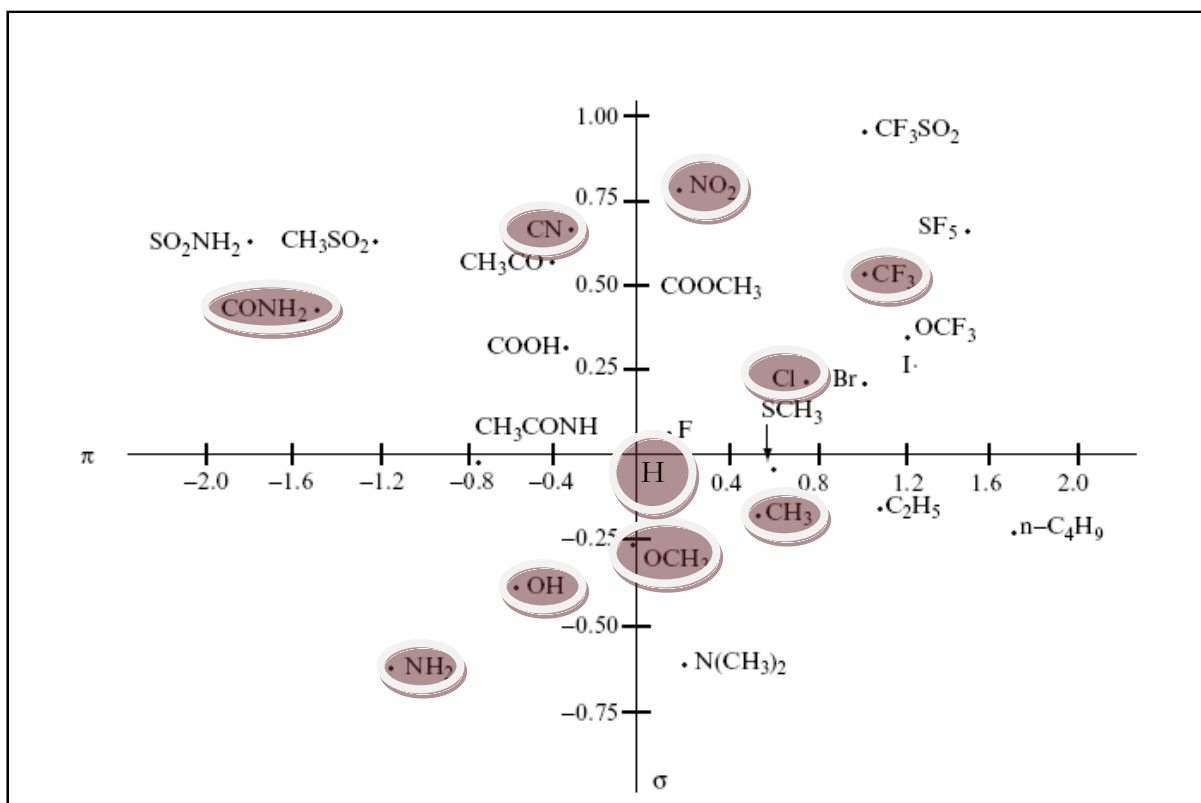


Figure 14: The Craig plot, with selected substituents highlighted. [1.91]

1.8.1 The substituent at the 7-position of the quinoline ring

Previous studies have shown that the 7-chloro group is a crucial feature for inhibition of β -haematin formation. [1.53, 1.57] Properties that have tentatively been proposed to be ideal for the group at the 7-position are moderate electron withdrawing and strongly lipophilic properties. [1.53] These properties strongly lean towards the halogens; hence the activity associated with the chloro-substituted quinolines. The selection of substituents from the Craig plot (Fig. 14) introduced at the 7-position of the quinoline ring presented an opportunity to clearly define the role of this group. Researchers have steered away from replacing the 7-chloro group as it has been found that this modification changes the pK_a of the molecule. The change in pK_a affects accumulation in

the food vacuole, and can impact negatively on the antiplasmodial activity of the molecule. [1.38]

Incorporating the various substituents formed the basis of a series of 4-aminoquinoline derivatives to demonstrate the effect of replacing the 7-Cl group with other substituents. The Cl atom, evident from its position on the Craig plot, as well as being electron withdrawing, contributes to the lipophilicity of CQ and its derivatives. Replacing this group will test the extent to which the hydrophilicity of the molecule can be improved whilst retaining its antiplasmodial activity. The obvious advantage with a more hydrophilic molecule is the reduction of interactions of the drug with undesirable targets such as the hERG K⁺ channel and CYP450s favoured by lipophilic and basic compounds, thus potentially affording a safer drug candidate.

Noteworthy, in a recent study the authors replaced the group at the 7-position of the quinoline ring with a more lipophilic group (Fig. 15). The authors used bulky groups to explore the effect of size and / or hydrophobicity at this position. In one series, they found that increasing hydrophobicity decreased antiplasmodial activity. [1.93] Additionally the authors did not find any stereo-electronic effects with any of the groups they used at the 7-position. The authors also observed that the less lipophilic compounds were generally more potent against the chloroquine sensitive (3D7) and chloroquine resistant (K1) strains of the malaria parasite. [1.93]

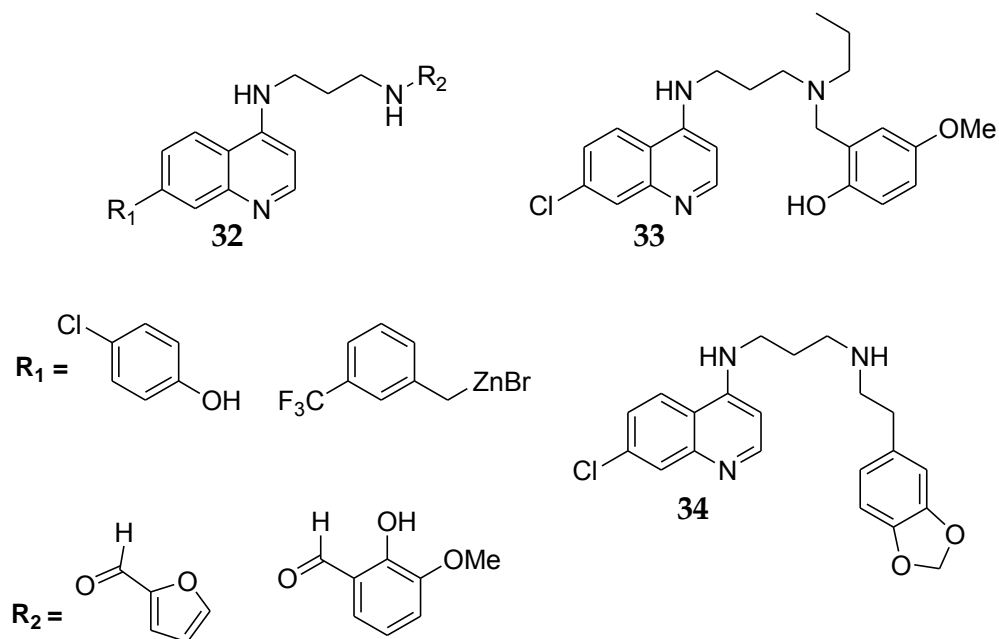


Figure 15: Examples of 4-amino quinoline molecules with hydrophobic groups (32) and different lateral chains (33) and (34) [1.93, 1.94]

1.9 Background to the current research question and hypothesis

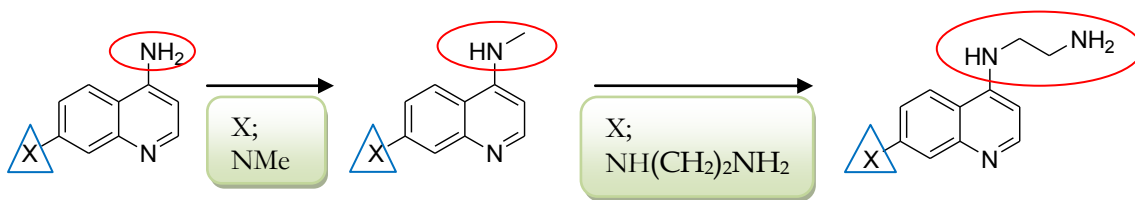
1.9.1 The fragment-based approach method

There have been studies purporting the definitive characteristics of the group at the 7-position of the quinoline ring. All these studies have proposed tentative properties for the group and as a result the panoramic SAR view of quinoline antimalarials, particularly regarding the role of the group at the 7-position, is still being debated. To elucidate the question of structural specificity of CQ-haematin binding, a set of 4-quinoline analogues were synthesised, the starting point being the 4-amino-7-X-quinoline (Fig. 16). From this starting point, the lateral chain on the 4-amino group was gradually built up starting with a -CH₃ group and then proceeding to an ethane-1,2-diamino group to observe its effect on haem binding and β -haematin inhibition. A fragment-based approach method used in this study is useful in that the building blocks, the quinoline moiety, the lateral chains

and the different substituents, can be scored independently and used to determine the next molecules in the series to be synthesised. [1.95] Used herein is the matched molecular pair fragment approach, where the average effect of a substituent is estimated by analyzing molecules obtained when the only change in the structure involved a single localized substituent. [1.95]

It has been shown that 3-, 5-, 6- and 8-aminoquinolines, the quinoline itself and other simple aminoquinolines do not bind strongly to haematin. [1.54] However, 2- and 4-aminoquinolines bind strongly to haematin in aqueous dimethyl sulfoxide (DMSO), with a binding constant of 4.49 ± 0.01 for 4-aminoquinoline that is comparable to some quinoline based antimalarial compounds. [1.53, 1.54] The inclusion of an alkyl chain at 2- or 4-amino groups or indeed a chloro group at the 7-position has little influence ($\log K$ 4.38 ± 0.01 and 4.38 ± 0.03 respectively) on the strength of the binding. This suggests the side chains do not interact with haematin. [1.54] The synthesis of 4-amino-7-X-quinoline (Fig. 16) ensured useful comparisons of the role of the group at the 7-position. The same substituents were then used in the syntheses of analogues that constitute methylamine and ethane-1,2-diamino lateral chains. In this regards the effect of the lateral chain attached to the quinoline moiety was investigated relative to haem binding and the inhibition of β -haematin formation.

The inhibition of β -haematin formation on the other hand presents a different picture as the presence or absence of a chloro group at the 7-position of the quinoline ring dictates whether there is inhibition or not. 4-Aminoquinoline has been found not to inhibit the formation of β -haematin while 4-amino-7-chloroquinoline does inhibit β -haematin although both these compounds have virtually identical haem binding constants ($\log K$ 4.49 ± 0.01 and 4.43 ± 0.01 for 4-aminoquinoline and 4-amino-7-chloroquinoline respectively). [1.53]



X = (a) CH₃; (b) CF₃; (c) Cl; (d) CONH₂; (e) CN; (f) H; (g) NH₂; (h) NO₂; (i) OCH₃; (j) OH

Figure 16: An illustration of molecular changes to estimate the contribution of the selected substituents on the structural specificity of CQ-haematin binding and inhibition of β -haematin formation.

The chloro group was therefore identified as a necessary though not sufficient component for the inhibition of β -haematin formation. The extent of the inhibition of β -haematin formation is partly influenced by the strength of association of haematin with the aminoquinoline molecule. [1.53] The more strongly the molecule binds with haem the more likely it is to inhibit the formation of β -haematin. By using different substituents with diverse steric and electronic properties, the characteristics of the group at the 7-position can be defined. In particular, this would be relevant in the context of the reason why the chloro group at the 7-position is important for inhibition and the effect of replacing it. Though it is known that a 4-aminoquinoline compound with the chloro group at 7-position will inhibit the formation of β -haematin, whether this influence is steric, electronic or both is unknown. The substituents chosen herein presented a wide range of groups with equally varied properties to investigate this point. Correlations between electronic properties and lipophilicity with haem binding or inhibition of β -haematin were investigated in order to understand the influence of the lateral chain and the group at the 7-position on these physicochemical properties on the 4-amino quinoline ring.

1.9.2 Phenylequine (PQ), pyridodibemequine (PDB) and chloroquine (CQ) lateral chains

To further investigate the physicochemical properties of the group at the 7-position of the quinoline ring relative to antiparasmodial activity, three lateral chain from known active compounds were chosen for attachment at the 4-position of the quinoline ring. These were *N*¹,*N*¹-diethylpentane-1,4-diamine, 2-(dimethylaminomethyl) benzylamine and *N*-(4-(aminomethyl)benzyl)-*N*-methyl-1-(pyridine-2-yl)methanamine, which will be referred to as chloroquine, phenylequine and pyridodibemequine analogue lateral chains respectively. Apart from their known antiparasmodial activity the lateral chains had other advantages, namely:

(i) Phenylequine (PQ) (**35**) (Fig. 17) contains the essential components for efficacy viz the quinoline nucleus structure and the weakly basic amino group. [1.96] Phenylequine and its analogues are relatively simple and attractive compounds to synthesise as quinoline based antimalarial drugs that circumvent chloroquine resistance. In a study to further investigate PQ, Blackie and co-workers synthesised a PQ analogue (**36**) (Fig. 17) by replacing the 7-chloro group with a hydrogen (H) atom. In an unrelated study Kaschula and others synthesised a CQ analogue (**37**) (Fig. 17) by replacing the 7-chloro group with H atom among other substituents. They found that the new compound could not inhibit β -haematin formation at the highest practicable concentrations. [1.53] Interestingly, as shown in the Table 1 below, the phenylequine and chloroquine analogues show considerable differences in antiparasmodial activity.

The phenylequine analogue (**36**) is more active than expected. In a study on the role of drug accumulation in the potency of antimalarials, Hawley and others found that the presence of a phenolic moiety in amodiaquine changed the characteristics of the

compound. The presence of the aromatic benzene ring increases the lipophilicity of the compound and decreases its basicity because it is an electron withdrawing group. [1.97]

In the present study, phenylequine lateral chain gave rise to analogues (**57a-j**) that form a series of potentially active antimalarial agents (Fig. 18). The effect of the different substituents at the 7-position together with a lateral chain known to have an additive effect in terms of antiplasmodial activity presented an opportunity to further investigate the SAR of quinoline antimalarial drugs.

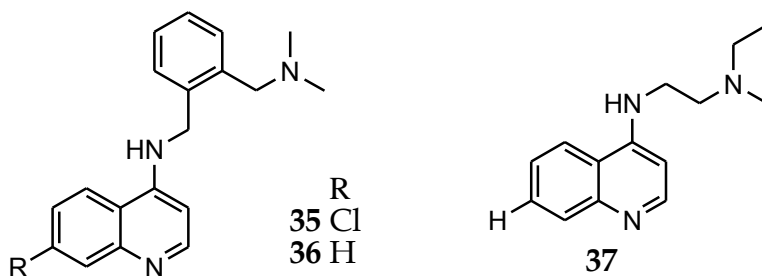
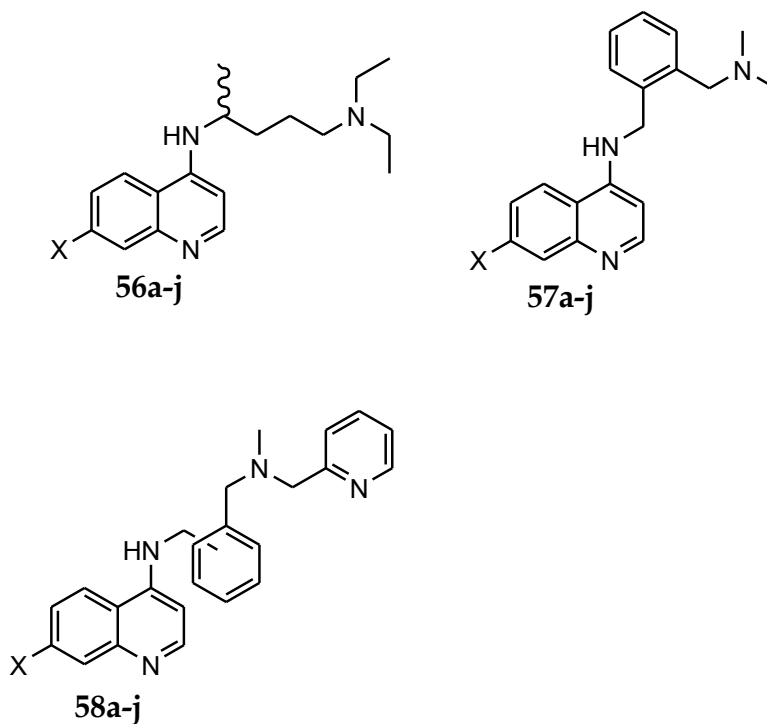


Figure 17: The structures of phenylequine (**35**), its analogue (**36**) and chloroquine analogue (**37**)

Table 1: Results from *in vitro* antiplasmodial testing on chloroquine sensitive (D10) and chloroquine resistant (K1) strains of *P. falciparum*. [1.41, 1.53]

Compound	IC ₅₀ (nM) [D10 Strain]	IC ₅₀ (nM) [K1 Strain]
Chloroquine (1)	22.9	352.6
Phenylequine (35)	13.2	6.9
Chloroquine analogue (36)	1060	-
Phenylequine analogue (37)	106.2	70.3

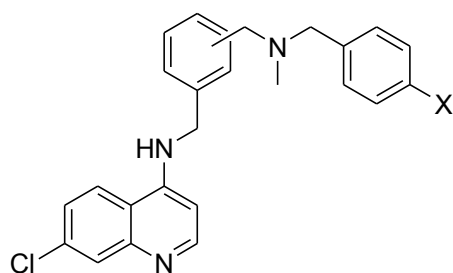


X = (a) CH₃; (b) CF₃; (c) Cl; (d) CONH₂; (e) CN; (f) H; (g) NH₂; (h) NO₂; (i) OCH₃; (j) OH

Figure 18: The proposed target molecules with CQ (56), PQ (57) and dibemequine analogue (58) lateral chains

(ii) Aminomethyl dibemethins are a series of compounds that were reported recently and shown to have resistance-reversing properties (Fig. 19). [1.98, 1.99] Their design was based on known resistance-reversers (Fig. 10) but these have a shortened linker, an aminomethyl chain, instead of a three carbon aminoalkyl chain positioned between the two aromatic rings. Dibemequines (DB) (Fig. 19) were identified to enhance biological activity through accumulation in the digestive food vacuole through pH trapping. In this study one of the aromatic rings in aminomethyl dibemethins was replaced by a pyridine ring (Fig. 18), thus potentially enhancing the basicity and reducing lipophilicity of the compounds. As a result of this change the target compounds were called pyridodibemequines (PDB). Additionally, the dibemethin analogues (Fig. 18) were

selected as potential resistance-reversers that would potentially target the resistant strains of the malaria parasite. Similar to the dibemaquines, the target compounds would potentially function as hybrid molecules that have a capability to deliver equal amounts of two compounds with one dose. This model provides both the potent antimalarial activity of the 4-aminoquinoline pharmacophore and the CQ resistance-reversing properties. [1.98, 1.99]



X = H; Cl; OCH₃; N(CH₃)₂

Figure 19: Dibemaquine resistance-reversing agents [1.98, 1.99]

(iii) The 2-amino-5-diethylaminopentane (CQ lateral chain) would allow for observations of how the different groups at the 7-position affect antiplasmodial activity in a well known series. Issues of cross-resistance with CQ were also investigated in this series of compounds.

There is a need to advance the understanding of structural specificity of quinolines with regards to the inhibition of β -haematin formation and antiplasmodial activity. The mechanism of action of 4-aminoquinolines described above and the mechanism through which parasites become resistant to the drug are not connected. Drug design can therefore still be based on this sub-structure while avoiding resistance.

1.10 Aims and objectives

1.10.1 Aims

The overall aims of the project were to synthesise and investigate potential β -haematin formation inhibitors as potential antimalarials. The underlying principle is that the physicochemical properties are as hydrophilic as possible without compromising activity. An attempt was made to define the role of the substituent at the 7-position. Consequently assisted in explaining the exact physicochemical effects involved (SAR) in haem binding and inhibition of β -haematin formation.

The Craig Plot derived substituents were introduced at the 7-position to answer the question why the chloro-group is essential at this position in quinoline ring of 4-amino quinolines to maintain antiplasmodial activity and the effect of replacing it.

1.10.2 Objectives

The objectives of the project were:

- (i) Synthesise 4-chloro-7-X-quinolines the precursors for quinoline analogues
- (ii) Synthesise 4-amino-7-X-quinolines, 4-amino methyl-7-X-quinolines and ethylenediamine-7-X-quinoline
- (iii) Synthesise phenylequine side chain for coupling with 4-chloro-7-X-quinolines
- (iv) Couple 4-chloro-7-X-quinolines with aminomethyl dibemethins analogues, phenylequine side chain and chloroquine side chain
- (v) Measure haem binding constants and determine inhibition of β -haematin formation (BHIA₅₀)

(vi) Use computer modeling to predict solubility and metabolic stability for phenylequine (PQ), chloroquine (CQ) and pyridodibemequine (PDB) analogues

(vii) Determine antiplasmodial activity for PQ, CQ and PDB analogues

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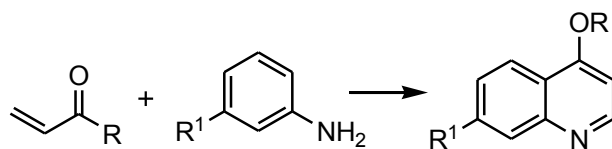
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2. SYNTHESIS

2.1 Background

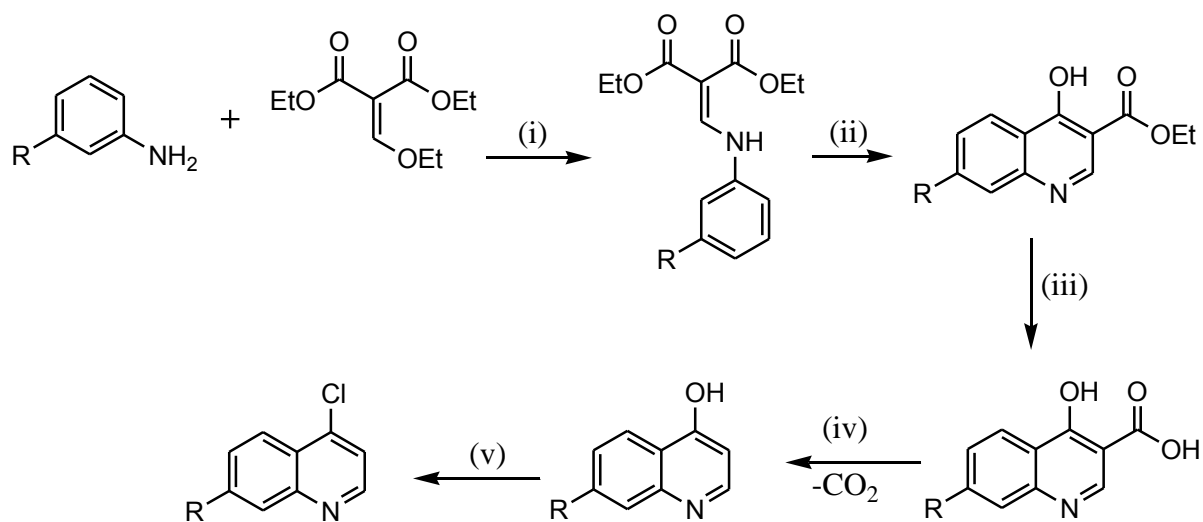
The quinoline moiety is important in medicinal chemistry and agrochemicals and is widely distributed in nature. [2.1] Quinoline derivatives are used as antitumor, antibacterial as well as antimalarial agents. As a result of this widespread usage, there are several methods for the synthesis of the quinoline moiety. Most of these established methods suffer from harsh reaction conditions and poor yields. Three are described below:

(i) The Skraup and Doebner-Milner method: This uses a variety of Lewis and Brønsted acids which can be corrosive. The reaction conditions used in the method are unfriendly to the environment by producing significant amounts of waste and require long reaction times. [2.2] The methods currently used in literature are variations of the original in which an aniline is heated with a glycerol such as ethyl acrylate serving as a source of a three-carbon fragment in the presence of an acid catalyst. A stable intermediate that forms then requires an oxidising agent, I_2 , to transform it into a fully aromatised heterocyclic compound after undergoing high temperature Friedel-Crafts acylation and cyclisation (Scheme 2.1). [2.3]



Scheme 2.1: Reagents and conditions: glycerol, H₂SO₄, I₂, reflux

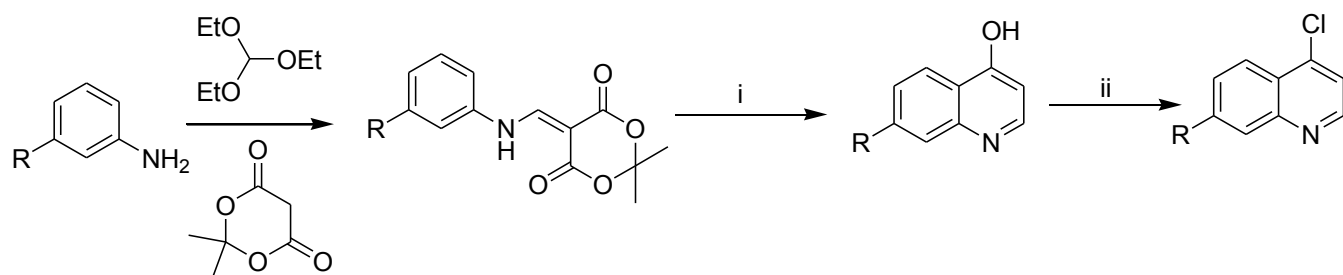
(ii) The Price and Roberts method: This has proved popular in recent studies. [2.4 - 2.7] The method involves the condensation of a substituted aniline with ethoxymethylenemalonic acid. The general synthetic route is shown below (Scheme 2.2).



Scheme 2.2: Reagents and conditions: (i) 110 °C, 45 min; (ii) Ph₂O 265°C, 45 min; (iii) NaOH (aq) 115°C, 30 min; (iv) Ph₂O 265°C, 30 min; (v) POCl₃ 140°C, 30 min.

The method, which is described in detail below (Scheme 2.4), involves a 4-step synthetic route to obtain the same product that the Meldrum's acid method (also mentioned below) obtains in two steps.

(iii) The Meldrum's acid method: This uses 2,2-dimethyl-1,3-dioxane-4,6-dione (Meldrum's acid) rather than the malonate ester used above in the Price and Roberts method.



Scheme 2.3: Reagents and conditions: (i). Diphenylether 265°C, 1 hr; (ii) POCl₃, 45 min.

Methods (ii) and (iii) have the disadvantage that they use extremely high temperatures (>250°C) and are messy. [2.3]

The wide distribution and importance of the quinoline motif dictates that the list above is not exhaustive. Microwave-assisted organic synthesis, [2.2] photocyclisation [2.1] are among other methods that have been previously described.

2.2 Synthesis of 4-chloro-7-X-substituted quinolines

The synthesis of 4-chloro-7-substituted quinolines was achieved using modification of the two [methods (ii) and (iii) above] previously reported methods. [2.3 - 2.6; 2.8] The choice of method was dependent on the substituent at the *meta* position of the aniline as described below. The 4-chloro-7-substituted quinolines were used as precursors for subsequent synthesis of target molecules. The target 7-substituted quinolines (Fig. 2.1) consisting of the substituents -CF₃ (**b**), -Cl (**c**) and -H (**f**), were available commercially while the rest were synthesised.

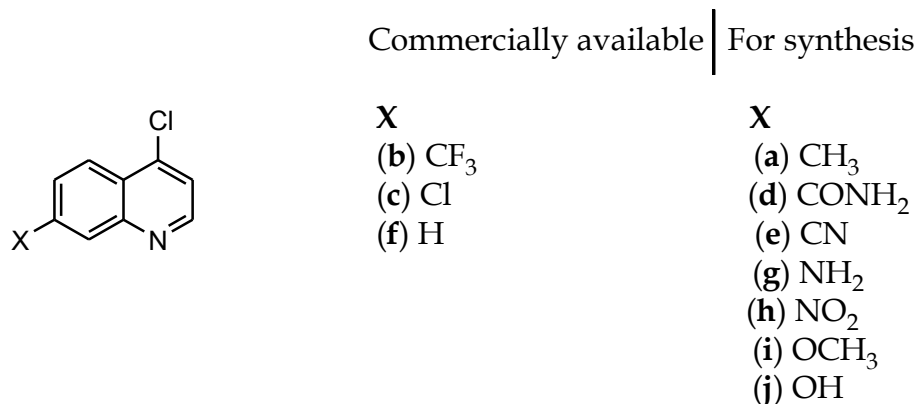
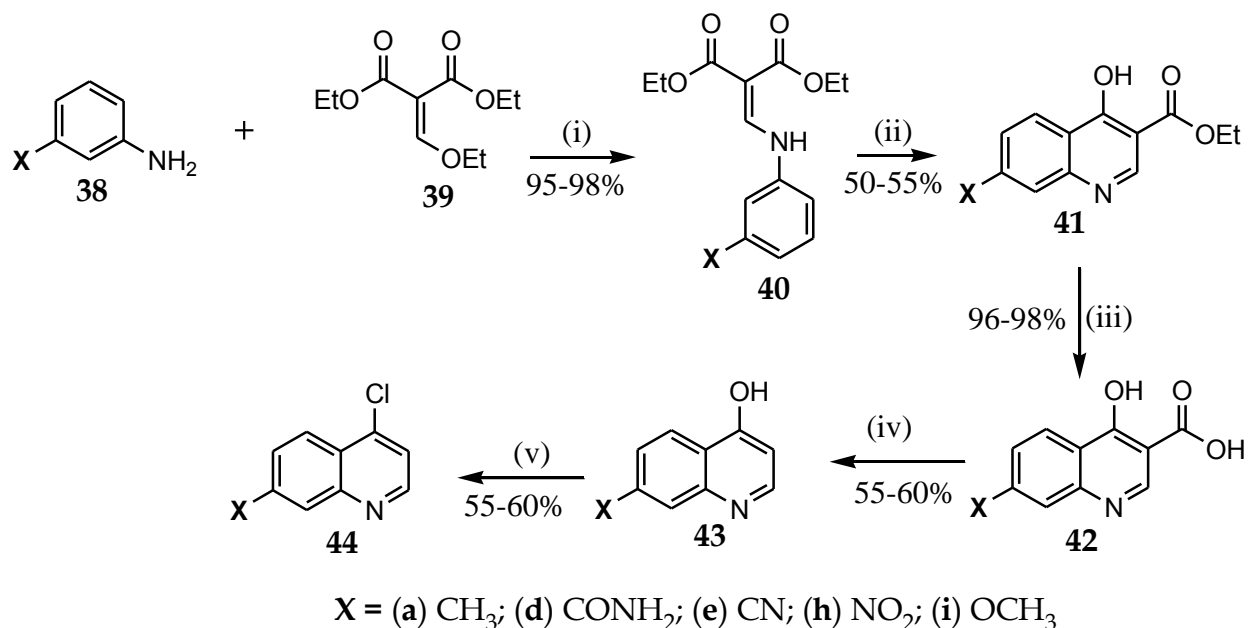


Figure 2.1: The general structure of 4-chloro-7-substituted quinoline compounds

The target compounds with the -NH₂ (g) substituent at the 7-position were obtained by the reduction of the -NO₂ (h) substituted compounds. The compounds with the -OH (j) substituent attached at the 7-position of the quinoline ring were obtained by demethylation of -OCH₃ (i) 7-substituted compounds.

2.2.1 The malonate ester synthesis route

Condensation of equimolar *m*-substituted anilines (**38a**, **38d**, **38e**, **38h** & **38i**) with diethyl methylene malonate (**39**) at 110 °C produced *N*-substituted acrylates (**40a**, **40d**, **40e**, **40h** & **40i**) with the elimination of ethanol. These were cyclised to quinoline esters (**41a**, **41d**, **41e**, **41h** & **41i**) by refluxing in phenyl ether at 250 - 260 °C for a maximum of 45 minutes (Scheme 2.4). On cyclisation, the two *ortho* positions (Fig 2.2) which are both reactive, can give regioisomeric products. To optimise yields and minimise the formation of undesired 5-isomeric products of the quinoline esters, the acrylates were added into an excess of already boiling phenyl ether.



Scheme 2.4: Reagents and conditions: (i) 110 °C, 45 min; (ii) Ph₂O 265 °C, 45 min; (iii) NaOH (aq) 115 °C, 30 min; (iv) Ph₂O 265 °C, 30 min; (v) POCl₃ 140 °C, 30 min.

Though the 5- and 7-isomers are almost always formed in the reaction, the product favours the substituent being in the 7-position rather than the 5-position possibly due to steric effects. The electronic properties of the substituent (**X**) are influential in the overall formation of the regioisomers. Electron releasing substituents (-OCH₃ and -CH₃) showed little or no evidence of the 5-substituted product. The more electron withdrawing substituents (-NO₂ and -CN) produced both the 5- and 7-position regioisomers in almost equimolar quantities.

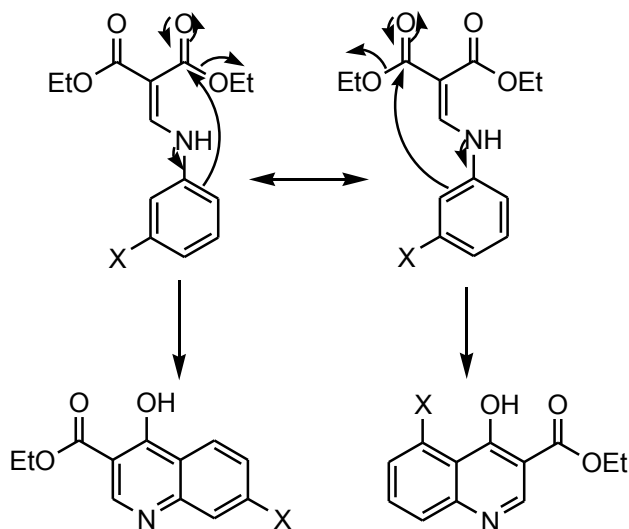


Figure 2.2: Possible regioisomers that can form from cyclisation of unsymmetrical acrylates.

The undesired products were selectively dissolved in boiling methanol. Compounds **41a**, **41d**, **41e**, **41h** & **41i** were isolated by filtration and recovered as solids. Alkaline hydrolysis of **41a**, **41d**, **41e**, **41h** & **41i** yielded the corresponding carboxylic acids which were decarboxylated (except **42h**) by refluxing in diphenyl ether. Subsequent treatment of the decarboxylated compounds with excess phosphorus oxychloride produced **44a**, **44d**, **44e**, & **44i** with yields ranging from 60 - 80% (Scheme 2.4).

When compound **42h** was decarboxylated using the above method, it was found to result in low yields and mainly starting material was recovered. The decarboxylation is thought to proceed initially by the formation of keto-enol tautomers of carboxylic acid (Fig. 2.3). When the key bonds break during heating, the acid proton is transferred to the carbonyl group and carbon dioxide is liberated (Fig. 2.3). In theory, the electron withdrawing nature of the $-\text{NO}_2$ substituent would be expected to favour the decarboxylation process as this would result in the weakening of the bond joining the carboxylic acid group to the quinoline nucleus. This was not observed and there are no literature explanations.

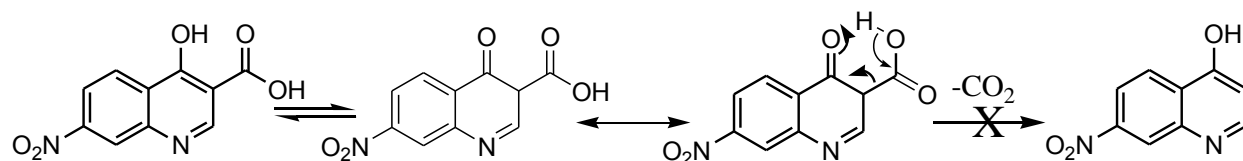
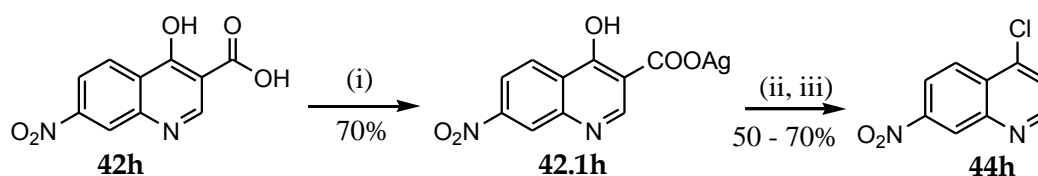


Figure 2.3: Decarboxylation of nitro compound (**42h**).

Investigation into the reasons for this anomalous behaviour was beyond the scope of the project. Nevertheless the decarboxylation was achieved using the method described by Baker *et al.* [2.9] A silver salt of 4-hydroxy-7-nitroquinoline-3-carboxylic acid was prepared prior to decarboxylation and treatment with phosphorus oxychloride (Scheme 2.5).



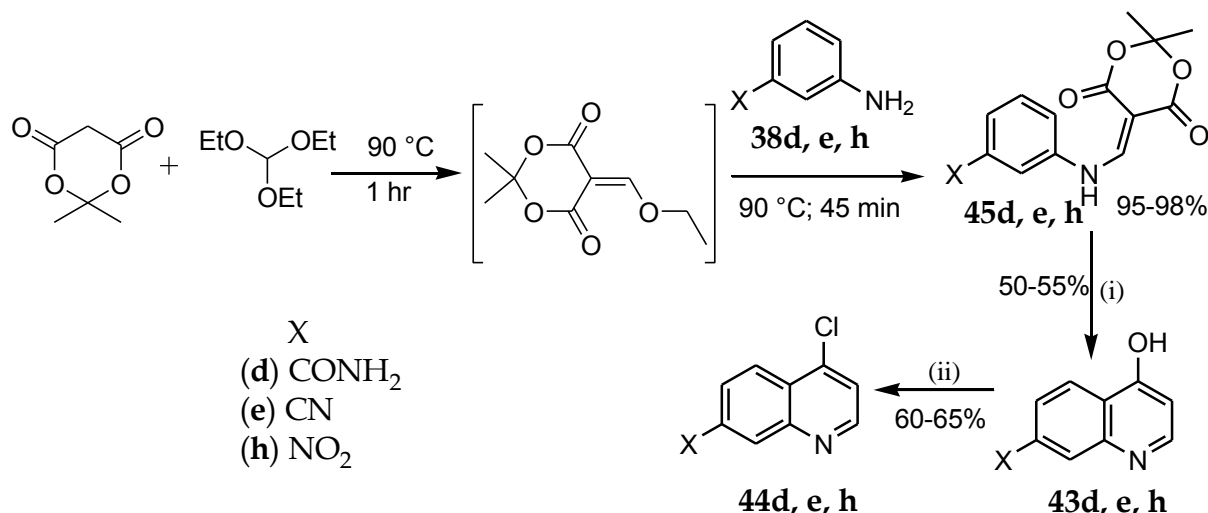
Scheme 2.5: Reagents and conditions: (i) 25% NH₃ (aq), AgNO₃, H₂O, reflux, 16 hr. (ii) Ph₂O, 265 °C, 45 min. (iii) POCl₃, 140 °C, 30 min.

In the Scheme 2.5 illustrated above, the silver salt was decarboxylated in the presence of heat giving the product as a grey solid. The solid was refluxed in ethanol for 5 hours, filtered whilst hot and concentrated to give **43h** as a yellow solid in modest yields of 50%. Upon treatment of **43h** with phosphorus oxychloride the desired product (**44h**) was obtained in good yield (70%).

2.2.2 The Meldrum's acid synthesis route

The decarboxylation of 4-hydroxy-7-nitroquinoline-3-carboxylic acid, described above, is a relatively low yielding reaction requiring long reaction times. The inclusion of the silver salt step in the reaction sequence adds to an already lengthy synthetic route. To address

this issue as well as the regioselectivity, particularly for other electron withdrawing groups (-CN, -NO₂ and -CONH₂) an alternative synthetic route was used (Scheme 2.6).



Scheme 2.6: Reagents and conditions: (i). Diphenylether 265 °C, 5 min; (ii) POCl₃, 140 °C, 30 min.

Meldrum's acid (2,2-dimethyl-1,3-dioxane-4,6-dione) was employed as a substitute for the malonate ester used in section 2.1(a). Meldrum's acid and triethyl-*ortho*-formate were heated for an hour at 90 °C. An intermediate, ethoxymethylene Meldrum's acid, which formed *in situ* was then reacted with the corresponding aniline (38d, 38e & 38h) in an addition-elimination reaction to afford the ene-amine 45d, 45e & 45h, precursors for the cyclisation step (Scheme 2.6). The reaction conditions used ensured excellent yields of greater than 90% for the initial step. The products (44d, 44e, 44h) were characterised and used in the next step without any further purification. A typical ¹H NMR spectrum of an ene-amine product is shown in Figure 2.4.

