Synthesis, Antimalarial Evaluation and Structure-Activity Relationship in a Series of Dibenzylmethylamine (Dibemethin) Derivatives of 4-Amino-7-Chloroquinoline

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By

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ABSTRACT

A series of twelve 4-amino-7-chloroquinolines containing (*N, N*-dibenzylmethylamine) dibemethin side chains attached to the amino group of the quinoline were synthesized by reaction of aminomethyl dibemethins with 4,7-dichloroquinoline to give "dibemethinoquines". The attachment between the quinoline ring and the side chain was varied from *ortho*, to *meta* to *para*. The aminomethyl dibemethin side chains were synthesized using the Staudinger reduction of azides or the reductive amination *via* oxime of an aldehyde. Derivatives in which the *para* substituent on the terminal phenyl ring were altered from the parent compound (X = H) by replacement with Cl, OCH_3 or NMe_2 group were also synthesized. The choice of substituents was based on the Topliss scheme. Analogues of these aminomethyl dibemethins in which their primary amino terminus was formylated were also synthesized *via* a single step reaction with ethyl formate to afford amidomethyl dibemethins. Thus, three classes of compounds; aminomethyl dibemethins, their formylated derivatives (amidomethyl dibemethins) and the proposed "reversed chloroquine" dibemethinoquines were successfully synthesized. Their *in vitro* biological activity was evaluated against chloroquine-sensitive (D10) as well as chloroquine-resistant (K1 or in some cases W2) strains of *Plasmodium falciparum*. *In vivo* activity of these compounds was also tested for two representative compounds against *P. yoelii nigeriensis*. These compounds showed significant *in vivo* antimalarial activity in the mouse model *P. yoelii nigeriensis*, although neither was as active as chloroquine diphosphate when administered by intra-peritoneal injection. This observed lower activity relative to chloroquine diphosphate could be ascribed to poor solubility of the free bases of these compounds as they were administered as suspensions, whereas chloroquine diphosphate was a solution. A preliminary cytotoxicity investigation of the prototype compound in Chinese hamster ovarian (CHO) cells showed that it exhibits highly selective activity against the malaria parasite.

Dibemethinoquine compounds exhibited activity against both chloroquine sensitive and resistant parasites *in vitro*. Their IC_{50} values in the K1 strain ranged between 24 nM and 1 μM, with one compound showing no activity even at 23 μM. These values compared to 284 nM and 29 nM for chloroquine in chloroquine resistant and chloroquine sensitive

parasites respectively. Thus the most active compound had an IC_{50} almost identical to chloroquine in the chloroquine sensitive strain. Two other compounds showed similarly strong activity. Structure-activity studies show that, as with other chloroquine analogues, the activity of dibemethinoquine compounds results from their ability to accumulate in the parasite digestive vacuole and inhibit haemozoin formation. In dibemethinoquines, this inherent activity appears to depend on both the site of attachment of the aminoquinoline to the dibemethin side chain and the identity of the group on the terminal phenyl ring of the dibemethin in a manner that is too complex to rationalize on the basis of the limited number of derivatives used in this study. However, correlation analyses using a structural descriptor, where the position of attachment of the aminoquinoline to the dibemethin side chain was numbered 2, 3 and 4 for *ortho*-, *meta*- and *para*- positions respectively showed log IC_{50} to significantly correlated with position in the order *ortho*- > *meta*- > *para*.

Finally, it was found that the aminomethyl dibemethin side chains themselves exhibit resistance-reversal activity. Surprisingly, findings in this study show that the arrangement of a three carbon aminoalkyl chain positioned between the aromatic rings proposed by Bhattacharjee *et al* (2002), as ideal for imipramine-like resistance reversers is not essential for chloroquine resistance reversal. All of the aminomethyl dibemethin compounds exhibit stronger activity at 1 µM concentration than their formylated derivative. However, at their respective IC_{10} concentrations, both the aminomethyl and amidomethyl dibemethin compounds show chloroquine resistance reversing properties. The reduced resistance reversing activity of the formylated compounds can probably be ascribed to decreased pH trapping within the digestive vacuole of the malaria parasite.

DECLARATION

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

Vincent Kudakwashe Zishiri

Dedication

To my late father O.J Zishiri.

Acknowledgements

I would like to extend my sincere and heartfelt gratitude to my supervisors, Professors Timothy Egan and Roger Hunter for their expert guidance throughout the course of this project.

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Lastly, special mention goes to my wife Elizabeth, daughter Idanai, immediate family and friends for their continued moral support.

All glory be to God

List of abbreviations

3D-QSAR	Three dimensional quantitative structure-activity relationships
Ar	Aromatic
ArH	Aromatic proton
Ar_{qua}	Quaternary aromatic carbon
BHIA	Beta-haematin inhibitory assay
$BHIA_{50}$	50% Inhibitory concentration (molar equivalent relative to chloroquine)
brs.	Broad singlet
t-BuLi	*tert*-butyllithium
CD_3OD	Deuteromethanol
$CDCl_3$	Deuterochloroform
CH_3CN	Acetonitrile
d	Doublet (in ^1H NMR)
dd	Doublet of doublets (in ^1H NMR)
DDT	Dichlorodiphenyltrichloroethane
DMF	Dimethylformamide
DMSO	Dimethyl sulphoxide
equiv.	Equivalents
EI	Electron ionisation
ESI	Electron spray ionisation
Et_2O	Diethyl ether
EtOAc	Ethyl acetate
EtOH	Ethanol
Fe(III)PPIX	Ferriprotoporphyrin IX
g	Grams
Hex	Hexane
hr.	Hour
Hz	Hertz
HRMS	High Resonance Mass Spectrometry
HRP	Histidine rich protein
IC_{50}	50% inhibitory concentration
IR	Infra Red spectroscopy

J	coupling constants
K_2CO_3	Potassium carbonate
LG	Leaving group
$LiAlH_4$	Lithium aluminium hydride
m	Multiplet (in ^1H NMR)
m	*Meta*
M^+	Molecular ion
MeOH	Methanol
m.p.	Melting point
mg	Milligram(s)
$MgSO_4$	Magnesium sulphate
MHz	Mega hertz (in NMR)
ml	Millilitre(s)
mmol	Millimole(s)
mol	Mole(s)
MeO	Methoxy
$NaCNBH_3$	Sodium cyanoborohydride
NaN_3	Sodium azide
Na_2SO_4	Sodium sulphate
NBS	*N*-bromosuccinimide
NEt_3	Triethylamine
nM	Nanomolar
NMe_2	Dimethylamino
NMP	*N*-methyl-2-pyrrolidine
NMR	Nuclear magnetic resonance
o	*Ortho*
OPG	O-protecting group
p	*Para*
PFCRT	*Plasmodium falciparum* chloroquine resistant transporter protein
Pgh	P-glycoprotein homologue
PPh_3	Triphenylphosphine
ppm	Parts per million
QSAR	Quantitative structure-activity relationships
R_f	Retention factor

rt	Room temperature
s	Singlet (in ^{1}H NMR)
SAR	Structure-activity relationships
TCTP	Translationally controlled tumor protein
THF	Tetrahydrofuran
TLC	Thin layer chromatography
µM	Micromolar
v/v	Volume by volume

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CHAPTER 1: INTRODUCTION

1.1 MALARIA AS A GLOBAL PROBLEM

Infectious diseases are today the largest cause of death in developing countries and amongst the young.[1] Malaria ranks fifth in terms of numbers of deaths due to infectious disease. According to the World Health Organization (WHO), between 300 and 500 million clinical cases of malaria occur every year.[2] Malaria is estimated to kill as many as 1 million people annually, with most of the deaths occurring among children under the age of six living in sub-Saharan Africa. It is estimated that over 40% of the world's population lives in malaria endemic areas, which are localized around the tropical and subtropical regions (Figure 1.1).

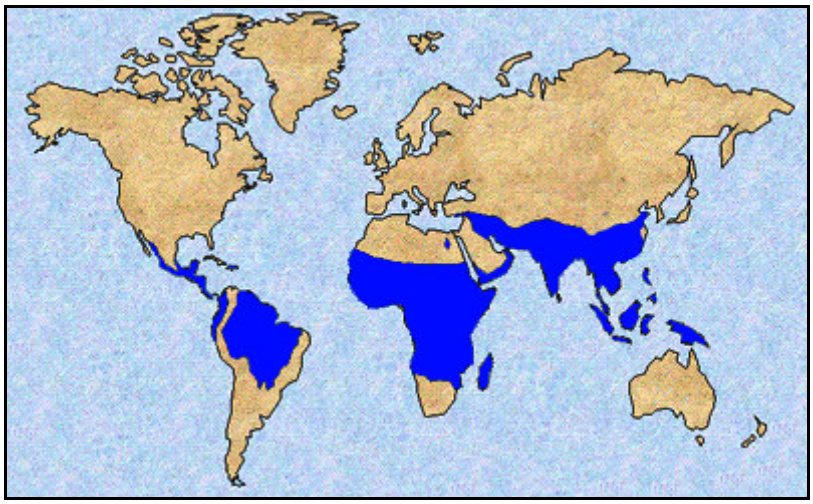


Figure 1.1: Global distribution of malaria transmission risk (www.cdc.gov/malaria/distribution).

The disease takes an economic toll as well because of reduced productivity, which is estimated to be responsible for an estimated average loss of 1.3% of economic growth annually in countries with intense transmission.[3]

Several organized efforts to control transmission of the disease have been made in the last 60 years; these include suppression of vector numbers therefore minimizing the

incidents of transmission, preventing contact between the host and an infected vector and / or the use of antimalarial drugs both in prophylaxis and treatment of the disease. Huge progress was made in reducing the number of malaria cases in the 1950's using these approaches. Coinciding with the discovery of the insecticide dichlorodiphenyltrichloroethane (DDT) and chloroquine chemotherapy, World Health Organization (WHO) embarked on a Global Malaria Eradication Campaign. The remarkable results were that malaria was eliminated completely from Taiwan, the Caribbean, the Balkans, parts of North Africa, in most parts of Europe, Northern Australia, a large portion of the South Pacific and huge drops in the malaria cases were observed in Sri Lanka and India[1]. By the mid-1960's however, resistance to DDT and chloroquine had emerged. In addition, the indiscriminate use of DDT as an insecticide had caused undesirable impacts on the environment; consequently, the eradication campaign was put on hold.

1.1.2 Magnitude of the malaria problem

Contrary to the rest of the world, the Global Eradication Campaign had very little impact in the Amazon basin and Sub-Saharan Africa where social and economic conditions meant the program was either not implemented or not sustainable.[1]

The downward trend in malaria-related mortality achieved during the 1950's has undergone a reversal since the late 1980's. This resurgence is confined mainly to underdeveloped countries where inadequate health systems are unable to provide rapid diagnosis and treatment. In Africa, malaria is the world's most important parasitic disease as it is directly responsible for one in five childhood deaths and indirectly contributes to illness and deaths from respiratory infections, diarrhoeal disease and malnutrition.[1]

The seriousness of the disease has been exacerbated by several factors, these include amongst others; the spread of the disease into areas previously free of malaria mostly as a result of changing land utilization.[4] Failure to implement control measures in conflict regions particularly in Africa and Southeast Asia has also contributed to the upsurgence of the disease in areas that it was previously under control.[4] Most of the antimalarial drugs available currently have been in use for decades, but their use is now severely limited by the emergence and spread of drug resistance, especially chloroquine. Hence, there is an urgent need to discover new antimalarial drugs to supplement this very limited armamentarium.[4]

1.1.3 History of malaria

The disease supposedly has its origins in the jungles of Africa, where it remains prevalent. The Romans named the disease “mal’ aria” as at that time the disease was attributed to the “bad air” surrounding stagnant waters.[1] Scientists were to later confirm that mosquitoes transmit the malaria parasite through their bite. Today, the malaria parasite is known to be transmitted by the female *Anopheles* mosquito; furthermore, of about 400 species of the *Anopheles* mosquito known, only about 60 transmit the disease.[5]

The antiquity of Malaria, which is caused by the protozoan parasites of the genus *Plasmodium*, is demonstrated by the host specificity of over 100 parasite species found in reptiles, birds and mammals. Of the four species of *plasmodia* that infect man; *P. vivax, P. malariae, P. ovale* and *P. falciparum*; it is *P. falciparum* that is the most dangerous and is responsible for almost all fatalities.[6]

1.2 Life cycle of the malaria parasite

The life cycle of *Plasmodium falciparum* is divided into 2 basic cycles, an asexual cycle in man and a sexual cycle in the *Anopheles* mosquito. In man the cycle can further be divided into a liver stage and a blood stage. The life cycle of the malaria parasite is illustrated in Figure 1.2 below. In the gut of an infected mosquito, sexual reproduction occurs where male and female gametes fuse to form a zygote that develops into an oocyst, and later into sporozoites. These sporozoites travel to the salivary glands where they wait to be discharged into the human host when the mosquito takes its blood meal. When an infected *Anopheles* mosquito bites, it releases malaria sporozoites into the blood stream of the human host. These sporozoites rapidly pass to the liver and penetrate its parenchymal cells where they multiply asexually 30 000 fold and form liver schizonts. The schizonts burst open the host cells and enter the blood stream as merozoites after a period known as the liver stage in the parasite’s life cycle in a process that takes 7-12 days.[7] The merozoites invade red blood cells and over the next 48 hours, develop into small rings contained within a parasitophorous vacuole (ring stage); they will metabolize haemoglobin (trophozoite stage) and replicate asexually (schizont stage) with subsequent rupturing of the cells.[8] Rupturing of the cells leads to further release of

merozoites into the blood stream hence increased parasitemia. While most of the trophozoites develop into blood schizonts during the blood stage of the parasites life cycle; some of the trophozoites develop into male and female gametocytes that may then be taken up by an *Anopheles* mosquito when it bites an infected person.

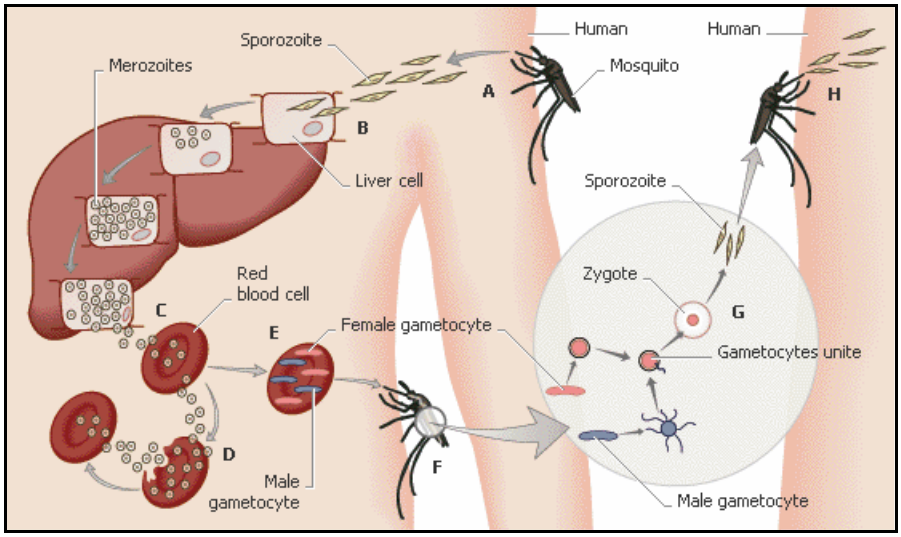


Figure 1.2: Life cycle of *P.falciparum.* (A) Mosquito bites human passing sporozoites into the human's bloodstream. (B) Sporozoites travel to the liver and undergo asexual reproduction forming merozoites. (C) Merozoites enter the bloodstream and infect red blood cells. (D) In red blood cells merozoites grow and divide (E) some merozoites develop into male and female gametocytes. (F) Another mosquito bites the infected human ingesting the gametocytes. (G) In the mosquito's stomach, the gametocytes mature to form sporozoites which travel to the mosquito's salivary glands. (H) If this mosquito bites another human, the cycle begins again (www.cdc.gov/malaria/biology).

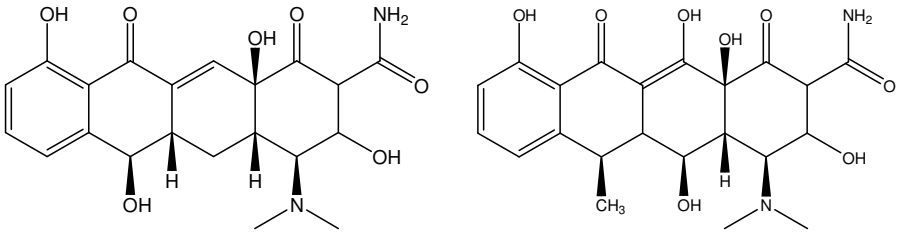
1.3 CLASSES OF ANTIMALARIALS

Antimalarial drugs can best be classified according to their mode of action as compounds acting on haem-detoxification, drugs generating an oxidative stress (endoperoxides), nucleic acid inhibitors or inhibitors of protein metabolism. These drugs

act by targeting different stages of the malaria life cycle,[9] although most of them act on the blood stage of development of the malaria parasite.

1.3.1 Inhibitors of protein synthesis

In this group are found the antibiotics (Figure 1.3), which have a long history in the treatment of malaria. Interest in the use of antibiotics to treat malaria re-emerged with the appearance of chloroquine-resistant and multi-drug resistant strains of *P. falciparum*. The tetracyclines cannot not be used alone for initial treatment of malaria, even when the parasite is sensitive towards them because their antimalarial activity is delayed. This delay is related to their mechanism of action. The tetracyclines act by inhibiting protein synthesis in the mitochondria and/or apicoplast.[10]



1.1 **1.2**

Figure 1.3: Chemical structure of tetracycline (**1.1**) and doxycycline (**1.2**).

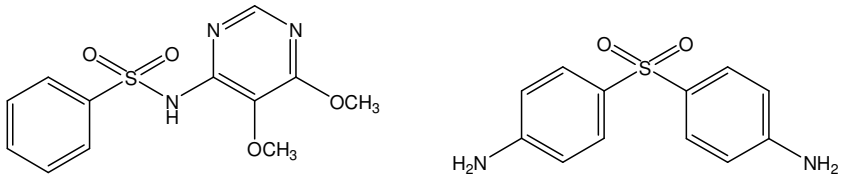
1.3.2 Nucleic acid inhibitors

Nucleic acid inhibitors are anti-folates and atovaquone. They exert their effect by inhibiting synthesis of parasitic pyrimidines and thus of parasitic DNA.[11]

1.3.2.1 Folate antagonists

Some of the most widely used antimalarial drugs belong to the folate antagonist class. This class of compounds act by inhibiting enzymes of the folate pathway, which consequently results in decreased pyrimidine synthesis, hence reduced DNA, serine, and methionine formation. They exerted their activity at all growing stages of the asexual

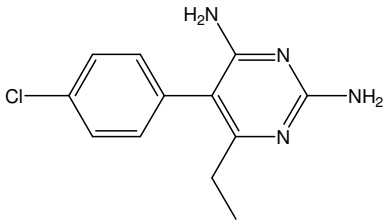
red blood cell cycle and on young gametocytes. Conventionally, antifolates are classified as type-1 and type-2 antifolates.



1.3 **1.4**

Figure 1.4: The DHPS inhibitors sulfadoxine (**1.3**) and dapsone (**1.4**).

Type-1 antifolates are sulfonamides and sulfones (Figure 1.4). They prevent the formation of dihydropteroate from hydroxymethyldihydropterin, itself a pyrophosphate derivative, in a process that is catalyzed by a bifunctional enzyme in *Plasmodia* known as dihydropteroate synthase (DHPS) by competing for the active site of this enzyme. Ultimately, this leads to reduced dihydropyrimidine synthesis and then DNA, serine and methionine formation.



1.5

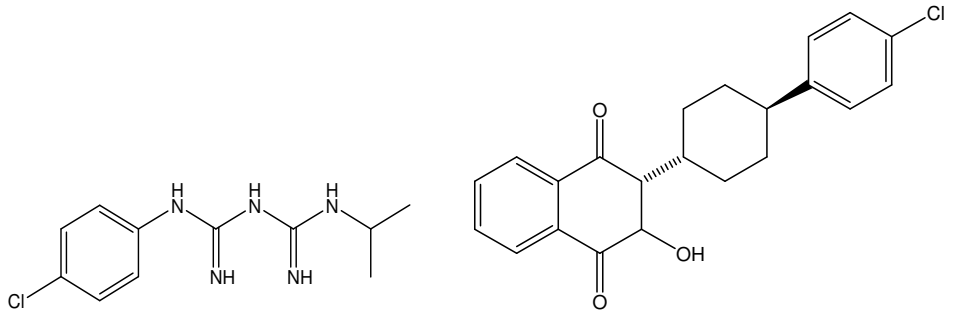
Figure 1.5: The DHFR inhibitor pyrimethamine (**1.5**).

Type-2 antifolates include pyrimethamine (Figure 1.5), quinazolines, biguanides and triazine metabolites. They act by inhibiting dihydrofolate reductase (DHFR), also a bifunctional enzyme in *Plasmodia* coupled with thymidylate synthase [TS]), thus preventing the NADPH-dependent reduction of H2folate (DHF) to H4folate (THF) by this enzyme. THF is a necessary cofactor for the biosynthesis of thymidylate, purine nucleotides, and certain amino acids. Unfortunately, resistance to these drugs is now widespread in Asia, India and Africa,[12,13]. Due to noticeable synergistic effects, a type-1 drug is usually used in combination with a type-2 drug with pyrimethamine-sulfadoxine

(SP) being the most widely used combination. The mechanism of resistance has been shown to be due to mutations in the genes of DHFR,[14] and DHPS.[15]

1.3.2.2 Atovaquone and proguanil

Atovaquone (Figure 1.6) is used in both treatment and prophylaxis of malaria. Despite a novel mode of action, resistance occurs readily when atovaquone is used alone, thus, it is used in a fixed-ratio combination with proguanil (Figure 1.6) as Malarone®. Atovaquone is thought to act on dihydroorotate dehydrogenase (DHODase), a critical enzyme in electron transport. Inhibition of DHODase blocks pyrimidine synthesis,[16,17]. Proguanil on the other hand is a pro-drug that inhibits parasite dihydrofolate reductase (DHFR) when converted to its active form cycloguanil.



1.6 **1.7**

Figure 1.6: The DHFR and DHODase inhibitors proguanil (**1.6**) and atovaquone (**1.7**) respectively.

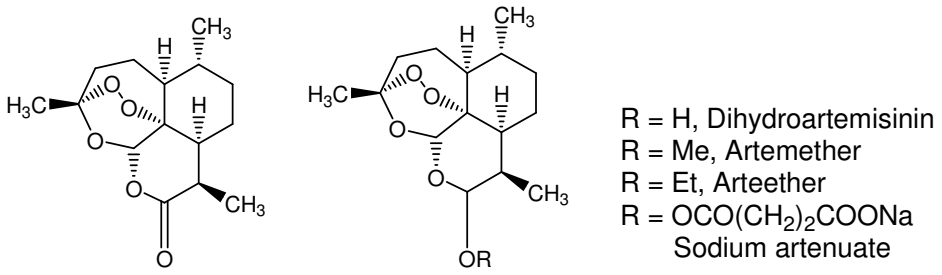
It is generally agreed that atovaquone acts on the mitochondrial electron transfer chain although more recently its activity and synergy with proguanil has been ascribed to its interfering with mitochondrial membrane potential. The two proposed mechanisms of action although not fully understood, are linked as the mitochondrial electron transport chain serves to generate this membrane potential.[10]

The combination of atovaquone and proguanil is very effective,[18] but the mechanism of such interaction remains largely unknown. One seemingly obvious explanation of synergy is that both atovaquone and cycloguanil act on enzymes involved in pyrimidine synthesis (DHODase and DHFR, respectively), however, this does not appear to be the

case because the combination is highly effective both in patients who do not convert proguanil to cycloguanil[19] and against highly cycloguanil-resistant parasites.[20]

1.3.3 Endoperoxides

This class of compounds comprises artemisinin, a sesquiterpene lactone that is naturally occurring in *Artemisia annua* [21] and its derivatives, which include artemether and sodium artesunate (Figure 1.10). Artemisinin has been used historically in China as a treatment of malaria [22]. Artemisinin-type compounds have activity throughout the phases of the asexual intra-erythrocytic schizogonic cycle, and also act on young gametocytes.



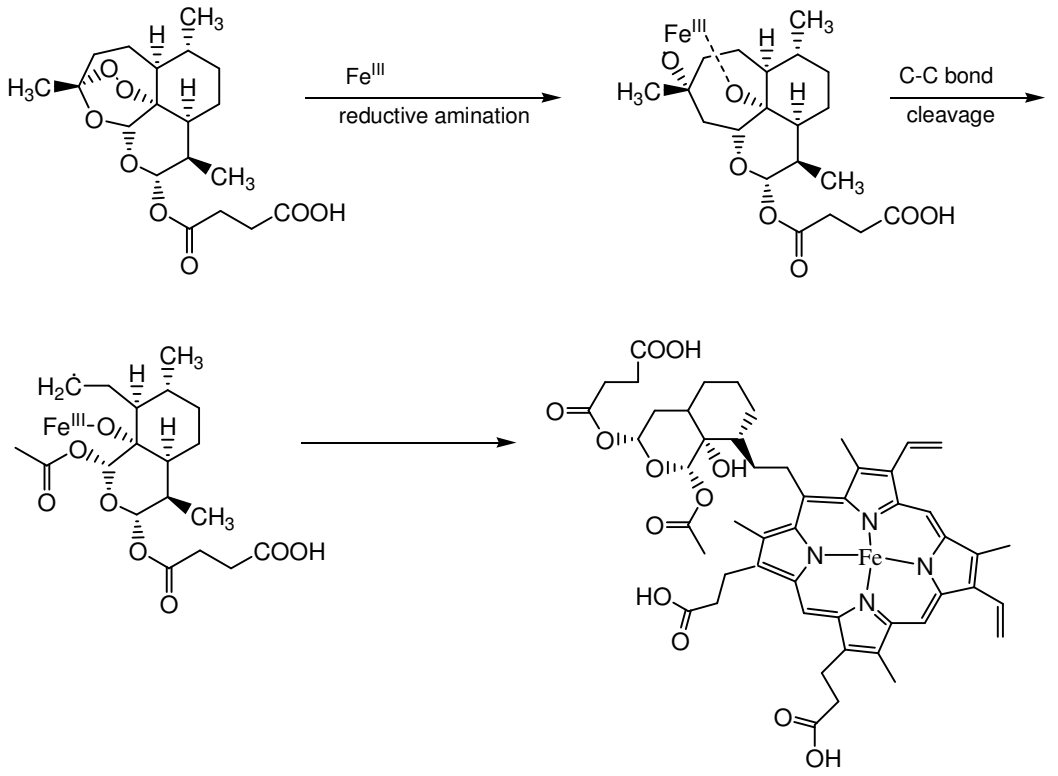
1.15 **1.16**

Figure 1.10: Artemisinin (1.15) and its semi-synthetic derivatives.

They achieve higher reduction rates of parasitemia per-cycle than any other drug known to date.[23] The semi-synthetic derivatives artemether and arteether are effective against both chloroquine-sensitive and chloroquine-resistant *P. falciparum* strains and are clinically used for cerebral malaria.

Although their mechanism of action is not fully understood, the prevailing hypothesis is that reductive cleavage of the O-O bond by Fe(III)-haem generates C-centred radicals which in turn would alkylate biomolecules leading to the death of the parasite.[24] Whilst it is generally accepted that Fe(II)PPIX induces extensive decomposition of artemisinin, there is no unequivocal evidence that this can occur in the highly oxidizing and acidic environment of the food vacuole. Early studies indicated that an “oxidative stress” could be involved,[25] but the nature of the oxidative stress remains unclear. The theory that

artemisinin acts by invoking oxidative stress is substantiated by observations of synergism with other oxidant drugs and antagonism by agents that lower oxidative stress [26]. However, peroxide-bridge compounds do not usually induce oxidative stress. For this type of compounds to induce oxidative stress they need to undergo a two step process (Scheme 1.1).



Scheme 1.1: Hypothetical mechanism for the radical alkylation of haem by artesunate.[24]

The first step would be the "activation" of the molecule *via* homolytic cleavage of the peroxide-bridge. This step presumably occurs in the presence of transition metal ions such as Fe(II)PPIX, although it is unclear whether haem iron alone is sufficient or whether additional reduced iron is needed. The second step would be the alkylation of putative protein target(s). The "cleavage" of the peroxide bond gives rise to the formation of an oxygen-centred and then a carbon-centred radical, followed by a ferryl-protoporphyrin IX=O species, and finally, an epoxide, which is a highly active alkylating [27] (Scheme1.1).The carbon-centred radicals[28] may alkylate either haem itself or some other proteins, such as translationally controlled tumor protein (TCTP)[29] or histidine-rich

protein. Translationally controlled tumor protein binds haem, hence possibly bringing artemisinin into the vicinity for reaction. It is not known whether the alkylation is specific for the TCTP homologue or whether any protein that binds haem can be similarly alkylated. It is also unclear whether haem-bound artemisinin can react with proteins that do not bind haem. This "carbon-centred radical theory" is not universally accepted.[30] Eckstein-Ludwig *et al*., (2003) have reported compelling evidence that artemisinins act *via* a Fe^{2+}-dependent activation mechanism.[31] In studies conducted on *Xenopus* oocytes, they demonstrated that PfATP6, the sarco / endoplasmic reticulum Ca^{2+}-ATPase (SERCA) orthologue of *Plasmodium falciparum* is inhibited by artemisinin with potency similar to that of thapsigargin (another sesquiterpene lactone and highly specific SERCA inhibitor) but not by quinine or chloroquine. The most probable answer as to why resistance has not developed for endoperoxide compounds is therefore that artemisinin compounds do not exert their antimalarial effects by hitting a single biological target but rather by simultaneously hitting several targets with very high precision and efficiency.[32] Hence, their mode of action could involve enzyme inhibition, lipid peroxidation, membrane damage through vital enzymes and some effect on haem detoxification.

1.3.4 Compounds acting on haem detoxification

This class of compounds includes some of the most common and widely used antimalarial drugs and can be separated into two types. Type-1 drugs, (Figure 1.7), are the 4-aminoquinolines (chloroquine), and Mannich-base (amodiaquine). Chloroquine was introduced in 1944-1945 and soon became the mainstay of malaria treatment and prevention because it was cheap, non-toxic and active against all strains of the malaria parasite [11]. Since two decades ago, the emergence and spread of the malaria parasite resistant strains has limited its use. Primaquine has been used with chloroquine against resistant strains of *P. vivax*, while amodiaquine due to its superior efficacy has been used for clearing parasitemia in cases of complicated malaria as well as against chloroquine-resistant strains.[33,34]

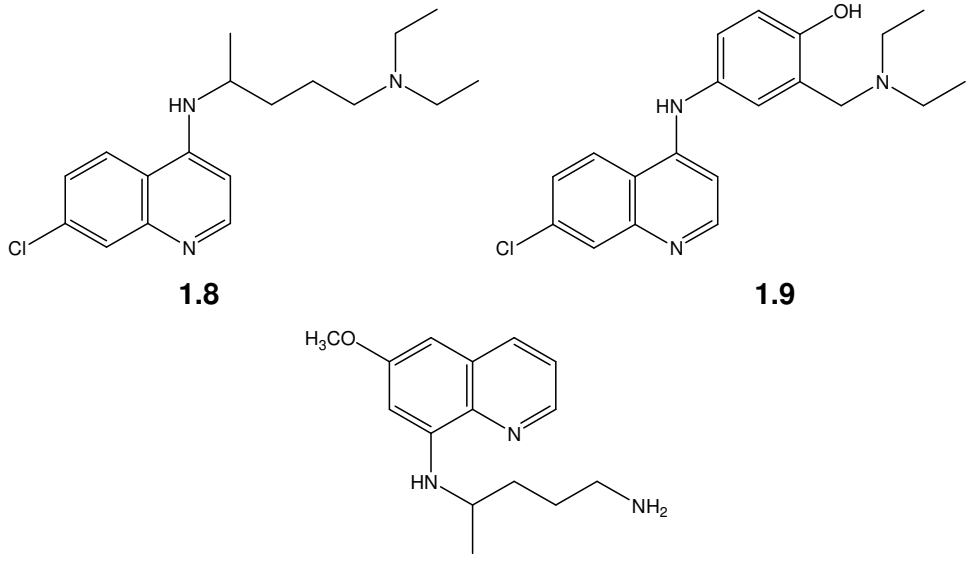


Figure 1.7: Chemical structures of chloroquine (**1.8**) amodiaquine (**1.9**) and primaquine (**1.10**).

Type-2 drugs are the aryl-amino alcohols (quinine and quinidine, mefloquine and halofantrine). Quinine and its isomers including quinidine (Figure 1.8) are found in the bark of the cinchona tree[35] and for almost three centuries quinine in its purified form or as a component of the cinchona bark extract was the only widely available medication for clinical use.

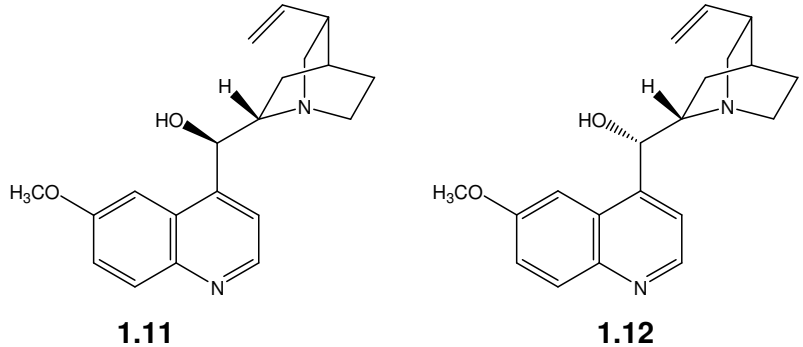
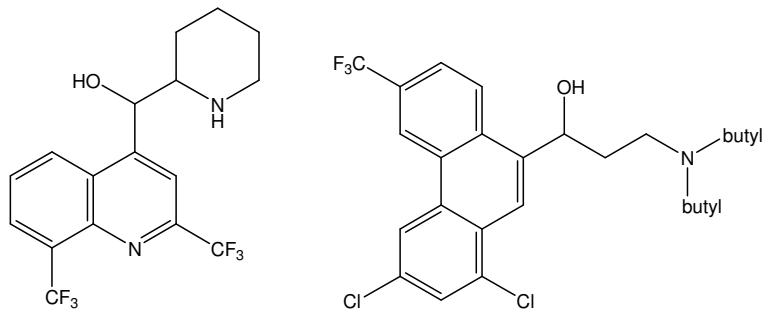


Figure 1.8: The chemical structures of quinine (**1.11**) and quinidine (**1.12**).

Mefloquine (Figure 1.9) is structurally related to quinine and its long half-life (14-21 days) has contributed to the rapid development of parasite resistance to this drug. Halofantrine (Figure 1.9) is effective against chloroquine-resistant parasites;[11] however, cardiotoxicity has limited its use a therapeutic drug.[11]



1.13 **1.14**

Figure 1.9: Chemical structures of mefloquine (**1.13**) and halofantrine (**1.14**).

In general, the two classes of drugs differ in that type-1 drugs are weak bases, can be diprotonated and are hydrophilic at neutral pH, whereas type-2 drugs are weaker bases and lipid soluble at neutral pH. In addition, the two groups appear to interact differently with their putative target[36] and show an inverse relationship with respect to parasite sensitivities. Various mechanisms have been proposed for the action of quinoline-based and related compounds. These include inhibition of protein synthesis,[37] inhibition of food vacuole lipase[38] and aspartic proteinase,[39] and inhibition of DNA and RNA synthesis.[40] The commonly accepted hypothesis is that quinoline-containing drugs act primarily in the food vacuole by preventing the detoxification of haem (a product of the digestion of haemoglobin - the parasites food source in the host red blood cells) and that chloroquine selectively accumulates in the parasite food vacuole due to its weak base properties. There is conflicting data as to whether such a mechanism would explain the mode of action of both chloroquine and the quinoline / phenanthrene methanols.[41] However, recent experiments using proteinase inhibitors and quinoline-containing drugs substantiate the view that interaction with haem is central to the activity of this class of compounds.[42]

1.4 HAEMOGLOBIN AND HAEMOZOIN FORMATION

During the blood stage of the malaria parasite's life cycle, the parasite utilizes host haemoglobin as its main food source[43] obtaining amino acids required for protein synthesis from degrading 25-75% of host haemoglobin in the occupied red blood cells.[44] In this process, large quantities of red blood cell cytoplasm are ingested by fluid phase endocytosis and transported in vesicles to a secondary lysosome known as the food vacuole.[6] The food vacuole contains at least three classes of proteinase enzymes, these are the three cysteine proteases, falcipain 1, 2 and 3[45] the four aspartic proteases plasmepsins I, II,[46] IV[47] and histoaspartic protease (HAP)[48,49,50] and a metallo pepetidase, facilysin[51] which break down haemoglobin. This haemoglobin degradation process is complicated by the release of ferrous haem which is rapidly oxidised to the ferric form known as haematin (aquaferriprotoporphyrin IX or H_2O-Fe(III)PPIX) (Figure 1.11). Solubilized haematin is cytotoxic and has been shown to damage biological membranes [52], [53], [54], [55] and inhibit a variety of enzymes[56] including vacuolar proteases.[39,57]

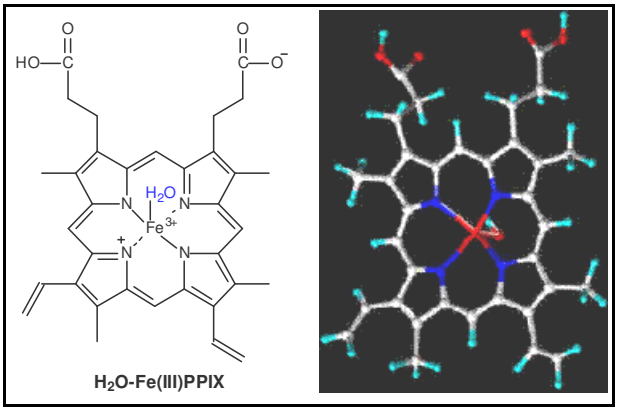


Figure 1.11: Chemical and molecular structure of haematin.[58]

In mammals Fe(III)PPIX is degraded *via* the haem-oxygenase / biliverdin reductase pathway.[59] The malaria parasite however, has evolved a pathway to detoxify haematin by conversion to the insoluble, black, granular substance known as malaria pigment or haemozoin (Figure 1.12). It has been shown that at least 95% of the haem released because of haemoglobin digestion is converted to haemozoin.[60]

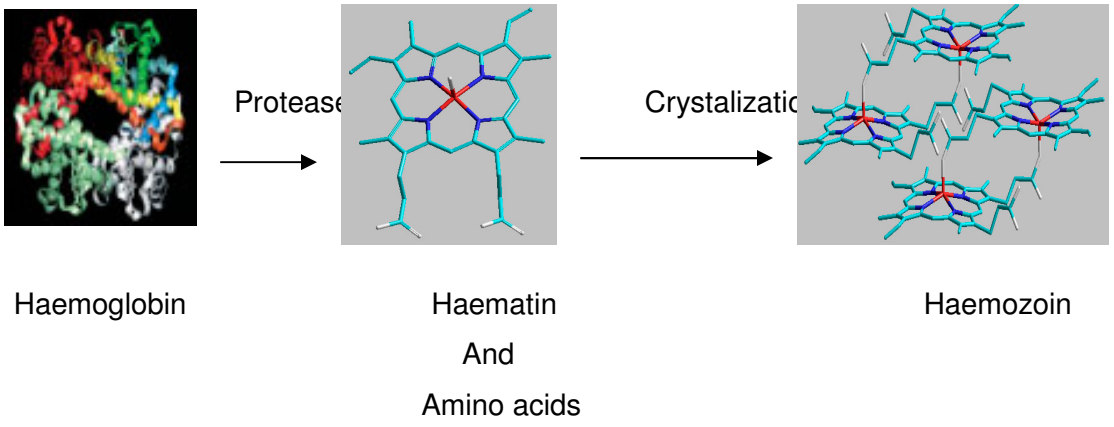


Figure 1.12: The haemoglobin degradation pathway and generation of haemozoin.[61]

Haematin crystallization is thought to occur when the negatively charged propionate group of one is drawn to the positively charged Fe(III) centre of the next producing the β-haematin precursor,[62] this initiates the assembly of the haemozoin crystal by the recurring assembly of haematin molecules in his manner.

Slater and Cerami suggested that haemozoin formation is enzyme catalysed *in vivo* and demonstrated *P. falciparum* trophozoites extracts promoting formation of its synthetic counterpart β-haematin,[63] However, this was questioned by Dorn *et al*. who noted that heat treatment of the extracts had little effect on the reaction and suggested the initiator to be some haem-derived material associated with haemozoin.[64] Bendrat *et al*. suggested that this reaction promoting material is a lipid contaminating the haemozoin extracts.[65] Sullivan *et al*. managed to identify and clone two histidine-rich proteins (HRP II and HRP III) in purified digestive vacuoles, which are similar to human histidine-rich glycoprotein (HRG) and are composed of histidine (34%), alanine (37%) and aspartic acid (10%).[66] These authors claim all the ferrihaems in the HRP II-Fe(III)PPIX complex are low spin and six coordinate with a two histidine ligands and that this complex is stable between pH 5.5 and 7.0. These results are at odds with the findings of Choi *et al*., who observed two types of haem-binding within HRP-II which causes conformational

changes around pH 6.[67] This protein has been found to promote β-haematin formation under physiological conditions when Fe(III)PPIX binding is weakened at low pH. These authors attributed this weak Fe(III)PPIX binding to a change in conformation of the protein which results in possible coordination of a carboxylate group from an aspartate amino acid.

Since findings on the mechanism of β-haematin formation have compared this process to biomineralisation, it is possible that the role of HRP *in vivo* will be to provide a scaffold on which nucleation and growth of the crystal can occur. However, recent findings show that parasite clones lacking the gene for HRP-1 or 2 form haemoglobin normally, ruling out a role for this protein. Haemozoin formation is not unique to the malaria parasite but has also been identified in other organisms that lack HRP but degrade haemoglobin where presumably it serves the same purpose. These organisms include amongst others, *Shistosoma mansoni* a worm in humans causing bilharzia[68] and *Haemoproteus colombae* a protozoan parasite common in birds. [69]

The weight of evidence has recently shifted away from histidine-rich protein II or III to lipid initiation.[70,71,62,72] Bendrat *et al*., (1995) initially proposed possible polar lipid initiation,[65] this hypothesis was substantiated by Dorn *et al*. (1995) and (1998).[64,73] Fitch also proposed a role for neutral lipids, but was not able to identify digestive vacuolar lipids,[70] while Tripathi and coworkers suggested a combination of proteins and lipids.[71,74] Recent work in *Plasmodium* linked neutral lipid bodies on both the interior and the exterior of digestive vacuoles with haem crystallization.[75,76,77] Based on the published electron micrographic evidence of intra-vacuolar lipid bodies, Pisciotta identified neutral lipids closely associated with hemozoin.[78] *In vitro* growth of β-hematin under biomimetic conditions indicate that this crystalline solid forms exceptionally efficiently in a lipid environment, [78,62] especially when Fe(III)PPIX is directly introduced at or near the interface between the lipid and aqueous solution.[62] The high rate at which solid crystalline β-hematin forms suggests that the lipid / water interface itself is probably sufficient to bring about haemozoin formation *in vivo*.[79]

1.5 MECHANISM OF ACTION OF QUINOLINE ANTIMALARIALS

Quinoline antimalarial drugs are widely used in chemotherapy of the disease. However, their effectiveness particularly that of chloroquine has been considerably reduced due to the advent of resistant strains of the parasite. Although the mechanism of action of this class of drugs is not unequivocally known, these drugs are believed to accumulate by a weak base mechanism in an acidic compartment found inside the parasite within the infected red blood cell known as the food vacuole. The currently held hypotheses, although not without its flaws, is that chloroquine interferes with conversion of haem into haemozoin thereby causing an accumulation of the potentially toxic haematin. However, the accumulation of haematin alone cannot be viewed as fatal to the parasite as the excess haematin could presumably be disposed by diffusion from the cell or possibly by reduced glutathione-mediated degradation[80] although there is no evidence that this actually occurs. The toxic event would therefore be the formation of a haem-chloroquine complex that would prevent haem degradation. Based on the morphological changes and specific drug accumulation within the food vacuole upon chloroquine administration, it would appear that the food vacuole is the primary site of drug activity. Various intravacuolar theories have been brought forward and shall be discussed. These include inhibition of enzymes inside the food vacuole, an increased vacuolar pH, and other hypotheses on interference with haematin detoxification.

1.5.1 INTRAVACUOLAR HYPOTHESES

1.5.1.1 Inhibition of Protease enzymes

It has been shown that chloroquine binding to Fe(III)PPIX decreases protein synthesis since Fe(III)PPIX was shown to stimulate protein synthesis in a trophozoite extract.[37] However, no evidence of haem involvement in protein synthesis in the parasite has been presented. Furthermore, the parasite could not possibly withstand the high concentrations of haem required to stimulate protein synthesis. Chloroquine has also been shown to inhibit partially purified aspartic protease activity from *P. falciparum* extracts[57] but again only at concentrations above the therapeutic range. Furthermore, haematin itself is a very good inhibitor of acid proteases (roughly 10 μM) and this activity is unchanged in the presence of chloroquine.[39]

The hypothesis that chloroquine interferes with the parasite's feeding mechanism have further been questioned by Ginsburg and Krugliak who noted that treatment of chloroquine-treated parasites with membrane permeable amino-acids, were unable to alleviate inhibition of parasite growth.[81]

1.5.1.2 Inhibition of Phospholipase enzymes

Vacuolar phospholipase enzymes have been identified within the food vacuole and are responsible for degrading endocytic vesicle membranes in order to release their haemoglobin content into the food vacuole. Chloroquine has been shown to inhibit phospholipase activity *in vitro*,[81] but at concentrations substantially higher than those likely to occur in the vacuole.

1.5.1.3 Increased vacuolar pH

A number of authors have suggested that chloroquine raises the pH of the food vacuole because of it weak base properties.[81] This would result in a homeostatic change in the food vacuole; consequently, the normal functions of the enzymes within this normally acidic compartment are disrupted.

This hypothesis is no longer accepted because it has been shown that not all weak bases are antimalarials, a case in point is the intrinsic antiplasmodial inactivity of 9-epiquinine, a stereoisomer of the potent antimalarial drug quinine in spite of both compounds possessing virtually identical weak base properties.

Further experiments using fluorescent dextrans to monitor the vacuolar pH have shown that alkalization is only observed at levels above the therapeutic concentration range of the antimalarial drugs.[82,83,84] This is presumably due to the ability of the vacuolar proton pumps to maintain an acidic pH even in the presence of a weak base such as chloroquine.[85] Nonetheless, weak base properties probably account in part for accumulation of drugs in the food vacuole.

1.5.1.4 Interference with haematin detoxification

Haemozoin inhibitors are thought to act at the formation of the unit head-to-tail haem crystal dimer by drug–haem complex competition or at the growing face of a large haemozoin crystal by drug–haem complex binding to prevent addition of more haem crystal dimers.[79] First indications of haem-drug complex formation were reported by

Cohen *et al*., (1964).[86] Later reports by other workers would suggest that chloroquine and related drugs act by forming a complex with haematin and in so doing inhibit the conversion of the toxic haematin to non-toxic haemozoin; a process developed by the parasite as part of its natural defence.[64,87,88,89] Additional interest in the haemozoin formation theory was centred on the fact that drugs such as chloroquine inhibit its formation under synthetic conditions, [88, 90,87,63] demonstrating that inhibition of haemozoin formation may be the basis of activity of these drugs.

