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Department of Chemistry

Antimalarials Based on the Arylpiperazine Privileged Substructure

By

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In the subject

CHEMISTRY

Supervisor:

Associate Professor Kelly Chibale

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ABSTRACT

Based on a previous study, arylpiperazines (2-chlorophenylpiperazine, 2-ethoxyphenylpiperazine and phenylpiperazine) were found to be significantly more potent against the chloroquine-resistant (K1) strain than against the chloroquine-sensitive (D10) strain. In other studies, 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) has been identified as a potential antimalarial agent for the inhibition of the 5-hydroxytryptamine type 1A receptor in *Plasmodium falciparum*. A number of arylpiperazines are also known to target this receptor in other systems.

Coupled with the potential role of arylpiperazines as replacements for the antimalarial 8-OH-DPAT, these results prompted a further investigation into the antiplasmodial properties of a broader range of simple unsubstituted and substituted arylpiperazines against a broader range of chloroquine-sensitive and chloroquine-resistant strains of *Plasmodium falciparum*. The results are reported in this thesis.

Arylpiperazines in which the terminal secondary amino group is unsubstituted were found to display a mefloquine-type behaviour in being significantly more potent against chloroquine-resistant (W2 and FCR3) strains than against the chloroquine-sensitive (D10 and NF54) strains. Substitution of the aforementioned amino group led to a dramatic drop in activity across all strains as well as the abolition of the preferential potency against resistant strains that was observed for the unsubstituted counterparts. This data suggests that unsubstituted arylpiperazines appear not to be sufficiently well recognised by the chloroquine resistance mechanism and may imply that the unsubstituted arypiperazines

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mechanistically act differently from chloroquine. On the other hand, 4-aminoquinoline-based heteroarylpiperazines in which the terminal secondary amino group is unsubstituted were found to be equally active against the chloroquine-resistant and chloroquine-sensitive strains, suggesting that chloroquine cross-resistance is not observed with these two 4-aminoquinolines. However, relative to other unsubstituted arylpiperazines, there is increased recognition of the two 4-aminoquinoline-based heteroarylpiperazines by the chloroquine resistance mechanism.

Increasing the length of the chain between the aforementioned amino and substituted aryl units led to an increase in antimalarial activity in both the chloroquine-resistant and chloroquine-sensitive strains.

Substitution of the simple aryl groups by biaryl and aryl ether moieties led to an increase in antiplasmodial activity. However, where the simple unsubstituted arylpiperazines has preferential potency against resistant strains, these biaryl piperazines had preferential potency against the chloroquine-sensitive strain.

These studies provide structural features that may determine the antimalarial activity of arylpiperazines.

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ABBREVIATIONS

AP	Aspartic protease
Ar	Aromatic
BBB	Blood brain barrier
BINAP	(R)-(+)-2,2'-bis(diphenylphosphino)1,1'-binaphthyl
2-CB	4-bromo-2,5-dimethoxyphenylamine
CP	Cysteine protease
DCM	Dichloromethane
DMSO	Dimethylsulphoxide
DNA	Deoxyribonucleic acid
DOI	1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane
Et	Ethyl
eq	Equivalent
Fe	Iron
GABA	Gamma aminobutyric acid
Hex	Hexane
H_2O-Fe(III)PPIX	Aquaferriprotoporphyrin IX
HRMS	High resolution mass spectroscopy
HRP-2	Histidine-rich protein II
hrs	Hours
HT	Hydroxytryptamine

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IC_{50}	Inhibitory concentration to inhibit 50% of enzyme activity or parasite growth
IR	Infrared
K	Kelvin
Leu	Leucine
LRMS	Low resolution mass spectroscopy
mol	Mole
Me	Methyl
MS	Mass spectroscopy
NMR	Nuclear magnetic resonance
8-OH-DPAT	8-hydroxy-2-(di-*n*-propylamino)tetralin
P	*Plasmodium*
RBC	Red blood cell
Ph	Phenyl
Phe	Phenylalanine
RI	Resistance Index
rt	Room temperature
SAR	Structure-activity relationship
S_N2	Nucleophilic bimolecular substitution
SP	Serine protease
TLC	Thin layer chromatography

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The following abbreviations are used in the EXPERIMENTAL chapter:

s	singlet
d	doublet
dd	doublet of doublets
t	triplet
q	quartet
quint	quintet
sex	sextet
m	multiplet
δ	chemical shift
Anal.	Analytical
cm^{-1}	wavelength
mp	melting point
m/z	mass to charge ratio
ppm	parts per million
R_f	retention factor

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CHAPTER 1

INTRODUCTION

1.1 Introduction to malaria

Malaria is the most widespread and serious parasitic disease. It mainly affects the tropical and subtropical areas of the world (Fig. 1.1)[1] and it is thought that two billion people are at risk. About 300-500 million people have malaria each year and from that 1.5-3 million people die. Malaria is the ninth most common cause of death owing to infectious disease. Many cases of malaria are found in Southeast Asia and South America but the worst affected area is Sub-Saharan Africa where 90% of all the cases are found. It has been found that the disease heavily affects the underdevelopment of the country and puts strain on the already limited health care facilities.

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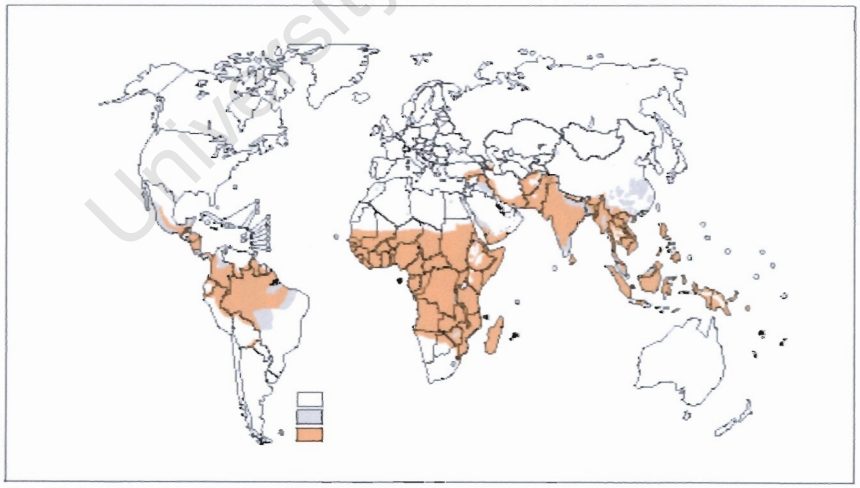

Above: World malaria situation. Malaria is endemic to tropical and subtropical regions.

Figure 1.1: Map of malaria infected regions[1]

1.2 Life cycle of the malaria parasite

Malaria found in humans is caused by four *Plasmodium* species[2]: *P. falciparum, P. vivax, P. malariae,* and *P. ovale*. *P. falciparum* is the most widespread of this infectious disease. *P. vivax* is the second most common causing a significant number of deaths. The female *Anopheles* mosquito is responsible for the majority of malaria transmission.

The life cycle (Fig. 1.2)[3] of *P. falciparum* has three stages: mosquito, liver and blood. In the gut of the mosquito there is a sexual reproduction of gametocytes (fusion of male and female cells). These then form zygotes, which bury themselves in the lining of the gut and develop into oocysts and then into sporozoites, which then migrate to the salivary glands. Multiple fissions involving a single cell are called schizogony and sporogony when zygotes are involved. When the mosquito bites the human host the sporozoites enter the blood stream and move to the liver and invade hepatocytes. They stay in the liver for about a week as schizonts (A schizont is formed by an asexual reproduction where the nucleus divides repeatedly giving a multinucleate cell or schizont) whilst the parasites multiply asexually. They then rupture the host's cells and enter the blood stream as merozoites (small cell with a single nucleus), which invade the red blood cells and enter the blood cycle that consists of ring, trophozoite and blood schizont stages. The asexual reproduction of merozoites is ongoing and thus there is an ever-increasing parasitaemia. Some merozoites develop into gametocytes and can be taken up by a mosquito when it bites the human host. This completes the life cycle.

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Symptoms of malaria are only observed once the cycle is in the blood stage, so drugs must have specific activity in this part of the cycle.

The signs of malaria are fever and chills, which are the non-specific signs. There are also complications such as cerebral malaria, anaemia, hypoglycaemia, renal failure and non-cardiac pulmonary oedema.

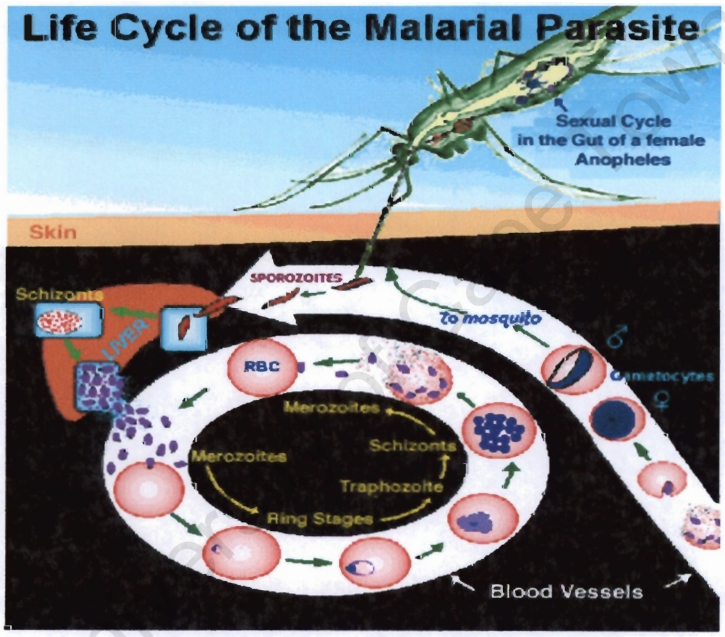

Figure 1.2: Life cycle of the malarial parasite[3]

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1.3 Biochemistry of the Food Vacuole

1.3.1 Haemoglobin degradation

1.3.1.1 Background

The trophozoite in the *P. falciparum* ingests and degrades host cell haemoglobin, Fig. 1.3.[4] Studies have shown that 60 - 80% of the haemoglobin that was originally in the red blood cell is digested.[5,6] The parasite uses amino acids derived from the haemoglobin in its own proteins.[7] It has been suggested that the parasite ingests the haemoglobin for three reasons:

1) as a food source
2) to make space for itself within the red blood cell.[8]
3) to maintain osmotic stability in the red blood cell.[9]

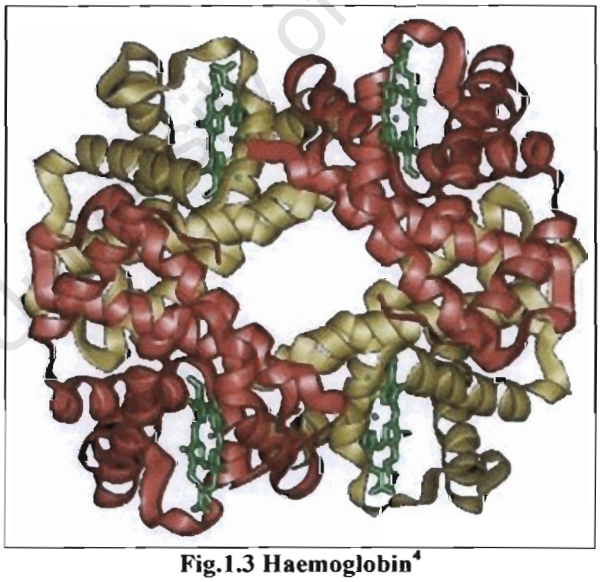

Fig.1.3 Haemoglobin[4]

Haemoglobin is taken up first by trophozoites and schizonts, which are the most metabolically active stages.[10]

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The parasite lives in the parasitophorous vacuole. The process of haemoglobin degradation involves the ingestion of red blood cell cytosol into the food vacuole via cytosome and transport vesicles, Fig. 1.4.[11] The food vacuole contains proteolytic enzymes which digest the haemoglobin, forming haem as a by-product. Haem is then converted into haematin, which is then converted into haemozoin. The peptides from the haemoglobin degradation are exported to the parasite cytosol and are further degraded to amino acids. The food vacuole is acidic and the pH is thought to be between 5 – 5.5. [12,13] Haem is converted into haemozoin, which is also known as malaria pigment, so as to prevent toxicity of haem in the parasite.[14] Processing of haem may also produce free iron which can be used for the biosynthesis of iron-containing proteins[15] and parasite-derived haem.[16] This is suggested by the observations in previous studies that show small amounts of iron are released from haem after incubation at the pH of the food vacuole.[10] From the above evidence it is shown that there are essential processes of haemoglobin catabolism and haem conversion, which are targets for antimalarial compounds.