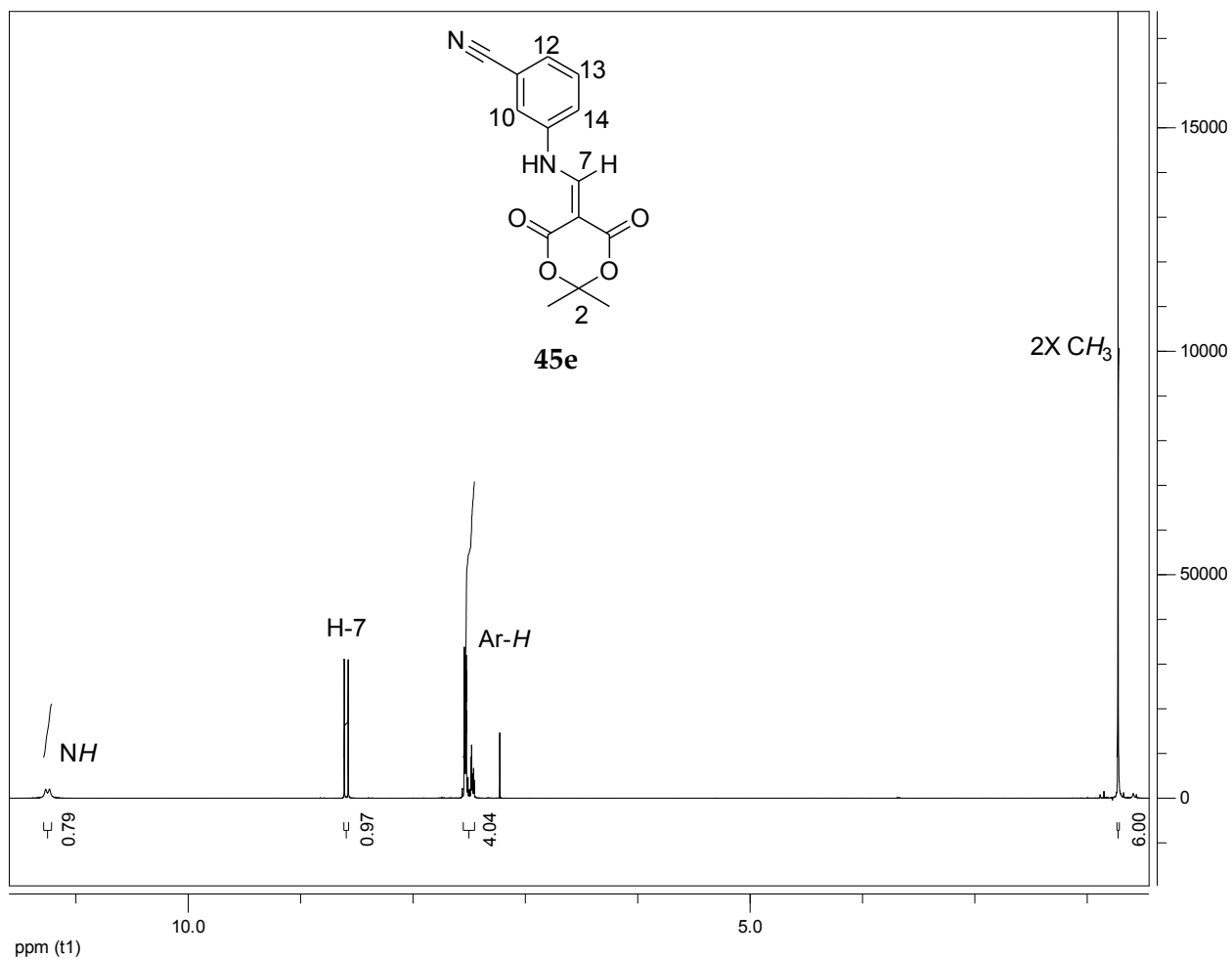


Figure 2.4: ¹H NMR spectrum for compound 45e in DMSO-d₆.

All the anilines used in this instance are strongly electron deficient because of the electron withdrawing properties of the *meta*-substituents (-CN, -NO₂ and -CONH₂). The reaction mechanism is thought to proceed as shown in Figure 2.5.

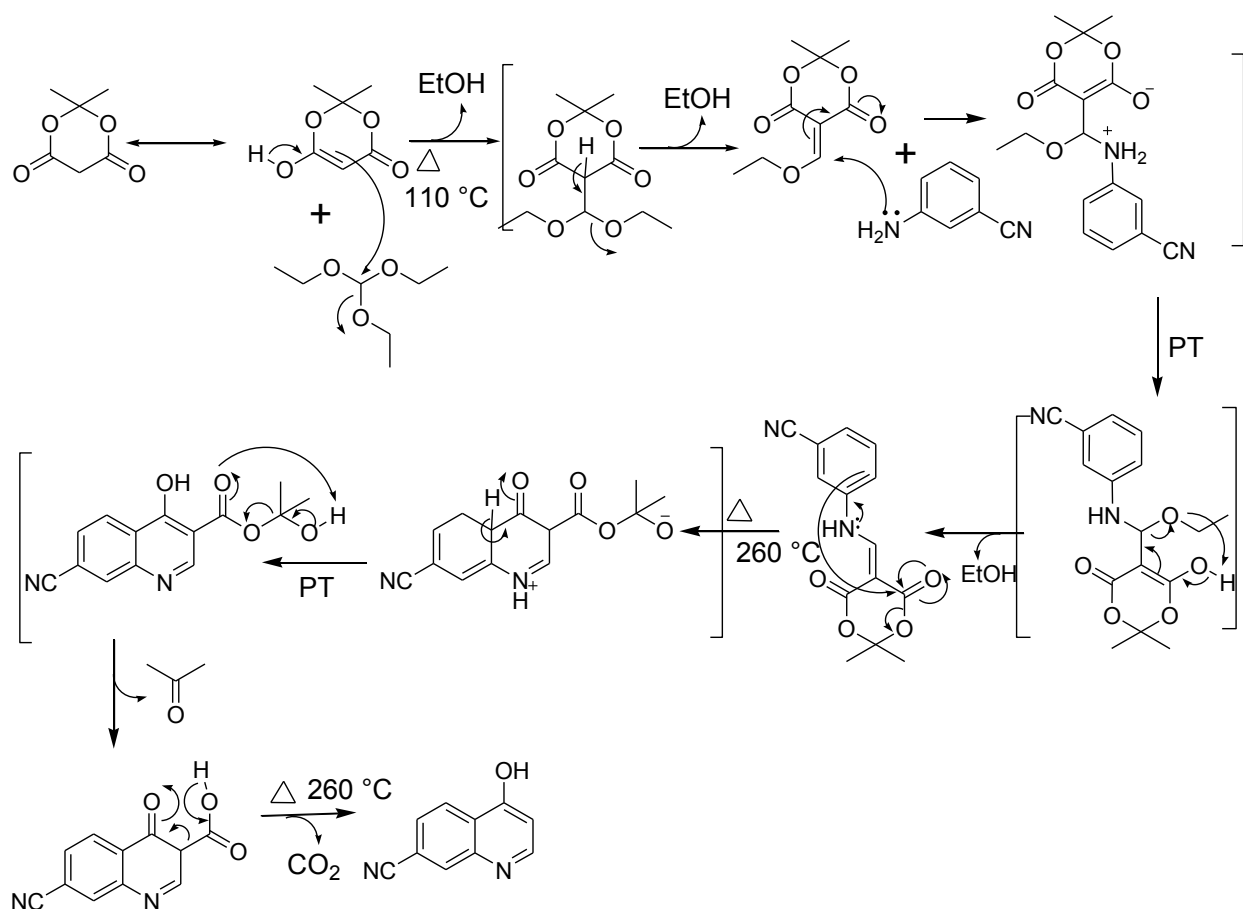


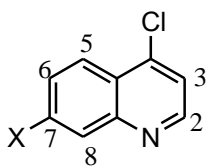
Figure 2.5: The proposed reaction mechanism for the formation of 4-chloro-7-X-quinoline via Meldrum's acid route [2.8]

Corresponding cyclised products were obtained by adding **45e** and **45h** to refluxing diphenylether over 5 minutes. Compound **45d** was found to be sensitive to the high temperature and was therefore added to refluxing phenyl ether and the mixture immediately cooled to room temperature. Treatment of **43d**, **43e** & **43h** with phosphorus oxychloride (Scheme 2.6) afforded target compounds **44d**, **44e** & **44h** which were purified by column chromatography. The yield for compounds **26d** was about 50% largely due to heat instability. Compounds **44e** & **44h** were obtained in reasonable yields of 60%.

The different aniline precursors showed varied reactivity characteristics that were reflected in the reaction times in the addition-elimination reaction step. On average the reaction was allowed to heat for 30 minutes before cooling to room temperature. The -CONH₂ substituent formed a precipitate after 10 minutes. The condensation products for both the malonate ester and Meldrum's acid reagents solidified upon cooling to room temperature. The -OCH₃ (i) substituent was the only exception and needed to be chilled in a liquid N₂/acetone slush bath in order to obtain precipitation. The products were all obtained in yields of greater than 90%.

Overall, the cyclisation step was the lowest yielding while the remaining steps were in the range of 60-70%. Characterisation of the compounds by proton NMR showed similar patterns although fingerprint differences are noticeable dependant on the group at the 7-position. Chemical shifts for C-7 of the different compounds are shown in Table 2.1, showing the effect of the different groups.

Table 2.1: Percentage yield and C-7 chemical shifts for 4-Chloro-7-X-substituted quinolines

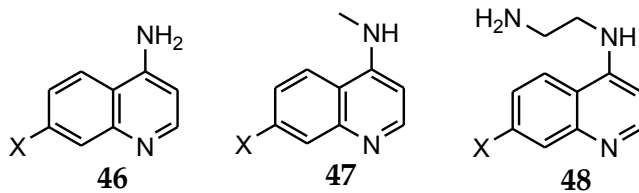


X= (a) CH₃; (d) CONH₂; (e) CN; (h) NO₂; (i) OCH₃ and (j) OH

Compound	% Yield	C-7 δ ppm
X		
(a) CH ₃	80	140.4
(d) CONH ₂	50	130.0
(e) CN	70	107.3
(h) NO ₂	60	152.4
(i) OCH ₃	80	161.3
(j) OH	70	160.1

2.3 Synthesis of 7-X-4-amino, 4-methyl amino and 4-ethylene diamine quinoline molecules

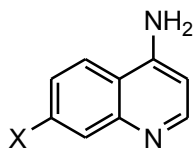
A fragment-based approach was used where simple short chain 4-aminoquinoline derivatives (Fig. 2.6) were synthesised. The starting point was the 4-amino-7-X-quinoline (**46a-j**) followed by 4-amino methyl-7-X-quinolines (**47a-j**) and the 4-ethylene diamine-7-X-quinolines (**48a-j**), Fig. 2.6. Starting with the 4-amino-7-X-quinoline, a simple 4-amino quinoline, the length of the lateral carbon chain was increased from zero to two carbons. The compounds were all synthesised via the nucleophilic substitution of the chloro group at the 4-position of the quinoline ring with the appropriate amine.



X= (a) CH₃; (b) CF₃; (c) Cl; (d) CONH₂; (e) CN; (f) H; (g) NH₂; (h) NO₂; (i) OCH₃; (j) OH

Figure 2.6: The general structures of the simple short chain 4-aminoquinoline molecules **46**, **47** and **48**.

2.3.1 Synthesis of 4-amino-7-X-substituted quinoline compounds (**46**)

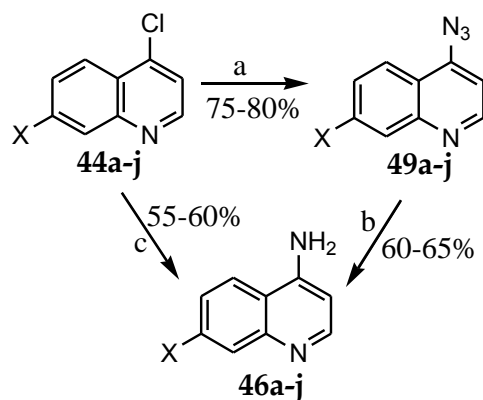


X= (a) CH₃; (b) CF₃; (c) Cl; (d) CONH₂; (e) CN; (f) H; (g) NH₂; (h) NO₂; (i) OCH₃; (j) OH

Figure 2.7: The 4-amino-7-X-quinoline compounds

The syntheses of 4-amino-7-X-quinolines (Fig. 2.7) were achieved using three different methods. The choice of method was dependant on the substituent at the 7-position.

(i) Microwave assisted organic synthesis (MAOS) was used for the substituents that are less electron withdrawing. Though hazardous because of the high temperatures and pressures, the method offered faster reaction times. Analogues **46a**, **46b**, **46c**, **46f** & **46i** were synthesised from the corresponding 4-chloro-7-X-quinoline (**44a-j**) using excess aqueous ammonia in the presence of a catalytic amount of zinc chloride (Scheme 2.7). The yields were in the range 55 - 60% suggesting the harsh conditions did not suite the reagents. The use of the Lewis acid zinc chloride (ZnCl₂) was to promote the reaction in order for it to go to completion as aqueous ammonia is not strongly nucleophilic.



X=(a) CH₃; (b) CF₃; (c) Cl; (d) CONH₂; (e) CN; (f) H; (g) NH₂; (h) NO₂; (i) OCH₃ and (j) OH

Scheme 2.7: Reagents and conditions: (a) NaN₃, H₂O/DMF, 90 °C, 2 hr; (b) Ph₃P, THF/H₂O, room temperature, 2 hr; (c) NH₃ (aq), ZnCl₂ 70 °C 8-12 hr or NH₃ (aq), ZnCl₂, Microwave (MW) set as follows: Method 1 of 2; P 20.5 bar, 160 °C, 40 min; Method 2 of 2; P 20.5 bar, 180 °C, 50 min.

The compounds were identified by proton (¹H) and carbon 13 (¹³C) NMR as well as high resolution mass spectroscopy. Further purification of the compounds was undertaken using preparative HPLC to ensure 98% or greater purity for all compounds. A typical ¹H NMR spectrum of the compound is shown (Fig. 2.8).

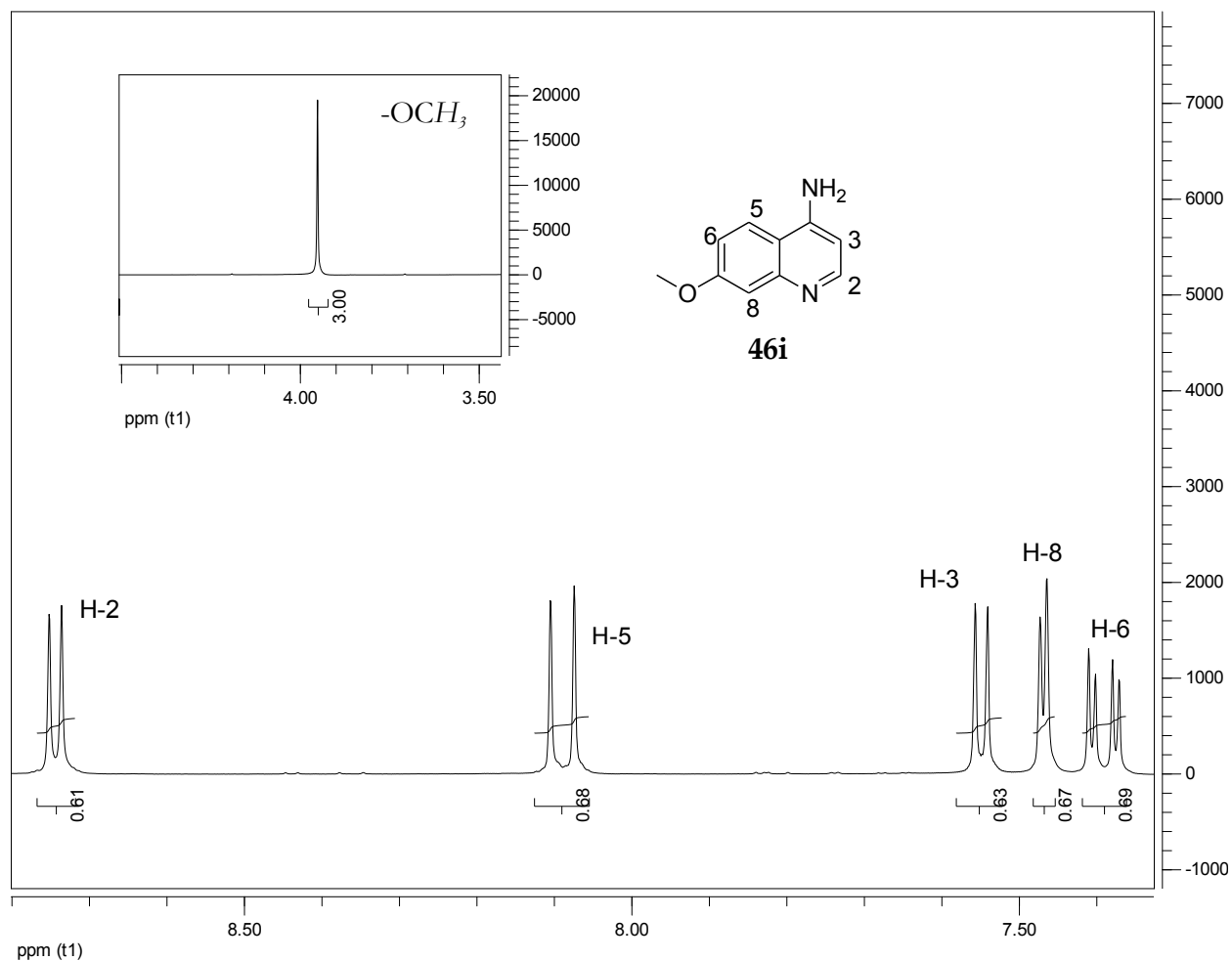


Figure 2.8: ¹H NMR spectrum of 4-amino-7-methoxyquinoline (**46i**) in DMSO-d₆ with methoxy protons shown in the insert

The harsh conditions in the microwave assisted synthesis, though necessary, were counterproductive in the synthesis of **46f**. In this case instead of **46f** being obtained, compound **43f** was obtained in quantitative yields (Fig. 2.9). This was unexpected and close analysis of other compounds that were synthesised using the same method showed no evidence of undesired product formation. There is a possibility that during the harsh

reaction conditions the ammonia evaporated from the vessel leaving water as the only available nucleophile (Fig. 2.9).

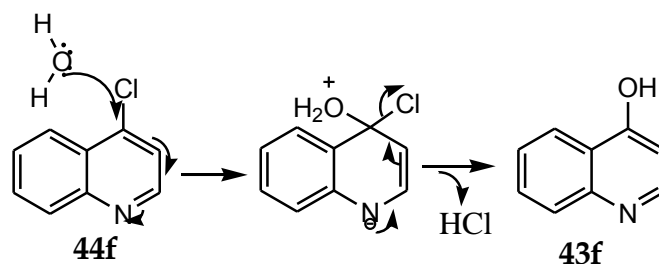


Figure 2.9: Proposed mechanism for the formation of compound **43f**

Compound **43f** was identified by proton (¹H) and carbon-13 (¹³C) NMR in addition high resolution mass spectroscopy. Carbon-13 (¹³C) NMR has a peak at 174 ppm indicative of a hydroxyl carbon (Fig. 2.10). The peak is absent in the target compound (insert).

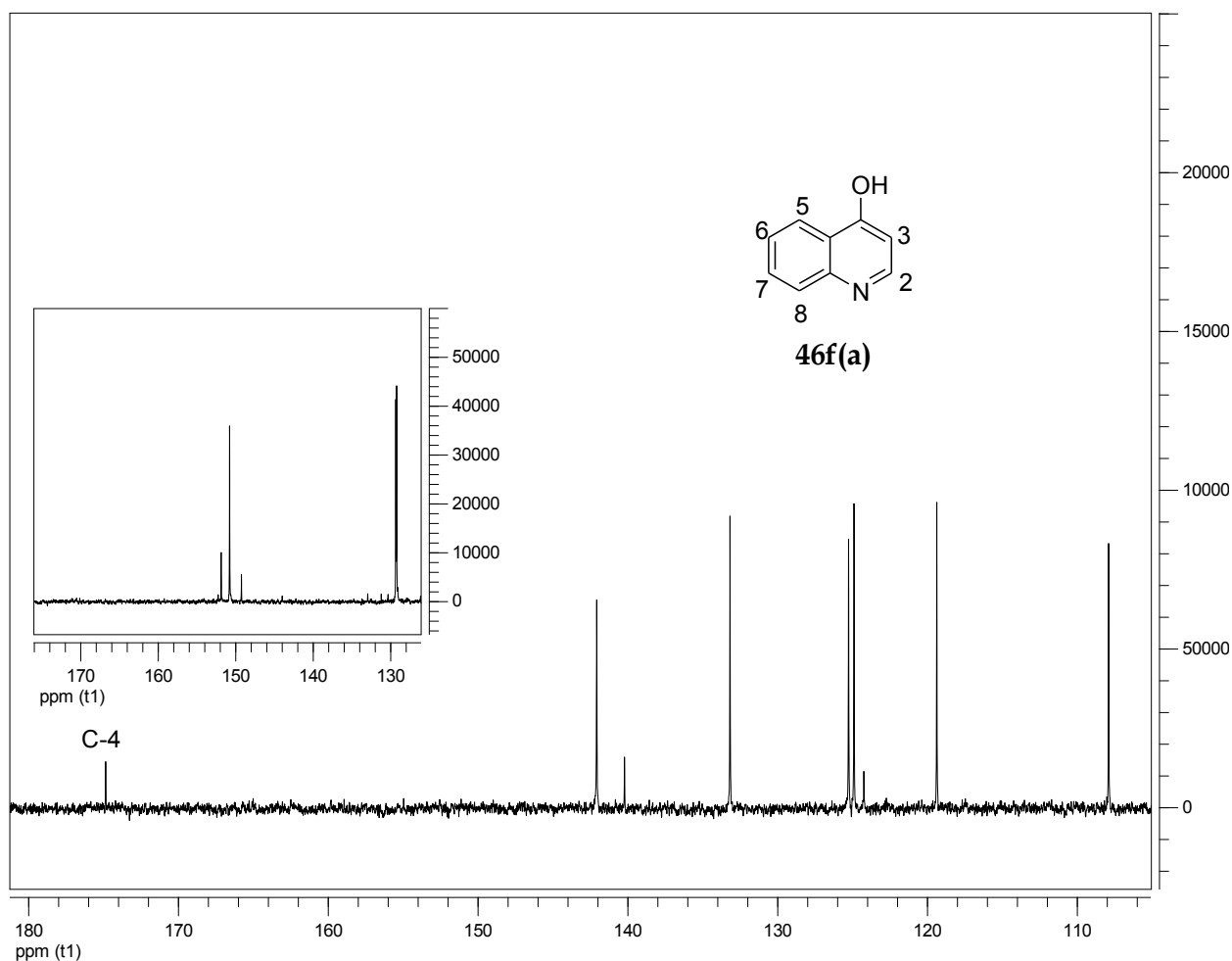


Figure 2.10 ^{13}C NMR spectrum of compound **46f (a)** and **46f** (insert) in DMSO-d_6 . The figure highlights the disappearance of hydroxyl carbon in insert.

(ii) An alternative method was to obtain the target compounds via the azide intermediate. Corresponding azide intermediates were prepared from compounds **44a-j** and sodium azide in a 50:50 $\text{H}_2\text{O}/\text{DMF}$ mixture for 2 hours at 90°C . In addition to ^1H and ^{13}C NMR the azides were confirmed by infrared (IR) spectroscopy by a prominent azide peak between $2160\text{-}2120\text{ cm}^{-1}$. Reduction of **49a-j** using triphenyl phosphine afforded **46a-j** in reasonable yields. The Staudinger reduction where an azide is reduced to an amine with triphenyl phosphine was used for this series of compounds. The

mechanism of the reaction involves an attack on the terminal nitrogen atom of the azide by the lone pair of electrons from the phosphorus on the triphenyl phosphine. This generates a phosphazide intermediate (I) which, after cyclisation results in the expulsion of N₂ to form iminophosphorane intermediate (II). In the second step, the amine is formed during the aqueous work up, where triphenylphosphine oxide is also lost as a by-product (Fig. 2.11).

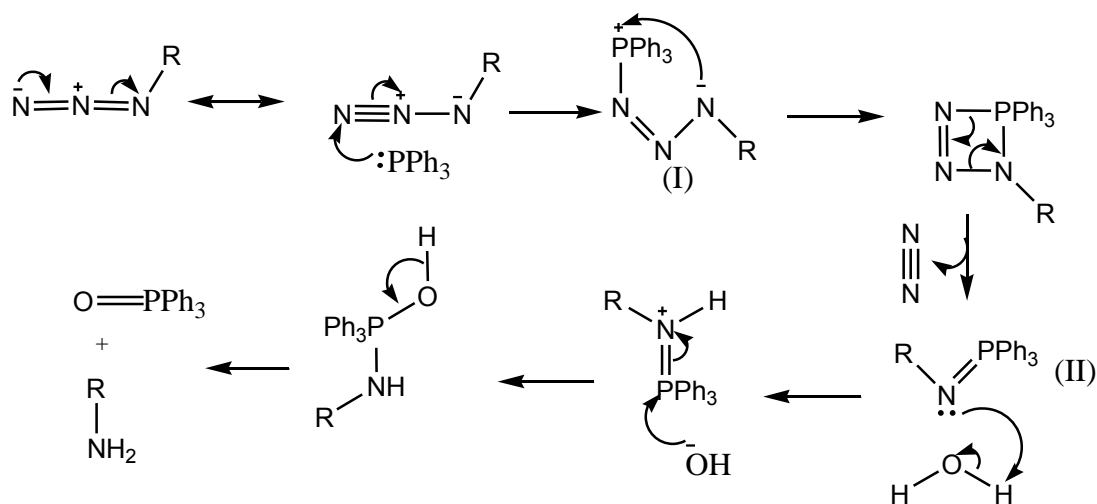


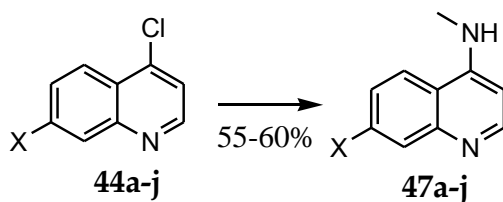
Figure 2.11: General mechanism of the Staudinger reduction

(iii) Conventional heating was used where microwave assisted synthesis proved too harsh. In this method the reagents were heated in a sealed tube at 90-110 °C for 4-6 hours with excess aqueous ammonia. Upon cooling to room temperature the solid was filtered and washed with 10% aqueous ammonia. This method was potentially hazardous because of the pressure build up in the sealed tube.

Characterisation of the 4-amino-7-X-quinolines was confirmed by use of high resolution mass spectrometry. ¹³C NMR showed a downfield shift for C-4 as a result of the amine group at that position.

2.3.2 Synthesis of 4-amino methyl-7-X-quinoline molecules

The methylamine series of compounds was synthesised using microwave technology with the methylamine substituting the chloro group at the C-4 position (Scheme 2.8). Milder conditions were also used for compounds **47a-j** where the 7-substituted quinolines were heated in a sealed tube at 70 °C for 6 hours. Average yields of 60% were obtained.



X=(a) CH₃; (b) CF₃; (c) Cl; (d) CONH₂; (e) CN; (f) H; (g) NH₂; (h) NO₂; (i) OCH₃ and (j) OH

Scheme 2.8: Reagents and conditions: Methyl amine, 120 °C, 6 hr.

Target compounds were obtained as solids after evaporating the solvent. ¹H NMR for the compounds showed a distinct doublet signal (with a coupling constant of ~4.7 Hz for all compounds) corresponding to three protons for the -CH₃ protons (Fig. 2.12), thus indicating -CH₃-NH proton coupling. However, the -NH proton peak was observed as a broad singlet signal, presumably an unresolved quartet, appearing at different chemical shifts.

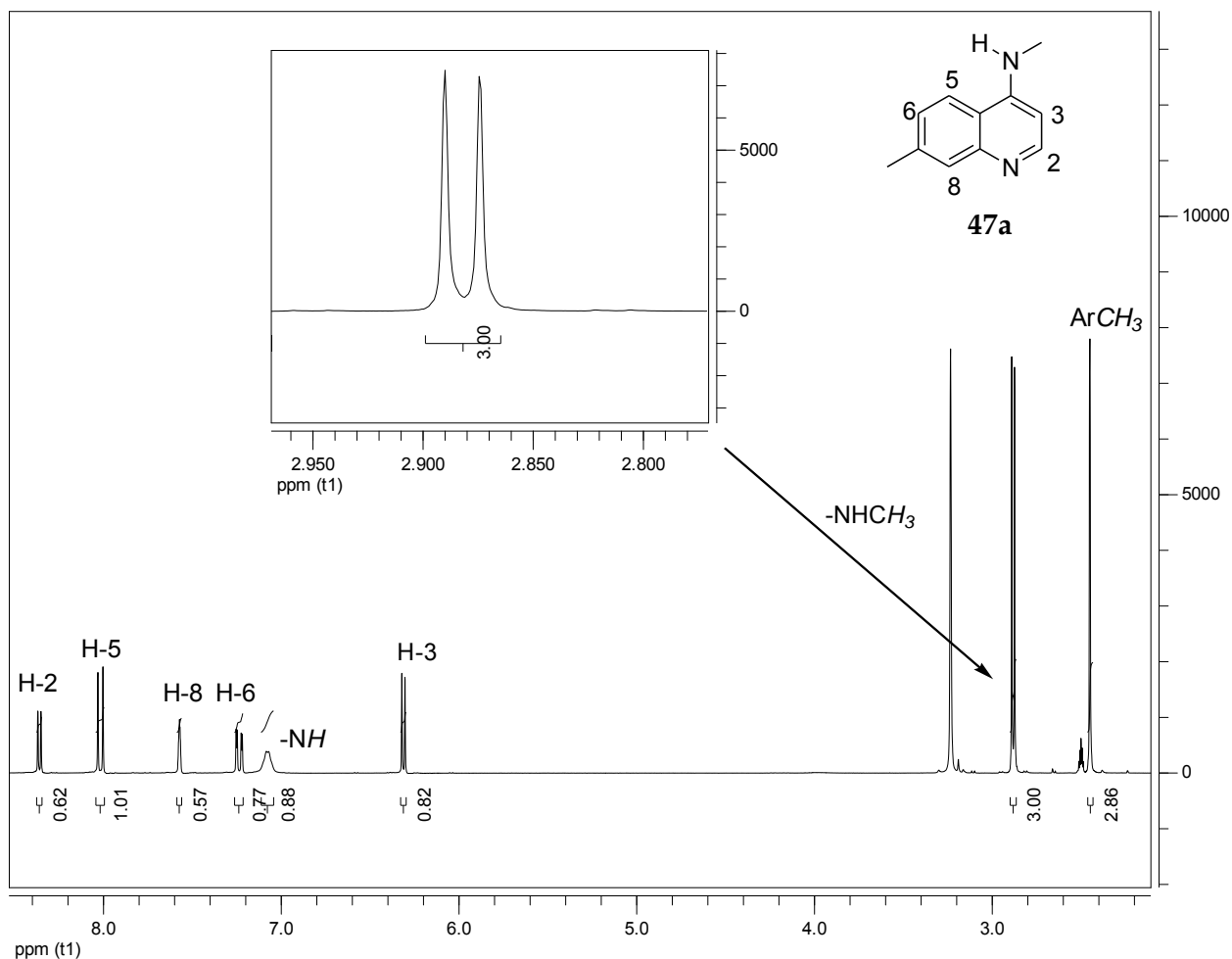
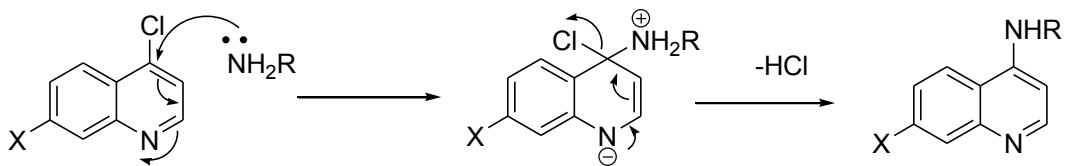


Figure 2.12: ^1H NMR spectrum of *N*,7-dimethylquinolin-4-amine (47a) in DMSO-d_6 .

In this series of compounds aromatic nucleophilic substitution of the chloro group by methylamine was achieved under relatively mild conditions. The reaction mechanism is the classic aromatic nucleophilic substitution. This involves the nucleophilic attack at the C-4 sp^2 carbon by the primary amino nitrogen and the expulsion of the chloride anion (Fig. 2.13). The quinoline nitrogen provides resonance stabilization of the intermediate and also influences regioselectivity. Work up involves removal of the solvent and where necessary purification using silica gel chromatography. To ensure greater than 98%

purity all the compounds were checked for purity using HPLC and where appropriate preparative HPLC was used in case the purity was below the required level.



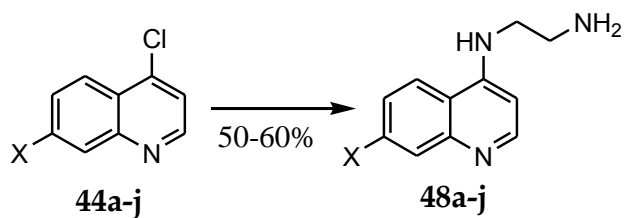
R = H, NH₂CH₃

X=(a) CH₃; (b) CF₃; (c) Cl; (d) CONH₂; (e) CN; (f) H; (h) NO₂; (i) OCH₃ and (j) OH

Figure 2.13: Mechanism for the formation of 4-amino-7-X-quinoline and 4-methyl amino-7-X-quinoline compounds

2.3.3 Synthesis of 4-(ethylene diamino)-7-X-quinoline molecules

Compounds **48a-j** were obtained via the nucleophilic aromatic substitution at C-4 of the quinoline ring of the 4-chloro group by ethane-1,2-diamine (Scheme 2.7). The 4-chloro-7-X-quinoline compounds were heated with excess ethane-1,2-diamine in a sealed tube for up to 6 hours and yields were generally low to poor. The compounds were purified by HPLC and characterisation was carried out using ¹H and ¹³C NMR and high resolution mass spectroscopy.



X=(a) CH₃; (b) CF₃; (c) Cl; (d) CONH₂; (e) CN; (f) H; (g) NH₂; (h) NO₂; (i) OCH₃ and (j) OH

Scheme 2.9: Reagents and conditions: Ethylene diamine, 130 °C, 3-6 hr.

The ^{13}C and ^1H NMR spectra of these compounds showed masking of the H-10 signal by the DMSO solvent peak for some compounds. A typical ^1H NMR is shown below (Fig. 2.14).

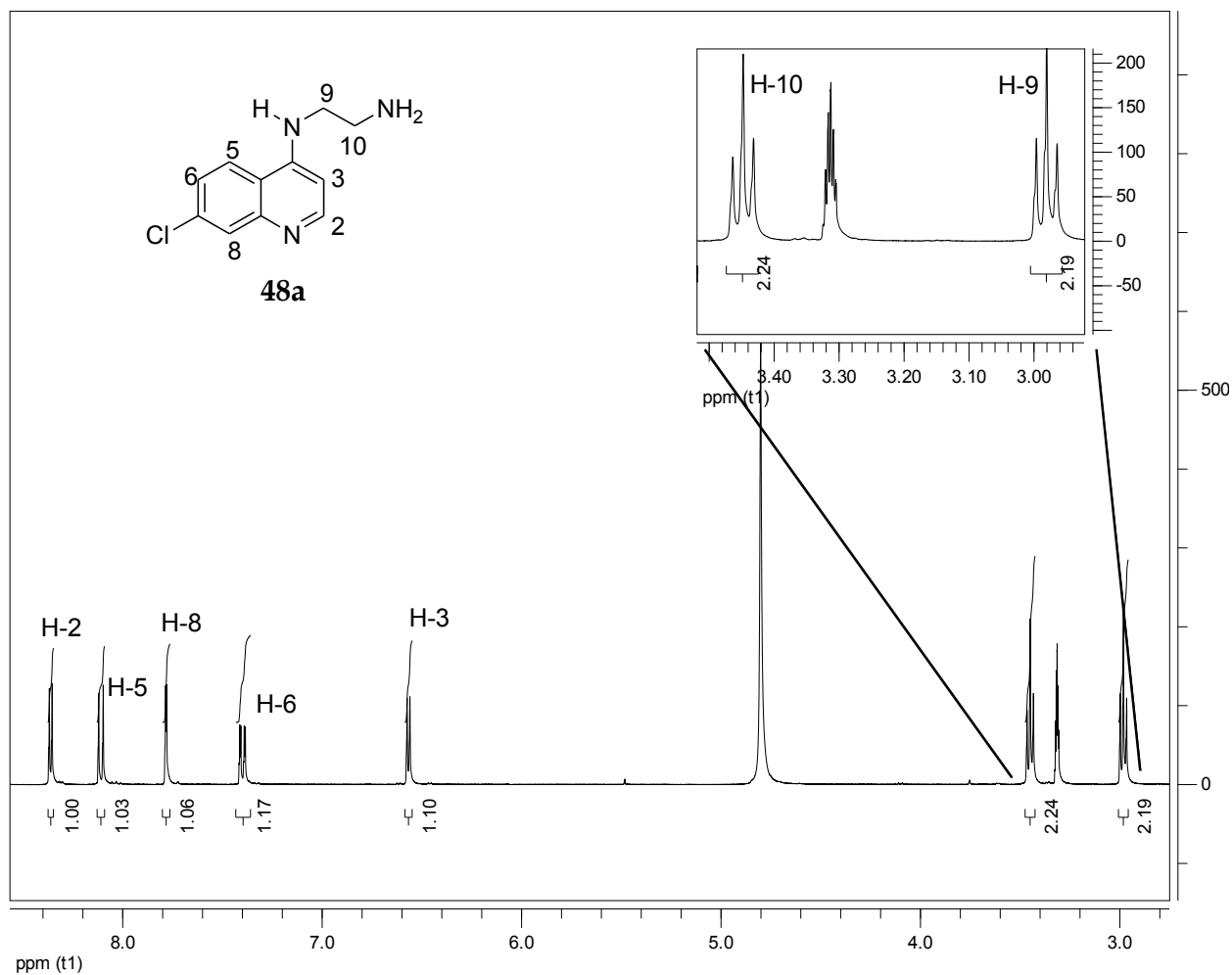


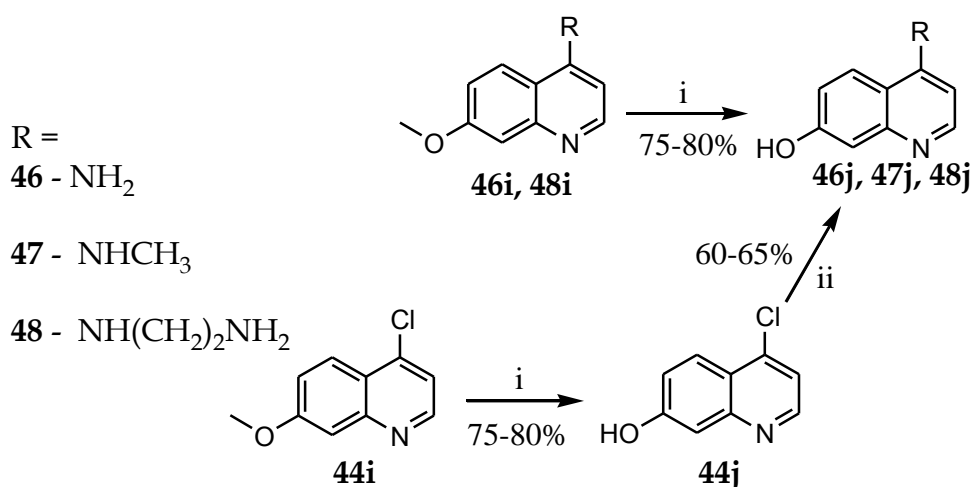
Figure 2.14: ^1H NMR spectrum of N^1 -(7-chloroquinolin-4-yl)ethane-1,2-diamine (**48c**) in DMSO-d_6

2.3.4 Demethylation reactions

Preparation of compounds **46j** & **48j** was achieved by BBr_3 -mediated demethylation of **28i** & **30j** respectively (Scheme 2.10). Compound **47j** was obtained by demethylation of **26i** prior to nucleophilic aromatic substitution at the C-4 position with methylamine.