Slater and Cerami put forward a proposal that a haem polymerase enzyme[91] catalyses haemozoin formation. It was further proposed that inhibition of this enzyme by chloroquine would result in a build up of the potentially toxic haem in the parasites food vacuole. However, Dorn *et al*., (1995) showed that haemozoin formation is not enzyme catalysed but is instead autocatalytic.[64] They also confirmed earlier observations by Egan *et al*., (1994) that haemozoin formation could be directly inhibited by chloroquine and suggested that this process is the target for chloroquine *in vivo*.[87]

Ginsburg *et al*., (1998) also proposed that chloroquine competitively inhibits degradation of haem by glutathione, thus allowing haem to accumulate in membranes.[92] Tilley and co-workers (1999) demonstrated the rapid decomposition of haem in the presence of H_2O_2 and suggested that chloroquine and quinacrine are efficient inhibitors of the peroxidative destruction of haem by complex formation.[93]

1.5.2 EXTRAVACUOLAR HYPOTHESES

Despite the background that the food vacuole appears to be the primary site of drug action. Various extravacuolar theories have also been brought forward and unsurprisingly, none has found widespread approval. These include DNA binding and inhibition of polyamine synthesis.

1.5.2.1 DNA binding

Chloroquine was originally thought to exert its antimalarial activity through DNA intercalation and hence inhibition of DNA replication and RNA synthesis ultimately leading to cell death. This hypothesis is no longer accepted on the basis that it has been demonstrated *in vitro* that chloroquine binds to DNA but at concentrations much higher (100 μM) than the pharmacological concentration (typically 10 nM).[91] Furthermore, this hypothesis does not explain why the quinoline antimalarial, mefloquine, which does not

bind to DNA,[94] is active. It has also been shown that there is no selectivity between the chloroquine-DNA interactions of host and that of parasite DNA.

1.5.2.2 Inhibition of Polyamine Synthesis

Quinoline antimalarial drugs have been reported to inhibit ornithine decarboxylase activity in trophozoite extracts of infected red blood cells.[95] Since ornithine decarboxylase enzymes are important in polyamine synthesis, it has been suggested that chloroquine may exert its antiplasmodial activity this way. There have been no further experiments pertaining to the effects of quinolines on this class of enzymes to give credence to this hypothesis. However, existing information does not explain why chloroquine is only active on the blood stage parasites as liver stage parasites also presumably have ornithine decarboxylase. Furthermore, using a functional proteomics approach to understand how quinoline drugs function and to provide a comprehensive description of quinoline-interacting proteins; Graves *et al*., (2001)[96] demonstrated several quinoline compounds to specifically target two human proteins, aldehyde dehydrogenase 1 (ALDH1) and quinone reductase 2 (QR2) but not to interact with *P. falciparum* proteins. These findings may well provide new insights into the mechanism of action of quinoline drugs.

1.6 STRUCTURE-ACTIVITY RELATIONSHIPS IN QUINOLINES

Various studies have shown that the activity of quinoline-based antimalarials is dependent on haemoglobin degradation.[97,98] This class of compounds is thought to act by complexing with haematin thus preventing the formation of haemozoin. A number of studies have shown that a wide range of quinoline and phenanthrene antimalarials and antiplasmodials inhibit synthetic haemozoin (β-haematin) formation *in vitro*.[73,64,87,89-104] Recent studies have thus investigated the structural requirements for association of quinolines with haematin. Egan *et al*., (2000) investigated structure-activity relationships in 21 chloroquine analogues and showed that the minimum structural requirements for drug association with haematin is substitution of the 2 or 4 position of the quinoline ring with an amino group (Figure 1.14).[102] Quinoline itself does form a complex with haematin but the association constant is thousand times weaker than that found for 2- and 4-aminoquinoline.

Attachment of an alkyl side-chain to the 2- and 4-amino group of aminoquinolines resulted in only a small change on the association constant. It was demonstrated that compounds that do not bind with haematin do not inhibit β-haematin formation and have essentially no antiplasmodial activity. Thus, the 4-aminoquinoline nucleus of chloroquine was identified to be a necessary requirement for antiplasmodial activity in this class of compounds. The molecular basis of specific interactions remains unknown, although it was proposed to be due to the ability of partial charges on quinoline to form strong interactions with opposite charges on haematin. Portela *et al*., (2003) supported this idea using molecular orbital calculations and suggested that the amino group connected to the quinolic ring corresponding to a positive electrostatic potential, interacts with the central negative zone of the haematin dimmer.[105] The negative potential located over the aromatic area of the quinoline ring also interacts with the peripheral positive area of the putative μ-oxo-dimer (Figure 1.13).

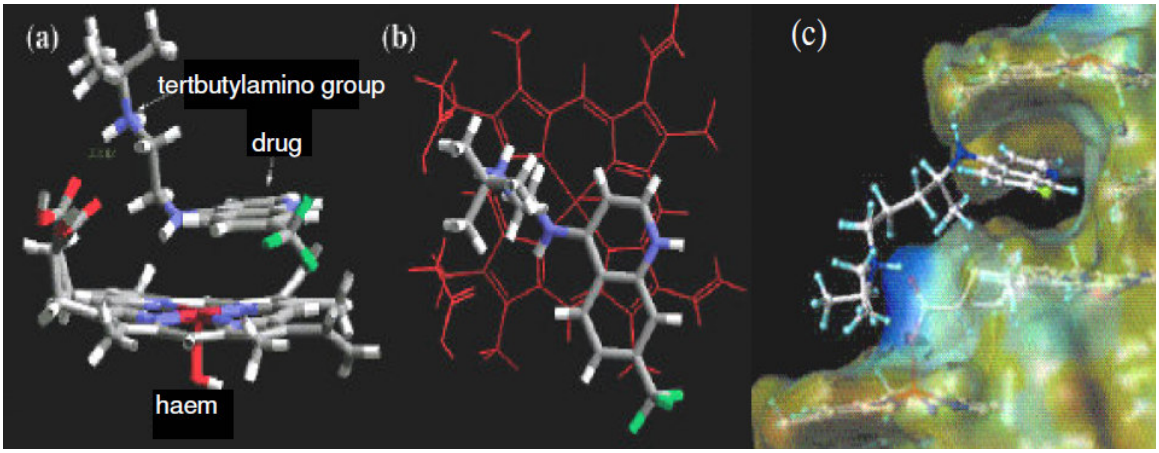
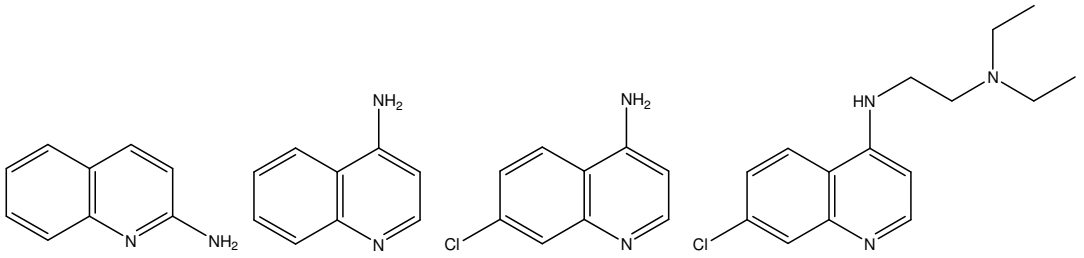


Figure1.13: (a) The side on view of the lowest energy complex of haematin with chloroquine showing the π-stacking interaction between the porphyrin ring of haematin and the quinoline ring of chloroquine. (b) The top down view of the chloroquine haematin interaction with all atoms of haematin displayed in red for clarity.[106] (c) The mode of interaction between haemozoin and chloroquine is illustrated with chloroquine interacting with the growing face of haemozoin.[107]

However, it is now clear that inhibition of β-haematin formation is not only a result of association with haematin. Quinolines including 9-epiquinine as well as certain 4-aminoquinolines have been shown not to inhibit β-haematin formation despite forming

complexes with haematin.[101,102] Egan *et al*., (2000) and Vippagunta *et al*., (1999) have shown that the key feature for inhibition of β-haematin formation for 4-aminoquinolines appears to be the presence of the 7-chloro group.[101,102] The identity of the group at this position was found to be the critical feature in determining the strength of inhibition of β-haematin formation. This conclusion has recently been supported by Cheruku *et al*., (2003) who have confirmed that the 4-amino-7-chloroquinoline substructure of chloroquine is essential for inhibition of β-haematin formation.[108] Replacement of the 7-chloro group with a bromo or nitro has been shown to result in complexes that are still active, while 7-amino and 7-hydro are not. This seems to suggest that groups that are π-electron withdrawing are successful inhibitors [101]. This is consistent with a recent report on the antiplasmodial activity of aminoquinolines with fluoro-, chloro-, bromo-, iodo-, trifluoromethyl-, and methoxy- substituents in the 7-position.[109] The fluoro- and methoxy-derivatives were found to be less active than chloro-, bromo-, iodo- and trifluoromethyl-derivatives. The efficacy of 4-aminoquinolines depends both on the strength of β-haematin inhibition and on the extent of accumulation of the drug in the food vacuole through pH trapping. The extent of pH trapping depends on the basicity of the quinoline ring and the side-chain amino group.



1.17 **1.18** **1.19** **1.20**

Log K = 4.23 ± 0.02 log K = 4.49 ± 0.01 log K = 4.43 ± 0.01 log K = 5.81± 0.01

Figure 1.14: A selection of 2-, and 4-aminoquinolines used to investigate SARs for haematin-quinoline association.[102]

Kaschula *et al*., (2002) reported that the pK_a is strongly dependent on the nature of the group at the 7-position on the quinoline ring in short chain analogues of chloroquine [110]. Groups that are electron-releasing such as NH_2 and OCH_3 raise pK_{a1} relative to that of

the 7-H derivative. By contrast, strongly electron-withdrawing groups such as NO_2 cause considerable decrease in pK_{a1}.

De *et al*., (1998) also found that the most active aminoquinolines were those with two or three carbon diaminoalkane side-chains especially those that have chloro-, bromo- or iodo- groups in the 7-position.[109] The authors also found that these aminoquinolines are active against chloroquine resistant *P. falciparum* because they avoid the mechanism responsible for resistance to chloroquine.[109] They suggested that the side-chain length (carbons between the two nitrogens in the diaminoalkane side-chain of 7-bromo- and 7-iodo- of 4-aminoquinolines) is the major determinant of activity against chloroquine resistant *P. falciparum* as it is with 7-chloro-4-aminoquinoline. The proposed structure activity relationship is shown in figure 1.15 below.

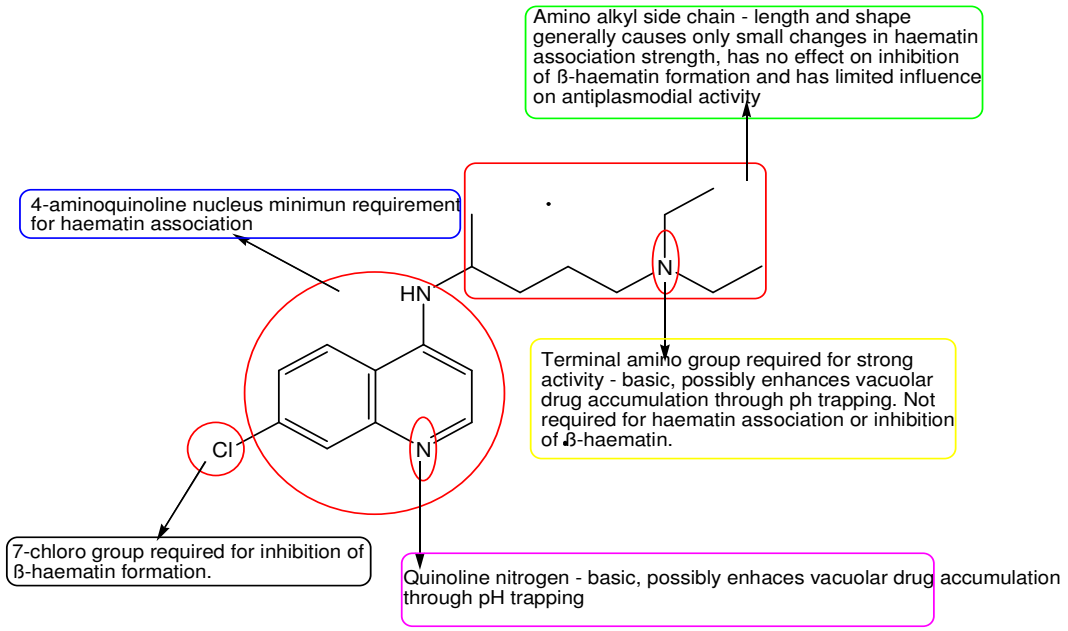


Figure 1.15: The proposed structure-activity relationships for chloroquine.[102]

1.7 MECHANISM OF CHLOROQUINE RESISTANCE

In general, resistance to a drug can be due to several mechanisms. Changes to the drug may make it less potent. Changes to the drug target may result in the drug not being able to exert its effect on the modified target. The target may be over-expressed thereby reducing the effect of the toxin. The drug may be denied access to its target by either a reduction in its accumulation or an increase in its efflux out of the cell or its site of action. In order to understand the occurrence of resistance towards quinoline drugs in *P. falciparum*, one needs to look at the problem of resistance from three fundamental points: Firstly, the physiological changes to the cell that give rise to resistant strains; secondly, the genetic mutations that cause resistance; and thirdly, the structure-activity relationships between chloroquine analogues and cross-resistance in chloroquine. Current evidence supports reduced accumulation as a basis of resistance to chloroquine. However, the manner in which this is achieved is widely debated and remains unresolved.

1.7.1 The physiological basis of chloroquine resistance

Chloroquine resistant strains of the malaria parasite accumulate less chloroquine than do sensitive parasites.[111,112] It is thought that this reduced accumulation is either a result of reduced uptake of the drug or an enhanced efflux of the drug from parasitized cells. The enhanced efflux hypothesis has found considerable support.[113,114]
The efflux mechanism has been shown to be influenced by glucose and thus proposed to be energy dependent.[115-118] However, this appears to be disproved by the observation by Bray *et al*. (1996) that deoxyglucose also stimulates chloroquine efflux, despite being unable to generate ATP.[119]

An alternative to the efflux mechanism is a decrease of drug uptake in the food vacuole as a result of an increase in pH at this site. An increase in food vacuole pH would be expected to cause a decrease in pH trapping of the drugs in the food vacuole. Early evidence suggested that chloroquine resistant parasites do indeed exhibit an increased digestive vacuole pH[120] but later studies could provide no definitive evidence to support this theory. Other studies have suggested that there is in fact a decrease in food vacuole pH in resistant parasites resulting in a decrease in haematin available as a target for chloroquine as a result of haematin aggregation and precipitation.[121] The latter study has been under criticism regarding interpretation of microscopic images used to determine the pH.[122]

Suggestions that a Na^+ / H^+ exchanger is involved in drug uptake[123,124] or that a Cl^- / HCO_3^- antiporter is responsible for maintaining pH gradients in the food vacuole[125] appear to have been disproved,[98, 126]or do not find strong support.[98]
In addition, general changes in pH are difficult to reconcile with the observation that closely related dibasic 4-aminoquinolines, differing only in side-chain length exhibit major differences in activity against chloroquine-resistant parasites.

The structural specificity of the resistance mechanism, for example, the well-known increased parasite sensitivity to quinine but decreased sensitivity to its enantiomer quinidine, suggests that a specific transporter-mediated mechanism is involved.[8]

1.7.2 The genetic basis of chloroquine resistance

Recently, mutations in the *pfcrt* gene of *P. falciparum* have been identified as the major player in the phenomenon of chloroquine resistance. The protein product of this gene, *Plasmodium falciparum* Chloroquine Resistant Transporter (PfCRT) is located primarily in the food vacuole membrane and is thought to be responsible for chloroquine resistance by transporting chloroquine out of its site of action. Mutations at codon 86 were found to be associated with chloroquine resistance in a number of isolates.[128-132] Later, little support for this correlation could be found in epidemiological studies [133-139] and a genetic cross between chloroquine-resistant and sensitive parasites showed no evidence of association with mutations in this gene at all.[140]

Plasmodium falciparum was also found to express a homologue of the human multi-drug resistant protein refered to as *Plasmodium falciparum* P-glycoprotein homologue-1 (PfPgh 1) coded for by the *pfmdr* 1 gene.[127] Although mutations in PfPgh 1 cannot confer chloroquine resistance alone, they can lead to increased chloroquine resistance through decreased drug accumulation.[141] Thus, PfPgh 1 mutations do appear to have a role in modulation of chloroquine resistance in at least some strains of *P. falciparum*.[142]

Martin and Kirk (2004) conducted a bioinformatics analysis of the *pfcrt* gene. This study indicates that the normal function of the protein products of this gene may be to export peptides from the food vacuole.[143] Recently, Zhang *et al*., (2004) have demonstrated that chloroquine binds directly to the transmembrane transporter protein PfCRT.[144] However,

no strong evidence has been brought forward to show that quinine is also bound although PfCRT is able to modulate quinine resistance. Cooper *et al*. (2002) have demonstrated that point mutations in PfCRT are associated with chloroquine resistance.[145] It has now been established that chloroquine resistant strains share a common K76T mutation in the *pfcrt* gene. The mutant (K76T) is characterized by a change of the charged amino acid, lysine, to an uncharged amino acid, threonine. This mutation is predicted to permit the positively charged diprotonated chloroquine molecule to pass through the food vacuole membrane down its concentration gradient. Another mutation associated with resistance involves a change in an asparagine to a serine (A220S) although this mutation does not confer resistance in the absence of the K76T mutation.

There is some evidence that mefloquine resistance may be related to an increase in *pfmdr*1 copy number,[146-149] while mutations at codon 86 are associated with increased sensitivity to Mefloquine.[147] Thus, there is evidence that multi-drug resistance to quinoline methanols may also be associated with the Pgh 1 protein.[150]
Interestingly, there have been reports of chloroquine resistant field isolates that do not possess the K76T mutation[151] and also some evidence has been reported of patients harboring the parasites with the K76T mutation that nonetheless respond to chloroquine treatment [152]. This may indicate that other proteins, including Pgh 1,[142] as well as patient immunity may also play a role in clinical failure of chloroquine.

1.8 Structure-activity relationships between chloroquine analogues and cross-resistance in chloroquine

Although the chloroquine resistance mechanism remains a subject of debate, resistance certainly arises from reduced drug accumulation rather than modifications to the target haem.[141] Various SARs between chloroquine analogues and drug resistance have been reported and it appears that the resistance mechanism is structure specific and seems to recognize the aminoalkyl side-chain of chloroquine. This was demonstrated by Krogstad *et al*. (1998) who showed that analogues of chloroquine with shortened (2-3 carbons long) or lengthened (10-12 carbons long) side-chains maintain full activity against chloroquine resistant parasites[153] (Figure 1.16), while those with chain lengths close to that of chloroquine have intermediate activity.[154]

It appears that a high degree of variability can be tolerated in the side-chain as can be seen from the diversity of the nature of side-chains (Figure 1.16).[154-157] In contrast, structural changes to the quinoline ring have shown little or no effect on the activity of chloroquine against both sensitive and resistant parasites.[109,121] Cross-resistance between chloroquine and quinoline methanols such as mefloquine is not ruled out,[146] but often an inverse relationship is observed with chloroquine-resistant parasites showing enhanced sensitivity to mefloquine.

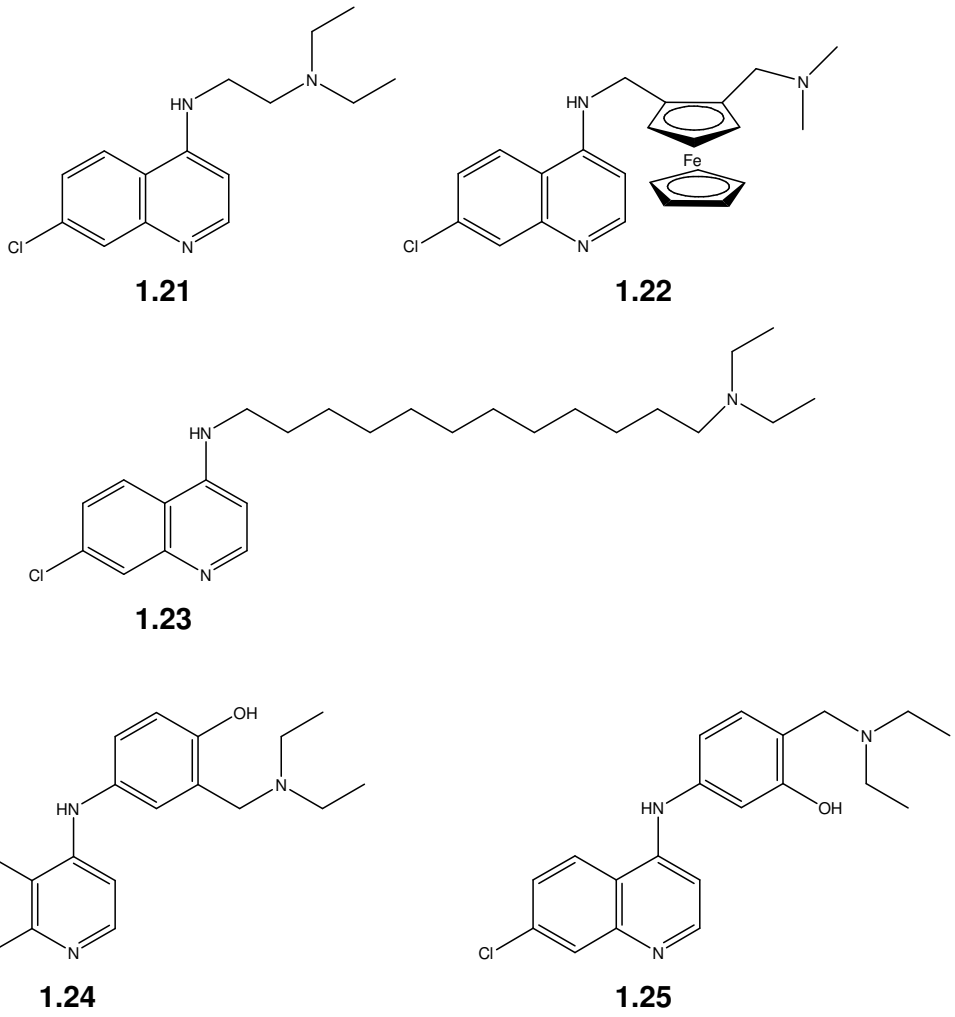


Figure 1.16: A selection of chloroquine analogues that are active against chloroquine-resistant parasites including short chain (**1.21**) and long chain (**1.23**) analogues,

analogues with side-chains containing bulky groups or aromatic rings [amodiaquine (**1.24**) and isoquine (**1.25**)] and metallated analogues [feroquine(**1.22**)].

These findings support proposals that resistance mechanisms in quinoline methanols are different to that of chloroquine resistance. The question as to whether structural changes to mefloquine can circumvent this type of resistance does not appear to have been investigated.[158]

1.9 Approaches to combat drug resistance

1.9.1 Drug rotation

There is evidence that parasites with the critical PfCRT K76 mutation are at a fitness disadvantage. For example, in Malawi where chloroquine had been replaced with sulfadoxine-pyrimethamine as a first-line therapy drug, it has been shown that after a sufficient period of nonuse the parasite's susceptibility to chloroquine is restored.[159] Similar observations have been made in Gabon, Vietnam, China and Thailand.[160] This could indicate that drug rotation is a useful approach to reversing resistance in a certain geographical location. However, there is evidence that recovery of chloroquine sensitivity arises from expansion of wild-type PfCRT in the population, rather than back-mutation.[161] This merely implies that re-expansion of resistant forms of the *pfcrt* gene is likely to spread rapidly upon resumption of drug pressure.[162] Furthermore, such reversal has not been seen in any South American strains.[163]

1.9.2 Combination therapy

The use of combination antimalarial therapy offers two important potential advantages. Firstly, the combination should improve efficacy providing additive or synergistic activity. Secondly, the combination should slow the progression of parasite resistance to the new drugs. Combination therapy is based on the assumption that while a mutation conferring resistance to a single drug might occur, the probability of simultaneous mutations conferring resistance to two drugs active against different targets is highly unlikely. Examples of successful combinations are sulfadoxine-pyrimethamine, chlorproguanil-dapsone (Lapdap)[164] and atovaquone-proguanil (Malarone®).[165] Attempts at combining chloroquine with other drugs, for example chloroquine-sulfadoxine-pyrimethamine has

been less successful.[166] However, combinations of other quinolines and related compounds have thrived. These include, mefloquine-artesunate (in Southeast Asia)[167] and lumefantrine-artemether (in Africa).[168] Combination therapy suffers a drawback when the elimination half-life of one member of the combination drug is very long or when the pharmacokinetics of the two drugs are not well matched.[169] Hastings and Watkins have described the process of resistance development as first passing through a stage of drug tolerance.[169,170] Further mutations then lead from tolerance to resistance. There is recent evidence that such a process could be unfolding with respect to artemisinin combination therapy. Sisowath *et al*., (2005) have investigated lumefantrine-artemether in Zanzibar and have reported a significant increase in *pfmdr*1 86N following drug treatment which may be a sign of developing lumefantrine tolerance.[168]

1.9.3 Redesigning of existing drugs

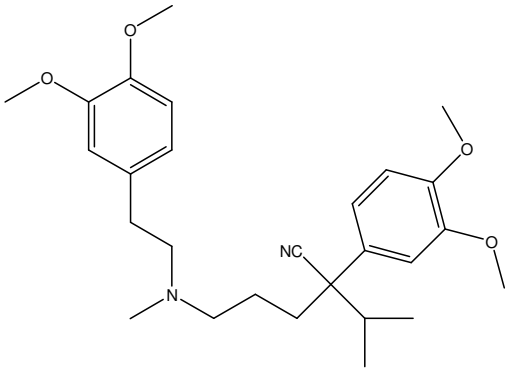
In this approach to combat emergence and spread of existing drug resistance, analogues of existing drugs are used in chemotherapy. The findings by Krogstad *et al*., (1998)[153] rationalize this approach. In their studies, Krogstad *et al*., (1998) showed that the chloroquine resistance mechanism is structure specific and appears to recognize the aminoalkyl side-chain of chloroquine.[153] In addition, Yuthavong and co-workers have shown that analogues of pyrimethamine in which the p*ara*-chloro group is moved to the *meta*-position are active against resistant strains of the parasite.[171] Increasing the flexibility of antifolate drug analogues also restores sensitivity of resistant strains as this allows the compound to again be able to fit into the drug binding site.[171] Furthermore, it has been pointed out that mutations occurring on enzymes are finite as many mutations will tend to reduce activity or result in an unstable protein.[171] In the case of quinoline compounds, analogues with shortened side-chains[154,155] or substantially altered side-chains containing a ferrocene group[156] have been shown to restore activity against resistant strains of the parasite.

1.9.4 RESISTANCE-REVERSING AGENTS

The use of resistance reversing agents has generated interest in malaria chemotherapy following the discovery of the chloroquine resistance-reversing properties of verapamil.[172] Since then, a wide and diverse range of compounds that also demonstrate the ability to reverse chloroquine-resistance and mefloquine-resistance has been described. These include other calcium channel blockers,[174-176] some tricyclic antidepressants[177-180] and antihistamines[181-183] (Figure 1.17).

A chloroquine resistance-reversing agent is a compound that lowers the IC_{50} value of chloroquine in chloroquine-resistant parasites to a value comparable to that of chloroquine-sensitive parasites. The substance is in itself not active against the parasite at the concentration at which it restores activity and it does not alter activity against chloroquine sensitive parasites. Chloroquine resistance-reversal in *Plasmodium falciparum* is therefore characterized by both a reduction of the chloroquine IC_{50} and the increased accumulation of chloroquine into chloroquine resistant strains specifically,[113,173].

Interestingly, chloroquine resistance-reversers seem to induce only a partial reversal of the resistant phenotype in the parasite. While they decrease the IC_{50} values in resistant strains to those found in sensitive strains, chloroquine never accumulates in resistant parasites to reach levels found in sensitive strains of the parasite.



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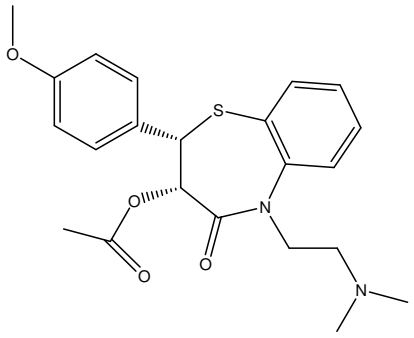

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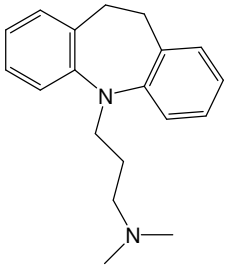

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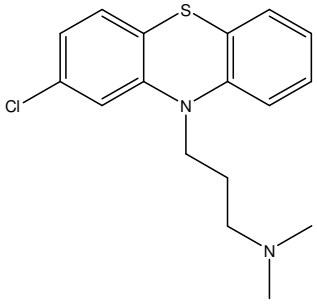

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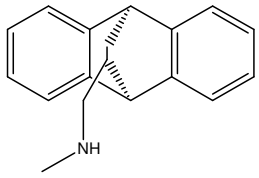

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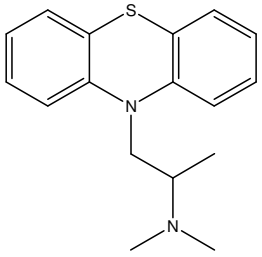

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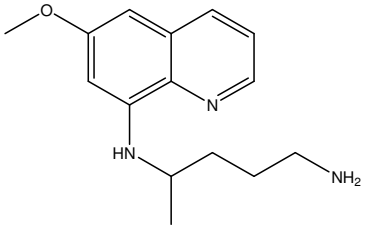

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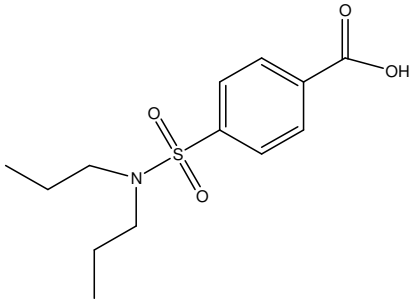

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Figure 1.17: A representative selection of compounds with reported chloroquine resistance-reversing activity. The compounds include known calcium channel blockers (**1.26**) and (**1.27**), antidepressants (**1.28**) and (**1.30**), antipsychotics (**1.29**) antihistamines (**1.31**) antimalarial (**1.32**) and an example of a non-typical chloroquine resistance-reverser (**1.33**).[141]

1.9.4.1 Mechanism of action of chloroquine resistance-reversers

Recent work has indicated that chloroquine resistance-reversal may involve direct competition of the resistance reverser for the chloroquine-binding site in PfCRT. A recent report by Lakshmanan *et al*., (2005) on the effects of *pfcrt* on chloroquine resistance, verapamil reversibility and activities of chloroquine analogues with shortened and lengthened side-chains has further elucidated these issues.[185] It appears that most chloroquine resistance-reversers bind to the same site on PFCRT as chloroquine, effectively blocking chloroquine binding by competition. The close structural relationships between chemosensitizors and chloroquine have been highlighted by Kalkinidis *et al*., (2002).[186] Further support for a direct competition is the fact that the aromatic nucleus of chloroquine, 4-amino-7-chloroquinoline is itself a resistance reverser.[186]

The binding site proposed by Alibert *et al*., (2002)[184] appears to have strict structural requirements. It readily accommodates chloroquine, but not analogues with shortened side-chains that are active against chloroquine-resistant parasites.[184] This is consistent with the observation that side-chain length of chloroquine analogues is important in determining the resistance profile to these drugs.[154] There are key mutations in *pfcrt* which appear to result in the formation of this binding site and of these mutations K76T is the most important. The K76T mutation is also shown to be the decisive factor in determining susceptibility to chloroquine analogues with different chain lengths. Overall accumulation of chloroquine and haem-binding to chloroquine could also be directly correlated with this key mutation.

A puzzling aspect of the study by Alibert *et al*. is that chloroquine resistance-reversal activity and increased chloroquine accumulation in the parasite do not appear to be directly correlated.[184] A possible explanation of this is that some dihydroanthracenes may interact with haem without inhibiting haemozoin formation, but competing with chloroquine and hence reducing its overall accumulation. In this regard, it is known that some quinolines can bind haem without inhibiting haemozoin formation.[102] It is also important to note that most resistance reversers work at micromolar concentrations which are much higher than the expected amount of free, non-haem bound chloroquine. A reason for this disparity is not clear but could be related to the reverser affinity for the chloroquine-binding site. Interestingly, some compounds are able to reverse the

resistance to both chloroquine and mefloquine, which is not consistent with the proposed strict structural specificity of the chloroquine-binding site. Furthermore, some compounds that reduce intracellular glutathione levels are also able to sensitize the parasites to chloroquine.[92]

1.9.4.2 Structure-activity relationships in chloroquine resistance-reversers

Previously, structure-activity relationship studies in chloroquine resistance-reversers were an area that was largely unexplored. However, in the last few years several articles have been published on SARs in resistance reversing agents.[184,187,188] A recent study by Lakshmanan *et al.*, (2005) also throws some light on the relationship between resistance reversal activity and PfCRT structure.[185]

Bhattacharjee *et al.*, (2002) conducted a 3D-QSAR study of 17 imipramine analogues.[187] The study revealed that the basic features for a compound to act as a resistance reverser are two hydrophobic aromatic rings and a hydrogen bond acceptor, preferably secondary or tertiary nitrogen in a specific geometric orientation (Figure 1.18). A two or three carbon bridge from the heterocyclic 7-membered ring to the N atom was shown to be optimal for activity. Nitrogen basicity, frontier orbital energies and lipophilicity were found to correlate with resistance reversing activity.

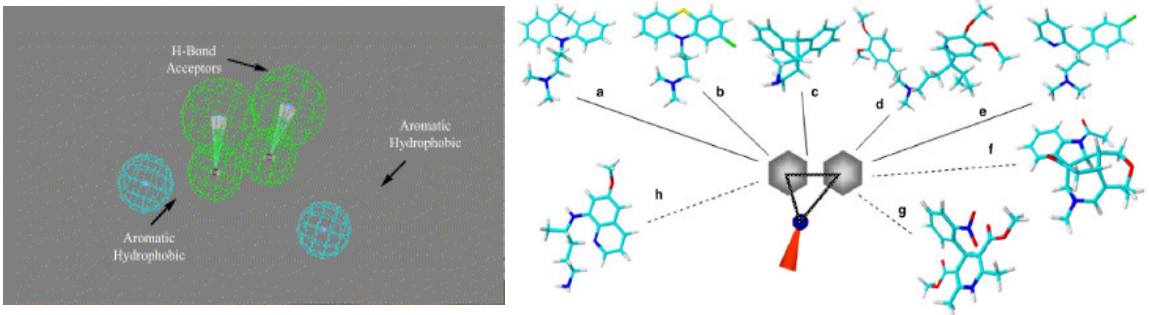


Figure 1.18: A schematic presentation of the pharmacophore for chloroquine resistance-reversal proposed by Bhattacharjee *et al.*, (2002) together with a series of representative compounds of various classes to illustrate the proposed pharmacophore. These include imipramine (a), chlorpromazine (b), a dihydroanthracene derivative (c), verapamil (d) and primaquine (h).

The study by Alibert *et al.*, (2002) conducted using a series of 28 novel rigid dihydroanthracene derivatives aided in establishing some possible characteristics of the binding site for resistance reversers.[184] Using a molecular modeling approach, the authors defined a putative interaction site for the N atom in which there is hydrogen bonding to a hydroxyl group of a serine residue and a salt bridge formed between the same protonated, positively charged N and an aspartate residue. The findings from this study are highlighted in Figure 1.19.

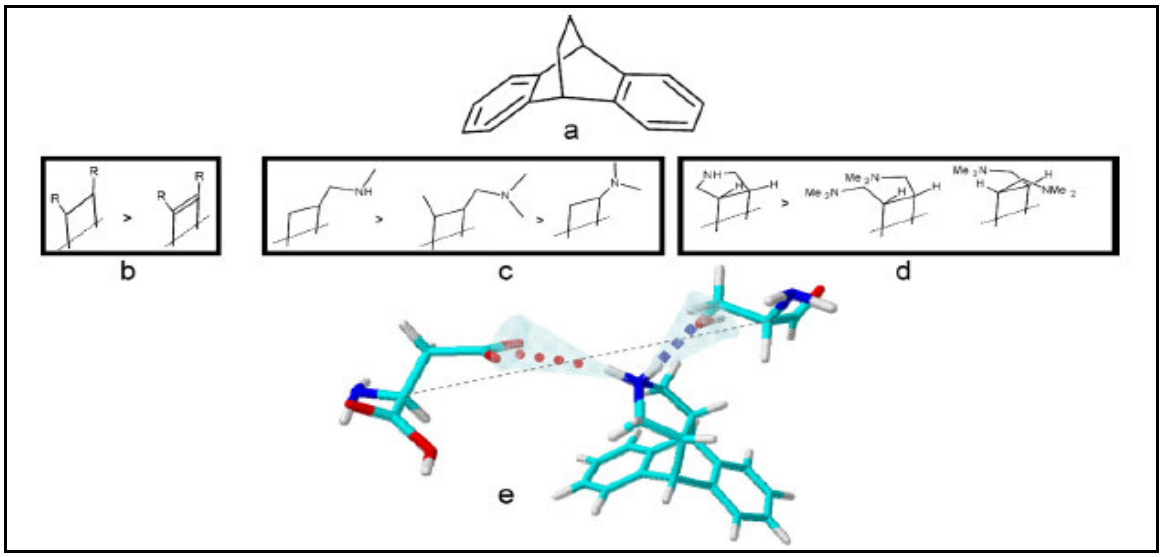


Figure 1.19: Schematic presentation of the SARs conducted by Alibert *et al.*, (2002) and the proposed chloroquine resistance-reverser-binding site. The SARs were conducted using the rigid 9, 10-dihyro-9, 10-ethano and etheno-anthracenes (a). Ethano-compounds were better resistance reversers than the etheno- compounds (b). Secondary amines were more active than tertiary amines while the N atom preferred the β-position in the chain (c). Cyclic amines were more active than acyclic structures (d). The binding site is depicted in (e). The amino group was proposed to hydrogen bond (blue dashes) to the hydroxyl group of a serine (or threonine) residue and to interact electrostatically (red dashes) with the carboxylate group of an aspartate (or glutamate) residue. The two residues were proposed to be 9.2 Å apart (dotted line).

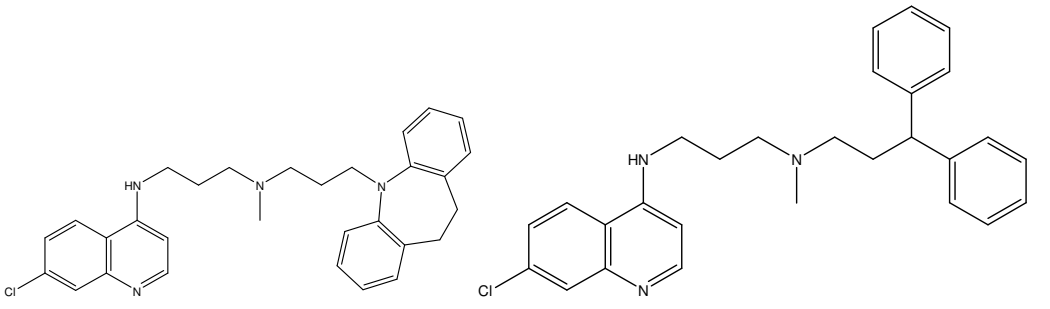
The mandatory requirement for the aromatic rings was illustrated by the inactivity of mecamylamine, which contains the bicyclic saturated ring system with a secondary N function, but lacks any aromatic rings. The authors suggested that the proposed binding

site is consistent with mutant PfCRT in chloroquine-resistant parasites that have alleles encoding a CVIET haplotype at residues 72–76. The functional groups on the glutamate and threonine residue side-chains would presumably form a site resembling that produced by the putative aspartate and serine residues in the hypothetical binding site generated by this study. [184]

1.9.4.3 "Reversed chloroquine" compounds

Burgess *et al*. (2006)[189] reported a highly innovative hybrid molecule that combines the pharmacophore of a 4-amino-7-chloroquinoline antimalarial with a resistance reversing group and called this class of compounds "reversed chloroquine". Such a design will deliver the resistance-reversing agent in a 1:1 ratio with the quinoline moiety. Thus, "reversed chloroquine" compounds provide the pharmacophore for haem-binding (4-aminoquinoline), inhibition of haemozoin formation (4-amino-7-chloroquinoline) and drug accumulation by pH trapping (a tertiary amino group in the side-chain) as well as the pharmacophore for a resistance reverser,[189] namely two suitably positioned aromatic groups with an amino group separated by a short chain.[191]

In addition to promoting drug accumulation in the food vacuole, it was speculated that the reversal agent component would interfere with export of the chloroquine moiety from the food vacuole by the mutated chloroquine-resistant PFCRT alluded to in Chapter 1.7.2. Together, these effects would allow a curative dose against chloroquine-resistant strains to be lower than that of its components given separately. These so-called "reversed-chloroquine" compounds demonstrated high potency against both sensitive and resistant strains of the malaria parasite *in vitro* and were shown to be active against *P. chabaudi* in mice with no signs of toxicity, although they have not yet been demonstrated to exhibit chloroquine resistance-reversal activity. Nonetheless, the approach is a novel one and could result in new active antimalarials that are not susceptible to development of chloroquine-like resistance. It must be emphasized conversely that in many cases alterations of the chloroquine side-chain has result in compounds that are active against chloroquine resistant strains of *P. falciparum*.[185] A representative selection of the "reversed chloroquine" compounds reported by Burgess *et al*., (2006) is illustrated in Figure 1.20 below.



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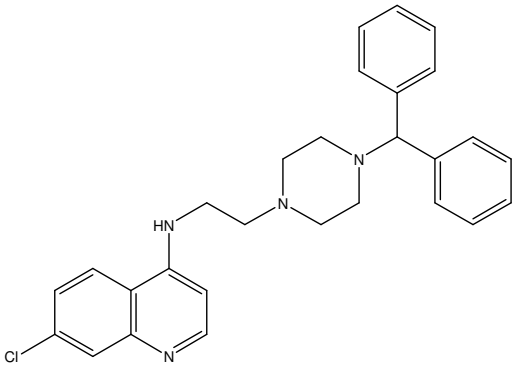

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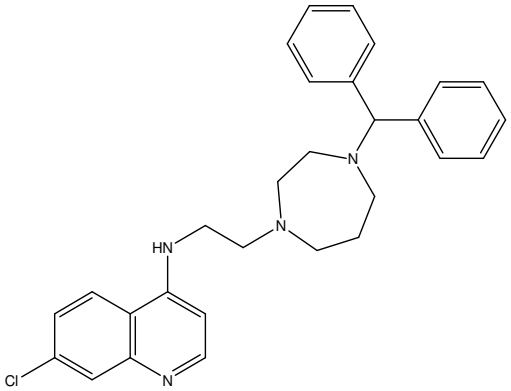

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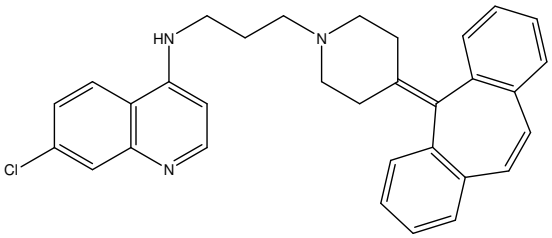

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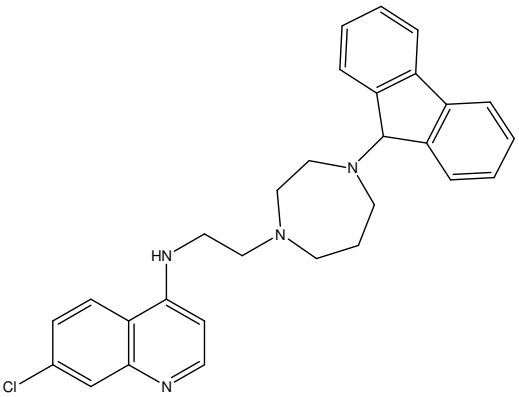

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Figure 1.20: A selection of "reversed chloroquine" compounds reported by Burgess *et al* (2006). All of the compounds shown exhibit activity against chloroquine-sensitive as well as resistant parasites.[189, 190]

1.10 OPTIMIZATION OF ACTIVITY IN DRUG DESIGN

The drug optimization process usually involves changing several characteristics of a compound, such as its shape, functionality (hydrogen bonding or ion pair interactions), lipophilicity and electronic characteristics. Historically, the experience and insight of the researcher as well as availability of starting material drove drug design. However, in the 1960's and 1970's, following the development of the Hansch method of structure-activity correlations, a more rational approach to drug design was employed and the method also brought the ability to generate Quantitative Structure-Activity Relationships (QSAR) with in analogue series.[192, 193]

The QSAR approach aims to take a set of analogues of an active compound, identify and quantify their physicochemical properties and determine which properties have a significant input to the activity of the molecule. Commonly used property descriptors are the substituent hydrophobicity constant, π (a measure of the substituent's hydrophobicity relative to hydrogen), the Hammett constant, δ (a measure of the substituent's electron withdrawing or donating characteristics) and the Taft's steric factor, E_s.[194] Through an appropriate mathematic derivation of a relationship between these and / or other properties and the level of activity, it should be possible to predict the biological activity of analogues. However, to generate a reliable correlation a relatively large number of compounds should be used in the initial analysis. Furthermore, it is not always easy to separate the significance of different parameters e.g., if activity varies across a homologous series of compounds in which an alkyl side-chain length is increased, it is difficult to decide whether this is due to the increase in chain length or lipophilicity or both.[193]

The optimization process therefore is a multifactorial process and needs to be broken down into a stepwise approach to provide focus for synthetic activities. One such step commonly faced is the identification of optimum substitution patterns on phenyl or hetero aryl rings which are regular integral features in lead compounds.