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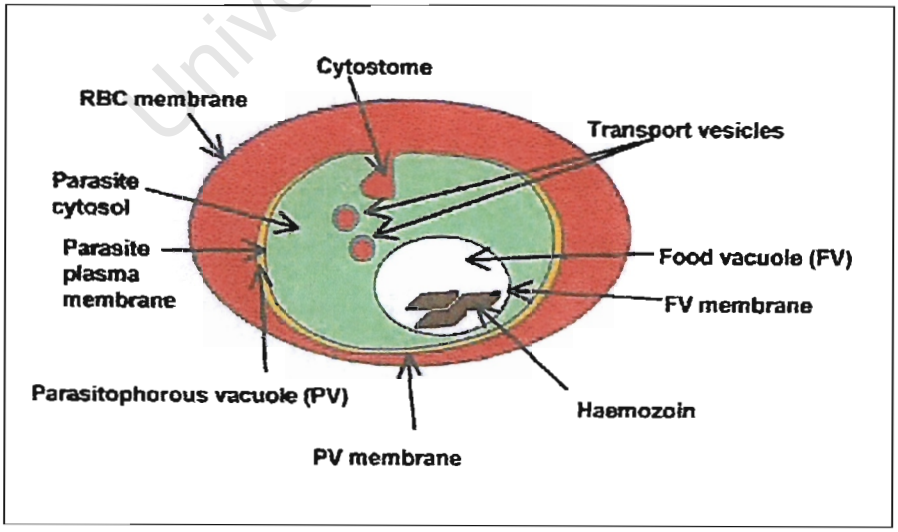

Fig1.4 A schematic representation of the parasitised red blood cell.[11]

1.3.1.2 Proteases and Haemoglobin

Haemoglobin degradation is a very efficient process, which involves at least three classes of enzymes that assist in the process. The three enzyme classes are:

1) Aspartic proteases namely: plasmepsins I, II and IV as well as a histo-aspartic protease.[17]
2) Cysteine proteases namely: falcipain 2 and 3.[18]
3) Zinc metalloprotease or falcilysin.[19]

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Plasmepsins I and II degrade the native haemoglobin and plasmepsin IV has weak proteolytic activity against the native protein and seems to act synergistically with plasmepsins I and II. The histo-aspartic protease is active against globin but not haemoglobin.[17]

Falcipain-2 is active against denatured globin and inactive against the native haemoglobin[20] as falcipain cannot degrade native haemoglobin without first reducing the conditions that denature the haemoglobin.[21] In other studies this reduction of conditions was predicted to be present in the food vacuole and the falcipain was observed to cleave the native haemoglobin.[22] The occurrence of the reducing conditions is controversial, as the redox potential of the food vacuole has not been measured. Haemoglobin undergoes autoxidation in the red blood cell cytoplasm. This causes oxidative damage[23,24], which can be prevented by antioxidants[25] that may be available at high concentrations so as to prevent haemoglobin denaturation in the food vacuole.[21]

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Falcilysin is active in cleaving the peptides, from denatured haemoglobin, to smaller fragments.[20]

The order in which the enzymes act is as follows. Plasmepsins I and II cleave the native haemoglobin, plasmepsin IV and histoaspartic protease further digest haemoglobin and form globin fragments. Falcipain-2 hydrolyses the globin fragments. Falcilysin proteolyses the peptides into oligomers.[17] Serine proteases then hydrolyse the oligomers into amino acids. This occurs in the parasite cytoplasm and is followed by the transport of the amino acids out of the food vacuole,[26] Fig.1.5[27] and Fig. 1.6.[28]

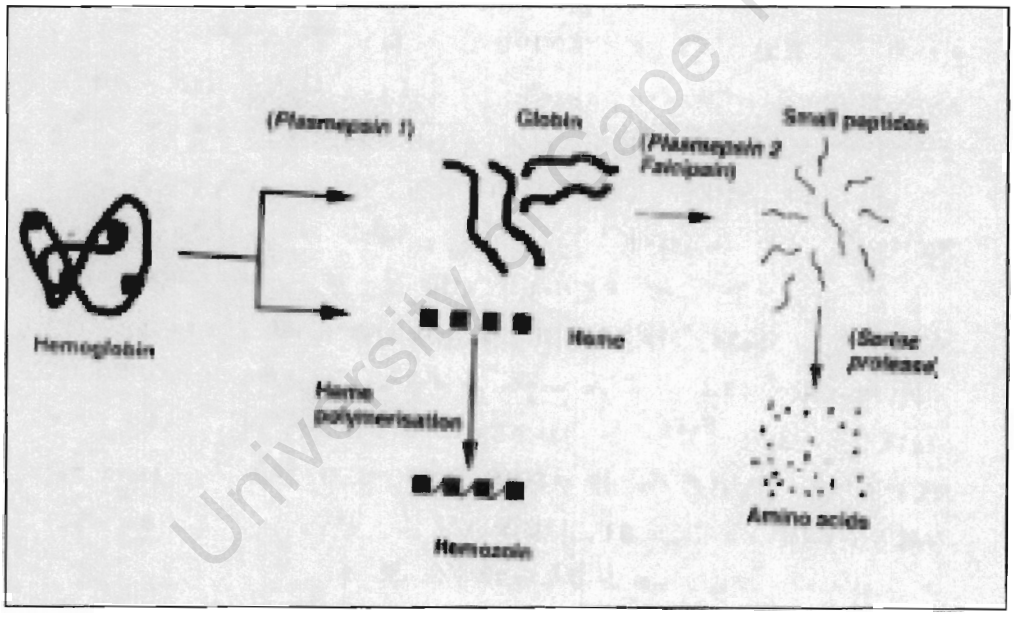

Fig.1.5: Outline of Haemoglobin degration in the red blood cell[27]

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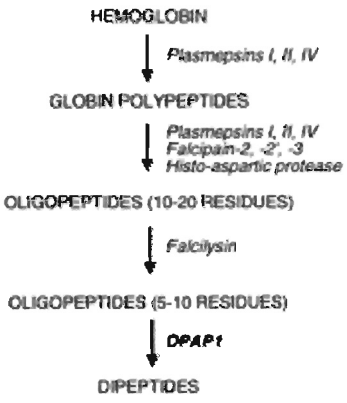

Fig. 1.6: Peptidases contributing to the degradation of haemoglobin inside the *P. falciparum* food vacuole[28]

Spectometric[15] and electorphoretic[29]methods are used to monitor the denaturation of haemoglobin when it is subjected to acidic pH, 4.0 – 4.5. This denaturation process is much slower in the food vacuole (pH 5.2 – 5.6). The activity of enzymes is essential for good separation of the haem and globin. From this a conclusion can be drawn that plasmepsins are required to denature haemoglobin for falcipain activity.[21] Plasmepsins I and II have at least 50 % of the total globin-degrading activity of the vacuole and under non-reducing assay conditions, they are capable of haemoglobin breakdown even when the cysteine protease is inactive.[21]

When plasmepsins I and II digest the haemoglobin *in vitro*, a total of 16 cleavages of about 30 amino acids in size are observed.[30]

The actual position for the aspartic protease attack is in the hinge region of the haemoglobin, which is between α33Phe and 34Leu. Other cleavages occur in other sites. Cleavage causes the tetramer to unravel and thus exposes other sites for proteolytic attack by aspartic and other proteases to continue the degradation.

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There is a difference between the native haemoglobin and the fragmented haemoglobin in that the aspartic protease has more selectivity towards the native haemoglobin than the fragmented haemoglobin. The same α33Phe and 34Leu cleavage is made, but other breaks appear at the same time in the fragmented haemoglobin, for example fragment 1 – 105, which is a peptide that appears from the cleavage at the 105 – 106 bond, is observed before the cleavage at 33 – 34.[31]

1.3.2 Haematin and Haemozoin

During the haemoglobin degradation, haem in the ferrous form is released as a by-product and autoxidised by O_2 to α haematin (aquaferriprotoporphyrin IX or H_2O-Fe(III)PPIX)[32], which is in the ferric form. The α haematin is toxic to the parasite, so it gets converted into a non-toxic form which is an insoluble pigment known as haemozoin, a highly insoluble crystalline substance. The majority of the α haematin is converted into haemozoin and the rest is degraded into free iron, but there may no evidence to support this as yet.

1.3.2.1 Haemozoin formation

As seen in the Fig1.5, haem is a by-product after proteolysis of haemoglobin. The iron of heme is oxidised from the ferrous +2 state to the ferric +3 state.[32] Due to haem being toxic to the parasite the haem is converted into haemozoin, which is an insoluble pigment.[32]

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It is not certain how haemozoin is formed in the malaria parasite. There are a number of hypotheses suggested that are listed below.

1) Enzymatic process[33]
2) Spontaneous formation[34]
3) Autocatalytic formation by preformed haemozoin[35]
4) Catalysis by lipids[36,37]
5) Nucleation or catalysis by histidine-rich protein II (HRP-2)[38]

HRP-2 is a histidine and alanine-rich protein, it is localized in several cell compartments including the parasite cytoplasm. The content, in HRP-2, of histidine is 34%, alanine is 10% and aspartic acid is 10 %.

Due to the lipids and HRP-2 reactions being too slow, it has been suggested that they are both involved in the haemozoin formation process.[39] The problem with HRP-2 is that it is not in the food vacuole but the red blood cell cytosol. Some of the HRP-2 is taken into the food vacuole, but this method of introducing a protein is inefficient, especially considering that the HRP-2 is supposed to catalyse the formation of haemozoin in the food vacuole.[40] Further evidence against the HRP-2, is the fact that a *P. falciparum* clone lacking the HRP-2 and HRP-3 still managed to form haemozoin.[41] In the defence of this hypothesis, the HRP-2-Fe(III)PPIX complex has peroxidatic activity which means the role of HRP-2 is to protect the parasite from oxidative effects of haematin.[42] Another factor is that a dendrimer model containing histidine containing a repeat peptide unit in HRP-2 is active in promoting β-haematin formation.[43] Thus the role of HRP-2 cannot be excluded.

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Haematin does not form stable complexes with carboxylates, unless they are at a very high concentration.[44] This observation led to the following assumption, that the carboxylate group is unable to displace the water or hydroxide from the coordination site of Fe(III)PPIX. Thus the coordination site of the Fe(III), in haemozoin, and propionate groups is unable to explain the stability. It was concluded that the overall thermodynamic stability was responsible for the stability resulting from the crystal lattice formation. This is not present in haematin.[45] If this hypothesis is correct then it implies that β-haematin exists as a solid crystalline material.

The role of the lipid nucleation, HRP-2 or other proteins as well as the catalysis of β-haematin formation appears mainly to shift the solubility limit of haematin to a higher concentration, thus inducing insoluble, crystalline haemozoin formation.[11]

As it is thought that the haemozoin formation process is facilitated in some way. Four processes were used to mediate the formation:

1) Haem polymerase[46]
2) Haem-derived material[35]
3) Phospholipids[37]
4) A nucleating template protein.[38]

Native haemozoin formation was originally thought to be catalysed by a parasite-dependent activity with a haem polymerase enzyme.[46] Acetonitrile extracts of native haem, that is free of detectable haem caused fast haem polymerization.[47] A similar fraction with phospholipid-like chromatographic properties could be readily extracted from β-haematin that was chemically synthesized from commercially available haem, by

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heating a haem chloride solution to 70°C at acid pH.[47] These properties supported the haem polymerization at 37 °C like haemozoin. It was then rationalized that if the activity was due to a haem-derived material as opposed to an enzyme, then it should be possible to extract a solubilised haem-polymerised activity from synthetic β-haematin.[35] This was proved by experiments that showed the acetonitrile extracts from synthetic β-haematin, trophozoite lysate and haemazoin can all support haem polymerization. Fast polymerization of haem is promoted by phospholipids and thus may play a role in the formation of haemozoin by malarial parasites.[35] The phospholipids account for the acetonitrile extracts but not whether the polymerization of haem in the malaria-infected erythrocytes is affected by the activity of these lipids.[35]

Another suggestion is that due to the rapid formation of the haemozoin polymer, catalysis may be involved.