Demethylation of **47i** unexpectedly resulted in compound **46i** being obtained as the major product. The product was confirmed by the presence of the methoxy signal at 3.86 ppm in ^1H NMR spectrum and absence of a singlet methyl signal.

The dealkylation method used has been reported previously. [2.10, 2.11] BBr_3 was added to a stirred solution of the respective compounds in dry DCM at $-80\text{ }^\circ\text{C}$. The mixture was stirred at room temperature and then left in a freezer for two days. Work-up involved re-cooling the mixture to $-80\text{ }^\circ\text{C}$ and carefully adding saturated NH_4Cl . Products were obtained as solids with yields of 60 - 80% and characterised fully using ^1H and ^{13}C NMR and high resolution mass spectroscopy.

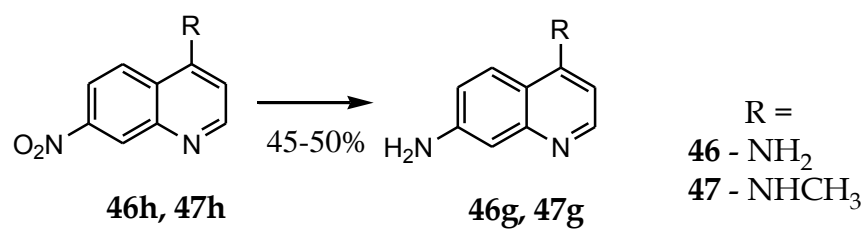


Scheme 2.10: Reagents and conditions: (i) BBr_3 , DCM, $-80\text{ }^\circ\text{C}$, 40 hr; (ii) Ethylene diamine, methylamine or NH_3 (aq), $90\text{ }^\circ\text{C}$, 3 - 6 hr.

Alternatively, the compounds were synthesised using the Meldrum's acid route. This way, the 4-chloro-7-hydroxy quinoline was synthesised, serving as a precursor for the final compounds.

2.3.5 Reduction of nitro compounds

Nitro compounds **46h**, **47h** & **48h** were transformed into their amine derivatives using stannous chloride (Scheme 2.11). Tin chloride dissolved in concentrated HCl was added slowly to the mixture of the nitro compounds dissolved in glacial acetic acid and heated to 60 °C for two hours. The products (**46g**, **47g**) were confirmed by ¹H and ¹³C NMR (Fig. 2.15) and high resolution mass spectroscopy.



Scheme 2.11: Reagents and conditions: SnCl₂, HCl, AcOH, 60 °C, 2 hr.

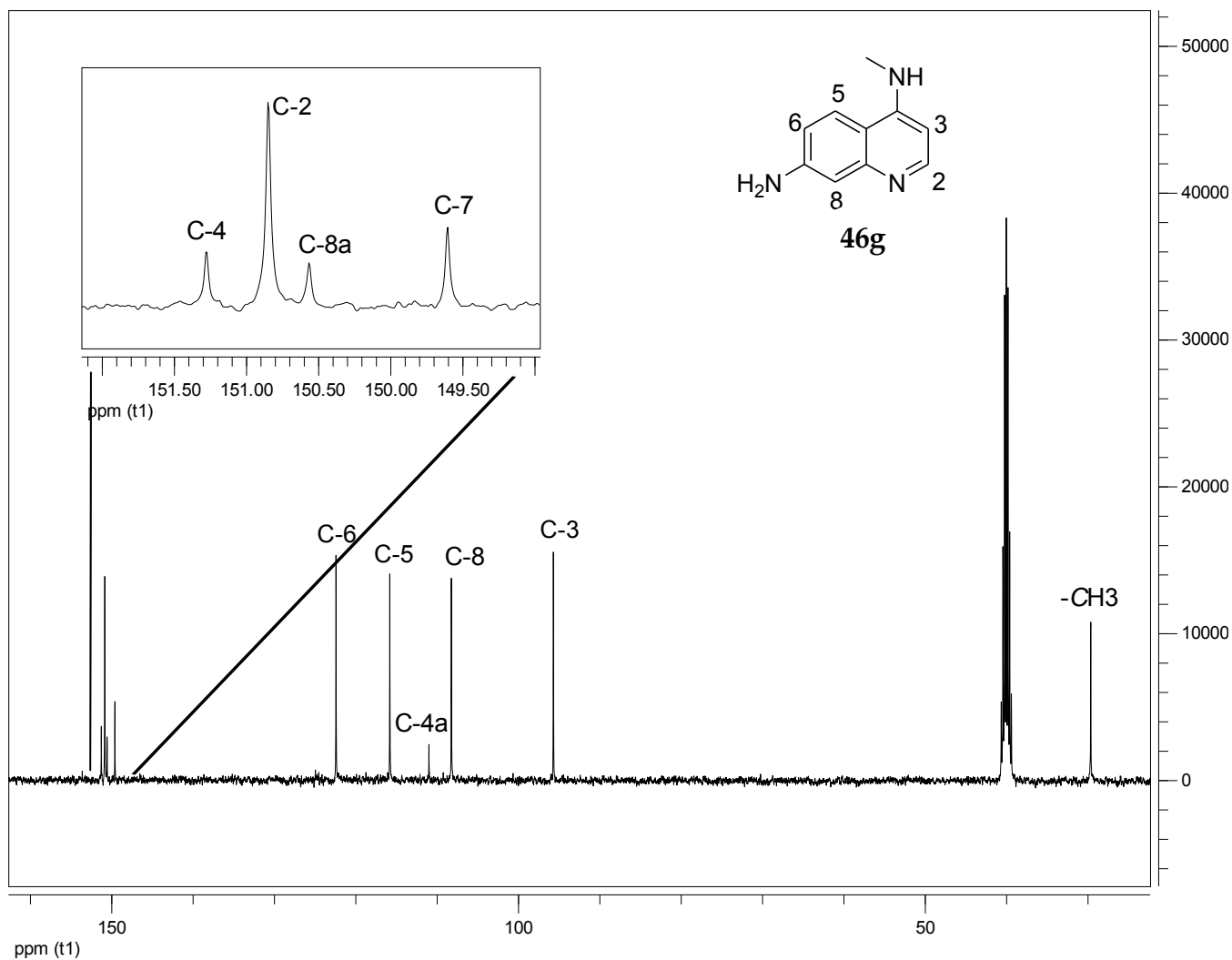
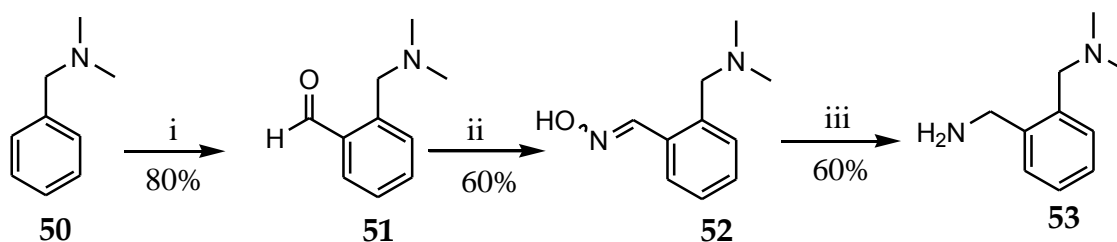


Figure 2.15: ^{13}C NMR spectrum of 4,7-diamino methyl quinoline (**46g**) in DMSO-d_6

2.4 Synthesis of 2-(dimethylaminomethyl) benzylamine (**53**) (Phenylequine side chain)

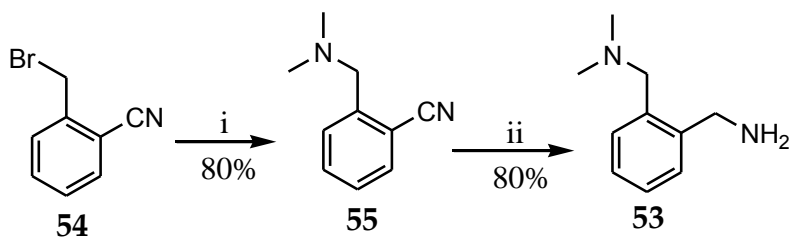
The synthesis of the phenylequine side chain (**53**) was based on an *ortho*-lithiation reaction followed by reductive amination using a known synthetic route. [2.12]. Commercially available compound **50** was transformed into an aldehyde **32**, which was in turn converted to an oxime (**52**). Intermediates **51** & **52** are known compounds and their characterisation was consistent with the reported literature. [2.13] The final product was obtained by reducing the oxime using lithium aluminium hydride (Scheme 2.12a).



Scheme 2.12a: Reagents and conditions: (i) *n*-BuLi, 12 hr, reflux under N₂, DMF 6 hr; (ii) NH₂OHHCl, NaOH, reflux 14 hr; (iii) THF, LiAlH₄, 15 hr reflux

The first step required abstraction of a proton for the formation of an *ortho*-lithiated species (Scheme 2.12a). This step could be carried out using either *tert*-butyllithium (*t*-BuLi) or *n*-butyllithium (*n*-BuLi) as the *ortho*-lithiating agent. The *t*-BuLi method had the advantage of a shorter reaction time (2 hr) but is hazardous because it is pyrophoric and therefore requires extreme care when handling. Though *n*-BuLi had a longer reaction time (12 hr), the yields were similar to those of *t*-BuLi and it required less effort in handling.

An alternative synthetic route has been reported previously (Scheme 2.12b). [2.14] It has the advantage of shorter reaction times, milder reaction conditions as well as improved yields. The synthesis of compound **53** was therefore reduced from three steps to two steps in overall yield of 80% using this method. Compound **54** is commercially available and the nucleophilic substitution of bromine with dimethylamine gave compound **55** in 80% yield. Reduction of compound **55** with lithium aluminium hydride afforded the target compound (**53**). Compound **53** is known and characterisation is consistent with recorded values. [2.12, 2.13]



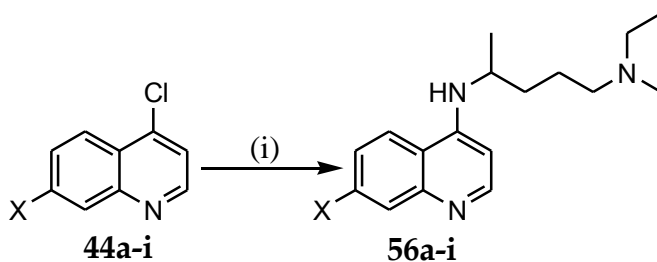
Scheme 2.12b: Reagents and conditions: (i) Dimethylamine [NH(CH₃)₂]; (ii) THF, LiAlH₄, 15 hr reflux

2.5 Synthesis of chloroquine (CQ), phenylequine (PQ) and pyridodibemequine (PDB) derivatives

The synthesis of the target molecules involved the aromatic nucleophilic substitution of the chloro group at position 4 with the appropriate lateral chain. The compounds were characterised using ¹H NMR, ¹³C NMR and high resolution mass spectroscopy. They were all purified using preparative HPLC to confirm their purity was greater than 98%.

2.5.1 Synthesis of *N*¹, *N*¹-diethylpentane-1,4-diamine-7-*X*-quinoline molecules

Compounds **56a-f**, **h** & **i** were synthesised using a previously reported method (Scheme 2.13). The 4-chloro-7-*X*-quinolines were heated in a sealed tube with *N*, *N*-diethylpentane-1, 4-diamine for up to 8 hours. The yields and percentage purity for the compounds are shown in Table 2.2.



X=(**a**) CH₃; (**b**) CF₃; (**c**) Cl; (**e**) CN; (**f**) H; (**h**) NO₂; (**i**)OCH₃

Scheme 2.13: Reagents and conditions: (i) *N*, *N*-Diethylpentane-1, 4-diamine, 120 °C, 4-8 hr.

The compounds were purified using preparative high pressure liquid chromatography (HPLC) after which analytical HPLC was used to ensure greater than 98% purity (Fig. 2.16). The compounds were eluted using the mobile phase $\text{NH}_4\text{CO}_3/\text{MeOH}$ pH 10.7 and $\text{H}_2\text{O}/\text{NH}_4\text{CO}_3$ pH 10.7 for 20 minutes. The ^1H and ^{13}C NMR of compounds **56a-i** were consistent with literature data of similar compounds. [2.4] A typical ^1H NMR spectrum is shown in Figure 2.17.

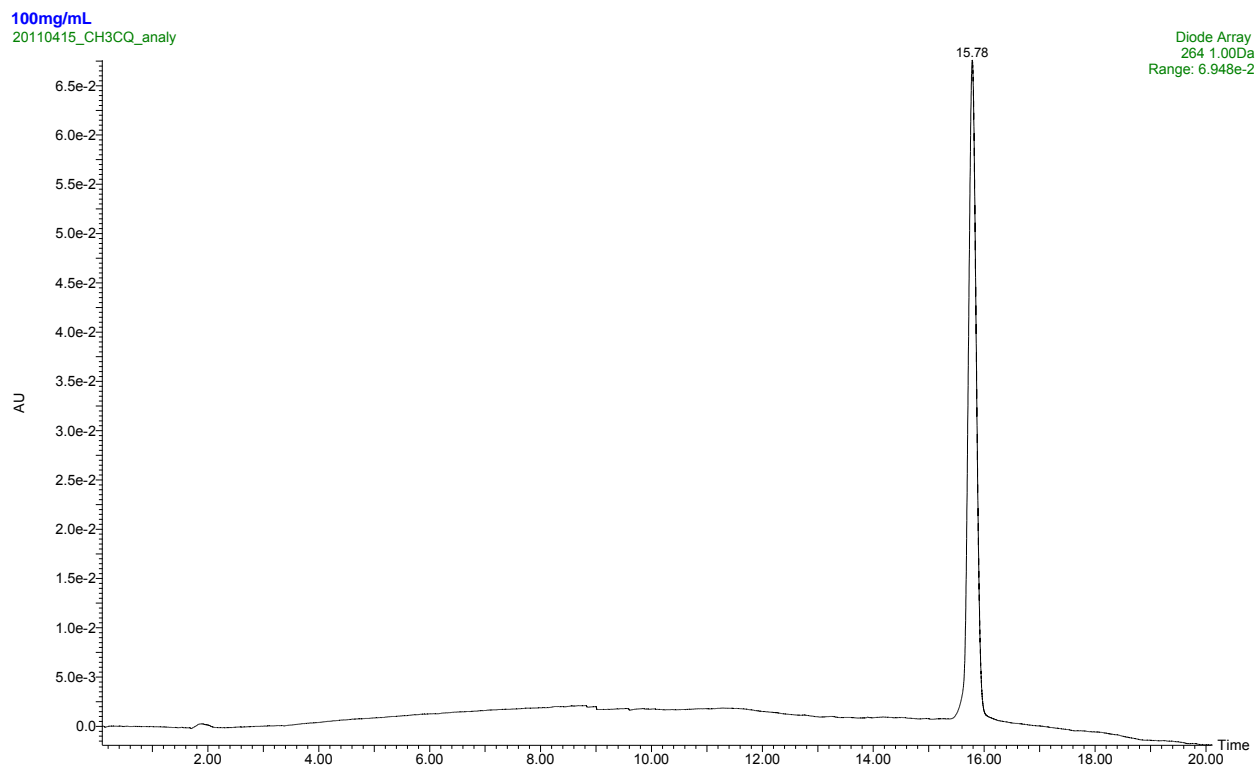


Figure 2.16: Analytical HPLC trace for compound **56a** using the mobile phase $\text{NH}_4\text{CO}_3/\text{MeOH}$ pH 10.7 and $\text{H}_2\text{O}/\text{NH}_4\text{CO}_3$ pH 10.7 for 20 minutes run at 1.2 mL/min.

Table 2.2: Yields and percentage purity for compounds **56a-i**

Compound (X)	% Yield	% Purity
(a) CH ₃	50	>98
(b) CF ₃	40	>98
(e) CN	30	>98
(f) H	55	>98
(h) NO ₂	20	>98
(i) OCH ₃	40	>98

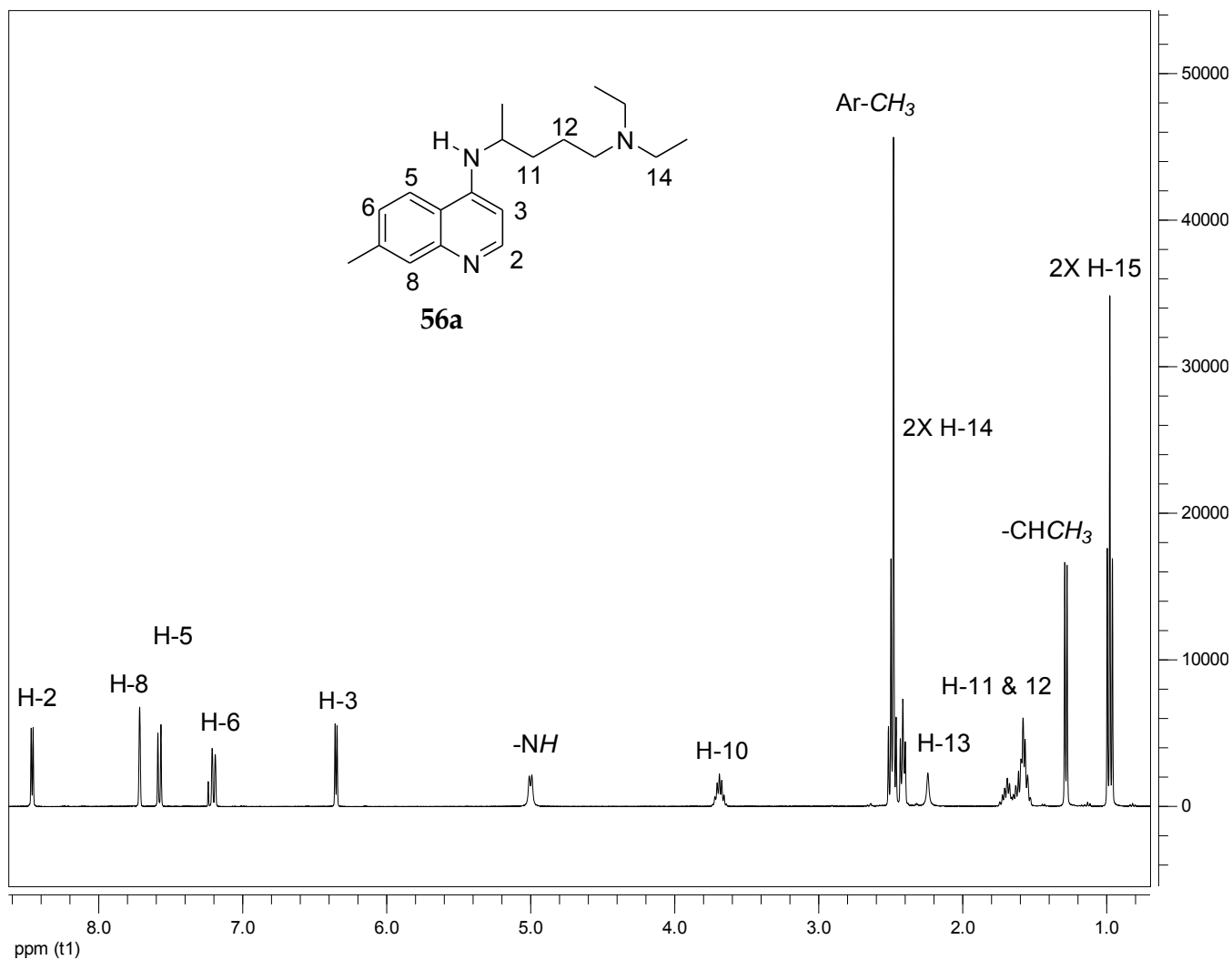
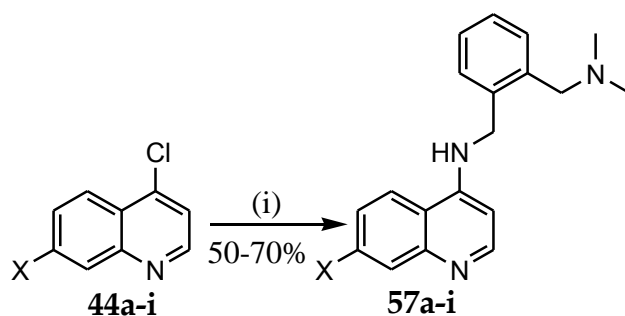


Figure 2.17: ¹H NMR spectrum of compound **56a** in CDCl₃

2.5.2 Synthesis of 4-(2-(dimethylaminomethyl) benzylamine)-7-X-quinolines (PQ derivatives)

This series of compounds was synthesised by nucleophilic substitution of the chloro group at position 4 by 2-(dimethylaminomethyl) benzylamine (**53**). The synthesis required the use of two bases, triethylamine (Et₃N) and potassium carbonate (K₂CO₃). The need for both bases is not clear but is known to improve the yields for this class of compounds.

The synthesis method used for this series of compounds has been previously reported. [2.13] Microwave assisted synthesis was also investigated for compound **57b** in an effort to reduce reaction times and eventually improve yields. Reaction time was set to 20 minutes at 150 °C, with microwave power set at 300 W. The results after several attempts using different conditions did not surpass the conventional yield of 40%. The lack of encouraging results from the first attempt on the -CF₃ substituted quinoline steered all other reactions towards conventional heating.



X=(a) CH₃; (b) CF₃; (c) Cl; (e) CN; (f) H; (h) NO₂; (i) OCH₃

Scheme 2.14: Reagents and conditions: (i) 2-(Dimethylaminomethyl) benzylamine, K₂CO₃, Et₃N, NMP, 112 °C, 15 hr.

Compounds **44a-i** and 2-(dimethylaminomethyl) benzylamine (**53**) (phenylequine side chain) were refluxed for 15 hours under an inert atmosphere (Scheme 2.14). Compounds **57a-i** were initially purified by silica gel chromatography and further purified using preparative HPLC to achieve greater than 98% purity. Characterisation by ¹H NMR, ¹³C NMR (Fig. 2.18) and high resolution mass spectrometry confirmed the compounds. The typical ¹H NMR spectrum for this class of compounds show H-2 and H-3 appearing as AB doublets with a coupling constant of 5.4 Hz. H-6 couples with H-5 and H-8 giving rise to a *dd* with coupling constants of 9.0 Hz and 2.2 Hz respectively. H-9 appeared as a doublet with a coupling constant of 4.9 Hz as a result of coupling to the -NH proton, itself

appearing as a broad singlet. The chemical shifts of the aromatic hydrogen atoms of the 2-(dimethylaminomethyl) benzylamine side chain appeared as multiplets in the aromatic region of the spectra. H-13 and H-14 on the other hand appeared as singlets at 3.50 ppm and 2.28 ppm respectively. The carbon spectra, in particular that of 7-trifluoromethyl substituted compound (**57b**) (Fig. 2.18) had distinctive quartets for C-7 (δ 128.19 - 131.15) and $-\text{CF}_3$ (δ 120.07 - 125.50). This is characteristic of fluorine splitting resulting in coupling constants above 250 Hz. The number of carbon atoms differed only with respect to the substituent at the 7-position of the quinoline ring.

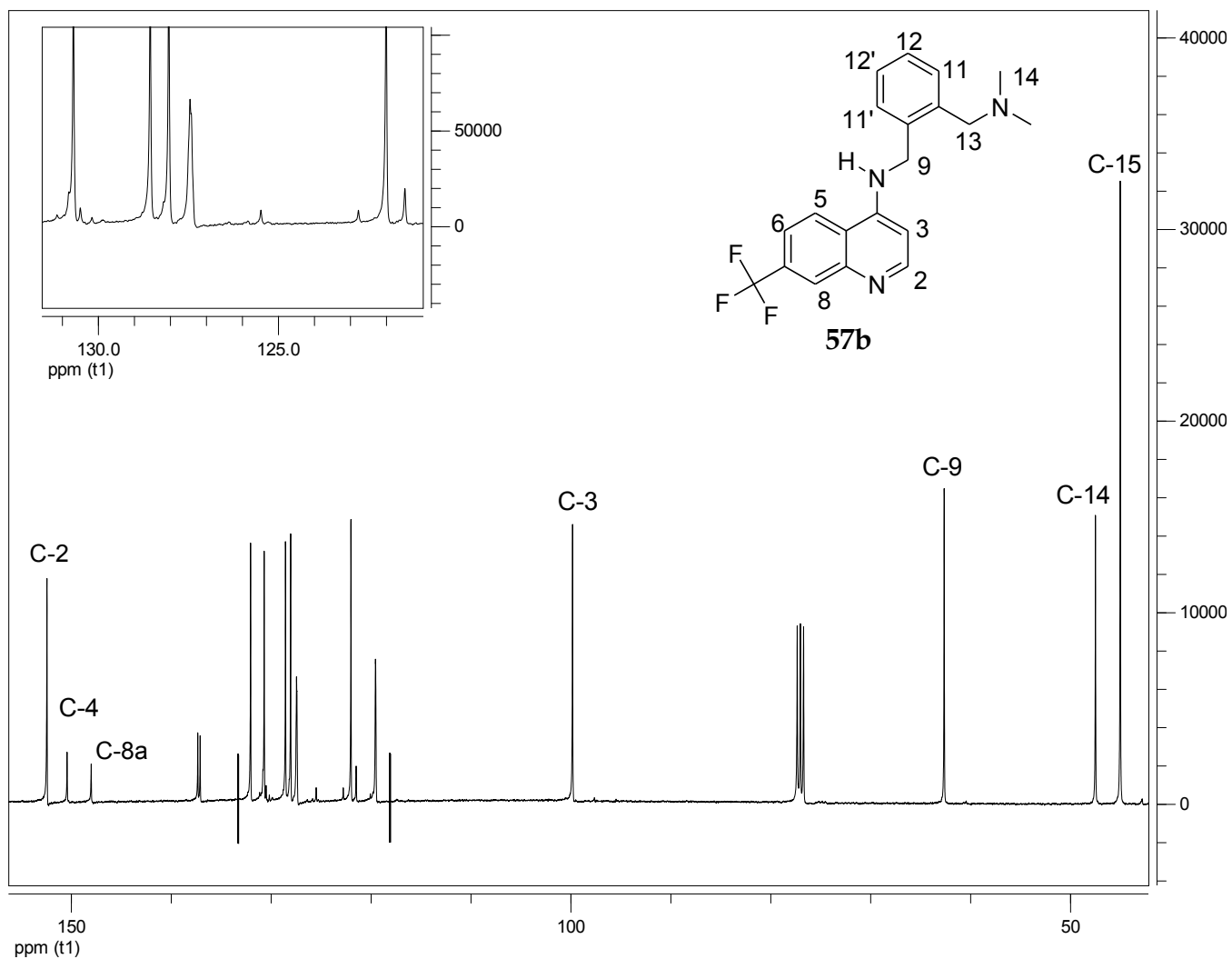


Figure 2.18a: ^{13}C NMR spectrum of (7-Trifluoro-quinolin-4-yl)-(2-dimethylaminomethyl benzylamine) (**57b**) in CDCl_3 . The insert is further expanded in Fig 2.18b to highlight the effect of splitting due to fluorine (F) in the molecule

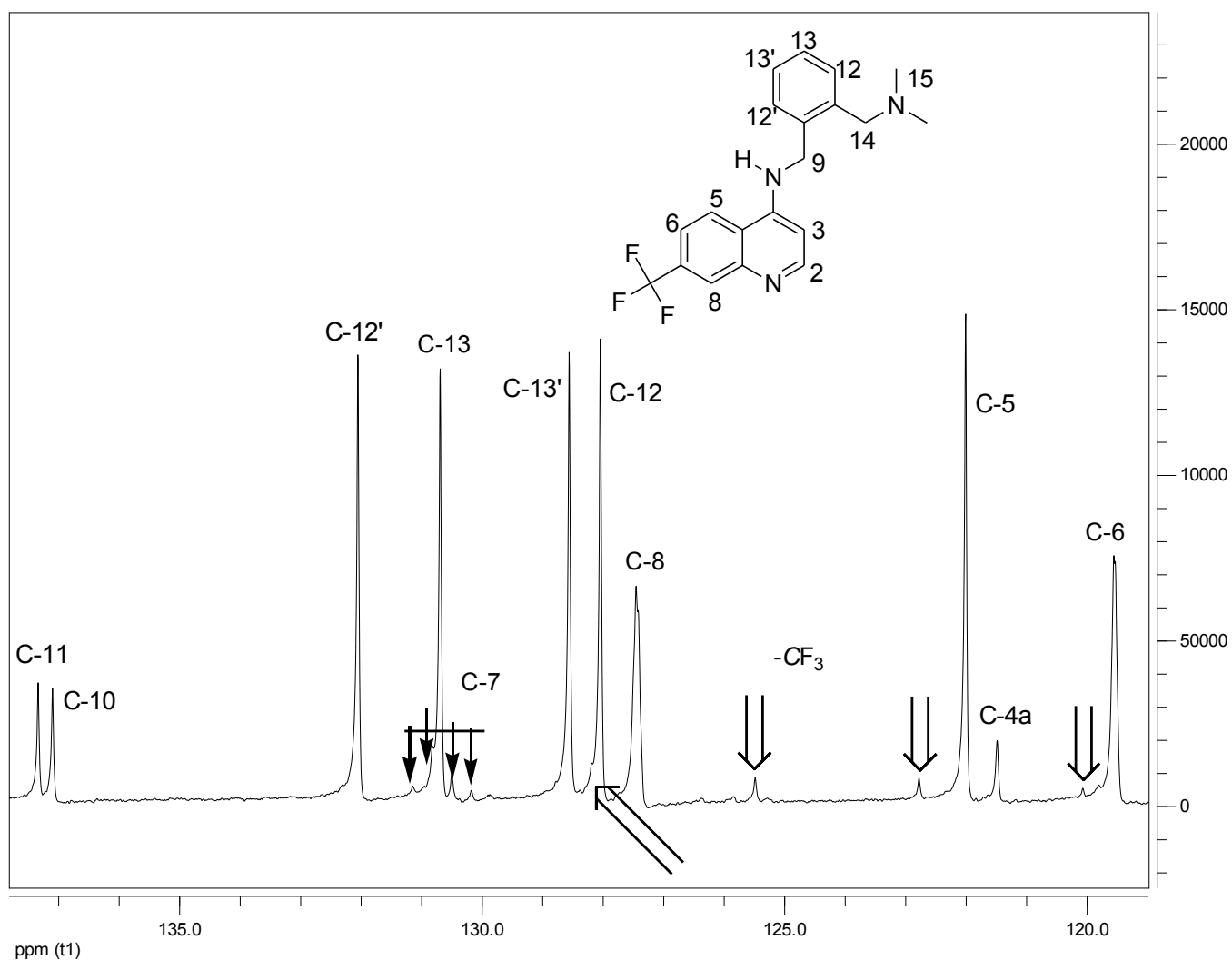
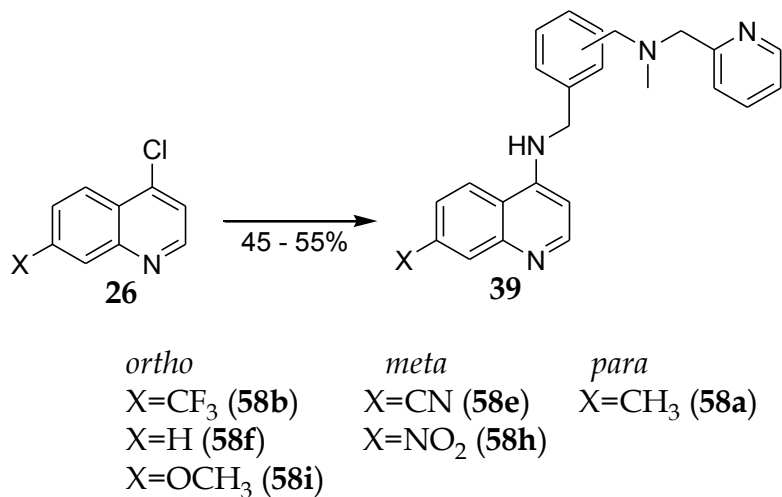


Figure 2.18b: ^{13}C NMR spectrum of (7-trifluoro-quinolin-4-yl)-(2-dimethylaminomethyl benzylamine) (**57b**) in CDCl_3 . The solid and wide arrows show a quartet for C-7 (δ 128.19 – 131.15) and $-\text{CF}_3$ (δ 120.07 – 125.50) respectively. They both have coupling constants above 250 Hz, characteristic for F splitting.

2.5.3 Synthesis of pyridodibemequine (PDB) analogues

The analogues in the PDB series (**58**) were synthesised as described above (Section 2.4.2). The PDB lateral chains were kindly donated by Dr. Mukesh Joshi in Professor T. J. Egan's laboratory. The target compounds were generally obtained with low yields as oils.

Purification was done by preparative HPLC to obtain all compounds with purity greater than 98%. As shown in Scheme 2.15 the series of compounds had comparable reaction times to the phenylequine derivatives.



Scheme 2.15: Reagents and conditions: K₂CO₃, Et₃N, NMP, 112 °C, 15 hr

The compounds were characterised by high resolution mass spectroscopy as well as ¹H and ¹³C NMR. The typical ¹H NMR of the compounds (Fig. 2.19) relative to the phenylequine derivatives above, differ by the inclusion of H-16 methylene protons as a singlet at δ 3.75 ppm. Singlet at δ 2.19 ppm represents H-15 methyl protons and there are 4 protons in the aromatic region due to H-19 to H-22. The rest of the chemical shifts of the proton and carbon NMR are as described above.

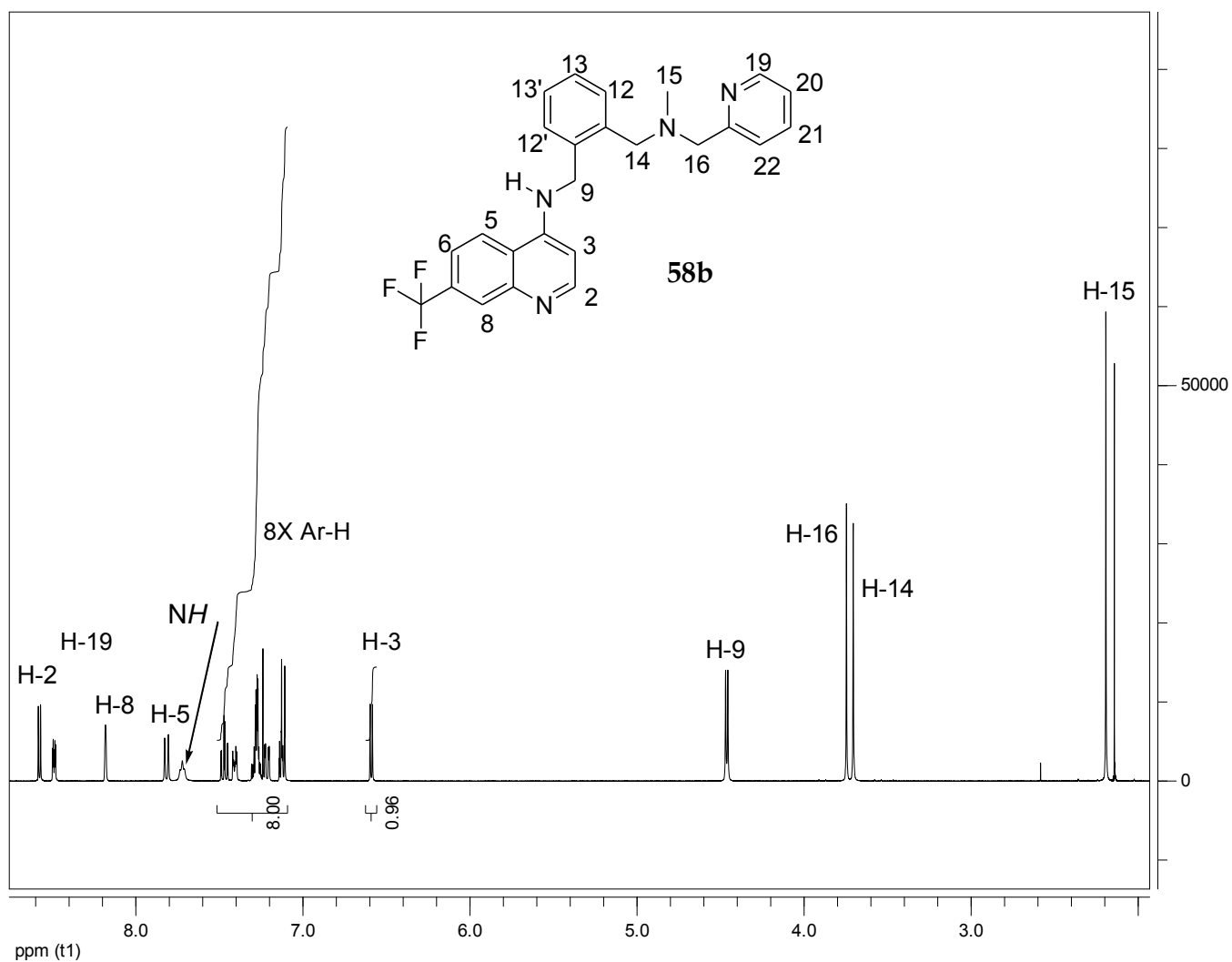


Figure 2.19: ¹H NMR spectrum of *N*-(2-((methyl(pyridine-2-yl-methyl)amino)benzyl)-7-(trifluoromethyl)quinoline-4-amine (**58b**) in CDCl₃.

2.6 Conclusion

The target set of compounds were synthesised, purified and characterised. Purity checks were performed by HPLC and where purity was less than 98%, preparative HPLC was carried out. The chemistry involved in the synthesis of these compounds followed a similar route. This involved the aromatic nucleophilic substitution of the chloro group at the 4-position by either -NH₂, methyl amine (NHCH₃) or ethylene diamine

($\text{NH}_2\text{CH}_2\text{CH}_2\text{NH}_2$). Synthesis of the CQ, PQ and PDB derivatives also followed a similar pattern apart from the addition of triethylamine and potassium carbonate (K_2CO_3). Yields, particularly for PDB were generally low (~50%).

2.7 References

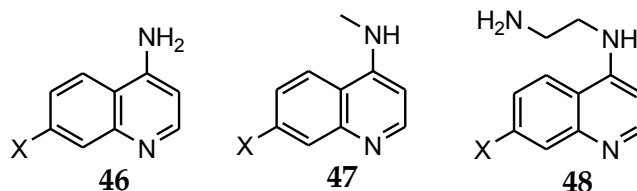
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3. STRUCTURE ACTIVITY RELATIONSHIPS OF SHORT CHAIN 4-AMINO-7-X-QUINOLINE MOLECULES

3.1 Background

The characteristics of the group at the 7-position of the 4-amino quinoline have been subject to investigation in various studies. [3.1 - 3.5] The studies focused on replacing the 7-chloro group to investigate whether the analogues retained antiparasmodial activity. They found analogues containing -NO₂, -Br and -I were active while those containing -CF₃, -F and -OCH₃ were found to be less active against the chloroquine sensitive (D10) strain of the malaria parasite. [3.3, 3.4] The poor β -haematin inhibitors (-OH and -NH₂ as the groups at the 7-position of the quinoline ring) were not found to have antiparasmodial activity against the malaria parasite. [3.3] In a similar study, Hwang *et al* found that substituting the 7-chloro group with a more hydrophobic group decreased antiparasmodial activity. [3.5] The studies employed short chain (2 - 4 carbon length) analogues as they had found that these were as active as their corresponding 7-chloro analogues. [3.4] In this study the identity of the group at the 7-position of the quinoline ring was investigated in relation to haem binding and β -haematin inhibition. In addition, the effect of the lateral chain on the extent of haem binding and β -haematin inhibition were investigated. Ray *et al* found that alteration of the lateral chain is important for antiparasmodial activity and that a range of chemical substitutions are tolerated at this position. [3.6] In this regard, a series of 4-amino-7-X-quinoline molecules were synthesised (Fig. 3.1). The X-substituents were chosen from a lipophilicity constant (π) versus Hammett constant (σ) Craig Plot (Fig. 3.1). A fragment approach was used starting with 4-amino-7-X-quinoline and then building up the lateral chain with methyl and ethylene diamine groups. The use of the Craig Plot was primarily to select substituents with diverse physicochemical properties. It has been reported that basic and lipophilic,

among other properties, compounds are more likely to fail as drug candidates because of their greater tendencies to interact with host biological systems, for example hERG and CYP450 enzymes. [3.7] Therefore, a substituent that is hydrophilic and still retains strong haem binding and is a good inhibitor of β -haematin formation would be potentially attractive in the development of a possibly less toxic antimalarial.