1.10.1 Topliss schemes

In the 1970's Topliss addressed some of the concerns arising form QSAR using non-mathematical schemes for analogue design.[195-197] The fundamental basis of the methods was the concept pioneered by Hansch,[192,193] that the primary influences on activity following introduction of a substituent resulted from lipohilic (π), electronic (δ) and steric (E_s) properties of the substituent. The first of these schemes used a decision tree approach which later became known as the Topliss tree.[194]

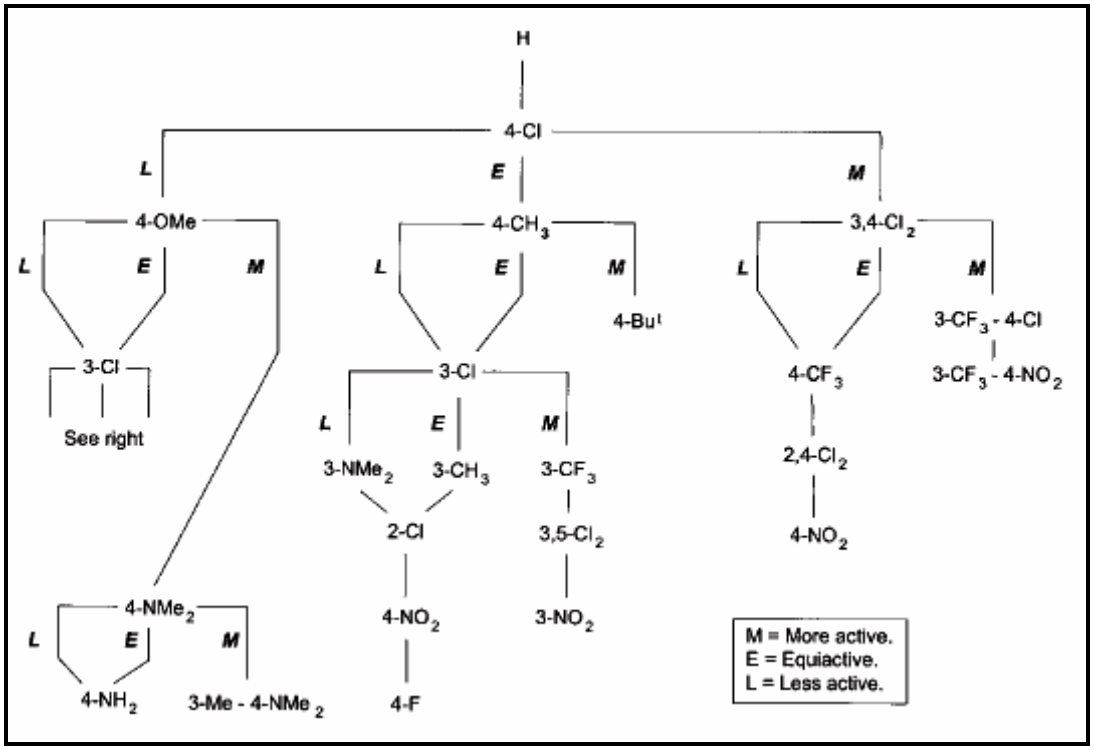


Figure 1.21: A simplified version of the Topliss tree.[194]

A stepwise procedure is used starting with the unsubstituted phenyl compound and proceeding first to the 4-chloro analogue. This substituent is lipophilic (+π) and electron withdrawing (+δ). The potencies of the two are compared and if the 4-chloro analogue is more active, this improved potency can the ascribed to a +π effect and a +δ effect or a combination of both. Then the 3, 4-dichloro compound is synthesized since in this analogue the summed +π and +δ values would be larger with prospects for improved potency. If the 4-chloro compound was equipotent, this could be due to a favorable +π

effect offset by an unfavorable -δ dependency. Then the 4-methyl analogue would be synthesized as this group possess both lipophilic and electron withdrawing properties. Similarly, if the 4-chloro analogue is less potent than the parent compound, one may conclude that activity is -π or -δ controlled. Then the 4-methoxy analogue would have to be made owing to the electron-releasing and marginal hydrophilic nature of this substituent. [194]

The Topliss scheme can also be applied in a batch wise manner in which the parent drug together with the 4-chloro, 3, 4-dichloro, 4-methyl and the 4-methoxy are all synthesized and their potency order compared to the order of the Topliss tree. From this activity pattern analysis a new selection can be made for the synthesis of potentially potent analogues.[194]

Both the stepwise and batchwise procedures have been successfully used in synthesis of more potent analogues including in the synthesis of a series of sulphonamide with carbonic anhydrase inhibitory activity[198] and a series of anti-inflammatory aryltetrazolyl propionic acids.[199]

1.11 AIMS

The overall aim of this project is to synthesize 4-amino-7-chloroquinoline antimalarials of high potency against chloroquine-resistant parasites, which possess potential resistance-reversing properties. Therefore, the target compounds are proposed to function as hybrid molecules ("reversed chloroquines") which provide both the potent antimalarial activity of chloroquine-like compounds with the added advantage of possessing chloroquine resistance-reversing properties.

1.12 OBJECTIVES

The design of the target compounds will be facilitated by 3 models that have been reviewed extensively above being; (i) The 3D-QSAR pharmacophore model for chloroquine resistance-reversal proposed by Bhattacharjee *et al.*, (2002); (ii) The SAR model based on findings by Egan *et al.*, (2000) that depicts the pharmacophore for antimalarial activity of quinoline antimalarials, and (iii) the Topliss scheme in an attempt to improve potency of the drugs.

Based on delineations from these three models, the target compounds will be synthesized, purified and characterized.

Their *in vitro and in vivo* antimalarial activity and cytotoxicity will be evaluated against chloroquine-sensitive as well as chloroquine-resistant strains of *P. falciparum*. Furthermore, the chloroquine resistance-reversing properties of the target compounds will also be evaluated

.

CHAPTER 2: SYNTHESIS

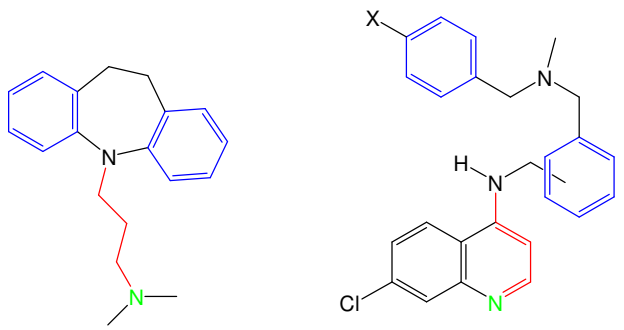
2.1 RATIONALE AND DESIGN

In the study described in this thesis, the model proposed by Egan *et al*., (2000)[102] together with work published by Bhattacharjee *et al*., (2002)[187] and Alibert *et al*., (2002)[184] facilitated the design of "reversed-chloroquine" compounds. The proposals made in these three models have been extensively reviewed above (Chapter 1.6 and Chapter 1.9.4).

In summary, Egan *et al*., (2000) highlighted the essential structural features in chloroquine-like compounds to be the 4-aminoquinoline nucleus, which was identified as the minimum requirement for haematin association; the 7-chloro group, which is optimal for inhibition of haemozoin formation; and the need for an amino group as a basic site in the side-chain to facilitate drug accumulation by pH trapping.[102]
For resistance reversal, Bhattacharjee *et al*., (2002) proposed the need to have two hydrophobic aromatic rings fused to a 7-membered ring containing a nitrogen atom bearing a three-carbon chain with a hydrogen-bond acceptor group at the terminal, preferably an amino group in imipramine-like resistance reversers.[200] It is interesting to note, as highlighted in Chapter 1.9, that other non imipramine-like resistance reversers were shown to contain many of the same features, typically the two aromatic rings and the chain with a basic N atom at the terminal and Alibert *et al*., (2002) gave an insight into the possible characteristics of the binding site for resistance reversers.

For this study, a comparison of the structures of chloroquine resistance-reversers shows that the linkage between the two aromatic rings is quite permissive and can range from a direct bond between rings to long flexible chains of six or seven atoms (**1.26**-**1.33**) (Chapter 1.94). Indeed, some resistance reversers such as primaquine (**1.10**) possess only a single aromatic ring system. Based on the resistance-reverser imipramine (**1.28**) in which the H-bond acceptor group (terminal N-atom) is linked to a ring system containing two hydrophobic aromatic rings *via* a 3-carbon chain (Figure 2.1), the target compounds (dibemethinoquines) were thus designed with a dibemethin side-chain linked to a hydrogen bond acceptor (quinoline N-atom) *via* a three-carbon linker involving the pyridine ring of the quinoline (Figure 2.1). In this manner, the target compounds were

perceived to meet the requirements for chloroquine resistance-reversal as proposed by Bhattacharjee *et al*., (2002). Thus, it was proposed in the current work that the dibemethinoquine molecule would exhibit resistance-reversing properties.

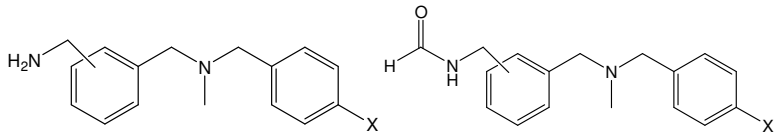


(1.28) **(6, 43 - 53)**

Figure 2.1: Chemical structure of imipramine (**1.28**) and the generic structure of dibemethinoquines (**6**, **43** – **53**).

In order to better understand the structure–activity relationships between resistance reversal and structure, the point of attachment of the Quinoline aminomethyl linker to the dibemethin was varied between *ortho*-, *meta*- and *para*- positions. Three different substituents (X) were introduced into the *para* position of the terminal aromatic ring in dibemethinoquines to investigate the effect of electronic and hydrophobic properties of groups attached to the dibemethin nucleus. The *para* position for the X-substituent was preferred over *ortho*- and *meta*- in order to minimize steric effects, while retaining mesomeric (hyper-conjugative) and inductive communication between X and the tetha joining the two aromatic rings. The selection of substituents (X) was based on the Topliss Scheme in which groups selected were Cl (hydrophobic/electron-withdrawing), OMe (electron-releasing, similar hydrophobicity to H) and NMe_2 (strongly electron-releasing/hydrophobic).

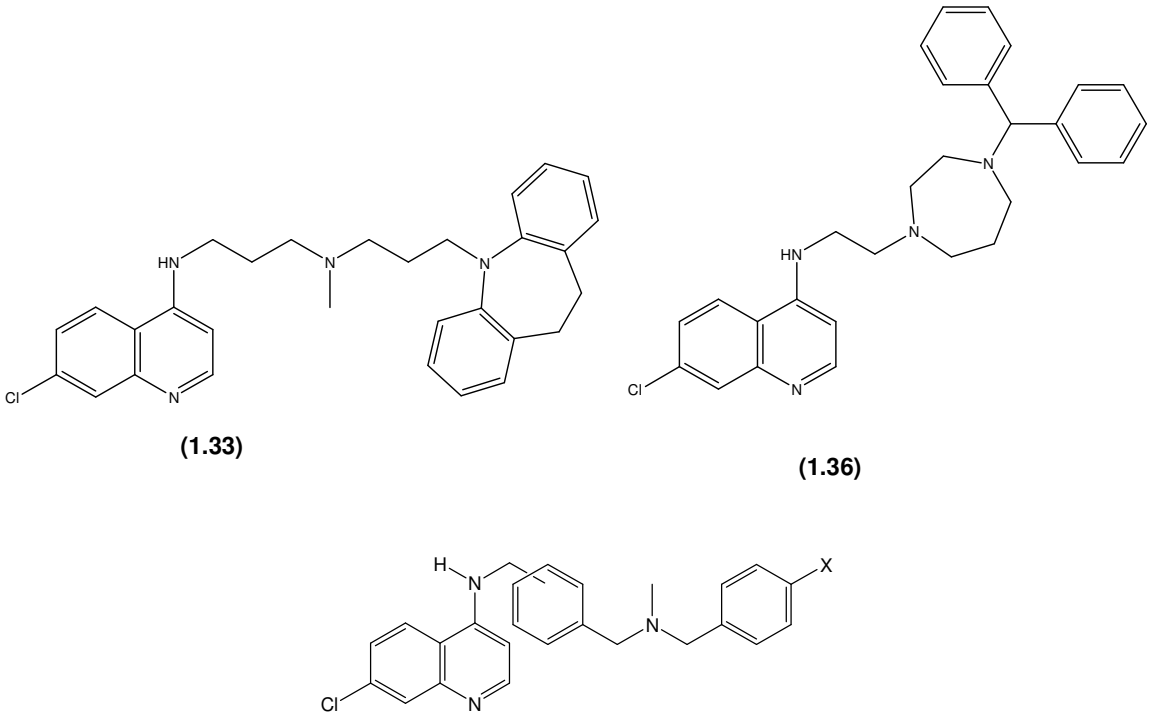
To investigate whether the side-chain itself might exhibit resistance-reversing activity and to probe structure–activity relationships between resistance reversal and side-chain structure, the dibemethin side-chain was designed with an aminomethyl chain on one phenyl ring. The primary amino terminal was also formylated to probe the effect, if any, of the basicity of this group (Figure 2.2).



(**4**, **21** – **31**) (**5**, **32** – **42**)

Figure 2.2: Generic structures of aminomethyl dibemethins (**4**, **21 – 31**) and amidomethyl dibemethins (**5**, **32** – **42**).

The basis of the "reversed chloroquine" idea reported by Burgess *et al*., (2006) is that the fundamental pharmacophore for haem-binding (4-aminoquinoline), inhibition of haemozoin formation (4-amino-7-chloroquinoline) and drug accumulation by pH trapping (a tertiary amino group in the side-chain) is preserved as is the pharmacophore for a resistance-reverser[190] (Figure 2.3), namely two suitably positioned aromatic groups with an amino group separated by a short chain.[190]



(1.33)

(1.36)

(**6, 43** – **53**)

Figure 2.3: Chemical structure of some reversed chloroquine compounds reported by Burgess *et al*., 2006 (**1.33** and **1.36**) and the generic structure of the target compounds (**6**, **43** – **53**).

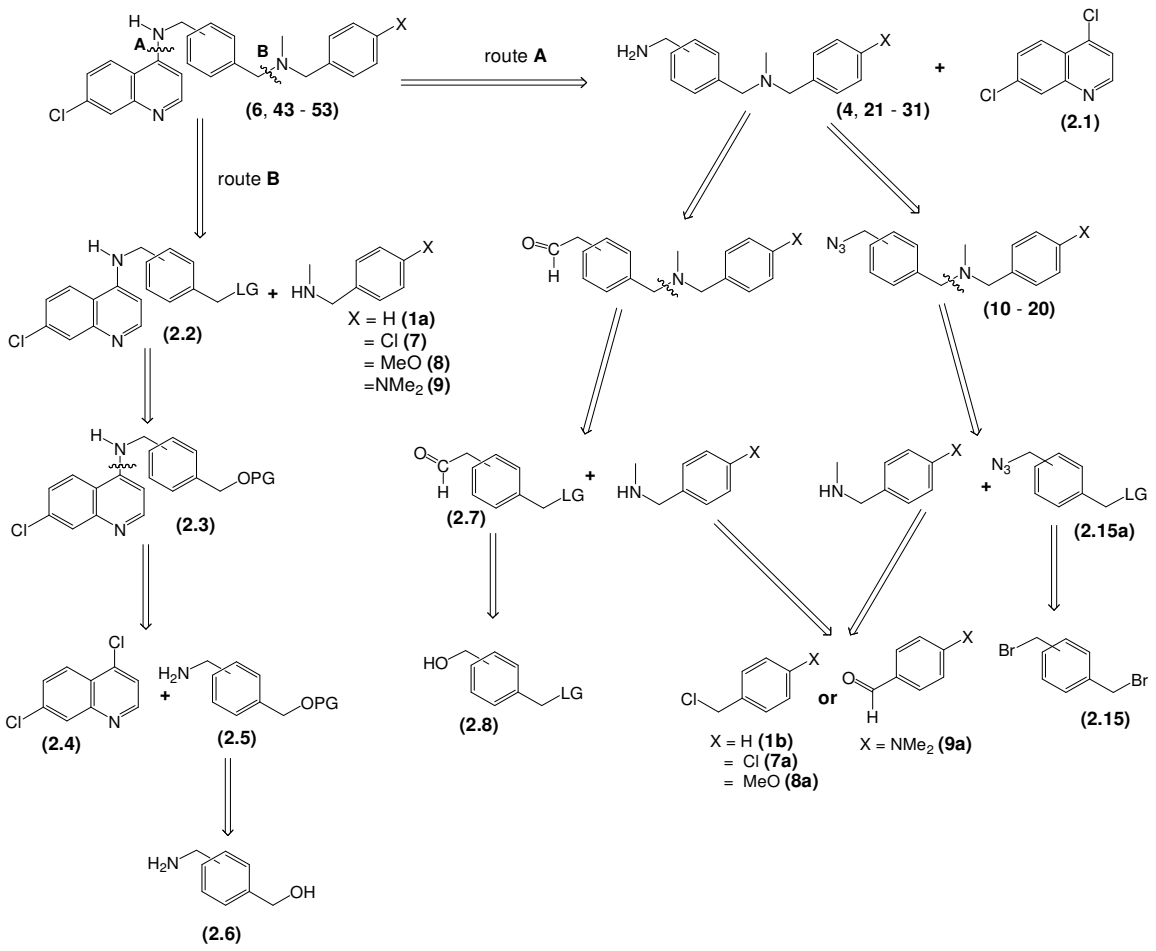
The target compounds in the current study would similarly possess the following characteristic features:

(i) A 4-amino-7-chloroquinoline antimalarial pharmacophore important for haem-binding and inhibition of haemozoin formation.
(ii) A dibemethin scaffold coupled with the H-bond acceptor (quinoline N-atom) for providing the resistance-reversing moiety.
(iii) Protonatable N atoms in the quinoline ring and the dibemethin side-chain for increasing drug accumulation in the food vacuole by pH trapping.

This was considered to fulfill the pharmacophore requirements of a “reversed chloroquine” molecule.

2.2 Retro-synthesis of the dibemethinoquines

Retrosynthetic analysis of the dibemethinoquine targets is shown in Scheme 2.1 below.



Scheme 2.1: Retro-synthesis of the target compounds.

The synthesis of dibemethinoquines was envisaged as attainable by considering a convergent synthetic route (route A) or a divergent synthetic route (route B) as outlined in Scheme 2.1 above. The convergent synthesis involved coupling of two pieces in which the side-chain aminomethyl group would be obtained *via* reductive amination of an aldehyde or the Staudinger reduction of an azide to give aminomethyl dibemethins (**4**, **21** - **31**). On the other hand, a divergent synthesis would involve alkylation of a suitably *p*-substituted benzyl-*N*-methylamine (**1a**, **7** – **9**) with a respective 4-aminoquinoline-*N*-

benzyl-halide (**2.2**). In this study, the convergent synthetic route was preferred as it entails considerably more reaction steps as shown in Scheme 2.1. Prior knowledge that incorporation of the quinoline moiety in chloroquine-like compounds is better achieved *via* nucleophilic substitution on the quinoline ring supported the resolute choice of 4, 7-dichloroquinoline (**2.1**) as the precursor to the quinoline moiety of dibemethinoquines. If quinoline is considered as a fused benzo[*b*]pyridine according to Hantzsch-Widman fusion nomenclature, its chemistry can be rationalised in terms of that of benzene and pyridine from which it is derived. Like pyridine, the quinoline pyridine ring is π-deficient due to the electron-withdrawing inductive and mesomeric effects of the nitrogen atom. These effects are pronounced at positions 2 and 4 (Figure 2.4). The reactivity of these positions is further increased by the presence of electron-withdrawing groups; for example, a halogen group at position 2 or 4 further activates these positions towards nucleophilic attack. 4, 7-Dichloroquinoline is therefore more reactive towards nucleophiles as a result of resonance stabilization of the reaction intermediate *via* the pyridine nitrogen atom and the presence of a second aromatic ring.

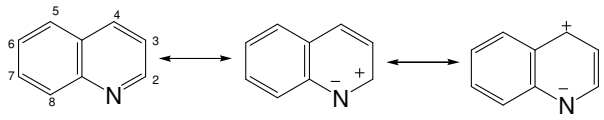
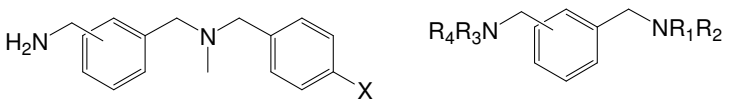


Figure 2.4: Resonance structures of quinoline ring, illustrating mesomeric effects with resulting preferred sites for nucleophilic attack.

Since changes at the 7-position of the quinoline nucleus of chloroquine have been shown not to produce compounds that are active against chloroquine-resistant parasites, the 7-chloroquinoline moiety of chloroquine was retained in dibemethinoquines (**6**, **43** – **53**) and commercially available 4, 7-dichloroquinoline (**2.1**) fulfilled this identity. Against this background, the key methodological challenge in the synthesis of dibemethinoquines was the synthesis of the side-chains (**4**, **21** – **31**). Aminomethyl dibemethins can be viewed as unsymmetrical *bis*-aminomethyl phenyldiamines with different substituents on each N (see (**2.7**) in Figure 2.5). The main challenge in their synthesis would be the timing and methods of forming the new C-N bonds.

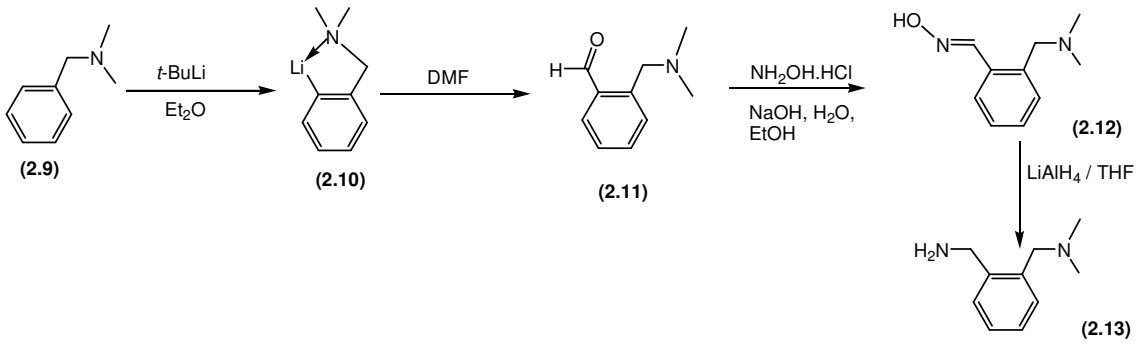


4, 21 - 31 **2.7**

Figure 2.5: General structure of dibemethins (**4**, **21**-**31**) and their condensed structure (**2.7**)

2.3 General routes to bis-aminomethyl phenyldiamines

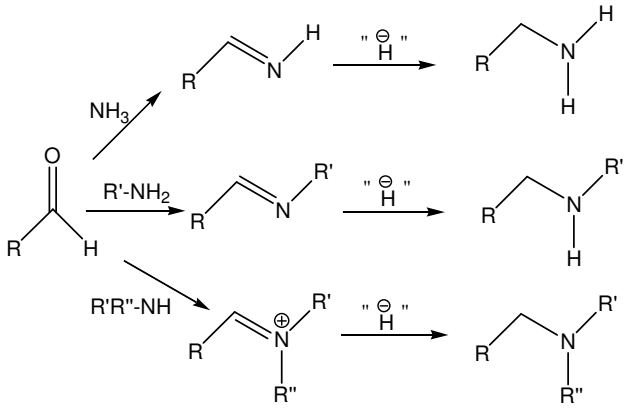
A number of classical methods have been employed in the formation of new C-N bonds. These include but are not restricted to: i) synthesis *via* azides by the Staudinger reaction; ii) from carboxylic acids in the Schmidt reaction; iii) from halides and hexamine in the Delepine reaction; iv) from primary halides using potassium phthalimide in the Gabriel synthesis; v) from primary alcohols using Mitsunobu and Staudinger reactions and *via* reductive amination of aldehydes and ketones. Evidently, the methods and precursors are diverse. In the literature, *bis*-aminomethyl phenyldiamines have been prepared by a variety of methods. Of particular importance to this project are the methods by Blackie *et al*., (2007)[200] and Rinke *et al*., (2001).[201]



Scheme 2.2: Route by Blackie *et al*., to the synthesis of *bis*-aminomethyl arylamines.

The first step in the method by Blackie *et al*. for synthesizing *bis*-aminomethyl phenyldiamines (Scheme 2.2) requires the synthesis of 2-dimethylaminomethylbenzaldehyde (**2.11**), which was prepared using *tert*-butyllithium (*t*-BuLi) and dimethylformamide (DMF) in a directed metallation / quenching sequence as shown in Scheme 2.2 above. Compound (**2.11**) was then reductively aminated *via* its benzylic oxime (**2.12**). Reductive amination is an important method in organic synthesis

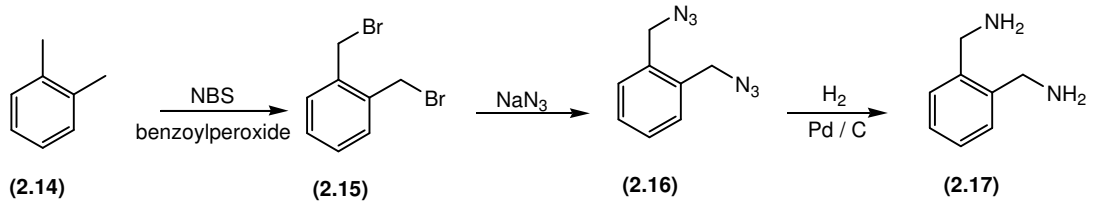
in which a carbonyl group is aminated. As illustrated in Scheme 2.3 below, the prototype reaction involves reaction of an aldehyde with ammonia to form an imine intermediate. Subsequent reduction of the imine by H_2, a Friedel catalyst or using a metallohydride yields a primary amine. Similarly, the reaction performed using a primary amine in place of ammonia yields a secondary amine while if a secondary amine is used the reaction proceeds *via* an iminium intermediate to give a tertiary amine.



Scheme 2.3: General mechanism for reductive amination.

The formation of primary amines *via* reductive amination of aldehydes using ammonia in the presence of the reductant is complicated by a competing reaction. As the primary amine begins to build up, it may react with the aldehyde to give an imine which is subsequently reduced to a secondary amine. For this reason, reductive amination *via* an oxime in which the condensation and reduction steps are separate is often the preferred route to the synthesis of primary amines from aldehyde or ketone sources.

An alternative to the method by Blackie *et al*., (2007)[200] is the method by Rinke *et al*., (2001)[201] (Scheme 2.4).

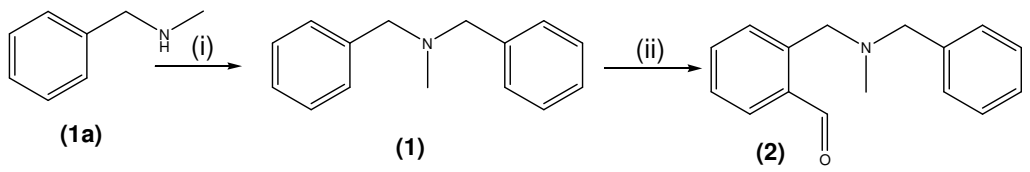


Scheme 2.4: Route by Rinke *et al*, to the synthesis of *bis*-aminomethyl phenyldiamines.

In this method, *o*-xylene was used as a starting point for the synthesis. Benzylic bromination with *N*-bromosuccinimide using benzoyl peroxide as radical initiator was followed by the substitution of the benzylic bromides with azide using sodium azide (2 equiv.). A degree of selective mono-substitution by azide can be achieved using a stoichiometric amount of azide. Other alkali azides, tetraalkylammonium azides, polymer-bound azides or silver azide have also been used to generate aliphatic or benzylic azides in the literature. Finally, reduction of the diazides using catalytic hydrogenation with Pd/C gave the desired diamines. $LiAlH_4$ or triphenylphosphine and H_2O (Staudinger process) have also been used to reduce azides to amines.

2.4 Synthesis of dibemethinoquines

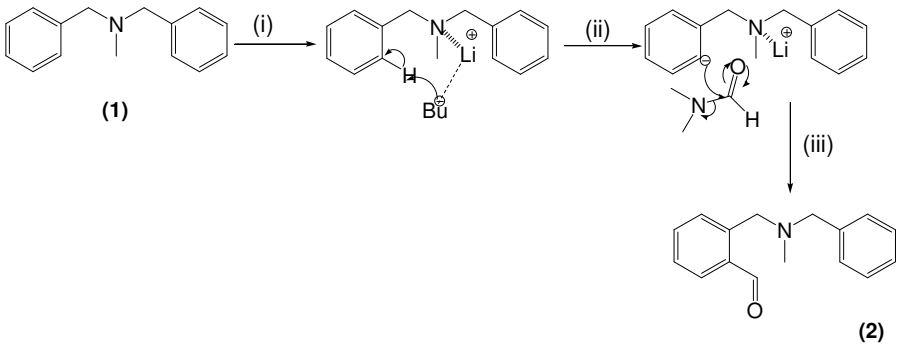
In this study, *o*-aminomethyl dibemethin (**4**) was synthesized in four steps using the method by Blackie *et al*. The first step in the synthetic route requires the synthesis of dibemethin (**1**) which was achieved *via* a nucleophilic substitution of benzyl bromide by *N*-benzyl-methylamine (**1a**) in CH_2Cl_2 (Scheme 2.5). Compound (**1**) is known, and was previously prepared by a similar method. Confirmation of the structure of (**1**) was provided by its ^1H NMR spectrum which revealed the following resonances: a 10-proton multiplet at δ 7.57 - 7.38 ppm assigned to the aromatic protons, a 4-proton singlet at δ 3.70 ppm for the symmetrical phenyl CH_2 and a 3-proton singlet at δ 2.37 ppm for the methyl protons. The correct number of carbons [as six] based on symmetry was observed in the ^{13}C spectrum of (**1**). As indicated in Scheme 2.5 below, *o*-formylation of dibemethin to afford (**2**) was achieved using *t*-BuLi and DMF.



(1a) **(1)** **(2)**

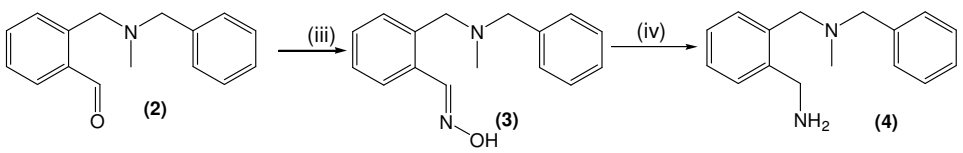
Scheme 2.5: Preparation of *o*-(*N*-benzyl-*N*-methylamino)methyl-benzaldehyde (**2**) . *Reagents and conditions*: (i) benzyl bromide, NEt_3, CH_2Cl_2, 2hrs, 67%; (ii) *t*-BuLi, Et_2O, -78 ºC, 30 minutes; DMF, r.t, 3h, 77%.

The mechanistic events that lead to the *o*-formylation of dibemethin are worthy of mention and are depicted in Scheme 2.6 below.



Scheme 2.6: The mechanistic events involved in the *o*-formylation of dibemethin (**1**) to afford (**2**). *Reagents and conditions*: (i) *t*-Butyllithium, anhydrous Et_2O, -78 ºC, 30 minutes; 0 ºC, 30 minutes. (ii) anhydrous DMF, rt, 3h, 77%.

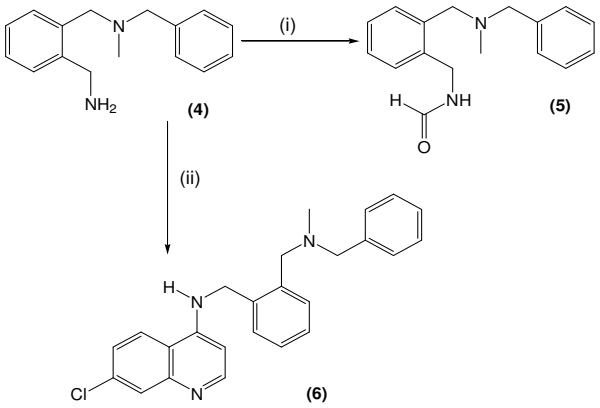
The proton at the *o*-position of dibemethin (**1**) is the most acidic owing to the presence and position of a nitrogen atom in this compound. *t*-Butyllithium was used to abstract the *ortho*-proton *via* an *ortho*-directed metallation involving the nitrogen resulting in the formation of an anionic intermediate which precipitated as a yellow lithio-salt. Caution was taken in this reaction as *t*-butyllithium reacts explosively with water. Consequently, anhydrous reagents and anaerobic conditions using a Schlenk line were employed. Subsequent addition of anhydrous DMF to the anion produced the benzaldehyde (**2**) in good yield (77%) following work-up and colunm chromatography. Its ^{1}H NMR and ^{13}C NMR spectra revealed peaks that were consistent with the target compound. Thus, a noteable change in the ^{1}H spectrum of (**2**) relative to (**1**) was the downfeild (deshielded) aromatic proton *ortho* to the newly inserted carbonyl group which was split into a dd, J = 1.5, 7.5 Hz, with *ortho* and *meta* aromatic couplings. Similarly, a formyl CHO proton appeared down-field as a singlet at δ 10.42 ppm. The IR spectrum of (**1**) also confirmed insertion of the carbonyl group by revealing a band at ν_{max} 1690 cm^{-1} due to (C=O), which was corroborated by a downfield resonance in the ^{13}C spectrum at δ 192.0 ppm for the formyl carbon.



Scheme 2.7: Preparation of *o*-aminomethyl dibemethin (**4**). *Reagent and conditions*: (iii) $NH_2OH.HCl$, $NaOH_{(aq)}$, EtOH, reflux, overnight, 85%; (iv) $LiAlH_4$, Et_2O, reflux overnight, 89%.

Subsequent reductive amination of the aldehyde (**2**) *via* its oxime according to the method by Blackie *et al*., (2007)[200] afforded *o*-aminomethyl dibemethin (Scheme 2.7). Thus, hydroxylammonium hydrochloride ($NH_2OH.HCl$) was basified with aqueous NaOH in ethanol. Subsequently, the free base was condensed *in situ* with the aldehyde (**2**) to afford oxime (**3**) in 85% yield after column chromatography (Scheme 2.7) as a mixture of geometrical isomers (~ 10: 1, with the *anti* assumed to be the major). The ^{13}C and ^{1}H NMR spectra of (**3**) revealed peaks that were consistent with the target compound. A noteable change in the ^{1}H NMR spectrum of (**3**) relative to (**2**) was the identity of a 1-proton broad-singlet at δ 1.29 ppm assigned to the OH group. Also, the oxime proton appeared less downfield compared to the formyl proton of (**2**), consistent with the substitution of oxygen with the less electronegative nitrogen The change in functionality at the *ortho*-position of dibemethin from aldehyde (**2**) to oxime (**3**) was confirmed in the IR spectrum of the latter by disappearance of the C=O band (v_{max}1690 cm^{-1}) and the emergence of a new C=N band at v_{max}1600 cm^{-1}. Reduction of the oxime was achieved by refluxing (**3**) in anhydrous diethylether in the presence of $LiAlH_4$ (Scheme 2.7). After the reaction had gone to completion, a saturated solution of aqueous Na_2SO_4 with a few drops of triethylamine was added to the reaction mixture and stirred for 30 minutes. The resultant precipitate was filtered through Celite and the residue washed with a solution of 5% triethylamine in THF. A small volume of toluene was used to facilitate the azeotropic removal of any water. This intricate work-up procedure was necessitated on account of the polar nature of *o*-aminomethyl dibemethin (**4**) owing to its primary amino terminus as revealed by TLC. This meant that a typical extractive work-up using an organic solvent and aqueous medium would not extract the organic product, as primary amines are known to be water-soluble. In this way, *o*-aminomethyl dibemethin (**4**) was afforded in 89% yield after purification by column chromatography using 100% ethyl acetate followed by EtOAc: MeOH: NEt_3 (95: 5: 5) as the mobile phase . To fulfill the aim of

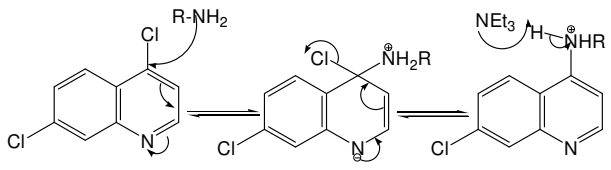
probing the effect of the basicity of the amino terminus of (**4**), a formyl derivative (**5**) was prepared lacking the basic primary amino group, this also served the purpose of characterization of (**4**). This was achieved by refluxing a solution of (**4**) in excess ethyl formate under an inert atmosphere overnight (Scheme 2.8). Unreacted ethyl formate was removed under reduced pressure to give a crude residue, which was purified by flash chromatography directly to afford *o*-amidomethyl dibemethin (**5**) as a formate ester in 92% yield and predominantly as an s-*trans* rotamer according to ^{1}H NMR and on steric grounds. Confirmation of the structure of (**5**) was provided by an additional downfield carbonyl carbon in its ^{13}C NMR, a formyl proton (singlet) in its ^{1}H NMR and an IR amide carbonyl stretch at v_{max} 1656 cm^{-1} The proposed "reversed chloroquine" compound, *o*-dibemethinoquine (**6**), was also afforded as a colourless crystalline solid in 27% yield following coupling (**4**) to 4, 7-dichloroquinoline (**2.1**) (DCQ coupling) (Scheme 2.8). ^{1}H NMR, ^{13}C NMR, HSQC, HMBC and IR spectroscopy were used to fully characterize (**4**), whose microanalytical (CHN) data confirmed molecular composition as: Found C, 74.56; H, 6.07; N, 10.08% when $C_{25}H_{24}N_3Cl$ requires C, 74.71; H, 6.02; N, 10.45%. Crystallographic analysis unequivocally confirmed attainment and structure of (**4**), the data of which is discussed in Section 2.7.



Scheme 2.8: Synthesis of *o*-amidomethyl dibemethin (**5**) and *o*-dibemethinoquine (**6**). *Reagents and conditions*: (i) ethyl formate, reflux overnight, 92%; (ii) 4, 7-DCQ, K_2CO_3, NEt_3, NMP, 90 ºC, 48h, 27%.

The DCQ coupling was carried out in a cyloaddition tube *via* reaction at 90℃ over 48 hours. The reaction was facilitated by the use of triethylamine and K_2CO_3 bases. The particular use of K_2CO_3 served to tweak the NH_2 protons which are acidic in nature

owing to their bonding to the more electronegative N atom. This increases the nucleophilicity of the nitrogen atom and augments DCQ couplings. The use of *N*-methyl-2-pyrrolidone (NMP) as a solvent has special place in these types of reactions as NMP is thought to propagate DCQ coupling by also H-bonding with the NH_2 hydrogens. The importance of this solvent is highlighted by the fact that attempts to perform the DCQ coupling using alternative high boiling solvents such as DMF or dioxane were unsuccessful. The high boiling point of NMP means that it cannot be removed on the rotorevaporator. However, it is miscible with water, hence a work-up was used involving extracting with ethyl acetate and washing the organic extracts several times with saturated brine to remove the NMP. Under these conditions, the product could still be extracted. Possibly the low yield reflected losses into the water. The mechanism of coupling is illustrated in Scheme 2.9.

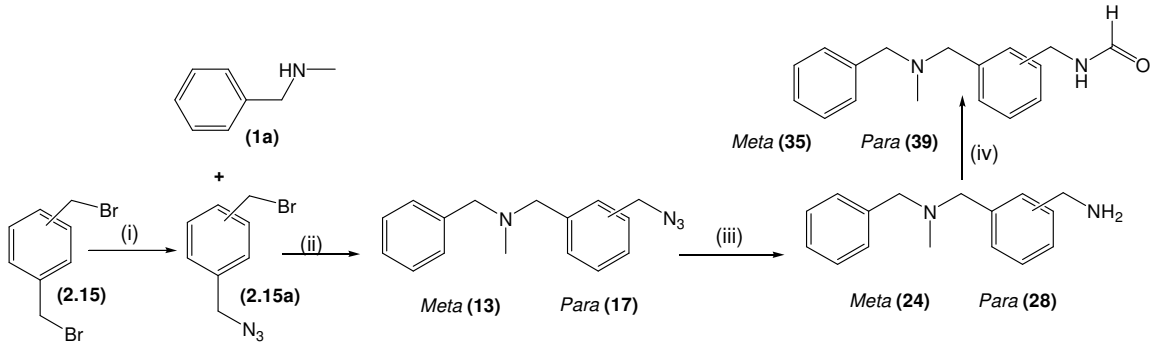


Scheme 2.9: General mechanism of DCQ couplings.

As discussed earlier, the C-2 and C-4 positions of quinoline are more susceptible to nucleophilic attack, which is enhanced by electronegative groups at these positions. The mechanism involves a classical aromatic nucleophilic substitution (S_NAr) mechanism in which nucleophilic attack by the primary amino nitrogen at the 4-position of 4, 7-dichloroquinoline is followed by elimination of the chloride anion. The observed regioselectivity is facilitated by the influence of the nitrogen atom *via* resonance stabilization of the intermediate.

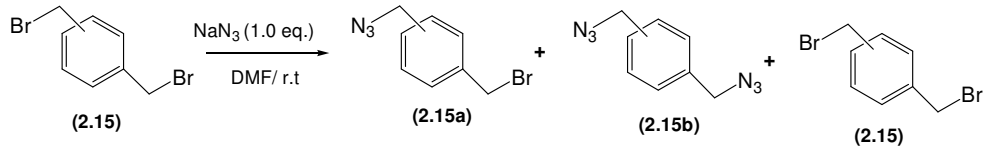
Although the procedure by Blackie *et al*., (2007) had been successfully employed in the synthesis of *o*-aminomethyl dibemethin (**4**) and subsequently (**5**) and (**6**), it could not be applied in the synthesis of *p*- and *m*-aminomethyl dibemethins owing to the regioselective nature of the formylation reaction (see Scheme 2.6). Furthermore, an alternative pathway to aminomethyl dibemethins that would avoid the complex reaction work-up associated with the $LiAlH_4$ reduction of oximes to amines discussed above was preferable. For these reasons, *meta* and *para*-aminomethyl dibemethin compounds (**24**,

28) were prepared in three steps based on modification to the method by Rinke *et al.* starting with a nucleophilic mono-substitution of commercially available *meta*- or *para*-*α, α'*- dibromoxylenes (**2.15**) with sodium azide ion as shown in Scheme 2.10 below. Owing to the structural similarities of the target compounds, characterization of all the compounds synthesized is discussed in Section 2.5.



Scheme 2.10: Preparation of *m*- and *p*-aminomethyl dibemethin (**24** and **28**) and their respective amidomethyl dibemethins (**35**) and (**39**) *Reagents and conditions*: (i) NaN_3 (1.0 equiv.), DMF, rt, overnight; (ii) K_2CO_3, CH_3CN, reflux overnight (**1a**); (iii) PPh_3, THF, H_2O, reflux overnight; (iv) ethyl formate, reflux overnight.

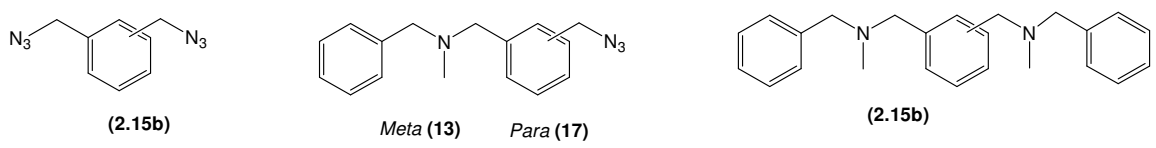
The drawback of the substitution reaction by azide is that using one equivalent of azide results in a mixture of the desired mono-substitution product (**2.15a**) as well as the di-substitution product (**2.15b**) and unreacted *m*, or *p*-dibromoxylene (**2.15**) as illustrated in Scheme 2.11 below.



Scheme 2.11: Mixture of products formed in the reaction of *α, α'*-dibromoxylene with NaN_3.

Based on TLC analysis, the mixture contained (**2.15a**), (**2.15b**) and (**2.15**) in a ratio of approximately (0.70): (0.15): (0.15) respectively. Separation of the products by column chromatography was complicated by the non-polar nature of these compounds.

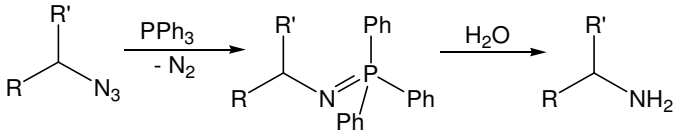
Therefore, the crude product of this reaction was used in the subsequent alkylation reaction by heating to reflux a solution of *N*-benzyl-methylamine (**1a**) in acetonitrile and the appropriate crude *m*, or *p*-*α, α'*-azido-bromoxylene overnight using K_2CO_3 as base (Scheme 2.10). After this reaction, the polarities of the products were significantly altered to permit purification by column chromatography since each product contained 0, 1, or 2 amino functionalities as the dominant polar functional group (Figure 2.6).



(2.15b) *Meta* **(13)** *Para* **(17)** **(2.15b)**

Figure 2.6: General structures of the products in the alkylation reaction of *N*-benzyl-methylamine (**1a**) and the mixture of products formed in the reaction of *α, α'*-dibromoxylene with NaN_3-see Scheme 2.11 above.

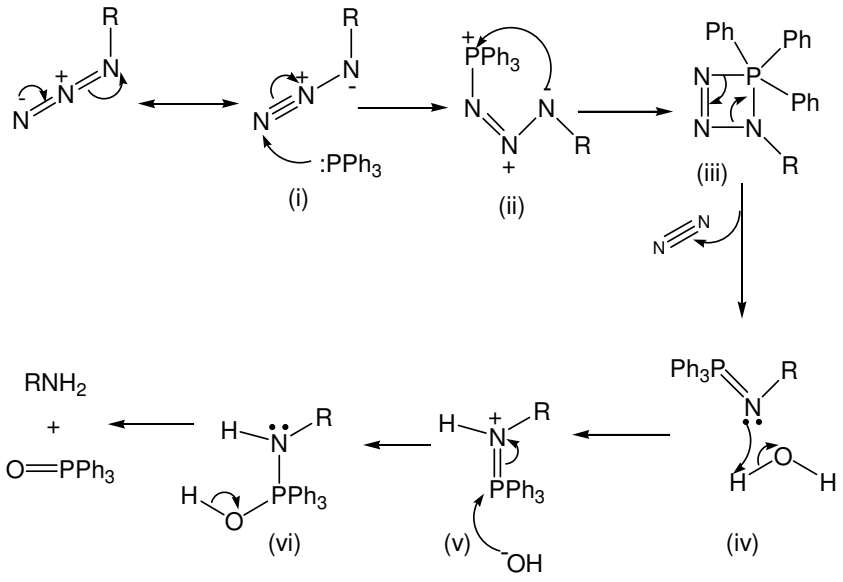
Yields of the azidomethyl dibemethins (**13**) or (**17**) were 57% and 59% respectively, which is in agreement with the observed yield for the mono-substituted azido-bromoxylenes (**2.15a**) on TLC in the azide substitution step assuming a high yield for the subsequent substitution reaction with (**1a**). Staudinger reduction of (**13**) or (**17**) gave the desired *m*-aminomethyl dibemethin (**24**) or *p*-aminomethyl dibemethin (**28**). This reaction involves nucleophilic attack on an organic azide by triphenylphosphine (PPh_3) to produce an iminophosphorane after nitrogen evolution (Scheme 2.12).



Scheme 2.12: General scheme of the Staudinger reduction of azides to primary amines.

The procedure involved adding PPh_3 to a solution of the respective azidomethyl dibemethin in THF and stirring for 30 minutes at room temperature. Water was then added and the reaction heated and allowed to reflux overnight. After the reaction had

gone to completion, water was removed by azeotroping with toluene. The crude product was purified directly by column chromatography to afford (**24**) and (**28**) in 88% and 89% yields respectively without the need for an extractive work-up, which was important in view of polarity. The mechanistic details of the Staudinger reduction of alkyl azides to primary amines are noteworthy and are illustrated in Scheme 2.13 below.

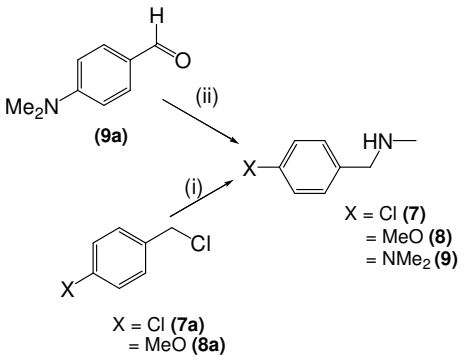


Scheme 2.13: General mechanism of the Staudinger reaction.

The resonance-stabilized azide is attacked by the lone pair of electrons on the phosphorus atom to give (ii) which cyclizes to intermediate (iii). Loss of a molecule of N_2 produces iminophosphorane intermediate (iv), which is hydrolysed to the product to yield amine and triphenylphosphine oxide as a by-product.