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1.3.2.2 Structure of haemozoin

The proposed structure of haemozoin is the co-ordination polymer of Fe(III)PPIX. The oxygen of the propionate group from one unit is an axial ligand to the five co-ordinate ferric iron of another unit. This structure has been proved with IR, Raman spectroscopies and EXAFS for the Fe-O (propionate) linkage.

Another structural model was suggested by Bohle *et al* [48]. A dimerisation of two linear polymer chains hydrogen bonded through the propionic group.

A third model was suggested by Pagola. It was proposed that haemozoin was a dimer of haem units that were linked by reciprocating iron-carboxylate bonds to a propionic side chain of each porphyrin. The dimers are hydrogen bonded into chains on an extended

crystal network, Fig 1.7.[49] When the crystal structure[49] was solved by x-ray powder diffraction, it was found that haemozoin was not a polymeric form of Fe(III)PPIX but a dimer and thus Pagola *et al*'s earlier proposed structure was correct.

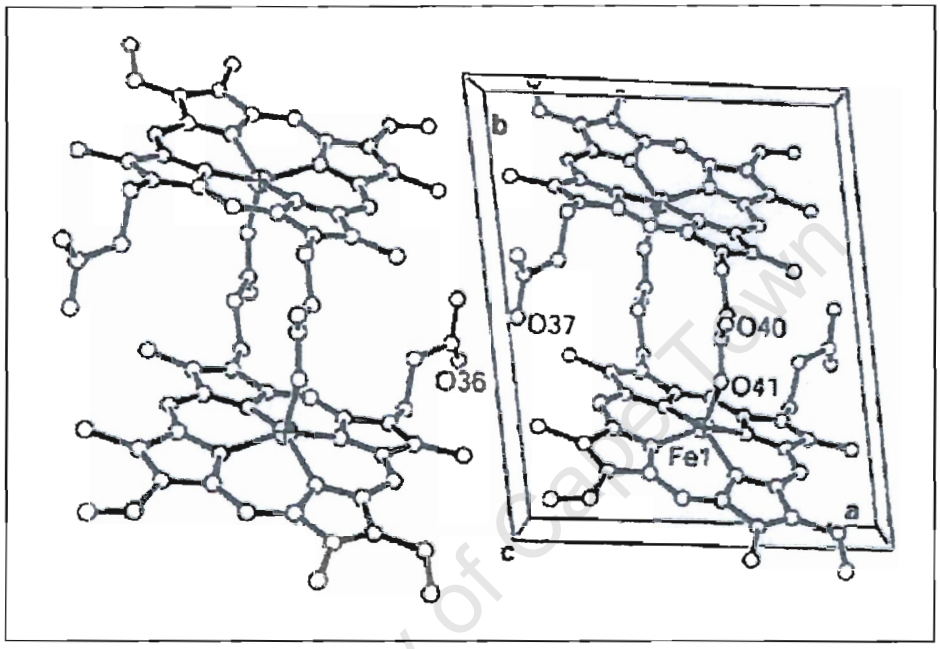

Fig.1.7 Molecular Structure of Haemozoin[49]

1.3.2.3 Structure of β-haematin

β-haematin is also called synthetic haemozoin. Structurally it is identical to natural haemozoin. It can be prepared synthetically by heating a haem chloride to 70°C at an acid pH in the presence of haemazoin or β-haematin.[35] Other methods include an acidic aqueous solution in the presence of organic acids,[50] lipids,[36,51] preformed β-haematin,[51,35] parasite extracts[33] or histidine rich protein 2[35] at 37°C or higher. Another method for the formation of β-haematin is in a non-aqueous solution of haemin (Cl-Fe(III)PPIX) from which HCl is abstracted by 2,6-lutidine in dry methanol.[52]

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Buller *et al*[53] have observed that a solid phase, which is closely related but not identical to β-haematin, may form and then disappear during the conversion of haematin to β-haematin. It is poorly crystalline but seems to have the same bonding.[54] It has been observed that two reciprocal β-haematin dimers can be formed, with propionate coordination to Fe(III); one with a center of inversion and the other without a center of inversion, Fig. 1.8[53] The dimer with the center of inversion is the β-haematin. From this Buller *et al* suggested that both dimers form initially but cannot be incorporated into the crystal. Thus the formation of β-haematin requires a rearrangement of the non-centrosymmetric (right dimer) into the centrosymmetric (left dimer) form. They have also suggested that the non-centrosymmetric isomer may be the reason the growth of β-heamatin crystals is limited.[53]

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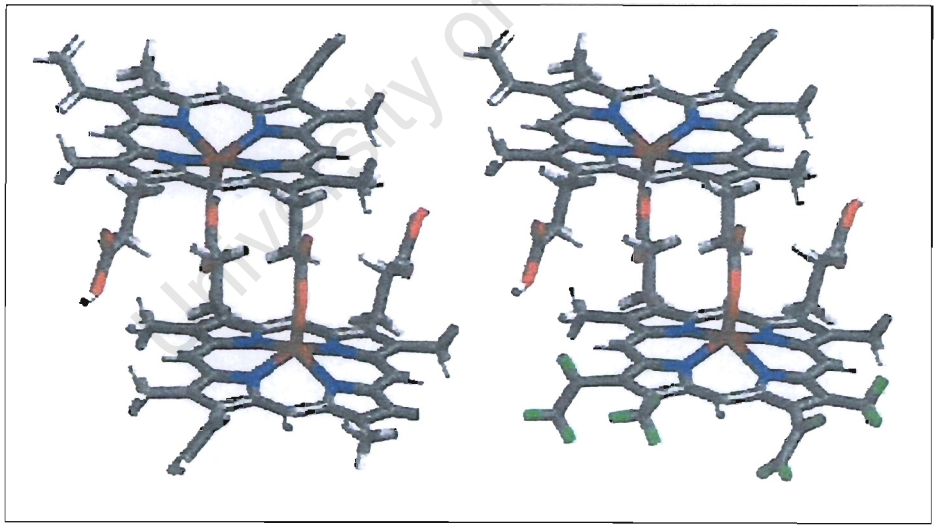

Fig. 1.8 Reciprocal dimers of Fe(III)PPIX with propionate coordination to Fe(III)[53]

1.4 Drug resistance

The disease has started to spread to new areas of the world due to land utilization being changed, global ecosystems and weather patterns changing due to global warming[32]. This has caused epidemics in the new areas; the area that has been affected the most is East Africa. As malaria has not been brought under control in Africa and Southeast Asia, areas that were free of malaria are now infected again. Another reason why malaria has spread is due to resistant strains of the parasite reducing the efficacy of most antimalarial drugs, for example chloroquine[32].

The definition of drug resistance in malaria is the "ability of a parasite strain to survive and/or to multiply despite the administration and absorption of a drug given in a dose equal to or higher than those usually recommended but within the limits of tolerance of the subject."[55]

1.5 Introduction to chemotherapy

According to Paul Erlich "the principal of chemotherapy was that a chemical could directly interfere with the proliferation of microorganisms at concentrations tolerated by the host. "[58] This was also known as the "magic bullet"[56] due to the chemical's capability of finding the microorganism and destroying it without affecting the host. This is achieved by the chemical showing greater toxicity to the microorganism than the cells of the host. This selectivity was shown as a chemotherapeutic index, showing the minimum effective dose of the drug against the maximum dose that can be tolerated by the host.[56] The therapeutic index, which is currently used, is the ratio of the unwanted effects of the drug relating to the wanted effects. It is also a measure of how safe a drug is, the larger

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the index the safer the drug. A more accurate definition is the dose levels of the drug that leads to 50% of cases being toxic. This is a better method than the chemotherapeutic index as it removes any unusual individual results/ anomalies.[56]

1.6 Antimalarial chemotherapy

The earliest antimicrobial agents came from the bark of the cinchona tree and extracts of the wormwood plant. Quinine was first isolated in 1820 from the cinchona bark. The screening of compounds has given rise to the development of synthetic antimalarials that are effective, for example chloroquine.[57]

It has been found in studies[57] that the 4-aminoquinoline antimalarials, for example chloroquine and amodiaquine, as well as the quinoline methanols, for example quinine and mefloquine, are dependent on the degradation of haemoglobin. The drug target of these antimalarials is thought to be one of these three sites: the inhibition of the proteinase enzymes, a peptide product of the haemoglobin or hematin. It is known that the 4-aminoquinolines and quinoline methanols form complexes with haematin.

The resistance to chloroquine has been a major setback in fighting the disease, as chloroquine was cheap, safe due to limited toxicity, an oral drug, has good absorption and tolerability and a highly efficacious drug. Other antimalarial drugs (Table 1) have been developed but they are not as safe or efficient as chloroquine and it has also been found that resistance to these compounds is increasing.[57]

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For example, quinine has slight toxic effects and some resistance to this drug is known. Mefloquine is expensive. Halofantrine has side effects of toxicity and is expensive. Sulfadoxine, pyrimethamine, cycloguanil or proguanil are ineffective against resistant parasites. Atovaquone has a recurrence of resistant parasites and primaquine is toxic. These drugs do have advantages though. Mefloquine has prophylactic uses. Halofantrine has strong activity and is usually effective against the resistant strain of *P. falciparum.* Sulfadoxine and pyrimethamine are inexpensive and have a synergistic effect when used in combination. Cycloguanil or proguanil are safe and have prophylactic uses. Atovaquone is safe and primaquine clears recurrent malaria in the liver and thus is the only drug used to cure *P. vivax* and *P. ovale.*[57]

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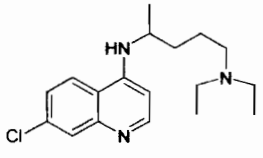
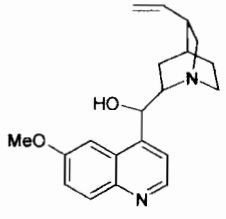
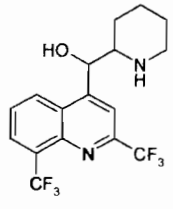
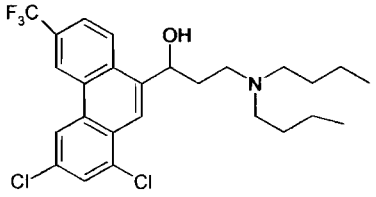
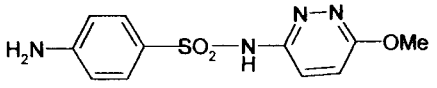
Table 1: Structure of Antimalarial Drugs

Name	Structure
Chloroquine	
Quinine	
Mefloquine	

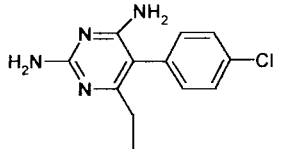
Halofantrine



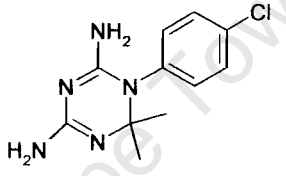
Sulfadoxine



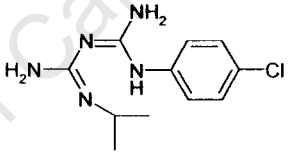
Pyrimethamine



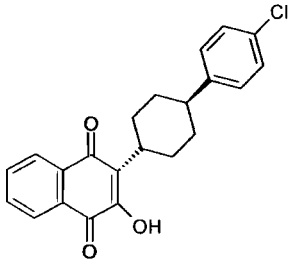
Cycloguanil



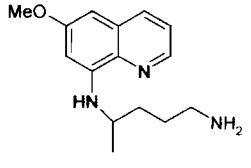
Proguanil



Atovaquone



Primaquine



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1.7 Current antimalarial research

Fig.1.9[27] shows where the drug effects can occur within the food vacuole. The first drug effect is to form a complex with haem, thus inhibiting haemozoin formation. The second drug effect is to inhibit the haem polymerisation, once again inhibiting the formation of haemozoin. The third drug effect is inhibition of haemoglobin degradation by proteases, plasmepsins I and II and falcipain. The fourth drug effect is the interaction with the haemozoin. The reason for this approach is that interaction between the drug and haemozoin would prevent haemozoin from growing further due to the growth sites being blocked by the drug.[27]