X = (a) CH₃; (b) CF₃; (c) Cl; (d) CONH₂; (e) CN (f) H; (g) NH₂; (h) NO₂ (i) OCH₃ and (j) OH

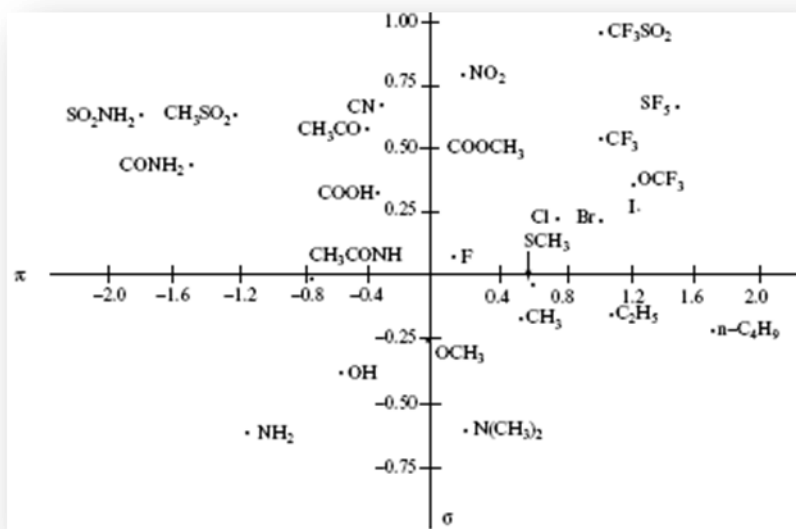


Figure 3.1: The 4-amino-7-X-quinoline compounds and a Craig Plot in which the Hammett constant (σ) is plotted against the lipophilicity constant (π)

3.2 EXPERIMENTAL METHODS

3.2.1 Haem binding constants

The binding constants between haematin and all the 4-amino-7-X-quinoline (**46**), 4-methylamino-7-X-quinoline (**47**) and 4-ethylene diamine-7-X-quinoline (**48**) compounds (short chain compounds), were determined by spectrophotometric titration according to literature methods. [3.1, 3.2] Bovine haemin (3.5 mg) was dissolved in dimethyl sulfoxide (DMSO) (5 mL) to obtain a stock solution (this was freshly prepared every day). A blank and a haematin solution were prepared in 2 mL cuvettes. The blank solution contained DMSO (0.80 mL), HEPES (0.20 mL) and water (1 mL), while the haematin solution had similar volumes with the addition of haemin solution (5 μ L) and DMSO (0.795 mL). This solvent system, containing 40% aq DMSO pH 7.4, has been found to control both the protonation state of haematin and drugs while maintaining haematin in a monomeric state. [3.8] It has also been shown in recent studies that at higher pH (pH 10) the solution forms μ -oxo dimer while aqueous media forms π - π dimer. [3.9] The two mixtures were titrated with a 2.0 mM solution of each compound at 25°C by monitoring the absorbance of the Soret band (402 nm). Blank measurements were performed simultaneously at the same wavelength to correct for the absorbance of the compounds. The data were fitted to the Equation 3.1 for a 1:1 complexation model using nonlinear least squares fitting, strictly following the procedure of Egan *et al.* [3.2] A_0 is the absorbance of haemin before addition of the drug, A_∞ is the absorbance for the drug-haematin adducts at saturation, A is the absorbance at each point of the titration, and K is the conditional association constant.

$$A = (A_0 + A_\infty \times K[C]) / (1 + K[C]) \quad \text{Equation 3.1}$$

3.2.2 Inhibition of β -haematin formation

The experiments were carried out in 96 well plates and done in triplicate using the method described by Ncokazi *et al.* [3.10] For each drug solution (0.168 M), 20 μ L were added to the first row of three wells and 10 μ L of solvent (DMSO) was added to the remainder of the wells. Serial dilution was carried out from the top to bottom of the plate by taking 10 μ L of the drug solution and mixing into the second well. This step was repeated till the second last well, at which point the remaining 10 μ L were discarded. Concentrations of the drug correspond to 10 - 0 (top to bottom of the plate) equivalents relative to haematin in the final mixture.

Haemin (27.39 mg) was dissolved in a 0.1 M solution of NaOH (25 mL) and mixed for about 5 minutes. 101 μ L of this solution was added to all wells. 10 μ L of 1.0 M HCl was then added to all wells and gently sonicated. 58.7 μ L of pre-incubated 9.7 M acetate buffer (60 °C for an hour) was added to all wells and gently shaken to mix the solution. The plate was then incubated at 60 °C for an hour in a water bath.

Following incubation, the reaction was stopped using 80 μ L of 30% pyridine solution, made up of 30% (v/v) pyridine and 10% (v/v) 0.02 M HEPES pH 7.5 in water. The solution was left to stand overnight to allow any solid material to settle. 38 μ L of the supernatant was aspirated to a new plate and 212 μ L of 30% pyridine solution added to each well. The plate was shaken gently to mix the solution and absorbances were read in a Thermo MultiSkan Spectrum plate reader at 405 nm. The values were fitted into a dose response curve using GraphPad Prism 3.0 to determine the number of drug equivalents required to inhibit formation of β -haematin by 50% (BHIA₅₀). [3.8] BHIA₅₀ values are reported as an average of three figures with standard error of the mean (SEM).

3.3 RESULTS

Haem binding and inhibition of β -haematin formation results for the 4-amino- (series 46), 4-amino methyl- (series 47) and 4-ethylene diamine-7-substituted (series 48) quinolines were obtained as described above.

3.3.1 Physicochemical results for 4-amino-7-X-quinoline molecules

In determining the haem binding constants, titrations for all series of compounds were done in triplicate and the standard error of the mean (SEM) calculated. Spectra obtained upon addition of compound were recorded. Examples are shown in Figure 3.2 (A) - (C). In a recent study Kuter *et al* found that changes in Fe(III)PPIX spectrum can be grouped into three categories: (i) where compounds such as pyridine and imidazole cause major band shifts typical of the formation of a low spin complex, (ii) *N*-donor ligands such as butylamine cause only slight changes in spectrum with either increase or decrease in intensity of the bands and (iii) compounds that are not able to coordinate to the Fe(III) centre for steric reasons and those that interact with PPIX cause hypochromism without any other changes in the spectrum, characteristic of π -stacking interactions. [3.11]

Figure 3.2 (A) shows the profile of 4-aminoquinoline-7-carbonitrile (46e), an example of a moderately strong haem binder (Table 3.1). The initial spectrum represents the absorbance of haem without the compound in the solution. The subsequent spectra represent the absorbance of haem after the compound has bound to a certain amount. The final intensities depend on the absorbance of the resulting complex; Figure 3.2 (A) is an example where the change from the initial spectrum is small.

Figure 3.2 (B) shows an example where the change in absorbance is large. Here, the intensities show significantly reduced absorbance of haem in the complex. Figure 3.2

(C) shows an example where the absorbance of haem greatly decreases in the quinoline complex.

The data from the titrations was corrected for dilution and was used to plot absorbance versus concentration curves (Fig. 3.3) which were fitted to a 1:1 association model to obtain binding constants (Tables 3.1, 3.2 and 3.3). Where the 1:1 association model did not produce a satisfactory curve, a 2:1 (ligand to haem) model was fitted using **Equation 3.2**. [3.2] The experiments were done in triplicate and therefore on calculation, the haem binding constants were reported as an average of the three experiments with a standard error of the mean (SEM).

$$A = A_0f_M + A_1f_{MQ} + A_2f_{M_2Q} \quad \text{Equation 3.2}$$

Where $f_M = M/[M]_T$; $f_{MQ} = MQ/[M]_T$ and $f_{M_2Q} = ([M]_T - M - MQ)/2[M]_T$

$$MQ = \left\{ - (1 + K_1[Q]_{\text{free}}) + \left[(1 + K_1[Q]_{\text{free}})^2 + 8K_1K_2[Q]_{\text{free}}[M]_T \right]^{1/2} / 4K_2 \right\}$$

$$M = \left\{ - (1 + K_1[Q]_{\text{free}}) + \left[(1 + K_1[Q]_{\text{free}})^2 + 8K_1K_2[Q]_{\text{free}}[M]_T \right]^{1/2} / 4K_1K_2[Q]_{\text{free}} \right\} \quad [3.2]$$

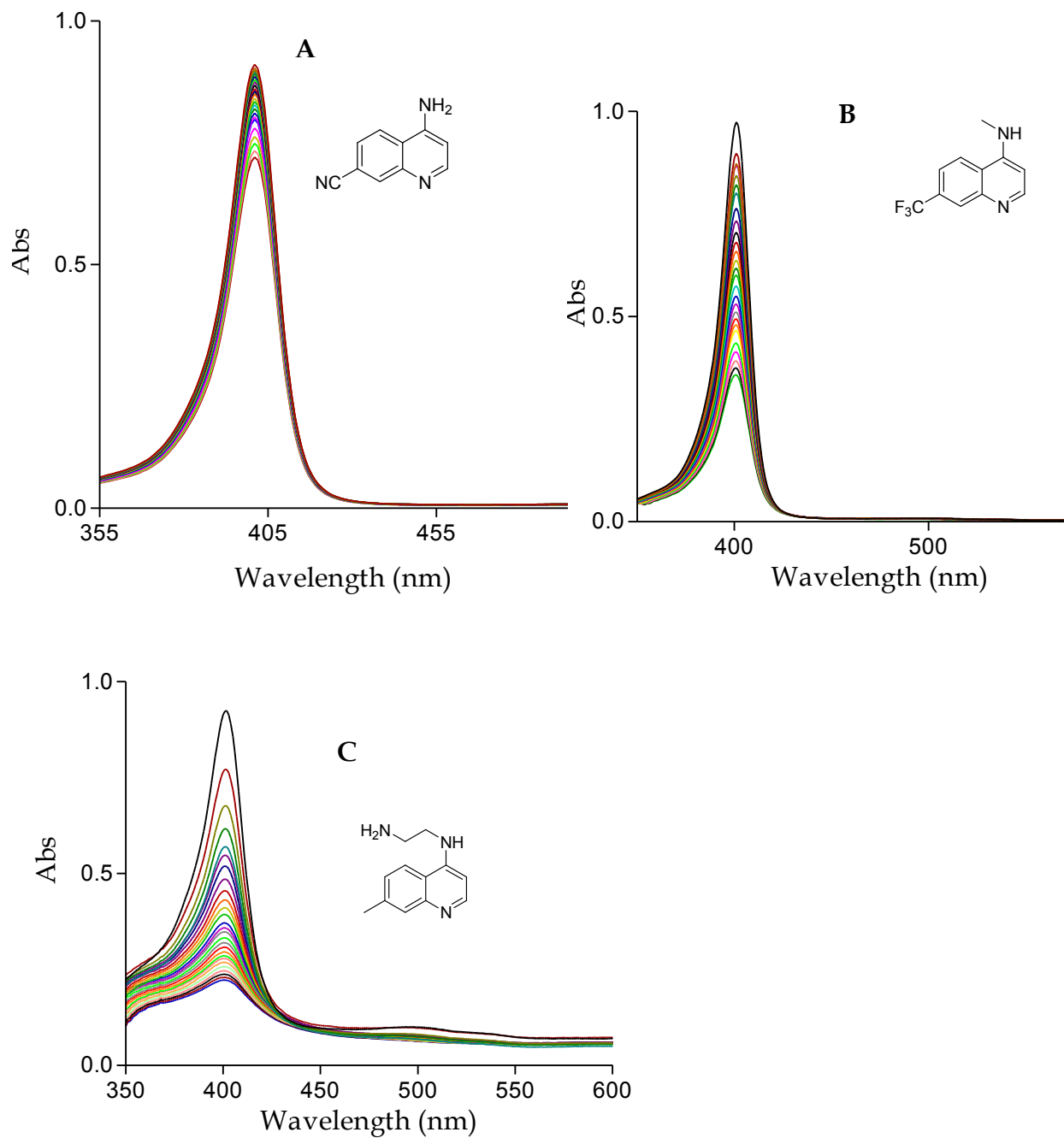


Figure 3.2: Spectrophotometric titrations for; **46e** (A), **47b** (B) and **48a** (C) done in 40% aqueous DMSO, pH 7.4 and temperature maintained at 25°C. Final concentration of haem is 2.7×10^{-6} M. The spectra are corrected for dilution.

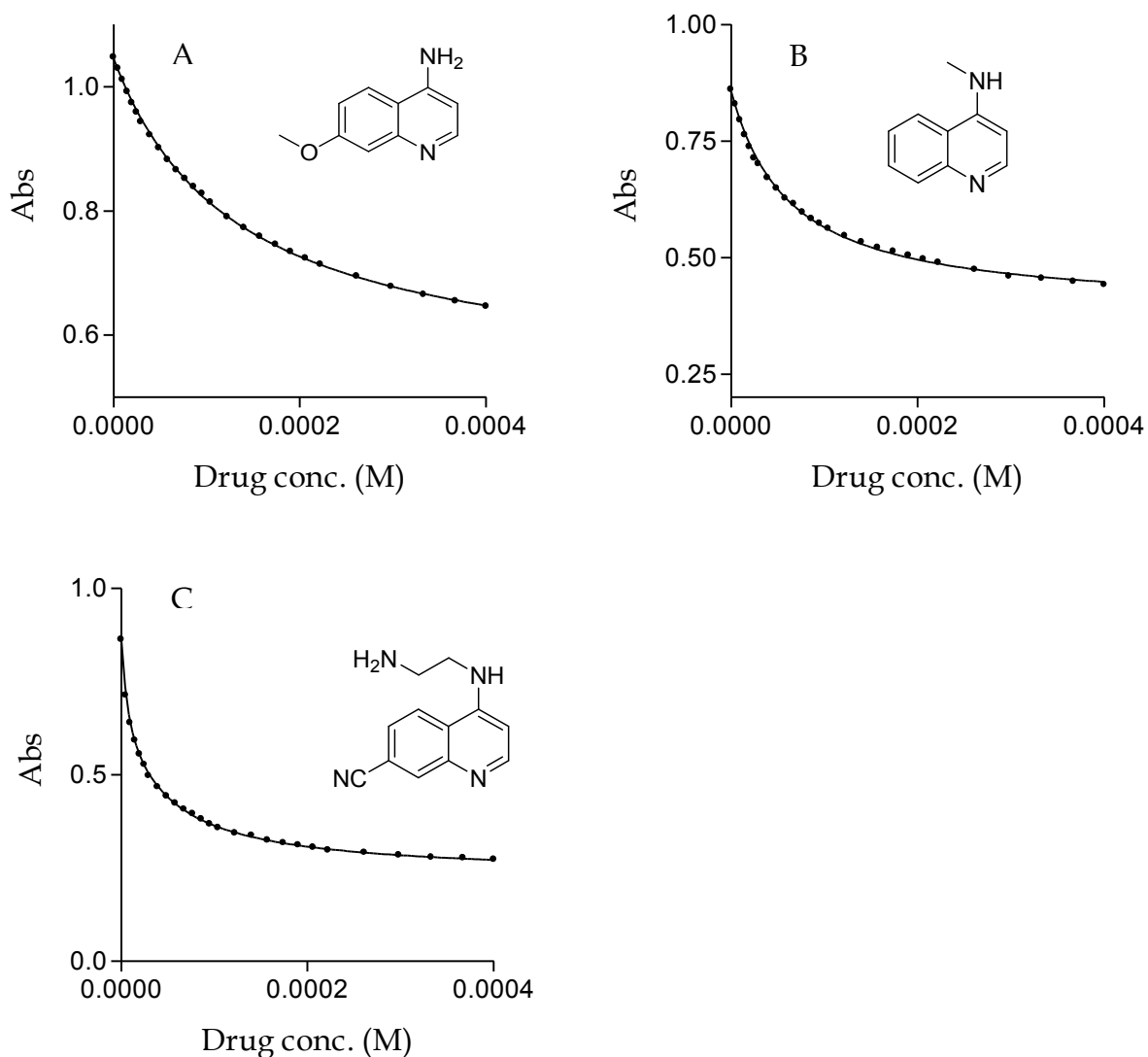


Figure 3.3: Typical plots of experimental data obtained from spectrophotometric titrations of haematin with the target molecules, fitted to a 1:1 model. The compounds selected are: (A) **47f**, (B) **46i** and (C) **48e**.

Values for the inhibition of β -haematin formation (BHIA₅₀) were obtained using the method outlined above of Egan *et al.* [3.2] BHIA₅₀ and Log *K* values for series **46**, **47** and **48** are listed in Tables 3.1, 3.2 and 3.3 respectively. Each value is reported as an average

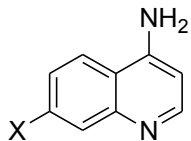
of three experiments with a standard error of the mean (SEM). The compounds that exceeded the experimental limit (>20) were considered non-inhibitors; these were **46g**, **46j**, **47g** and **47j**.

Log K values across all tables reflected a similar pattern where compounds containing electron withdrawing compounds, with the exception of **46d** and **47d** ($-\text{CONH}_2$), were found to be stronger haem binders than compounds with electron releasing groups.

BHIA₅₀ for compounds with electron releasing groups, where $X = -\text{CH}_3$, $-\text{NH}_2$, $-\text{OCH}_3$ and $-\text{OH}$ were found to be either poor or non-inhibitors of β -haematin formation. Compound **47e**, electron withdrawing and less lipophilic, has the best value (4.5 ± 0.3) for β -haematin inhibition.

Table 3.3 shows a list of inhibition of β -haematin formation (BHIA₅₀) and Log K values for the ethylene diamine-7- X -substituted quinoline molecules. Log K and β -haematin values follow the trend where compounds with electron withdrawing groups (**48b**, **48c**, **48e** and **48h**) show better β -haematin inhibition activity relative to compounds with electron releasing groups (**48a**, **48i** and **48j**).

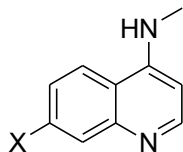
Table 3.1: Haem binding constants (Log *K*) and inhibition of β -haematin formation (BHIA₅₀) of 4-amino-7-*X*-quinoline analogues



Compound (<i>X</i>)	Log <i>K</i>	β -Haematin inhibition (BHIA ₅₀ ^a)	
(46a)	CH ₃	4.17 ± 0.03	10.8 ± 0.6
(46b)	CF ₃	4.41 ± 0.01	5.6 ± 0.7
(46c)	Cl	5.01 ± 0.16	5.8 ± 0.9
(46d)	CONH ₂	3.76 ± 0.12	10.3 ± 0.4
(46e)	CN	4.32 ± 0.28	6.1 ± 0.9
(46f)	H	3.83 ± 0.02	10.0 ± 0.3
(46g)	NH ₂	3.44 ± 0.20	No inhibition
(46h)	NO ₂	4.57 ± 0.11	5.7 ± 0.6
(46i)	OCH ₃	4.24 ± 0.02	15.6 ± 0.5
(46j)	OH	3.53 ± 0.15	No inhibition

^a Equivalents of compound relative to haematin

Table 3.2: 4-Amino methyl-7-X-quinoline molecules binding constants (Log *K*) and inhibition of β -haematin formation (BHIA₅₀) values



Compound (X)	Log <i>K</i>	β -Haematin inhibition (BHIA ₅₀ ^a)
(47a) CH ₃	4.01 ± 0.01	11.1 ± 0.9
(47b) CF ₃	4.18 ± 0.02	5.0 ± 0.4
(47c) Cl	4.62 ± 0.03	5.7 ± 0.5
(47d) CONH ₂	3.43 ± 0.07	10.1 ± 0.5
(47e) CN	4.42 ± 0.03	4.5 ± 0.3
(47f) H	3.92 ± 0.01	14.9 ± 0.3
(47g) NH ₂	4.04 ± 0.01	No inhibition
(47h) NO ₂	4.32 ± 0.08	5.5 ± 0.3
(47i) OCH ₃	3.71 ± 0.03	10.4 ± 0.3
(47j) OH	3.84 ± 0.02	No inhibition

^a Equivalents of compound relative to haematin

Table 3.3: 4-Amino ethylene diamine-7-X-quinoline haem binding and inhibition of β -haematin formation (BHIA₅₀) values

Compound (X)	Log K	β -Haematin inhibition (BHIA ₅₀ ^a)	
(48a)	CH ₃	4.35 ± 0.01	10.24 ± 0.25
(48b)	CF ₃	4.70 ± 0.01	5.35 ± 0.22
(48c)	Cl	5.10 ± 0.01	5.44 ± 0.52
(48e)	CN	4.47 ± 0.03	6.29 ± 0.84
(48f)	H	4.38 ± 0.03	10.56 ± 0.36
(48h)	NO ₂	4.48 ± 0.07	5.29 ± 0.35
(48i)	OCH ₃	4.47 ± 0.01	10.64 ± 0.29
(48j)	OH	4.69 ± 0.02	17.83 ± 0.01

^a Equivalents of compound relative to haematin

3.4 DISCUSSION

The fragment-based approach (*Chapter 1, Section 1.9.1, page 31*) used in this study was designed to elucidate the structure activity relationships of the selected series of molecules. This investigation also clarified, where possible, the relationship between haem binding and inhibition of β -haematin formation. The approach adopted in this study was to establish both the effect of the individual substituents at the 7-position of the quinoline ring as well as the contribution of the lateral chain in the relationship

between the two mentioned physicochemical effects. To this end, the undecorated 4-amino quinoline derivatives, 4-amino-7-X-quinoline, were used as the starting point. The side chain on the 4-amino group was then gradually built up to observe its effect, starting with a -CH₃ group and then proceeding to an ethane-1,2-diamino group. The binding constants and the inhibition of β -haematin formation values were correlated. Two parameters, π and σ , using substituents selected from a Craig Plot (Fig. 3.1) were individually correlated to the Log K and Log BHIA₅₀ of the short chain series of molecules. Statistical correlations were performed on EXCEL and GraphPad Prism 3.0 computer programmes. The use of the simple 4-amino-7-X-quinoline molecules was also used to confirm or discount the quinoline ring as the primary component for the inhibition of β -haematin formation as previously suggested. [3.1, 3.2] In their study, Kaschula *et al* highlighted the importance of pH trapping in aminoquinoline antimalarials and subsequently that of pK_a. [3.1] The study suggests the pK_as of 4-aminoquinolines are generally high and therefore in the conditions for determining haem binding and inhibition of β -haematin formation (pH 7.4 and 5) the compounds are likely to be fully protonated.

3.4.1 Structure activity relationships

3.3.1.1 The haem binding properties of 4-amino-7-X-quinolines

The binding constants seem to follow a pattern according to the position of the substituent in the Craig Plot. Compounds **46b**, **46c** and **46h** all of which are in the top right hand quadrant (high lipophilicity and electron withdrawing) have binding constants within a similar range (within one Log unit), 4.17 ± 0.03 , 5.01 ± 0.16 and 4.57 ± 0.11 respectively. Compound **46h** which contains the most electron withdrawing substituent (-NO₂) in that quadrant has the second strongest binding constant (Log K 4.57 ± 0.11) after 4-amino-7-chloroquinoline (**46c**) (Log K 5.01 ± 0.16). Compound **46h** is also less lipophilic than the other compounds in the quadrant.

Compound **46e**, which has the most electron withdrawing hydrophilic substituent (-CN,) (top left hand quadrant of the Craig Plot) showed good affinity to haem (Log K 4.32 ± 0.28). The strongly electron withdrawing -CN group has a binding and Hammett constants similar to that of the -NO₂ compound, though -CN is somewhat more hydrophilic. In the same quadrant, but with a rather lower haem binding constant, is **46d** with a value of 3.76 ± 0.12 . This compound is much more hydrophilic than **46e**. 4,7-Diamino quinoline (**46g**) and 4-aminoquinolin-7-ol (**46j**) in the bottom left quadrant of the Craig Plot, have the lowest haem binding constants, Log K 3.44 ± 0.20 and 3.53 ± 0.15 respectively. 4-Amino-7-methylquinoline (**46a**) in the bottom right quadrant of the Craig Plot also shows weak binding to haem (Log K 4.17 ± 0.03). The substituents (-CH₃, -NH₂ and -OH) are electron releasing and their low affinity to haem is consistent with previous studies that predicted that the group at the 7-position of the quinoline ring should be moderately strong electron withdrawing to bind strongly to haem. [3.1] Quinoline-4-amine (**46f**), the unsubstituted analogue, in the centre of the Plot, has an average binding constant 3.83 ± 0.02 .

Log K values were correlated with the lipophilicity constant (π) and Hammett constant (σ), the two parameters used in the Craig Plot (Fig. 3.1). There was no correlation between this series (4-amino-7-X-quinoline) of compounds and the Hammett constant. However, a significant correlation was observed against the lipophilicity constant (Fig. 3.4). Calculated Log K conformed to Equation 3.3:

$$\text{Log } K = 0.44\pi + 4.22 \qquad \text{Equation 3.3}$$

The graph has an r^2 value of 0.50 and P value of 0.02. This correlation suggests that for this series of molecules, lipophilicity, rather than electron withdrawing properties, of the compounds is the prominent feature in haem binding.

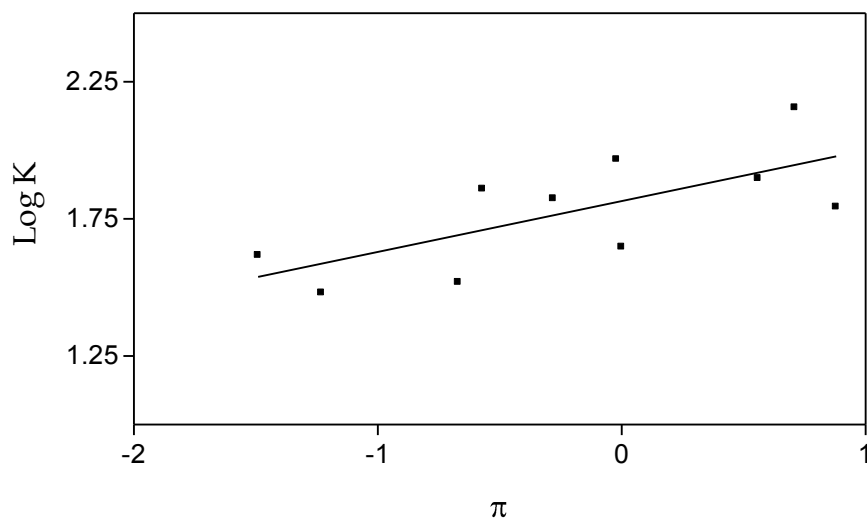


Figure 3.4: Correlation between lipophilicity constant and Log K for the 4-amino-7-X-quinoline series of molecules

The 4-amino-7-X-quinoline series of compounds provide the simplest 4-amino quinoline analogues to investigate the contribution of the 7-position substituents without the confounding effects of the lateral chain. The 4-amino group is not thought to be involved in the strength of binding and therefore the contribution of the 7-substituent may be isolated. In this class of molecules replacement of the -Cl group at the 7-position of the quinoline ring did not decrease the binding constants for all cases as greatly as in the case of previously investigated analogues. [3.1] There was a relatively small decrease in binding constants for the electron withdrawing compounds in the same quadrant as the -Cl substituted compounds, namely the -CF₃ and -NO₂ substituted compounds. The electron donating substituents (-OH and -NH₂) differ with lower binding constants, to a similar extent.

3.4.1.2 Inhibition of β -haematin formation by 4-amino-7-X-quinoline analogues

Inhibition of β -haematin formation is strongest for 4-amino-7-trifluoromethylquinoline (**46b**), 4-amino-7-chloroquinoline (**46c**) and 4-amino-7-nitroquinoline (**46h**). These compounds, with BHIA₅₀ values of 5.6 ± 0.7 , 5.8 ± 0.9 and 5.7 ± 0.6 respectively, are all in the top right hand quadrant (electron withdrawing and lipophilic) of the Craig Plot. The compound 4-aminoquinoline-7-carbonitrile (**46e**) in the left hand quadrant (electron withdrawing and hydrophilic) is roughly as active (6.1 ± 0.9) as the more lipophilic compounds. On the other hand, 4-aminoquinoline-7-carboxamide (**46d**) in the same quadrant is much less active. The molecules in the bottom half of the Craig Plot behave as predicted, exhibiting either weak or no inhibition. Compounds **46g** and **46j** are classed as non inhibitors as their BHIA_{50s} exceeded the experimental limit (20 equivalents).

There were no correlations observed between β -haematin inhibition and either lipophilicity constant or Hammett constants alone or in combination. This suggests there are additional factors contributing to the inhibition of β -haematin formation.

3.4.1.3 Haem binding and the 4-amino-methyl-7-X-quinoline molecules

The haem binding constants were obtained in the same way as mentioned above. The results are shown in Table 3.2 for the 4-amino-methyl-7-X-quinoline compounds. In this series of molecules, an amino methyl group is attached to the 4-position of the quinoline ring thereby extending the length of the lateral chain by one carbon atom. Maintaining all other positions constant, any changes in the activity of the molecule would be attributed to this change and therefore the contribution of the lateral chain can be evaluated.

The changes in haem binding from the 4-amino-7-X-quinoline (series **46**) to the 4-amino methyl-7-X-quinoline (series **47**) molecules show no particular trend. The molecules with

electron withdrawing substituents generally had better binding constants than those with electron releasing ones (negative Hammett constants) in series **47** molecules. The electron releasing substituents falling in the bottom two quadrants of the Craig Plot suggest electron releasing properties are not suitable characteristics for haem binding as suggested by Egan *et al.* [3.12] One possibility for this is that, generally, the quinoline family of drugs are thought to exert their antimalarial activity by forming drug: haem complexes mediated by π - π interactions. [3.13] Electron releasing groups would make the quinoline ring electron rich which might disrupt factors such as the distribution of electron density necessary for forming an active π - π complex. [3.13]

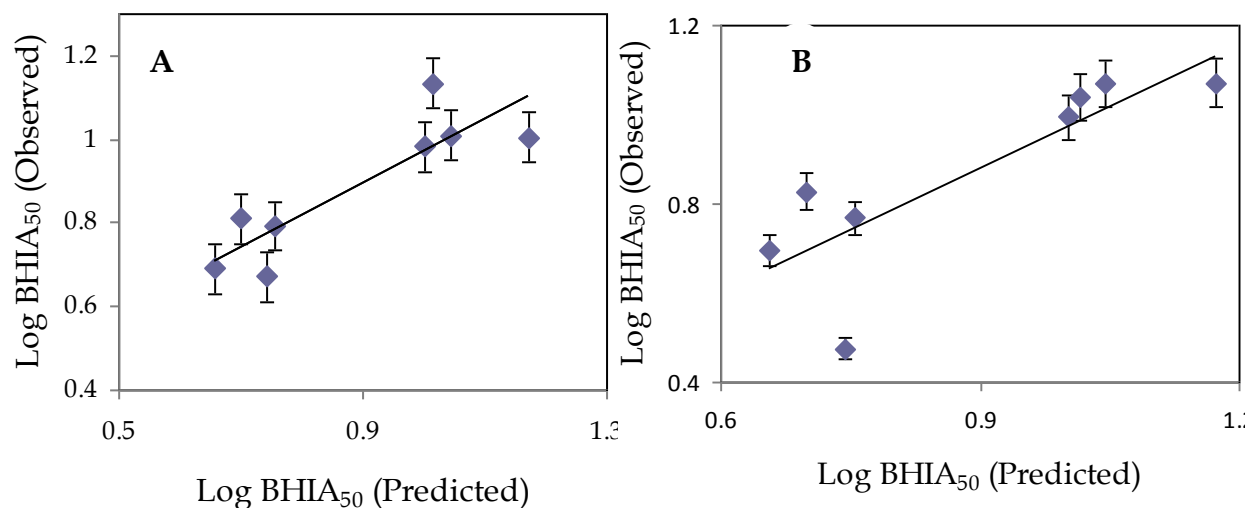
3.4.1.4 Inhibition of β -haematin formation and the 4-amino methyl-7-X-quinoline molecules

The inhibition of β -haematin formation experiments were carried out in triplicate as described above. Molecules with substituents in the top right hand quadrant of the Craig Plot (Fig. 3.1) are more active than molecules with substituents in any other quadrant for inhibition of β -haematin formation. The compounds with electron releasing substituents (bottom half of the Craig Plot) remain poorly active. Again, the electron releasing properties of a substituent at the 7-position of the quinoline ring does not appear to be favoured for the inhibition of β -haematin formation and haem binding for these types of molecules, consistent with findings from previous studies. [3.1, 3.12]

The difference between series **46** BHIA₅₀ values and those of series **47** show no obvious pattern with the addition of a methyl group onto the core (no lateral chain). This suggests the lateral chain has no significant influence on the inhibition of β -haematin formation. Those compounds that are electron releasing showed little or no inhibition at all. Compounds **47g** and **47j**, -NH₂ and -OH 7-substituted molecules respectively, showed no inhibition at all. Both substituents are in the lower left hand quadrant (electron releasing

and hydrophilic) of the Craig Plot. Compound **47d** in the top left hand quadrant was found to be a weak inhibitor along with **47f** (-H) located in the middle of the Craig Plot. Compounds **47e** and **47h** with electron withdrawing substituents (-CN and -NO₂ respectively), had relatively good BHIA₅₀ values along with the chloro-substituted compound (**47c**) also an electron withdrawing substituent, though to a lesser extent.

A strong correlation was found between BHIA₅₀ and both Log *K* and Hammett constant in combination (either σ_p or σ_m) (Fig. 3.5), for the 4-methyl amino-7-X-quinoline series of molecules.



	4-methyl amino-7-X-quinoline (-NMe)	
	Log BHIA ₅₀ vs log K and σ_m	Log BHIA ₅₀ vs log K and σ_p
	(A)	(B)
r^2	0.84	0.76
P value	0.01	0.01
F [F_{CRIT}^{95} (5.79)]	15.66	16.41
Deviation from zero	Significant	Significant
Equation	$-0.53\sigma_m - 0.15\log K + 1.66$	$-0.33\sigma_p - 0.19\log K + 1.77$

Figure 3.5: Graphs and statistical data showing correlations between BHIA₅₀ with Log K and σ_m or σ_p for 4-methyl amino-7-X-quinoline series of molecules

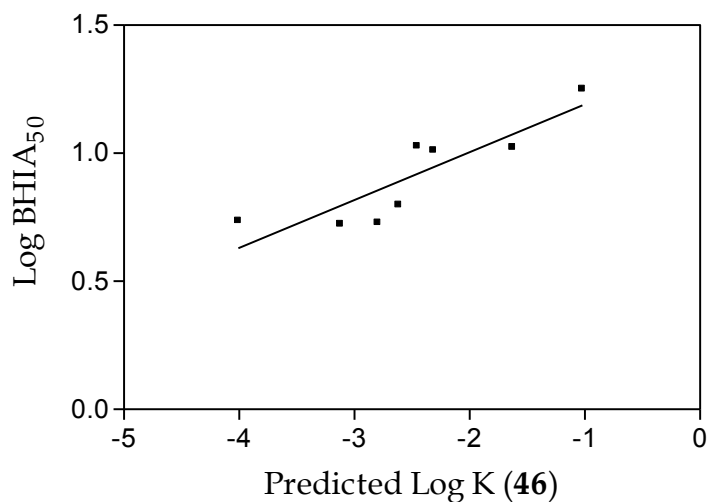
The dependence of inhibition of β -haematin on electron withdrawing properties of the group at the 7-position suggest the electronic requirements necessary to be adopted by the quinoline ring to form strongly β -haematin inhibitory complexes. They are similar to those described in previous studies. [3.14 - 3.16]

3.4.1.5 Haem binding properties of 4-(ethylene diamino)-7-X-quinoline series of molecules

The difference between binding constant values for 4-amino-7-X-quinoline (series **46**) molecules and 4-(ethylene diamino)-7-X-quinoline molecules (series **48**) shows weaker variation. With the exception of **48f/46f** and **48j/46j** where $\Delta\text{Log } K$ is -0.55 and -1.16 respectively, the differences range from 0.09 to 0.29 log units indicating a similar trend between the two series. The binding constant values increase for 4-(ethylene diamino)-7-X-quinoline (series **48**) molecules relative to the 4-methyl amino-7-X-quinoline (series **47**) molecules with differences ranging from 0.05 to 0.85 log units, this indicates greater variation between the two series. Predictably, the compounds with substituents in the top half of the Craig Plot [**48b** (CF_3), **48c** (-Cl), **48e** (-CN) and **48h** ($-\text{NO}_2$)] bound more strongly than those in the bottom half. The lateral chain, being the only modification made on the molecules, appears to play an important positive role in the extent of haem binding in this series.

3.4.1.6 Inhibition of β -haematin formation and 4-ethylene diamino-7-X-quinoline molecules

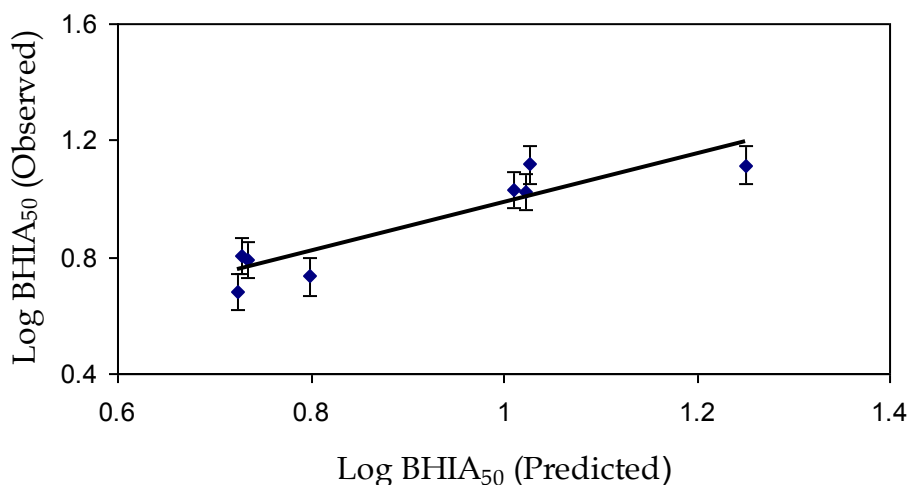
Compound **48e**, in the top left hand quadrant of the Craig Plot, has comparable inhibition of β -haematin formation (BHIA_{50}) value (6.3 ± 0.8) to **48b** ($-\text{CF}_3$), **48c** (-Cl) and **48h** ($-\text{NO}_2$). Among these substituents in the top half of the Craig Plot $-\text{CN}$ (**48e**) is least lipophilic of them. There does not appear to be any significant differences between BHIA_{50} for series **48** and that of series **47**. This suggests the lateral chain does not influence the inhibition of β -haematin. This can be illustrated by the good correlation between $\text{Log } K$ constants for the undecorated series **46** and log BHIA_{50} for series **48** (Fig. 3.6). Furthermore, there seems to be a correlation, albeit not statistically significant, between $\text{Log } K$ (series **46**) and Log BHIA_{50} for series **47** [r^2 0.50, P value 0.05, F_{STAT} 5.94 (F_{CRIT}^{95} 5.99)].



Log K vs Log BHIA ₅₀	
r ²	0.76
P value	0.005
F [F _{CRIT} ⁹⁵ (5.99)]	19.05
Deviation from zero	Significant
Equation	-2.02BHIA ₅₀ + 6.10

Figure 3.6: Correlation between Log K for series **46** and Log BHIA₅₀ for series **48**

As described earlier (*Chapter 3*, section 3.3.1.5) the inhibition of β -haematin shows dependence on haem binding and electronic properties of the group at the 7-position of the quinoline ring. This is further illustrated in the correlation between BHIA₅₀ with Log K and σ_p observed in series **48** molecules (Fig. 3.7).



4-(ethylene diamino)-7-X-quinoline [-NH(CH ₂) ₂ NH ₂]	
Log BHIA ₅₀ vs log K and σ_p	
r ²	0.84
P value	0.004
F [F _{CRIT} ⁹⁵ (5.79)]	20.07
Deviation from zero	Significant
Equation	$-0.41\sigma_p - 0.19\log K + 1.86$

Figure 3.7: The graph and statistical data showing the dependence of the inhibition of β -haematin formation on haem binding (Log K) and electronic features (σ_p) of the group at the 7-position of the quinoline ring for 4-(ethylene diamino)-7-X-quinoline

3.5 Conclusion

Compounds with electron withdrawing substituents, namely -NO₂, -CN, -Cl and -CF₃, were found to be strong inhibitors of β -haematin formation. Additionally, the hydrophilic and electron withdrawing compounds **46e**, **47e** and **48e** (7-CN substituted) were found to show strong inhibition of β -haematin. Possibly due to its much bigger

size and far greater hydrophilicity, $-\text{CONH}_2$ was found to be only a weak inhibitor. Electron releasing groups were found to be poor inhibitors or non-inhibitors of β -haematin formation.