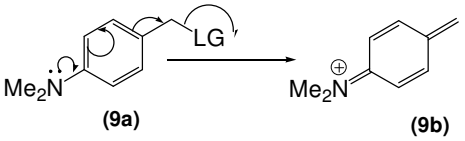
Subsequently, formylated derivates of aminomethyl dibemethins (**35**) and (**39**) (Scheme 2.10) were prepared as with compound (**5**) using excess ethyl formate and as predominantly s-*trans* rotamers according to ^{1}H NMR and on steric grounds in 95% and 89% yield respectively. Finally, *m*-dibemethinoquine and *p*-dibemethinoquine were afforded in 27% and 24% yield respectively following DCQ coupling of their respective aminomethyl dibemethins at 120 ℃ overnight. The azide strategy applied to the synthesis of *m*-dibemethinoquine and *p*-dibemethinoquine provided good overall yields, and its versatility meant that it could be applied to the synthesis of the 4-chloro-, 4-methoxy- and 4-dimethylamino- analogues of the parent compounds (Scheme 2.16) by simply changing the 4-substitutent of *N*-benzyl-methylamine (**1a**). For synthesis of these

amines, a nucleophilic substitution reaction between methylamine and the respective 4-chlorobenzyl chloride or 4-methoxybenzyl chloride (**7a**, **8a**) furnished the respective *p*-substituted *N*-benzyl-methylamine (**7**) and (**8**) as shown in Scheme 2.14 in good yields of 81% and 87% respectively. By comparison, (**9**) had to be prepared *via* a reductive amination sequence involving reaction of *N, N*-dimethylaminobenzaldehyde (**9a**) in acetonitrile with a 40% v/v methylamine solution in water followed by addition of $NaCNBH_3$ to reduce the imine and give (**9**) in 73% yield after column chromatography.



Scheme 2.14: Preparation of *p*-substituted benzyl-*N*-methylamine (**7** – **9**). *Reagents and conditions*: (i) 40% $MeNH_2$ / H_2O, THF, rt, overnight; (ii) 40% $MeNH_2$ / H_2O, CH_3CN, 0 °C, 2h; $NaCNBH_3$, r.t ,4hrs.

Reductive amination of (**9a**) to (**9**) was necessary because synthesis *via* nucleophilic substitution as was done in the X = Cl and MeO analogue was not viable because *p*-dimethylaminobenzyl chloride is unstable. An OPG or LG group in place of an aldehyde functionality results in the chemically unstable quinone-like intermediates as shown in Scheme 2.15 resulting in the formation of polymers.



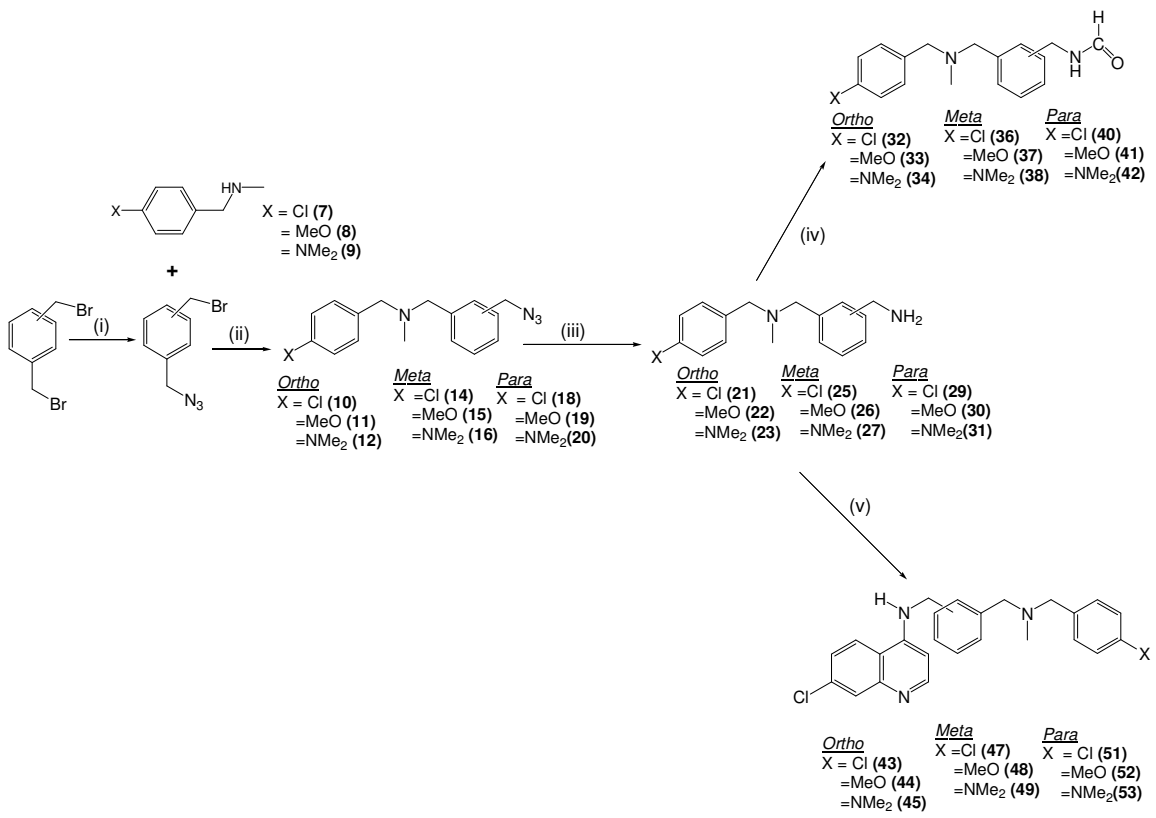
Scheme 2.15: Formation of quinone-like compounds in dimethylamino benzyl halides.

The 4-chloro-, 4-methoxy- and 4-*N*,*N*-dimethylamino- analogues of the parent aminomethyl dibemethins (**21** – **23**, **25** – **27** and **29** – **31**) were thus synthesized using

the modified method by Rinke *et al.,* as outlined in Scheme 2.16 to afford the target compounds in good yield (Table 2.1). Of note was the use of the azide strategy throughout without having to use the directed *ortho*-metallation strategy.

Table 2.1: Yields of compounds synthesized using the modified method of Rinke *et al.*

Reaction Step	X	Ortho	Meta	Para
Substitution of azido-benzyl bromides with (**7**) –(**9**)	Cl	58% (**10**)	61% (**14**)	63% (**18**)
	MeO	64% (**11**)	61% (**15**)	57% (**19**)
	NMe_2	57% (**12**)	61% (**16**)	63% (**20**)
Staudinger reduction	Cl	93% (**21**)	93% (**25**)	93% (**29**)
	MeO	83% (**22**)	88% (**26**)	89% (**30**)
	NMe_2	83% (**23**)	81% (**27**)	89% (**31**)
Formylation	Cl	89% (**32**)	84% (**36**)	86% (**40**)
	MeO	79% (**33**)	76% (**37**)	79% (**41**)
	NMe_2	82% (**34**)	89% (**38**)	78% (**42**)
DCQ coupling	Cl	31% (**43**)	27% (**47**)	21% (**51**)
	MeO	21% (**44**)	20% (**48**)	24% (**52**)
	NMe_2	18% (**45**)	22% (**49**)	26% (**53**)



Scheme 2.16: Route to the synthesis of 4-Cl, 4-MeO and 4-NMe_2- analogues of the parent dibemethinoquines. *Reagents and conditions*: (i) NaN_3, DMF, r.t, overnight (ii) K_2CO_3, CH_3CN, reflux overnight; (iii) PPh_3, THF, H_2O, reflux overnight; (iv) ethyl formate, reflux overnight; (v) 4, 7-DCQ, K_2CO_3, NEt_3, NMP, 120°C overnight.

2.5 CHARACTERIZATIONS

2.5.1 Characterisation of azidomethyl dibemethins

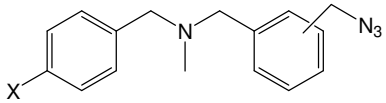
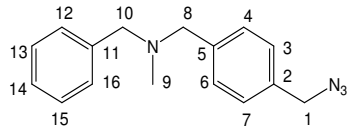


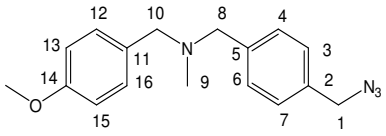
Figure 2.7: Generic structure of azidomethyl dibemethins (X = H, Cl, MeO and NMe_2).

The intermediates from each step were characterised using NMR, IR, CHN and / or high resolution mass spectrometry. The ^1H NMR spectra obtained for the azidomethyl dibemethins were consistent with the proposed structures. In this regard, three

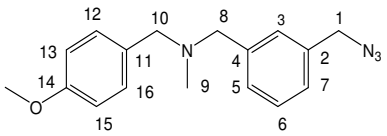
methylene singlets were observed throughout with the one for H-1 (see Figure 2.8) always downfield to those of H-8 and H-10 owing to the deshielding effect of the azido group. The most notable differences between the parent compounds (4-H) and their 4-Cl, 4-MeO, and 4-NMe_2 substituted analogues were the changes in chemical shift and the multiplicities of the four protons in the *p*-substituted aromatic ring bearing X. In the 4-MeO and 4-NMe_2 substituted derivatives (**11**, **12**, **15**, **16**, **19**, **20**), the two pairs of protons appeared as AB doublets. For example, in (**19**), the four protons in the *p*-methoxybenzyl-*N*-methylamine ring appeared as a 2-proton doublet at δ 7.43 ppm (J = 8.4 Hz) assigned to H-12 / 16 and a 2-proton doublet at δ 6.92 ppm (J = 8.4 Hz) assigned to H-13 / 15. The same protons appeared as part of an 8 or 9-proton multiplet in the 4-Cl and 4-H analogues respectively (**10**, **13**, **14**, **17**, and **18**).



(**17**)



(**19**)



(**15**)

Figure 2.8: Structure and numbering of compounds (**15**, **17** and **19**).

Differences were also noted in the protons on the ring bearing the azidomethyl group depending on whether the azidomethyl group was on the *ortho*-, *meta*- or *para*-position. In *meta* compounds e.g. (**15**), H-3 of the azidomethyl-benzyl-ring was the most deshielded of the aromatic rings protons owing to the inductive effect of the two ortho groups.

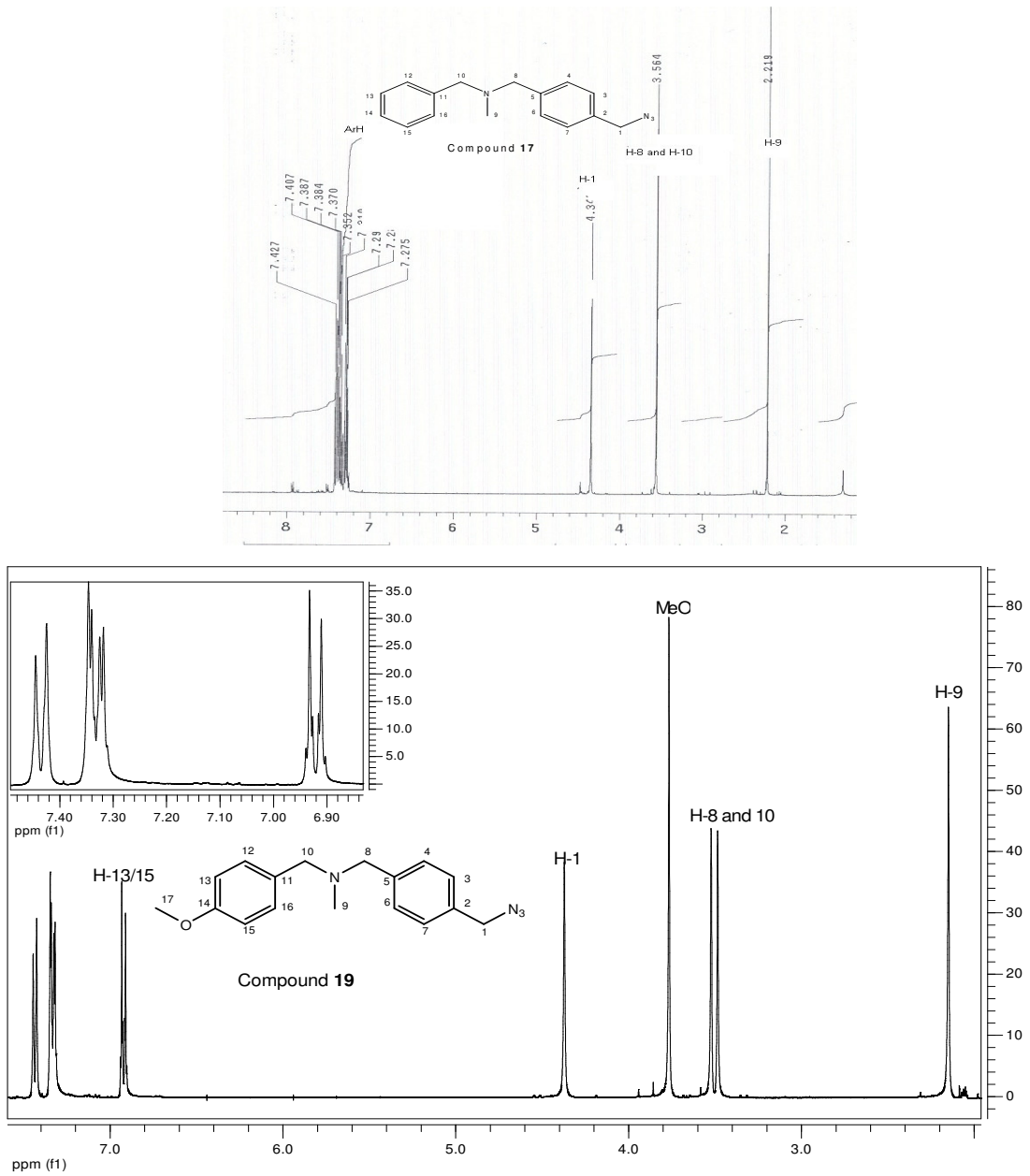


Figure 2.9: ^{1}H NMR spectra of compound **17** ($CDCl_3$, 400 MHz) and its 4-MeO analogue **19** ($CDCl_3$, 400 MHz).

The aromatic region in the spectrum of (**17**) and (**19**) have been expanded in Figure 2.9 above to highlight the typical differences caused by the nature of the substituent (X) at the *para*-position of the ring of azidomethyl dibemethins. The upfield shielding seen in H-13 / 15 of X = MeO and NMe_2 compounds was due to the mesomeric releasing effect of these groups. A summary of the different chemical shifts, multiplicities and coupling

constants of H-12 / 16 and H-13 / 15 in azidomethyl dibemethins is shown in Table 2.2 below.

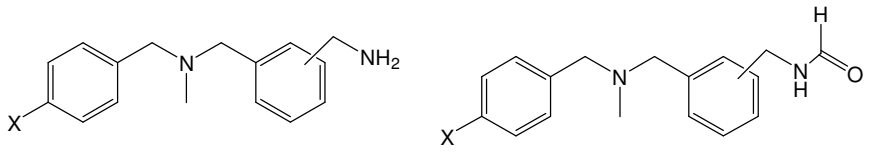
Table 2.2: Chemical shifts multiplicities and coupling constants of H-12 / 16 and H-13 / 16 in azidomethyl dibemethins

Compound	X	Position	H	*δ* ppm	multiplicity	*J* (Hz)
10	Cl	*o*	12/16 13/15	7.39-7.26	m	
11	MeO	*o*	12/16	7.29	d	8.9
			13/15	6.92	d	8.9
12	NMe_2	*o*	12/16	7.53	d	8.7
			13/15	6.90	d	8.7
13	H	*m*	12/16 13/15	7.40-7.14	m	
14	Cl	*m*	12/16 13/15	7.36-7.31	m	
15	MeO	*m*	12/16	7.28	d	8.4
			13/15	6.87	d	8.4
16	NMe_2	*m*	12/16	7.22	d	8.9
			13/15	6.73	d	8.9
17	H	*p*	12/16 13/15	7.43-7.28	m	
18	Cl	*p*	12/16 13/15	7.44-7.31	m	
19	MeO	*p*	12/16	7.44	d	8.4
			13/15	6.92	d	8.4
20	NMe_2	*p*	12/16	7.23	d	8.6
			13/15	6.72	d	8.6

The ^{13}C NMR data of all the compounds was in agreement with the expected values for the different types of carbon atoms. A notable observation in the ^{13}C spectra of these compounds is that C-12 and C-16 have the same chemical shift and appear as a single

peak as do C-13 and C-15, owing to C-2 symmetry. Thus, three high-field methylenes and one methyl resonance were always observed, as well as four quaternary carbons regardless of substitution patterns. Unambiguous assignments were made for all the alkyl protons and carbons as well as most carbons of the *p*-substituted benzyl-*N*-methylamine ring using HSQC and HMBC. High resolution mass spectrometry confirmed the molecular formulae of the proposed structures.

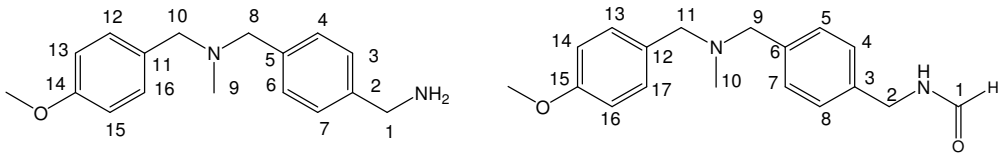
2.5.2 Characterisation of aminomethyl dibemethins and amidomethyl dibemethins



(**4**, **21** – **31**) (**5**, **32** – **42**)

Figure 2.10: Generic structures of aminomethyl dibemethins and amidomethyl dibemethins (X = H, Cl, MeO and NMe_2).

These target compounds were also fully characterised by NMR, IR and high resolution mass spectrometry. The IR spectra of the aminomethyl dibemethins (**21** – **31**) indicated a characteristic peak for a primary amine (NH_2) at v_{max} 3200 cm^{-1}. By comparison, the IR spectra of amidomethyl dibemethins (**32** – **42**) revealed the emergence of a characteristic band for C=O (1660 cm^{-1}) and the disappearance of the NH_2 band. The ^1H NMR of a representative compound ((**30**) see Figure 2.11) reveals the following key signals: a 4-proton multiplet at δ 7.32 - 7.27 ppm assigned to ArH-3, 4, 6 and 7 (see Figure 2.10), a 2-proton doublet at δ 7.23 ppm (*J* = 8.8 Hz) assigned to H-12 / 16, and a corresponding 2-proton doublet at δ 6.85 ppm (*J* = 8.8 Hz) assigned to H-13 / 15. A 2-proton singlet at δ 3.83 ppm was assigned to H-1 and a 3-proton singlet at δ 3.77 assigned to OMe. The identities of H-8 and H-10 were unequivocally assigned using HMBC as a 2-proton singlet at δ 3.46 ppm for H-8 and a 2-proton singlet at δ 3.43 ppm for H-10. The 3-proton singlet at δ 2.13 was assigned to H-9 and a broad singlet at δ 4.53 ppm to NH_2.



(**30**) (**41**)

Figure 2.11: Structure and numbering of compound (**30**) and (**41**).

Conversely, the ^{1}H NMR of the formylated derivative of this compound, (**41**) showed the emergence of two new signals being a 1-proton broad singlet at δ 8.21 ppm assigned to H-1 and a 1-proton broad singlet at δ 6.06 ppm assigned to the amide NH group. It is also interesting to note that H-1, which appeared as a 2-proton singlet at δ 3.83 ppm in (**30**) now numbered H-2 in (**41**) had shifted further downfield owing to the deshielding effect of the adjacent amido group and was now observed as a 2-proton doublet at δ 4.44 ppm (J = 5.7). The characteristic splitting patterns and chemical shifts that were observed for protons in azidomethyl dibemethins (**10** – **20**), which were discussed previously were also largely maintained in aminomethyl dibemethins (**21** – **31**), and amidomethyl dibemethins (**32** – **42**). The ^{13}C data of the target compounds were consistent with the proposed structures. The major difference between the ^{13}C spectra of aminomethyl dibemethins and their corresponding formamides as expected was an additional peak in the spectra of the latter owing to the newly inserted C-1. C-1 was characteristically observed downfield around δ160.0 ppm. A conspicuous observation in both the ^{13}C NMR and ^{1}H NMR of amidomethyl dibemethins were the presence of minor isomer peaks in the spectra as shown in an expanded insert of compound (**41**) in Figure 2.12 below. This observation is attributed to the presence of non-interconverting *cis* and *trans* isomers around the amide bond as rotamers, with the *trans* dominating on steric grounds (ratio ~ 1: 8 for *cis*: *trans* isomers) see H-2 in compound (**41**) in Figure 2.12. This pattern of differences and similarities was generally observed between aminomethyl dibemethins and their corresponding amidomethyl dibemethins.

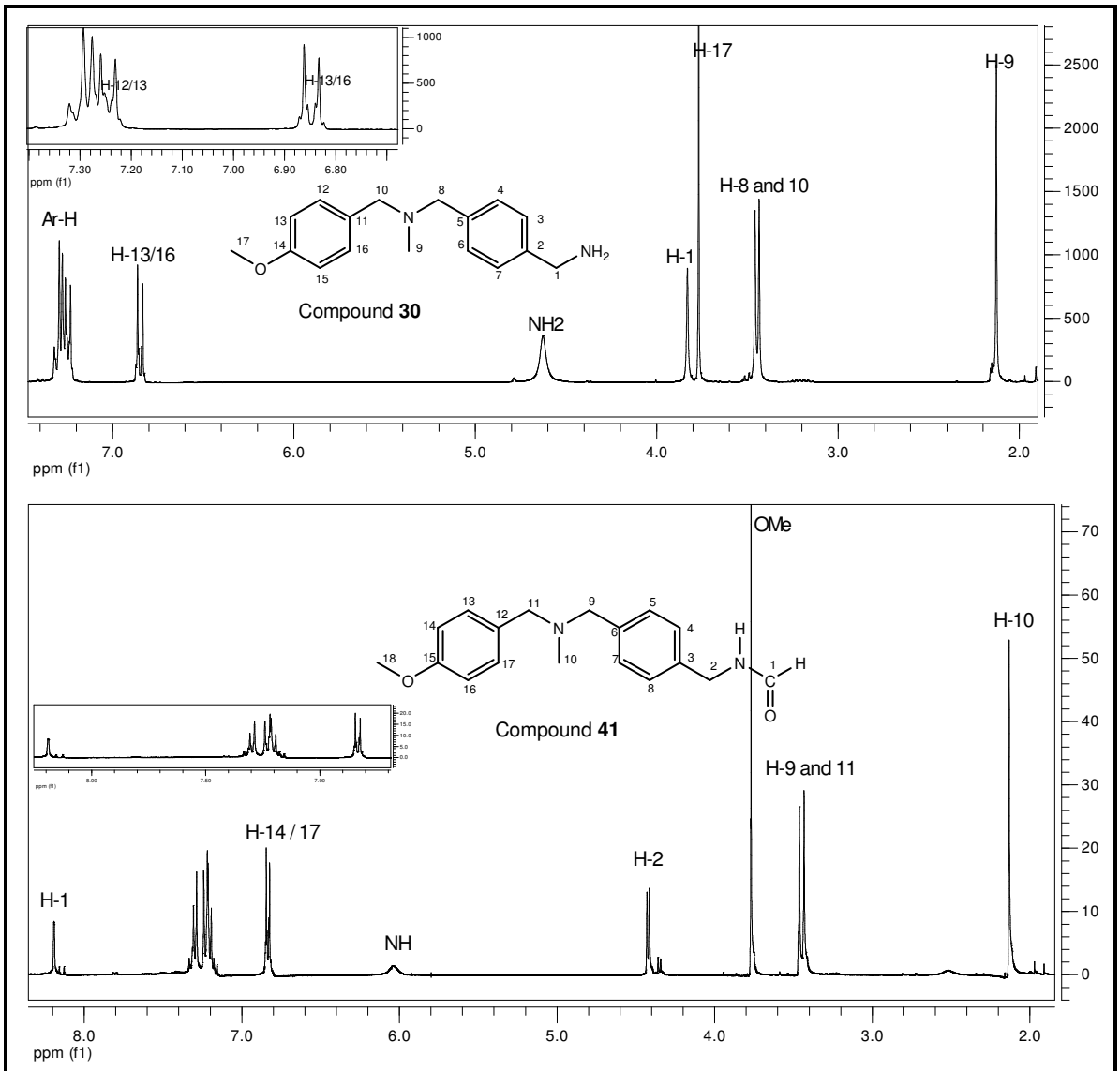


Figure 2.12: ^{1}H NMR spectra of compound (**30**) ($CDCl_3$, 300 MHz) and its formylated derivative (**41**) ($CDCl_3$, 400 MHz).

2.5.3 Characterisation of dibemethinoquines

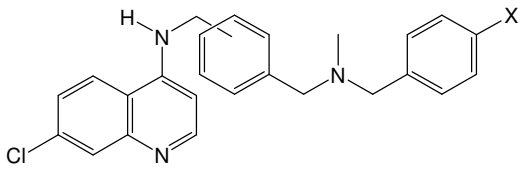


Figure 2.13: Generic structure of dibemethinoquines (X = H, Cl, MeO and NMe_2).

These target compounds were also fully characterised by NMR, IR and high resolution mass spectrometry where oils were obtained, or microanalysis (CHN) for solid compounds. Representative coupling patterns were observed in the ^{1}H NMR spectrum of (**52**) (Figure 2.14) for the quinoline ring. Thus, H-1 and H-2 appeared as an AB doublet pair with a coupling constant of 5.7 Hz, while in the benzene quinoline ring, H-5, H-6 and H-8 gave rise to a distinctive triad of d, dd, d respectively with *ortho* and *meta* coupling constants of *J* = 9.0 Hz and 2.4 Hz respectively. H-8 always resonated downfield to the other two owing to the deshielding effect of the pyridine nitrogen The NH proton appeared as a broad singlet at δ 5.77, while H-10 split into a doublet with *J* = 4.8 Hz by coupling to this proton. From the HSQC spectrum, the quinoline CH carbons could be assigned and the quaternary carbons were also identified (eight in total) based on reduced size due to relaxation. The characteristic splitting patterns and chemical shifts of the H and C atoms in the aminomethyl dibemethin side-chain of dibemethinoquines appeared as discussed above. The correct number of carbons for each target (22 carbons for **52**), taking phenyl-group symmetry into account, was always observed.

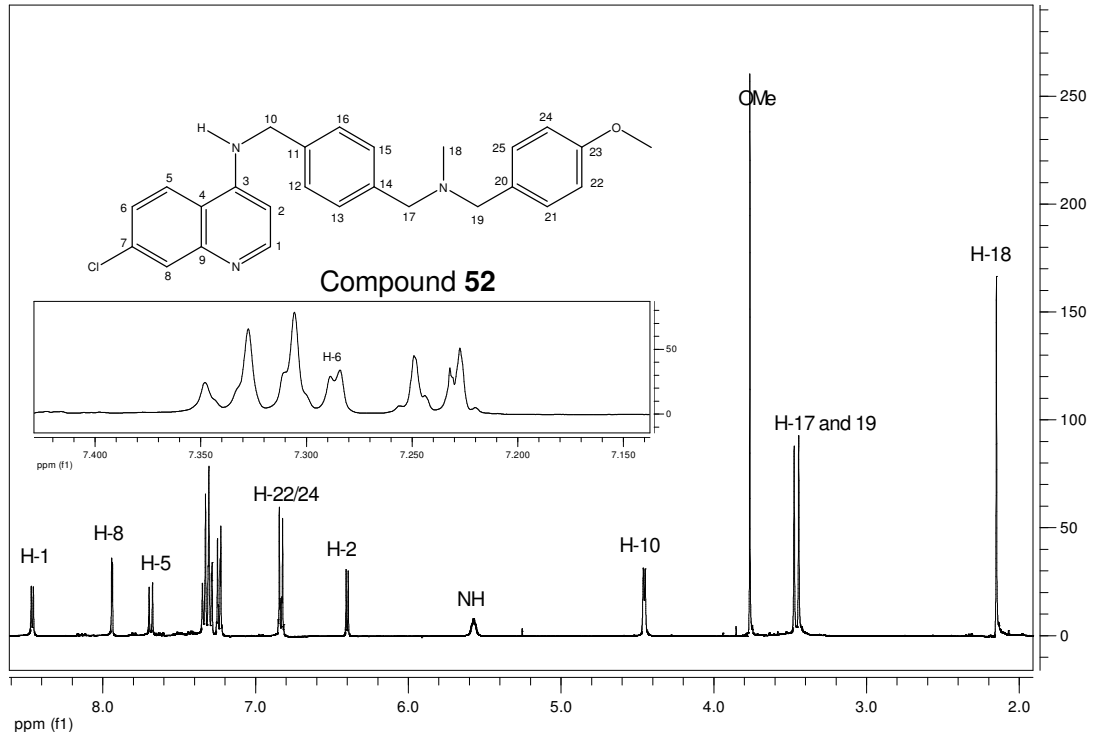


Figure 2.14: ^{1}H NMR ($CDCl_3$, 400 MHz) spectra of compound (**52**)

High resolution mass spectrometry (EI or ESI) or microanalysis (CHN) was sucessfuly used to confirm the molecular formulae and composition of the proposed structures except for compound (**47**) and (**51**) where there was not enough material to run a full CHN analysis. Where possible, i.e. in solid cases, CHN on a crystallized sample was preferred. The results of these analyses are tabulated in Table 2.3 below.

Table 2.3: HRMS and microanalysis data of dibemethinoquines (**6, 32 – 42**).

Comp	M.P (°C) (EtOAc:Hex)	Microanalysis (C.H.N)					
		Found (%)			Calculated (%)		
		C	H	N	C	H	N
6	101-103	74.56	6.07	10.08	74.71	6.02	10.45
46	103-104	74.53	5.86	9.87	74.71	6.02	10.45
50	120-122	74.35	5.88	10.0	74.71	6.02	10.45
		HRMS (M^+) (g mol^{-1})					
43	oil	436.1344			436.1347		
44	oil	432.1860			432.1860		
45	oil	445.2172			445.2159		
47	oil	436.1335			436.1347		
48	oil	432.1825			432.1843		
49	oil	445.2138			445.2159		
51	oil	436.1335			436.1347		
52	oil	432.1835			432.1843		
53	oil	445.2171			445.2159		

2.6 Crystallographic analysis

Crystals of prototype compound (**6**) were grown for structure determination and its crystal structure is depicted in Figure 2.14 below.

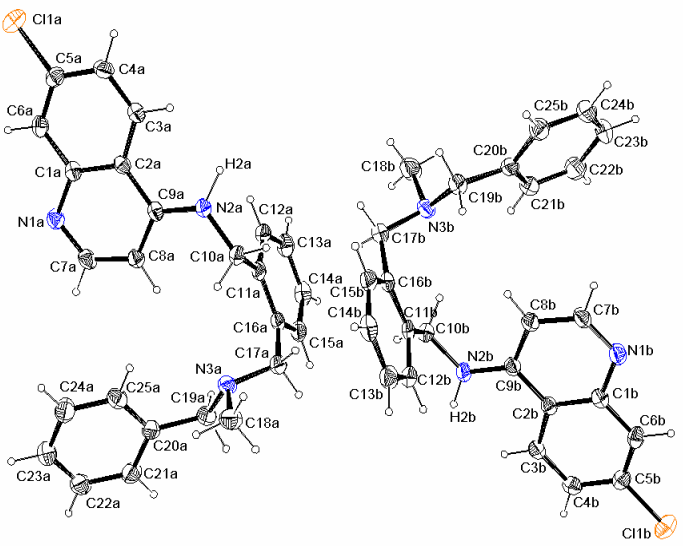


Figure 2.15: An ellipsoidal model at 50% probability level, showing the atomic numbering scheme.

Compound (**6**) crystallises in a monoclinic unit cell in the space group $P2_1/c$ with two crystallographically independent molecules per unit. The plane of the *o*-benzyl-*N*-methylamine- ring (aromatic ring A) (see Figure 2.16) is almost perpendicular to the plane of the quinoline ring at an angle of 88.1° (torsion angle C9-N2-C10-C11) while the plane of aromatic ring B is off the plane of aromatic ring A at an angle of 70.6° (torsion angle C16- C17-N3-C19). Aromatic ring B is bent towards the quinoline ring where the plane of the former ring is off the plane of the quinoline ring at an angle of 49° (Figure 2.16). The bond angle of N3-C17-C16 is 114.9° while N3-C19-C20 has a bond angle of 113.7°. The *syn*-like conformational arrangement of the quinoline and terminal aromatic ring is significant, suggesting intramolecular communication (*via* π-π stacking), as opposed to a sterically more favoured *anti*-arrangement. This could have implications for the biological activity and physicochemical properties of this compound.

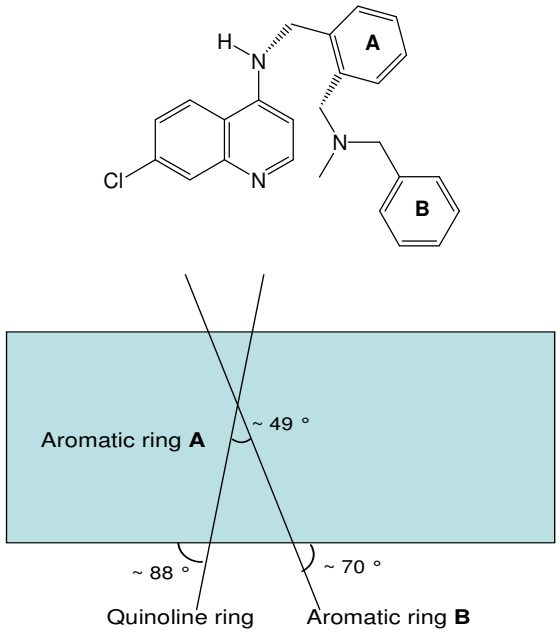


Figure 2.16: Edge-on view of the planes of aromatic ring A relative to quinoline ring and aromatic ring B.

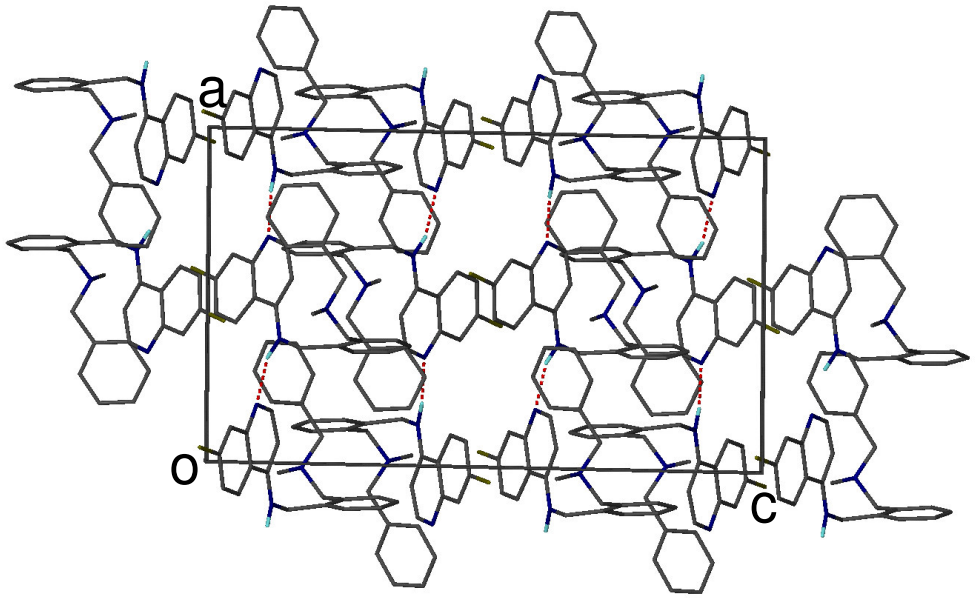


Figure 2.17: A projection viewed down [010]. All hydrogens except the amino hydrogens are omitted for clarity. The N-H···N hydrogen bonds are shown as dotted lines.

Hydrogen bonding in the crystal structure of (**6**) is illustrated in Figure 2.17 above which identifies that the structure is stabilised by intermolecular hydrogen bond interactions N-

H···N, formed between the donor secondary amino group NH and the quinoline nitrogen atom. Thus one-dimensional hydrogen bonding networks are formed *via* N2A-H2A···N1B and N2B-H2B···N1A interactions. Hydrogen bond distances are indicated in Table 2.4.

Table 2.4: Hydrogen bond distances and angles in the crystal structure of (**6**)

Atoms	D···A (Å)	D-H (Å)	H···A (Å)	D-H···A (Å)
N(2B)-H(2B)...N(1A)	2.938	1.01	1.98	158.0
N(2A)-H(2A)...N(1B)	2.972	1.05	1.95	164.9

Since the essential pharmacophore for chloroquine resistance-reversal in imipramine-like compounds was proposed to be two aromatic rings at a specific geometric orientation together with a nitrogen atom. To obtain a spatial delineation of this configuration, the bond lengths between the terminal amino-group in imipramine and the two aromatic rings as well as the distance between aromatic rings themselves was measured and compared with those in compound (**6**). Significantly, it was found that the bond length between the respective terminal amino group and the centre of the corresponding aromatic ring A was fairly similar in imipramine and compound (**6**), differing by about 0.3 Å (Figure 2.18). A comparable bond length between the respective terminal amino group and the centre of the corresponding aromatic ring B was also observed.

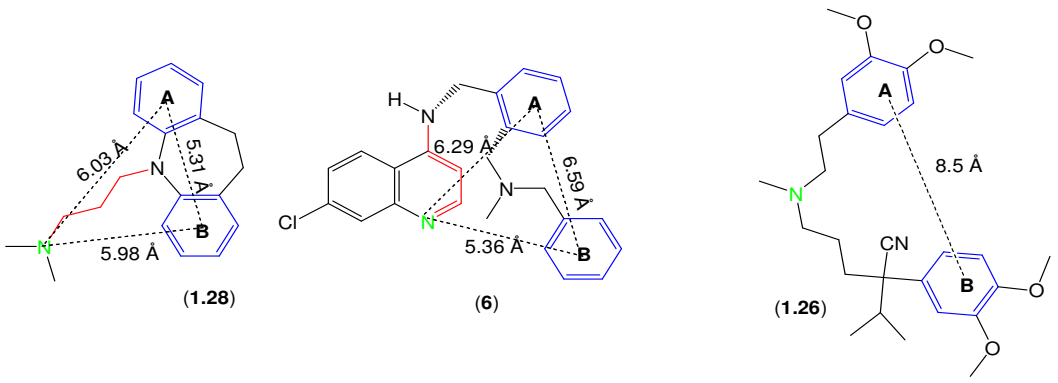


Figure 2.18: Comparison of bond lengths between the two aromatic rings and the respective N-atom in imipramine (**1.28**) and (**6**) and the two aromatic rings in verapamil (**1.26**).

Although the distance between the centres of the respective two aromatic rings A and B differed significantly between imipramine (**1.28**) and compound (**6**), it is important to note

that in the chloroquine resistance-reverser verapamil, the distance between the centres of the two aromatic rings is larger than in both imipramine and compound (**6**). This suggests that a variable spatial arrangement of the two aromatic rings is tolerated at the active site of chloroquine resistance-reversing agents, and that compound (**6**) may well indeed possess the necessary properties for chloroquine resistance-reversal. The crystal data of (**6**) is tabulated in Table 2.5 below.

Table 2.4: Crystal data of compound (**6**)

Empirical formula	C_{25} H_{24} Cl N_3
Formula weight	401.92
Crystal system, space group	Monoclinic, $P2_1/c$
a (Å)	13.1065(2)
b (Å)	15.7570(3)
c (Å)	21.7792(5)
α (°)	90
β (°)	90.5650(10)
γ (°)	90
V $(Å)^3$	4497.60(15)
Z	8
λ (Mo-Kα) (Å)	0.71073
F (000)	1696
Crystal size (mm)	0.16 x 0.11 x 0.09
Range scanned θ (°)	3.02 - 25.37
Range of indices	h: ±15 k: ±18 l: ±26
No. reflections collected	60271
No. unique reflections	8207
S	1.039
R_1	0.0945,
wR_2	0.1118
Δρ excursions /e.$Å^3$	-0.2, 0.242

2.7 Conclusion

Three classes of compounds, the proposed side-chains of the resistance reversering quinolines (aminomethyl dibemethins), their formylated derivatives (amidomethyl dibemethins) and the proposed “reversed chloroquine” compounds (dibemethinoquines) have been successfully synthesized.

Aminomethyl dibemethins were synthesized using the Staudinger reduction of azides or the reductive amination *via* oxime of an aldehyde in good yields. The precursors to these compounds were also successfully synthesized and purified in good yields.

Amidomethyl dibemethins were all synthesized *via* a single-step reaction, which involved refluxing the respective aminomethyl dibemethins in excess ethyl formate to obtain the product in near quantitative yield, albeit as rotamers. Dibemethinoquines were also obtained, although in low yields, by DCQ coupling reactions. All the target compounds were fully characterized using NMR, IR and high resolution mass spectrometry or microanalysis. The crystal structure of a representative compound (**6**) was also elucidated. A *syn*-like conformational arrangement of the quinoline and terminal aromatic ring was observed and thought to have implications in biological activity of dibemethinoquine compounds, as the conformation appears to conform to the resistance reverserpharmacophore

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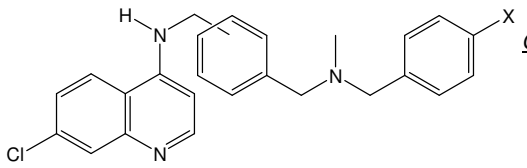
CHAPTER 3: QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIPS IN DIBEMETHINOQUINES

3.1 BACKGROUND

The advent of drug resistant strains of *P. falciparum* has reduced the efficacy of current antimalarial drugs including some mainstay compounds like chloroquine. Since the emergence of resistant strains of the malaria parasite, scientists have tried with limited success to produce cheap and effective 4-aminoquinoline alternatives to chloroquine. The discovery of the chloroquine resistance-reversing properties of the calcium channel blocker verapamil opened up interest in a new class of compounds known as resistance reversing agents. Since then, more compounds of diverse chemical structures have been identified as chloroquine resistance-reversering agents. However, the usefulness of these agents as chemosensitizers is impaired by their profound antipsychotic, antihistaminic or cardiovascular effects. This is further complicated by the fact that the effective dose of these compounds as chemosensitizers is generally close to or higher than their other clinical applications [188]. Against this background, there is an urgent need to find new and innovative drugs to combat chloroquine resistance in *P. falciparum.* In this regard, chloroquine analogues which are active against chloroquine-resistant parasites including short chain (**1.21**) and long chain (**1.23**) analogues, analogues with side-chains containing bulky groups or aromatic rings (**1.24**) and isoquine (**1.25**) and metallated analogues (**1.22**) have been synthesized,[154-157] but most have not found use in malaria chemotherapy. The introduction of advanced techniques such as molecular modeling and 3D-QSAR as tools in the search for new drugs has proved supportive in this drive. Using these methods, SAR models have been established based on the structures and activities of known chemosensitizers as well as existing chloroquine analogues. SAR models help identify essential features in a class of compounds. These findings have found particular application in the synthesis of highly potent drug targets.

In this study, a novel class of analogous compounds (dibemethinoquines, Figure 3.1) hypothesized to be potent antimalarial compounds against both sensitive and resistant strains of *P. falciparum* coupled with the added advantage of potentially being able to reverse resistance was synthesized.



Ortho	*Meta*	*Para*
X =H**(6)**	X =H**(46)**	X =H**(50)**
=Cl **(43)**	=Cl **(47)**	=Cl **(51)**
=MeO **(44)**	=MeO **(48)**	=MeO **(52)**
=NMe_2 **(45)**	=NMe_2 **(49)**	=NMe_2**(53)**

Figure 3.1: Chemical structure of dibemethinoquines (**6**, **43 - 53**).

By varying the point of attachment of the dibemethin side-chain to the quinoline ring between *ortho*, *meta* and *para* and also introducing three different substituents (X = Cl, MeO and NMe_2) to the side-chain of the parent compounds (X = H), a quantitative structure-activity relationship could be elucidated based on physicochemical properties and biological activities of these compounds.

3.2 EXPERIMENTAL METHODS

3.2.1 Synthesis of dibemethinoquines

The target compounds were synthesized in a single step by reaction of the appropriate dibemethin with excess commercially available 4, 7-dichloroquinoline in anhydrous *N*-methyl-2-pyrrolidone as outlined in Chapter 2.4. All the compounds were fully characterized by infrared, ^{1}H and ^{13}C NMR and mass spectrometry when oils and elemental combustion analysis when solids. Melting points of solid compounds were also determined. Experimental details of the compounds are outlined in Chapter 6.

3.2.2 β-haematin inhibition

The IC_{50} values for β-haematin inhibition were determined by the method of Ncokazi and Egan using a pyridine based 96-well plate assay method [202]. The method relies on the fact that 5 % aqueous pyridine dissolves haematin, but not β-haematin at pH 7.5. The extent of inhibition is then characterized by measuring the intensity of the monomeric pyridine-haematin complex at 405 nm.

3.2.3 pK_a determinations

The pK_a values of compounds (**6, 43**– **53**) were determined by pH titration. The values were determined by Dr Kanyile Ncokazi (Department of Chemistry, University of Cape Town).

3.2.4 Calculations of log *P* and log *D*

Log *P* and log *D* at the physiological pH (7.4) and vacuolar pH (5.0) were calculated from the observed pK_a values using the ClogP computer program.

3.2.5 Antiplasmodial and cytotoxicity testing

Antiplasmodial and cytotoxicity tests were conducted by the division of Pharmacology, Department of Medicine, University of Cape Town (courtesy of Prof. P Smith).The *in vitro* antiplasmodial activities of the compounds were tested against the chloroquine sensitive D10 and chloroquine resistant K1 strains of parasite. The prototype (**6**) was subjected to cytotoxicity testing in Chinese hamster ovarian (CHO) cells. Compounds (**6**) and (**50**) were also tested against *P. yoelii nigeriensis* in a mouse model by intra-peritoneal injection at 5 and 20 mg/kg and compared to chloroquine diphosphate at the same doses.

3.2.6 Statistical correlations

The statistical correlations were performed using the computer programs EXCEL and GPAPHPAD PRISM 3.0. In all cases where the p value was < 0.05, the correlations were considered to be significant.

3.3 RESULTS

The biological results obtained for dibemethinoquines are presented in Table 3.1 below. The table shows resonance constants (R), measured acid dissociation constant (pK_a) values, β-haematin inhibitory activities ($BHIA_{50}$) and *in vitro* antimalarial activities (IC_{50}) versus the D10 and K1 strains of *P. falciparum* and the resistance index (RI) of compounds.

Table 3.1: The resonance constants (R), measured acid dissociation constant (pK_a) values, β-haematin inhibitory activities ($BHIA_{50}$), *in vitro* antimalarial activities (IC_{50}) versus the D10 and K1 strains of *P. falciparum* and resistance index (RI) of compounds **6, 43** – **53**.

	Code	R	pK_{a1}	pK_{a2}	$BHIA_{50}$[a]	IC_{50} D10/ nM	IC_{50} K1/ nM	RI[d]
6	VZ1	0	7.57	9.85	1.12	138.3	187.9	1.4
46	VZ2	0	7.63	9.77	0.69	53.3	65.0	1.2
50	VZ3	0	7.56	9.90	0.32	26.5	24.5	0.9
43	2VZ1	–0.15	7.44	9.60	1.35	ND[b]	1127.5	–
47	2VZ2	–0.15	7.55	9.89	0.66	131.4	84.9	0.6
51	2VZ3	–0.15	7.44	9.74	0.34	ND[b]	ND[c]	–
44	3VZ1	–0.51	7.47	9.85	0.46	175.2	134.3	0.8
48	3VZ2	–0.51	7.38	9.67	0.52	90.5	116.8	1.3
52	3VZ3	–0.51	7.44	9.70	1.44	129.9	87.8	0.7
45	4VZ1	–0.92	7.40	9.71	0.48	177.7	153.3	0.9
49	4VZ2	–0.92	7.36	9.67	0.45	ND[b]	280.9	–
53	4VZ3	–0.92	7.33	9.66	0.41	47.8	37.0	0.8
CQ[e]		–	8.4	10.8	1.91	29.4	285	9.7

[a] Equivalents relative to haematin, [b] not determined, IC_{50} > 100 ng/ml, [c] not determined, IC_{50} > 10 μg/ml, [d] RI = IC_{50} (K1) / IC_{50} (D10), [e] chloroquine.