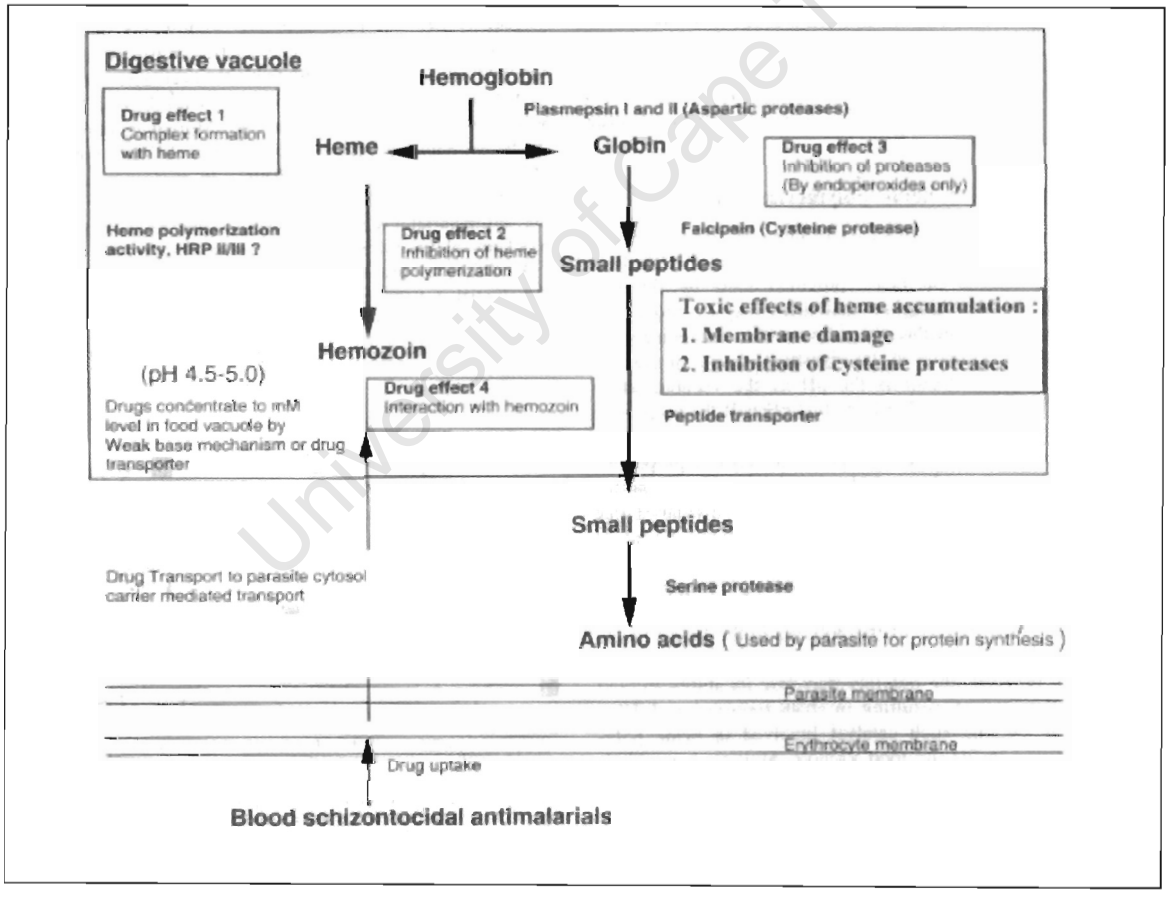

Fig. 1.9: Effects of Drugs within the food vacuole[27]

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1.7.1 Malarial proteases as targets for chemotherapy

Some of the research that is currently ongoing is focused on malarial proteases as targets for chemotherapy. The reason the proteases are a target is because in the life cycle (Fig 1.3) the merozoites invade erythrocytes, which then develop into trophozoites and then into schizonts. These schizonts rupture the erythrocytes releasing more merozoites. So the proteases seem to be required for rupture and invasion of erythrocytes by merozoite stage parasites and for the degradation of haemoglobin by the intraerythrocytic trophozoites.[57]

Figure 1.10 shows the protease targets in the erythrocytic malaria parasite. CP refers to cysteine protease, SP to serine protease and AP to aspartic protease. These proteases are used at different stages as chemotherapeutic targets.[57]

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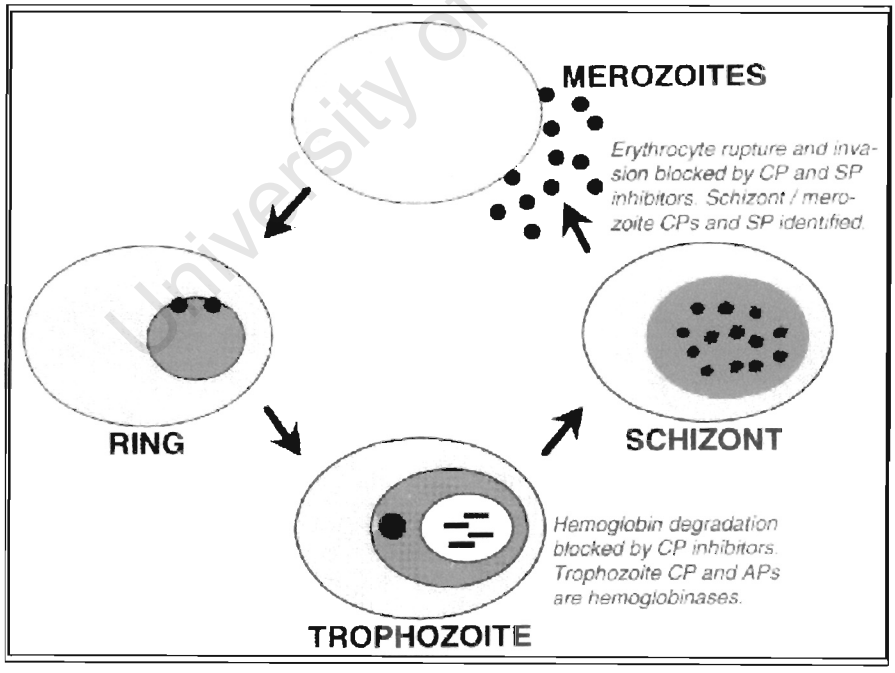

Fig. 1.10. Protease targets in erythrocytic malarial parasites[57]

1.7.1.1 Aspartic proteases

There are four aspartic proteases that have been identified and characterised in the food vacuole, plasmepsins I, II, IV and histo-aspartic protease (HAP). These proteases have an acidic pH and share sequence homology with other aspartic proteases. Plasmepsin I inhibitors could prevent the degrading of native haemoglobin into denatured haemoglobin. Plasmepsin II prefers the denatured haemoglobin as a substrate. Thus the plasmepsin II inhibitors could prevent the further digestion of haemoglobin, which forms the globin and haem fragments. If the initial step is inhibited the plasmepsin I would not be able to cleave the haemoglobin and unravel the tetramer.

1.7.1.2 Cysteine proteases

Cysteine protease inhibitors can prevent the denaturation of the haemoglobin molecules and block the separation of haem from globin when incubated with cultured malarial parasites. This was proved due to the fact that the majority of haemoglobin that had accumulated in the parasites appeared to be partially denatured. This implies that the cysteine protease falcipain is involved in haemoglobin degradation. The falcipain has been shown to hydrolyse globins, because inhibitors of this cysteine protease have been shown to inhibit the hydrolysis of globins.[29] The partial block in haemoglobin denaturation likely contributes to the antiplasmodial effects of cysteine protease inhibitors. The retention of haem in haemoglobin has also been evaluated in protease inhibitor-treated parasites. Due to the accumulated haemoglobin in parasites, a large amount of haem was seen to be retained. Falcipain thus has two important roles:

1) separating haem moieties from haemoglobin

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2) haemoglobin degradation.

From studies it has been shown that the inhibition of falcipain activity starves the parasite of amino acids. Falcipain inhibition may also limit supplies of iron that are required for the synthesis of iron-containing proteins,[15] including ribonucleotide reductase,[58] and for the synthesis of parasite-derived haem.[16] The studies have also shown that a block in the separation of haem from globin may allow the persistence of haem-globin complexes. These complexes would be expected to limit the haemozoin formation. This has been shown to occur with specific cysteine protease inhibitors and antimalarials, for example chloroquine and artemisinin. Free haem is toxic to malaria parasites, the antimalarial effect of chloroquine has been justified by haem-chloroquine complexation.[60] Heamoglobin complexes that accumulate under these conditions may be toxic to the parasite.

All of the above implies that cysteine proteases are potential targets for antimalarial chemotherapy.

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1.7.2 Inhibition of haemozoin formation

Quinoline based antimalarials inhibit haemozoin formation by forming complexes with haematin. These complexes block haemozoin formation and prevent the removal of haematin from the parasite.

It is believed that the complexes occur due to the π-π interactions between the quinolines and haematin. This means that there is a co-planar interaction between the aromatic ring in the quinoline compound and the porphyrin unit.[32]

It is also thought that a dimer of haematin and the quinoline interact through hydrophobic interactions. A good example of such a quinoline compound is chloroquine. Fig. 1.11 demonstrates the moieties in chloroquine that are important in β-haematin formation.[32]

It has also been found that in cultured parasites, haemazoin formation can be inhibited by the antimalarial pepstatin.[60]

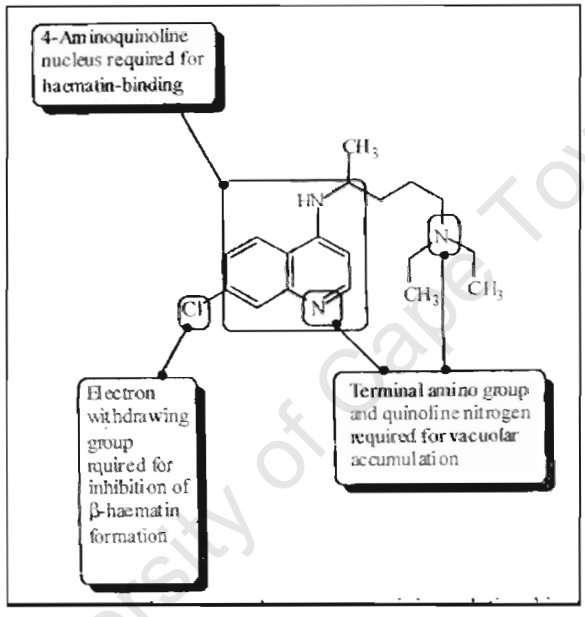

Fig. 1.11 Structure-activity relationship of Chloroquine[32]

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1.7.3 5-Hydroxytryptamine receptor as a target for antimalarial chemotherapy

Some serotonin receptor agonists were found to have antimalarial activity during the screening of plant extracts, which are used in traditional Polynesian medicine as well as other natural products for antimicrobial and antiviral activity[61]. The importance of this receptor is due to the fact that when inhibited, membrane channels on the parasitized erythrocyte are blocked thereby providing new treatments for the *Plasmodium falciparum* strain of malaria. Three serotonin receptor agonists have been found to markedly inhibit

the growth of *P. falciparum*. These agonists are 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) (**1**), 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) (**2**) and 4-bromo-2,5-dimethoxyphenylamine (2-CB) (**3**), the IC_{50} values found were 0.4, 0.7 and 1.5 μM respectively, fig 1.12. The inhibition of *P. falciparum* growth was correlated to the affinity of the serotonin receptor agonists for the $5HT_{1A}$ receptor. Serotonin receptor antagonists have lower antimalarial activity, which is not correlated with the serotonin receptor affinity.[61]

The antiparasitic activity of 8-OH-DPAT was found to be specific to a chloroquine-sensitive strain and not cytotoxic. When this agonist and chloroquine were combined, the activity was found to be in the nanomolar concentrations against the chloroquine-resistant parasites.[61]

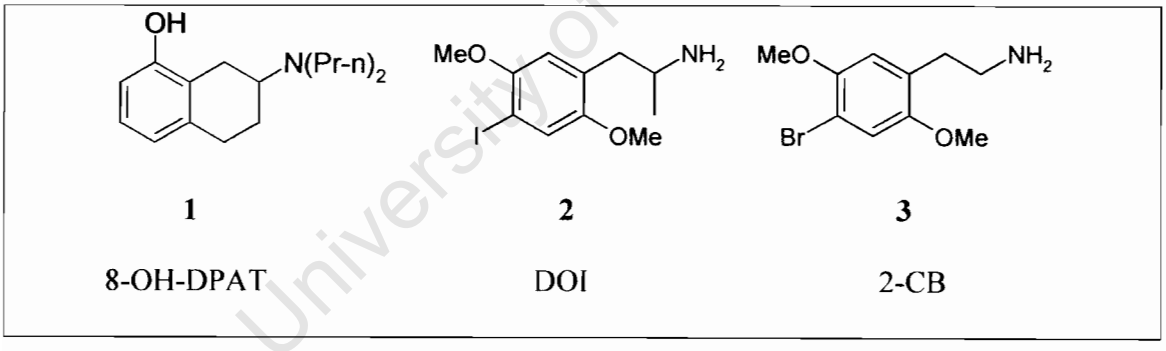

Fig. 1.12 Structures of serotonin agonists

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CHAPTER 2

ARYLPIPERAZINES AS PRIVILEGED SUBSTRUCTURES FOR ANTIMALARIAL DRUG DISCOVERY

2.1 Background

2.1.1 Privileged substructures

A privileged structure was first defined by B.E. Evans *et al* in 1988, as a "singular molecular framework able to provide ligands for diverse receptors".[62,63] The use of privileged structures or substructures for drug discovery is popular in medicinal and combinatorial chemistry. A privileged structure can also be defined as a molecular framework that binds to various proteinaceous surfaces with high affinity. Libraries of compounds can be synthesized around a scaffold based on privileged structures and cross-screened in various disease models.