BHIA₅₀ was found to correlate with Log K and σ_p in combination for both series **47** (r^2 0.76, P value 0.01) and **48** (r^2 0.84, P value 0.004). It was also found to correlate with Log K and σ_m in combination for series **47** (r^2 0.84 P value 0.01). These findings suggest the relevance of the properties of the group at the 7-position to effect β -haematin inhibition. Haem binding (Log K) and Hammett constant (σ) for the group at the 7-position appear to independently influence β -haematin inhibition. These findings are in agreement with previous studies that found electron withdrawing groups at the 7-position were essential for β -haematin inhibition. [3.1, 3.2] This study has also demonstrated that lipophilicity can be substantially reduced without losing the ability to inhibit β -haematin formation.

Electron withdrawing groups were also found to bind strongly to haem. The hydrophilic $-\text{CONH}_2$ was found to be a weaker haem binder as were compounds with electron releasing or donating groups ($-\text{CH}_3$, OCH_3 , NH_2 and OH). The un-substituted compounds (**46f**, **47f** and **48f**) were also found to be weaker haem binders. Lipophilicity was found to be an important factor in haem binding, confirmed by a statistically significant correlation (r^2 0.50 and P value 0.02) between Log K and lipophilicity constant for series **46**.

3.5.1 The effect of the lateral chain on haem binding and β -haematin inhibition

The lateral chain was found to play a positive role in haem binding. An increase in the length of the lateral chain was found to result in an increase in Log K , the increases were however dependent on the substituent at the 7-position of the quinoline ring.

The extension of the lateral chain from the simple 4-amino-7-X-quinoline (**28**) by the -CH₃ group and further by ethane-1,2-diamino groups was not found to have a significant impact on β -haematin inhibition.

A correlation was found between Log BHIA₅₀ for 4-ethylene diamino-7-X-quinoline compounds (series **48**) and Log *K* values for 4-amino-7-X-quinoline compounds (series **46**). Further, a suggestion of a weak, although not statistically significant, correlation between Log *K* (series **46**) and Log BHIA₅₀ for series **47** was also found (r^2 0.50, *P* value 0.05, *F*_{STAT} 5.94, *F*_{CRIT}⁹⁵ 5.99). This suggests the 4-amino quinoline sub-structure is essential though not sufficient for β -haematin inhibition, as previously reported. [3.1, 3.12]

The compounds in the top right half of the Craig Plot fared better in binding and β -haematin inhibition than compounds with substituents in the bottom half as the lateral chain was increased. The -CN substituent in the top left hand quadrant of the Craig Plot (electron withdrawing and hydrophilic) showed promising results and was deemed worthy of further investigation as a possible substituent for an active antimalarial.

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4. BIOLOGICAL AND OTHER PROPERTIES OF CHLOROQUINE, PHENYLEQUINE AND PYRIDODIBEMEQUINE ANALOGUES

4.1 Background

The β -haematin inhibition ($BHIA_{50}$) and Log K values evaluated in *Chapter 3* showed electron withdrawing compounds, with the exception of $-\text{CONH}_2$, as the best β -haematin inhibitors and strongest haem binders. Since inhibition of β -haematin formation and haem binding have been shown to be essential, albeit not sufficient for antiplasmodial activity, [4.8] the electron withdrawing substituents emerged as promising candidates for further investigation. This was because they may potentially possess antiplasmodial activity similar to the chloro analogues. While chloroquine remains a bench mark for future drugs, the lipophilic nature of many of its analogues need to be reduced because such compounds are known to possess undesirable off-target effects. The $-\text{CN}$ substituent being in the top left hand quadrant of the π versus σ Craig Plot (hydrophilic and electron withdrawing), offers potentially improved physicochemical properties with regard to toxicity for a quinoline based compound.

To explore this, 4-amino quinoline derivatives were synthesised based on CQ and other compounds with proven biological activity, viz phenylequine and dibemequine (Fig. 4.1). [4.2, 4.3, 4.11] In this study one of the aminomethyl debemethin phenyl rings was replaced by a pyridine ring to increase basicity and reduce lipophilicity. The target compounds were tested for *in vitro* antiplasmodial activity against chloroquine sensitive (NF54) and chloroquine resistant (K1) strains of the malaria parasite *Plasmodium falciparum*. The use of specific lateral chains are instructive in elucidating the extent of antiplasmodial activity particularly when combined with the different substituents

attached at the 7-position. The choice of the lateral chain was influenced by previous studies carried out on 4-aminoquinoline analogues. [4.2, 4.3]

Inhibition of β -haematin formation tests were done placing particular interest on the -CN substituted compounds. These were considered the potentially most active and most hydrophilic 4-aminoquinoline molecules in this class. Owing to the electron withdrawing nature the -CN substituent, compounds **56e**, **57e** and **58e** were predicted to be good β -haematin inhibitors. Further, in support of favourable physicochemical properties of the -CN substituent, the predicted Log P values show compounds **56e** (4.29), **57e** (2.53) and **58e** (3.20) have the lowest values in their respective series (Fig. 4.1).

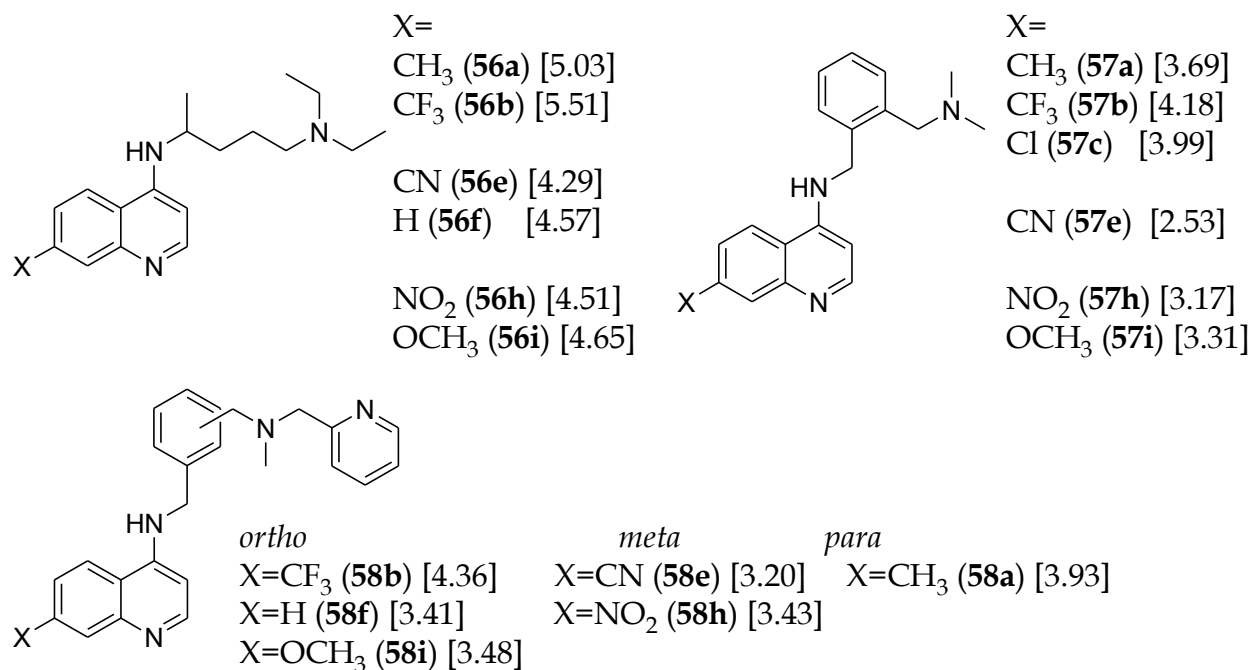


Figure 4.1: The target compounds tested against chloroquine sensitive (NF54) and chloroquine resistant (K1) strains of the malaria parasite and their predicted Log P values

The target compounds (Fig. 4.1) were also projected onto computer models using the computer program Volsurf+ to predict solubility and metabolic stability as a preliminary study of their potential as drug candidates. VolSurf+ is a computational procedure designed by the company Molecular Discovery that creates molecular descriptors which are particularly relevant to and used in ADME predictions [4.10].

4.2 Methods

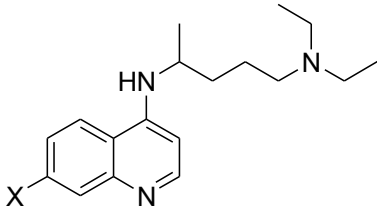
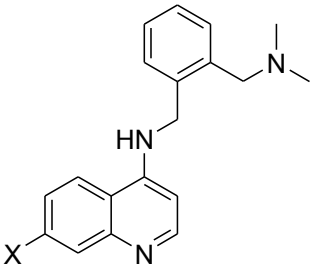
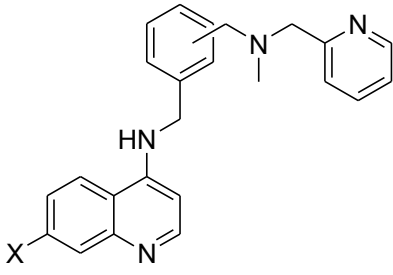
All compounds synthesised were sent to the Swiss Tropical and Public Health Institute in Switzerland for *in vitro* antiplasmodial testing. *In vitro* activity against erythrocytic stages of *P. falciparum* were determined using the chloroquine sensitive (NF54) and chloroquine resistant (K1) strains of the malaria parasite.

Inhibition of β -haematin formation (BHIA₅₀) were carried out as described above (Chapter 3 section 3.2.2 page 88).

4.3 Results

The antiplasmodial results of the target molecules (Fig. 4.1) are presented in Table 4.1. The table shows IC₅₀ values against chloroquine sensitive (NF54) and chloroquine resistant (K1) strains of the malaria parasite. Also reported is the Resistance Index (RI), which is the ratio of the IC₅₀ in NF54 against the IC₅₀ in K1 strain of the malaria parasite.

Table 4.1: Antiplasmodial activity IC₅₀ values of 'drug-like' analogues against sensitive (NF54) and resistant (K1) strains of the malaria parasite and their Resistance Indices (RI).

												
Compound		Strain			Strain		Strain		Strain		Strain	
Code	X	NF54 IC ₅₀ (nM)	K1 IC ₅₀ (nM)	RI	Code	NF54 IC ₅₀ (nM)	K1 IC ₅₀ (nM)	RI	Code	NF54 IC ₅₀ (nM)	K1 IC ₅₀ (nM)	RI
56a	CH₃	64.79	NA ¹	-	57a	25.54	31.76	1.24	58a	98.56	320.39	3.25
56b	CF₃	13.86	97.48	7.03	57b	NA ¹	NA ¹	-	58b	113.87	344.81	3.03
	Cl				57c	4.91	7.67	1.56				
56e	CN	54.76	456.29	8.33	57e	8.22	14.22	1.73	58e	93.52	168.37	1.80
56f	H	192.69	NA ¹	-								
56h	NO₂	26.63	208.52	7.83	57h	44.89	60.05	1.34	58h	77.39	178.25	2.30
56i	OCH₃	72.91	NA ¹	-	57i	105.78	203.32	1.92				
Chloroquine		13.44	300.43	22.35	¹ NA = Not Active (> 1 μM)							

The IC₅₀ values shown in the Table 4.1 all represent activities less than 1 μM against both the sensitive (NF54) and the resistant (K1) strains of the malaria parasite. Compounds that fell outside this limit were considered not active (NA). Phenylequine (**38c**) had the best IC₅₀, 4.91 nM and 7.67 nM against the sensitive (NF54) and resistant (K1) strains respectively (lit. Blackie *et al* IC₅₀ 6.9 nM and 13.2 nM for D10 and K1 strains respectively). [4.3] This series of compounds generally showed better activity against both strains. Compound **56b** had the best IC₅₀ 13.86 nM and 97.48 nM against the sensitive and resistant strains respectively in the series of compounds with the chloroquine side chain. Compound **58h** showed the best activity (77.39 nM) against the sensitive strain while **58e** had the best activity (168.37 nM) against the resistant strain for the pyridodibemequine series **58**.

Inhibition of β-haematin formation (BHIA₅₀) was determined for the 7-CN substituted compounds (**37e**, **38e** and **39e**) (Table 4.2). BHIA₅₀ is reported in molar equivalents of the compound that causes 50% inhibition relative to haematin. Compound **57e** and **58e** show good BHIA₅₀ (0.4 ± 0.2 and 0.6 ± 0.2 respectively) while **56e** (5.0 ± 0.4) had the lowest BHIA₅₀.

Table 4.2: Experimentally determined β-Haematin inhibition (BHIA₅₀) activities for compounds 37e, 38e and 39e

Compound(X)		β-Haematin inhibition (BHIA ₅₀)
56e	CN	5.0 ± 0.4
57e	CN	0.4 ± 0.2
58e	CN	0.6 ± 0.2
Chloroquine		1.9 ± 0.3

4.4 Discussion

To investigate the effect of the group at the 7-position on antiplasmodial activity of the series **56**, **57** and **58** (Fig. 4.1) were synthesised. The different lateral chains on these molecules would also help to determine the effect of the side chain as it is an important determinant of the resistance of the malaria parasite to CQ [4.1].

Compounds **56e**, **57e** and **58e**, all electron withdrawing and hydrophilic, show good antiplasmodial activity with IC₅₀ values ranging from 8.22 nM (**57e**) to 93.52 nM (**58e**) against NF54. Activities for the same compounds in the K1 strain range from 14.22 nM (**57e**) to 456.29 nM (**56e**). Compounds with electron withdrawing groups generally showed better activity relative to those with electron releasing groups.

Compounds with electron releasing groups fared much worse than those with electron withdrawing groups in antiplasmodial activities. **57a** showed good activity, 25.54 nM and 31.76 nM against NF54 and K1 respectively. This series (**57**) of compounds showed the best activities relative to other two series (**56** and **58**). There is no significant cross-resistance observed in series **57** and **58**. There is however, considerable cross-resistance observed for series **56** as may be expected since these compounds contain the chloroquine side chain.

The resistance index (RI) was used as a measure of the antiplasmodial activities of the compounds in the chloroquine resistant strain (K1) relative to their antiplasmodial activities in chloroquine sensitive (NF54) strain of the malaria parasite. The RI was found to be low in series **57** indicating the antiplasmodial activities in the K1 strain for these compounds are comparable with those of NF54 [4.9]. Series **58** had moderate RI values ranging from 1.80 (**58e**) to 3.25 (**58a**). The chloroquine analogues (series **56**) had the worst

(highest) RI values ranging from 7.03 (**56b**) to 8.33 (**56e**), this was as expected as this series showed the highest cross-resistance.

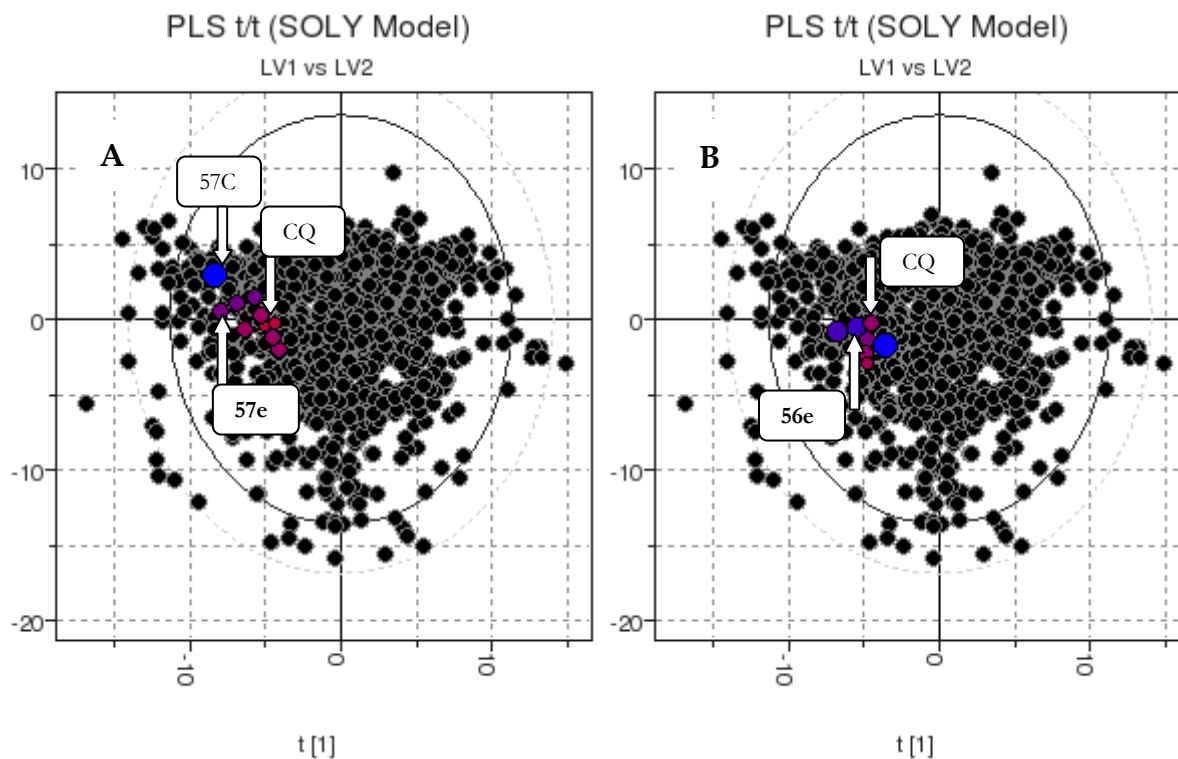
The inhibition of β -haematin formation (BHIA₅₀) values (Table 4.2) show compound **56e** (5.0 ± 0.4) as a poorer inhibitor relative to chloroquine (1.9 ± 0.3). The other compounds (**57e** and **58e**) perform better than chloroquine revealing that these compounds are potential good candidates as antimalarials and that their mechanism of action probably involves inhibition of haemozoin formation.

4.4.1 ADME properties of synthesised 4-amino quinoline derivatives

In silico ADME prediction models and tools complement *in vitro* and *in vivo* ADME experiments in drug discovery [4.4]. In this study, the compounds that showed potential by retaining antiplasmodial activity while being less lipophilic than the known quinoline antimalarials, were given particular scrutiny *in silico* predictions as a preliminary investigation of their potential for further drug development. VolSurf+ was applied to predict the physicochemical and ADME parameters of the compounds, such as intrinsic aqueous solubility at pH 5 and pH 7.5. The results are shown in Figures 4.2 and 4.3 below.

The computer programme VolSurf+ has a data set generated from hundreds of training set compounds. These form the model onto which test set compounds are projected. The programme uses the visible colour spectrum to indicate satisfactory and undesirable predictions, blue being good and red indicating poor predicted properties. In the plots herein (Fig. 4.2), the test compounds are coloured and compounds from the training set are black. Good solubility is represented by colour and size of the dots. As colour moves from blue to red and as the size of the dots decrease, solubility also decreases.

Compounds **56e** and **57e** show moderate to good solubility, this is illustrated in Figures 4.3B and 4.3C. Predicted solubility for **58e** is poor (Fig. 4.2c), this is also confirmed in Fig. 4.3A which shows a plot of Log solubility against Log D at pH 5 or 7.5 (explained below). With exception of series **58**, other compounds, apart from **56h**, **57c** and **58h** in the test set, did not compare better than compounds with the -CN substituent.



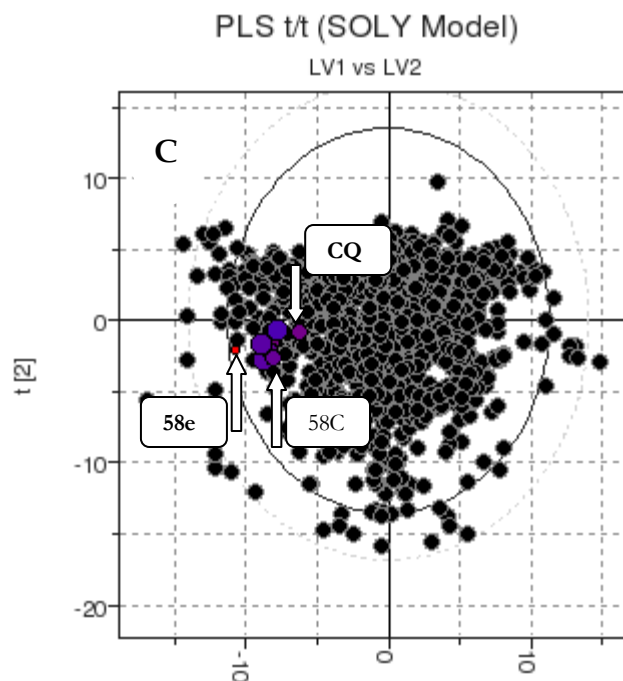
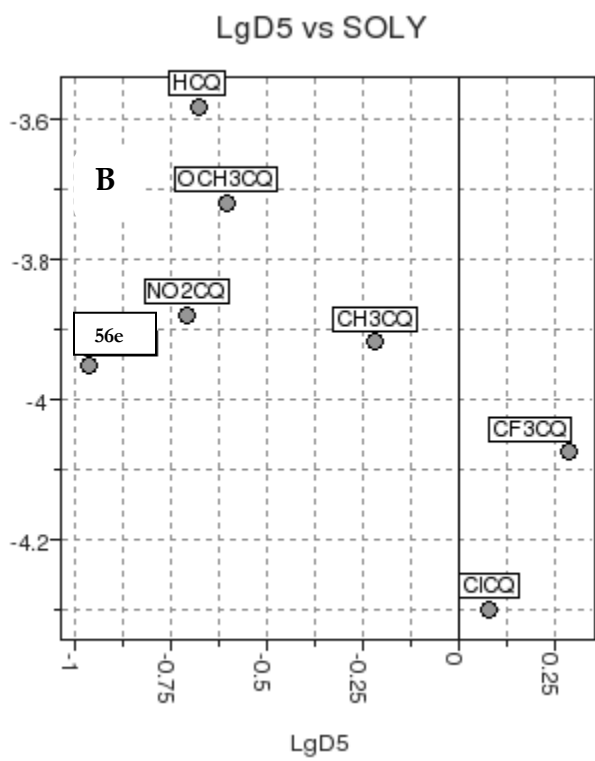
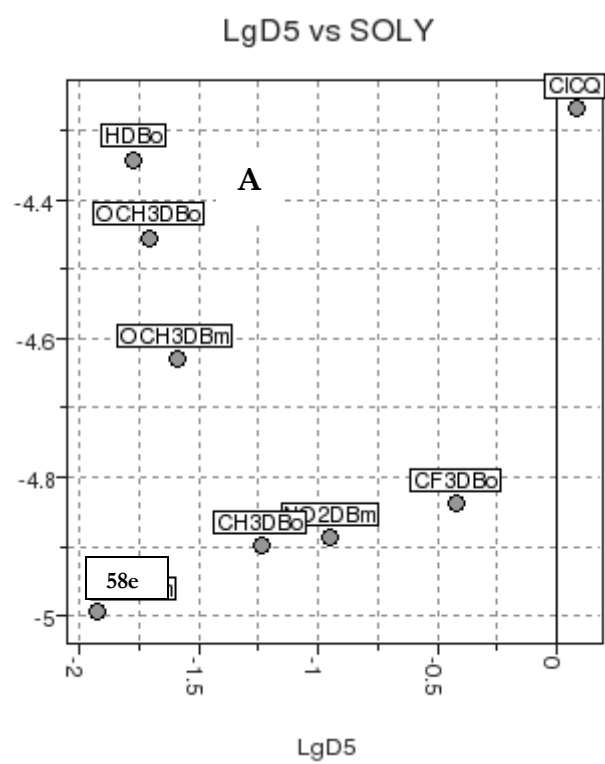


Figure 4.2: Predicted solubility plots for series (A); 57, (B) 56 and (C) 58 projected onto a partial least squares (PLS) solubility model.

Figures 4.3(A), (B) and (C) are plots of predicted log solubility against distribution coefficient (Log D) at pH 5.00 [Figs. 4.3 (A) and (C)] and at pH 7.5 [Fig. 4.3 (B)]. In all cases in these plots ClCQ represents chloroquine while CQ, PQ and DB for series 56, 57 and 58 respectively are preceded by the substituents at the 7-position of the 4-amino quinoline ring.



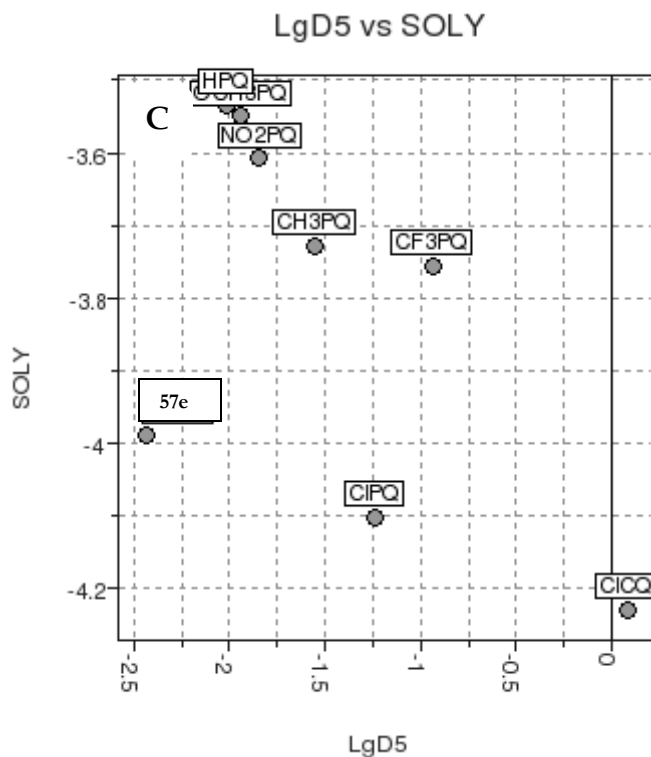
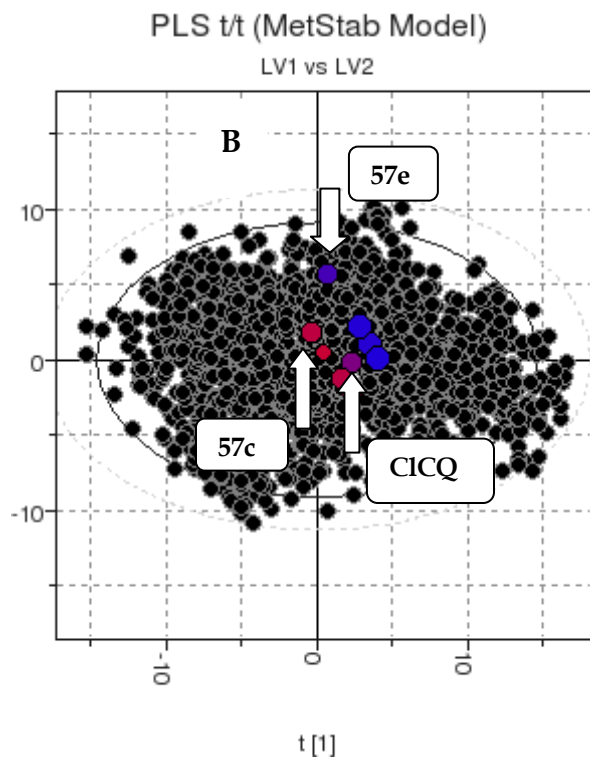
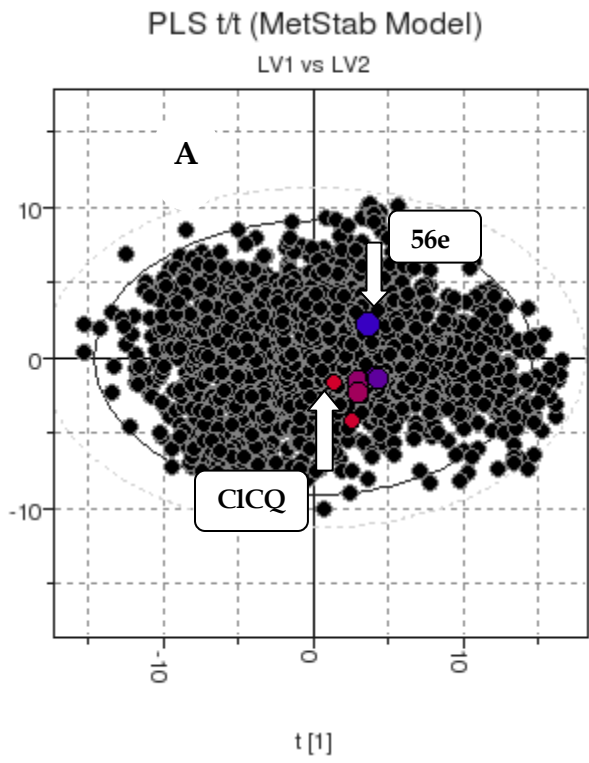


Figure 4.3: Plots showing predicted solubility (Log SOLY) against Log D at pH 5.00 and 7.50: (A) profile for series 58; (B) profile for series 56 and (C) profile for series 57.

Metabolic stability was predicted for series 56, 57 and 58 set of compounds (Fig. 4.4), colour and size of the dots are used to depict the metabolic stability of the compounds. Blue indicates good stability while red suggests poor metabolic stability. Metabolic stability decreases as the size of the dots decrease. The compounds not labeled either showed poor antiparasmodial activity relative to -CN substituted compounds or were predicted to be metabolically unstable or a combination of both. Chloroquine is used as a guide since its metabolic stability is known. In the three figures [Fig. 4.4 (A), (B) and (C)] the chloro substituted compound is used for comparison.

The prediction suggests the -CN 7-substituted compounds are likely to be more stable than the -Cl substituted equivalents.



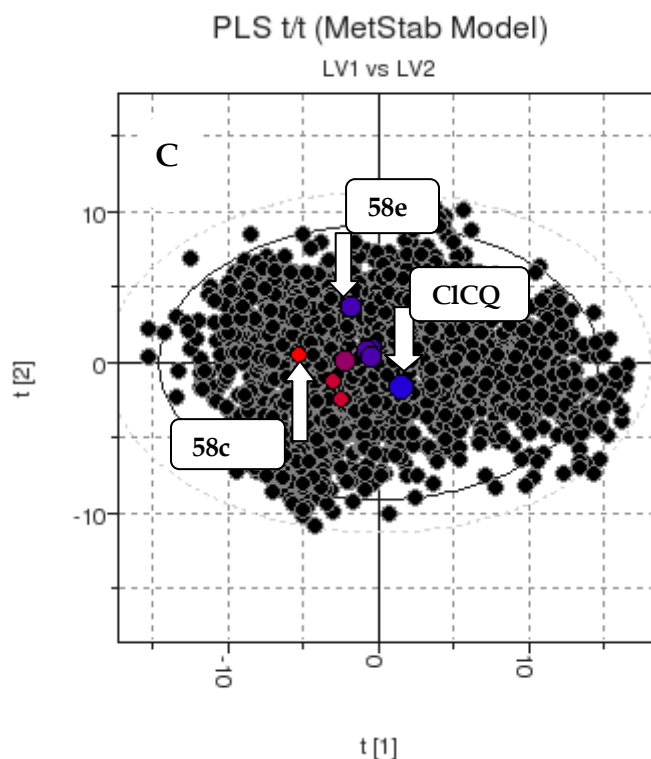


Figure 4.4: Predicted metabolic stabilities for series 56 (A), 57 (B) and 58 (C) set of molecules. ClDB and 38c are the chloro analogues in the respective series of molecules and ClCQ represents chloroquine.

It is worth mentioning that the predictions above are yet to be substantiated with experimental results. It has nonetheless been shown that it is possible to synthesise a less lipophilic analogue of chloroquine that retains activity.

4.5 Conclusion

The compounds at the top half of the Craig Plot were generally found to have good antiplasmodial activity against NF54 and K1 strains of the malaria parasite in these series CQ, PQ and DB. In particular, compounds 56e, 57e and 58e were all found to have good antiplasmodial activity. The attraction to these compounds is their physicochemical

properties which are potentially suitable for producing safer 4-aminoquinoline antimalarials.

The *in silico* predictions showed the compounds with least lipophilic substituent (-CN) had promising results that could be a foundation for further work.

4.6 References

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5. CONCLUSIONS AND FUTURE WORK

5.1 Conclusions

In summary, this project set out to (i) investigate the effect of replacing the 7-chloro group in 4-aminoquinoline, (ii) look into the effect of the lateral chain at the 4-position of the quinoline ring on haem binding and inhibition of β -haematin formation and (iii) ascertain whether those compounds with substituents are good haem binders and inhibitors of β -haematin formation. In particular, the question of whether those that are electron withdrawing and hydrophilic, retain antiplasmodial activity when incorporated into known active compound series was investigated.

It was found that the compounds with a group at the 7-position of the quinoline ring that is electron withdrawing and, in the case of $-\text{CN}$ hydrophilic, were good haem binders. The substituents ($-\text{CN}$, $-\text{NO}_2$ and $-\text{CF}_3$) found in the top half of the Hammett constant (σ) versus lipophilicity constant (π) Craig Plot (Fig. 3.1 *Chapter 3* section 3.1 page 87) were found to effectively replace the chloro group and retain good haem binding activity. The substituents in the bottom half of the Craig Plot gave compounds that were found to be weaker haem binders. Haem binding constants were found to have improved in the compounds with an ethane-1,2-diamine group relative to the core (4-amino-7-X-substituted quinoline). This suggests some participation of the chain in binding. A correlation was found between the haem binding constant (σ) and the lipophilicity constant (π) for the simple 4-amino-7-X-quinoline series of molecules. This is in agreement with previous studies that have shown the extent of haem binding depends on the lipophilicity of the group at the 7-position of the 4-aminoquinoline ring [5.1].

Previous studies have emphasised the importance of the 7-chloro group in the 4-aminoquinoline molecules in the inhibition of β -haematin formation [5.1, 5.2]. This study found that the chloro group can be replaced by other electron withdrawing groups retaining or improving β -haematin inhibition. This was confirmation of earlier work from a more limited series of compounds [5.1]. Further, and more significantly, compounds containing the hydrophilic -CN substituent were also found to be good inhibitors of β -haematin formation, thus presenting a substituent with favourable properties (hydrophilic and β -haematin inhibitor) for inclusion in an antimalarial drug. Compounds **37e**, **38e** and **39e**, all of which contain the -CN substituent, were found to inhibit the formation of β -haematin. It was also found that the electron releasing or donating groups produced compounds that were poor inhibitors of β -haematin formation. A strong correlation was found between BHIA₅₀ and Log *K* combined with the Hammett constant (σ_m or σ_p) suggesting electronic properties of the group at the 7-position of the quinoline ring and haem binding are independently important for β -haematin inhibition.

In three series of compounds namely, **56**, **57** and **58**, molecules with substituents in the top half of the Craig Plot were found to have better antiplasmodial activity relative to those with electron releasing substituents. The hydrophilic -CN analogues, **56e**, **57e** and **58e** (Fig. 5.1) also showed good antiplasmodial activity, demonstrating that such compounds have potential as antimalarials.

In silico predictions showed the -CN substituted compounds may have better metabolic stability than their -Cl equivalents. This stems from fact that a less lipophilic compound will have reduced binding with the lipophilic active site of metabolic enzymes such as CYP450. Predicted solubility for compounds **56e** and **57e** was found to be better than that of chloroquine, but **58e** is expected to have relatively poor solubility.

The -CN and -CF₃ groups may be used as alternative electron-withdrawing substituents instead of halogens. The two groups have comparable electronic effects, but -CN will increase the overall hydrophilicity of the compound relative to CF₃.

5.2 Future work

The *in silico* predictions need to be supplemented with experimental data where the -CN substituted compounds are tested for metabolic stability and solubility. It would be worthwhile in the immediate future to expose the active and hydrophilic compounds (**56e**, **57e** and **58e**) to a wider range of drug sensitive and resistant parasite strains for *in vitro* testing. This would test the robustness of the compounds and ensure they retain activity across the cell lines. The strains that maybe available are, *P. berghei*, *P. yoelii*, 3D7, D10, W2 among others. These studies can then be followed up with *in vivo* testing where mouse models are used to test the efficacy of the compounds.

Additionally, -CN substituted compounds maybe synthesised to increase the library of the hydrophilic compounds and build on the findings of this study. These can then be used in association with recently reported resistance reversing chloroquine derivatives (Fig 5.1).

The compound library may also contain the phenylequine side chain which was found to exhibit the best activity among the series synthesised. This lateral chain can be used together with selected substituents from the Craig Plot that would act by inhibiting the formation of β -haematin.

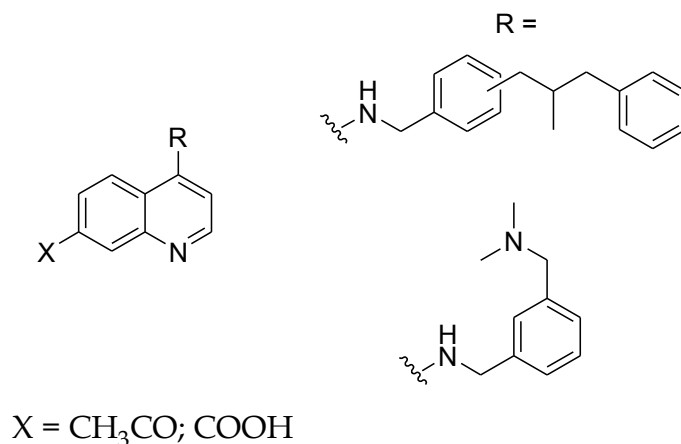


Figure 5.1: Proposed structures with hydrophilic groups that could inhibit β -haematin formation

The 4-aminoquinoline compounds have had a long and successful history. This study has shown that these can still be used to fight resistance. The hydrophilic compounds **57e** and **58e** were found to have good antiplasmodial activity against both sensitive and resistant strains of the malaria parasite. Consequently it has been shown that an electron withdrawing and hydrophilic substituent may be used to replace the 7-chloro group and still retain antiplasmodial activity. This may yet have a profound impact on the continuing quest to develop a safer antimalarial.

5.3 References

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6. EXPERIMENTAL

6.1 General procedures

Chemicals and solvents were purchased from Sigma-Aldrich Chemical Company or Fluka. ^1H NMR and ^{13}C NMR were recorded on a Bruker Avance (400 MHz) or Varian EM (300 MHz) spectrometer. Chemical shifts (δ) are expressed in parts per million (ppm) relative to tetramethylsilane (TMS) as the internal standard recorded in the deuterated solvent indicated. Signal splitting patterns are described as singlet (s), broad singlet (br s), doublet (d), doublet (dd), triplet (t) or multiplet (m). Coupling constants are reported in Hertz (Hz). High resolution electrospray measurements were performed on a Bruker Daltonis MicroOTOF mass spectrometer. Column chromatography was carried out using Silica gel 60 from Sigma-Aldrich.

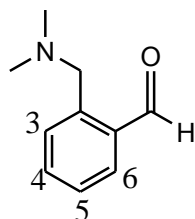
Diethyl ether was dried by distillation with calcium hydride as the drying agent. THF was distilled from sodium wire and benzophenone. Benzene was distilled over sodium wire.

The purity of final compounds was controlled by high performance liquid chromatography (HPLC). This was performed on spectra system equipped with two types of HPLC columns, the xBridge columns (USA) C18 5 μm , 4,6 mm \times 150 mm, 100A analytical column and xBridge column (USA) Prep C18, 5 μm , 19 mm \times 250 mm preparative column. Compounds were dissolved in DMSO and water and injected through a 50 μL loop. The solvent system used was; mobile phase A: $\text{NH}_4\text{CO}_3/\text{H}_2\text{O}$ pH 10.4 and mobile phase B: $\text{NH}_4\text{CO}_3/\text{MeOH}$ pH 10.4 with a flow rate of 1.2 mL/min for

analytical and 20 mL/min for preparative methods with 20 min per run. The chromatographic purities of all the compounds were >98%.

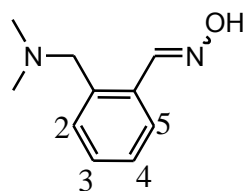
6.2 Characterisation

2-(Dimethylaminomethyl) benzaldehyde (51)



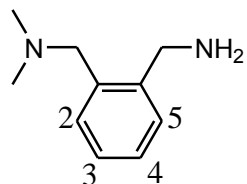
N,N-Dimethylaminomethylbenzene (6.0 ml, 39 mmol) was placed in a heat dried two neck round bottom flask under an atmosphere of nitrogen followed by freshly distilled diethyl ether (80 mL). *N*-Butyllithium was then added slowly. The mixture was allowed to stir at room temperature overnight. Anhydrous *N,N*-dimethylformamide (DMF) (3.2 mL, 39.9 mmol) was added slowly to the mixture and allowed to stir for a further 6 hours. 20 mL of diethyl ether was used to dilute the mixture and approximately 20 mL of deionised water was used to quench the reaction. The mixture was extracted into diethyl ether, washed twice with brine and dried over sodium sulphate. The solvent was then removed under reduced pressure. The product was purified by silica gel chromatography as a reddish oil (2.78 g, 42%). Solvent system: silica/hexane: diethyl ether: triethylamine (8:1:1). NMR - δ_{H} (400 MHz; CDCl_3) 2.25 (6H, s, CH_3), 3.74 (2H, s, CH_2), 7.40 (2H, m, Ar-H), 7.52 (1H, td, J 1.5, 7.4Hz, Ar-H), 7.88 (1H, dd, J 1.4, 7.6Hz, Ar-H), and 10.43 (1H, s, CHO). δ_{C} (100 MHz; CDCl_3) 45.1 ($2 \times -\text{CH}_3$), 60.9 ($-\text{CH}_2$), 127.7 (C-5), 129.4 (C-3), 130.4 (C-6), 133.1 (C-2), 135.1 (C-4), 141.7 (C-1) and 192.1 (CHO).