The pK_{a1}, which corresponds to the quinoline heteroaromatic N atom, was found to lie in a narrow range from 7.33 (**53**) to 7.63 (**46**) while pK_{a2} which corresponds to the basic tertiary amino group in the dibemethin side-chain ranged from 9.6 (**43**) to 9.9 (**50**). The IC_{50} values for β-haematin inhibition covered a somewhat larger range, with the most potent inhibitor (**50**) having an IC_{50} of 0.32, while the least active (**52**) exhibited a value of 1.44.

In the D10 strain, activities were not determined above 100 ng/ml while in the K1 strain measurements were not performed above 10 μg/ml. With these cutoff values, three compounds (**43**, **51** and **49**) were inactive against the D10 strain, while one (**51**) was

inactive in the K1 strain. Of the remaining compounds (**43**) and (**49**) had the weakest measured IC_{50} values in the K1 strain. The IC_{50} values ranged from 27 nM (**50**) to 178 nM (**49**) in the D10 strain and from 24 nM (**50**) to 1178 nM (**43**). The resistance index, a ratio of IC_{50} in the K1 strain to that in the D10 strain ranged between 0.7 (**52**) and 1.4 (**6**). This variation is probably not statistically significant and reflects the essentially complete retention of activity in resistant parasites by these compounds.

3.3.1 Determination of log *P* and log *D*

The accumulation of an ionizable compound in cells is determined by its partition coefficient (P) or distribution coefficient (D) and is usually expressed as logarithms (log *P* and log *D* respectively). Calculations of log *P* and log *D* are useful as measures of hydrophilicity or hydrophobicity of a compound and aid in estimating distribution of a compound within the cell or the body. The partition coefficient is a ratio of concentrations of un-ionized compound between two immiscible solvents normally aqueous (water) and organic (octanol). For partially ionized compounds, the partition coefficient between two phases at any fixed pH is called the distribution coefficient (D). It is therefore the ratio of the sum of the concentrations of all forms of the compound (ionized plus neutral) in each of the two phases. The difference between log *P* and log *D* is therefore determined by the percentage of the drug ionized at different pH values and is given by Equation 1 for a basic compound [203].

$$\log D = \log P - \log [1 + 10^{(pKa-pH)}] \qquad \text{(Equation 1)}$$

When the difference between pK_a and pH is more than 1.0 log unit, subtraction of this difference from log *P* will furnish log *D* (Hansch and Leo, 1995). This makes it possible to calculate[204] log *D* from knowledge of log *P*, pK_a and pH.

In the case of a drug possessing two basic centres, as in the case of dibemethinoquines, the correction needed in log *P* to obtain log *D* at a given pH involves the additive contribution of both ionized species, and Equation 1 must be modified to Equation 2.[205]

$$\log D = \log P - \log [1 + 10^{(pKa1 - pH)} + 10^{(pKa1 + pKa2 - 2pH)}] \qquad \text{(Equation 2)}$$

In this study Log *P* values were obtained using the Clog*P* program and the log *D* calculated using Equation 2. The program provides a useful method of computing

approximate log *P* values for compounds of known structure. This program is widely used in approximating log *P* values although it has limitations in that it does not take into account some differences due to conformational isomerism.[206]

The Log *P* and Log *D* values of dibemethinoquines are listed in Table 3.2 below.

Table 3.2: The calculated log *P* and log *D* values of dibemethinoquines

Compound	Code	log *P*	log $D_{5.0}$	log $D_{7.4}$
6	VZ1	5.01	-2.41	2.39
46	VZ2	4.90	-2.50	2.30
50	VZ3	4.90	-2.56	2.24
43	2VZ1	5.61	-1.43	3.36
47	2VZ2	5.50	-1.94	2.86
44	3VZ1	4.93	-2.39	2.41
48	3VZ2	4.82	-2.23	2.57
52	3VZ3	4.82	-2.32	2.48
45	4VZ1	5.12	-1.99	2.81
49	4VZ2	5.01	-2.02	2.78
53	4VZ3	5.01	-1.98	2.81

No quantitative relationships were found between log D and antiplasmodial activity in dibemethinoquines due to their similar pKa and hence log *D* values. However, in agreement with the occurrence of aromatic hydrophobic rings in their side-chain structure, all dibemethinoquine compounds showed the expected enhanced hydrophobic properties based on the calculated log *P* (5.06 ± 0.2) compared with chloroquine (4.72). The greatly increased lipophilicity of dibemethinoquine compounds compared to chloroquine is demonstrated by their higher log $D_{7.4}$ (2.64 ± 0.3) relative to chloroquine (0.96). The distribution coefficient (log *D*) at pH 7.4 affords some insight into the potential membrane transfer capabilities of these drugs. Significantly, chloroquine-resistance mechanisms have been shown to be related to drug lipophilicity, less lipophilic 4-aminoquinolines being relatively less effective against resistant strains [119].

3.3.2 Dependence of pK_a on the identity of the terminal group on the side-chain.

Although the pK_a values for compounds (**6, 43** – **53**) vary over only a very small range of about 0.3 log units, a statistically significant correlation with the physical characteristics of the functional group attached to the terminal phenyl ring of the side-chain is observed (Figure 3.2). Unexpectedly, both pK_{a1} and pK_{a2} increase as the functional group becomes less resonance releasing (i.e. as the parameter R becomes more positive). One would expect that an electron-releasing group would strengthen the $N–H^+$ bonds, making deprotonation more difficult and raising rather than decreasing the pK_a. Furthermore, the quinoline N atom is separated from the functional group on the terminal phenyl ring by fifteen bonds and even the dibemethin N atom is separated from this group by six bonds. This means that the influence of the group on pK_a must be a through space interaction. The crystal structure of (**6**) illustrates that these molecules can adopt a folded structure in which the quinoline comes into relatively close contact with the terminal phenyl ring of the side-chain. Although less-likely, the observed drop in pK_{a1} in dibemethinoquines by ~1 unit compared to the reported pK_{a1} of chloroquine can be thought to be due to increased steric hindrance to protonation of the heteroaromatic quinoline N owing to this folded configuration of dibemethinoquines.

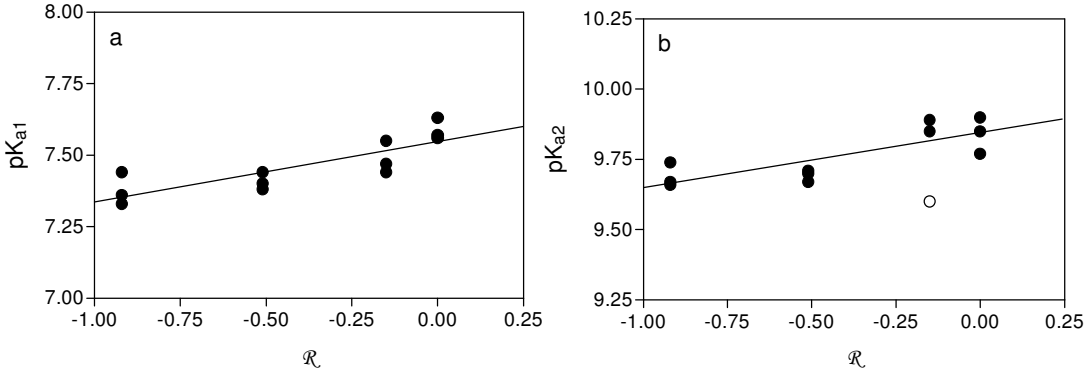


Figure 3.2: Correlations between pK_a values and resonance constants (R) of the group attached to the terminal phenyl ring (see Table 3.1). (**a**) Statistically significant correlation for the quinoline N, pK_{a1} (r^2 = 0.69, P = 0.0008). (**b**) Correlation for the dibemethin tertiary amino group, pK_{a2}. In this case the correlation is not as good, but is still statistically significant if all of the data points are included (r^2 = 0.35, P = 0.042). Omission of a single data point (open circle) for compound (**43**) improves the fit considerably (r^2 = 0.65, P = 0.0028).

Without a more detailed investigation of such intramolecular interactions, the trend in pK_a values is unlikely to be rationalized.

Using Equation 3 the predicted accumulation ratio of (**6, 43** – **53**) in the digestive vacuole of the parasite (VAR) can be calculated.[207]

$$VAR = \frac{[Q]_v}{[Q]_e} = \frac{1+10^{pKa1-pH_v}+10^{pKa1+pKa2-2pH_v}}{1+10^{pKa1-pH_e}+10^{pKa1+pKa2-2pH_e}} \qquad \text{Equation 3}$$

Here $[Q]_v$ is the concentration of the compound in the digestive vacuole of the parasite, $[Q]_e$ is its concentration in the extracellular medium, pH_v is the digestive vacuole pH (taken as 5, the midpoint between two recent estimates of 4.8 and 5.2) and pH_e is the pH of the external medium (7.4). Values are given in Table 4. An accumulation normalized IC_{50} for antimalarial activity can be calculated by multiplying the observed IC_{50} by the VAR value for each compound. As 4-aminoquinolines are believed to act by inhibiting haemozoin formation in the digestive vacuole, these numbers may be more relevant than the observed IC_{50}. They are therefore reported in Table 3.3 below.

Table 3.3: Calculated vacuolar accumulation ratio (VAR) and vacuolar accumulation normalized IC_{50} against the K1 strain of *P. falciparum in vitro* ($VAR \cdot IC_{50}$).

	VAR[a]	$VAR \cdot IC_{50}$ / mM
6	15029	2.82
46	15843	1.03
50	14892	0.37
43	13173	14.85
47	14753	1.25
44	13620	1.83
48	12318	1.44
52	13181	1.16
45	12608	1.93
49	12031	3.38
53	11601	0.43

[a] according to eq. 1, assuming a vacuolar pH of 5.0

3.3.3 Correlations of biological activity and β-haematin inhibition.

In the case of compounds (**6**), (**43**), (**46**), (**47**), (**50**) and (**51**) the β-haematin inhibition activity increases markedly in going from the *ortho*- to *meta*- to *para*- derivatives. However, this pattern is reversed in the series (**44**), (**48**) and (**52**) and no significant differences are seen in compounds (**45**), (**49**) and (**53**). Thus, the abilities of these compounds to inhibit haemozoin formation appears to depend on both the site of attachment of the aminoquinoline to the dibemethin side-chain and the identity of the group on the terminal phenyl ring of the dibemethin in a manner that is too complex to rationalize on the basis of the limited number of derivatives used in this study.

As IC_{50} values were not determined for three of the compounds against the D10 strain of parasite, while values were obtained for eleven of the twelve compounds against the K1 strain, the K1 IC_{50} data has been used for structure-activity relationship analysis. Plots of log IC_{50} for *in vitro* antimalarial activity versus log IC_{50} for inhibition of β-haematin formation (log $BHIA_{50}$) indicate that there is a general decrease in biological activity as β-haematin inhibition activity decreases (Figure 3.3a). However, the trend is not statistically significant unless the data for compound (**52**) is omitted. The correlation is only marginally improved if the antimalarial activity is normalized for accumulation in the digestive vacuole (Figure 3.3b). The correlation is again only statistically significant if compound (**52**) is omitted. While this may suggest that either the $BHIA_{50}$ or the IC_{50} value for (**52**) is an outlier, it is more likely an indication that other factors play a role in biological activity in addition to vacuolar accumulation and haemozoin inhibition. By contrast to a previous study of short-chain analogues of chloroquine in which vacuolar accumulation and β-haematin inhibition alone correlated with activity,[208] in the present study the structure of the side-chain is not kept constant. Therefore, a correlation analysis in which a structural descriptor (the position number 2, 3 and 4 for *ortho*-, *meta*- and *para*- positions respectively) was incorporated. The log IC_{50} is significantly correlated with position in the order *ortho*- > *meta*- > *para* (Fig. 3.3c). This correlation improves slightly if the accumulation normalized IC_{50} is used (r^2 = 0.55 and P = 0.0087 versus r^2 = 0.52 and P = 0.012 without normalization). When the structural descriptor was used in the correlation together with the log $BHIA_{50}$ the correlation with accumulation normalized log IC_{50} is greatly improved (Figure 3.3d). When vacuolar

accumulation is treated as a third variable in multiple correlation analysis with the observed log IC_{50} values the correlation improves even further (Figure 3.3e).

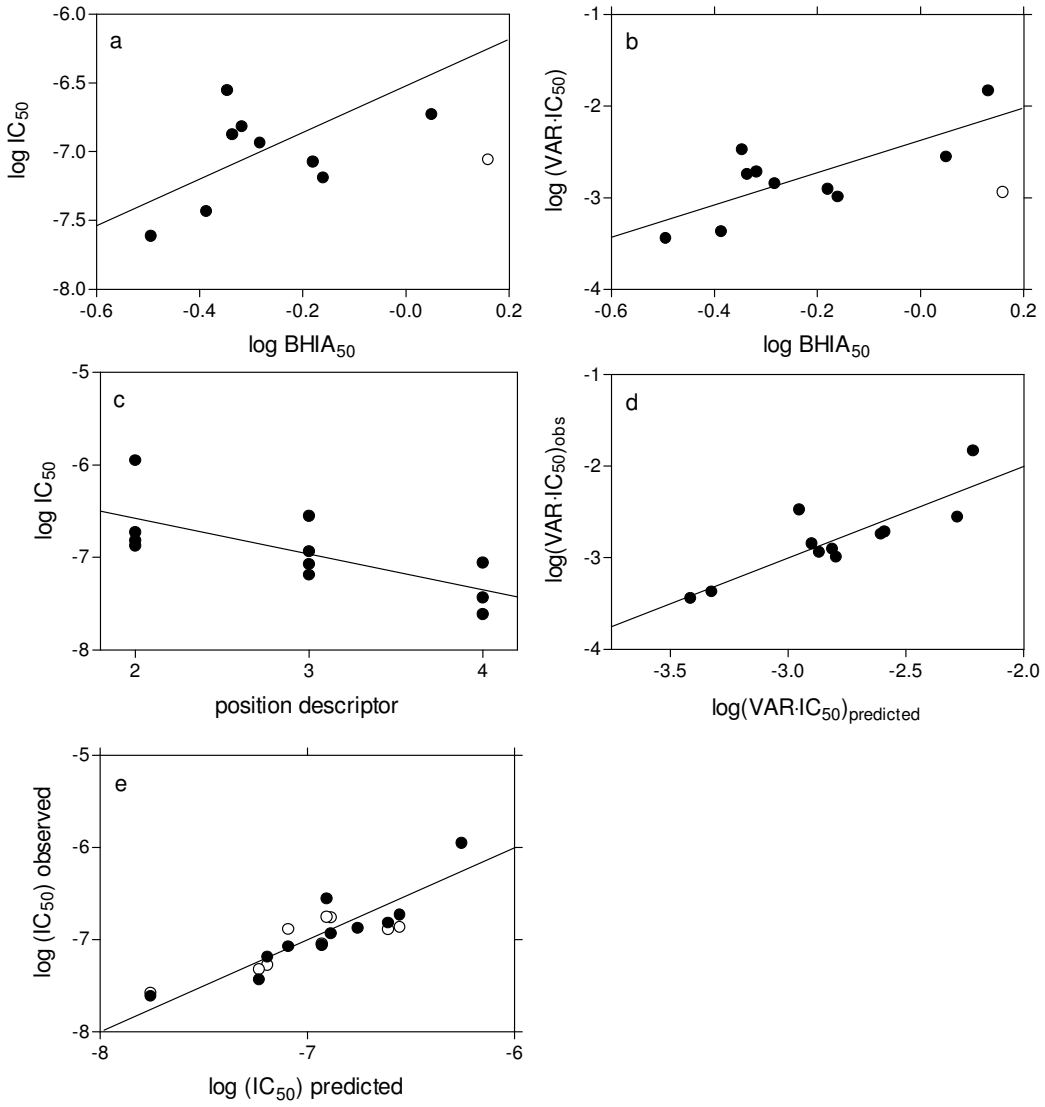


Figure 3.3: Correlations of biological activity (IC_{50}) with vacuolar accumulation ratio (VAR), IC_{50} for β-haematin inhibition activity ($BHIA_{50}$) and a molecular structure descriptor (*ortho-*, *meta-* or *para-* indicated by 2, 3 or 4 respectively and referring to compounds (**6**), (**43**), (**44**), and (**45** – **49**); and (**50** – **53**) respectively). (**a**) Linear correlation between log IC_{50} and log $BHIA_{50}$ is not statistically significant (r^2 = 0.30, P = 0.083) unless the point for compound (**52**) is omitted (open circle) which considerably improves the correlation (r^2 = 0.50, P = 0.021). (**b**) Linear correlation between the vacuolar accumulation normalized log IC_{50} (log VAR·IC_{50}) and log $BHIA_{50}$ is also not

statistically significant (r^2 = 0.33, P = 0.064) unless the point for compound (**52**) is omitted (open circle) which again considerably improves the correlation (r^2 = 0.51, P = 0.012). (**c**) Statistically significant linear correlation between log IC_{50} and the structural descriptor (r^2 = 0.52, P = 0.012). (**d**) Multiple linear correlation between the log of the vacuolar accumulation normalized IC_{50} (log VAR·IC_{50}) and both log $BHIA_{50}$ and the structural descriptor conform to the equation log VAR·IC_{50} = 0.84 × log $BHIA_{50}$ − 0.34 × *position descriptor* − 1.65. F = 10.3 > F_{crit} = 8.65 at the 99% confidence level. (**e**) Multiple linear correlation of log IC_{50} with VAR, log $BHIA_{50}$ and *position descriptor*. Here the data conform to the equation log IC_{50} = 0.95 × log $BHIA_{50}$ – 0.35 × *position descriptor* – 3.87 × log VAR + 10.24. F = 9.70 > 8.45 at the 99 % confidence level.

The factors affecting the biological activities of this series of compounds are in agreement with previous studies on short chain chloroquine analogues, [110] with the additional observation of an independent influence of side-chain structure. The origin of this latter effect is unknown. It could indicate enhanced uptake of the *para*- substituted compounds, or more likely there is a decrease in the concentration of the quinoline available to inhibit haemozoin formation arising from interaction with constituents of the cell or culture medium which binds the *ortho*- compounds most strongly. In any event, both haemozoin inhibition and vacuolar accumulation are confirmed to be significant for biological activity.

It is noteworthy that there is no indication of any significant cross-resistance with chloroquine in this series of compounds in the K1 strain of parasite. When the available IC_{50} values are plotted on the same correlation graph as those of the K1 strain (Figure 3.3e) the points clearly fall on the same correlation line. The maintenance of activity against chloroquine resistant parasites could be a result of the resistance reversing side-chain, or merely owing to the altered structure of the side-chain, which makes it no longer susceptible to the resistance machinery of the digestive vacuole.

3.3.4 Cytotoxicity of (6) and evaluation of compounds (4) and (6) against *Plasmodium yoelii nigeriensis* (NS) *in vivo* in adult BalbC white mice

The prototype compound (**6**) was subjected to cytotoxicity testing in Chinese hamster ovarian (CHO) cells. The IC_{50} in this system was found to be 32 μM which is 232 times higher than the IC_{50} in the D10 strain of *P. falciparum* and 170 times greater than that in the K1 strain. Thus, compound (**6**) exhibits highly selective activity against the malaria parasite.

Compounds (**6**) and (**50**) were also tested against *P. yoelii nigeriensis* in a mouse model by intra-peritoneal injection at 5 mg/kg and 20 mg/kg and compared to chloroquine diphosphate at the same doses. These doses were chosen since the compound proved as effective as chloroquine *in vitro* and thus the same test doses as chloroquine were used *in vivo.* Typically, with *Plasmodium yoelii* (NS), a 5 mg/kg dose of chloroquine in 100μl phosphate-buffered saline (PBS) produces a moderate parasite clearance in the bloodstream; a 20 mg/kg dose produces an almost complete clearance of circulating parasites. Each test group (5 mice) was challenged using the 4-day suppressive test as described by Peters *et al*, (1975) with modifications. Identical procedures were used for both the 5 mg/kg and 20 mg/kg experiments. The results of the *in vivo* antimalarial activity of compounds (**6**) and (**50**) are tabulated in Table 3.4 below.

Table 3.4: *In vivo* antimalarial activities of suspensions of compounds (**6**) and (**50**) administered to adult BalbC white mice infected with *P. yoelii nigeriensis* by intra-peritoneal injection.

	Mean parasitaemia 5 mg/kg	% Range 5 mg/kg	Mean parasitaemia 20 mg/kg	% Range 20 mg/kg
CQ[a]	6.1%	4-8	<1%	ND
6	23.45%	8.75-34.72	2.76%*	1.30-4.05*
50	20.07%	17.71-32.85	3.13%	1.99-3.86

*Total range from 4 mice only, following one death overnight after infection on Day 0.

As indicated in Table 3.4, both compounds were less effective than chloroquine, although substantial activity is observed at 20 mg/kg. As both compounds were administered as suspensions of their free bases rather than solutions, as opposed to the

chloroquine salt which was fully dissolved, the somewhat lower activity of these two compounds *in vivo* may just reflect lower bioavailability owing to the method of administration. Neither compound appeared to exert any obvious acute toxicity on the mice.

3.4 Conclusion

The series of novel 4-amino-quinolines with chloroquine resistance-reversing dibemethin side-chains attached *via* a methylene bridge at the *ortho*-, *meta*- or *para*- position on the dibemethin exhibit activity against chloroquine-sensitive and resistant parasites. Three compounds, (**46**), (**50**) and (**53**) show promising levels of *in vitro* antimalarial activity, with compound (**50**) in particular being more active than chloroquine against chloroquine-sensitive parasites. Both the prototype compound (**6**) and compound (**50**) have been shown to have significant *in vivo* antimalarial activity in the mouse model *P. yoelii nigeriensis*, although neither was as active as chloroquine diphosphate when administered intra-peritoneal. Preparation of salts of these compounds and improved formulation would probably improve *in vivo* activity considerably, as both (**6**) and (**50**) were administered as suspensions, whereas chloroquine diphosphate was a solution. A preliminary cytotoxicity investigation of compound (**6**) suggests that toxicity is acceptably low relative to antimalarial activity. Structure activity studies show that, as with other chloroquine analogues, the activity of this class of compound results from their ability to accumulate in the parasite digestive vacuole and inhibit haemozoin formation.

CHAPTER 4: RESISTANCE REVERSING POTENTIAL OF THE AMINOMETHYL DIBEMETHIN SIDE-CHAIN OF DIBEMETHINOQUINE COMPOUNDS

4.1 BACKGROUND

The structural makeup of the "reversed chloroquine" hybrid compounds reported by Burgess *et al*., (2006),[189] helped shape the structural delineation of dibemethinoquines. As discussed earlier, "reversed chloroquine" compounds combine the fundamental pharmacophore for haem-binding and inhibition of haemozoin formation (4-amino-7-chloroquinoline) and the pharmacophore for a resistance reverser.

Since dibemethinoquines are proposed to fall in the class of "reversed chloroquine" compounds, then the earlier observed antimalarial activity of these compounds against resistant strains of *P. falciparum* could possibly be ascribed to their aminomethyl dibemethin side-chains whose two aromatic hydrophobic ring structure coupled with the presence of protonatable N-atoms in the side-chain, although not inline with the spartial delinations proposed in the Bhattacharjee *et al*., (2002) model, had successfully conferred the chloroquine resistance-reversing entity of this type of compounds. If this hypothesis is true, then aminomethyl dibemethins can be viewed as a new class of chloroquine resistance-reversing agents.

A chloroquine resistance-reversing agent should restore chloroquine activity at concentrations at which it has little or no inherent biological activity. To explore the potential of aminomethyl dibemethins as chloroquine resistance-reversing agents, *in vitro* chloroquine resistance-reversing activity was tested against the W2 chloroquine resistant strain of the malaria parasite. Furthermore, to probe the effect, if any, of the primary amino group of these compounds, their formylated analogues were prepared and tested similarly. β-haematin inhibitory activity tests were also conducted to determine if the mode of action of the weak antimalarial activity of these compounds is *via* interference with the haem detoxification pathway of the malaria parasite as has been reported in some resistance modulating agents,[209]

4.2 EXPERIMENTAL METHODS

4.2.1 Synthesis of aminomethyl and amidomethyl dibemethins

As discussed in Chapter 2, *o*-aminomethyl dibemethin (**4**) was synthesized in four steps through a reductive amination *via* the oxime route using the method by Blackie *et al*., (2007).[200] All the other aminomethyl dibemethins were synthesized *via* the Staudinger reduction of azides using a modified synthetic route to the method by Rinke *et al*., (2001).[201] The formylated derivatives of aminomethyl dibemethins (amidomethyl dibemethins) were all synthesized in a single step reaction of the appropriate aminomethyl dibemethin with excess ethyl formate. All the target compounds were fully characterized by infrared, ^{1}H and ^{13}C NMR and mass spectrometry. Experimental details of the compounds are outlined in Chapter 6.

4.2.2 β-haematin inhibition

The IC_{50} values for β-haematin inhibition were determined by the method of Ncokazi and Egan (2005) using a pyridine based 96-well plate method.[202]

4.2.3 Antiplasmodial and chloroquine resistance-reversal testing

The antiplasmodial and chloroquine resistance-reversal activity of aminomethyl and amidomethyl dibemethins was tested against the chloroquine resistant W2 strain of parasite with the compound concentration fixed at 1 μM and also with each fixed at its individual $IC_{10.}$ This work was conducted at the Division of Pharmacology, Department of Medicine, University of Cape Town (courtesy of Prof. P Smith).

4.3 RESULTS

4.3.1 β-haematin formation

All the aminomethyl dibemethin compounds and their formylated derivatives were tested for their β-haematin inhibitory activity using 96-well plate method of Ncokazi and Egan (2005).[202] None of the compounds demonstrated β-haematin inhibitory activity *in vitro*.

4.3.2 Antimalarial activity

Since a chloroquine resistance-reversing agent restores chloroquine activity at concentrations where it has little or no inherent biological activity itself, it was necessary to first test the *in vitro* antimalarial activities of aminomethyl dibemethin compounds (**4**, **21** – **31**) and amidomethyl dibemethin compounds (**5**, **32** – **42**) against the chloroquine resistant W2 strain of parasite. The IC_{50} and IC_{10} values are reported in Table 4.1 below. Each compound was then tested in combination with chloroquine. In each case, testing was carried out with the compound concentration fixed at 1 μM and also with each fixed at its individual IC_{10}. This permits both direct comparison of the relative resistance reversing activities of the compounds as well as providing information on the maximal resistance reversing activity under conditions in which the compound has little inherent antimalarial activity. These data are reported in Table 4.2.

Table 4.1: The *in vitro* IC_{50} and IC_{10} values of aminomethyl dibemethin compounds (**4**, **21** – **31**) and amidomethyl dibemethin compounds (**5**, **32** – **42**), against the chloroquine resistant W2 strain of *P. falciparum*.

Compound Number	Compound Code	IC_{50} / μM
4	VZ1A	4.2
24	VZ2A	3.4
28	VZ3A	39.0
21	2VZ1A	6.1
25	2VZ2A	27.9
29	2VZ3A	59.8
22	3VZ1A	ND
26	3VZ2A	5.3
30	3VZ3A	50.5
23	4VZ1A	21.0
27	4VZ2A	30.4
31	4VZ3A	14.5
5	VZ1F	10.1
35	VZ2F	25.5
39	VZ3F	5.4
32	2VZ1F	4.8
36	2VZ2F	18.4
40	2VZ3F	27.2
33	3VZ1F	31.6
37	3VZ2F	45.7
41	3VZ3F	43.7
34	4VZ1F	53.9
38	4VZ2F	ND
42	4VZ3F	37.3

Table 4.2: The IC_{50} of chloroquine (nM) in the presence of 1 μM concentration and the IC_{10} of aminomethyl dibemethin compounds (**4**, **21** – **31**) and amidomethyl dibemethin compounds (**5**, **32** – **42**) and the corresponding resistance modification index in the chloroquine resistant W2 strain.

Compound	Code	At 1 μM compound	RMI[a]	At the IC_{10}	RMI[a]
–	**–**	165.9[b]	1.00	144.9	-
4	VZ1A	4	0.024	**ND**	**-**
24	VZ2A	-[c]	**-**	3.70	0.0255
28	VZ3A	26.8	0.162	**ND**	**-**
21	2VZ1A	4	0.024	4.53	0.031
25	2VZ2A	44.3	0.267	64.05	0.442
29	2VZ3A	29.7	0.179	83.66	0.577
22	3VZ1A	47.1	0.284	95.61	0.660
26	3VZ2A	36.9	0.222	38.38	0.265
30	3VZ3A	36.9	0.222	52.40	0.362
23	4VZ1A	45.7	0.275	59.91	0.413
27	4VZ2A	29.7	0.179	4.53	0.031
31	4VZ3A	27.1	0.163	8.44	0.058
5	VZ1F	114	0.687	68.49	0.473
35	VZ2F	115	0.693	41.96	0.290
39	VZ3F	166	**1**	59.91	0.413
32	2VZ1F	138	0.832	43.87	0.303
36	2VZ2F	138	0.832	24.59	0.170
40	2VZ3F	138	0.832	24.59	0.170
33	3VZ1F	134	0.808	5.90	0.041
37	3VZ2F	164	**0.989**	51.26	0.354
41	3VZ3F	162	**0.976**	40.13	0.277
34	4VZ1F	146	0.880	61.26	0.423
38	4VZ2F	-[c]	**-**	**ND**	**-**
42	4VZ3F	187	**1.127**	70.02	0.483

[a] IC_{50} of CQ in presence of compound/IC_{50} of CQ in its absence, [b] in presence of CQ alone, [c] compound too toxic to parasite to test.

The data shows (Table 4.1) that none of the dibemethins tested exhibit *in vitro* antimalarial IC_{50} values at concentrations below 1 μM. On the other hand, all show significant resistance reversing activity. With most compounds bringing about restoration of chloroquine activity in the W2 strain of chloroquine resistant parasite at their IC_{10} values to within about 2 – 3 times the IC_{50} in the D10 strain. Only compounds **22** and **29** show weak resistance reversing activity at their IC_{10} values, restoring chloroquine activity to about 5 – 6 times the IC_{50} in the D10 strain.

Among compounds (**4**, **21** – **31**), there is little difference in their chemosensitizing activity except for compounds (**4)** and (**21**) which are exceptionally active, probably because they possess some direct toxicity to the parasite. When comparing the chemosensitizing activities of the whole set of these compounds at the same concentration (1 μM), the dominant effect appears to be formylation of the primary amino group. At this concentration the activity of the formylated compound is almost abolished. Only (**5**) and (**35**) retain any resistance reversing activity. By contrast, all of the compounds with a basic primary amino group exhibit strong activity at this concentration. The likely explanation for this observation is that the dibasic compounds (**4**, **21** – **31**) accumulate in the digestive vacuole of the parasite, the site of drug action of chloroquine [210] through pH trapping to a far higher concentration than the monobasic compounds (**5**, **32** – **42**). Comparing the activities of the dibemethin compounds at their respective IC_{10} concentrations shows that all, including the formylated series (**5**, **32** – **42**) are active chloroquine resistance-reversers. The fact that formylation of the amino group does not abolish the resistance reversing properties of these compounds demonstrates that the N atom at this position need not possess basic properties in order to exhibit chloroquine resistance-reversing activity.

4.4 DISCUSSION

The data obtained in this study gives an insight into the structural characteristics that are associated with chloroquine resistance-reversing activity. The ability to interact with haem was shown not to be a necessary feature of resistance reversing activity. As expected, this confirms that haem-binding is not directly related to resistance reversing activity. By contrast to the proposal by Bhattacharjee *et al*., (2002) [187], the two aromatic rings linked by a 1-carbon chain to an N atom provide resistance reversal activity

although the position of the N atom appears to be less critical than earlier studies suggest. Furthermore, all of the compounds with a basic primary amino group exhibit stronger activity at this concentration than their formylated derivative. Indeed, it has previously been shown that accumulation at the site of action is critical for resistance reversing activity.

A possible explanation for the observed resistance reversing properties of these compounds is that the aromatic rings in aminomethyl dibemethins may function by mimicking the molecular structure of the chloroquine ring system in a manner that allows specific interaction with the resistance apparatus in chloroquine resistant parasites. This would be consistent with a model in which the chemosensitizer binds directly to the chloroquine resistance transporter PfCRT on the vacuolar side of the membrane, competing with chloroquine.[61] Alternatively, the aminomethyl dibemethins may bind to the chloroquine resistance transporter protein and indirectly affect chloroquine accumulation by altering the proton gradient across the food vacuole membrane [211], [155]. Yet a third possibility is that if the resistance transporter functions by exporting chloroquine from its site of action, aminomethyl dibemethins may bind closely with the quinoline ring of chloroquine *via* π-π interactions. The resultant compound is more hydrophobic than chloroquine and may be less efficiently exported from the food vacuole although this seems less likely. In this light, it is interesting to note that a direct correlation has been observed between hydrophobicity and activity against chloroquine resistant parasites in a series of quinoline antimalarials.[207]

4.5 CONCLUSIONS

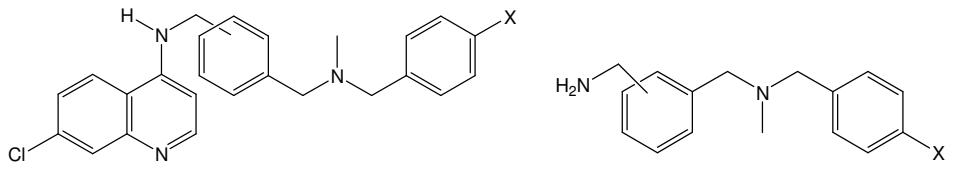
The aminomethyl derivatives of dibemethin represent a new class of chloroquine resistance-reversing agent in *Plasmodium falciparum*. A significant finding is that the need for a three-carbon aminoalkyl chain positioned between the aromatic rings as proposed by Bhattacharjee *et al*., (2002),[187] is not essential for resistance reversal. Finally, ability to pH trap in the digestive vacuole appears to greatly enhance resistance-reversing activity, while stereospecific factors seem to play an important role in the relative activities of closely related compounds.

CHAPTER 5: OVERALL CONCLUSCIONS AND FUTURE WORK

5.1 Overall conclusions

The principle aim of this project was to synthesize chloroquine-like antimalarial compounds of high potency with the inherent ability to switch-off drug resistance should it develop, based on the principle of the "reversed chloroquine" hybrid molecule reported by Burgess *et al* (2006).[189] The structural makeup and synthesis of the target compounds (dibemethinoquines) was guided by 3 models being; (i) a SAR model based on findings by Egan *et al*., (2000),[102] (ii) a 3D-QSAR pharmacophore model for chloroquine resistance-reversal proposed by Bhattacharjee *et al*., (2002)[187] and (iii) the Topliss scheme[193] to select suitable substituents in an attempt to synthesize analogues of superior activity to the parent compounds.

By possessing two hydrophobic aromatic rings (the aminomethyl dibemethin side-chain) and a hydrogen bond acceptor (quinoline N-atom) in agreement with the requirements of the proposal by Bhattacharjee *et al*., (2002), dibemethinoquines were hypothesized to act as resistance-reversing compounds. Furthermore, the 4-amino-7-chloroquinoline moiety provided the focus for haem-binding and inhibition of haemozoin formation thus fulfilling the requirements in the SAR model proposal by Egan *et al*., (2000) for the activity of chloroquine-like compounds (Figure 5.1) and the Topliss scheme was used in the choice of substituent (X).



(**6, 43** – **53**) (**4, 21** – **31**)

Figure 5.1: Chemical structures of dibemethinoquines (**6, 43** – **53**) and aminomethyl dibemethins (**4**, **21 – 31**).

To better understand the structure–activity relationships of resistance reversal on side-chain structure, the influence of the position of connection between the 4-amino-7-

chloroquinoline moiety and the dibemethin side-chain was probed by varying the connection between *ortho*-, *meta*- and *para*- positions. Furthermore, analogues of the parent dibemethinoquine compounds (X = H) were prepared by introducing substituents on the *para* position of the benzyl-*N*-methylamine ring of the side-chain. The choice of substituents was based on the Topliss Scheme where the Cl (hydrophobic/electron-withdrawing), OMe (electron-releasing, similar hydrophobicity to H) and NMe_2 (strongly electron-releasing/hydrophobic) groups were selected.

The IC_{50} values of dibemethinoquine compounds were almost identical in the chloroquine-sensitive D10 and chloroquine-resistant K1 strains of malaria parasite *in vitro*. Three compounds, (**46**), (**50**) and (**53**) showed promising levels of *in vitro* antimalarial activity, with compound (**50**) having an IC_{50} almost identical to chloroquine in the chloroquine sensitive strain. Compound (**6**) and (**50**) were tested in a mouse model for *in vivo* activity against *P. yoelii nigeriensis*. Although active, they were less active than chloroquine. However, as they were administered by i.p. injection of the free bases (versus the diphosphate salt of chloroquine), the lower activity can be ascribed to poor water solubility. Indeed, these compounds were noted to be suspensions, whereas chloroquine diphosphate was clearly a solution. No obvious acute adverse effects were observed in the mice and cytotoxicity tests on the prototype compound (**6**) showed that this class of compound is selectively active against malaria parasites. All the dibemethinoquines were shown to inhibit β-hematin (synthetic haemozoin) formation *in vitro* and a quantitative structure activity relationship between digestive vacuole accumulation normalized IC_{50} (determined from pK_a values and the Henderson-Hasselbach equation) and both β-hematin inhibition strength and a structural descriptor of the dibemethin moiety (taking into account *ortho*-, *meta*- or *para*-substitution) was found. This strongly supports the hypothesis that dibemethinoquines act by inhibiting haemozoin formation in the parasite.

Somewhat surprisingly, the aminomethyl dibemethin compounds used as side-chains all showed strong chloroquine resistance-reversing (chemosensitizing) activity in the chloroquine resistant W2 strain of *Plasmodium falciparum* cultured *in vitro* when present at 1 μM concentration or at their IC_{10} concentration. Their N-formylated derivatives (amidomethyl dibemethins) also showed resistance reversing activity, but only at their significantly higher IC_{10} concentrations. The fact that formylation of the terminal amino

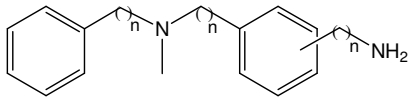
group does not abolish the resistance reversing properties of these compounds demonstrates that the N atom at this position does not need to be basic in order to exhibit chloroquine resistance-reversing activity. However, the fact that the aminomethyl dibemethin compounds exhibit stronger activity at 1 μM concentration than their formylated derivatives highlights the observation that the ability to accumulate in the digestive vacuole by pH trapping enhances resistance-reversing activity. The resistance reversing activity observed in aminomethyl and amidomethyl dibemethins demonstrates that the amino group need not be attached to the two aromatic rings *via* a three or four carbon chain for a compound to exhibit chloroquine resistance-reversing properties as has been suggested by previous QSAR studies.

5.2 FUTURE WORK

An extensive study of the magnitude of chemosensitization of chloroquine resistant parasites by these novel classes of resistance reversers and "reversed chloroquine" compounds will have to be performed by isobologram analysis. This will aid in establishing whether the interaction of these new compounds with chloroquine is synergistic, additive or antagonistic and hence to establish whether the active quinoline derivatives themselves actually reverse chloroquine resistance.

In view of the fact that free-bases of dibemethinoquines showed solubility problems both in the in *vitro* and in the in *vivo* antimalarial tests, preparation of salts of these compounds and / or improved formulation is essential to improve biological tests results and to determine their potential as new drugs.

In this study, it was found that aminomethyl dibemethin compounds exhibit chloroquine resistance-reversing activity. In future studies, it would be worthwhile synthesizing analogues of these compounds with longer alkyl chains separating the two aromatic rings as illustrated in Figure 5.2 below.

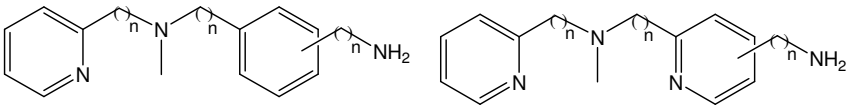


Where n = 1, 2, 3 e.t.c

Figure 5.2: Chemical structures of proposed new aminomethyl dibemethin synthetic targets with elongated alkyl chains between N and the two aromatic rings.

Synthesis of such compounds would afford the opportunity to further explore structure-activity relationships in this class of compounds with regard to resistance reversal.

Basicity of aminomethyl dibemethin compounds was identified as having a bearing on activity by improving accumulation *via* pH trapping. It would be interesting to observe resistance reversing properties of analogues of these compounds where one or both of the aromatic rings is replace by a heteroaromatic ring such as pyridine (Figure 5.3)



Where n = 1, 2, 3 etc

Figure 5.3: Chemical structures of proposed new aminomethyl dibemethin synthetic targets with elongated alkyl chains between N and the two aromatic rings and with one or both or the benzene rings replaced by pyridine.

Such compounds would in essence retain the aromatic nature of these regions while more importantly increasing both the overall basicity of the compound and the number of H-bond acceptors within the molecule. The improved H-bond acceptor properties of this type of compound may possibly assist in the drug binding to the resistance transporter protein.

Similar changes to aminomethyl dibemethins can be effected to the side-chains of a new class of potential “reversed chloroquine” compounds (Figure 5.4).This will offer novel

compounds of potentially superior activity based on findings on the dibemethinoquine compounds.

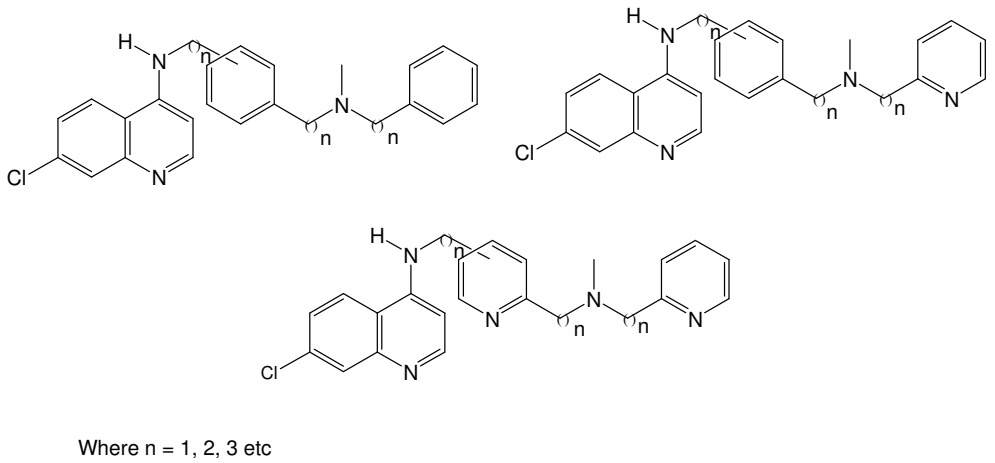


Figure 5.4: Chemical structures of proposed new dibemethinoquine synthetic targets with elongated alkyl chains between N and the two aromatic rings and / or with one or both or the benzene rings replaced by pyridine.

CHAPTER 6: EXPERIMENTAL

6.1 General Procedure

All solvents were freshly distilled. Dichloromethane was distilled over phosphorus pentoxide under nitrogen. Tetrahydrofuran was distilled under nitrogen and dried over sodium wire with benzophenone. Other reagents were purified according to standard procedures.[317] All reagents were purchased from Aldrich or Merck. Low temperature reactions were carried out using liquid nitrogen in acetone (-78 ºC) or a slurry of water and ice (0 ºC).

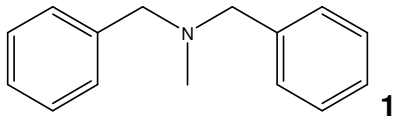
Thin layer chromatography (TLC) was used to monitor reactions using aluminium-backed Merck silica-gel 60 F_{254} plates. Compounds on TLC were observed by a combination of ultra-violet light, iodine vapour, or by spraying with a 2.5% solution of anisaldehyde in a mixture of sulfuric acid and ethanol (1:10 v/v) and then heating at 150 ºC. Column chromatography was performed using silica-gel 60 mesh (Merck 7734).

Melting points were measured on a Reichert-Jung Thermovar hot-stage microscope and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer Paragon 1000 FT-IR spectrometer on sodium chloride plates. Elemental analyses were performed using a Fisons EA 110 CHN elemental analyzer. High-resolution mass spectrometry was performed at The University of the Witwatersrand using a VG70-SEQ micromass spectrometer or at The School of Chemistry, University of Stellenbosch on an API Q-TOF Ultima machine. All spectra were recorded in Electron Ionisation mode, unless otherwise stated.

Nuclear Magnetic Resonance spectra were recorded on either a Varian Unity 400 (at 399.95 MHz for ^{1}H and 100.58 MHz for ^{13}C) or a Varian VXR-300 (at 300.08 MHz for ^{1}H and 75.5 MHz for ^{13}C) spectrometer in deuterated chloroform unless otherwise stated. Chemical shifts (δ) were recorded using residual chloroform (δ 7.26 in ^{1}H NMR and δ 77.00 in ^{13}C NMR) as internal standards. All chemical shifts are reported in ppm and *J* values are quoted in Hz.

6.2 CHARACTERIZATION

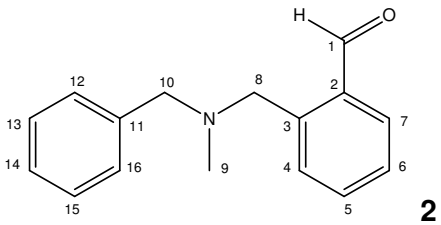
N, N-Dibenzylmethylamine



1

To a stirred solution of *N, N*-benzylmethylamine (10.6 ml, 82.5 mmol) in DCM (50 ml) and triethylamine (17.2 ml, 124 mmol) at 0 ºC under an atmosphere of nitrogen, was added benzyl bromide (9.80 ml, 82.5 mmol) slowly and the reaction allowed to progress for 2 hours after which time a white precipitate had formed. The reaction mixture was worked up by the addition of a saturated solution of aqueous Na_2CO_3 and the product extracted into ethyl acetate (3 × 50 ml), the organic extracts dried over anhydrous $MgSO_4$ and the solvent removed under reduced pressure to give a crude product (17 g), which was chromatographed on silica-gel (170 g) using 10% ethyl acetate in hexane as eluent. The product was dried *in vacuo* to give **1** (11.6 g, 67%) as a pale yellow oil; δ_H (300 MHz, $CDCl_3$) 7.57 - 7.38 (10H, m, ArH), 3.70 (4H, s, C*H*$_2$), 2.37 (3H, s, C*H*$_3$); δ_C ($CDCl_3$, 75.5 MHz) 139.2 (Ar_{qu}), 128.8 (Ar_{ortho}), 128.1 (Ar_{meta}), 126.8 (Ar_{para}), 61.8 (*C*H_2), 42.1 (*C*H_3).

o – (*N*-Benzyl-*N*-methylamino)methyl-benzaldehyde

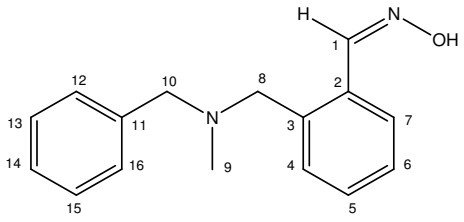


2

t-Butyllithium (10.0 ml, 1.7 M, 17.0 mmol) was slowly added dropwise to a stirred solution of **1** (3.00 g, 14.2 mmol) in anhydrous diethyl ether (100 ml) at -78 ºC under an atmosphere of nitrogen. The mixture was warmed up to 0 ºC and allowed to stir until the colour of the mixture turned from orange to pale-yellow and a white precipitate of the lithio-salt had formed. Anhydrous DMF (1.10 ml, 14.3 mmol) was then slowly added

dropwise at 0 ºC and the reaction allowed to progress to room temperature while stirring for 3 hours after which time the reaction mixture was clear of precipitate. Diethyl ether was evaporated off on the rotary evaporator, the resulting solid was worked up with deionised water and the product extracted into ethyl acetate (3 × 100 ml). The organic extracts were dried over anhydrous $MgSO_4$ and the solvent removed under reduced pressure to give a crude product (3.20 g), which was chromatographed on silica-gel using mixtures of ethyl acetate: hexane (0:100) to (5: 95) as eluent. The product was dried *in vacuo* to give **2** (2.61 g, 77%) as a yellow oil; ν_{max}/cm^{-1} ($CHCl_3$) 3066, 3029, 3010, 2949, 2880, 2843, 2793, 1689, 1600; δ_H ($CDCl_3$, 300 MHz) 10.42 (1H, s, H-1), 7.92 (1H, dd, *J* = 1.5, 7.5 Hz, H-7), 7.50 - 7.23 (8H, m, ArH), 3.84 (2H, s, H-8), 3.53 (2H, s, H-10), 2.13 (3H, s, H-9); δ_C ($CDCl_3$, 75.5 MHz) 192.0 (C-1), 141.7 (Ar_{qu}), 138.5 (Ar_{qu}), 134.9 (Ar_{qu}), 133.0 (Ar_{C-H}), 130.4 (C-4), 128.9 (C-7), 128.8 (C-12 / 16), 128.1 (C-13 / 15), 127.6 (Ar_{C-H}), 127.0 (Ar_{C-H}), 61.8 (C-10), 58.9 (C-8), 41.6 (C-9); HRMS (ES): Found 239.13034 (M^+). $C_{16}H_{17}NO$ (M^+) requires 239.13101.