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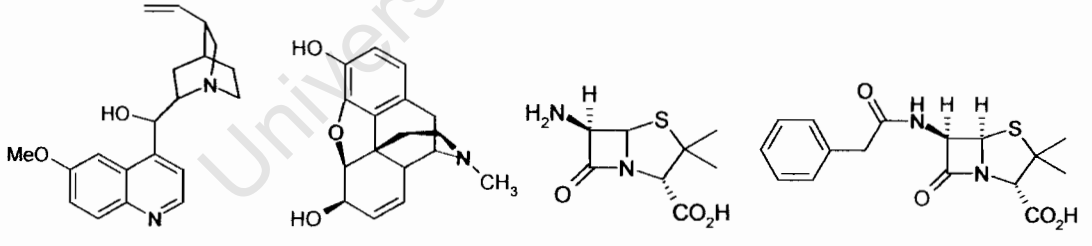
There are two types of privileged structures. Natural products and organic scaffolds.[62]

2.1.1.1 Natural products

Natural products have been a good source of important lead compounds, especially against infectious diseases. Quinine (**4**) is an alkaloid and the most important lead compound against malaria. It was used as a template for the discovery of chloroquine and mefloquine.

Other examples of natural products with medicinal properties are morphine (**5**) and penicillin (**6a**). Morphine is found in poppy seeds and is an analgesic, but has serious side effects, such as constipation, respiratory depression and addiction. Other compounds, similar to morphine, have been synthesized in an attempt to reduce the side effects as chemical modifications can have a huge impact on activity.[56]

Alexander Fleming discovered penicillin after he noticed that bacterial cultures were killed by mold. Penicillin is one of the most effective antibacterial agents, as well as one of the safest, ever found. But it has not been able to kill all known bacteria, in fact over time some bacterial strains have become resistant.[56] Penicillin analogues have been synthesized and penicillin G (**6b**) is one of these analogues. Penicillin G is non-toxic although it has disadvantages, for example allergic reactions. It is also ineffective when taken orally.



4 Quinine

5 Morphine

6a Penicillin

6b Penicillin G

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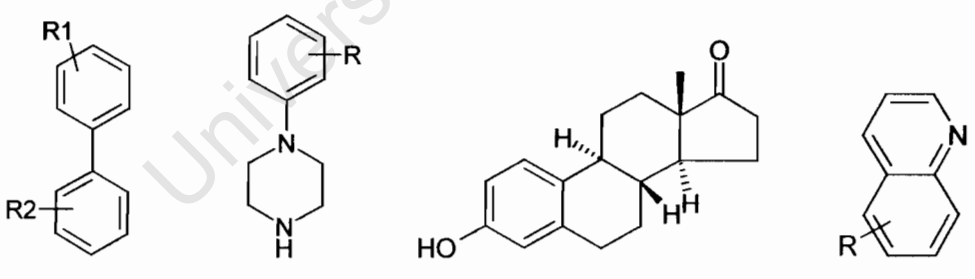
2.1.1.2 Organic scaffolds

When using an organic scaffold, a library of compounds is synthesized based on one core scaffold. This library is then screened against different receptors to, hopefully, obtain several active compounds. Organic scaffolds are not structures in their own right as they include only a subsection of the molecule. Natural products dominate the organic scaffolds, as the scaffold is based on the active framework of the natural product. The privileged structure can achieve selectivity and specificity, with the receptor, depending on the side chain attached to the scaffold.[62]

Some examples of organic scaffolds include biphenyls (**7**), arylpiperazines (**8**), steroids, such as oestrone (**9**), and quinolines (**10**). In this chapter arylpiperazines will be discussed in greater detail.



7 Biphenyls

8 Arylpiperazines

9 Oestrone

10 Quinolines

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2.1.2 Arylpiperazines as Drug Leads

Arylpiperazines have a wide variety of receptors.[62] The arylpiperazine substructure has been found in a large number of compounds that have pharmaceutical properties. The MDL Drug Data Report in 2001 showed 2271 drugs with phenylpiperazine structures, 65 of which were in Phase II clinical trials in 23 therapeutic fields.[62] These structures included compounds that were used as antibacterials, antidepressants, antivirals, antifungals, antitussives and serotonin receptor (5HT) antagonists to name a few. The arylpiperazine substructure is active against the majority of the serotonin receptors.[62] An example of an antidepressant with a phenylpiperazine structure is nefozodone (**11**), also known as serzone. Unfortunately due to adverse effects, including liver failure, this drug has been discontinued.[64] Nefozodone was also found to be a $5HT_{2A}$ receptor antagonist.[65] Another example of an antidepressant is trazodone (**12**).[67] Two examples of antifungal drugs that have the phenylpiperazine moiety are itraconazole (**13**), also known as sporanox, and saperconazole (**14**).[65] One example of an antiviral is R-61837 (3-methoxy-6-[4-methylphenyl]-1-piperazinyl]pyridazine) (**15**)[65] while levodrapropizine (**16**)[65] is an example of an antitussive agent.

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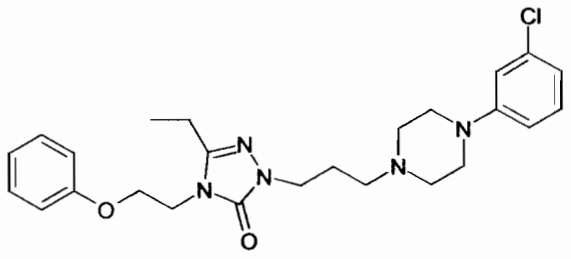
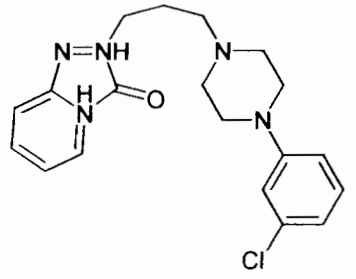


11
Nefozodone



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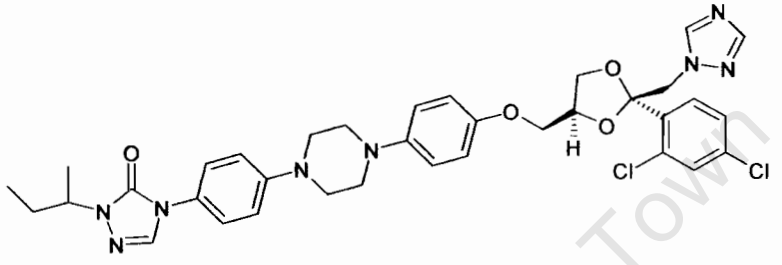
Trazodone



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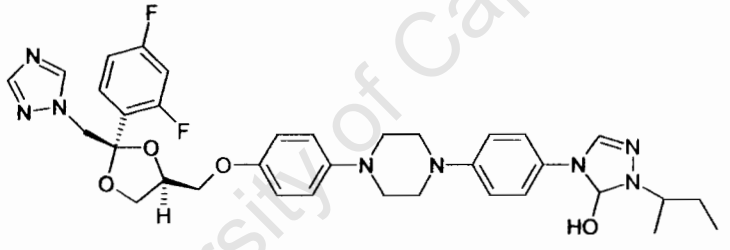
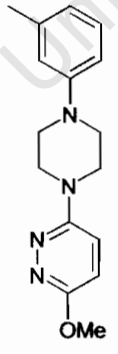
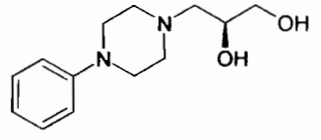
Itraconazole



14
Saperconazole



15 (R-61837)



16 (Levodropropizine)

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2.1.3 Arylpiperazines as Agonists for the Serotonin Receptor

Serotonin or 5-hydroxytryptamine (5HT) is a neurotransmitter, or a chemical messenger[56], in the central nervous system. There are seven main types of serotonin receptors, which can be separated into subtypes. These 5-HT receptors are mostly postsynaptic (a synapse is a connection between two neurons, postsynaptic refers to the second receiving cell.)[64] except for 5-HT1A and 5-HT1B, which are mostly presynaptic (the cell that releases the neurotransmitter)[64]; they also modulate the release of serotonin.[67]

Serotonin interacts with a range of membrane-bound receptors. These 5HT receptors are found in the central and peripheral nervous system, nonneuronal tissues and cardiovascular system. It controls the central functions and has a role in the central nervous system.[68]

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2.1.3.1 Biosynthesis of serotonin

The biosynthesis of serotonin from tryptophan is a two-step process (Fig 2.1). The L-tryptophan is hydroxylated, with tryptophan hydrolase as an enzyme, into 5-hydroxytryptophan. This process also requires tetrahydrobiopterine, oxygen, NADPH2 and a metal (copper or iron). The 5-hydroxytryptophan undergoes a decarboxylation to give serotonin. This process also requires an enzyme, L-aromatic amino acid decarboxylase, as a catalyst and pyridoxal-phosphate as a coenzyme.

Tryptophan is ingested daily in about 0.5g – 1g quantities. Of this only 0.2g is required and only a portion of that is converted into serotonin. The biosynthesis of serotonin depends on the amount of tryptophan that crosses the blood brain barrier (BBB). There are two factors that decrease the amount of tryptophan that can cross the blood brain barrier;

1) The tryptophan has to be free in the plasma in order to cross the BBB, decrease of the free ratio decreases penetration.
2) Other amino acids also cross the barrier. This causes competition between them and thus limits the tryptophan that crosses the barrier.[56]

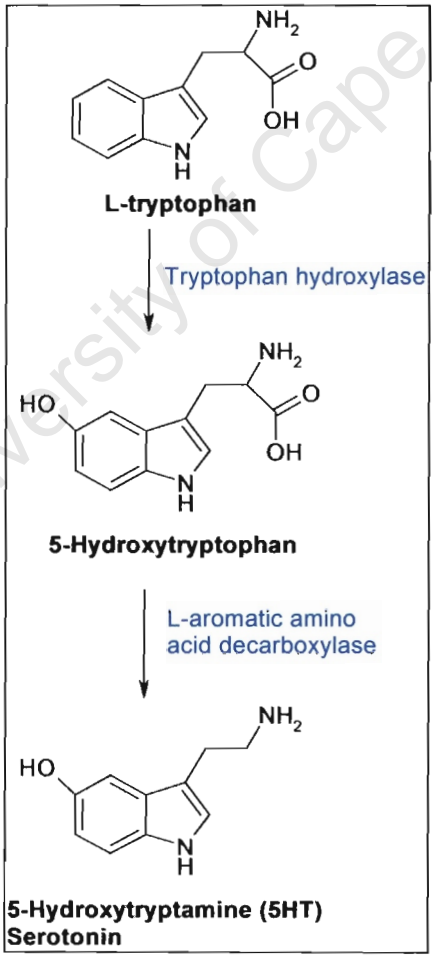

Fig 2.1 Biosynthesis of serotonin

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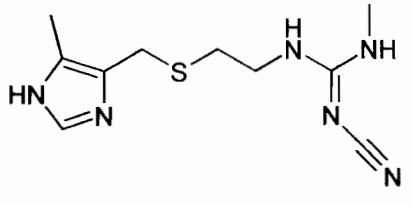
2.2 Role of receptors

Receptors are one of the main targets for drugs. Chemical messengers, or neurotransmitters, come from the nerve cell and interact with the receptor, which is a protein embedded in the cell membrane. The result of this interaction causes ions to flow across the cell membrane and/or enzymes to switch on or off in the target cell. This then results in a biological response. As this process is started by a chemical messenger it means that other chemicals, for example drugs, can interfere in the process. There are four different types of such drugs namely: antagonists, agonists, partial agonists and inverse agonists.[56]

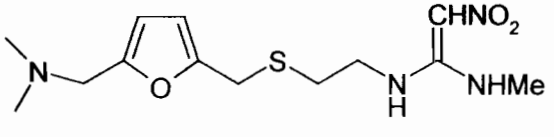
2.2.1 Antagonist

An antagonist is a drug that interacts with a receptor to block either the agonist or the natural messengers, this interction occurs when there are too many messengers released and the target cell cannot deal with all the messengers.[56] For example, cimetidine (**17**) inhibits the H2-receptor and therefore inhibits gastric acid release. Cimetidine, which is also known as Tagamet, was marketed in the UK in 1976 and was the biggest selling anti-ulcer drug until 1988 when ranitidine (**18**) was developed.