2-(Dimethylaminomethyl) benzaldehyde oxime (52)



2-(Dimethylaminomethyl) benzaldehyde (1.16 g; 7.13 mmol) was placed in a round bottom flask followed by 75 mL of absolute ethanol and hydroxylamine hydrochloride (1.38 g; 20.10 mmol). A solution of sodium hydroxide (1.60 g; 39.96 mmol in 20 mL deionised water) was then added to the mixture. The mixture was allowed to heat under reflux (100 °C) under nitrogen for 16 hours. The mixture was allowed to cool to room temperature. HCl was added until the mixture had a pH of about 7 (neutralized). The mixture was quenched with deionised water. The compound was extracted into dichloromethane (DCM) and washed with brine and dried over sodium sulphate. The solvent was removed under reduced pressure and dried under high vacuum. The product was isolated as reddish brown oil (72.95 mg, 57%). NMR - δ_{H} (400 MHz; CDCl_3) 2.31 (1H, s, OH), 2.34 (6H, s, 2 \times CH_3), 3.63 (2H, s, $-\text{CH}_2$), 7.32 (3H, m, Ar-H), 7.73 (1H, dd, J 2.4, 6.6Hz, Ar-H), and 8.55 (1H, s, $\text{N}=\text{CH}$). δ_{C} (100 MHz; CDCl_3) 45.2 (2 \times $-\text{CH}_3$), 61.8 ($-\text{CH}_2$), 127.0 (C-4), 128.0 (C-2), 129.3 (C-3), 131.2 (C-1), 148.8 ($\text{N}=\text{CH}$).

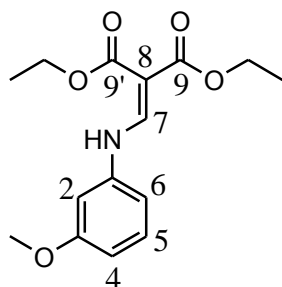
2-(Dimethylaminomethyl) benzylamine (53)



2-(Dimethylaminomethyl) benzaldehyde oxime (0.70 g, 4.10 mmol) was weighed and placed in a round bottom flask. 50 mL of anhydrous THF was added followed by lithium aluminium hydride (77.8 mg, 20.50 mmol). The mixture was heated under reflux under nitrogen for 15 hrs at 100 °C. The mixture was allowed to cool to room temperature

before diluting with diethyl ether and a saturated solution of brine. The product was extracted into diethyl ether and dried over potassium carbonate. The solvent was removed under reduced pressure before drying the product *in vacuo*. The product was isolated as reddish brown oil (65.88 mg, 98%). NMR - δ_{H} (400 MHz; CDCl_3) 2.22 (6H, s, 2 \times CH_3), 3.44 (2H, s, NH_2), 3.85 (2H, s, CH_2), 7.25 (4H, m, Ar - H). δ_{C} (100 MHz; CDCl_3) 45.1 (2 \times CH_3), 51.0 ($-\text{CH}_2\text{NH}_2$), 62.5 ($-\text{CH}_2\text{NCH}_3\text{CH}_3$), 126.4 (C-3), 127.8 (C-5), 128.6 (C-4), 130.7 (C-2), 136.7 (C-1), 143.3 (C-6).

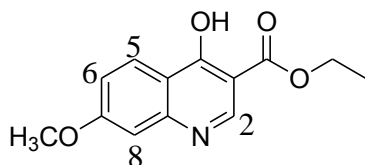
Diethyl 2-((3-methoxyphenylamino) methylene) malonate (40i)



3-Methoxyaniline (3.40 mL; 30.00 mmol) and diethyl ethoxymethylenemalonate (6.50 mL; 30.00 mmol) were mixed together and heated to 110 °C for one hour, liberating ethanol. The mixture was cooled to room temperature forming brown oil. The oil was then cooled in a cold bath composed of acetone, ice and liquid nitrogen resulting in the formation of a white solid. The solid was dissolved in ether and the same cold bath used to cool the mixture. White crystals were formed, filtered and dried to give **40i** (6.30 g, 69%) as a white powdery solid, mp 40-41 °C. δ_{H} (400 MHz; CDCl_3) 1.33 (3H, t, J 7.1 Hz, CH_3), 1.38 (3H, t, J 7.1 Hz, CH_3) 3.82 (3H, s, OCH_3), 4.25 (2H, q, J 7.1 Hz, CH_2), 4.31 (2H, q, J 7.1 Hz, CH_2), 6.66 (1H, t, J 2.1 Hz, H-2), 6.69 (1H, dd, J 1.7, 8.3 Hz, H-6), 6.73 (1H, dd, J 1.5, 8.0 Hz, H-4), 7.26 (1H, t, J 8.1 Hz, H-5), 8.50 (1H, d, J 13.7 Hz, $\text{C}=\text{CH}-\text{NH}$), 10.95 (1H, d, J 13.6 Hz, NH). δ_{C} (100 MHz; CDCl_3) 14.3 (CH_2CH_3), 14.4 (CH_2CH_3), 55.4 ($-\text{OCH}_3$), 60.0 (CH_2CH_3),

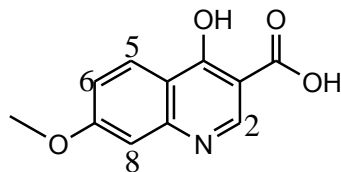
60.3 (CH₂CH₃), 93.7 (C-8), 103.3 (C-2), 109.4 (C-4), 110.3 (C-6), 130.7 (C-5), 140.5 (C-1), 151.8 (C-7), 160.9 (C-3), 166.7 (C-9'), 169.0 (C-9).

Ethyl-4-hydroxy-7-methoxyquinoline-3-carboxylate (**41i**)



41i (6.30 g, 30.00 mmol) was suspended in boiling phenyl ether (100 mL) and refluxed at 260 °C for an hour. The resulting quinoline ester was cooled to room temperature, diluted with petroleum ether and filtered. The solid was washed with large volume of petroleum ether and dried to yield (4.40 g, 86%), mp 273-274 °C (lit. Lauer *et al* 1946, 275 °C). δ_{H} (400 MHz; DMSO) 1.28 (3H, t, *J* 7.1, CH₃), 3.87 (3H, s, OCH₃), 4.21 (2H, q, *J* 7.1 Hz, CH₂), 7.13 (2H, m, Ar-H), 8.06 (1H, d, *J* 8.5 Hz, H-5), 8.42 (1H, s, H-2), 11.88 (1H, s, OH). δ_{C} (100 MHz; DMSO) 14.7 (-CH₂CH₃), 55.9 (-OCH₃), 59.9 (-CH₂CH₃), 100.8 (C-3), 109.1 (C-8), 113.4 (C-4a), 114.5 (C-6), 127.3 (C-5), 128.0 (C-2), 139.3 (C-8a), 145.0 (C-4), 162.4 (C-7), 162.9 (COO-).

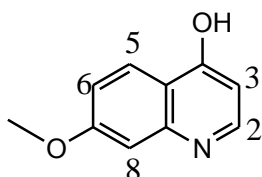
4-Hydroxy-7-methoxyquinoline-3-carboxylic acid (**42i**)



41i (4.40 g, 17.80 mmol) was hydrolysed by refluxing in 2N NaOH at 110 °C for an hour. The mixture was cooled to room temperature and acidified to pH 3-4 using 2M HCl. The resulting white precipitate was filtered, washed with water and dried to give **42i** (2.50 g, 63%), mp 272 °C (lit. Lauer *et al* 1946, 257 - 260 °C). δ_{H} (400 MHz; DMSO) 3.90 (3H, s, OCH₃), 7.17 (1H, dd, *J* 2.4, 9.0 Hz, H-6), 7.29 (1H, d, *J* 2.3 Hz, H-8), 8.16 (1H, d, *J* 9.0 Hz, H-

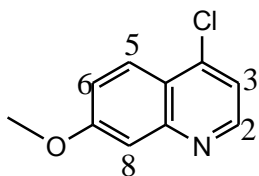
5), 8.72 (1H, s, H-2), 13.68 (1H, s, Ar-OH), 15.50 (1H, bs, -COOH). δ_C (100 MHz; DMSO) 56.3 (Ar-OCH₃), 101.1 (C-3), 107.8 (C-8), 116.6 (C-4a), 118.9 (C-6), 127.3 (C-5), 142.0 (C-2), 145.0 (C-8a), 163.8 (C-4), 166.9 (C-7), 178.1 (COOH).

7-methoxyquinolin-4-ol (**43i**)



42i (4.90 g, 22.40 mmol) was added slowly to already boiling phenyl ether and allowed to reflux at 260°C for an hour. After cooling to room temperature the solid was filtered, washed with petroleum ether and dried. **43i** (3.60 g, 91%) was obtained as a brown powdery solid, mp 209-211 °C (lit. Lauer *et al* 1946, 215 °C). δ_H (400 MHz; DMSO) 3.83 (3H, s, OCH₃), 5.92 (1H, d, *J* 7.4, H-3), 6.89 (1H, dd, *J* 2.3, 8.9 Hz, H-6), 6.97 (1H, d, *J* 2.0 Hz, H-8), 7.77 (1H, d, *J* 7.2 Hz, H-2), 7.97 (1H, d, *J* 8.9 Hz, H-5), 11.74 (1H, br s, Ar-OH). δ_C (100 MHz; DMSO) 55.9 (Ar-OCH₃), 99.8 (C-8), 109.0 (C-3), 113.4 (C-4a), 120.8 (C-6), 127.2 (C-5), 139.4 (C-8a), 142.4 (C-2), 162.3 (C-7), 176.9 (C-4).

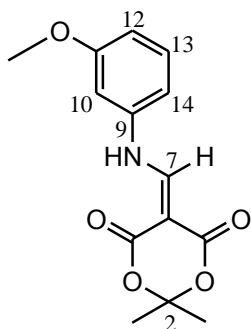
4-Chloro-7-methoxyquinoline (**44i**)



43i (3.60 g, 20.40 mmol) was refluxed in phosphorus oxychloride at 140 °C for 20 minutes. The mixture was then cooled to room temperature and excess phosphorus oxychloride evaporated leaving a slurry mixture. Ice cold sodium hydroxide was added and the precipitated solid was filtered, washed with water and dried. **44i** (2.20 g, 56%) was

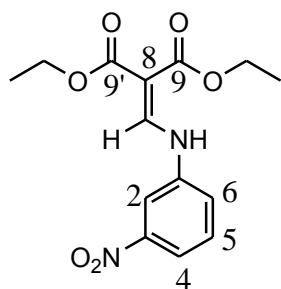
obtained as a brown solid, mp. 84 - 85 °C (lit. Lauer *et al* 1946, 82 - 83 °C). δ_{H} (400 MHz; DMSO) 3.93 (3H, s, OCH₃), 7.39 (1H, dd, *J* 2.5, 9.2Hz, H-6), 7.46 (1H, d, *J* 2.3Hz, H-8), 7.56 (1H, d, *J* 4.7Hz, H-3), 8.08 (1H, d, *J* 9.2Hz, H-5), 8.74 (1H, d, *J* 4.8Hz, H-2). δ_{C} (100 MHz; DMSO) 55.58 (OCH₃), 107.62 (C-8), 119.19 (C-3), 120.73 (C-6), 121.60 (C-4a), 125.26 (C-5), 142.36 (C-4), 150.91 (C-8a), 150.99 (C-3), 161.33 (C-7).

5-((3-Methoxyphenylamino)methylene)-2,2-dimethyl-1,3-dioxane-4,6-dione (45i)



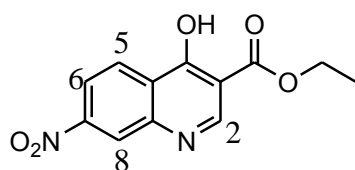
A solution of 2,2-dimethyl-1,3-dioxane-4,6-dione (10.39 g, 72.07 mmol) and triethyl orthoformate (11.20 mL, 65.52 mmol) in ethanol (15 mL) were heated at 90 °C for an hour. 3-methoxyaniline (9.97 mL, 72.06 mmol) in ethanol (40 mL) was added and the mixture was refluxed for a further 2 hours and allowed to cool to room temperature. Solid that formed was recovered by filtration and washed thoroughly with ice cold ethanol and dried to afford the target compound as a yellow solid (14.69 g, 95%). δ_{H} (400 MHz; CDCl₃) 1.73 (6H, s, 2 × CH₃), 3.82 (3H, s, OCH₃), 6.79 (3H, m, Ar-H), 7.31 (1H, t, *J* 8.1 Hz, H-Ar), 8.61 (1H, d, *J* 14.3 Hz, -NHCH), 11.19 (1H, d, *J* 13.9 Hz, -NH). δ_{C} (100.6 MHz; CDCl₃) 26.99 (2×CH₃), 55.38 (OCH₃), 87.2 (C-2), 103.9 (C-10), 105.2 (C-12), 110.2 (C-14), 112.5 (C-13), 130.9 (C-5), 138.9 (C-9), 152.5 (C-7), 161.0 (C-11), 163.5 (C-4), 165.5 (C-6).

Diethyl-2-((3-nitrophenylamino) methylene) malonate (40h)



3-Nitroaniline (7.00 g, 50.69 mmol) and diethyl ethoxymethylenemalonate (10.00 mL, 49.90 mmol) were mixed and heated slowly to 110 °C for one hour. The brown oily mixture was cooled to room temperature resulting in formation of a white solid. White solid was suspended in hexane, triturated and filtered. (40h) was obtained as a white solid (14.87 g, 95%), mp 82 - 83 °C (lit. Vippagunta *et al* 1999, 81 - 82 °C). δ_{H} (400 MHz; CDCl₃) 1.39 (3H, t, *J* 7.1 Hz, CH₃), 1.35 (3H, t, *J* 7.1 Hz, CH₃), 4.33 (2H, q, *J* 7.1 Hz, CH₂), 4.29 (2H, q, *J* 7.1 Hz, CH₂), 7.44 (1H, dd, *J* 1.3, 8.1 Hz, H-6), 7.56 (1H, t, *J* 8.1 Hz, H-5), 7.98 (2H, m, Ar-H), 8.51 (1H, d, *J* 13.2 Hz, H-7), 11.16 (1H, d, *J* 12.7 Hz, NH). δ_{C} (100 MHz; DMSO) 14.2 (-CH₂CH₃), 14.4 (-CH₂CH₃), 60.4 (-CH₂CH₃), 60.7 (-CH₂CH₃), 96.2 (C-8), 111.5 (C-2), 119.0 (C-4), 122.6 (C-6), 130.8 (C-5), 140.6 (C-1), 149.4 (C-3), 150.5 (C-7), 165.2 (C-9), 168.7 (C-9).

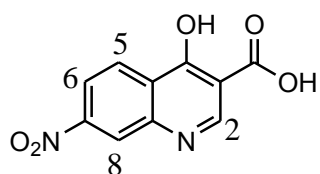
Ethyl-4-hydroxy-7-quinoline-3-carboxylate (41h)



40h (11.23 g; 36.42 mmol) was added to already refluxing phenyl ether (100 mL) and allowed to reflux at 260 °C for an hour. The mixture was allowed to cool to room temperature, diluted with petroleum ether and filtered. The solid was washed with an

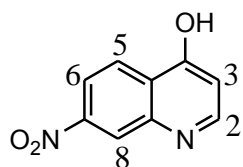
ethyl acetate and petroleum ether mixture (2:1) and dried to give **41h** (6.51 g, 68%) as a yellow solid mp 304 - 306 °C (lit. Vippagunta *et al* 1999, 290 - 296 °C). δ_{H} (400 MHz; DMSO) 1.30 (3H, t, J 7.1 Hz, CH_3), 4.23 (2H, q, 7.1 Hz, CH_2), 8.10 (1H, dd, J 2.2, 8.9 Hz, H-6), 8.37 (1H, d, J 8.9 Hz, H-5), 8.48 (1H, d, J 2.2 Hz, H-8), 8.61 (1H, s, H-2), 8.71 (1H, s, OH). δ_{C} (100 MHz; DMSO) 14.7 (CH_2CH_3), 60.2 (CH_2CH_3), 112.1 (C-3), 118.4 (C-8), 119.2 (C-4a), 122.3 (C-6), 125.2 (C-5), 149.2 (C-8a), 152.9 (C-2), 156.2 (C-7), 163.2 (C-4), 164.8 (COOH).

4-hydroxy-7-nitroquinoline-3-carboxylic acid (**42h**)



41h (5.28 g; 20.15 mmol) was suspended in 2 M NaOH (80 mL) and refluxed at 110 °C for 45 minutes. Progress of the reaction was monitored by solubilisation of the product. The mixture was cooled to room temperature and acidified (pH 3-4) with 2 M HCl. The precipitate was filtered, washed with water and dried to give **42h** (4.13 g, 88%) as a brown solid, mp 265-267 °C (lit. Vippagunta *et al* 1999, 274 - 275 °C). δ_{H} (400 MHz; DMSO) 8.27 (1H, dd, J 2.2, 8.9 Hz, H-6), 8.51 (1H, d, J 8.9 Hz, H-5), 8.68 (1H, d, J 2.1 Hz, H-8), 9.04 (1H, s, H-2). δ_{C} (100 MHz; DMSO) 109.8 (C-3), 116.1 (C-8), 119.8 (C-4a), 123.1 (C-6), 128.1 (C-5), 146.2 (C-8a), 147.3 (C-2), 150.6 (C-7), 165.9 (C-4), 178.0 (COOH).

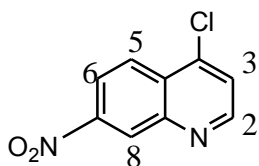
7-nitroquinolin-4-ol (**43h**)



42h was decarboxylated using a previously reported method (Baker *et al.* 1946). To **42h** (4.13 g, 17.40 mmol) in 25 mL of deionised water at 100 °C, NH_3 (aq) (0.32 g, 18.79 mmol)

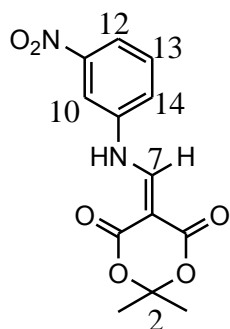
was added followed by a saturated solution of AgNO₃ (2.99 g, 17.60 mmol). The mixture was refluxed at 100 °C for 16 hours. The mixture was cooled to room temperature, filtered, washed with water and dried to give (4.41 g, 62%) beige powdery solid silver salt of **42h**. Solid was added slowly to already refluxing phenyl ether (100 mL) and refluxed at 260 °C with vigorous stirring for 45 minutes, cooled to room temperature, filtered and dried. Product was then refluxed in ethanol (80 mL) at 90 °C for 5 hours. Mixture was filtered while still hot and solvent removed under reduced pressure to give a yellow powder of **43h** (0.29 g, 14%), mp >300 °C (lit. Vippagunta *et al* 1999, 314 - 317 °C). δ_{H} (400 MHz; DMSO) 6.17 (1H, d, *J* 7.3 Hz, H-3), 8.03 (1H, dd, *J* 2.2, 8.9 Hz, H-6), 8.09 (1H, 7.5 Hz, H-2), 8.29 (1H, d, *J* 8.8 Hz, H-5), 8.43 (1H, d, *J* 2.0 Hz, H-8). δ_{C} (100 MHz; DMSO) 116.4 (C-3), 118.4 (C-8), 123.2 (C-4a), 127.2 (C-6), 129.8 (C-5), 148.1 (C-8a), 149.7 (C-2), 156.5 (C-7), 165.9 (C-4).

4-Chloro-7-nitroquinoline (44h)



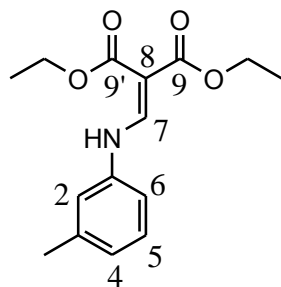
43h (0.39 g; 2.05 mmol) was refluxed in phosphorus oxychloride (5 mL) at 140 °C for 30 minutes and allowed to cool to room temperature. Mixture was chilled in an ice bath while ice cooled 2M NaOH was used to neutralize the solution. **44h** was obtained as a yellow powder (0.37 g, 98%) mp 169-172 °C (lit. Vippagunta *et al* 1999, 156 - 160 °C). δ_{H} (400 MHz; DMSO) 7.60 (1H, d, *J* 4.7 Hz, H-3), 8.16 (1H, dd, *J* 1.7, 8.7 Hz, H-6), 8.36 (1H, d, *J* 8.8 Hz, H-5), 8.51 (1H, d, *J* 1.6 Hz, H-8), 8.88 (d, 1H, *J* 4.6 Hz, H-2). δ_{C} (100 MHz; DMSO) 117.1 (C-8), 120.4 (C-6), 124.5 (C-3), 126.0 (C-5), 130.2 (C-4a), 134.3 (C-4), 137.5 (C-8a), 149.3 (C-2), 152.4 (C-7).

2,2-Dimethyl-5-((3-nitrophenylamino)methylene)-1,3-dioxane-4,6-dione (45h)



To a solution of 3-nitroaniline (7.00 g, 50.71 mmol) in ethanol (60 mL) were added 2,2-dimethyl-1,3-dioxane-4,6-dione (10.00 g, 69.38 mmol) then triethyl orthoformate (11.20 mL, 65.52 mmol). The mixture was refluxed for 2 hours and then allowed to cool to room temperature. Solid that formed was recovered by filtration and washed thoroughly with ice cold ethanol and dried to afford **45h** as a beige solid (14.69 g, 95%), mp 215-217 °C. δ_{H} (400 MHz; DMSO) 1.74 (6H, s, 2×CH₃), 7.54 (1H, ddd, *J* 1.0, 2.3, 8.1Hz, H-12), 7.61 (1H, t, *J* 8.1Hz, H-13), 8.10 (2H, m, Ar-H) 8.66 (1H, d, *J* 13.9Hz, NHCH), 11.34 (1H, d, *J* 14.0Hz, NH). δ_{C} (100.6 MHz; DMSO) 26.4 (2×CH₃), 87.9 (C-2), 104.1 (C-10), 114.4 (C-12), 120.8 (C-14), 125.3 (C-13), 130.6 (C-5), 139.3 (C-9), 148.3 (C-11), 153.7 (C-7), 162.4 (C-4), 163.3 (C-6).

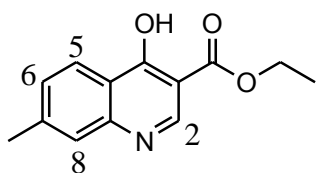
Diethyl-2-((*m*-tolylamino) methylene) malonate (40a)



m-Toluidine (8 mL, 73.84 mmol) and diethyl ethoxymethylenemalonate (**39**) (15 mL, 74.90 mmol) were mixed and heated to 110 °C for 45 minutes. Cooling to room temperature resulted in yellow oil that was placed in an ice/acetone cold bath left in a cold room (4

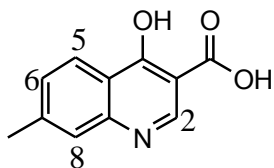
°C) for 30 minutes. The solid that formed was crystallized with hexane to give **40a** (14.81 g, 65%) as white feathery solid, mp 40-41 °C. δ_{H} (400 MHz; CDCl_3) 1.30 (3H, t, J 7.1 Hz, CH_3), 1.35 (3H, t, J 7.1 Hz, CH_3), 2.33 (3H, s, CH_3), 4.23 (2H, q, J 7.2 Hz, CH_2), 4.28 (2H, q, J 7.3 Hz, CH_2), 6.92 (3H, m, Ar-H), 7.22 (1H, t, J 8.0 Hz, H-5), 8.49 (1H, d, J 13.6 Hz, H-7), 10.91 (1H, d, J 13.8 Hz, NH). δ_{C} (100MHz; CDCl_3) 14.3 (CH_2CH_3), 14.4 (CH_2CH_3), 21.4 (Ar- CH_3), 60.0 (CH_2CH_3), 60.3 (CH_2CH_3), 98.5 (C-8), 114.3 (C-6), 117.9 (C-2), 125.7 (C-4), 129.6 (C-5), 139.3 (C-3), 151.9 (C-1), 166.9 (C-7), 169.0 (C-9).

Ethyl-4-hydroxy-7-methylquinoline-3-carboxylate (**41a**)



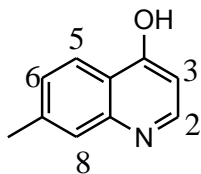
40a (14.81 g, 53.39 mmol) was added to already refluxing (265 °C) phenyl ether (100 mL). The mixture was left to reflux for 30 minutes and then cooled to room temperature. The solid that formed was filtered and washed thoroughly with hexane to give **41a** (5.64 g, 23%) as a white powder, mp 258-261 °C. δ_{H} (400 MHz; CDCl_3) 1.29 (3H, t, J 7.1 Hz, CH_3), 2.44 (3H, s, CH_3), 4.22 (2H, q, J 7.1 Hz, CH_2), 7.24 (1H, d, J 8.3, 1.0 Hz, H-6), 7.41 (1H, d, J 1.4 Hz, H-8), 8.07 (1H, d, J 8.2 Hz, H-5), 8.47 (1H, s, H-2), 12.05 (1H, br s, OH). δ_{C} (100MHz; CDCl_3) 14.7 ($-\text{CH}_2\text{CH}_3$), 21.6 (Ar- CH_3), 59.9 ($-\text{CH}_2\text{CH}_3$), 117.2 (C-3), 118.5 (C-4a), 126.1 (C-5), 126.6 (C-8), 131.7 (C-6), 143.1 (C-7), 143.6 (C-2), 144.8 (C-8a), 166.3 (Ar-COH), 173.7 ($-\text{COO}-$).

4-Hydroxy-7-methylquinoline-3-carboxylic acid (**42a**)



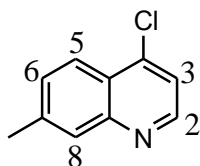
41a (4.31 g, 18.65 mmol) was then hydrolysed by refluxing in 2M sodium hydroxide (80 mL) and monitoring completion of the reaction by solubilisation (~30 minutes). Upon cooling to room temperature, pH of the mixture was adjusted to about 4 with 2M HCl. The resultant solid was filtered, washed with water and dried. **42a** (3.50 g, 92%) was obtained as a white solid, mp 254-255 °C. δ_{H} (400 MHz; CDCl_3) 2.52 (3H, s, CH_3), 6.83 (1H, dd, J 2.4, 9.0 Hz, H-6), 7.29 (1H, d, J 1.7 Hz, H-8), 7.89 (1H, d, J 9.1 Hz, H-5), 8.73 (1H, s, H-2), 13.85 (1H, br s, Ar-OH). δ_{C} (100 MHz; CDCl_3) 21.9 (Ar- CH_3), 107.8 (C-3), 119.2 (C-4a), 122.8 (C-5), 125.3 (C-8), 128.3 (C-6), 133.5 (C-7), 140.1 (C-2), 145.0 (C-8a), 166.9 (C-4), 178.5 (Ar-COOH).

7-Methylquinolin-4-ol (**43a**)



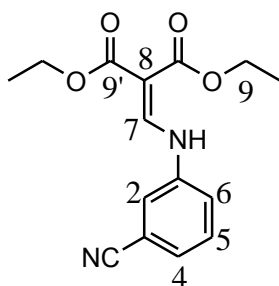
42a (3.50 g, 17.24 mmol) was decarboxylated by adding it to already refluxing phenyl ether (100 mL) and allowed to reflux for 45 minutes. After cooling to room temperature, the solid was filtered and washed with hexane to give **43a** (2.59 g, 94%) as a white solid, mp 200-202 °C. δ_{H} (400 MHz; CDCl_3) 3.24 (3H, s, CH_3), 5.85 (1H, d, J 7.4 Hz, H-3), 6.80 (1H, dd, J 2.5, 8.9 Hz, H-6), 6.91 (1H, d, J 2.5 Hz, H-8), 7.69 (1H, d, J 8.9 Hz, H-5), 7.89 (1H, d, J 7.1 Hz, H-2), 11.74 (1H, br s, OH). δ_{C} (100 MHz; CDCl_3) 21.7 (Ar- CH_3), 109.0 (C-3), 118.0 (C-4a), 124.3 (C-5), 125.2 (C-6), 125.4 (C-8), 139.5 (C-7), 140.7 (C-8a), 142.0 (C-2), 177.3 (C-4).

4-Chloro-7-methylquinoline (44a)



43a (2.59 g, 16.23 mmol) was added to phosphorus oxychloride (6 mL) and heated at 140 °C for an hour. The mixture was then poured onto ice, neutralised with 2M NaOH resulting in a solid that was filtered, washed with water and dried to afford **44a** (0.41 g, 14%) as an oil. δ_{H} (400 MHz; CDCl_3) 2.08 (1H, s, CH_3), 7.90 (1H, d, J 4.7 Hz, H-3), 8.20 (1H, d, J 1.5 Hz, H-8), 8.33 (1H, dd, J 1.3, 7.3 Hz, H-6), 8.41 (dd, 1H, J 7.6 Hz), 8.94 (1H, d, J 4.7 Hz, H-2). δ_{C} (100 MHz; CDCl_3) 22.1 ($-\text{CH}_3$), 103.1 (C-3), 119.5 (C-5), 123.0 (C-4a), 125.6 (C-8), 128.6 (C-6), 139.2 (C-4), 140.4 (C-7), 145.4 (C-8a), 145.6 (C-2).

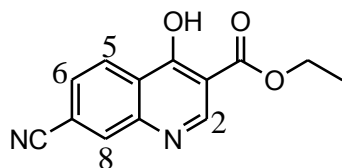
Diethyl-2-((3-cyanophenylamino) methylene) malonate (40e)



3-Aminobenzonitrile (4.42 g, 37.42 mmol) and diethyl ethoxymethylenemalonate (**39**) (7.5 mL, 37.42 mmol) were mixed and heated at 110 °C for 45 minutes. Cooling to room temperature resulted in a solid which was then filtered, washed with hexane and dried to give **40e** (10.42 g, 97%) as a white solid, mp 112-113 °C. δ_{H} (400 MHz; CDCl_3) 1.26 (3H, t, J 7.1 Hz, CH_3), 1.29 (3H, t, J 7.1 Hz, CH_3), 4.17 (2H, q, J 7.1 Hz, CH_2), 4.22 (2H, q, J 7.1 Hz, CH_2), 7.25 (1H, m, Ar-H), 7.31 (1H, m, Ar-H), 7.33 (1H, t, J 1.2 Hz, H-2), 7.38 (1H, m, Ar-H), 8.34 (1H, d, J 13.3 Hz, H-7), 10.95 (1H, d, J 13.1 Hz, NH). δ_{C} (100 MHz; CDCl_3) 14.2 (CH_2-CH_3), 14.4 (CH_2-CH_3), 60.4 ($-\text{CH}_2\text{CH}_3$), 60.7 ($-\text{CH}_2\text{CH}_3$), 96.0 (C-8), 114.1 (C-3), 117.9

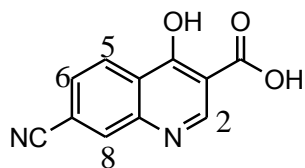
(Ar-CN), 119.9 (C-6), 121.2 (C-2), 127.9 (C-4), 130.8 (C-5), 140.3 (C-1), 160.6 (C-7), 165.2 (C-9).

Ethyl-7-cyano-4-hydroxyquinoline-3-carboxylate (**41e**)



40e (10.24 g, 35.55 mmol) was added slowly to already boiling phenyl ether (100 mL) at 280 °C and allowed to reflux for an hour. The mixture was cooled to room temperature resulting in formation of a solid which was washed with hexane and dried. The solid was then added to hot methanol to selectively dissolve ethyl-5-cyano-4-hydroxyquinoline-3-carboxylate. Precipitate was recovered by filtration to give **41e** (3.92 g, 49%) as a white solid, mp 290-292 °C. δ_{H} (400 MHz; CDCl_3) 1.23 (3H, t, J 6.9 Hz, CH_3), 4.18 (2H, q, J 7.1 Hz, CH_2), 7.64 (1H, d, J 8.3 Hz, H-5), 7.75 (1H, d, J 1.5, H-8), 7.81 (1H, dd, J 2.0, 7.5 Hz, H-6), 8.50 (1H, s, H-2), 12.25 (1H, br s, OH). δ_{C} (100 MHz; DMSO) 14.7 ($-\text{CH}_2\text{CH}_3$), 60.3 ($-\text{CH}_2\text{CH}_3$), 110.1 (C-3), 124.3 (C-7), 127.0 (Ar-CN), 127.7 (C-4a), 132.6 (C-5), 133.4 (C-6), 140.5 (C-8), 145.4 (C-8a), 146.2 (C-2), 164.8 (C-4), 172.0 ($-\text{COOH}$).

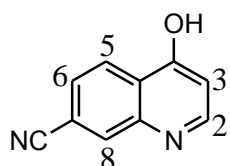
7-Cyano-4-hydroxyquinoline-3-carboxylic acid (**42e**)



41e (3.92 g, 16.19 mmol) was suspended in 2 M sodium hydroxide solution (100 mL) and heated to reflux (112 °C). Completion of the reaction was monitored by dissolution of the solid (~30 minutes). The mixture was cooled to room temperature and pH adjusted to around 4 with 2 M aqueous HCl. The solid precipitate was filtered, washed with water

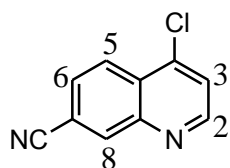
and dried to give **42e** (3.23 g, 94%) as a white powder, 276-78 °C. δ_{H} (400 MHz; DMSO) 7.88 (1H, d, J 1.8, H-8), 8.03 (1H, dd, J 0.9, 8.4 Hz, H-6), 8.37 (1H, d, J 8.5 Hz, H-5), 8.95 (1H, s, H-2), 13.66 (1H, bs, Ar-H), 15.04 (1H, br s, COOH). δ_{C} (100 MHz; DMSO) 109.0 (C-3), 120.2 (C-7), 121.7 (Ar-CN), 125.4 (C-4a), 126.0 (C-5), 126.2 (C-6), 133.7 (C-8), 134.8 (C-8a), 145.2 (C-3), 166.4 (C-4), 178.5 (-COOH).

4-Hydroxyquinoline-7-carbonitrile (**43e**)



42e (3.23 g, 15.16 mmol) was added slowly to boiling phenyl ether (100 mL) and allowed to reflux for an hour. After cooling to room temperature, the solid that formed was filtered and washed thoroughly with hexane and recrystallised from ethanol. **43e** (2.00 g, 78%) was obtained as a white powder mp 203-205 °C. δ_{H} (400 MHz; CDCl_3) 6.11 (1H, d, J 7.4 Hz, H-3), 7.93 (1H, d, J 7.3 Hz, H-5), 8.02 (1H, dd, J 1.5, 8.4 Hz, H-6), 8.15 (1H, d, J 1.2 Hz, H-8), 8.37 (1H, d, J 8.4 Hz, H-2), 12.34 (1H, br s, OH). δ_{C} (100 MHz; DMSO) 123.6 (C-3), 124.8 (C-7), 125.5 (Ar-CN), 126.2 (C-4a), 127.8 (C-5), 131.6 (C-6), 136.3 (C-8), 148.5 (C-8a), 152.0 (C-2), 167.2 (C-4).

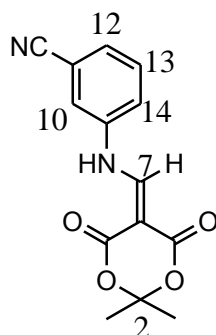
4-Chloroquinoline-7-carbonitrile (**44e**)



43e (2.00 g, 9.34 mmol) was treated with excess phosphorus oxychloride (6 mL) and heated at 140 °C for an hour. The mixture was then left to cool to room temperature, added into ice and neutralised with 2 M NaOH. The resultant precipitate was filtered,

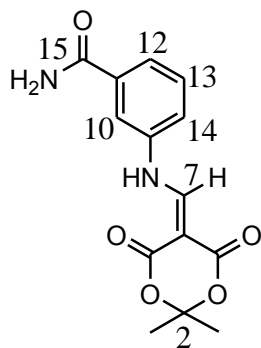
washed with water and dried. **44e** (1.31g, 74%) was obtained as white powder, mp 152-154 °C. δ_{H} (400 MHz; CDCl_3) 7.60 (1H, d, J 4.7 Hz, H-3), 8.17 (1H, dd, J 2.0, 8.4 Hz, H-6), 8.36 (1H, d, J 9.1 Hz, H-5), 8.51 (1H, d, J 1.9 Hz, H-8), 8.88 (1H, d, J 4.6 Hz, H-2). δ_{C} (100 MHz; DMSO) 107.3 (C-7), 118.8 (Ar-CN), 124.9 (C-3), 125.5 (C-5), 130.5 (C-6), 136.3 (C-4a), 139.0 (C-8), 140.0 (C-4), 149.2 (C-8a), 152.2 (C-2).

3-((2,2-Dimethyl-4,6-dioxo-1,3-dioxan-5-ylidene)methylamino)benzonitrile (**45e**)



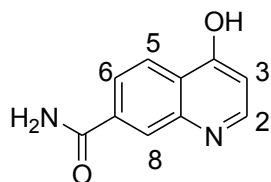
To a solution of 3-aminobenzonitrile (4.70 g, 39.85 mmol) in ethanol (50 mL) were added 2,2-dimethyl-1,3-dioxane-4,6-dione (7.50 g, 52.45 mmol) then triethyl orthoformate (9.00 mL, 52.65 mmol). The mixture was refluxed for 2 hours and then allowed to cool to room temperature. Solid that formed was recovered by filtration and washed thoroughly with ice cold ethanol and dried to afford **45e** as a white solid (10.62 g, 97%). δ_{H} (400 MHz; DMSO) 1.73 (6H, s, $2 \times \text{CH}_3$), 7.47 (2H, m, Ar-H), 7.53 (2H, m, Ar-H), 8.59 (1H, d, J 14.0 Hz, NHCH), 11.25 (1H, d, J 14.1 Hz, NH). δ_{C} (100 MHz; DMSO) 27.0 ($-\text{CH}_3$), 83.3 (C-2), 104.7 (C-11), 112.8 ($-\text{CN}$), 118.6 (C-14), 123.2 (C-10), 124.6 (C-12), 130.0 (C-13), 131.2 (C-5), 140.1 (C-9), 154.2 (C-7), 163.0 (C-6), 164.2 (C-4).

3-((2,2-Dimethyl-4-6-dioxo-1,3-dioxan-5-ylidene)methylamino)benzamide (45d)



To a solution of 3-aminobenzamide (4.12 g, 30.28 mmol) in ethanol (30 mL) were added 2,2-dimethyl-1,3-dioxane-4,6-dione (5.51 g, 38.25 mmol) then triethyl orthoformate (5.60 mL, 32.76 mmol). Mixture was refluxed for 2 hours and allowed to cool to room temperature. Solid that formed was recovered by filtration and washed thoroughly with ice cold ethanol and dried to afford **45d** as a white solid (8.42 g, 96%), mp 221-222 °C. δ_{H} (400 MHz; DMSO) 1.69 (6H, s, 2 × CH₃), 7.46 (1H, br s, H-10), 7.51 (1H, t, *J* 7.9 Hz, H-13), 7.71 (1H, dd, *J* 2.2, 8.0 Hz, H-12), 7.76 (1H, d, *J* 7.8 Hz, H-14), 8.03 (2H, m, NH₂), 8.69 (1H, d, *J* 13.7 Hz, NHCH), 11.31 (1H, d, *J* 13.5, NH). δ_{C} (100 MHz; CDCl₃) 27.0 (Ar-CH₃), 87.5 (C-2), 104.7 (C-10), 117.9 (C-12), 122.6 (C-14), 125.8 (C-13), 130.1 (C-11), 136.2 (C-5), 139.0 (C-9), 153.9 (C-7), 163.2 (C-6), 164.4 (C-4), 167.4 (C-15).