***o*-(*N*-Benzyl-*N*-methylamino)methyl-benzaldehyde oxime**

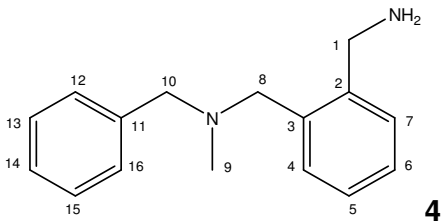


3

To a stirred solution of **2** (2.50 g, 10.4 mmol) in absolute ethanol (50 ml), was added hydroxylammonium chloride (1.97 g, 28.3 mmol) followed by a solution of NaOH (2.26 g, 56.5 mmol) in deionised water(3 ml). The reaction was allowed to reflux overnight under an atmosphere of nitrogen. The mixture was then worked up with deionised water and extracted with ethyl acetate (3 x 50 ml). The organic extracts were dried over anhydrous $MgSO_4$ and the solvent evaporated under reduced pressure. The crude product was chromatographed on silica-gel using a mixture of ethyl acetate: hexane (10:90) as eluent. The product was dried *in vacuo* to give **3** (2.25 g, 85%) as a pale-yellow oil; ν_{max}/cm^{-1} ($CHCl_3$) 3305, 3065, 3028, 3011, 2950, 2881, 2841, 2791, 1601; δ_H ($CDCl_3$, 300 MHz) 8.69 (1H, s, H-1), 7.80 (1H, dd, *J* = 1.7, 6.5 Hz, H-7), 7.35 - 7.25 (8H, m, ArH), 3.64 (2H, s, H-8), 3.54 (2H, s, H-10), 2.15 (3H, s, H-9), 1.29 (1H, br s, OH); δ_C ($CDCl_3$,

75 MHz) 149.2 (C-1), 138.8 (Ar_{qu}), 137.7 (Ar_{qu}), 131.4 (Ar_{qu}), 130.7 (Ar_{C-H}), 129.4 (Ar_{C-H}), 129.1 (C-12 / 6), 128.3 (C-13 / 15), 127.6 (Ar_{C-H}), 127.0 (Ar_{C-H}), 126.4 (C-7), 62.1 (C-10), 60.0 (C-8), 41.8 (C-9); HRMS (ES): Found 254.14271 (M^+). $C_{16}H_{18}N_2O$ (M^+) requires 254.14191.

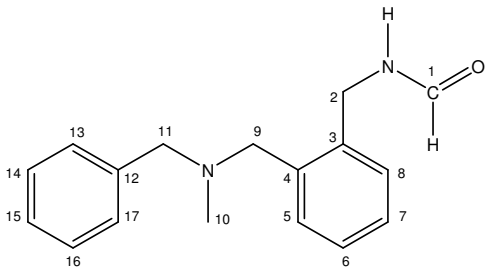
***o*-[(*N*-Benzyl-*N*-methyl)aminomethyl]-benzylamine**



4

To a stirred solution of **3** (1.00 g, 3.90 mmol) in anhydrous diethyl ether (10 ml), was added $LiAlH_4$ (75 mg, 1.96 mmol) slowly and the reaction allowed to reflux overnight under an atmosphere of nitrogen. After the reaction mixture was allowed to cool to room temperature, a saturated solution of aqueous Na_2SO_4 (10 ml) with a few drops of triethylamine was added to the reaction mixture. This mixture was stirred for 30 minutes and the resultant precipitate filtered through Celite, the residue washed with a solution of 5% triethylamine in THF (100 ml). The solvent was removed under reduced pressure with a small volume of toluene being added to facilitate the azeotropic removal of any water. The crude product was purified by column chromatography using 100% ethyl acetate followed by EtOAc: MeOH: NEt_3 (90: 5: 5) as eluent to give **4** (0.83 g, 89%) as a dark-orange oil; v_{max}/cm^{-1} (DMSO) 3523 - 3422, 3287, 2791, 2143, 2050, 1973, 1711, 1663, 1579, 1491; δ_H ($CDCl_3$, 300 MHz) 7.32 - 7.23 (9H, m, ArH), 6.88 (2H, br s, NH_2), 3.94 (2H, s, H-1), 3.58 (2H, s, H-8), 3.57 (2H, s, H-10), 2.07 (3H, s, H-9); δ_C ($CDCl_3$, 75.5 MHz) 137.5 (Ar_{qu}), 137.1 (Ar_{qu}), 136.8 (Ar_{qu}), 131.3 (C-7), 130.7 (C-4), 129.5 (C-12 / 16); 128.5 (C-13 / 15); 128.4 (Ar_{C-H}), 128.0 (Ar_{C-H}), 127.4 (Ar_{C-H}), 62.3 (C-10), 60.8 (C-8), 43.0 (C-1), 40.9 (C-9); HRMS (ES): Found 240.16025 (M^+). $C_{16}H_{20}N_2$ (M^+) requires 240.16265.

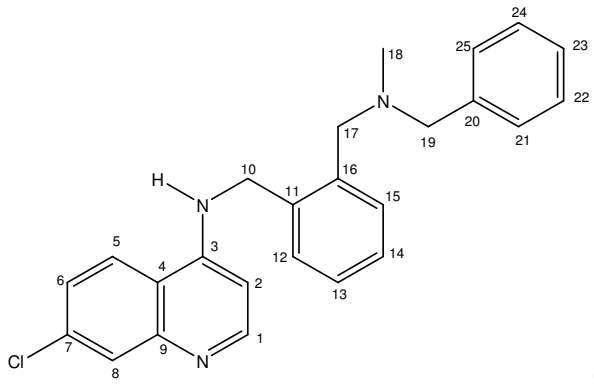
s-*trans*-*N*-[{2-(*N*-Benzyl-*N*-methylaminomethyl)phenyl}methyl] formamide



5

A stirred solution of **4** (0.35 g, 1.46 mmol) in excess ethyl formate (5.75 ml, 72.5 mmol) under N_2 was refluxed overnight. The excess ethyl formate was removed under reduced pressure and the crude product purified by column chromatography using mixtures of ethyl acetate: hexane (50:50) to (95:5) as eluent to give **5** (0.36 g, 92%) as a thick-yellow oil and predominantly (> 90%) as a s-*trans* rotamer; ν_{max}/cm^{-1} (DMSO) 3621 - 3211, 2595, 2148, 2059, 1979, 1907, 1656; δ_H ($CDCl_3$, 300 MHz) 8.33 (1H, br s, NH), 8.08 (1H, s, H-1), 7.41 - 7.22 (9H, m, ArH), 4.44 (2H, d, *J* = 5.7 Hz, H-2), 3.58 (2H, s, H-9), 3.55 (2H, s, H-11), 2.12 (3H, s, H-10); δ_C ($CDCl_3$, 75.5 MHz) 160.3 (C-1), 137.9 (Ar_{qu}), 137.8 (Ar_{qu}), 136.7 (Ar_{qu}), 131.7 (C-8), 130.6 (C-5), 129.4 (C-13 / 17); 128.6 (Ar_{C-H}), 128.5 (C-14 / 16); 127.7 (Ar_{C-H}), 127.6 (Ar_{C-H}), 62.2 (C-9), 61.4 (C-11), 42.0 (C-10), 41.3 (C-2); HRMS (ES): Found 268.15875 (M^+). $C_{17}H_{20}N_2O$ (M^+) requires 268.15756.

***N*-[{2-(*N*-Benzyl-*N*-methylaminomethyl)phenyl}methyl]-7-chloro-4-quinolinamine**



6

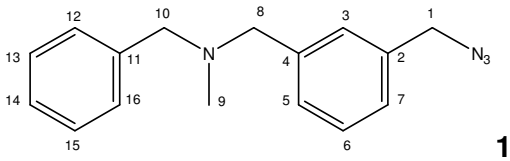
To a stirred solution of **4** (0.42 g, 1.73 mmol) in anhydrous *N*-methyl-2-pyrrolidone (2 ml) under N_2, were added triethylamine (1.21 ml, 8.67 mmol), K_2CO_3 (0.48 g, 3.47 mmol) and 4, 7-dichloroquinoline (1.72, 8.67 mmol) and the mixture was heated at 90 °C for 48

Hours. After the mixture was allowed to cool to room temperature it was poured into saturated brine (20 ml) and extracted with ethyl acetate (3 × 50 ml). The combined organic extracts were further washed 5 times with saturated brine to ensure removal of any traces of the pyrrolidone, before being dried over anhydrous Na_2SO_4 and filtered. Following solvent removal, the resulting crude product was dried under reduced pressure and purified by silica-gel chromatography using mixtures of ethyl acetate: hexane (30:70) to (80:20) as eluent to give **6** (0.26 g, 37%) as a white crystalline solid, m.p. (EtOAc:Hex) 101-103 ºC; v_{max}/cm^{-1} (DMSO) 3583, 3384 - 3256, 2595, 2140, 2050, 1975, 1659, 1580; δ_H ($CDCl_3$, 300 MHz) 8.53 (1H, d, *J* = 5.1 Hz, H-1), 7.89 (1H, d, *J* = 2.0 Hz, H-8), 7.49 - 7.19 (10H, m, Ar H and H-5), 6.85 (1H, dd, *J* = 2.0, 9.0 Hz, H-6), 6.52 (1H, d, *J* = 5.1 Hz, H-2), 4.43 (2H, d, *J* = 5.1 Hz, H-10), 3.61 (2H, s, H-17), 3.58 (2H, s, H-19), 2.14 (3H, s, H-18), 1.27 (1H, s, NH); δ_C ($CDCl_3$, 75.5 MHz) 152.0 (C-1), 150.2 (Ar_{qu}), 149.3 (Ar_{qu}), 137.4 (Ar_{qu}), 137.2 (Ar_{qu}), 137.1 (Ar_{qu}), 134.5 (Ar_{qu}), 132.0 (C-15), 130.5 (Ar_{C-H}), 129.9 (C-12), 129.9 (Ar_{C-H}),128.4 (C-21 / 25), 128.4 (C-22 / 24), 127.9 (C-8), 127.6 (Ar_{C-H}), 124.7 (C-6), 122.2 (C-5), 117.8 (Ar_{qu}), 99.0 (C-2), 61.9 (C-17), 61.0 (C-19), 46.6 (C-10), 41.9 (C-18); (Found: C, 74.56; H, 6.07; N, 10.08%. $C_{25}H_{24}N_3Cl$ requires C, 74.71; H, 6.02; N, 10.45%).

General procedure for synthesis of the *α, α'*-2, 3 and 4 azido-bromo xylenes (GP1)

Sodium azide (1.0 equiv.) was added to a stirred solution of the appropriate *α, α'*-dibromoxylene (*o*, *m* or *p*) in anhydrous DMF under N_2, and the reaction allowed stirring at room temperature overnight. The reaction mixture was diluted with ethyl acetate (100 ml) and the organic layer washed with saturated brine (3 x 50 ml), dried ($MgSO_4$) and concentrated *in vacuo*. The crude product was obtained as a mixture of the mono- and di-substituted as well as the unreacted *α, α'*-dibromoxylene, with approximately 65% of the crude product being the desired mono-substituted azidobromoxylene as evaluated by TLC and overall yields of the two steps. This crude product was used without further purification.

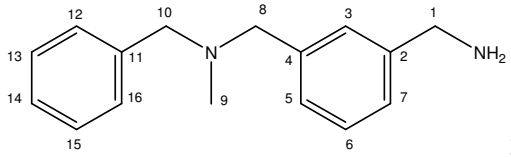
***N*-[{3-(Azidomethyl)phenyl}methyl]-*N*-benzylmethylamine**



13

To a stirred solution of crude *meta- α, α'* -azidobromoxylene (0.22 g, 0.99 mmol) synthesized as outlined in GP1 and K_2CO_3 (0.27 g, 2.00 mmol) in anhydrous acetonitrile (25 ml) at 0 °C, was added *N*-benzylmethylamine (0.19 ml, 1.49 mmol) and the reaction heated and allowed to progress under reflux overnight. Acetonitrile was then removed under reduced pressure, saturated aqueous Na_2CO_3 added and the organic product extracted into EtOAc (3 × 100 ml). Drying ($MgSO_4$) of the organic extracts and concentration *in vacuo* furnished a residue, which was purified by column chromatography using mixtures of ethyl acetate: hexane (10:90) to (20:80) as eluent to give **13** (0.15 g, 57% over 2 steps.) as a yellow oil; δ_H ($CDCl_3$, 300 MHz) 7.40 - 7.14 (9H, m, ArH), 4.35 (2H, s, H-1), 3.57 (2H, s, H-8), 3.57 (2H, s, H-10), 2.22 (3H, s, H-9); δ_C ($CDCl_3$, 75.5 MHz) 140.0 (Ar_{qu}), 138.9 (Ar_{qu}), 135.3 (Ar_{qu}), 128.9 ($Ar_{C\text{-}H}$), 128.9 (C-12 / 16), 128.7 ($Ar_{C\text{-}H}$), 128.7 ($Ar_{C\text{-}H}$), 128.2 (C-13 / 15), 127.0 ($Ar_{C\text{-}H}$), 126.8 ($Ar_{C\text{-}H}$), 61.8 (C-8), 61.5 (C-10), 54.8 (C-1), 42.1 (C-9); HRMS (ES): Found 266.14947 (M^+). $C_{16}H_{18}N_4$ (M^+) requires 266.15361.

***m*-[(*N*-Benzyl-*N*-methyl)aminomethyl]-benzylamine**

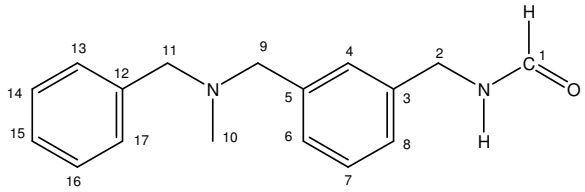


24

To a stirred solution of **13** (0.44 g, 1.65 mmol) in THF (4.50 ml) under N_2, was added PPh_3 (0.44 g, 1.66 mmol) and the reaction mixture allowed to stir for 30 minutes at room temperature. Water was added (1.50 ml, 83.3 mmol) and the reaction heated at reflux for 6 hours. The reaction was then cooled to room temperature and solvent removed on the rotorevaporator with the water removed by azeotroping with toluene. The crude product was purified directly by column chromatography using EtOAc (100%) followed by EtOAc:

MeOH: NEt_3 (90: 5: 5) as eluent to give **24** (0.35 g, 88%) as a dark-yellow oil; v_{max}/cm^{-1} (DMSO) 3567 - 3374, 3273, 2840, 2784, 2149, 2056, 1968, 1711, 1661, 1579; δ_H (CD_3OD, 300 MHz) 7.60 (1H, s, H-3), 7.44 - 7.24 (8H, m, ArH), 4.10 (2H, s, H-1), 3.67 (2H, s, H-8), 3.67 (2H, s, H-10), 2.23 (3H, s, H-9); δ_C (CD_3OD, 75.5 MHz) 139.6 (Ar_{qu}), 138.3 (Ar_{qu}), 136.1 (Ar_{qu}), 131.0 (Ar_{C-H}), 131.0 (Ar_{C-H}), 130.7 (C-12 / 16), 130.1 (Ar_{C-H}), 129.5 (C-13 / 15), 129.1 (Ar_{C-H}), 128.7 (Ar_{C-H}), 62.6 (C-8), 62.1 (C-10), 44.5 (C-1), 41.9 (C-9); HRMS (ES): Found 239.13034 (M^+). $C_{16}H_{17}NO$ (M^+) requires 239.13101.

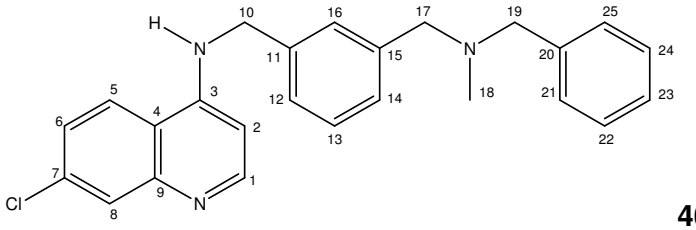
s-*trans*-*N*-[{3-(*N*-Benzyl-*N*-methylaminomethyl)phenyl}methyl] formamide



35

A stirred solution of **24** (0.15 g, 0.63 mmol) in excess ethyl formate (2.50 ml, 31.5 mmol) under N_2 was refluxed overnight. The excess ethyl formate was removed under reduced pressure and the crude product purified by column chromatography using mixtures of ethyl acetate: hexane (50:50) to (95:5) as eluent to give **35** (0.16 g, 95%) as a thick-yellow oil and predominantly as a s-*trans* rotamer; v_{max}/cm^{-1} (DMSO) 3591 - 3275, 2587, 2321, 2137, 2070, 1988, 1904, 1655 ; δ_H ($CDCl_3$, 300 MHz) 8.25 (1H, s, H-1), 7.38 - 7.16 (9H, m, ArH), 6.02 (1H, br s, NH), 4.48 (2H, d, *J* = 5.9 Hz, H-2), 3.57 (2H, s, H-9), 3.54 (2H, s, H-11), 2.20 (3H, s, H-10); δ_C ($CDCl_3$, 75.5 MHz) 161.0 (C-1), 139.3 (Ar_{qu}), 138.3 (Ar_{qu}), 137.7 (Ar_{qu}), 129.1 (C-13 / 17), 128.7 (Ar_{C-H}), 128.4 (Ar_{C-H}), 128.4 (Ar_{C-H}), 128.3 (C-14 / 16), 127.2 (Ar_{C-H}), 126.6 (Ar_{C-H}), 61.8 (C-9), 61.5 (C-11), 42.1 (C-2), 42.0 (C-10); HRMS (ES): Found 268.15763 (M^+). $C_{17}H_{20}N_2O$ (M^+) requires 268.15756.

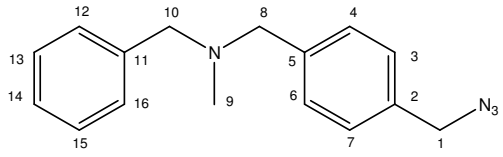
***N*-[{3-(*N*-Benzyl-*N*-methylaminomethyl)phenyl}methyl]-7-chloro-4-quinolinamine**



46

To a stirred solution of **24** (0.50 g, 2.08 mmol) in anhydrous *N*-methyl-2-pyrrolidone (5 ml) under N_2, were added triethylamine (1.45 ml, 10.4 mmol), K_2CO_3 (0.57 g, 4.16 mmol) and 4,7-dichloroquinoline (2.06 g, 10.4 mmol). The mixture was heated under pressure in a cyclo-addition tube at 130 °C overnight. After the mixture was allowed to cool to room temperature, it was poured into saturated brine (20 ml) and extracted with ethyl acetate (3 × 50 ml). The organic layer was further washed with saturated brine (5 × 50 ml) to ensure removal of any traces of the pyrrolidone. The organic layer was dried (Na_2SO_4) and concentrated *in vacuo* to afford a crude product, which was purified by column chromatography using mixtures of ethyl acetate: hexane (50:50) to (90:10) as eluent to give **46** (0.19 g, 23%) as a colourless solid, m.p. (EtOAc:Hex) 103-104 ºC; ν_{max}/cm^{-1} (DMSO) 3582, 3395 - 3279, 2596, 2143, 2060, 1972, 1660, 1579; δ_H ($CDCl_3$, 300 MHz) 8.51 (1H, d, *J* = 5.1 Hz, H-1), 7.98 (1H, d, *J* = 2.6 Hz, H-8), 7.69 (1H, d, *J* = 9.0 Hz, H-5), 7.37 (1H, dd, *J* = 2.6, 9.0 Hz, H-6), 7.34 - 7.20 (9H, m, ArH), 6.45 (1H, d, *J* = 5.1 Hz, H-2), 5.43 (1H, br t, *J* = 5.1 Hz, NH), 4.51 (2H, d, *J* = 5.1 Hz, H-10), 3.53 (2H, s, H-17), 3.51 (2H, s, H-19), 2.19 (3H, s, N-CH_3); δ_C ($CDCl_3$, 75.5 MHz) 152.1 (C-1), 149.5 (Ar_{qu}), 149.2 (Ar_{qu}), 140.4 (Ar_{qu}), 139.1 (Ar_{qu}), 137.2 (Ar_{qu}), 134.9 (Ar_{qu}), 128.9 (Ar_{C-H}), 128.8 (Ar_{C-H}), 128.8 (C-21 / 25), 128.5 (Ar_{C-H}), 128.2 (C-22 / 24); 127.9 (C-8), 126.9 (Ar_{C-H}), 126.1 (Ar_{C-H}), 125.4 (C-6), 120.9 (C-5), 117.2 (Ar_{qu}), 99.7 (C-2), 61.9 (C-17), 61.6 (C-19), 47.6 (C-10), 42.3 (N-CH_3). (Found: C, 74.53; H, 5.76; N, 9.87%. $C_{25}H_{24}N_3Cl$ requires C, 74.71; H, 6.02; N, 10.45%).

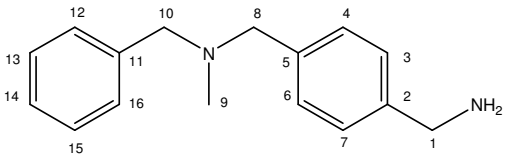
***N*-[{4-(Azidomethyl)phenyl}methyl]-*N*-benzylmethylamine**



17

To a stirred solution of crude *para- α, α'* -azidobromoxylene (0.50 g, 2.25 mmol), synthesized as outlined in GP1 and K_2CO_3 (0.61, 4.50 mmol) in anhydrous acetonitrile (50 ml) at 0 °C, was added *N*-benzylmethylamine (0.43 ml, 3.40 mmol) and the reaction heated and allowed to progress under reflux overnight. Acetonitrile was then removed under reduced pressure, saturated aqueous Na_2CO_3 added and the organic product extracted into EtOAc (3 × 100 ml). Drying ($MgSO_4$) of the organic extracts and concentration *in vacuo* furnished a residue, which was purified by column chromatography using mixtures of ethyl acetate: hexane (10:90) to (20:80) as eluent to give **17** (0.35 g, 59% over 2 steps.) as a yellow oil; δ_H ($CDCl_3$, 400 MHz) 7.43 - 7.26 (9H, m, ArH), 4.35 (1H, s, H-1), 3.56 (2H, s, H-8), 3.56 (2H, s, H-10), 2.22 (3H, s, H-9); δ_C ($CDCl_3$, 100.6 MHz) 139.7 (Ar_{qu}), 139.2 (Ar_{qu}), 134.1 (Ar_{qu}), 129.4 (2 x Ar_{C-H}), 129.0 (2 x Ar_{C-H}), 128.3 (2 x Ar_{C-H}), 128.2 (2 x Ar_{C-H}), 127.0 (Ar_{C-H}), 61.9 (C-8), 61.5 (C-10), 54.7 (C-1), 42.1 (C-9); HRMS (ESI): Found 267.1605(M^+ +1). $C_{16}H_{18}N_4$ (M^+ +1) requires 267.1610.

***p*-[(*N*-Benzyl-*N*-methyl)aminomethyl]-benzylamine**

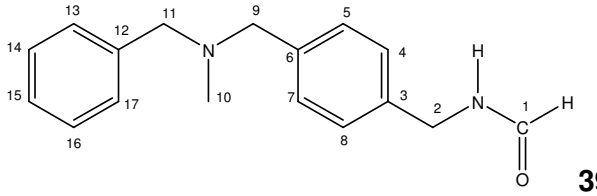


28

To a stirred solution of **17** (0.70 g, 2.63 mmol) in THF (7.20 ml) under N_2, was added PPh_3 (0.84 g, 3.16 mmol) and the reaction mixture allowed to stir for 30 minutes at room temperature. Water (2.39 ml, 133 mmol) was added and the reaction heated at reflux for 6 hours. The reaction was then cooled to room temperature and solvent removed on the rotorevaporator with the water removed by azeotroping with toluene. The crude product was purified directly by column chromatography using EtOAc (100%) followed by EtOAc:

MeOH: NEt_3 (90: 5: 5) as eluent to give **28** (0.56 g, 89%) as a dark-yellow oil; v_{max}/cm^{-1} (DMSO) 3531 - 3408, 3273, 2131, 2057, 1973, 1659, 1612, 1512; δ_H (CD_3OD, 400 MHz) 7.21 – 7.10 (9H, m, ArH), 3.73 (2H, s, H-1), 3.38 (2H, s, H-8), 3.37 (2H, s, H-10), 2.03 (3H, s, H-9); δ_C (CD_3OD, 100.6 MHz) 140.3 (Ar_{qu}), 139.8 (Ar_{qu}), 139.2 (Ar_{qu}), 130.7 (C-13 / 15), 130.3 (C-12 / 16); 129.3 (C-4 / 6), 128.8 (C-3 / 7), 128.3 (C-14), 62.7 (C-8), 62.4 (C-10), 45.9 (C-1), 42.4 (C-9); HRMS (ES): Found 240.16265 (M^+). $C_{16}H_{20}N_2$ (M^+) requires 240.16164.

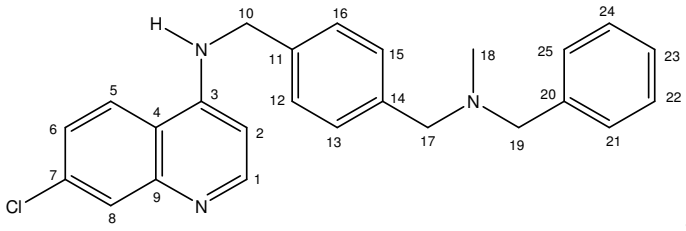
s-*trans*-*N*-[{4-(*N*-Benzyl-*N*-methylaminomethyl)phenyl}methyl] formamide



39

A stirred solution of **28** (150 mg, 0.63 mmol) in excess ethyl formate (2.50 ml, 31.5 mmol) under N_2 was refluxed overnight. The excess ethyl formate was removed under reduced pressure and the crude product purified by column chromatography using mixtures of ethyl acetate: hexane (50:50) to (95:5) as eluent to give **39** (149 mg, 89%) as a thick-yellow oil and predominantly as a s-*trans* rotamer; v_{max}/cm^{-1} (DMSO) 3532 - 3413, 3300, 2582, 2363, 2325, 2262, 2215, 2101, 1994, 1903; δ_H ($CDCl_3$, 400 MHz) 8.15 (1H, s, H-1), 7.34 - 7.20 (9H, s, ArH), 6.36 (1H, br s, NH), 4.41 (2H, d, *J* = 5.9 Hz, H-2), 3.50 (2H, s, H-9), 3.49 (2H, s, H-11), 2.17 (3H, s, H-10); δ_C ($CDCl_3$, 100.6 MHz) 161.2 (C-1), 139.2 (Ar_{qu}), 138.8 (Ar_{qu}), 136.4 (Ar_{qu}), 129.3 (C-14 / 16), 128.9 (C-13 / 17), 128.3 (C-5 / 7), 127.7 (C-4 / 8), 127.0 (C-15), 61.8 (C-11), 61.5 (C-9), 42.2 (C-2), 41.9 (C-10); HRMS (ES). Found 268.15756 (M^+). $C_{17}H_{20}N_2O$ (M^+) requires 268.15741.

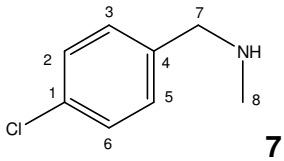
***N*-[{4-(*N*-Benzyl-*N*-methylaminomethyl)phenyl}methyl]-7-chloro-4-quinolinamine**



50

To a stirred solution of **28** (0.35 g, 1.46 mmol) in anhydrous *N*-methyl-2-pyrrolidone (3.5 ml) under N_2, were added triethylamine (1.02 ml, 7.25 mmol), K_2CO_3 (0.57 g, 4.16 mmol) and 4,7-dichloroquinoline (2.06 g, 10.4 mmol). The mixture was heated under pressure in a cyclo-addition tube at 90 °C for 48 hours. After the mixture was allowed to cool to room temperature it was poured into saturated brine (20 ml) and extracted with ethyl acetate (3 × 50 ml). The organic layer was further washed with saturated brine (5 × 50 ml) to ensure removal of any traces of the pyrrolidone. The organic layer was dried (Na_2SO_4), concentrated *in vacuo* and the resulting crude product was purified by column chromatography using mixtures of ethyl acetate: hexane (50:50) to (90:10) as eluent to give **50** (0.19 g, 32%) as a colourless solid, m.p. (EtOAc:Hex) 120-122 ºC; v_{max}/cm^{-1} (DMSO) 3603, 3394 - 3225, 2598, 2349, 2140, 2056, 1969, 1903, 1657; δ_H ($CDCl_3$, 300 MHz) 8.53 (1H, d, *J* = 5.1 Hz, H-1), 7.98 (1H, d, *J* = 1.9 Hz, H-8), 7.68 (1H, d, *J* = 8.3 Hz, H-5), 7.41 - 7.22 (9H, m, ArH), 7.28 (1H, dd, *J* = 1.9, 8.3 Hz, H-6), 6.46 (1H, d, *J* = 5.1 Hz, H-2), 5.29 (1H, br s, NH), 4.49 (2H, d, *J* = 5.1 Hz, H-10), 3.53 (2H, s, H-17), 3.51 (2H, s, H-19), 2.19 (3H, s, H-18); δ_C ($CDCl_3$, 75.5 MHz) 152.1 (C-1), 149.5 (Ar_{qu}), 149.2 (Ar_{qu}), 139.5 (Ar_{qu}), 139.3 (Ar_{qu}), 135.8 (Ar_{qu}), 134.9 (Ar_{qu}), 129.5 (C-21 / 25), 129.0 (C-23), 128.8 (C-22 / 24), 128.2 (C-13 / 15), 127.6 (C-12 / 16), 127.0 (C-8), 125.5 (C-6), 120.9 (C-5), 117.1 (Ar_{qu}), 99.6 (C-2), 61.9 (C-17), 61.4 (C-19), 47.4 (C-10), 42.3 (C-18). (Found: C, 74.35; H, 5.88; N, 10.00%. $C_{25}H_{24}N_3Cl$ requires C, 74.71; H, 6.02; N, 10.45%).

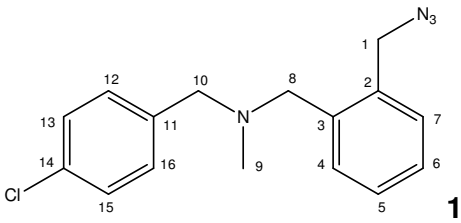
(4-Chlorobenzyl)methylamine



7

To a stirred solution of 4-chlorobenzyl chloride (3.00 g, 18.6 mmol) in THF (300 ml) under N_2 at 0 °C, was added methylamine solution in water (40% v/v) (9.0 ml, 93.4 mmol) and the reaction allowed to progress at room temperature overnight. The solvent was removed on the rotorevaporator with the water removed by azeotroping with toluene. The crude product was purified directly by column chromatography using EtOAc (100%) followed by EtOAc: MeOH: NEt_3 (92: 5: 3) as eluent to give **7** (2.35 g, 81%) as a yellow oil; δ_H ($CDCl_3$, 300 MHz) 7.25 - 7.18 (4H, m, ArH), 3.66 (2H, s, H-7), 2.38 (3H, s, H-8), 1.65 (1H, s, NH); δ_C ($CDCl_3$, 100.6 MHz) 138.6 (Ar_{qu}), 132.6 (Ar_{qu}), 129.5 (C-2 / 6), 128.4 (C-3 / 5), 55.2 (C-7), 35.8 (C-8); HRMS (ESI): Found 156.0580 (M^+). $C_8H_{10}NCl$ (M^+) requires 156.0556.

***N*-[{2-(Azidomethyl)phenyl}methyl]-*N*-(4-chlorobenzyl)methylamine**

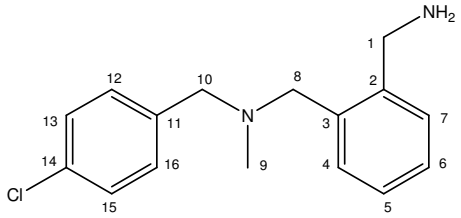


10

To a stirred solution of **7** (0.60 g, 3.85 mmol) and K_2CO_3 (0.71 g, 5.14 mmol.) in anhydrous acetonitrile (150 ml) under N_2 at 0 °C, was added crude *ortho- α, α'* - azidobromoxylene (0.58 g, 2.57 mmol), synthesized as outlined in GP1 and the reaction heated and allowed to progress under reflux for 6 hours. Acetonitrile was then removed under reduced pressure, saturated aqueous Na_2CO_3 added and the organic product extracted into EtOAc (3 × 100 ml). Drying ($MgSO_4$) of the organic extracts and concentration *in vacuo* furnished a residue, which was purified by column chromatography using mixtures of ethyl acetate: hexane (5:95) to (20:80) as eluent to

give **10** (0.45 g, 58% over 2 steps.) as a yellow oil; δ_H ($CDCl_3$, 400 MHz) 7.39 - 7.26 (9H, m, ArH), 4.55 (2H, s, H-1), 3.58 (2H, s, H-8), 3.50 (2H, s, H-10), 2.13 (3H, s, H-9); δ_C ($CDCl_3$, 75.5 MHz) 137.4 (Ar_{qu}), 137.1 (Ar_{qu}), 134.7 (Ar_{qu}), 132.7 (Ar_{qu}), 130.7 (Ar_{C-H}), 130.2 (C-12 / 16), 129.6 (Ar_{C-H}), 128.3 (C-13 / 15), 128.1 (Ar_{C-H}), 127.7 (Ar_{C-H}), 61.5 (C-10), 59.9 (C-8), 52.0 (C-1), 41.9 (C-9); HRMS (ESI): Found 301.1220 (M^+). $C_{16}H_{18}N_4Cl$ (M^+) requires 301.1211.

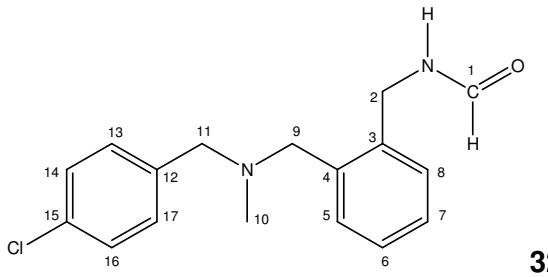
***o*-[(*N*-4-Chlorobenzyl-*N*-methyl)aminomethyl]-benzylamine**



21

To a stirred solution of **10** (0.40 g, 1.33 mmol) in THF (3.60 ml) under N_2, was added PPh_3 (0.42 g, 1.60 mmol) and the reaction mixture allowed to stir for 30 minutes at room temperature. Water was added (1.20 ml, 66.7 mmol) and the reaction heated at reflux for 6 hours. The reaction was then cooled to room temperature and solvent removed on the rotorevaporator with the water removed by azeotroping with toluene. The crude product was purified directly by column chromatography using EtOAc (100%) followed by EtOAc: MeOH: NEt_3 (90: 5: 5) as eluent to give **21** (0.34 g, 93%) as a yellow oil; δ_H ($CDCl_3$, 300 MHz) 7.35 - 7.16 (9H, m, ArH), 3.87 (2H, s, H-1), 3.55 (2H, s, H-8), 3.52 (2H, s, H-10), 2.07 (3H, s, H-9); δ_C ($CDCl_3$, 75.5 MHz) 141.3 (Ar_{qu}), 136.7 (Ar_{qu}), 136.4 (Ar_{qu}), 133.0 (Ar_{qu}), 131.1 (Ar_{C-H}), 130.6 (C-12 / 16), 129.4 (Ar_{C-H}), 128.5 (C-13 / 15), 128.2 (Ar_{C-H}), 127.1 (Ar_{C-H}), 61.7 (C-10), 60.7 (C-8), 44.1 (C-1), 41.4 (C-9); HRMS (ESI): Found 275.1315 (M^+). $C_{16}H_{20}N_2Cl$ (M^+) requires 275.1324.

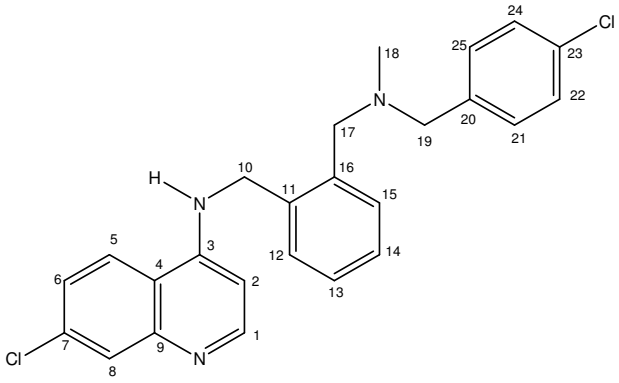
s-*trans*-*N*-[{2-(*N*-*p*-Chlorobenzyl-*N*-methylaminomethyl)phenyl}methyl] formamide



32

A stirred solution of **21** (100 mg, 0.36 mmol) in excess ethyl formate (1.44 ml, 18.3 mmol) under N_2 was heated to reflux overnight. The excess ethyl formate was removed under reduced pressure and the crude product purified by column chromatography using mixtures of ethyl acetate: hexane (50:50) to (100%) as eluent to give **32** (98 mg, 89%) as a thick-cream paste and predominantly as a s-*trans* rotamer; ν_{max}/cm^{-1} (DMSO) 3627-3228, 2597, 2146, 2060, 1976, 1903, 1661; δ_H ($CDCl_3$, 300 MHz) 8.09 (1H, s, H-1), 8.05 (1H, br s, NH), 7.39 - 7.14 (9H, m, ArH), 4.65 (2H, d, *J* = 5.4 Hz, H-2), 3.57 (2H, s, H-9), 3.51 (2H, s, H-11), 2.10 (3H, s, H-10); δ_C ($CDCl_3$, 75.5 MHz) 160.3 (C-1), 137.7 (Ar_{qu}), 136.5 (Ar_{qu}), 136.4 (Ar_{qu}), 133.3 (Ar_{qu}), 131.6 (Ar_{C-H}), 130.6 (C-13 / 17), 130.4 (Ar_{C-H}), 128.6 (C-14 / 16), 128.6 (Ar_{C-H}), 128.5 (Ar_{C-H}), 127.7 (Ar_{C-H}), 61.4 (C-11), 61.1 (C-9), 41.2 (C-10), 40.5 (C-2); HRMS (ESI): Found 303.1264 (M^+). $C_{17}H_{20}N_2OCl$ (M^+) requires 303.1247.

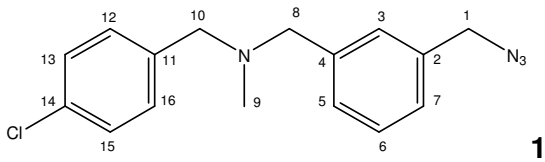
***N*-[{2-(*N*-*p*-Chlorobenzyl-*N*-methylaminomethyl)phenyl}methyl]-7-chloro-4-quinolinamine**



43

To a stirred solution of **21** (0.20 g, 0.73 mmol) in anhydrous *N*-methyl-2-pyrrolidone (2 ml) under N_2, were added triethylamine (0.58 ml, 4.17 mmol), K_2CO_3 (0.23 g, 1.46 mmol) and 4,7-dichloroquinoline (0.82 g, 4.14 mmol). The mixture was heated under pressure in a cyclo-addition tube at 120 °C overnight. After the mixture was allowed to cool to room temperature, it was poured into saturated brine (20 ml) and extracted with ethyl acetate (3 × 50 ml). The organic layer was further washed with saturated brine (5 × 50 ml) to ensure removal of any traces of the pyrrolidone. The organic layer was dried (Na_2SO_4) and concentrated *in vacuo* to afford a crude product, which was purified by column chromatography using mixtures of ethyl acetate: hexane (50:50) to (80:10) as eluent to give **43** (98 mg, 31%) as a yellow oil; ν_{max}/cm^{-1} (DMSO) 3595, 3378-3231, 2142, 2067, 1975, 1657; δ_H ($CDCl_3$, 300 MHz) 8.53 (1H, d, *J* = 5.4 Hz, H-1), 7.91 (1H, d, *J* = 2.4 Hz, H-8), 7.41 - 7.12 (8H, m, ArH), 7.26 (1H, d, *J* = 9.0 Hz, H-5), 6.98 (1H, dd, *J* = 2.4, 9.0 Hz, H-6), 6.52 (1H, d, *J* = 5.4 Hz, H-2), 5.29 (1H, s, NH), 4.44 (2H, d, *J* = 3.0 Hz, H-10), 3.60 (2H, s, H-17), 3.54 (2H, s, H-19), 2.16 (3H, s, H-18); δ_C ($CDCl_3$, 75.5 MHz) 151.9 (C-1), 150.1 (Ar_{qu}), 149.1 (Ar_{qu}), 137.1 (Ar_{qu}), 136.9 (Ar_{qu}), 135.9 (Ar_{qu}), 134.8 (Ar_{qu}), 133.5 (Ar_{qu}), 132.0 ((Ar_{C-H}), 131.1 (C-21 / 25), 130.5 (Ar_{C-H}), 128.6 (C-22 / 24), 128.5 (Ar_{C-H}), 128.4 (Ar_{C-H}), 128.0 (C-8), 124.9 (C-6), 121.8 (C-5), 118.0 (Ar_{qu}), 99.1 (C-2), 61.4 (C-19), 60.8 (C-17), 46.5 (C-10), 42.0 (C-18); HRMS (ESI): Found 436.1347 (M^+). $C_{16}H_{20}N_2Cl$ (M^+) requires 436.1344.

***N*-[{3-(Azidomethyl)phenyl}methyl]-*N*-(4-chlorobenzyl)methylamine**

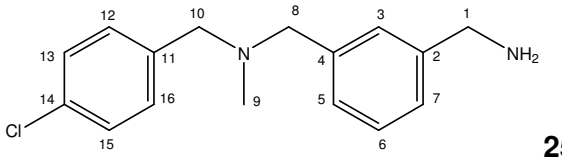


14

To a stirred solution of **7** (0.80 g, 5.13 mmol) and K_2CO_3 (0.95 g, 6.85 mmol) in anhydrous acetonitrile (200 ml) at 0 °C, was added crude *meta-α, α'* -azidobromoxylene (0.77 g, 3.43 mmol), synthesized as outlined in GP1, and the reaction heated and allowed to progress under reflux for 6 hours. Acetonitrile was then removed under reduced pressure, saturated aqueous Na_2CO_3 added and the organic product extracted into EtOAc (3 × 100 ml). Drying ($MgSO_4$) of the organic extracts and concentration *in vacuo* furnished a residue, which was purified by column chromatography using

mixtures of ethyl acetate: hexane (5:95) to (20:80) as eluent to give **14** (0.63 g, 61% over 2 steps.) as a yellow oil; δ_H ($CDCl_3$, 300 MHz) 7.36 - 7.31 (7H, m, ArH), 7.22 (1H, m, H-3), 4.35 (1H, s, H-1), 3.54 (2H, s, H-8), 3.50 (2H, s, H-10), 2.19 (3H, s, H-9); δ_C ($CDCl_3$, 75.5 MHz) 139.4 (Ar_{qu}), 137.7 (Ar_{qu}), 135.3 (Ar_{qu}), 132.6 (Ar_{qu}), 130.1 (C-12 / 16), 128.8 (C-13 / 15), 128.7 (Ar_{C-H}), 128.6 (Ar_{C-H}), 128.3 (Ar_{C-H}), 126.9 (Ar_{C-H}), 61.5 (C-8), 61.0 (C-10), 54.7 (C-1), 42.1 (C-9); HRMS (ESI): Found 301.1220 (M^+). $C_{16}H_{18}N_4$ Cl (M^+) requires 301.1221.

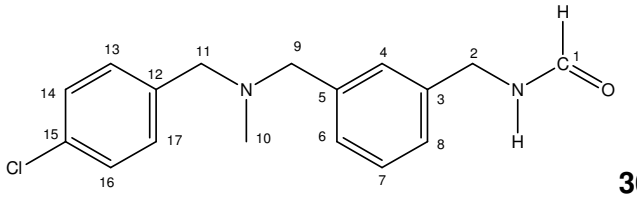
***m*-[(*N*-4-Chlorobenzyl-*N*-methyl)aminomethyl]-benzylamine**



25

To a stirred solution of **20** (0.60 g, 2.00 mmol) in THF (5.40 ml) under N_2, was added PPh_3 (0.63 g, 2.40 mmol) and the reaction mixture allowed to stir for 30 minutes at room temperature. Water was added (1.80 ml, 100 mmol) and the reaction heated at reflux for 6 hours. The reaction was then cooled to room temperature and solvent removed on the rotorevaporator with the water removed by azeotroping with toluene. The crude product was purified directly by column chromatography using EtOAc (100%) followed by EtOAc: MeOH: NEt_3 (90: 5: 5) as eluent to give **25** (0.51 g, 93%) as a light-orange oil; ν_{max}/cm^{-1} (DMSO) 3547-3399, 3271, 2835, 2783, 2144, 2048, 1707, 1663, 1605, 1491; δ_H ($CDCl_3$, 300 MHz) 7.31 - 7.18 (8H, m, ArH), 3.85 (2H, s, H-1), 3.49 (2H, s, H-8), 3.46 (2H, s, H-10), 3.02 (2H, br s, NH_2), 2.15 (3H, s, H-9); δ_C ($CDCl_3$, 75.5 MHz) 142.0 (Ar_{qu}), 139.3 (Ar_{qu}), 137.7 (Ar_{qu}), 132.4 (Ar_{qu}), 130.0 (C-12 / 16), 128.3 (Ar_{C-H}), 125.8 (Ar_{C-H}), 128.2 (C-13 / 15), 127.6 (Ar_{C-H}), 127.4 (Ar_{C-H}), 61.6 (C-8), 61.0 (C-10), 45.8 (C-1), 42.0 (C-9); HRMS (ESI): Found 275.1315 (M^+). $C_{16}H_{20}N_2Cl$ (M^+) requires 275.1316.