17

Cimetidine



18

Ranitidine (Zantac)

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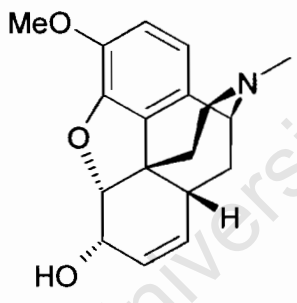
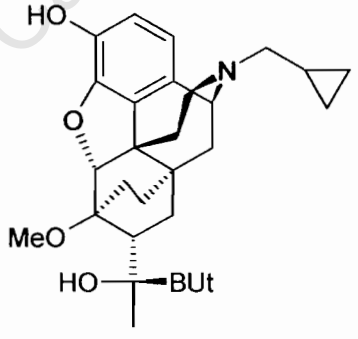
2.2.2 Partial agonist

A partial agonist is a drug that acts as an agonist and produces a biological response, but this response is not as strong as the effect of a full agonist. A partial agonist also acts like an antagonist by blocking the agonist.[56] Two examples of partial agonists are codeine (**19**) and buprenorphine (**20**). Codeine is an analgesic, but has a lower efficacy than morphine. Its analgesic effect is due to the demethylation to morphine in the liver. It has a significant antitussive effect and induces constipation. Buprenorphine is a lipid soluble compound and acts as a partial agonist. It also has less efficacy than morphine, which leads to the re-emergence of pain in patients. However, it has a longer duration of action compared to morphine and is more emetic. It can also induce dysphoria.



19

Codeine



20

Buprenorphine

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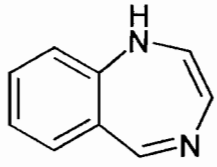
2.2.3 Inverse agonist

An inverse agonist acts like an antagonist, it binds to the receptor like an agonist but does not produce a biological response and then blocks the normal chemical messenger from binding.[56] Benzodiazepine (**21**) is an example of an inverse agonist. Such compounds

decrease gamma aminobutyric acid (GABA is an amino acid that is in the central nervous system and acts as an inhibitory neurotransmitter) potency. This class of compounds can also act as agonists (by increasing the inhibitory transmitter GABA potency) and as antagonists (by blocking the action of the agonist).



21

Benzodiazepines

The agonists reduce anxiety, muscle tension, convulsions, vigilance and memory. The inverse agonists increase these effects and the antagonist prevents the effects of both types of agonists by blocking their access to the receptor. Fig. 2.2 shows the other benzodiazepine receptor-ligand interactions.

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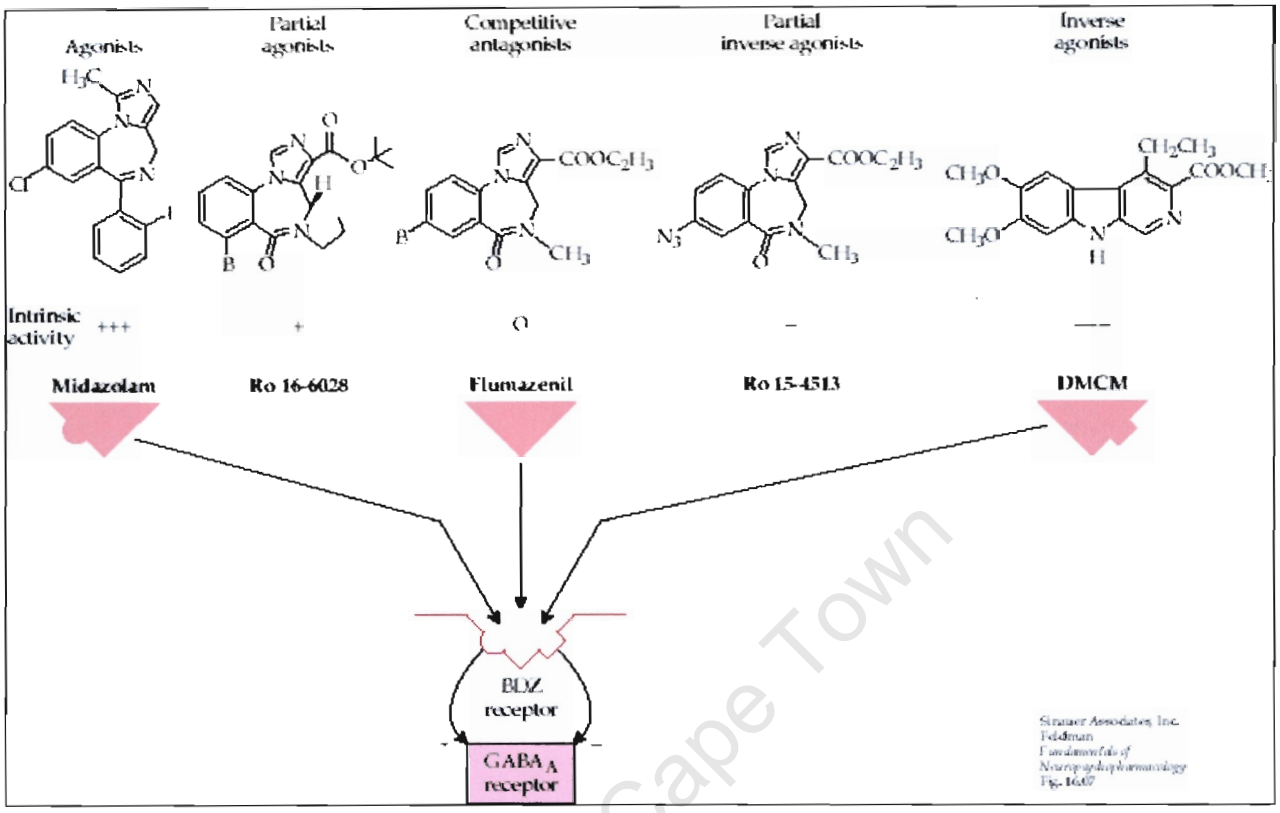
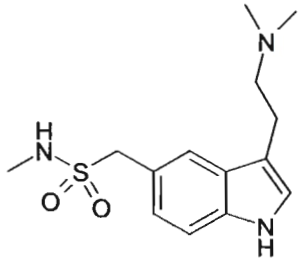


Fig. 2.2 Benzodiazepine receptor-ligand interactions.

2.2.4 Agonist

An agonist is a drug that produces a response at a particular receptor. An agonist is also used when there are too few messengers released and the target cell becomes a bit slow and unproductive.[56] An example of an agonist at the 5-HT_1 receptor is Sumatriptan (**22**).



(22)

Sumatriptan

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2.2.5 Designing an agonist

The drugs that are designed should mimic the natural neurotransmitters. Once it is known what binding groups are present in the receptor the drug can be designed to interact with these groups. There are three criteria to consider when designing an agonist.[56]

1) The correct binding groups should be present in the drug.
2) The binding groups must be in the correct position.
3) The drug must have the correct size to fit in the binding site.

2.3 Arylpiperazines as Potential Antimalarial Agents

2.3.1 5HT receptor agonist: 8-hydroxy-2-(di-*n*-propylamino)tetralin as an Antimalarial Agent

In recent studies, 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT)[69] (**1**) has been identified as a good compound for the inhibition of the 5-hydroxytryptamine type 1A ($5HT_{1A}$) receptor.[61] This is important in malaria as it could lead to new treatments for *Plasmodium falciparum* malaria due to a recent finding that 8-OH-DPAT inhibits the growth of *P. falciparum* by blocking a membrane channel on parasitized erythrocytes.[62] The use of 8-OH-DPAT as an anti-malarial is unlikely due to the neurological side effects that are possible. This is known as the serotonin syndrome. However, the serotonin receptor agonists may be useful as a way of characterizing the membrane transport properties of the malaria parasite and for new lead compounds in the treatment of malaria. This *P. falciparum* receptor could be a significant target for malaria chemotherapy.

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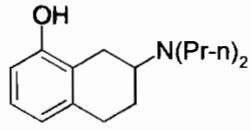

1

8-OH-DPAT

2.3.2 Background to MSc project undertaken

In a previous study, totarol (**23**) that has been isolated from a large variety of plants and shown to have a potent *in vitro* antibacterial activity was used as a scaffold to synthesise a series of β-amino alcohol derivatives.[70] As part of the antiplasmodial screening of the target amino alcohol derivatives of totarol, the starting arylpiperazines, morpholine and piperidine amines were also tested. Among the amines tested, the arylpiperazines phenylpiperazine (**24**), 2-chlorophenylpiperazine (**25**) and 2-ethoxyphenylpiperazine (**26**) were found to be significantly more potent against a chloroquine-resistant (K1) strain than against a chloroquine-sensitive (D10) strain. The presence of a chloro and ethoxy group in the *ortho* position of phenylpiperazine delivered a 2-fold increase in potency against both D10 and K1. In the same assay the 7-chloro-4-aminoquinoline-based piperazine (**27**) was found to be almost equipotent against both strains, a result noted to be in marked contrast to the aforementioned three arylpiperazines. Coupled with the potential role of arylpiperazines as replacements for the antimalarial 8-OH-DPAT, these results prompted a further investigation into the antiplamodial properties of a broader range of simple unsubstituted and substituted arylpiperazines against a broader range of chloroquine-sensitive and chloroquine-resistant strains of *Plasmodium falciparum*.

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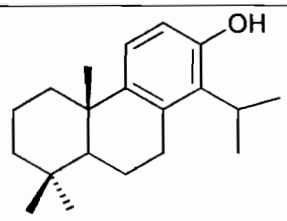
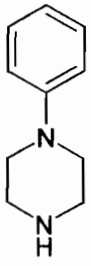


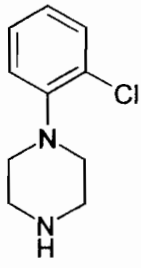
23

Totarol



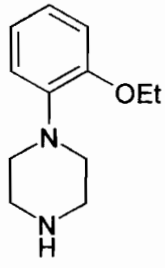
24

Phenylpiperazine



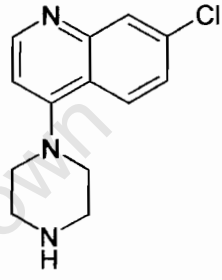
25

2-chlorophenyl piperazine



26

2-ethoxyphenyl piperazine



27

7-chloro-4-aminoquinoline piperazine

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2.4 Objective of this project

To carry out a preliminary structure-activity relationship study of arylpiperazines as antimalarial agents against chloroquine-sensitive and chloroquine-resistant strains of the malaria parasite *Plasmodium falciparum.*

2.5 Aims for this project

1) Synthesis and preliminary structure-activity relationship studies of the target exploratory molecules shown in Fig 2.3.