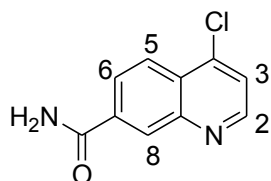
4-Hydroxyquinoline-7-carboxamide (43d)



45b (4.22 g, 14.54 mmol) was added slowly to boiling phenyl ether (100 mL) and allowed to reflux for 5 minutes. After cooling to room temperature, the solid that formed was filtered and washed thoroughly with hexane and recrystallised from ethanol. **43b** (1.45 g, 53%) was obtained as a white powder mp >300 °C. δ_{H} (400 MHz; CDCl₃) 6.07 (1H, d, *J* 7.1

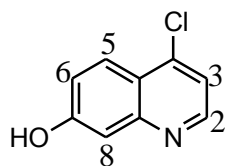
Hz, H-3), 7.78 (1H, d, *J* 8.3 Hz, H-6), 7.91 (1H, d, *J* 8.3 Hz, H-5), 8.14 (1H, d, *J* 7.3 Hz, H-2), 8.24 (1H, s, H-8). δ_C (100 MHz; CDCl₃) 109.9 (C-3), 120.8 (C-4a), 123.6 (C-5), 125.9 (C-6), 128.4 (C-8), 130.7 (C-7), 135.5 (C-8a), 140.9 (C-2), 167.8 (C-4), 177.2 (-CONH₂).

4-Chloroquinoline-7-carboxamide (**44d**)



44d (1.05 g, 5.58 mmol) was treated with excess phosphorus oxychloride (4 mL) and heated at 140 °C for 30 minutes. The mixture was then left to cool to room temperature, added into ice and neutralised with 2 M NaOH. The resultant precipitate was filtered, washed with water and dried. **44d** (60 mg, 52%) was obtained as white powder, mp 289 - 291 °C. δ_H (400 MHz; CDCl₃) 6.08 (1H, d, *J* 7.4 Hz, H-3), 7.73 (1H, dd, *J* 1.6, 8.4 Hz, H-6), 7.93 (1H, d, *J* 8.4 Hz, H-5), 8.02 (1H, d, *J* 1.4 Hz, H-8), 8.13 (1H, d, *J* 7.5 Hz, H-2). δ_C (100 MHz; CDCl₃) 119.1 (C-3), 123.2 (C-4a), 124.7 (C-5), 127.1 (C-6), 127.9 (C-8), 130.0 (C-7), 131.2 (C-8a), 140.0 (C-4), 154.2 (C-2), 164.1 (-CONH₂).

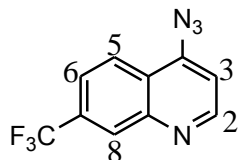
4-Chloroquinolin-7-ol (**44j**)



Solution of 4-chloro-7-methoxyquinoline (**44i**) (1.77 g, 0.91 mmol) in dry DCM (15 mL) under an inert atmosphere was cooled to -84 °C using a slush bath of EtOAC and liquid N₂. BBr₃ (4 eqv.) was added slowly to the mixture and the solution allowed to stir for an hour. Mixture was then left in a freezer for 48 hours then re-cooled to -84 °C and neutralised with K₂CO₃. Solid that formed was washed with water and dried **44j** (1.31 g,

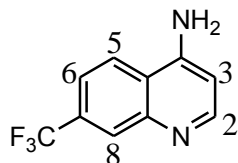
74%) was obtained as white powder, mp 78-79 °C. δ_{H} (400 MHz; DMSO) 7.33 (2H, m), 7.51 (1H, d, J 4.8 Hz, H-2), 8.06 (1H, d, J 9.7 Hz, H-5), 8.69 (1H, d, J 4.8 Hz, H-3), 10.55 (1H, br s, Ar-OH). δ_{C} (100 MHz; DMSO) 110.8 (C-8), 119.1 (C-3), 120.2 (C-5), 121.2 (C-4a), 125.5 (C-6), 141.6 (C-4), 150.8 (C-8a), 150.9 (C-2), 160.1 (C-7).

4-Azido-7-(trifluoromethyl)quinoline (49b)



A solution of 4-chloro-7-trifluoromethylquinoline (1.95 g, 8.42 mmol) and NaN₃ (2.19 g, 33.68 mmol) in a mixture DMF/H₂O (160 mL/60 mL) was heated at 90 °C for 2 hr. After the solution was cooled, a saturated solution of NH₄Cl (500 mL) was added and the mixture extracted with CHCl₃ (3 × 150 mL). The extract was dried over MgSO₄ and concentrated to dryness to give the azido compound (1.66 g, 85%) as a white solid. NMR - δ_{H} (400 MHz; DMSO) 6.13 (1H, d, J 7.4 Hz, H-3), 7.63 (1H, dd, J 1.5, 8.3 Hz, H-6), 7.96 (1H, d, J 7.5 Hz, H-2), 8.01 (1H, br s, H-8), 8.22 (1H, d, J 8.3 Hz, H-5). δ_{C} (100 MHz; DMSO) 109.7 (C-6), 109.9 (C-3), 117.9 (-CF₃), 123.3 (C-8), 124.7 (C-5), 126.4 (C-4a), 131.2 (C-7), 131.6 (C-4), 139.6 (C-8a), 140.4 (C-2).

7-(trifluoromethyl) quinoline-4-amine (46b)



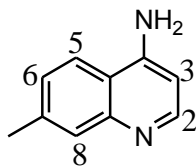
A solution of **49b** (1.00 g, 4.20 mmol) and triphenylphosphine (1.32 g, 5.03 mmol) was stirred at room temperature for 2 hr in a THF/H₂O mixture (1:1). After concentration, 1 M HCl (100 mL) was added and the mixture extracted with ether (3 × 100 mL). The aqueous layer was then made alkaline by 1 M NaOH and extracted with CHCl₃ (3 × 250

mL). The extract was dried over MgSO_4 and concentrated to dryness to give the amino compound **46b** as a white solid (80 mg, 80%), mp 221-222 °C. NMR - δ_{H} (400 MHz; DMSO) 6.67 (2H, br s, $-\text{NH}_2$), 6.76 (1H, d, J 2.4 Hz, H-3), 6.81 (1H, dd, J 1.9, 8.1 Hz, H-6), 7.06 (1H, d, J 1.5 Hz, H-8), 7.68 (1H, d, J 8.1 Hz, H-5), 7.80 (1H, d, J 2.4 Hz, H-2). δ_{C} (100 MHz; DMSO) 108.0 (C-3), 118.6 (C-6), 121.9 (C-5), 127.0 (Ar- CF_3), 129.7 (C-5), 129.8 (C-4a), 132.5 (C-7), 132.6 (C-8), 133.9 (C-2), 141.7 (C-4). HPLC t_{R} = 13.27 min, Purity - 98%. HRMS (ESI) m/z 213.0644 [M^+ $\text{C}_{10}\text{H}_8\text{N}_2\text{F}_3$, requires 213.0640].

General synthesis of compounds 46a-j

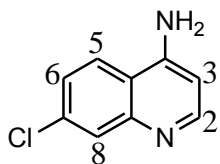
Compounds **44a-j** were heated in a sealed tube with excess aqueous ammonia at 70°C for 4 hours. The mixtures were cooled to room temperature and solids filtered. Solids were then washed with 5% NH_3 (aq) and dried. Compounds were purified by silica gel chromatography and preparative HPLC where purity was below 98%.

7-Methylquinolin-4-amine (46a)



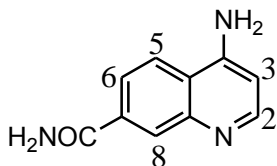
Following the general procedure above **46a** was obtained as a white solid (90 mg, 80%), mp 77-78°C. NMR - δ_{H} (400 MHz; DMSO) 2.41 (3H, s, Ar- CH_3), 6.53 (1H, d, J 5.4 Hz, H-3), 7.12 (1H, dd, J 1.6, 8.9 Hz, H-6), 7.30 (2H, br s, Ar- NH_2), 8.02 (1H, d, J 1.6 Hz, H-8), 7.99 (1H, d, J 9.0 Hz, H-5), 8.30 (1H, d, J 5.4 Hz, H-3). δ_{C} (400 MHz; DMSO) 21.7 (Ar- CH_3), 109.0 (C-3), 117.9 (C-4a), 122.8 (C-5), 125.4 (C-6), 126.3 (C-8), 127.2 (C-7), 139.6 (C-8a), 142.2 (C-2), 150.0 (C-4). HPLC t_{R} = 11.08 min, Purity - 98%. HRMS (ESI) m/z 158.0844 [M^+ $\text{C}_{10}\text{H}_{11}\text{N}_2$, requires 158.0842].

7-Chloroquinolin-4-amine (46c)



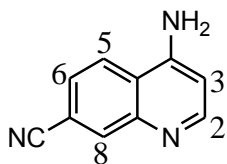
Following the general procedure **46c** was obtained as a white solid (100 mg, 60%). mp 147-148 °C (lit. Price *et al* 1946, 105 - 110 °C). NMR - δ_{H} (400 MHz; DMSO) 6.86 (1H, d, J 5.5 Hz, H-3), 7.34 (1H, dd, J 2.2 Hz, 9.0 Hz, H-6), 7.75 (1H, d, 1H, J 2.2 Hz, H-8), 8.20 (1H, d, J 9.0 Hz, H-5), 8.35 (1H, d, J 5.5 Hz, H-2). δ_{C} (100 MHz; DMSO) 110.0 (C-3), 124.5 (C-4a), 129.7 (C-5), 129.8 (C-6), 132.7 (C-8), 132.8 (C-7), 133.1 (C-8a), 151.8 (C-2), 156.5 (C-4). HPLC t_{R} = 12.52 min, Purity - 98%. HRMS (ESI) m/z 179.0364 [M^+ , $\text{C}_9\text{H}_8\text{ClN}_2$ requires 179.0376].

4-Aminoquinolin-7-carboxamide (46d)



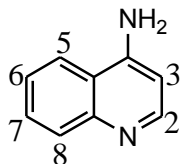
Following the general procedure **46d** was obtained as a white solid (50 mg, 40%). mp 132-134 °C. NMR - δ_{H} (400 MHz; DMSO) 5.12 (2H, br s, $-\text{NH}_2$), 6.66 (1H, d, J 5.1 Hz, H-3), 7.59 7.80 (1H, d, J 8.8 Hz, H-6), 8.34 (1H, d, J 8.8 Hz, H-5), 8.42 (1H, s, H-8), 8.56 (1H, d, J 5.1 Hz, H-2). δ_{C} (100 MHz; DMSO) 116.8 (C-3), 121.8 (C-4a), 124.9 (C-5), 125.3 (C-6), 126.8 (C-8), 127.7 (C-7), 143.1 (C-8a), 153.2 (C-2), 153.6 (C-4), 156.3 ($-\text{CONH}_2$). HPLC t_{R} = 9.51 min, Purity - 98%. HRMS (ESI) m/z 187.1980 [M^+ $\text{C}_{10}\text{H}_9\text{N}_3\text{O}$, 187.1969].

4-Aminoquinolin-7-carbonitrile (**46e**)



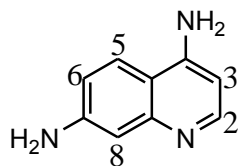
Following the general procedure **46e** was obtained as a white solid (60 mg, 65%), mp 258-59 °C. NMR - δ_{H} (400 MHz; DMSO) 7.57 (2H, br s, NH), 7.83 (1H, d, J 4.7 Hz, H-2), 8.20 (1H, d, J 8.8 Hz, H-5), 8.27 (1H, dd, J 0.7, 8.7 Hz, H-6), 8.65 (1H, d, J 0.7 Hz, H-8), 8.93 (1H, d, J 4.7 Hz, H-3). δ_{C} (100 MHz; DMSO) 123.2 (C-7), 124.4 (-CN), 127.1 (C-4a), 127.4 (C-5), 129.3 (C-6), 136.6 (C-3), 141.6 (C-8), 148.6 (C-8a), 151.8 (C-2), 167.5 (C-4). HPLC t_{R} = 11.57 min, Purity - 98%. HRMS (ESI) m/z 169.0640 [M^+ $\text{C}_{10}\text{H}_8\text{N}_3$ 169.0752]

Quinolin-4-amine (**46f**)



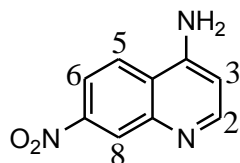
Following the general procedure **46f** was obtained as a white solid (65 mg, 55%) mp 103-104 °C (lit. Den Hertog *et al* 1967, 151 - 152 °C). NMR - δ_{H} (400 MHz; DMSO) 6.54 (1H, d, J 5.2 Hz, H-3), 6.72 (1H, br s, NH), 7.37 (1H, t, J 7.6 Hz, H-6), 7.58 (1H, t, J 7.6 Hz, H-7), 7.75 (1H, d, J 8.5 Hz, H-8), 8.14 (1H, d, J 8.4 Hz, H-5), 8.30 (1H, d, J 5.1 Hz, H-2). δ_{C} (100 MHz; DMSO) 102.8 (C-3), 119.1 (C-6), 122.8 (C-4a), 123.9 (C-5), 129.2 (C-8), 129.3 (C-7), 149.3 (C-8a), 150.8 (C-2), 151.9 (C-4). HPLC t_{R} = 10.18 min, Purity - 98%. HRMS (ESI) m/z 143.9557 [M^+ $\text{C}_9\text{H}_9\text{N}_2$, requires 144.0687].

Quinolin-4,7-diamine (**46g**)



Stannous chloride (SnCl_4) (0.68 g, 2.61 mmol) dissolved in concentrated hydrochloric acid (3 mL) was added slowly to a stirred solution of **46h** (0.09 g, 0.57 mmol) in glacial acetic acid (10 mL). The mixture was stirred at 60°C for 2 hr. After the mixture was cooled, acetone (30 mL) was added and it was stirred vigorously. Precipitate was collected by filtration, washed with acetone, and suspended in water (100 mL). The suspension was made basic (pH 12) with sodium hydroxide and the product was extracted with chloroform (5 × 50 mL). The crude product was purified by on silica gel chromatography with chloroform-methanol (10:1) mixture containing 0.5% triethylamine. Solvent was evaporated to obtain **46g** (65 mg, 70%) as a yellow solid mp 225-227°C. NMR - δ_{H} (400 MHz; DMSO) 8.00 (1H, d, J 4.7Hz, H-3), 8.46 (1H, m, H-6), 8.86 (1H, d, J 1.4Hz, H-8), 9.05 (1H, d, J 4.7Hz, H-2). δ_{C} (100 MHz; DMSO) 120.99 (C-3), 124.17 (C-8), 124.86 (C-4a), 126.08 (C-5), 128.70 (C-6), 141.26 (C-7), 147.26 (C-8a), 148.26 (C-2), 152.86 (C-4). HPLC t_{R} = 9.51 min, Purity - 98%. HRMS (ESI) m/z 159.0796 [M^+ $\text{C}_9\text{H}_9\text{N}_3$, 159.0781].

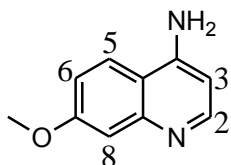
7-Nitroquinolin-4-amine (**46h**)



Following the general procedure **46h** was obtained a yellow solid (40 mg, 62%) mp 150-151 °C. NMR - δ_{H} (400 MHz; DMSO) 7.95 (1H, d, J 4.7 Hz, H-3), 7.99 (1H, d, J 8.0 Hz, H-5), 8.18 (1H, d, J 1.1 Hz, H-8) 8.38 (1H, dd, J 1.1, 8.5 Hz, H-6), 8.99 (1H, d, J 4.7 Hz, H-3).

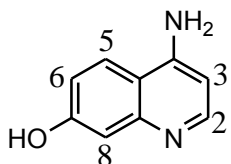
δ_C (100 MHz; DMSO): 117.1 (C-3), 124.5 (C-8), 125.9 (C-6), 130.2 (C-5), 134.3 (C-4a), 137.5 (C-8a), 146.3 (C-2), 149.3 (C-4), 152.4 (C-7). HPLC t_R = 11.69 min, Purity - 98%. HRMS (ESI) m/z 190.0580 [M^+ C₉H₈N₃O₂, requires 190.0580].

7-Methoxyquinolin-4-amine (46i)



Following the general procedure **46i** was obtained as a white solid (100 mg, 60%), mp 194-196 °C. NMR - δ_H (400 MHz; DMSO) 3.86 (3H, s, OCH₃), 6.42 (1H, d, J 5.2 Hz, H-3), 6.57 (2H, bs, Ar-NH₂), 7.01 (1H, dd, J 2.7, 9.2 Hz, H-6), 7.27 (d, 1H, J 2.6 Hz), 8.04 (1H, d, J 9.2 Hz, H-6), 8.26 (1H, d, J 5.2 Hz, H-2). δ_C (100 MHz; DMSO) 55.6 (CH₃), 101.8 (C-8), 107.8 (C-3), 113.6 (C-4a), 115.9 (C-6), 124.1 (C-5), 150.8 (C-3), 152.0 (C-4), 160.2 (C-7). HPLC t_R = 11.66 min, Purity - 98%. HRMS (ESI) m/z 175.0873 [M^+ , C₁₀H₁₁N₂O 175.0871].

4-Aminoquinolin-7-ol (46j)



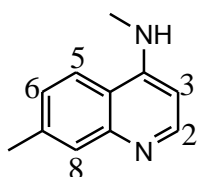
Solution of 7-methoxyquinolin-4-amine (**46i**) (75.48 mg, 0.43 mmol) in dry DCM (15 mL) under an inert atmosphere was cooled to -84°C using a slush bath of EtOAc and liquid N₂. BBr₃ (4 eqv.) was added slowly to the mixture and the solution allowed to stir for an hour. Mixture was then left in a freezer for 48 hours then re-cooled to -84 °C and neutralised with K₂CO₃. Solid that formed was washed with water and dried **46j** (52.84 mg, 70%) was obtained as cream solid mp 151-153 °C. NMR - δ_H (400 MHz; DMSO) 6.34 (1H, d, J 5.2 Hz, H-3), 6.48 (2H, br s, -NH₂), 6.90 (1H, dd, J 2.5, 9.0 Hz, H-6), 7.00 (1H, d, J

2.4 Hz, H-8), 7.95 (1H, d, *J* 9.0 Hz, H-5), 8.14 (1H, d, *J* 5.2 Hz, H-2). δ_C (100 MHz; DMSO) 100.4 (C-3), 110.0 (C-8), 115.5 (C-6), 123.3 (C-5), 150.0 (C-8a), 150.4 (C-2), 151.1 (C-4), 157.9 (C-7). HPLC t_R = 3.16 min, Purity - 98%. HRMS (ESI) m/z 160.0637 [M^+ C₉H₈N₂, 160.0811].

General synthesis for compounds 47a-j

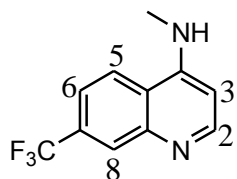
Compounds **44a-j** and excess methylamine were heated in sealed tubes at 90 °C for 6 hours. The mixtures were allowed to cool to room temperature and concentrated under reduced pressure. Compounds were then purified by silica gel chromatography using MeOH: EtOAc: Et₃N (1:98:1).

N,7-Dimethylquinolin-4amine (47a)



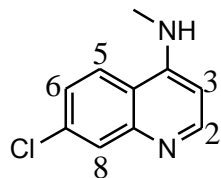
By the general procedure, the title compound was obtained as a white solid (80 mg, 65%), mp 133-135 °C. NMR - δ_H (400 MHz; DMSO) 2.51 (3H, s, Ar-CH₃), 3.07 (3H, d, *J* 4.6 Hz, NHCH₃), 6.66 (1H, d, *J* 7.0 Hz, H-2), 7.49 (1H, dd, *J* 1.5, 8.7 Hz, H-6), 7.75 (1H, br s, H-8), 8.43 (1H, d, *J* 6.9 Hz, H-3), 8.45 (1H, d, *J* 8.7 Hz, H-5), 9.34 (1H, br s, NHCH₃). δ_C (100 MHz; DMSO) 20.9 (Ar-CH₃), 29.1 (-NHCH₃), 97.2 (C-3), 116.4 (C-4a), 121.4 (C-5), 125.7 (C-6), 127.1 (C-8), 138.4 (C-7), 147.3 (C-8a), 149.8 (C-3), 151.1 (C-4). HPLC t_R = 10.60 min, Purity - 98%. HRMS (ESI) m/z 173.1062 [M^+ C₁₁H₁₃N₂, requires 173.1079].

N-Methyl-7-(trifluoromethyl) quinolin-4-amine (47b)



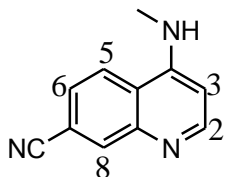
By the general procedure, the title compound was obtained as a white solid (70 mg, 60%), mp 227-229 °C. NMR - δ_{H} (400 MHz; DMSO) 2.87 (3H, d, J 4.7 Hz, NHCH_3), 6.47 (1H, d, J 5.4 Hz, H-3), 7.51 (1H, d, J 4.3 Hz, NHCH_3), 7.64 (1H, dd, J 1.9 Hz, J 8.8 Hz, H-6), 8.04 (1H, br s, H-8), 8.34 (1H, d, J 8.8 Hz, 5), 8.48 (1H, d, J 5.3 Hz, H-2). δ_{C} (100 MHz; DMSO) 29.8 ($-\text{NHCH}_3$), 100.0 (C-3), 119.4 (C-6), 121.3 (C-5), 124.1 (C-4a), 126.8 (Ar- CF_3), 126.8 (C-8), 129.4 (C-7), 147.9 (C-8a), 151.3 (C-2), 152.8 (C-4). HPLC t_{R} = 9.49 min, purity - 98%. HRMS (ESI) m/z 227.0782 [M^+ $\text{C}_{10}\text{H}_{10}\text{N}_2\text{F}_3$, requires 227.0796].

7-Chloro-N-methylquinolin-4-amine (47c)



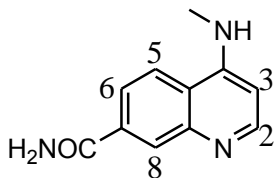
By the general procedure, the title compound was obtained as a white solid (100 mg, 70%), mp 235-237 °C. NMR - δ_{H} (400 MHz; DMSO) 2.89 (3H, d, J 4.4 Hz, NHCH_3), 6.40 (1H, d, J 5.4 Hz, H-3), 7.36 (1H, br s, NH), 7.42 (1H, dd, J 2.3 Hz, 9.0 Hz, H-6), 7.79 (1H, d, J 2.2 Hz, H-8), 8.18 (1H, d, J 9.0 Hz, H-5), 8.42 (1H, d, J 5.4 Hz, H-2). δ_{C} (100 MHz; DMSO) 29.1 (C-11), 98.2 (C-3), 117.3 (C-4a), 123.6 (C-5), 123.9 (C-6), 127.3 (C-8), 133.2 (C-7), 148.8 (C-8a), 150.8 (C-2), 151.8 (C-4). HPLC t_{R} = 12.51 min, purity - 98%. HRMS (ESI) m/z 193.0517 [M^+ $\text{C}_{10}\text{H}_{10}\text{N}_2\text{Cl}$, requires 193.0533].

4-(Methylamino)quinolin-7-carbonitrile (47e)



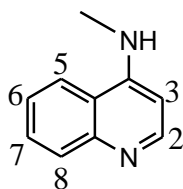
By the general procedure, the title compound was obtained as a white solid (90 mg, 65%), mp 252-254 °C. NMR - δ_{H} (400 MHz; DMSO) 2.87 (3H, d, J 4.7 Hz, -NHCH₃), 6.47 (1H, d, J 5.4 Hz, H-3), 7.51 (1H, d, J 4.4 Hz, Ar-NH), 7.68 (1H, dd, J 1.7, 8.7 Hz, H-6), 8.22 (1H, d, J 1.7 Hz, H-8), 8.28 (1H, d, J 8.7 Hz, H-5), 8.48 (1H, d, J 5.4 Hz, H-2). δ_{C} (100 MHz; DMSO) 29.8 (-NHCH₃), 100.3 (C-7), 111.9 (Ar-CN), 119.2 (C-3), 121.8 (C-5), 124.0 (C-4a), 125.0 (C-6), 135.0 (C-8), 147.7 (C-8a), 151.2 (C-2), 153.0 (C-4). HPLC t_{R} = 9.51 min, purity - 98%. HRMS (ESI) m/z 184.0874 [M^+ C₁₁H₁₀N₃, requires 184.0796].

4-(Methylamino)quinolin-7-carboxamide (47d)



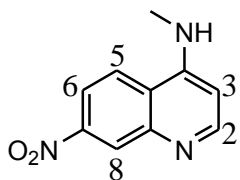
By the general procedure, the title compound was obtained as a white solid (60 mg, 50%), mp 165-166 °C. NMR - δ_{H} (400 MHz; DMSO) 2.82 (1H, d, J 4.6 Hz, NHCH₃), 5.88 (1H, d, J 3.9 Hz, NHCH₃), 6.68 (1H, d, 1H, J 5.5 Hz, H-2), 7.69 (1H, d, J 8.4 Hz, H-5), 7.91 (1H, d, J 0.8 Hz, H-8), 8.06 (1H, dd, J 1.3, 8.5 Hz, H-6), 8.52 (1H, d, J 5.4 Hz, H-3). δ_{C} (100 MHz; DMSO) 31.1 (NHCH₃), 102.7 (C-3), 110.4 (C-4a), 123.0 (C-5), 128.0 (C-6), 135.0 (C-8), 147.3 (C-7), 149.6 (C-8a), 149.8 (C-2), 151.9 (C-4). HPLC t_{R} = 9.58 min, purity - 98%. HRMS (ESI) m/z 201.0700 [M^+ C₁₁H₁₂N₃O, requires 201.0902].

N-Methylquinolin-4-amine (47f)



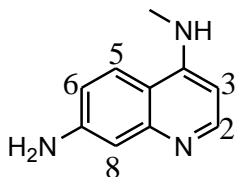
By the general procedure, the title compound was obtained as a white solid (80 mg, 65%), mp. 180-181 °C (Watanabe *et al* 1980, 231 - 232 °C). NMR - δ_{H} (400 MHz; DMSO) 2.97 (1H, d, J 2.8 Hz, -NHCH₃), 6.55 (1H, d, J 6.4 Hz, H-3), 7.52 (1H, t, J 7.7 Hz, H-6), 7.74 (1H, t, J 8.3 Hz, H-7), 7.87 (1H, d, J 8.5 Hz, H-8), 8.18 (1H, bs, Ar-NH), 8.36 (1H, d, J 8.5 Hz, H-5), 8.43 (1H, d, J 6.3 Hz, H-2). δ_{C} (100 MHz; DMSO) 30.1 (-NHCH₃), 98.3 (C-3), 118.0 (C-5), 123.0 (C-4a), 124.1 (C-6), 125.9 (C-7), 131.8 (C-8), 142.3 (C-8a), 145.1 (C-2), 154.4 (C-4). HPLC t_{R} = 9.53 min, purity - 98%. HRMS (ESI) m/z 159.0923 [M^+ C₁₀H₁₁N₂, requires 159.0922].

N-Methyl-7-nitroquinolin-4-amine (47h)



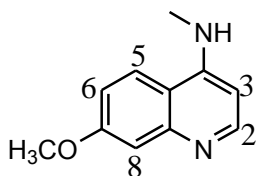
By the general procedure, title compound was obtained as a yellow solid (40 mg, 55%), mp >330 °C. NMR - δ_{H} (400 MHz; DMSO) 2.94 (3H, s, CH₃), 6.55 (1H, d, J 5.3 Hz, H-3), 8.10 (1H, dd, J 2.0, 9.2 Hz, H-6), 8.48 (1H, d, J 9.2 Hz, H-5), 8.54 (1H, d, J 1.8 Hz, H-8), 8.57 (1H, d, J 5.3 Hz, H-2). δ_{C} (100 MHz; DMSO) 31.1 (CH₃), 102.7 (C-3), 110.5 (C-8), 123.0 (C-6), 128.0 (C-5), 135.0 (C-4a), 147.3 (C-8a), 149.6 (C-3), 149.8 (C-7), 151.9 (C-4). HPLC t_{R} = 5.30 min, purity - 98%. HRMS (ESI) m/z 204.0792 [M^+ C₁₀H₁₀N₃O₂, requires 204.0773].

***N*⁴-Methylquinolin-4,7-diamine (47g)**



Stannous chloride (SnCl_4) (0.87 g, 4.57 mmol) dissolved in concentrated hydrochloric acid (3 mL) was added slowly to a stirred solution of **47h** (0.12 g, 0.57 mmol) in glacial acetic acid (20 mL). The mixture was stirred at 60 °C for 2 hr. After the mixture was cooled, acetone (50 mL) was added and it was stirred vigorously. Precipitate was collected by filtration, washed with acetone, and suspended in water (250 mL). The suspension was made basic (pH 12) with sodium hydroxide and the product was extracted with chloroform (5 × 50 mL). The crude product was purified by on a silica gel chromatography with chloroform-methanol (10:1) mixture containing 0.5% triethylamine. Solvent was evaporated to obtain **47g** (84 mg, 70%) as a yellow solid mp 283-285 °C. NMR - δ_{H} (400 MHz; DMSO) 2.91 (3H, d, J 4.7Hz, $-\text{NHCH}_3$), 6.53 (1H, d, J 5.4Hz, H-3), 7.45 (1H, bs, $-\text{NHCH}_3$), 8.08 (1H, dd, J 2.3, 9.2Hz, H-6), 8.36 (1H, d, J 9.2Hz, H-5), 8.52 (1H, d, J 2.3Hz, H-8), 8.54 (1H, d, J 5.3Hz, H-2). δ_{C} (100 MHz; DMSO) 29.8 ($-\text{NHCH}_3$), 100.7 (C-3), 117.1 (C-8), 123.1 (C-4a), 124.5 (C-5), 124.9 (C-6), 148.0 (C-7), 150.3 (C-8a), 151.5 (C-2), 153.6 (C-4). HPLC t_{R} = 7.86 min, purity - 98%. HRMS (ESI) m/z 174.1031 [M^+ $\text{C}_{10}\text{H}_{12}\text{N}_3$ requires 174.1029].

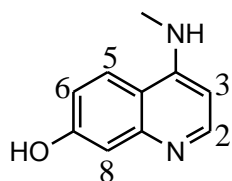
7-Methoxy-*N*-methylquinolin-4-amine (47i)



By the general procedure, the title compound was obtained as a white solid (100 mg, 60%), mp 75-78 °C. NMR - δ_{H} (400 MHz; DMSO) 2.94 (3H, br s, $-\text{NHCH}_3$), 3.85 (3H, s, Ar-

OCH₃), 6.44 (1H, d, *J* 6.4 Hz, H-3), 7.13 (1H, dd, *J* 2.6, 9.2 Hz, H-6), 7.25 (1H, d, *J* 2.6 Hz, H-8), 8.22 (1H, d, *J* 9.3 Hz, H-5), 8.26 (1H, s, Ar-NHCH₃), 8.33 (1H, d, *J* 6.4 Hz, H-2). δ_C (100 MHz; DMSO) 30.0 (-NHCH₃), 56.1 (Ar-OCH₃), 103.7 (C-8), 112.3 (C-3), 117.1 (C-4a), 124.5 (C-6), 144.3 (C-5), 146.0 (C-8a), 154.3 (C-2), 161.8 (C-4), 164.8 (C-7). HPLC t_R = 8.91 min, purity - 98%. HRMS (ESI) m/z 189.1026 [M^+ C₁₁H₁₃N₂O, requires 189.1028].

4-(Methylamino) quinolin-7-ol (**47j**)

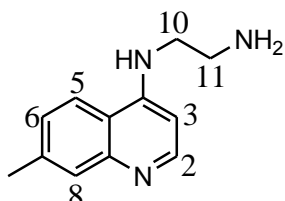


A solution of 4-chloro-7-methoxyquinoline (**46i**) (82.41 mg, 0.43 mmol) in dry DCM (15 mL) in a sealed tube under an inert atmosphere was cooled to -84 °C using a slush bath of EtOAc and liquid N₂. BBr₃ (4 eqv.) was added slowly to the mixture and the solution allowed to stir for an hour. Mixture was then left in a freezer for 48 hours then re-cooled to -84 °C and neutralised with K₂CO₃. Solid that formed was washed with water and dried to 4-chloroquinolin-7-ol which was heated in a sealed tube with excess methylamine at 90 °C for 6 hours. Mixture was allowed to cool to room temperature and concentrated under reduced pressure. **47j** (57.69 mg, 70%) was obtained as cream solid mp 200-201 °C. NMR - δ_H (400 MHz; DMSO) 2.94 (3H, s, Ar-CH₃), 6.44 (1H, d, *J* 6.4 Hz, H-3), 7.13 (1H, dd, *J* 2.6, 9.2 Hz, H-6), 7.25 (1H, d, *J* 2.6 Hz, H-8), 8.22 (1H, d, *J* 9.3 Hz, H-5), 8.26 (1H, br s, -NH) 8.33 (1H, d, *J* 6.4 Hz, H-2), 8.42 (1H, s, -OH). δ_C (100 MHz; DMSO) 29.7 (-CH₃), 96.7, (C-3), 110.5 (C-8), 112.8 (C-4a), 116.4 (C-6), 123.3 (C-5), 149.9 (C-8a), 150.8 (C-2), 151.7 (C-4), 158.6 (C-7). HPLC t_R = 10.58 min, purity - 98%. HRMS (ESI) m/z 175.0871 [M^+ C₁₀H₁₁N₂O₃, requires 175.0803].

General synthesis of ethylene diamine series

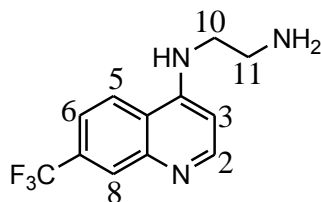
4-Chloro-7-substituted quinoline (1 eqv.) and excess (5 eqv.) ethylene diamine were heated in a sealed tube at 120 °C for 4-6 hours. The reaction mixture was cooled to room temperature and a mixture of 1 M NaOH and CHCl₃ (1:4) was added to the mixture. The organic layer was washed with brine and dried with Na₂SO₄. Solvent was removed and resulting solid purified by column chromatography and further purified by HPLC where purity was found to be below 98%.

N¹-(7-methylquinolin-4-yl)ethane-1,2-diamine (48a)



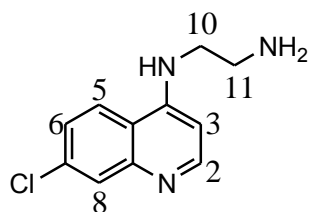
By the general procedure, the title compound was obtained as a white solid (80 mg, 50%). NMR - δ_{H} (400 MHz; DMSO) 2.44 (3H, s, Ar-CH₃), 2.83 (3H, t, *J* 6.3 Hz, H-10), 3.24 (2H, t, *J* 6.2 Hz, H-11), 6.38 (1H, d, *J* 5.3 Hz, H-3), 6.87 (1H, br s, Ar-NH-), 7.22 (1H, dd, *J* 1.3, 8.5 Hz, H-6), 7.55 (1H, br s, H-8), 8.08 (1H, d, *J* 8.5 Hz, H-5), 8.32 (1H, d, *J* 5.3 Hz, H-2). δ_{C} (100 MHz; DMSO) 21.6 (Ar-CH₃), 38.7 (C-11), 41.8 (C-10), 98.5 (C-3), 122.5 (C-4a), 124.5 (C-5), 129.0 (C-6), 129.5 (C-8), 139.4 (C-7), 145.8 (C-8a), 148.7 (C-2), 150.2 (C-4). HPLC *t_R* = 11.79 min, purity - 98%. HRMS (ESI) *m/z* 202.1350 [*M*⁺ C₁₂H₁₆N₃, requires 202.1344].

***N*¹-(7-(trifluoromethyl)quinolin-4-yl)ethane-1,2-diamine (48b)**



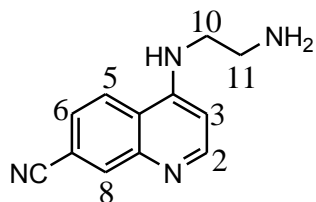
By the general procedure, title compound was obtained as a white solid (30mg, 45%), mp. 267-269°C. NMR - δ_{H} (400 MHz; DMSO). 2.86 (2H, t, *J* 6.3 Hz, H-11), 6.59 (1H, d, *J* 5.4 Hz, H-3), 7.40 (1H, br s, -NH), 7.65 (1H, d, *J* 8.1 Hz, H-6), 8.06 (1H, br s, H-5), 8.46 (1H, br s, H-8), 8.48 (1H, d, *J* 5.4 Hz, H-2). δ_{C} (100 MHz; DMSO) 40.3 (C-11), 45.3 (C-10), 100.2 (C-3), 119.2 (C-5), 119.2 (C-4a), 121.4 (C-6), 124.4 (-CF₃), 126.7 (C-8), 126.8 (C-7), 147.9 (C-8a), 150.6 (C-2), 152.7 (C-4). HPLC *t_R* = 13.16 min, purity - 98%. HRMS (ESI) *m/z* 256.1060 [*M*⁺ C₁₂H₁₂N₃F₃, requires 256.1062].

***N*¹-(7-chloroquinolin-4-yl)ethane-1,2-diamine (48c)**



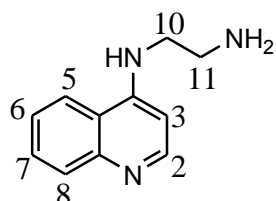
By the general procedure, the title compound was obtained as a yellow solid (90 mg, 60%), mp 136-137 °C (lit. Peck *et al* 1959, 137 - 139 °C). NMR - δ_{H} (400 MHz; DMSO) 2.98 (2H, t, *J* 6.4 Hz, H-11), 3.45 (2H, t, *J* 6.4 Hz, H-10), 6.57 (1H, d, *J* 5.7 Hz, H-3), 7.40 (1H, dd, *J* 2.1, 9.0 Hz, H-6), 7.78 (1H, d, *J* 2.1 Hz, H-8), 8.11 (1H, d, *J* 9.0 Hz, H-5), 8.36 (1H, d, *J* 5.6 Hz, H-2). δ_{C} (100 MHz; DMSO) 40.9 (C-11), 45.3 (C-10), 99.7 (C-3), 118.8 (C-4a), 124.3 (C-5), 126.1 (C-6), 127.7 (C-8), 136.4 (C-7), 149.8 (C-8a), 152.5 (C-2), 152.9 (C-4). HPLC *t_R* = 12.51 min, purity - 98%. HRMS (ESI) *m/z* 222.0802 [*M*⁺ C₁₁H₁₃ClN₃, requires 222.0798].

4-(2-Aminoethylamino)quinoline-7-carbonitrile (48e)



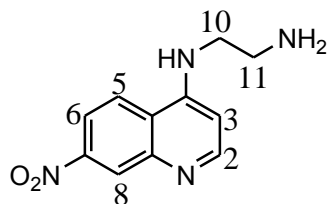
By the general procedure, the title compound was obtained as a white solid (60 mg, 50%), mp 161-163 °C. NMR - δ_{H} (400 MHz; DMSO) 2.81 (3H, t, J 6.4 Hz, H-11), 3.26 (3H, t, J 5.3 Hz, H-10), 6.57 (1H, d, J 5.5 Hz, H-3), 7.35 (1H, br s, Ar-NH), 7.68 (1H, dd, J 1.7, 8.7 Hz, H-6), 8.22 (1H, d, J 1.7 Hz, H-8), 8.39 (1H, d, J 8.7 Hz, H-5), 8.46 (1H, d, J 5.4 Hz, H-3). δ_{C} (100 MHz; DMSO) 46.3 (C-10), 100.6 (C-7), 111.8 (Ar-CN), 119.2 (C-3), 121.9 (C-5), 124.4 (C-4a), 124.9 (C-6), 135.1 (C-8), 147.9 (C-8a), 150.6 (C-2), 152.9 (C-4). HPLC t_{R} = 12.92 min, purity - 98%. HRMS (ESI) m/z 213.1156 [M^+ $\text{C}_{12}\text{H}_{13}\text{N}_4$, requires 213.1140].