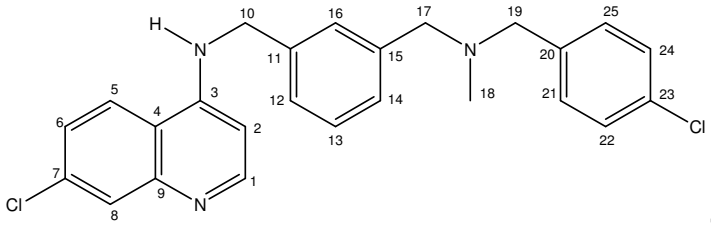
s-*trans*-*N*-[{3-(*N*-*p*-Chlorobenzyl-*N*-methylaminomethyl)phenyl}methyl] formamide



36

A stirred solution of **25** (100 g, 0.36 mmol) in excess ethyl formate (2.88 ml, 36.5 mmol) under N_2 was heated and allowed to reflux overnight. The excess ethyl formate was removed under reduced pressure and the crude product purified by column chromatography using mixtures of ethyl acetate: hexane (50:50) to (100%) as eluent to give **36** (93 mg, 84%) as a thick-yellow oil and predominantly as a s-*trans* rotamer; v_{max}/cm^{-1} (DMSO) 3628-3257, 2592, 2147, 2065, 1982, 1906, 1658; δ_H ($CDCl_3$, 400 MHz) 8.25 (1H, s, H-1), 7.26 - 7.23 (7H, m, ArH), 7.15 (1H, m, H-4), 6.05 (1H, br s, NH), 4.42 (2H, d, *J* = 6.0 Hz, H-2), 3.48 (2H, s, H-9), 3.43 (2H, s, H-11), 2.13 (3H, s, H-10); δ_C ($CDCl_3$, 75.5 MHz) 161.0 (C-1), 140.0 (Ar_{qu}), 137.7 (Ar_{qu}), 137.6 (Ar_{qu}), 132.6 (Ar_{qu}), 130.1 (C-13 / 17), 128.7 (Ar_{C-H}), 128.3 (C-14 / 16), 128.1 (Ar_{C-H}), 128.1 (Ar_{C-H}), 126.4 (Ar_{C-H}), 61.6 (C-9), 61.1 (C-11), 42.2 (C-2), 42.1 (C-10); HRMS (ESI): Found 303.1264 (M^+). $C_{17}H_{20}N_2OCl$ (M^+) requires 303.1258.

***N*-[{3-(*N*-*p*-Chlorobenzyl-*N*-methylaminomethyl)phenyl}methyl]-7-chloro-4-quinolinamine**

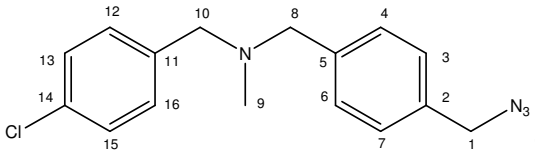


47

To a stirred solution of **25** (0.45 g, 1.64 mmol) in anhydrous *N*-methyl-2-pyrrolidone (4 ml) under N_2, were added triethylamine (1.16 ml, 8.32 mmol), K_2CO_3 (0.46 g, 3.33 mmol) and 4,7-dichloroquinoline (1.65 g, 8.32 mmol). The mixture was heated under pressure in a cyclo-addition tube at 120 °C overnight. After the mixture was allowed to cool to room temperature, it was poured into saturated brine (20 ml) and extracted with ethyl acetate (3 × 50 ml). The organic layer was further washed with saturated brine (5 × 50

ml) to ensure removal of any traces of the pyrrolidone. The organic layer was dried (Na_2SO_4) and concentrated *in vacuo* to afford a crude product, which was purified by column chromatography using mixtures of ethyl acetate: hexane (50:50) to (90:10) as eluent to give **47** (0.19 g, 27%) as a colourless solid, m.p. (DCM:Hex) 103-104 ºC; ν_{max}/cm^{-1} (DMSO) 3620, 3372-3193, 2594, 2345, 2150, 2054, 1971, 1911, 1676, 1626; δ_H ($CDCl_3$, 300 MHz) 8.48 (1H, d, *J* = 5.3 Hz, H-1), 7.97 (1H, d, *J* = 2.4 Hz, H-8), 7.16 (1H, d, *J* = 8.7 Hz, H-5), 7.37 - 7.23 (9H, m, H-6 and ArH), 6.42 (1H, d, *J* = 5.3 Hz, H-2), 5.62 (1H, br s, NH), 4.51 (2H, d, *J* = 4.8 Hz, H-10), 3.50 (2H, s, H-17), 3.45 (2H, s, H-19), 2.15 (3H, s, H-18); δ_C ($CDCl_3$, 75.5 MHz) 151.5 (C-1), 149.8 (Ar_{qu}), 149.8 (Ar_{qu}), 140.1 (Ar_{qu}), 137.6 (Ar_{qu}), 137.2 (Ar_{qu}), 135.1 (Ar_{qu}), 132.6 (Ar_{qu}), 130.0 (C-21 / 25), 128.9 (C-8), 128.4 (Ar_{C-H}), 128.4 (Ar_{C-H}), 128.3 (C-22 / 24), 127.8 (Ar_{C-H}), 126.2 (Ar_{C-H}), 125.5 (C-6), 121.2 (C-5), 117.1 (Ar_{qu}), 99.6 (C-2), 61.6 (C-17), 61.0 (C-19), 47.5 (C-10), 42.2 (C-18); HRMS (ESI): Found 436.1335 (M^+). $C_{16}H_{20}N_2Cl$ (M^+) requires 436.1347.

***N*-[{4-(Azidomethyl)phenyl}methyl]-*N*-(4-chlorobenzyl)methylamine**

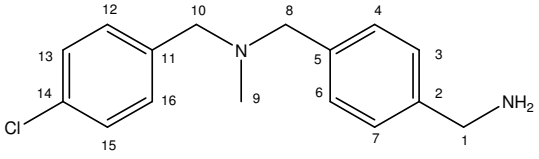


18

To a stirred solution of **7** (1.00 g, 6.41 mmol) and K_2CO_3 (1.19 g, 8.60 mmol.) in anhydrous acetonitrile (300 ml) at 0 °C, was added crude *para- α, α'* -azidobromoxylene (0.96 g, 4.25 mmol) synthesized as outlined in GP1, and the reaction heated and allowed to progress under reflux for 6 hours. Acetonitrile was then removed under reduced pressure, saturated aqueous Na_2CO_3 added and the organic product extracted into EtOAc (3 × 100 ml). Drying ($MgSO_4$) of the organic extracts and concentration *in vacuo* furnished a residue, which was purified by column chromatography using mixtures of ethyl acetate: hexane (5:95) to (20:80) as eluent to afford **18** (0.83 g, 63% over 2 steps.) as a light-yellow oil; δ_H ($CDCl_3$, 400 MHz) 7.44 - 7.31 (8H, m, ArH), 4.35 (2H, s, H-1), 3.56 (2H, s, H-8), 3.52 (2H, s, H-10), 2.22 (3H, s, H-9); δ_C ($CDCl_3$, 75.5 MHz) 139.3 (Ar_{qu}), 137.7 (Ar_{qu}), 134.0 (Ar_{qu}), 132.4 (Ar_{qu}), 130.0 (C-13 / 15); 129.1 (C-12

/ 16), 128.2 (C-4 / 6), 128.0 (C-3 / 7); 61.3 (C-8), 61.0 (C-10), 54.4 (C-1), 42.0 (C-9); HRMS (ESI): Found 301.1220 (M^+). $C_{16}H_{18}N_4Cl$ (M^+) requires 301.1223.

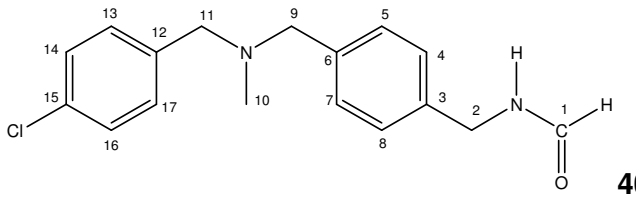
***p*-[(*N*-4-Chlorobenzyl-*N*-methyl)aminomethyl]-benzylamine**



29

To a stirred solution of **18** (0.80g, 2.67 mmol) in THF (7.20 ml) under N_2, was added PPh_3 (0.92 g, 3.20 mmol) and the reaction mixture allowed to stir for 30 minutes at room temperature. Water (2.40 ml, 133 mmol) was added and the reaction heated at reflux for 6 hours. The reaction was then cooled to room temperature and solvent removed on the rotorevaporator with the water removed by azeotroping with toluene. The crude product was purified directly by column chromatography using EtOAc (100%) followed by EtOAc: MeOH: NEt_3 (90: 5: 5) as eluent to give **29** (0.68 g, 93%) as a dark-orange oil; ν_{max}/cm^{-1} (DMSO) 3551-3388, 3287, 2790, 2138, 2063, 1957, 1708, 1662, 1513, 1490; δ_H ($CDCl_3$, 300 MHz) 7.32 - 7.24 (8H, m, ArH), 3.83 (2H, s, H-1), 3.48 (2H, s, H-8), 3.44 (2H, s, H-10), 3.05 (2H, br s, NH_2), 2.14 (2H, s, H-9); δ_C ($CDCl_3$, 75.5 MHz) 137.7 (Ar_{qu}), 132.3 (Ar_{qu}), 131.7 (Ar_{qu}), 129.9 (C-13 / 15), 128.9 (C-12 / 16), 128.1 (C-4 / 6); 127.7 (Ar_{qu}), 127.0 (C-3 / 7), 61.3 (C-8), 60.7 (C-10), 45.5 (C-1), 41.9 (C-9); HRMS (ESI): Found 275.1315 (M^+). $C_{16}H_{20}N_2Cl$ (M^+) requires 275.1326.

s-*trans*-*N*-[{4-(*N*-*p*-Chlorobenzyl-*N*-methylaminomethyl)phenyl}methyl] formamide

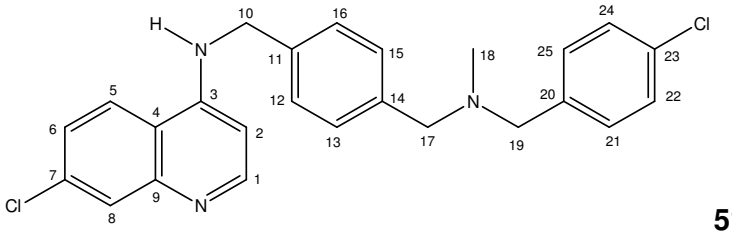


40

A stirred solution of **29** (100 mg, 0.36 mmol) in excess ethyl formate (2.88 ml, 36.5 mmol) under N_2 was refluxed overnight. The excess ethyl formate was removed under reduced pressure and the crude product purified by column chromatography using

mixtures of ethyl acetate: hexane (50:50) to (100%) as eluent to give **40** (94 mg, 86%) as a thick-yellow oil and predominantly as a s-*trans* rotamer; ν_{max}/cm^{-1} (DMSO) 3630 - 3180, 2592, 2143, 2053, 1972, 1909, 1659; δ_H ($CDCl_3$, 400 MHz) 8.21 (1H, s, H-1), 7.31 - 7.14 (8H, m, ArH), 6.15 (1H, br s, NH), 4.40 (2H, d, *J* = 4.5 Hz, H-2), 3.45 (2H, s, H-9), 3.42 (2H, s, H-11), 2.11 (3H, s, H-10); δ_C ($CDCl_3$, 75.5 MHz) 161.0 (C-1), 138.6 (Ar_{qu}), 137.7 (Ar_{qu}), 136.3 (Ar_{qu}), 132.5 (Ar_{qu}), 130.0 (C-14 / 16), 129.3 (C-13 / 17), 128.3 (C-5 / 7), 127.6 (C-4 / 8), 61.3 (C-9), 61.0 (C-11), 42.1 (C-10), 41.8 (C-2); HRMS (ESI): Found 303.1264 (M^+). $C_{17}H_{20}N_2OCl$ (M^+) requires 303.1257.

N-[{4-(*N*-*p*-Chlorobenzyl-*N*-methylaminomethyl)phenyl}methyl]-7-chloro-4-quinolinamine

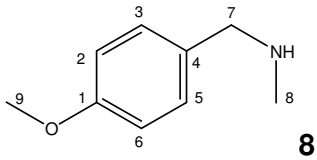


51

To a stirred solution of **29** (0.57 g, 2.08 mmol) in anhydrous *N*-methyl-2-pyrrolidone (6 ml)) under N_2, were added triethylamine (1.45 ml, 10.4 mmol), K_2CO_3 (0.57 g, 4.16 mmol) and 4,7-dichloroquinoline (2.06 g, 10.40 mmol). The mixture was heated under pressure in a cyclo-addition tube at 120 °C overnight. After the mixture was allowed to cool to room temperature it was poured into saturated brine (20 ml) and extracted with ethyl acetate (3 × 50 ml). The organic layer was further washed with saturated brine (5 × 50 ml) to ensure removal of any traces of the pyrrolidone. The organic layer was dried (Na_2SO_4), concentrated *in vacuo* and the resulting crude product was purified by column chromatography using mixtures of ethyl acetate: hexane (50:50) to (90:10) as eluent to give **51** (0.19 g, 21%) as a colourless crystalline solid, m.p. (DCM:Hex) 101-104 ºC; ν_{max}/cm^{-1} (DMSO) 3607, 3362-3195, 2344, 2146, 2059, 1947, 1905, 1677, 1627; δ_H ($CDCl_3$, 300 MHz) 8.47 (1H, d, *J* = 5.4 Hz, H-1), 7.95 (1H, d, *J* = 1.8 Hz, H-8), 7.37 - 7.25 (1H, d, *J* = 9.0 Hz, H-5), 7.32 (9H, m, H-6 and ArH), 6.40 (1H, d, *J* = 5.4 Hz, H-2), 5.73 (1H, br s, NH), 4.48 (2H, d, *J* = 3.9 Hz, H-10), 3.50 (2H, s, H-17), 3.47 (2H, s, H-19), 2.17 (3H, s, H-18); δ_C ($CDCl_3$, 75.5 MHz) 151.6 (C-1), 149.8 (Ar_{qu}), 149.8 (Ar_{qu}), 139.0 (Ar_{qu}), 137.8 (Ar_{qu}), 135.8 (Ar_{qu}), 135.0 (Ar_{qu}), 130.1 (C-21 / 25), 129.4 (C-22 / 24), 128.4

(Ar_{qu}), 128.3 (C-13 / 15), 127.5 (C-8), 127.5 (C-12 / 16), 125.4 (C-6), 121.2 (C-5), 117.1 (Ar_{qu}), 99.5 (C-2), 61.4 (C-17), 61.0 (C-19), 47.3 (C-10), 42.2 (C-18); HRMS (ESI): Found 436.1335 (M^+). $C_{16}H_{20}N_2Cl$ (M^+) requires 436.1347.

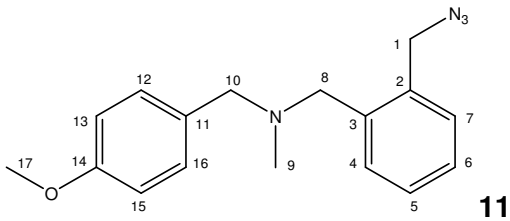
(4-Methoxybenzyl)methylamine



8

To a stirred solution of 4-methoxybenzyl chloride (3.00 g, 19.7 mmol) in THF (350 ml) under N_2 at 0 °C, was added methylamine solution in water (40% v/v) (9.30 ml, 95.9 mmol) and the reaction allowed to progress at room temperature overnight. The solvent was removed on the rotorevaporator with the water removed by azeotroping with toluene. The crude product was purified directly by column chromatography using EtOAc (100%) followed by EtOAc: MeOH: NEt_3 (92: 5: 3) as eluent to give **8** (2.61 g, 87%) as a yellow oil; δ_H ($CDCl_3$, 300 MHz) 7.27 (2H, d, *J* = 6.6 Hz, H-3 / 5), 6.87 (2H, d, *J* = 6.6 Hz, H-2 / 6), 3.75 (3H, s, H-9), 3.64 (2H, s, H-7), 2.33 (3H, s, H-8); δ_C ($CDCl_3$, 75.5 MHz) 159.0 (Ar_{qu}), 132.5 (Ar_{qu}), 129.6 (C-3 / 5), 113.8 (C-2 / 6), 54.9 (C-9 / C-7), 35.1 (C-8); HRMS (ESI): Found 152.1070 (M^+). $C_9H_{14}NO$ (M^+) requires 152.1070.

***N*-[{2-(Azidomethyl)phenyl}methyl]-*N*-(4-Methoxybenzyl)methylamine**

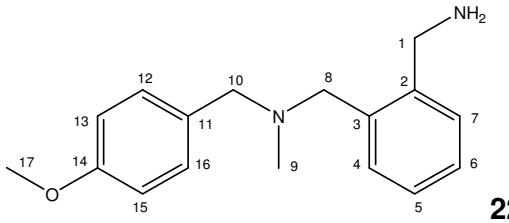


11

To a stirred solution of **8** (1.00 g, 6.61 mmol) and K_2CO_3 (1.22 g, 8.87 mmol.) in anhydrous acetonitrile (300 ml) at 0 °C, was added crude *ortho- α, α'* -azidobromoxylene (0.99 g, 4.38 mmol) synthesized as outlined in GP1, and the reaction heated and allowed to progress under reflux for 6 hours. Acetonitrile was then removed under reduced pressure, saturated aqueous Na_2CO_3 added and the organic product extracted

into EtOAc (3 × 100 ml). Drying ($MgSO_4$) of the organic extracts and concentration *in vacuo* furnished a residue, which was purified by column chromatography using mixtures of ethyl acetate: hexane (10:90) to (25:75) as eluent to give **11** (0.83 g, 64% over 2 steps.) as a yellow oil; δ_H ($CDCl_3$, 300 MHz) 7.42 - 7.31 (4H, m, ArH), 7.29 (2H, d, *J* = 8.9 Hz, H-12 / 16), 6.91 (2H, d, *J* = 8.9 Hz, H-13 / 15), 4.56 (2H, s, H-1), 3.83 (3H, s, H-17), 3.57 (2H, s, H-8), 3.51 (2H, s, H-10), 2.15 (3H, s, H-9); δ_C ($CDCl_3$, 75.5 MHz) 159.1 (Ar_{qu}), 137.8 (Ar_{qu}), 135.1 (Ar_{qu}), 131.3 (Ar_{qu}), 131.1 (Ar_{C-H}), 130.5 (C-12 / 16), 129.7 (Ar_{C-H}), 128.3 (Ar_{C-H}), 128.3 (Ar_{C-H}), 114.0 (C-13 / 15), 62.1 (C-8), 60.0 (C-10), 55.5 (C-17), 52.2 (C-1), 42.3 (C-9); HRMS (ESI): Found 297.1715 (M^+). $C_{17}H_{21}N_4O$ (M^+) requires 297.1727.

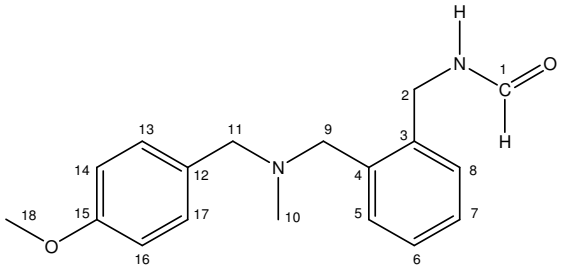
***o*-[(*N*-4-Methoxybenzyl-*N*-methyl)aminomethyl]-benzylamine**



22

To a stirred solution of **11** (0.80 g, 2.70 mmol) in THF (7.29 ml) under N_2, was added PPh_3 (0.85 g, 3.24 mmol) and the reaction mixture allowed to stir for 30 minutes at room temperature. Water (2.45 ml, 135 mmol) was added and the reaction heated at reflux for 6 hours. The reaction was then cooled to room temperature and the solvent removed on the rotorevaporator with the water removed by azeotroping with toluene. The crude product was purified directly by column chromatography using EtOAc (100%) followed by EtOAc: MeOH: NEt_3 (93: 5: 2) as eluent to give **22** (0.61 g, 83%) as a light-brown oil; v_{max}/cm^{-1} (DMSO) 3592-3324, 2593, 2142, 2061, 1981, 1664, 1510; δ_H ($CDCl_3$, 300 MHz) 7.32 - 7.15 (6H, m, ArH), 6.83 (2H, d, *J* = 8.4 Hz, H-13 /15), 3.85 (2H, br s, H-1), 3.77 (3H, s, H-17), 3.53 (2H, s, H-8), 3.49 (2H, s, H-10), 3.40 (2H, br s, NH_2), 2.09 (3H, s, H-9); δ_C ($CDCl_3$, 75.5 MHz) 158.7 (Ar_{qu}), 142.1 (Ar_{qu}), 136.7 (Ar_{qu}), 131.0 (Ar_{C-H}), 130.5 (Ar_{qu}), 130.4 (C-12 / 16), 129.1 (Ar_{C-H}), 128.0 (Ar_{C-H}), 127.0 (Ar_{C-H}), 113.6 (C-13 / 15), 62.0 (C-8), 60.5 (C-10), 55.1 (C-17), 44.3 (C-1), 41.4 (C-9).

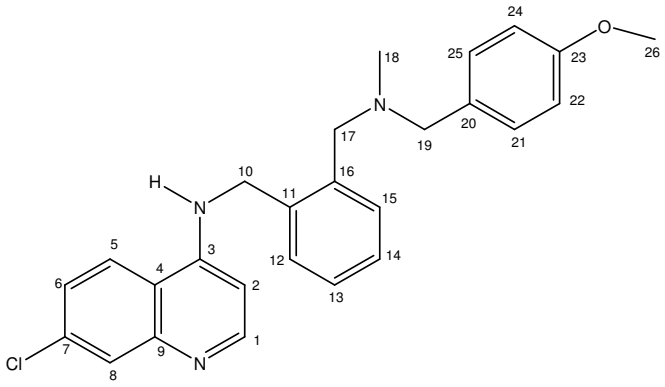
s-*trans*-*N*-[{2-(*N*-4-Methoxybenzyl-*N*-methylaminomethyl)phenyl}methyl] formamide



33

A stirred solution of **22** (100 mg, 0.37 mmol) in excess ethyl formate (2.92 ml, 37.0 mmol) under N_2 was heated to reflux overnight. The excess ethyl formate was removed under reduced pressure and the crude product purified by column chromatography using mixtures of hexane: ethyl acetate: methanol (50:50:0) to (0: 97:3) as eluent to give **33** (87 mg, 79%) as a thick-yellow oil and predominantly as a s-*trans* rotamer; ν_{max}/cm^{-1} (DMSO) 3593-3257, 2593, 2146, 2063, 1978, 1905, 1658; δ_H ($CDCl_3$, 400 MHz) 8.50 (1H, br s, NH), 8.15 (1H, s, H-1), 7.39 - 7.12 (6H, m, ArH), 7.14 (2H, d, *J* = 8.8 Hz, H-13 / 17), 6.85 (2H, d, *J* = 8.8 Hz, H-14 / 16), 4.43 (2H, d, *J* = 6.0 Hz, H-2), 3.80 (3H, s, H-18), 3.55 (2H, s, H-9), 3.49 (2H, s, H-11), 2.09 (3H, s, H-10); δ_C ($CDCl_3$, 100.6 MHz) 160.6 (C-1), 159.3 (Ar_{qu}), 138.1 (Ar_{qu}), 137.1 (Ar_{qu}), 131.9 (Ar_{C-H}), 131.0 (Ar_{qu}), 130.8 (C-13 / 17), 130.0 (Ar_{C-H}), 128.8 (Ar_{C-H}), 127.9 (Ar_{C-H}), 114.1 (C-14 / 16), 61.4 (C-9), 61.8 (C-11), 55.5 (C-18), 41.3 (C-2), 41.0 (C-10); HRMS (ESI): Found 299.1760 (M^+). $C_{18}H_{23}N_2O_2$ (M^+) requires 299.1756.

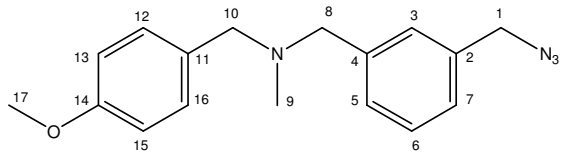
7-Chloro-*N*-[{2-(*N*-*p*-methoxybenzyl-*N*-methylaminomethyl)phenyl}methyl]-4-quinolinamine



44

To a stirred solution of **22** (0.35 g, 1.30 mmol) in anhydrous *N*-methyl-2-pyrrolidone (3.50 ml) under N_2, were added triethylamine (0.91 ml, 6.50 mmol), K_2CO_3 (0.54 g, 3.90 mmol) and 4, 7-dichloroquinoline (2.06 g, 10.4 mmol). The mixture was heated under pressure in a cyclo-addition tube at 120 °C overnight. After the mixture was allowed to cool to room temperature, it was poured into saturated brine (20 ml) and extracted with ethyl acetate (3 × 50 ml). The organic layer was further washed with saturated brine (5 × 50 ml) to ensure removal of any traces of the pyrrolidone. The organic layer was dried (Na_2SO_4), concentrated *in vacuo* and the resulting crude product was purified by column chromatography using mixtures of ethyl acetate: hexane (50:50) to (100:0) as eluent to give **44** (0.12 g, 21%) as a dark-yellow oil; ν_{max}/cm^{-1} (DMSO) 3618, 3376-3177, 2633, 2595, 2548, 2149, 2059, 1972, 1903, 1681; δ_H ($CDCl_3$, 400 MHz) 8.53 (1H, d, *J* = 5.4 Hz, H-1), 7.91 (1H, d, *J* = 2.3 Hz, H-8), 7.62 (1H, s, NH), 7.34 – 7.31 (4H, m, ArH), 7.27 (1H, d, *J* = 9.0 Hz, H-5), 7.09 (2H, d, *J* = 8.9 Hz, H-21 / 25), 6.92 (1H, dd, *J* = 2.3, 9.0 Hz, H-6), 6.75 (2H, d, *J* = 8.9 Hz, H-22 / 24), 6.53 (1H, d, *J* = 5.4 Hz, H-2), 4.42 (2H, s, H-10), 3.77 (3H, s, H-26), 3.61 (2H, s, H-17), 3.53 (2H, s, H-19), 2.16 (3H, s, H-18); δ_C ($CDCl_3$, 75.5 MHz) 159.1 (Ar_{qu}), 151.7 (C-1), 150.3 (Ar_{qu}), 149.0 (Ar_{qu}), 137.2 (Ar_{qu}), 137.1 (Ar_{C-H}), 134.6 (Ar_{qu}), 132.0 (Ar_{C-H}), 131.0 (C-21 / 25), 130.5 (Ar_{qu}), 129.3 (Ar_{qu}), 128.3 (Ar_{C-H}), 128.1 (Ar_{C-H}), 127.9 (C-8), 124.7 (C-6), 122.3 (C-5), 117.8 (Ar_{qu}), 113.8 (C-22 / 24), 99.0 (C-2), 61.5 (C-17), 60.9 (C-19), 55.2 (C-26), 46.5 (C-10), 41.7 (C-18); HRMS (ESI): Found 432.1860 (M^+). $C_{26}H_{27}N_3OCl$ (M^+) requires 432.1860.

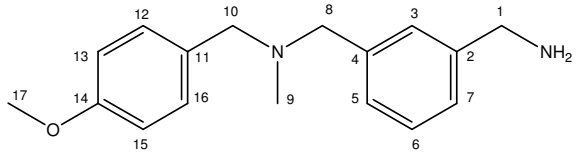
***N*-[{3-(Azidomethyl)phenyl}methyl]-*N*-(4-methoxybenzyl)methylamine**



15

To a stirred solution of **8** (1.00 g, 6.61 mmol) and K_2CO_3 (1.22 g, 8.87 mmol.) in anhydrous acetonitrile (300 ml) at 0 °C, was added crude *meta- α, α'* -azidobromoxylene (0.99 g, 4.38 mmol) synthesized as outlined in GP1, and the reaction heated and allowed to progress under reflux for 5 hours. Acetonitrile was then removed under reduced pressure, saturated aqueous Na_2CO_3 added and the organic product extracted into EtOAc (3 × 100 ml). Drying ($MgSO_4$) of the organic extracts and concentration *in vacuo* furnished a residue, which was purified by column chromatography using mixtures of ethyl acetate: hexane (10:90) to (25:75) as eluent to give **15** (0.79 g, 61% over 2 steps.) as a yellow oil; δ_H ($CDCl_3$, 300 MHz) 7.35 - 7.18 (4H, m, ArH), 7.28 (2H, d, *J* = 8.4 Hz, H-12 / 16), 6.87 (2H, d, *J* = 8.4 Hz, H-13 / 15), 4.34 (2H, s, H-1), 3.81 (3H, s, H-17), 3.53 (2H, s, H-8), 3.49 (2H, s, H-10), 2.18 (3H, s, H-9); δ_C ($CDCl_3$, 75.5 MHz) 158.7 (Ar_{qu}), 140.1 (Ar_{qu}), 135.3 (Ar_{qu}), 131.0 (Ar_{qu}), 130.1 (C-12 / 16), 128.9 (Ar_{C-H}), 128.7 (Ar_{C-H}), 128.7 (Ar_{C-H}), 126.8 (Ar_{C-H}), 113.6 (C-13 / 15), 61.3 (C-8), 61.2 (C-10), 55.2 (C-17), 54.8 (C-1), 42.0 (C-9); HRMS (ESI): Found 297.1715 (M^+). $C_{17}H_{21}N_4O$ (M^+) requires 297.1716.

***m*-[(*N*-4-Methoxybenzyl-*N*-methyl)aminomethyl]-benzylamine**

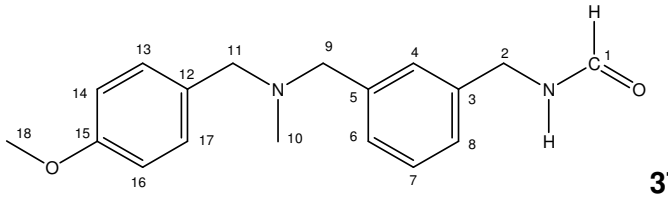


26

To a stirred solution of **15** (0.75 g, 2.53 mmol) in THF (7.0 ml) under N_2, was added PPh_3 (0.80 g, 3.04 mmol) and the reaction mixture allowed to stir for 30 minutes at room temperature. Water was added (2.30 ml, 127.0 mmol) and the reaction heated at reflux for 6 hours. The reaction was then cooled to room temperature and solvent removed on the rotorevaporator with the water removed by azeotroping with toluene. The crude

product was purified directly by column chromatography using EtOAc (100%) followed by EtOAc: MeOH: NEt_3 (93: 5: 2) as eluent to give **26** (0.60 g, 88%) as a dark-yellow oil; v_{max}/cm^{-1} (DMSO) 3531- 3410, 3286, 2137, 2060, 1986, 1661, 1611, 1511; δ_H ($CDCl_3$, 400 MHz) 7.29 - 6.80 (8H, m, ArH), 4.53 (2H, br s, NH_2), 3.73 (2H, s, H-1), 3.77 (3H, s, H-18), 3.46 (2H, s, H-8), 3.43 (2H, s, H-10), 2.13 (3H, s, H-9); HRMS (ESI): Found 271.1810 (M^+). $C_{17}H_{23}N_2O$ (M^+) requires 271.1813.

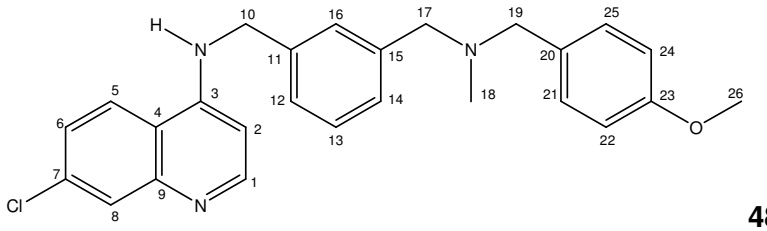
s-*trans*-*N*-[{3-(*N*-4-Methoxybenzyl-*N*-methylaminomethyl)phenyl}methyl] formamide



37

A stirred solution of **26** (100 mg, 0.37 mmol) in excess ethyl formate (2.92 ml, 37.03 mmol) under N_2 was heated and allowed to reflux overnight. The excess ethyl formate was removed under reduced pressure and the crude product purified by column chromatography using mixtures of hexane: ethyl acetate: methanol (50:50:0) to (0:97:3) as eluent to give **37** (84 mg, 76%) as a thick-yellow oil and predominantly as a s-*trans* rotamer; v_{max}/cm^{-1} (DMSO) 3599-3278, 2592, 2140, 2066, 1982, 1904, 1660; δ_H ($CDCl_3$, 400 MHz) 8.28 (1H, s, H-1), 7.33 - 7.17 (6H, m, ArH), 6.87 (2H, d, *J* = 8.8 Hz, H-14 / 16), 5.85 (1H, br s, NH), 4.50 (2H, d, *J* = 6.4 Hz, H-2), 3.81 (3H, s, H-18), 2.19 (2H, s, H-9), 2.18 (2H, s, H-11), 2.18 (3H, s, H-10); δ_C ($CDCl_3$, 75.5 MHz) 160.9 (C-1), 158.7 (Ar_{qu}), 140.1 (Ar_{qu}), 137.5 (Ar_{qu}), 131.1 (Ar_{qu}), 130.1 (C-13 / 17), 128.7 (Ar_{C-H}), 128.3 (Ar_{C-H}), 128.3 (Ar_{C-H}), 126.4 (Ar_{C-H}), 113.6 (C-14 / 16), 61.4 (C-11), 61.3 (C-9), 55.2 (C-18), 42.1 (C-2), 42.1 (C-10); HRMS (ESI): Found 299.1760 (M^+). $C_{18}H_{23}N_2O_2$ (M^+) requires 299.1746.

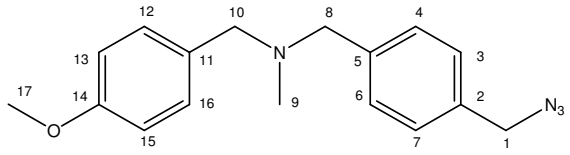
7-Chloro-*N*-[{3-(*N*-*p*-methoxybenzyl-*N*-methylaminomethyl)phenyl}methyl]-4-quinolinamine



48

To a stirred solution of **26** (0.40 g, 1.48 mmol) in anhydrous *N*-methyl-2-pyrrolidone (4.0 ml) under N_2, were added triethylamine (1.04 ml, 7.40 mmol), K_2CO_3 (0.61 g, 4.44 mmol) and 4, 7-dichloroquinoline (1.47 g, 7.40 mmol). The mixture was heated under pressure in a cyclo-addition tube at 120 °C overnight. After the mixture was allowed to cool to room temperature, it was poured into saturated brine (20 ml) and extracted with ethyl acetate (3 × 50 ml). The organic layer was further washed with saturated brine (5 × 50 ml) to ensure removal of any traces of the pyrrolidone. The organic layer was dried (Na_2SO_4), concentrated *in vacuo* and the resulting crude product was purified by column chromatography using mixtures of ethyl acetate: hexane (50:50) to (90:10) as eluent to give **48** (0.13 g, 20%) as a light-brown oil; ν_{max}/cm^{-1} (DMSO) 3600, 3363 - 3169, 2599, 2350, 2148, 2059, 1973, 1907, 1679, 1618; δ_H ($CDCl_3$, 300 MHz) 8.51 (1H, d, *J* = 5.4 Hz, H-1), 7.98 (1H, d, *J* = 2.0 Hz, H-8), 7.70 (1H, d, *J* = 9.0 Hz, H-5), 7.40 - 7.24 (4H, m, ArH), 7.36 (1H, dd, *J* = 2.0, 9.0 Hz, H-6), 7.21 (2H, d, *J* = 8.7 Hz, H-21 / 25), 6.80 (2H, d, *J* = 8.7 Hz, H-22 / 24), 6.45 (1H, d, *J* = 5.4 Hz, H-2), 5.44 (1H, br s, NH), 4.51 (2H, d, *J* = 5.4 Hz, H-10), 3.78 (3H, s, H-26), 3.50 (2H, s, H-17), 3.45 (2H, s, H-19), 2.17 (3H, s, H-18); δ_C ($CDCl_3$, 75.5 MHz) 158.6 (Ar_{qu}), 152.0 (C-1), 149.6 (Ar_{qu}), 149.1 (Ar_{qu}), 140.4 (Ar_{qu}), 137.2 (Ar_{qu}), 134.9 (Ar_{qu}), 131.0 (Ar_{qu}), 130.0 (C-21 / 25), 128.8 (C-8), 128.7 (Ar_{C-H}), 128.5 (Ar_{C-H}), 128.0 (Ar_{C-H}), 126.1 (Ar_{C-H}), 125.4 (C-6), 121.1 (C-5), 117.2 (Ar_{qu}), 113.6 (C-22 / 24), 99.6 (C-2), 61.4 (C-17), 61.2 (C-19), 55.2 (C-26), 47.5 (C-10), 42.1 (C-18); HRMS (ESI): Found 432.1843 (M^+). $C_{26}H_{27}N_3OCl$ (M^+) requires 432.1825.

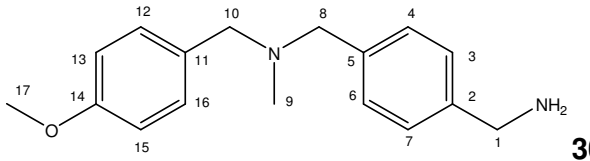
***N*-[{4-(Azidomethyl)phenyl}methyl]-*N*-(4-methoxybenzyl)methylamine**



19

To a stirred solution of **8** (1.00 g, 6.61 mmol) and K_2CO_3 (1.22 g, 8.87 mmol.) in anhydrous acetonitrile (300 ml) at 0 °C, was added crude *para- α, α'* -azidobromoxylene (0.99 g, 4.38 mmol), synthesized as outlined in GP1 and the reaction heated and allowed to progress under reflux for 6 hours. Acetonitrile was then removed under reduced pressure, saturated aqueous Na_2CO_3 added and the organic product extracted into EtOAc (3 × 100 ml). Drying ($MgSO_4$) of the organic extracts and concentration *in vacuo* furnished a residue, which was purified by column chromatography using mixtures of ethyl acetate: hexane (10:90) to (25:75) as eluent to afford **19** (0.74 g, 57% over 2 steps.) as a yellow oil; δ_H ($CDCl_3$, 400 MHz) 7.43 (2H, d, *J* = 8.4 Hz, H-12 / 16), 7.35 - 7.32 (4H, m, ArH), 6.92 (2H, d, *J* = 8.4 Hz, H-13 / 15), 4.37 (2H, s, H-1), 3.77 (3H, s, H-17), 3.52 (2H, s, H-8), 3.48 (2H, s, H-10), 2.15 (3H, s, H-9); δ_C ($CDCl_3$, 75.5 MHz) 159.2 (Ar_{qu}), 140.2 (Ar_{qu}), 134.6 (Ar_{qu}), 131.6 (Ar_{qu}), 130.2 (C-4 / 6), 128.5 (C-3 / 7), 129.4 (C-12 / 16), 113.9 (C-13 / 15), 61.4 (C-8), 61.4 (C-10), 54.9 (C-17), 54.4 (C-1), 41.8 (C-9); HRMS (ESI): Found 297.1715 (M^+). $C_{17}H_{21}N_4O$ (M^+) requires 297.1706.

***p*-[(*N*-4-Methoxybenzyl-*N*-methyl)aminomethyl]-benzylamine**

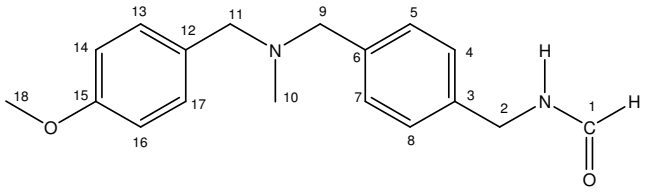


30

To a stirred solution of **19** (0.70 g, 2.36 mmol) in THF (6.40 ml) under N_2, was added PPh_3 (0.74 g, 2.84 mmol) and the reaction mixture allowed to stir for 30 minutes at room temperature. Water (2.1 ml, 118 mmol) was added and the reaction heated at reflux for 6 hours. The reaction was then cooled to room temperature and solvent removed on the rotorevaporator with the water removed by azeotroping with toluene. The crude product was purified directly by column chromatography using EtOAc (100%) followed by EtOAc:

MeOH: NEt_3 (92: 5: 3) as eluent to give **30** (0.57 g, 89%) as a light-brown oil; v_{max}/cm^{-1} (DMSO) 3374-3272, 2833, 2785, 2145, 2056, 1978, 1903, 1662, 1610, 1583; δ_H ($CDCl_3$, 300 MHz) 7.32 - 7.27 (4H, m, ArH), 7.23 (2H, d, *J* = 8.8 Hz, H-12 / 16), 6.85 (2H, d, *J* = 8.8 Hz, H-13 / 15), 4.53 (2H, br s, NH_2), 3.83 (2H, s, H-1), 3.77 (3H, s, H-17), 3.46 (2H, s, H-8), 3.43 (2H, s, H-10), 2.13 (3H, s, H-9); δ_C ($CDCl_3$, 75.5 MHz) 158.5 (Ar_{qu}), 138.6 (Ar_{qu}), 138.4 (Ar_{qu}), 131.0 (Ar_{qu}), 129.9 (C-12 / 16), 129.1 (C-4 / 6), 127.4 (C-3 / 7), 113.5 (C-13 / 17), 61.1 (C-8), 61.0 (C-10), 55.1 (C-17), 44.9 (C-1), 41.8 (C-9).

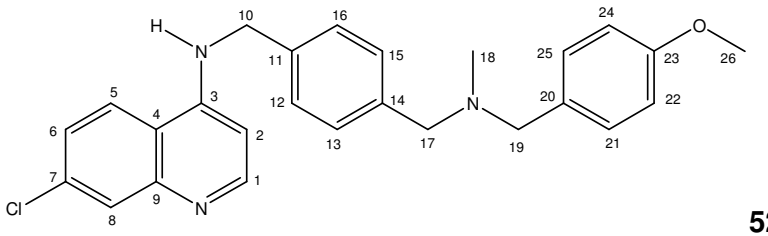
s-*trans*-*N*-[{4-(*N*-4-Methoxybenzyl-*N*-methylaminomethyl)phenyl}methyl] formamide



41

A stirred solution of **30** (100 mg, 0.37 mmol) in excess ethyl formate (2.90 ml, 37.0 mmol) under N_2 was refluxed overnight. The excess ethyl formate was removed under reduced pressure and the crude product purified by column chromatography using mixtures of hexane: ethyl acetate: methanol (50:50:0) to (0:97:3) as eluent to give **41** (87 mg, 79%) as a thick-yellow oil and predominantly as a s-*trans* rotamer; v_{max}/cm^{-1} (DMSO) 3582- 3295, 2595, 2137, 2067, 1983, 1906, 1660; δ_H ($CDCl_3$, 400 MHz) 8.21 (1H, br s, H-1), 7.35 - 7.17 (6H, m, ArH), 6.85 (2H, d, *J* = 8.7 Hz, H-14 / 16), 6.06 (1H, br s, NH), 4.44 (2H, d, *J* = 5.7 Hz, H-2), 3.77 (3H, s, H-17), 3.48 (2H, s, H-9), 3.45 (2H, s, H-11), 2.15 (3H, s, H-10); δ_C ($CDCl_3$, 75.5 MHz) 161.0 (C-1), 158.6 (Ar_{qu}), 138.8 (Ar_{qu}), 136.2 (Ar_{qu}), 131.0 (Ar_{qu}), 130.0 (C-13 / 15), 129.3 (C-5 / 7), 127.7 (C-4 / 8), 113.6 (C-14 / 16), 61.1 (C-9), 61.1 (C-11), 55.2 (C-18), 42.0 (C-2 / 10), 41.9 (C-10 / 2); HRMS (ESI): Found 299.1760 (M^+). $C_{18}H_{23}N_2O_2$ (M^+) requires 299.1754.

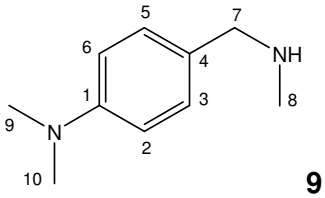
7-Chloro-*N*-[{4-(*N*-*p*-Methoxybenzyl-*N*-methylaminomethyl)phenyl}methyl]-4-quinolinamine



52

To a stirred solution of **30** (0.50 g, 1.85 mmol) in anhydrous *N*-methyl-2-pyrrolidone (5.00 ml) under N_2, were added triethylamine (1.30 ml, 9.25 mmol), K_2CO_3 (0.77 g, 5.55 mmol) and 4,7-dichloroquinoline (1.84 g, 9.25 mmol). The mixture was heated under pressure in a cyclo-addition tube at 120 °C overnight. After the mixture was allowed to cool to room temperature it was poured into saturated brine (20 ml) and extracted with ethyl acetate (3 × 50 ml). The organic layer was further washed with saturated brine (5 × 50 ml) to ensure removal of any traces of the pyrrolidone. The organic layer was dried (Na_2SO_4), concentrated *in vacuo* and the resulting crude product was purified by column chromatography using mixtures of ethyl acetate: hexane (50:50) to (100:0) as eluent to give **52** (0.19 g, 24%) as a dark-yellow oil; ν_{max}/cm^{-1} (DMSO) 3589, 3365 - 3255, 2148, 2059, 1977, 1910, 1661; δ_H ($CDCl_3$, 400 MHz) 8.46 (1H, d, *J* = 5.7 Hz, H-1), 7.94 (1H, d, *J* = 2.1 Hz, H-8), 7.69 (1H, d, *J* = 9.0 Hz, H-5), 7.38 - 7.25 (7H, m, H-6 and ArH), 6.86 (2H, d, *J* = 8.7 Hz, H-22 / 24), 6.40 (1H, d, *J* = 5.7 Hz, H-2), 5.57 (1H, br s, NH), 4.49 (2H, d, *J* = 4.8 Hz, H-10), 3.76 (3H, s, H-26), 3.47 (2H, s, H-17), 3.44 (2H, s, H-19), 2.15 (3H, s, H-18); δ_C ($CDCl_3$, 100.6 MHz) 158.7 (Ar_{qu}), 151.5 (C-1), 149.9 (Ar_{qu}), 148.4 (Ar_{qu}), 139.4 (Ar_{qu}), 135.7 (Ar_{qu}), 135.2 (Ar_{qu}), 131.1 (Ar_{qu}), 130.1 (C-22 / 24), 129.6 (C-13 / 15), 128.3 (C-8), 127.6 (C-12 / 16), 125.6 (C-6), 121.2 (C-5), 117.1 (Ar_{qu}), 113.7 (C-21 / 25), 99.5 (C-2), 61.2 (C-17), 61.2 (C-19), 55.3 (C-26), 47.4 (C-10), 42.1 (C-18); HRMS (ESI): Found 432.1843 (M^+). $C_{26}H_{27}N_3OCl$ (M^+) requires 432.1835.

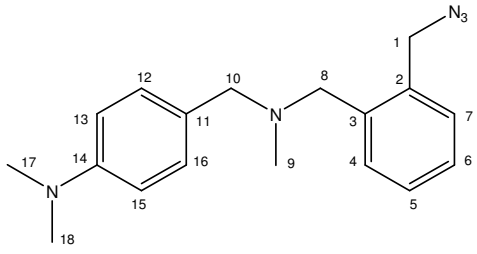
(4-*N, N*-Dimethylaminobenzyl)-methylamine



9

To a stirred solution of 4-*N, N*-dimethylaminobenzaldehyde (3.00 g, 18.3 mmol) in acetonitrile (400 ml) under N_2 at 0 ℃ was added methylamine solution in water (40% v/v) (10.0 ml, 103 mmol) and the reaction allowed to progress for 2 hours. $NaCNBH_3$ (3.46 g, 64.1 mmol) was then added and the reaction allowed to progress at room temperature for 4 hours. The solvent was removed on the rotorevaporator with the water removed by azeotroping with toluene. The crude product was purified directly by column chromatography using EtOAc (100%) followed by EtOAc: MeOH: NEt_3 (92: 5: 3) as eluent to give **9** (2.20 g, 73%) as a yellow oil; δ_H ($CDCl_3$, 300 MHz) 7.17 (2H, d, *J* = 8.7 Hz, H-3 / 5), 6.70 (2H, d, *J* = 8.7 Hz, H-2 / 6), 3.59 (2H, s, H-7), 2.88 (6H, s, H-9 / 10), 2.34 (3H, s, H-8); δ_C ($CDCl_3$, 100.6 MHz) 150.0 (Ar_{qu}), 129.1 (Ar_{qu}), 129.1 (C-3 / 5), 112.7 (C-2 / 6), 55.6 (C-7), 40.3 (C-8), 35.6 (C-9 / 10); HRMS (ESI): Found 165.1392 (M^+ +1). $C_{10}H_{17}N_2$ (M^+ +1) requires 165.1390.