2) Characterization of the synthesized compounds by spectroscopic and analytical techniques.

3) Evaluation of the biological activity of the compounds in collaboration with appropriate laboratories.

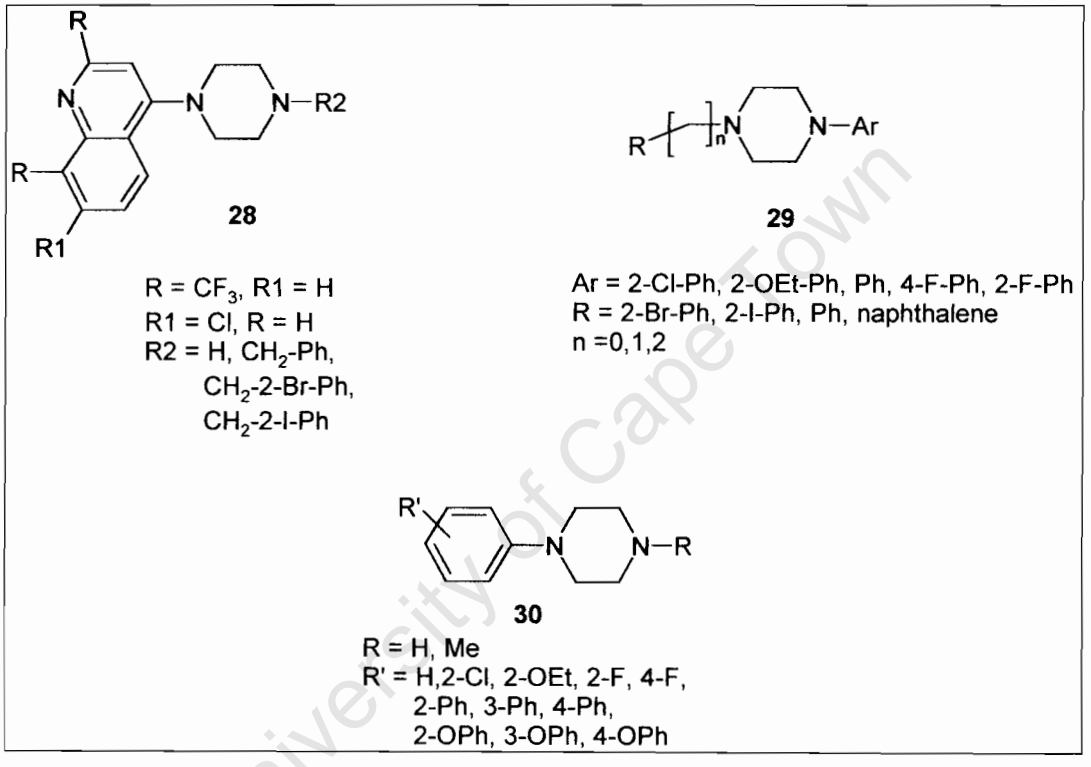

Fig. 2.3 Target compounds

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CHAPTER 3

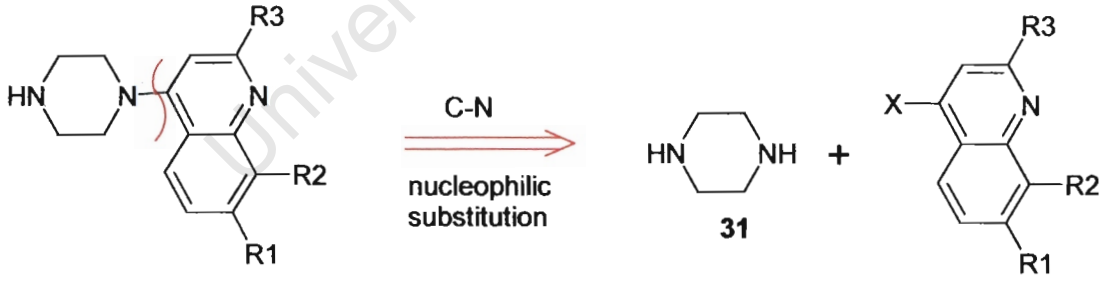
RESULTS AND DISCUSSION:

SYNTHESIS OF EXPLORATORY TARGET COMPOUNDS

3.1 Synthesis of arylpiperazines (CMP10 and CMP15) *via* nucleophilic substitution[71]

3.1.1 Retrosynthetic analysis

From the retrosynthetic analysis (**Scheme 3.1**), it can be seen that the 7-chloro-4-piperazin-1-yl-quinoline (**CMP10**) and 2,8-bis(trifluoromethyl)-4-piperazin-1-yl-quinoline (**CMP15**) can be prepared *via* a one step nucleophilic substitution from commercially available starting materials, piperazine (**31**), 4,7-dichloroquinoline and 4-bromo-2,8-bis(trifluoromethyl)-quinoline (**32**).



CMP10 R1 = Cl, R2 = R3 = H
CMP15 R1 = H, R2 = R3 = CF_3

Scheme 3.1: Retrosynthetic analysis

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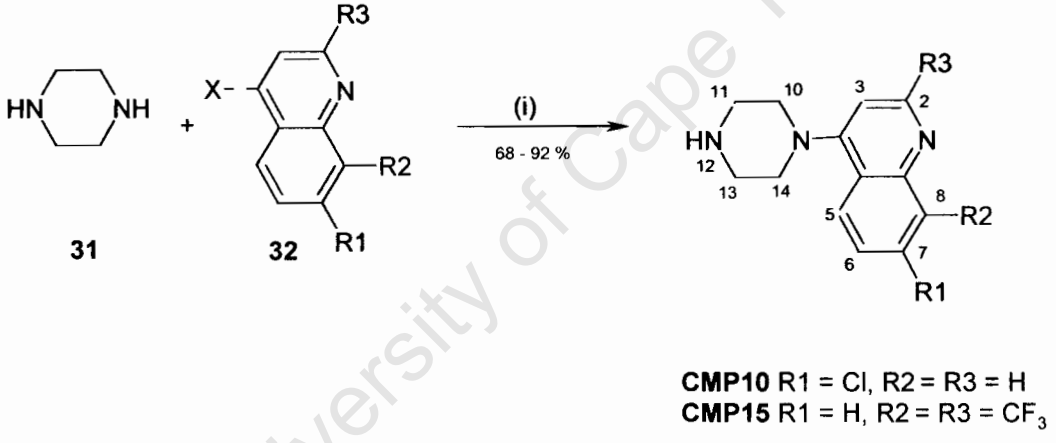
3.1.2 Synthesis[71]

The compounds (**CMP10, CMP15**) were synthesized *via* a nucleophilic substitution reaction by refluxing a mixture of piperazine (**31**), halogenated quinoline (**32**), potassium carbonate and triethylamine in *N*-methyl-2-pyrrolidinone at 135°C for 4 hours under nitrogen, **scheme 3.2**. Column chromatography was used to purify the crude products, with 10% methanol in dichloromethane as eluent to deliver the target compounds 7-chloro-4-piperazin-1-yl-quinoline (**CMP10**) and 2,8-bis(trifluoromethyl)-4-piperazin-1-yl-quinoline (**CMP15**) in 68% and 92% yield, respectively.



Scheme 3.2: *Reagents and Conditions:* (i) K_2CO_3, Et_3N, NMP, 135°C, 4 hrs

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The structures of these compounds were confirmed using ^{1}H and ^{13}C NMR as well as infra-red spectroscopy, mass spectroscopy and micro-analysis. In deuterated chloroform the spectroscopic indicators in the proton NMR spectra for **CMP10** are the doublets at 6.8 ppm for H3 (*J* 5.12 Hz), 7.9 ppm for H5 (*J* 9.15 Hz), 8.0 ppm for H8 (*J* 2.01 Hz) and 8.7 ppm for H2 (*J* 4.94 Hz) and the doublet of doublets at 7.4 ppm for H6 (*J* 9.15, 2.11 Hz). The indicators for **CMP15** are the doublets at 8.1 ppm for H5 (*J* 7.32 Hz) and 8.4

ppm for H7 (*J* 7.63 Hz), the singlet at 7.2 ppm for H3 and the triplet at 7.6 ppm for H6 (*J* 7.94 Hz). The broad singlet for the piperazine methylene groups (H10, H11, H13, H14) at 3.1 ppm and the singlet for the NH proton (H12) at 1.9 ppm for (**CMP10**) and 1.7 ppm for (**CMP15**) were further indicators.

3.1.3 Mechanism of nucleophilic substitution

In **scheme 3.3**, the quinoline (**32**) moiety requires no more than its own in-built capacity for electron withdrawal and is therefore attacked by a nucleophile, such as piperazine (**31**), at the electrophilic 4-halo (eg. chloro) position, which is closer to the electron withdrawing quinoline nitrogen atom, compared to the 7-halo (eg. chloro) position. In this case where R3, R2 = CF_3 and X = Br, the carbon at the 4-bromo position is the most electrophilic due to the aforementioned quinoline nitrogen. Thus, nucleophilic attack occurs at this position. Since the reaction follows a second order rate law, there is some formal resemblance to S_N2 reactions. However, it follows a typical S_NAr mechanism. The carbon atom undergoing attack by the nucleophilic amino group is sp^2 hybridised. The interaction of the nitrogen lone pair is with an antibonding π-aromatic orbital orthogonal to the plane of the C-Cl bond. This part of the mechanism is similar to that of a Michael (1,4-conjugate) reaction, which is then followed by an elimination reaction from intermediate (**33**) to deliver the target compounds after loss of HX from intermediate (**34**).

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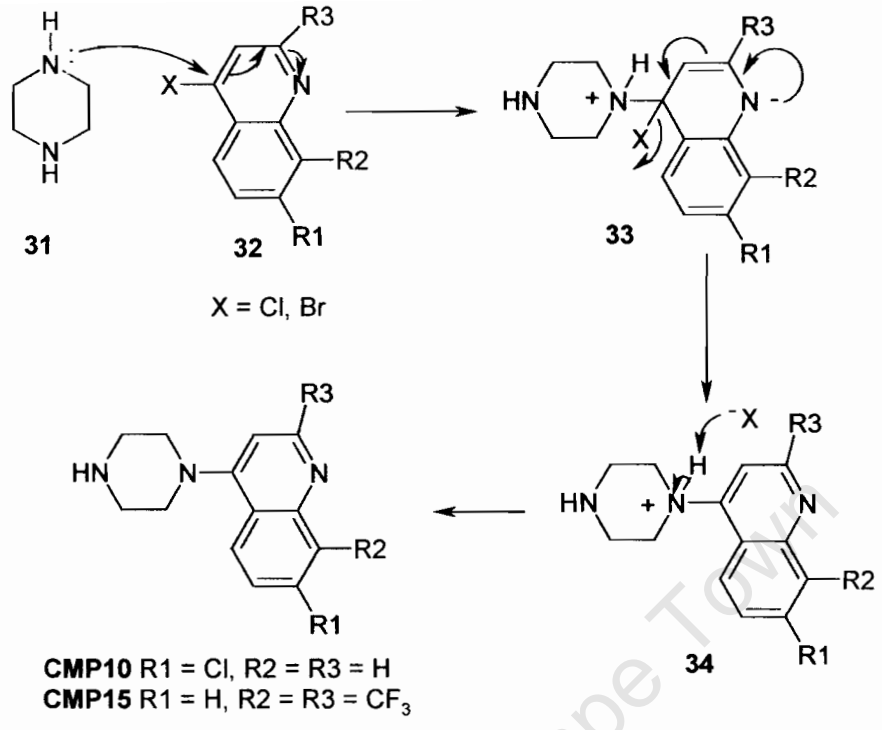

Scheme 3.3: Mechanism of Nucleophilic Substitution of quinoline halides by an amine

3.2 Synthesis of arylpiperazines (CMP1 – CMP9, CMP19, CMP24 – CMP29 and CMP31) *via* Reductive amination[72]

3.2.1 Retrosynthetic analysis

Scheme 3.4 depicts a retrosynthetic analysis of target compounds (**35**) *via* a one-step reductive amination of aldehydes or ketones (**36**).