N-(Quinolin-4-yl)ethane-1,2-diamine (48f)



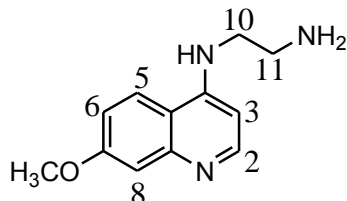
By the general procedure, title compound was obtained as a white solid (90mg, 55%), mp 170-172°C. NMR - δ_{H} (400 MHz; DMSO) 2.86 (2H, t, J 6.3Hz, H-11), 3.28 (2H, t, J 6.4Hz, H-10), 3.64 (1H, br s, -NH), 6.47 (1H, d, J 5.4Hz, H-3), 7.41 (1H, dt, J 1.3, 8.1Hz, H-6), 7.60 (1H, m, H-7), 7.78 (1H, td, J 1.0, 5.0, 8.3Hz, H-5), 8.19 (1H, t, J 7.7Hz, H-8), 8.38 (1H, d, J 5.3Hz, H-2). δ_{C} (100 MHz; DMSO) 40.8 (C-11), 45.8 (C-10), 98.0 (C-3), 121.3 (C-5), 123.4 (C-4a), 123.6 (C-6), 128.3 (C-8), 128.9 (C-7), 148.2 (C-8a), 149.6 (C-2), 150.4 (C-4). HPLC t_{R} = 13.71 min, purity - 98%. HRMS (ESI) m/z 188.1188 [M^+ $\text{C}_{11}\text{H}_{13}\text{N}_3$, requires 188.1192].

***N*¹-(7-nitroquinolin-4-yl)ethane-1,2-diamine (48h)**



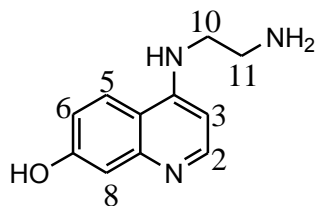
By the general procedure, the title compound was obtained as a yellow solid (90 mg, 55%), mp 170-172 °C. NMR - δ_{H} (400 MHz; DMSO) 2.82 (2H, t, *J* 6.1 Hz, H-11), 3.22 (2H, t, *J* 6.1 Hz, H-10), 6.79 (1H, d, *J* 5.5 Hz, H-3), 7.74 (1H, d, *J* 8.4 Hz, H-5), 7.91 (1H, d, *J* 1.2 Hz, H-8), 8.09 (1H, dd, *J* 1.3, 8.5 Hz, H-6), 8.54 (1H, d, *J* 5.5 Hz, H-2). δ_{C} (100 MHz; DMSO) 45.9 (C-10), 103.2 (C-3), 110.6 (C-8), 122.5 (C-6), 128.1 (C-5), 134.6 (C-4a), 147.4 (C-8a), 148.8 (C-2), 149.8 (C-7), 151.8 (C-4). HPLC t_{R} = 10.38 min, purity - 98%. HRMS (ESI) *m/z* 256.97 [M^+ C₁₁H₁₃N₄O₂, requires 256.08].

***N*¹-(7-Methoxyquinolin-4-yl)ethane-1,2-diamine (48i)**



By the general procedure, the title compound was obtained as a beige solid (50 mg, 50%), mp 309-312 °C. NMR - δ_{H} (400 MHz; DMSO) 2.79 (2H, t, *J* 5.9 Hz, H-11), 3.06 (2H, t, *J* 5.8 Hz, H-10), 3.88 (3H, s, Ar-OCH₃), 5.56 (1H, s, Ar-NH), 6.31 (1H, d, *J* 5.4 Hz, H-3), 7.03 (1H, dd, *J* 2.6, 9.1 Hz, H-6), 7.30 (1H, d, *J* 2.4 Hz, H-8), 7.65 (1H, d, *J* 9.2 Hz, H-5), 8.43 (1H, d, *J* 5.3 Hz, H-2). δ_{C} (100 MHz; DMSO) 14.2 (-OCH₃), 60.97 (C-11), 94.7 (C-12), 116.2 (C-8), 120.3 (C-6), 123.1 (C-3), 130.1 (C-4a), 135.3 (C-5), 139.8 (C-8a), 151.3 (C-2), 165.6 (C-4), 168.4 (C-7). HPLC t_{R} = 11.32 min, purity - 98%. HRMS (ESI) *m/z* 218.1293 [M^+ C₁₂H₁₆N₃O, requires 218.1299].

4-(2-Aminoethylamino)quinolin-7-ol (48j)

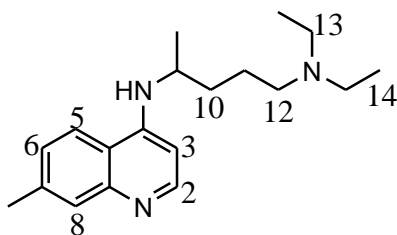


By the general procedure, the title compound was obtained as a white solid (20 mg, 55%), mp 172-174 °C. NMR - δ_{H} (400 MHz; DMSO). 3.05 (2H, br s, H-11), 3.92 (2H, br s, H-10), 6.63 (1H, d, J 7.0 Hz, H-3), 7.28 (1H, dd, J 2.2, 9.3 Hz, H-6), 7.35 (1H, d, J 2.0 Hz, H-8), 8.44 (1H, d, J 6.9 Hz, H-2), 8.48 (1H, d, J 9.3 Hz, H-5), 9.21 (1H, br s, OH). δ_{C} (100 MHz; DMSO) 38.0 (C-11), 56.3 (C-10), 111.8 (C-3), 115.2 (C-8), 119.0 (C-6), 122.7 (C-6), 129.1 (C-5), 147.9 (C-8a), 149.4 (C-2), 155.0 (C-4), 157.9 (C-7). HPLC t_{R} = 6.32 min, purity - 98%. HRMS (ESI) m/z 203.1061 [M^+ $\text{C}_{11}\text{H}_{14}\text{N}_3\text{O}$, requires 203.1059].

General synthesis of N^1, N^1 -diethylpentane-1,4-diamine-7-X-quinolines

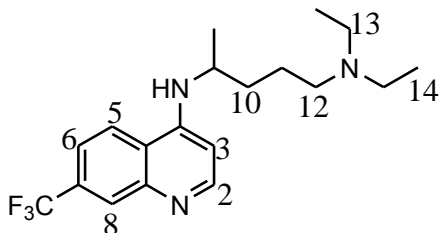
4-Chloro-7-substituted quinoline (1 eqv.) and N^1, N^1 -diethylpentane-1,4-diamine (4 eqv.) were mixed and heated at 90 °C for an hour under an inert atmosphere. Temperature was then increased to 140 °C for 15 hours after which mixture was cooled to room temperature. A saturated solution of potassium carbonate was added to the mixture and product extracted several times with dichloromethane. Organic fractions were combined, dried over MgSO_4 and concentrated. Crude product was purified on preparative silica gel TLC plates eluting with ethyl acetate: hexane: triethylamine (6:3:1). Further purification was performed on preparative HPLC.

***N*¹, *N*¹-diethyl-*N*⁴-(7-methylquinolin-4-yl) pentane-1, 4-diamine (56a)**



By the general procedure, the title compound was obtained as a yellow solid (0.94 g, 78%), mp 98-99 °C. NMR data δ_{H} (400 MHz; CDCl_3) 1.04 (6H, t, J 7.1 Hz, 2 x H-14), 1.34 (3H, d, J 6.3 Hz, $\text{NHCH}(\text{CH}_3)\text{CH}_2$), 1.67 (4H, m, H-10 & 11), 2.54 (3H, s, Ar- CH_3), 2.56 (6H, m, H-12 & 13), 3.74 (1H, m, H-9), 5.12 (1H, d, NH , J 6.8 Hz), 6.41 (1H, d, J 5.3 Hz, H-3), 7.26 (1H, d, J 8.4 Hz, H-6), 7.64 (1H, d, J 8.5 Hz, H-5), 7.77 (1H, s, H-8), 8.51 (1H, d, J 5.3 Hz, H-2). δ_{C} (100 MHz; CDCl_3) 11.5 (2 x C-14), 20.3 (- $\text{NHCH}(\text{CH}_3)\text{CH}_2$ -), 21.5 (Ar- CH_3), 24.0 (C-11), 34.7 (C-10), 46.8 (C-9), 48.2 (C-13), 52.7 (C-12), 98.5 (C-3), 116.8 (C-4a), 119.1 (C-5), 126.4 (C-6), 129.1 (C-8), 138.9 (C-7), 148.8 (C-8a), 148.9 (C-2), 151.0 (C-4). HPLC t_{R} = 15.78 min, purity - 98%. HRMS (ESI) m/z 300.2440 [M^+ $\text{C}_{19}\text{H}_{30}\text{N}_3$, requires 300.2451].

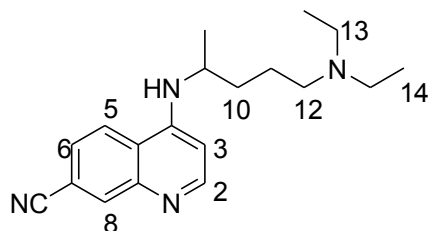
***N*¹, *N*¹-diethyl-*N*⁴-(7-(trifluoromethyl)quinolin-4-yl) pentane-1, 4-diamine (56b)**



By the general procedure, the title compound was obtained as yellow oil (36 mg, 40%). NMR data δ_{H} (400 MHz; CDCl_3) 0.90 (6H, t, J 7.2 Hz, 2 x CH_3), 0.95 [3H, d, J 6.3 Hz, $\text{NHCH}(\text{CH}_3)\text{CH}_2$], 1.41 [4H, m, 2 x CH_2 (- $(\text{CH}_3)\text{CCH}_2\text{CH}_2$ -)], 2.40 (6H, q, J 7.2 Hz, 3 x CH_2 [- $\text{CH}_2\text{NCH}_2\text{CH}_2$ -]), 2.78 [1H, m, $\text{NH-CH}(\text{CH}_3)\text{-CH}_2$], 5.28 (1H, d, J 8.1 Hz, NH), 6.40 (1H, d, J 5.4 Hz, H-3), 7.44 (1H, dd, J 2.1, 8.8 Hz, H-6), 7.72 (1H, d, J 1.8 Hz, H-8), 8.26 (1H, d, J 8.8 Hz, H-5), 8.77 (1H, d, J 4.7 Hz, H-2). δ_{C} (100 MHz; CDCl_3) 11.4 (C-14), 20.1

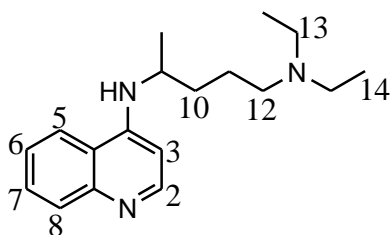
[NHCH(CH₃)CH₂], 23.9 (C-11), 34.5 (C-10), 46.9 (C-9), 48.4 (C-13), 52.6 (C-12), 100.3 (C-3), 119.7 (C-6), 119.8 (C-4a), 120.7 (C-5), 121.0 (-CF₃), 127.7 (C-8), 127.7 (C-7), 148.0 (C-8a), 148.9 (C-2), 152.2 (C-4). HPLC *t*_R = 15.30 min, purity - 98%. HRMS (ESI) *m/z* 354.2162 [M⁺ C₁₉H₂₇F₃N₃, requires 254.2157].

***N*¹, *N*¹-diethyl-*N*⁴-(7-cyanoquinolin-4-yl) pentane-1, 4-diamine (56e)**



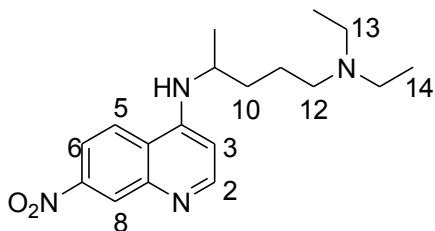
By the general procedure, the title compound was obtained as oil (20 mg, 30%). NMR data δ_{H} (400 MHz; CDCl₃) 0.98 (6H, t, *J* 7.2 Hz, 2 × H-14), 1.30 [3H, d, *J* 6.3 Hz, -CH(CH₃)CH₂-], 1.68 (4H, m, H-10 & 11), 2.43 (2H, t, *J* 6.8 Hz, H-12), 2.50 (4H, q, *J* 7.2 Hz, 2 × H-13), 3.69 (1H, m, H-9), 5.46 (1H, br s, NH), 6.50 (1H, d, *J* 5.8 Hz, H-3), 7.51 (1H, dd, *J* 1.7, 8.7 Hz, H-6), 7.82 (1H, d, *J* 8.6 Hz, H-5), 8.27 (1H, d, *J* 1.6 Hz, H-8), 8.57 (1H, d, *J* 5.4 Hz, H-2). δ_{C} (100 MHz; CDCl₃) 11.3 (C-14), 20.0 (-CH(CH₃)CH₂-), 23.9 (C-11), 34.5 (C-10), 46.9 (C-9), 48.5 (C-13), 52.5 (C-12), 100.8 (C-3), 112.5 (C-7), 118.6 (-CN), 121.4 (C-4a), 121.4 (C-5), 124.9 (C-6), 135.7 (C-8), 147.9 (C-8a), 148.9 (C-2), 152.6 (C-4). HPLC *t*_R = 15.10 min, purity - 98%. HRMS (ESI) *m/z* 311.2236 [M⁺ C₁₉H₂₇N₄, requires 311.2236].

***N*¹, *N*¹-diethyl-*N*⁴-(quinolin-4-yl) pentane-1, 4-diamine (56f)**



By the general procedure, the title compound was obtained as a yellow oil (24 mg, 65%). δ_{H} (400 MHz; CDCl_3) 0.95 [6H, t, J 7.1 Hz, 2 \times (H-14) CH_3], 1.25 [3H, d, J 6.3 Hz, $\text{NHCH}(\text{CH}_3)\text{CH}_2$], 1.57 [4H, m, 2 \times CH_2 (H-10 & 11)], 2.44 [6H, m, 3 \times CH_2 (H-12 & 13)], 3.66 (1H, m, H-9), 5.04 (1H, d, J 7.4 Hz, NH), 6.36 (1H, d, J 5.2 Hz, H-3), 7.33 (1H, m, Ar-H), 7.54 (1H, m, Ar-H), 7.66 (2H, m, Ar-H), 8.46 (1H, d, J 5.4 Hz, H-2). δ_{C} (100 MHz; CDCl_3) 11.4 (2 \times C-14), 20.3 (NHCHCH_3), 23.8 (C-11), 34.6 (C-10), 46.9 (C-9), 48.2 (C-13), 52.7 (C-12), 99.0 (C-3), 119.4 (C-6), 124.4 (C-8), 128.9 (C-7), 130.0 (C-8a), 149.0 (C-2), 151.0 (C-4). HPLC t_{R} = 15.08 min, purity - 98%. HRMS (ESI) m/z 286.2283 [M^+ $\text{C}_{18}\text{H}_{28}\text{N}_3$, requires 286.2295].

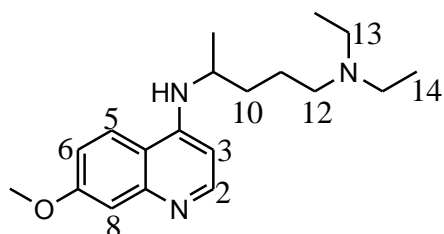
***N*¹, *N*¹-diethyl-*N*⁴-(7-nitroquinolin-4-yl) pentane-1, 4-diamine (56h)**



By the general procedure, the title compound was obtained as a yellow oil (25 mg, 20%). NMR δ_{H} (400 MHz; CDCl_3) 1.00 (6H, t, J 7.1 Hz 2 \times H-14), 1.32 [3H, d, J 6.3 Hz, - $\text{CH}(\text{CH}_3)\text{CH}_2$ -], 1.66 (4H, m, H-10 & 11), 2.45 (2H, t, J 6.8 Hz, H-12), 2.52 (4H, q, J 7.1 Hz, H-13), 3.72 (1H, m, H-9), 5.59 (1H, br s, NH), 6.53 (1H, d, J 5.5 Hz, H-3), 7.87 (1H, d, J 9.2 Hz, H-5), 8.12 (1H, dd, J 2.4, 9.2 Hz, H-6), 8.63 (1H, d, J 5.4 Hz, H-2), 8.81 (1H, d, J 2.4 Hz, H-8). δ_{C} (100 MHz; CDCl_3) 42.7 (C-14), 47.9 [$\text{CH}(\text{CH}_3)\text{CH}_2$ -], 62.8 (C-10), 63.7 (C-11),

101.9 (C-9), 118.0 (C-12), 121.3 (C-3), 122.3 (C-8), 126.1 (C-6), 126.7 (C-5), 128.5 (C-4a), 129.0 (C-8a), 136.7 (C-2), 149.0 (C-7), 153.8 (C-4). HPLC t_R = 15.70 min, purity - 98%. HRMS (ESI) m/z 331.2134 [M^+ C₁₈H₂₇N₄O₂, requires 331.2133].

***N*¹, *N*¹-diethyl-*N*⁴-(7-methoxyquinolin-4-yl) pentane-1, 4-diamine (56i)**



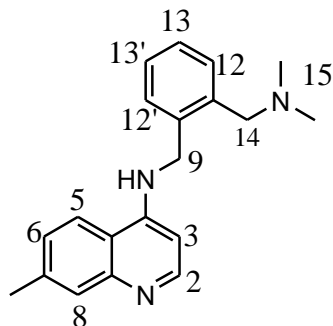
By the general procedure, the title compound was obtained as a yellow oil (39 mg, 60%). NMR data δ_H (400 MHz; CDCl₃) 1.01 (6H, t, J 7.2 Hz, 2 \times CH₃), 1.31 [3H, d, J 6.3 Hz, -CH(CH₃)CH₂-], 1.63 (4H, m, H-10 & 11), 2.54 (6H, m, 3 \times CH₂ H-12 & 13), 3.71 (1H, m, H-10), 3.92 (3H, s, OCH₃), 5.06 (1H, d, J 7.0 Hz, NH), 6.34 (1H, d, J 5.5 Hz, H-3), 7.04 (1H, dd, J 2.7, 9.2 Hz, H-6), 7.32 (1H, d, J 2.6 Hz, H-8), 7.61 (1H, d, J 9.2 Hz, H-5), 8.45 (1H, d, J 5.5 Hz, H-2). δ_C (100 MHz; CDCl₃) 11.4 (C-14), 20.4 [NH-CH(CH₃)CH₂], 23.8 (C-11), 34.7 (C-10), 46.8 (C-9), 48.2 (C-13), 52.6 (C-12), 55.4 (-OCH₃), 98.0 (C-3), 108.1 (C-8), 113.3 (C-6), 116.7 (C-4a), 120.8 (C-5), 149.2 (C-8a), 150.3 (C-2), 151.1 (C-4), 160.3 (C-7). HPLC t_R = 15.43 min, purity - 98%. HRMS (ESI) m/z 316.2389 [M^+ C₁₉H₃₀N₃O, requires 316.2392].

General synthesis of 4-(2-(dimethylaminomethyl) benzylamine)-7-X-quinolines

2-(Dimethylaminomethyl) benzylamine (**53**) (1 eqv.), 4-chloro-7-X-quinoline (1.1 eqv.), potassium carbonate (2 eqv.), anhydrous triethylamine (6 eqv.) and anhydrous *N*-methyl-2-pyrrolidinone (NMP) (6 mL) were placed in a 25 mL flask with a stirrer and allowed to heat under reflux and nitrogen for 15 hrs at 100 °C. The mixture was allowed to cool to room temperature before diluting with ethyl acetate. The mixture was washed several times with brine and the organic layer dried over sodium sulphate, filtered and solvent removed under reduced pressure. The product was purified using silica gel

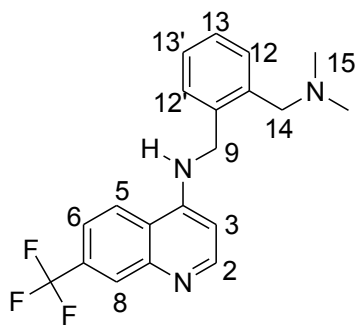
chromatography (ethyl acetate: hexane: Et₃N, 45:50:5). Further purification was carried out on preparative HPLC.

N-(2-((Dimethylamino)methyl)benzyl)-7-methylquinolin-4-amine (57a)



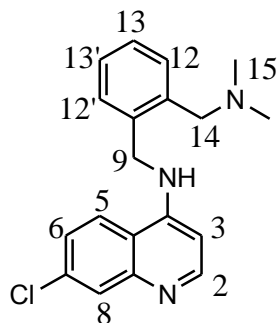
By the general procedure, the title compound was obtained as white solid (20 mg, 35%), mp 80-83 °C. NMR δ_{H} (400 MHz; CDCl₃) 2.28 (6H, s, 2 × CH₃), 2.49 (3H, s, Ar-CH₃), 3.50 (2H, s, H-14), 4.48 (2H, d, *J* 5.0 Hz, H-9), 6.52 (1H, d, *J* 5.5 Hz, H-3), 7.19 (1H, dd, *J* 1.7, 8.6 Hz, H-6), 7.29 (3H, m, Ar-H), 7.42 (1H, m, Ar-H), 7.59 (1H, d, *J* 8.6 Hz, H-5), 7.77 (1H, br s, NH), 8.27 (1H, br s, H-8), 8.51 (1H, d, *J* 5.5 Hz, H-2). δ_{C} (100 MHz; CDCl₃) 21.5 (Ar-CH₃), 45.0 (C-15), 47.3 (C-9), 62.6 (C-14), 98.0 (C-3), 117.3 (C-4a), 120.4 (C-5), 126.5 (C-6), 127.9 (C-13), 128.0 (C-12'), 128.5 (C-13'), 130.6 (C-12), 131.9 (C-8), 137.2 (C-11), 137.5 (C-7), 139.4 (C-10), 147.8 (C-8a), 150.1 (C-2), 151.0 (C-4). HPLC t_{R} = 15.57 min, purity - 98%. HRMS (ESI) m/z 306.1970 [M⁺ C₂₀H₂₄N₃, requires 306.1984].

(7-Trifluoro-quinolin-4-yl)-(2-dimethylaminomethyl benzylamine) (57b)



By the general procedure, the title compound was obtained as white solid (80 mg, 60%), mp 165- 166 °C. NMR data - δ_{H} (400 MHz; CDCl_3) 2.30 (6H, s, CH_3), 3.15 (2H, s, CH_2 , H-14), 4.48 (2H, s, CH_2 , H-9), 6.64 (1H, d, J 5.4 Hz, H-3), 7.30 (3H, m, Ar-H), 7.43 (1H, m, Ar-H), 7.51 (1H, dd, J 1.7, 8.8 Hz, H-6), 7.80 (1H, d, J 8.8 Hz, H-5), 8.23 (1H, br s, H-8), 8.50 (1H, br s, NH), 8.64 (1H, d, J 5.4 Hz, H-2); δ_{C} (100 MHz; CDCl_3) 45.0, (C-15), 47.5 (C-14), 62.6 (C-9), 99.8 (C-3), 119.6 (C-6), 121.5 (C-4a), 122.8 (C-5), 124.0 ($-\text{CF}_3$), 127.5 (C-8), 128.1 (C-12), 128.6 (C-13'), 130.7 (C-7), 130.7 (C-13), 132.1 (C-12), 137.1 (C-10), 137.3 (C-11), 148.0 (C-4), 150.4 (C8a), 152.4 (C-2). HPLC t_{R} = 14.08 min, purity - 98%. HRMS (ESI) m/z 359.1609 [M^+ $\text{C}_{20}\text{H}_{21}\text{F}_3\text{N}_3$, requires 359.1611].

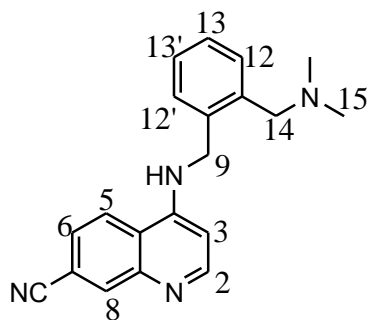
7-Chloro-N-(2-((dimethylamino)methyl)benzyl)quinoline-4-amine (57c)



By the general procedure, the title compound was obtained as white solid (60 mg, 60%), mp 187-188 °C. NMR data - δ_{H} (400 MHz; CDCl_3) 2.27 (6H, s, $2 \times \text{CH}_3$), 3.49 (2H, s, H-14), 4.45 (2H, d, J 4.7 Hz, H-9), 6.54 (1H, d, J 5.4 Hz, H-3), 7.30 (4H, m, Ar-H), 7.42 (1H, m,

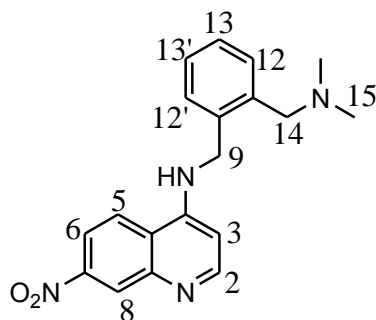
Ar-H), 7.62 (1H, d, *J* 9.0 Hz, H-5), 7.92 (1H, d, *J* 2.1 Hz, H-8), 8.33 (1H, br s, NH), 8.55 (1H, d, *J* 5.4 Hz, H-2). δ_C (100 MHz; CDCl₃) 45.0 (CH₃), 47.5 (C-9), 62.6 (C-14), 98.8 (C-3), 117.9 (C-4a), 122.2 (C-5), 125.0 (C-12'), 127.0 (C-11), 128.0 (C-12), 128.6 (C-6), 130.7 (C-12'), 132.0 (C-8), 135.0 (C-13), 137.0 (C-7), 137.3 (C-10), 148.6 (C-8a), 150.9 (C-2), 151.4 (C-4). HPLC *t_R* = 14.56 min, purity - 98%. HRMS (ESI) *m/z* 325.1338 [M⁺ C₁₉H₂₁ClN₃, 325.1345].

4-(2-((Dimethylamino)methyl)benzylamino)quinoline-7-carbonitrile (57e)



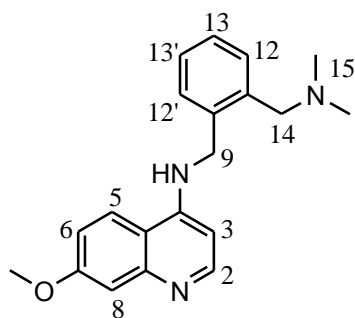
By the general procedure, the title compound was obtained as a white solid (20 mg, 30%), mp 153-154°C. NMR δ_H (400 MHz; CDCl₃) 2.27 (6H, s, H-15), 3.49 (2H, s, H-14), 4.45 (2H, d, *J* 3.9 Hz, H-9), 6.62 (1H, d, *J* 5.4 Hz, H-3), 7.30 (3H, m, Ar-H), 7.40 (1H, m, Ar-H), 7.46 (1H, dd, *J* 1.7, 8.7 Hz, H-6), 7.75 (1H, d, *J* 8.7 Hz, H-5), 8.26 (1H, d, *J* 1.4 Hz, H-8), 8.56 (1H br s, NH), 8.63 (1H, d, *J* 5.4 Hz, H-2). δ_C (100 MHz; CDCl₃) 45.0 (C-15), 47.6 (C-9), 62.6 (C-14), 100.3 (C-3), 112.3 (C-7), 118.7 (-CN), 122.3 (C-5), 124.7 (C-6), 128.1 (C-12'), 128.6 (C-13), 130.7 (C-13'), 132.1 (C-12), 135.5 (C-11), 137.0 (C-8), 137.2 (C-10), 147.9 (C-8a), 150.3 (C-2), 152.8 (C-4). HPLC *t_R* = 15.55 min, purity - 98%. HRMS (ESI) *m/z* 317.1766 [M⁺ C₂₀H₂₁N₄, requires 317.1753].

N-(2-((Dimethylamino)methyl)benzyl)-7-nitroquinolin-4-amine (57h)



By the general procedure, the title compound was obtained as yellow solid (40 mg, 40%), mp 141-143 °C. NMR δ_{H} (400 MHz; CDCl_3) 2.28 (6H, s, H-15), 3.50 (2H, s, H-14), 4.46 (2H, d, J 3.4 Hz, H-9), 6.66 (1H, d, J 5.4 Hz, H-3), 7.31 (3H, m, Ar-H), 7.41 (1H, m, Ar-H), 7.80 (1H, d, J 9.2 Hz, H-5), 8.07 (1H, dd, J 2.4 Hz, 9.2 Hz, H-6), 8.67 (1H, d, J 5.4 Hz, H-2), 8.79 (1H, d, J 2.3 Hz, H-8). δ_{C} (100 MHz; CDCl_3) 45.1 (C-15), 47.9 (C-9), 62.8 (C-14), 100.9 (C-3), 117.1 (C-117.6 (C-6), 122.8 (C-5), 123.8 (C-4a), 125.7 (C-8), 128.5 (C-12), 131.4 (C-13'), 132.3 (C-13), 145.2 (C-8a), 152.1 (C-2), 153.3 (C-7), 154.8 (C-4). HPLC t_{R} = 16.32 min, purity - 98%. HRMS (ESI) m/z 337.1665 [M^+ $\text{C}_{19}\text{H}_{21}\text{N}_4\text{O}_2$, requires 337.1668].

N, (2(Dimethylamino) methyl) benzyl)-7-methoxyquinolin-4-amine (57i)



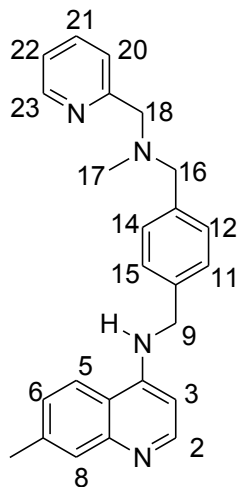
By the general procedure, the title compound was obtained as a white solid (60 mg, 60%), mp 218-220 °C. NMR δ_{H} (400 MHz; CDCl_3) 2.28 (6H, s, $2 \times \text{CH}_3$), 3.50 (2H, s, H-14), 3.92 (3H, s, Ar- OCH_3), 4.48 (2H, d, J 4.9 Hz, H-9), 6.50 (1H, d, J 5.7 Hz, H-3), 7.00 (1H, dd, J 2.6, 9.2 Hz, H-6), 7.30 (3H, m, Ar-H), 7.39 (1H, d, J 2.6 Hz, H-8), 7.42 (1H, m, Ar-H),

7.58 (1H, d, *J* 9.2 Hz, H-5), 8.46 (1H, d, *J* 5.7 Hz, H-2). δ_c (100 MHz; CDCl₃) 45.0 (CH₃), 47.4 (C-9), 55.5 (OCH₃), 62.6 (C-14), 97.5 (C-3), 106.7 (C-4a), 116.9 (C-6), 121.9 (C-5), 128.0 (C-13), 130.6 (C-12), 131.9 (C-11), 137.1 (C-10), 137.3 (C-8a), 149.6 (C-2), 151.4 (C-4), 160.7 (C-7). HPLC t_R = 14.71 min, purity - 98%. HRMS (ESI) m/z 322.1919 [M^+ C₂₀H₂₄N₃O, requires 322.1917].

General synthesis of PDB series of compounds

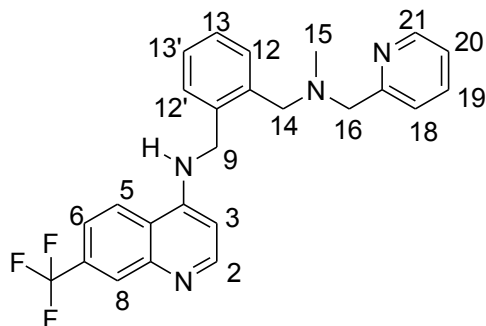
To a stirred solution of the corresponding 4-chloro-7-X-quinoline (**26**) compound in anhydrous N-methyl-2-pyrrolidone (NMP) (5 mL) were added K₂CO₃ (3 eqv.), Et₃N (3 eqv.) and the corresponding amine (1 eqv.). The amines used were *m*-[(*N*-benzyl-*N*-methyl)aminomethyl]-benzylamine, *o*, *m* & *p*-*N*-[(aminomethyl)benzyl]-*N*-methyl-1-(pyridine-2-yl-methanamine. The mixtures were heated at 120 °C for 15 hours under an inert atmosphere. The mixtures were then cooled to room temperature and diluted with ethyl acetate (30 mL). The mixtures were washed 10 times with a solution of brine. The organic layers were dried with Mg₂SO₄ and concentrated to afford the crude products which were purified using preparative thin layer chromatography plates using mixture ethyl acetate and hexane (90:10) as the solvent. Products were further purified with preparative HPLC using MeOH/NH₄CO₃ pH 10.7 and H₂O/NH₄CO₃ pH 10.7 as the mobile phase and chromatographic purities of all compounds were >98%.

**7-Methyl-N-(4-((methyl(pyridine-2-ylmethyl)amino)methyl)benzyl)quinoline-4-amine
(58a)**



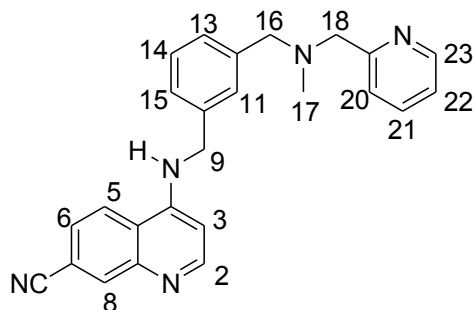
By the general procedure, the title compound was obtained as a white solid (20 mg, 30%), mp 73-76 °C. NMR δ_{H} (400 MHz; CDCl_3) 2.24 (3H, s, H-17), 2.49 (3H, s, Ar- CH_3), 3.58 (2H, s, 16), 3.68 (2H, s, 18), 4.48 (2H, d, J 5.0 Hz, H-9), 5.32 (1H, br s, NH), 6.39 (1H, d, J 5.3 Hz, H-3), 7.13 (1H, m, Ar-H), 7.33 (2H, m, Ar-H), 7.39 (1H, m, Ar-H), 7.47 (1H, m, Ar-H), 7.62 (1H, d, J 8.8 Hz, H-6), 7.76 (1H, s, H-8), 7.88 (1H, m, H-20), 8.10 (1H, d, J 8.6 Hz, H-5), 8.48 (1H, m, H-21), 8.52 (1H, d, J 5.1 Hz, H-2), 8.71 (1H, d, J 4.7 Hz, H-23). δ_{C} (100 MHz; CDCl_3) 21.7 (C-17), 42.5 (Ar- CH_3), 47.4 (C-9), 61.7 (C-16), 63.4 (C-18), 98.9 (C-3), 116.6 (C-4a), 119.7 (C-5), 120.5 (C-22), 121.9 (C-20), 122.9 (C-15), 123.8 (C-11), 126.9 (C-6), 127.6 (C-12), 128.8 (C-14), 128.9 (C-8), 129.1 (C-13), 129.5 (C-21), 129.9 (C-7), 136.4 (C-8a), 138.9 (C-10), 140.9 (C-23), 149.0 (C-2), 149.8 (C-4), 150.8 (C-19). HPLC t_{R} = 15.36 min, 10 - 100%B/20 min. HRMS (ESI) m/z 383.2225 [M^+ $\text{C}_{25}\text{H}_{27}\text{N}_4$, requires 383.2236].

***N*-2-((Methyl(pyridine-2-yl-methyl)amino)benzyl)-7-(trifluoromethyl)quinoline-4-amine (58b)**



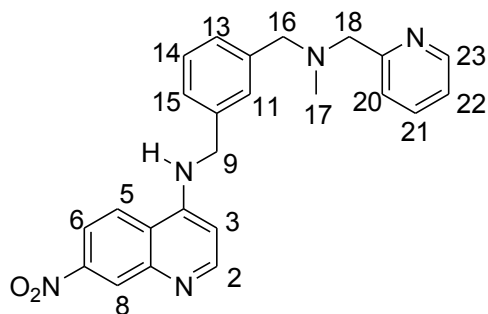
By the general procedure, the title compound was obtained as a white solid (60 mg, 60%), mp 81-83 °C. NMR δ_{H} (400 MHz; CDCl_3) 2.19 (3H, s, H-15), 3.71 (2H, s, H-14), 3.75 (2H, s, H-16), 4.46 (2H, d, J 5.3 Hz, H-9), 6.59 (1H, d, J 5.4 Hz, H-3), 7.12 (2H, m, 12 & 12'), 7.21 (1H, dd, J 1.9, 8.8 Hz, H-6), 7.28 (3H, m, H-13, 13' & 18), 7.41 (1H, m, H-19), 7.47 (1H, m, H-20), 7.72 (1H, br t, J 5.3 Hz, NH), 7.82 (1H, d, J 8.8 Hz, H-5), 8.18 (1H, br s, H-8), 8.49 (1H, m, H-21), 8.58 (1H, d, J 5.4 Hz, H-2). δ_{C} (100 MHz; CDCl_3) 42.2 (C-15), 46.6 (C-9), 61.5 (C-14), 63.9 (C-16), 100.0 (C-3), 119.4 (C-6), 119.4 (C-20), 122.5 (C-4a), 122.3 (C-5), 124.0 (-CF₃), 127.3 (C-13), 127.3 (C-12'), 127.9 (C-13'), 128.3 (C-8), 130.6 (C-12), 132.1 (C-7), 136.4 (C-11), 137.1 (C-19), 137.2 (C-10), 148.0 (C-8a), 149.5 (C-21), 150.2 (C-2), 152.3 (C-4), 157.9 (C-17). HPLC t_{R} = 16.57 min, 10 - 100%B/20 min. HRMS (ESI) m/z 437.1953 [M^+ C₂₅H₂₄F₃N₄, requires 437.1973].

4-(3-((Methyl(pyridine-2-ylmethyl)amino)methyl)benzylamine)quinoline-7-carbonitrile (58e)



By the general procedure, the title compound was obtained as a white solid (30 mg, 40%), mp 259-261 °C. NMR δ_{H} (400 MHz; CDCl_3) 2.23 (3H, s, H-17), 3.59 (2H, s, H-16), 3.66 (2H, s, H-18), 4.51 (2H, d, J 5.2 Hz, H-9), 5.43 (1H, br t, J 5.0 Hz, NH), 6.54 (1H, d, J 5.4 Hz, H-3), 7.11 (1H, m, Ar-H), 7.25 (1H, dd, J 1.6, 4.7 Hz, H-6), 7.32 (2H, m, Ar-H), 7.40 (2H, m, Ar-H), 7.56 (2H, m, Ar-H), 7.84 (1H, d, J 8.5 Hz, H-5), 8.32 (1H, d, J 1.3 Hz, H-8), 8.49 (1H, ddd, J 0.9, 1.8, 4.9 Hz, H-23) 8.59 (1H, d, J 5.3 Hz, H-2). δ_{C} (100 MHz; CDCl_3) 42.6 (C-17), 47.7 (C-9), 61.9 (C-16), 63.3 (C-18), 101.3 (C-7), 112.7 (-CN), 118.5 (C-3), 121.2 (C-22), 122.0 (C-5), 122.9 (C-4a), 125.4 (C-6), 126.3 (C-20), 128.0 (C-13), 128.1 (C-15), 128.8 (C-14), 129.0 (C-11), 135.8 (C-12), 136.4 (C-8), 136.8 (C-21), 140.1 (C-10), 147.7 (C-8a), 149.1 (C-23), 149.2 (C-2), 152.8 (C-4), 159.4 (C-19). HPLC t_{R} = 13.51 min, purity - 98%. HRMS (ESI) m/z 393.1953 [M^+ $\text{C}_{25}\text{H}_{24}\text{N}_5$, requires 393.1976].

***N*-3-((Methyl(pyridine-2-ylmethyl)amino)methyl)benzyl)-7-nitroquinolin-4-amine
(58h)**



By the general procedure, the title compound was obtained as a yellow solid (20 mg, 30%), mp 116-119 °C. NMR δ_{H} (400 MHz; CDCl_3) 2.24 (3H, s, H-17), 3.60 (2H, s, H-16), 3.67 (2H, s, H-18), 4.53 (2H, d, J 5.1 Hz, H-9), 5.42 (1H, br t, J 4.6 Hz, NH), 6.57 (1H, d, J 5.4 Hz, H-3), 7.12 (1H, m, Ar-H), 7.27 (1H, dd, J 1.6, 4.6 Hz, H-13), 7.34 (2H, m, Ar-H), 7.41 (2H, m, Ar-H), 7.58 (1H, m, H-20), 7.88 (1H, d, J 9.2 Hz, H-5), 8.15 (1H, dd, J 2.4, 9.2 Hz, H-6), 8.50 (1H, m, H-23), 8.64 (1H, d, J 5.2 Hz, H-2), 8.85 (1H, d, J 2.3 Hz, H-8). δ_{C} (100 MHz; CDCl_3) 42.7 (C-17), 47.9 (C-9), 61.8 (C-16), 63.7 (C-18), 101.8 (C-3), 118.5 (C-6), 121.9 (C-5), 122.3 (C-22), 124.2 (C-20), 125.7 (C-15), 126.1 (C-8), 126.7 (C-13), 128.5 (C-14), 129.4 (C-11), 136.7 (C-21), 146.5 (C-23), 153.3 (C-2). HPLC t_{R} = 15.27 min, purity - 98%. HRMS (ESI) m/z 414.1930 [M^+ $\text{C}_{24}\text{H}_{24}\text{N}_5\text{O}_2$, requires 414.1925].