***N*-[{2-(Azidomethyl)phenyl}methyl]-*N*-(4-Dimethylaminobenzyl)methylamine**

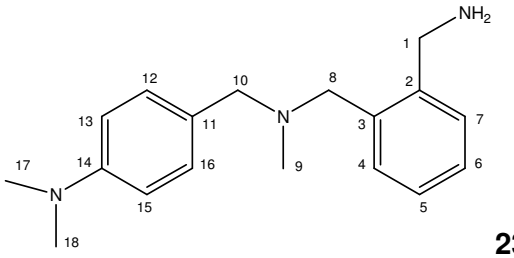


12

To a stirred solution of **9** (1.00 g, 6.06 mmol) and K_2CO_3 (1.25 g, 9.09 mmol) in anhydrous acetonitrile (150 ml) at 0 °C, was added crude *ortho- α, α'* -azidobromoxylene (1.14 g, 5.05 mmol), synthesized as outlined in GP1 and the reaction heated and allowed to progress under reflux for 6 hours. Acetonitrile was then removed under reduced pressure, saturated aqueous Na_2CO_3 added and the organic product extracted

into EtOAc (3 × 100 ml). Drying ($MgSO_4$) of the organic extracts and concentration *in vacuo* furnished a residue, which was purified by column chromatography using mixtures of ethyl acetate: hexane (05:95) to (30:70) as eluent to give **12** (0.89 g, 57% over 2 steps.) as a yellow oil; δ_H ($CDCl_3$, 400 MHz) 7.48 - 7.42 (4H, m, ArH), 7.40 (2H, d, *J* = 8.7 Hz , H-12 / 16), 6.90 (2H, d, *J* = 8.7 Hz, H-13 / 15), 4.69 (2H, s, H-1), 3.69 (2H, s, H-8), 3.64 (2H, s, H-10), 3.09 (6H, s, C-17 / 18), 2.31 (3H, s, H-9); δ_C ($CDCl_3$, 75 MHz) 149.7 (Ar_{qu}), 137.4 (Ar_{qu}), 134.7 (Ar_{qu}),130.5 (Ar_{C-H}), 129.8 (C-12 / 16), 129.7 (Ar_{C-H}), 127.6 (Ar_{C-H}), 127.2 (Ar_{C-H}), 126.4 (Ar_{qu}), 112.2 (C-13 / 15), 61.8 (C-10), 59.4 (C-8), 51.6 (C-1), 41.7 (C-9), 40.3 (C-17 / 18); HRMS (ESI): Found 310.2032 (M^+). $C_{18}H_{24}N_5$ (M^+) requires 310.2047.

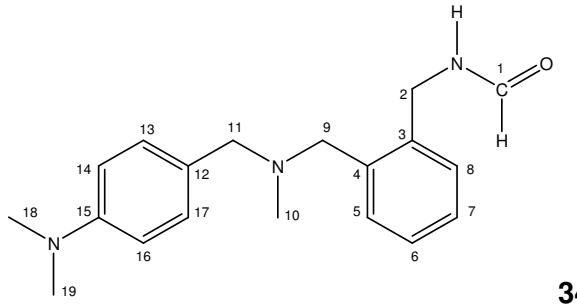
***o*-[(*N*-4-Dimethylaminobenzyl-*N*-methyl)aminomethyl]-benzylamine**



23

To a stirred solution of **12** (0.80 g, 2.58 mmol) in THF (7.20 ml) under N_2, was added PPh_3 (0.81 g, 3.10 mmol) and the reaction mixture allowed to stir for 30 minutes at room temperature. Water (2.40 ml, 133 mmol) was added and the reaction heated at reflux for 6 hours. The reaction was then cooled to room temperature and the solvent removed on the rotorevaporator with the water removed by azeotroping with toluene. The crude product was purified directly by column chromatography using EtOAc (100%) followed by EtOAc: MeOH: NEt_3 (93: 5: 2) as eluent to give **23** (0.61 g, 83%) as a light-brown oil; v_{max}/cm^{-1} (DMSO) 3550 - 3401, 3293, 2132, 2068, 1980, 1663, 1613, 1522; δ_H ($CDCl_3$, 300 MHz) 7.31 - 7.17 (6H, m, ArH), 6.71 (2H, d, *J* = 9.0 Hz, H-13 / 15), 4.05 (2H, br s, NH_2), 3.85 (2H, s, H-1), 3.53 (2H, s, H-8), 3.48 (2H, s, H-10), 2.92 (6H, s, H-17 / 18), 2.07 (3H, s, H-9); δ_C ($CDCl_3$, 75.5 MHz) 149.9 (Ar_{qu}), 141.3 (Ar_{qu}), 137.0 (Ar_{qu}), 131.1 (Ar_{C-H}), 130.3 (C-12 / 16), 129.5 (Ar_{C-H}), 128.0 (Ar_{C-H}), 127.0 (Ar_{C-H}), 125.8 (Ar_{qu}), 112.5 (C-13 / 15), 62.1 (C-8), 60.5 (C-10), 44.2 (C-1), 41.2 (C-9), 40.6 (C-17 / 18); HRMS (ESI): Found 284.2127 (M^+). $C_{18}H_{26}N_3$ (M^+) requires 284.2123.

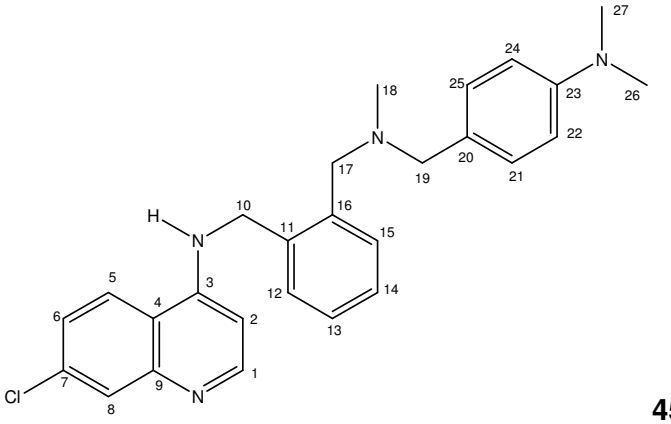
s-*trans*-*N*-[{2-(*N*-4-Dimethylaminobenzyl-*N*-methylaminomethyl)phenyl}methyl] formamide



34

A stirred solution of **23** (100 mg, 0.35 mmol) in excess ethyl formate (3.00 ml, 38.1 mmol) under N_2 was heated to reflux overnight. The excess ethyl formate was removed under reduced pressure and the crude product purified by column chromatography using mixtures of hexane: ethyl acetate: methanol (50:50:0) to (0: 93:7) as eluent to give **34** (90 mg, 82%) as a thick-yellow oil and predominantly as a s-*trans* rotamer; v_{max}/cm^{-1} (DMSO) 3606 - 3245, 2587, 2147, 2067, 1981, 1903, 1659; δ_H ($CDCl_3$, 400 MHz) 8.71 (1H, br s, NH), 8.08 (1H, s, H-1), 7.39 - 7.18 (4H, m, ArH), 7.09 (2H, d, *J* = 8.2 Hz, H-13 / 17), 6.68 (2H, d, *J* = 8.2 Hz, H-14 / 16), 4.42 (2H, d, *J* = 5.2 Hz, H-2), 3.55 (2H, s, H-9), 3.46 (2H, s, H-11), 2.93 (6H, s, H-18 / 19), 2.08 (3H, s, H-10); δ_C ($CDCl_3$, 75.5 MHz) 160.6 (C-1), 160.6 (Ar_{qu}), 138.2 (Ar_{qu}), 137.0 (Ar_{qu}), 132.0 (Ar_{C-H}), 130.9 (Ar_{C-H}), 130.6 (C-13 / 17), 129.4 (Ar_{qu}), 128.7 (Ar_{C-H}), 127.8 (Ar_{C-H}), 112.6 (C-14 / 16), 62.0 (C-11), 61.4 (C-9), 41.2 (C-2), 41.0 (C-10), 40.8 (C-18 / 19); HRMS (ESI): Found 312.2076 (M^+). $C_{19}H_{26}N_3O$ (M^+) requires 312.2070.

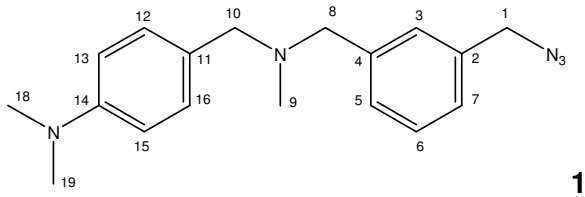
7-Chloro-*N*-[{2-(*N*-*p*-dimethylaminobenzyl-*N*-methylaminomethyl)phenyl}methyl]-4-quinolinamine



45

To a stirred solution of **23** (400 mg, 1.41 mmol) in anhydrous *N*-methyl-2-pyrrolidone (4.00 ml) under N_2, were added triethylamine (1.00 ml, 7.19 mmol), K_2CO_3 (0.58 g, 4.23 mmol) and 4, 7-dichloroquinoline (1.39 g, 7.04 mmol). The mixture was heated under pressure in a cyclo-addition tube at 120 °C overnight. After the mixture was allowed to cool to room temperature it was poured into saturated brine (20 ml) and extracted with ethyl acetate (3 × 50 ml). The organic layer was further washed with saturated brine (5 × 50 ml) to ensure removal of any traces of the pyrrolidone. The organic layer was dried (Na_2SO_4), concentrated *in vacuo* and the resulting crude product was purified by column chromatography using mixtures of hexane: ethyl acetate: methanol (50:50:0) to (0:90:10) as eluent to give **45** (112 mg, 18%) as a dark-yellow oil; v_{max}/cm^{-1} (DMSO) 3633, 3360 - 3104, 2706, 2641, 2598, 2342, 2147, 2056, 1970, 1901; δ_H ($CDCl_3$, 300 MHz) 8.54 (1H, d, *J* = 5.4 Hz, H-1), 7.89 (1H, d, *J* = 2.1 Hz, H-8), 7.79 (1H, br s, NH), 7.42 (1H, m, ArH), 7.31 (3H, m, ArH), 7.24 (1H, d, *J* = 9.0 Hz, H-5), 7.04 (2H, d, *J* = 8.6 Hz, H-21 / 25), 6.88 (1H, dd, *J* = 2.1, 9.0 Hz, H-6), 6.57 (2H, d, *J* = 8.6 Hz, H-22 / 24), 6.54 (1H, d, *J* = 5.4 Hz, H-2), 4.41 (2H, s, H-10), 3.61 (2H, s, H-17), 3.50 (2H, s, H-19), 2.92 (6H, s, H-26 / 27), 2.14 (3H, s, H-18); δ_C ($CDCl_3$, 75.5 MHz) 152.0 (C-1), 150.3 (Ar_{qu}), 150.0 (Ar_{qu}), 149.2 (Ar_{qu}), 137.4 (Ar_{qu},), 137.4 (Ar_{qu},), 134.4 (Ar_{qu}), 132.0 (Ar_{C-H}), 130.8 (C-21 / 25), 130.5 (Ar_{C-H}), 128.2 (C-8), 128.0 (Ar_{C-H}), 127.8 (Ar_{C-H}), 124.8 (Ar_{qu}), 124.7 (C-6), 122.6 (C-5), 117.9 (Ar_{qu}), 112.3 (C-22 / 24), 98.8 (C-2), 61.6 (C-19), 61.0 (C-17), 46.5 (C-10), 41.5 (C-18), 40.4 (C-26 / 27); HRMS (ESI): Found 445.2159 (M^+). $C_{27}H_{30}N_4Cl$ (M^+) requires 445.2172.

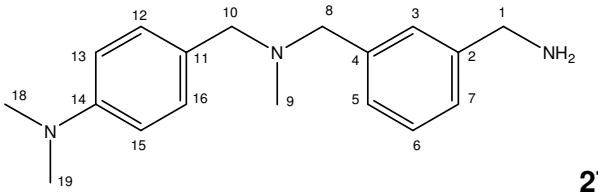
***N*-[{3-(Azidomethyl)phenyl}methyl]-*N*-(4-Dimethylaminobenzyl)methylamine**



16

To a stirred solution of **9** (1.00 g, 6.06 mmol) and K_2CO_3 (1.25 g, 9.09 mmol) in anhydrous acetonitrile (150 ml) at 0 °C, was added crude *meta- α, α'* -azidobromoxylene (1.14 g, 5.05 mmol), synthesized as outlined in GP1 and the reaction heated and allowed to progress under reflux for 6 hours. Acetonitrile was then removed under reduced pressure, saturated aqueous Na_2CO_3 added and the organic product extracted into EtOAc (3 × 100 ml). Drying ($MgSO_4$) of the organic extracts and concentration *in vacuo* furnished a residue, which was purified by column chromatography using mixtures of ethyl acetate: hexane (05:95) to (30:70) as eluent to give **16** (0.95 g, 61% over 2 steps.) as a yellow oil; δ_H ($CDCl_3$, 300 MHz) 7.35 - 7.26 (4H, m, ArH), 7.23 (2H, d, *J* = 8.9 Hz, H-12 / 16), 6. 73 (2H, d, *J* = 8.9 Hz, H-13 / 15), 4.34 (2H, s, H-1), 3.52 (2H, s, H-8), 3.47 (2H, s, H-10), 2.94 (6H, s, H-18 / 19), 2.19 (3H, s, H-9); δ_C ($CDCl_3$, 75.5 MHz) 149.9 (Ar_{qu}), 140.4 (Ar_{qu}), 135.2 (Ar_{qu}), 129.9 (C-12 / 16), 128.9 (Ar_{C-H}), 128.7 (Ar_{C-H}), 128.6 (Ar_{C-H}), 126.8 (Ar_{qu}), 126.7 (Ar_{C-H}), 113.6 (C-13 / 15), 61.4 (C-8), 61.2 (C-10), 54.8 (C-1), 42.0 (C-9), 40.7 (C-18 / 19); HRMS (ESI): Found 310.2032 (M^+). $C_{18}H_{24}N_5$ (M^+) requires 310.2042.

***m*-[(*N*-4-Dimethylaminobenzyl-*N*-methyl)aminomethyl]-benzylamine**

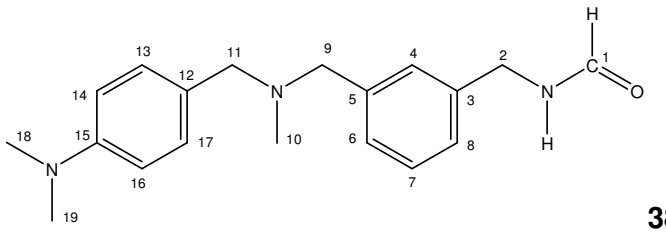


27

To a stirred solution of **16** (0.80 g, 2.58 mmol) in THF (7.20 ml) under N_2, was added PPh_3 (0.81 g, 3.10 mmol) and the reaction mixture allowed to stir for 30 minutes at room temperature. Water (2.40 ml, 133 mmol) was added and the reaction heated at reflux for

6 hours. The reaction was then cooled to room temperature and solvent removed on the rotorevaporator with the water removed by azeotroping with toluene. The crude product was purified directly by column chromatography using EtOAc (100%) followed by EtOAc: MeOH: NEt_3 (93: 5: 2) as eluent to give **27** (0.59 g, 81%) as a light-brown oil; v_{max}/cm^{-1} (DMSO) 3531- 3410, 3286, 2137, 2060, 1986, 1661, 1611, 1511; δ_H ($CDCl_3$, 300 MHz) 7.29 – 7.18 (6H, m, ArH), 6.86 (2H, d, *J* = 8.7 Hz, H-13 / 15), 3.86 (2H, s, H-1), 3.79 (6H, s, H-18 / 19), 3.49 (2H, s, H-8), 3.47 (2H, s, H-10), 2.50 (2H, br s, NH_2), 2.16 (3H, s, H-9); δ_C ($CDCl_3$, 75.5 MHz) 158.6 (Ar_{qu}), 142.4 (Ar_{qu}), 139.6 (Ar_{qu}), 131.1 (Ar_{qu}), 130.0 (C-12 / 16), 128.3 (Ar_{C-H}), 127.6 (Ar_{C-H}), 127.5 (Ar_{C-H}), 125.7 (Ar_{C-H}), 113.5 (C-13 / 15), 61.5 (C-10), 61.2 (C-8), 55.1 (C-18 / 19), 46.1 (C-1), 42.0 (C-9).

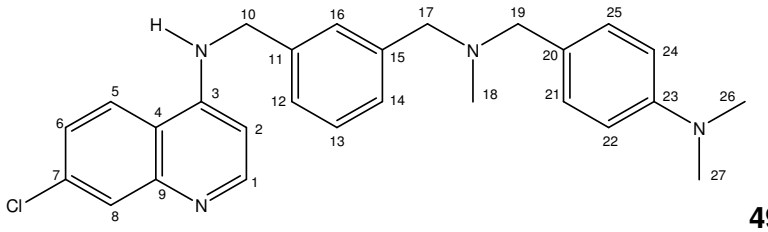
s-*trans*-*N*-[{3-(*N*-4-Dimethylaminobenzyl-*N*-methylaminomethyl)phenyl}methyl] formamide



38

A stirred solution of **27** (100 mg, 0.35 mmol) in excess ethyl formate (3.00 ml, 38.1 mmol) under N_2 was heated to reflux overnight. The excess ethyl formate was removed under reduced pressure and the crude product purified by column chromatography using mixtures of hexane: ethyl acetate: methanol (50:50:0) to (0: 93:7) as eluent to give **38** (97 mg, 89%) as a thick-yellow oil and predominantly as a s-*trans* rotamer; v_{max}/cm^{-1} (DMSO) 3599-3278, 2592, 2140, 2066, 1982, 1904, 1660; δ_H ($CDCl_3$, 400 MHz) 8.21 (1H, s, H-1), 7.30 - 7.27 (3H, m, ArH), 7.21 (2H, d, *J* = 8.8 Hz, H-13 / 17), 7.15 (1H, m, H-4), 6.72 (2H, d, *J* = 8.8 Hz, H-14 / 16), 6.22 (1H, br s, NH), 4.45 (2H, d, *J* = 6.0 Hz, H-2), 3.49 (2H, s, H-9), 3.46 (2H, s, H-11), 2.94 (6H, s, H-18 / 19), 2.17 (3H, s, H-10); δ_C ($CDCl_3$, 100.6 MHz) 161.1 (C-1), 149.9 (Ar_{qu}), 140.1 (Ar_{qu}), 137.5 (Ar_{qu}), 130.0 (C-13 / 17), 129.9 (Ar_{qu}), 128.6 (Ar_{C-H}), 128.4 (Ar_{C-H}), 128.4 (Ar_{C-H}), 126.4 (Ar_{C-H}), 113.6 (C-14 / 16), 61.4 (C-9), 61.3 (C-11), 42.0 (C-2), 42.0 (C-10), 40.7 (C-18 / 19); HRMS (ESI): Found 312.2063 (M^+). $C_{19}H_{26}N_3O$ (M^+) requires 312.2063.

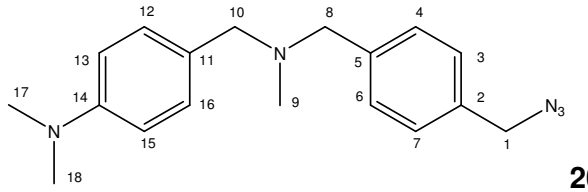
7-Chloro-*N*-[{3-(*N*-*p*-Dimethylaminobenzyl-*N*-methylaminomethyl)phenyl}methyl]-4-quinolinamine



49

To a stirred solution of **27** (0.40 g, 1.41 mmol) in anhydrous *N*-methyl-2-pyrrolidone (4.00 ml) under N_2, were added triethylamine (1.00 ml, 7.19 mmol), K_2CO_3 (0.58 g, 4.23 mmol) and 4,7-dichloroquinoline (1.39 g, 7.04 mmol). The mixture was heated under pressure in a cyclo-addition tube at 120 °C overnight. After the mixture was allowed to cool to room temperature it was poured into saturated brine (20 ml) and extracted with ethyl acetate (3 × 50 ml). The organic layer was further washed with saturated brine (5 × 50 ml) to ensure removal of any traces of the pyrrolidone. The organic layer was dried (Na_2SO_4), concentrated *in vacuo* and the resulting crude product was purified by column chromatography using mixtures of hexane: ethyl acetate: methanol (50:50:0) to (0:90:10) as eluent to give **49** (0.14 g, 22%) as a dark-yellow oil; ν_{max}/cm^{-1} (DMSO) 3363 - 3169, 2599, 2350, 2148, 2059, 1973, 1907, 1679, 1618; δ_H ($CDCl_3$, 400 MHz) 8.47 (1H, d, *J* = 5.2 Hz, H-1), 7.95 (1H, d, *J* = 2.0 Hz, H-8), 7.70 (1H, d, *J* = 8.8 Hz, H-5), 7.38 - 7.21 (4H, m, ArH), 7.31 (1H, dd, *J* = 2.0, 8.8 Hz, H-6), 7.13 (2H, d, *J* = 8.6 Hz, H-21 / 25), 6.61 (2H, d, *J* = 8.6 Hz, H-22 / 24), 6.41 (1H, d, *J* = 5.2 Hz, H-2), 5.58 (1H, br s, NH), 4.47 (2H, d, *J* = 4.8 Hz, H-10), 3.47 (2H, s, H-17), 3.40 (2H, s, H-19), 2.88 (6H, s, H-26 / 27), 2.15 (3H, s, H-18); δ_C ($CDCl_3$, 100.6 MHz) 152.4 (Ar_{qu}), 152.0 (C-1), 149.9 (Ar_{qu}), 149.6 (Ar_{qu}), 140.7 (Ar_{qu}), 137.2 (Ar_{qu}), 135.0 (Ar_{qu}), 130.1 (Ar_{qu}), 129.8 (C-21 / 25), 128.8 (C-8), 128.8 (Ar_{C-H}), 128.6 (Ar_{C-H}), 128.1 (Ar_{C-H}), 126.1 (Ar_{C-H}), 125.5 (C-6), 121.0 (C-5), 117.2 (Ar_{qu}), 112.5 (C-22 / 24), 99.7 (C-2), 61.3 (C-17), 61.3 (C-19), 47.7 (C-10), 42.2 (C-19), 40.7 (C-26 / 27); HRMS (ESI): Found 445.2159 (M^+). $C_{27}H_{30}N_4Cl$ (M^+) requires 445.2138.

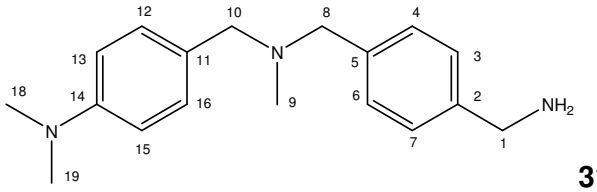
***N*-[{4-(Azidomethyl)phenyl}methyl]-*N*-(4-Dimethylaminobenzyl)methylamine**



20

To a stirred solution of **9** (1.00 g, 6.06 mmol) and K_2CO_3 (1.25 g, 9.09 mmol) in anhydrous acetonitrile (150 ml) at 0 °C, was added crude *para- α, α'* -azidobromoxylene (1.14 g, 5.05 mmol), synthesized as outlined in GP1 and the reaction heated and allowed to progress under reflux for 6 hours. Acetonitrile was then removed under reduced pressure, saturated aqueous Na_2CO_3 added and the organic product extracted into EtOAc (3 × 100 ml). Drying ($MgSO_4$) of the organic extracts and concentration *in vacuo* furnished a residue, which was purified by column chromatography using mixtures of ethyl acetate: hexane (5:95) to (30:70) as eluent to give **20** (0.99 g, 63% over 2 steps.) as a yellow oil; δ_H ($CDCl_3$, 400 MHz) 7.40 - 7.25 (4H, m, ArH), 7.23 (2H, d, J = 8.6 Hz, H-12/ 16), 6.72 (2H, d, J = 8.6 Hz, H-13 / 17), 4.32 (2H, s, H-1), 3.51 (2H, s, H-8), 3.46 (2H, s, H-10), 2.92 (6H, s, H-17 / 19), 2.15 (3H, s, H-9); δ_C ($CDCl_3$, 75.5 MHz) 149.8 (Ar_{qu}), 139.9 (Ar_{qu}), 133.8 (Ar_{qu}), 129.8 (Ar_{C-H}), 129.3 (C-12 / 16), 128.1 (Ar_{C-H}), 126.9 (Ar_{qu}), 112.5 (C-13 / 15), 61.4 (C-10), 61.1 (C-8), 54.6 (C-1), 42.1 (C-9), 40.7 (C-17 / 18); HRMS (ESI): Found 310.2032 (M^+). $C_{18}H_{24}N_5$ (M^+) requires 310.2024.

***p*-[(*N*-4-Dimethylaminobenzyl-*N*-methyl)aminomethyl]-benzylamine**

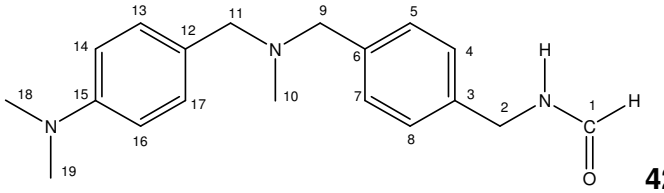


31

To a stirred solution of **20** (0.80 g, 2.58 mmol) in THF (7.20 ml) under N_2, was added PPh_3 (0.81 g, 3.10 mmol) and the reaction mixture allowed to stir for 30 minutes at room temperature. Water (2.40 ml, 133 mmol) was added and the reaction heated at reflux for 6 hours. The reaction was then cooled to room temperature and the solvent removed on

the rotorevaporator with the water removed by azeotroping with toluene. The crude product was purified directly by column chromatography using EtOAc (100%) followed by EtOAc: MeOH: NEt_3 (93: 5: 2) as eluent to give **31** as a light-brown oil (0.65 g, 89%); v_{max}/cm^{-1} (DMSO) 3545 - 3385, 3285, 2582, 2141, 2066, 1985, 1904, 1661, 1521; δ_H ($CDCl_3$, 400 MHz) 7.31 – 7.21 (4H, m, ArH), 7.19 (2H, d, *J* = 8.8 Hz, H-12 / 16), 6.69 (2H, d, *J* = 8.8 Hz, H-13 / 15), 3.81 (2H, s, H-1), 3.46 (2H, s, H-8), 3.43 (2H, s, H-10), 2.91 (6H, s, NMe_2), 2.49 (2H, br s, NH_2), 2.14 (3H, s, N-CH_3); δ_C ($CDCl_3$, 75.5 MHz) 149.8 (Ar_{qu}),141.2 (Ar_{qu}), 138.0 (Ar_{qu}), 129.8 (C-4 / 6), 129.2 (C-12 /16), 127.0 (C-3 / 7); 126.8 (Ar_{qu}), 112.5 (C-13 / 15), 61.2 (C-8 or C-10), 61.1 (C-8 or C-10), 46.0 (C-1), 41.9 (C-9), 40.7 (C-18 / 19).

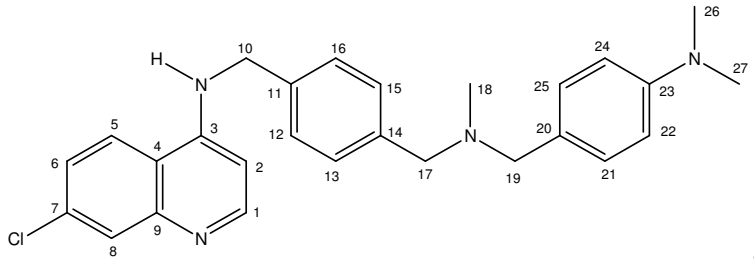
s-*trans*-*N*-[{4-(*N*-4-Dimethylaminobenzyl-*N*-methylaminomethyl)phenyl}methyl] formamide



42

A stirred solution of **31** (100 mg, 0.35 mmol) in excess ethyl formate (3.00 ml, 38.1 mmol) under N_2 was heated to reflux overnight. The excess ethyl formate was removed under reduced pressure and the crude product purified by column chromatography using mixtures of hexane: ethyl acetate: methanol (50:50:0) to (0: 93:7) as eluent to give **42** (85 mg, 78%) as a thick-yellow oil and predominantly as a s-*trans* rotamer; v_{max}/cm^{-1} (DMSO) 3615 - 3235, 2595, 2142, 2061, 1973, 1901, 1657; δ_H ($CDCl_3$, 400 MHz) 8.16 (1H, s, H-1), 7.32 -7.20 (4H, m, ArH), 7.19 (2H, d, *J* = 8.8 Hz, H-13 / 17), 6.69 (2H, d, *J* = 8.8 Hz, H-14 / 16), 5.41 (1H, br s, NH), 4.41 (2H, d, *J* = 5.7 Hz, H-2), 3.51 (2H, s, H-9), 3.47 (2H, s, H-11), 2.90 (6H, s, H-18 / 19), 2.14 (3H, s, H-10); δ_C ($CDCl_3$, 75.5 MHz) 161.1 (C-1), 149.9 (Ar_{qu}), 138.0 (Ar_{qu}), 136.4 (Ar_{qu}), 130.0 (C-5 / 7), 129.5 (C-13 / 17), 127.6 (C-4 / 8) , 125.6 (Ar_{qu}), 112.4 (C-14 / 16), 60.9 (C-9 or 11), 60.6 (C-9 or 11), 41.8 (C-2), 41.5 (C-10), 40.6 (C-18 / 19); HRMS (ESI): Found 312.2089 (M^+). $C_{19}H_{26}N_3O$ (M^+) requires 312.2070.

7-Chloro-*N*-[{4-(*N*-*p*-dimethylaminobenzyl-*N*-methylaminomethyl)phenyl}methyl]-4-quinolinamine



53

To a stirred solution of **31** (0.40 g, 1.41 mmol) in anhydrous *N*-methyl-2-pyrrolidone (4.00 ml) under N_2, were added triethylamine (1.00 ml, 7.19 mmol), K_2CO_3 (0.58 g, 4.23 mmol) and 4,7-dichloroquinoline (1.39 g, 7.04 mmol). The mixture was heated under pressure in a cyclo-addition tube at 120 °C overnight. After the mixture was allowed to cool to room temperature it was poured into saturated brine (20 ml) and extracted with ethyl acetate (3 × 50 ml). The organic layer was further washed with saturated brine (5 × 50 ml) to ensure removal of any traces of the pyrrolidone. The organic layer was dried (Na_2SO_4), concentrated *in vacuo* and the resulting crude product was purified by column chromatography using mixtures of hexane: ethyl acetate: methanol (50:50:0) to (0:90:10) as eluent to give **53** (0.16 g, 26%) as a dark-yellow oil; ν_{max}/cm^{-1} (DMSO) 3616, 3378 - 3212, 2596, 2150, 2062, 1977, 1904, 1674, 1635; δ_H ($CDCl_3$, 400 MHz) 8.43 (1H, d, *J* = 5.4 Hz, H-1), 7.92 (1H, d, *J* = 2.0 Hz, H-8), 7.70 (1H, d, *J* = 9.0 Hz, H-5), 7.34 - 7.28 (4H, m, ArH), 7.26 (1H, dd, *J* = 2.4, 9.0 Hz, H-6), 7.19 (2H, d, *J* = 8.4 Hz, H-21 / 25), 6.68 (2H, d, *J* = 8.4 Hz, H-22 / 24), 6.37 (1H, d, *J* = 5.4 Hz, H-2), 5.82 (1H, br s, NH), 4.43 (2H, d, *J* = 4.8 Hz, H-10), 3.46 (2H, s, H-17), 3.42 (2H, s, H-19), 2.89 (6H, s, H-26 / 27), 2.15 (3H, s, H-18); δ_C ($CDCl_3$, 75.5 MHz) 151.7 (C-1), 149.7 (Ar_{qu}), 149.7 (Ar_{qu}), 148.8 (Ar_{qu}), 139.2 (Ar_{qu}), 135.6 (Ar_{qu}), 134.8 (Ar_{qu}), 129.7 (C-13 / 15), 129.3 (C-21 / 25), 128.3 (C-8), 127.3 (C-12 / 16), 126.7 (Ar_{qu}), 125.2 (C-6), 121.3 (C-5), 117.1 (Ar_{qu}), 112.4 (C-22 / 24), 99.5 (C-2), 61.2 (C-17 or 19), 61.0 (C-17 or 19), 47.1 (C-10), 42.0 (C-18), 40.6 (C-26 / 27); HRMS (ESI): Found 445.2159 (M^+). $C_{27}H_{30}N_4Cl$ (M^+) requires 445.2171.

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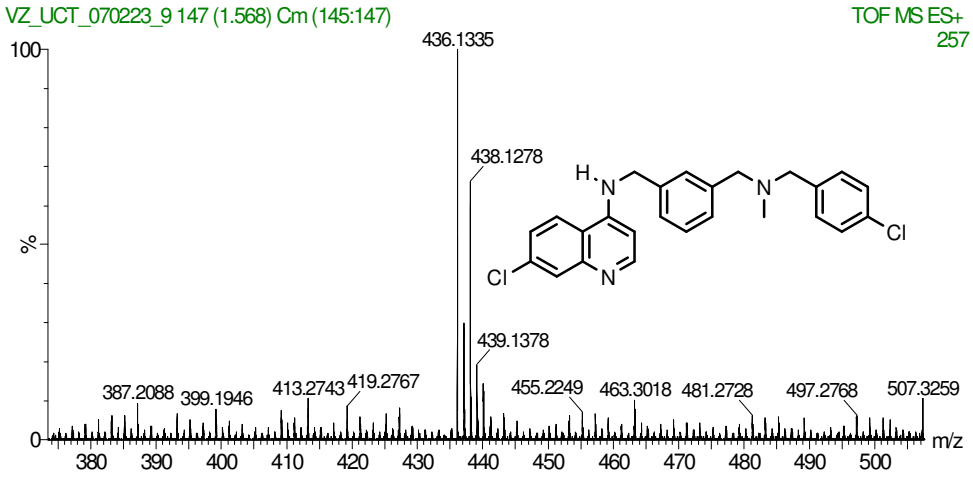
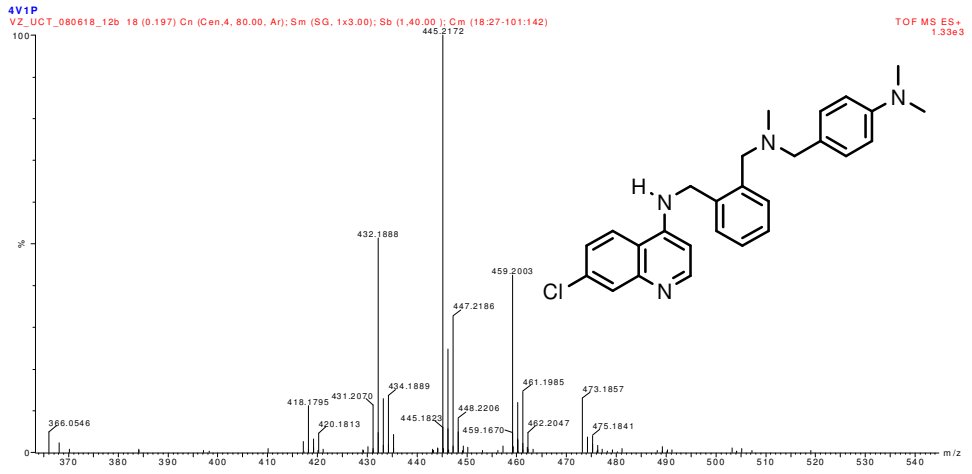


HRMS (ES) spectra for **(47)**: Found 436.1335 (M^+). $C_{25}H_{24}N_3Cl_2$ requires 436.1347.



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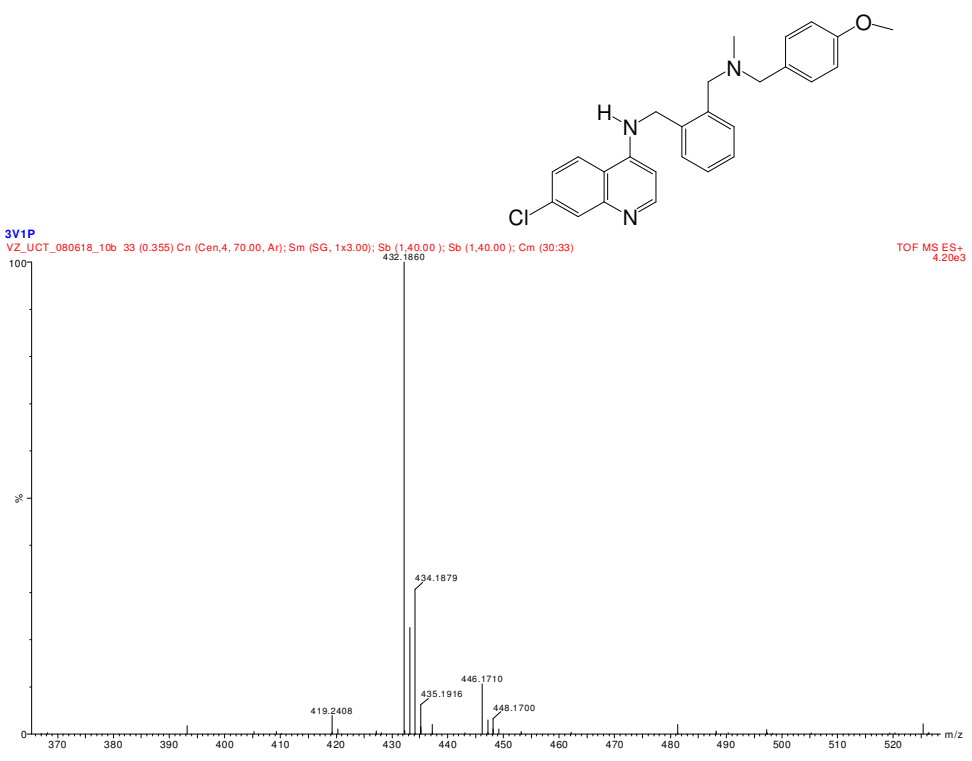

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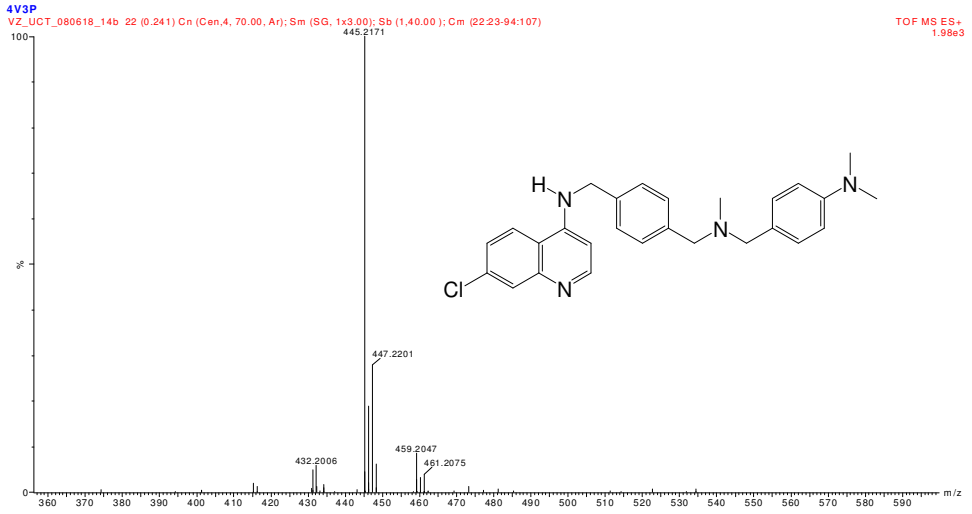

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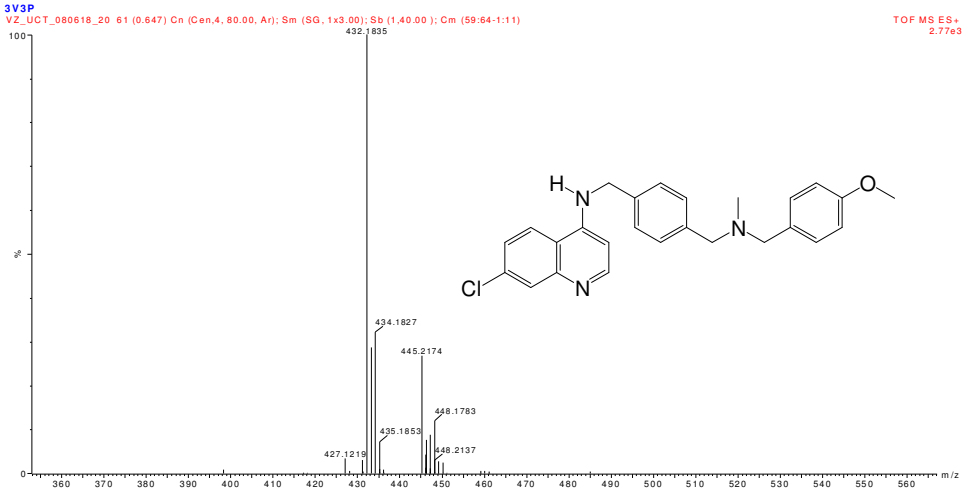

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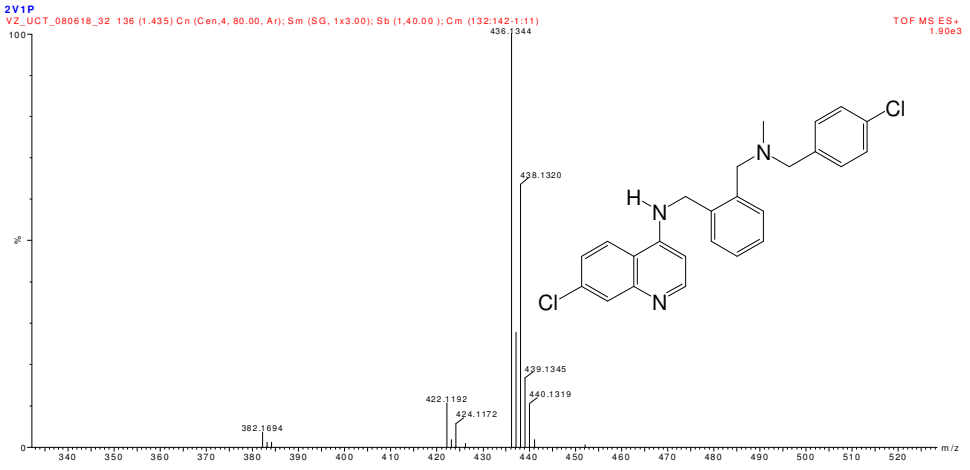

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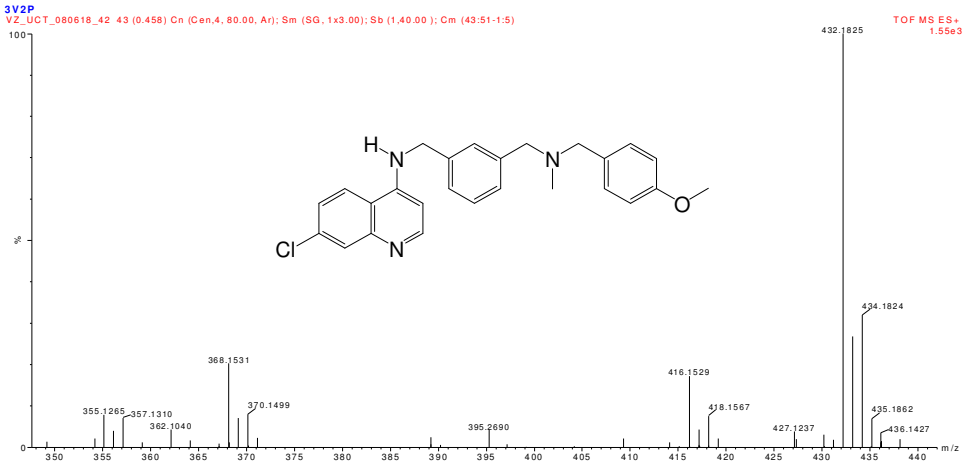

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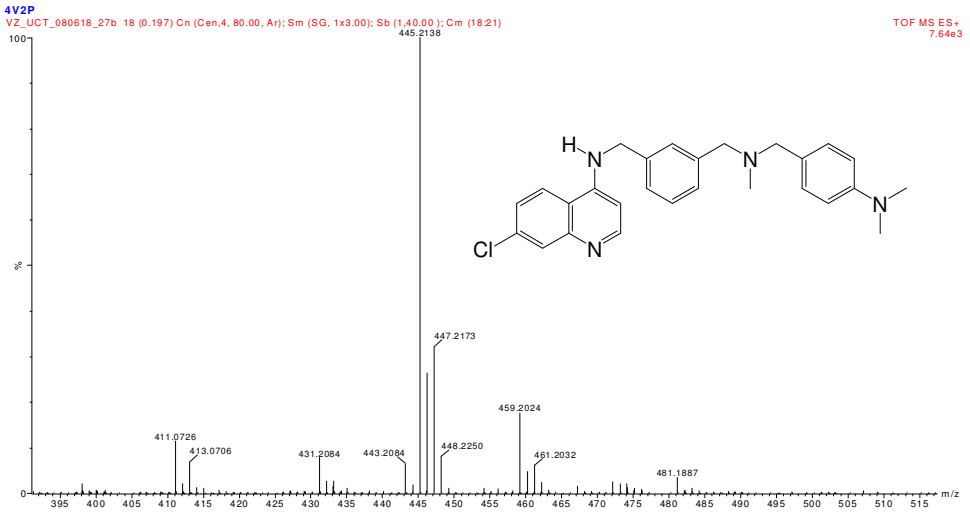

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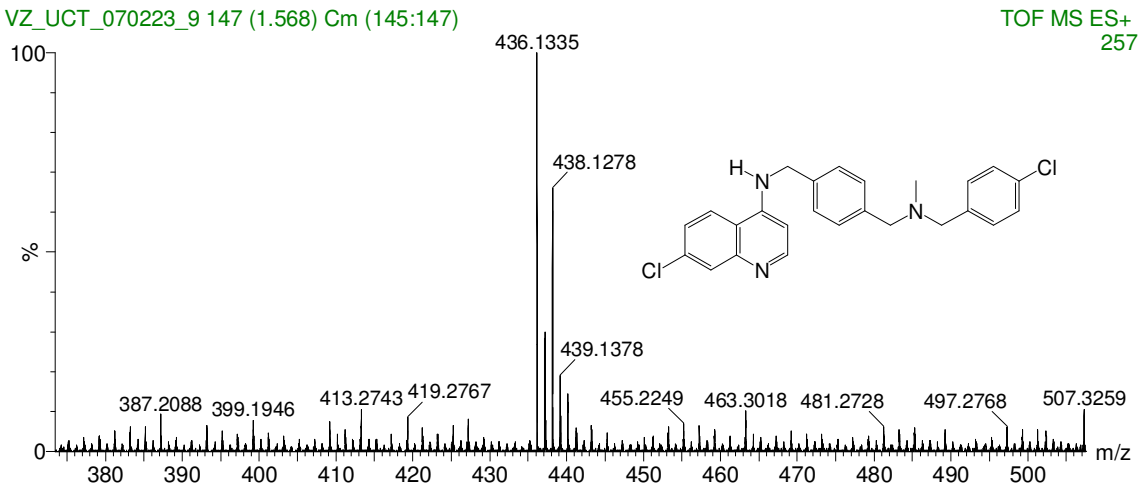

HRMS (ES) spectra for **(51)**: Found 436.1335 (M^+). $C_{25}H_{24}N_3Cl_2$ requires 436.1347.