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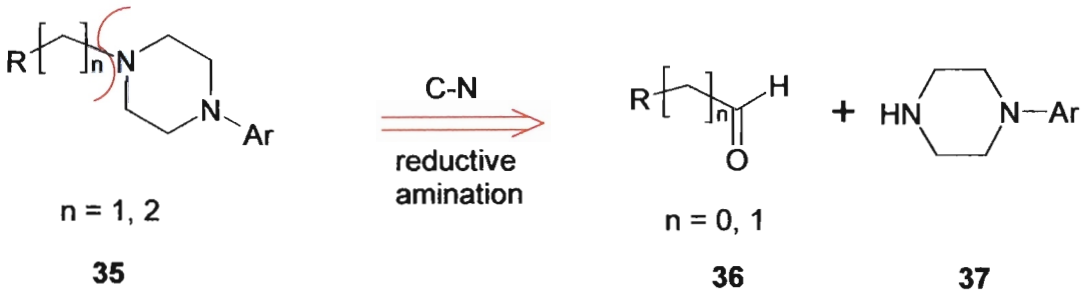

Scheme 3.4: Retrosynthetic analysis of substituted piperazines (35)

3.2.2 Synthesis [72]

The target compounds (**35**) were synthesized by stirring a mixture of carbonyl compounds (**36**) and aryl piperazine (**37**) in anhydrous methanol at 25°C for 4 hours under nitrogen, followed by addition of sodium cyanoborohydride to reduce the intermediate iminium ion (**38**) and stirring for a further 2 hours, **scheme 3.5**. Once the solvent was removed under reduced pressure, the residue was dissolved in 1N hydrochloric acid and washed with diethyl ether to remove any excess aldehyde that may have remained from the first step of the synthesis. The aqueous layer was neutralized with aqueous sodium carbonate, and the organic layer extracted. Column chromatography was used to purify the crude products.

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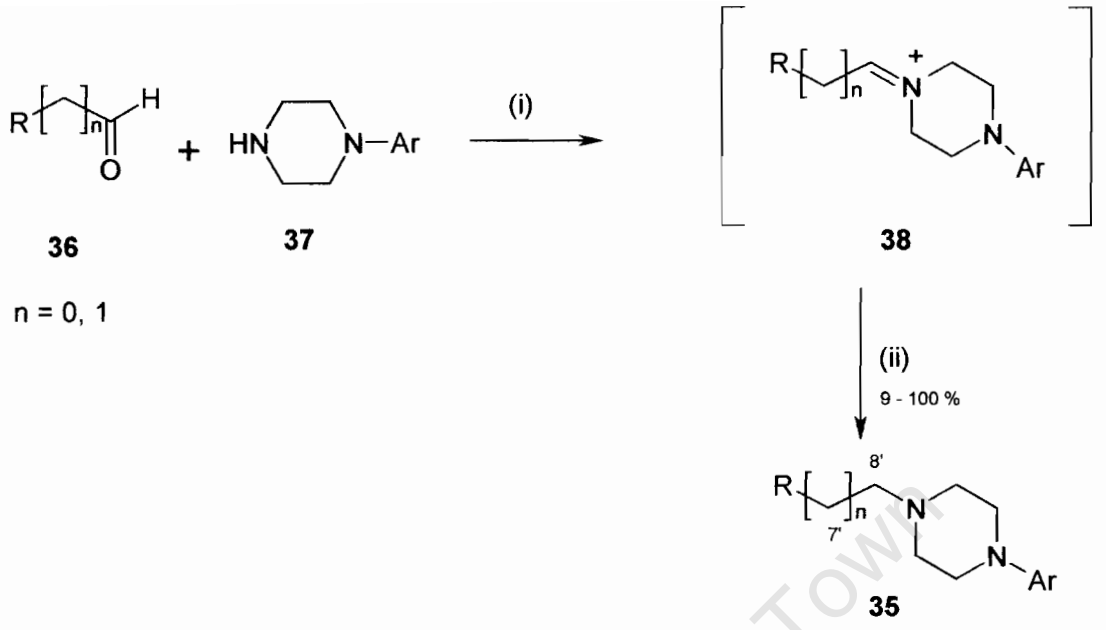

Scheme 3.5: *Reagents and Conditions:* (i) MeOH, 25°C, 4 hrs (ii) $NaBH_3CN$, 25°C, 2 hrs

The structure of these compounds was confirmed using ^1H and ^{13}C NMR as well as infra-red spectroscopy, mass spectroscopy and micro-analysis. The key spectroscopic indicators in the proton NMR spectra in deuterated chloroform were, for n=0, the piperazine methylene groups at 2.6 and 3.2 ppm and the CH_2 group at 2.2 ppm (Hα). For n=1, the key spectroscopic indicators were the piperazine methylene groups at 2.6 and 3.2 ppm and the two CH_2 peaks at 2.7 ppm for H8' and 2.8 ppm for H7'. For compound **CMP19** the ferrocenic ring had singlet CH peaks at 3.4 – 4.2 ppm (H8 – H17). The reductive amination products and their percentage yields are given in **table 3.1**.

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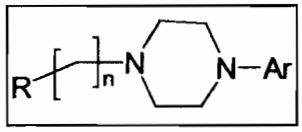
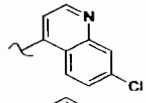
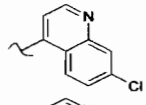
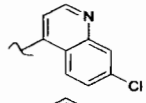
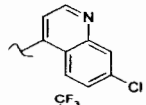
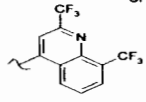
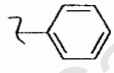
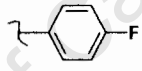
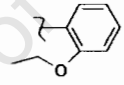
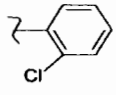
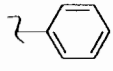
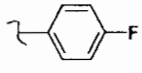
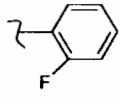
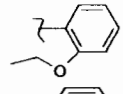
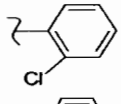
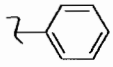
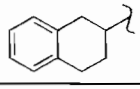
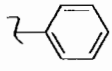
Table 3.1: Reductive amination products and their yields



COMPOUND (35)	R	Ar	n	Yield %
CMP1			1	54
CMP2			1	64
CMP3			1	100
CMP4			1	27
CMP5			1	59
CMP6			1	42
CMP7			1	35
CMP8			1	64
CMP9			1	38
CMP24			2	11
CMP25			2	13
CMP26			2	9
CMP27			2	26
CMP28			2	13
CMP29			1	18
CMP31			0	27

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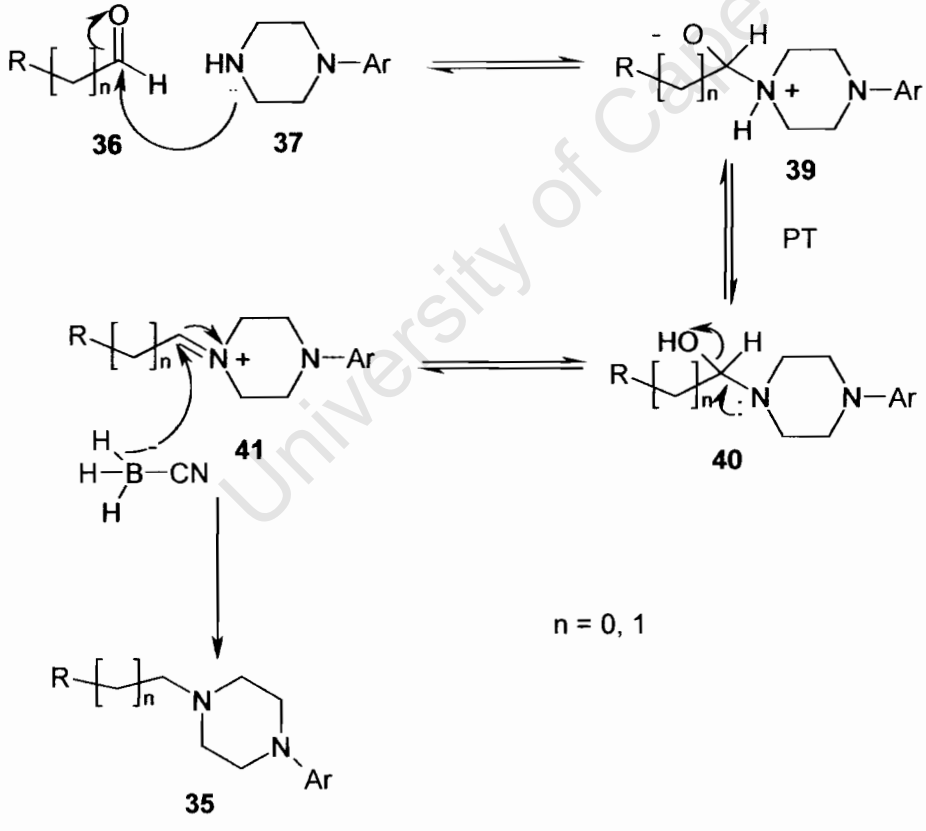
3.2.3 Mechanism of Reductive Amination

In **scheme 3.6**, the mechanism for the reductive amination is shown for aldehydes (**36**). Nucleophilic attack of the piperazine (**37**) NH on to the carbonyl group of (**36**) generates intermediate (**39**). A proton transfer then occurs to give aminal (**40**), from which the iminium ion (**41**) is generated after loss of a hydroxyl (OH) group. Reduction of the iminium ion intermediate with sodium cyanoborohydride then results in product (**35**) formation.



Scheme 3.6: Mechanism of reductive amination reaction

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3.2.4 Spectral Analysis of target compounds

^{1}H-NMR spectra of **CMP2**, Fig.3.1, and **CMP27**, Fig.3.2, are shown below. The quinoline protons and piperazine methylene protons were easily assignable, due to their position and multiplicities.

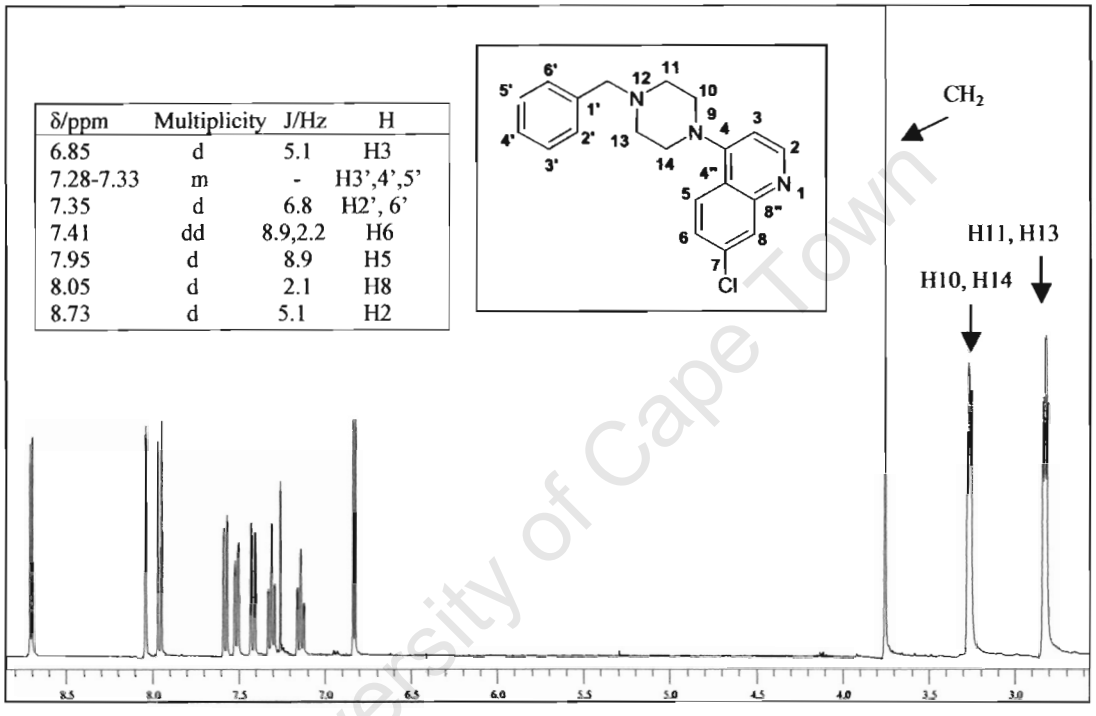

Fig.3.1: ^{1}H-NMR of CMP2 in $CDCl_3$ as the deuterated NMR solvent

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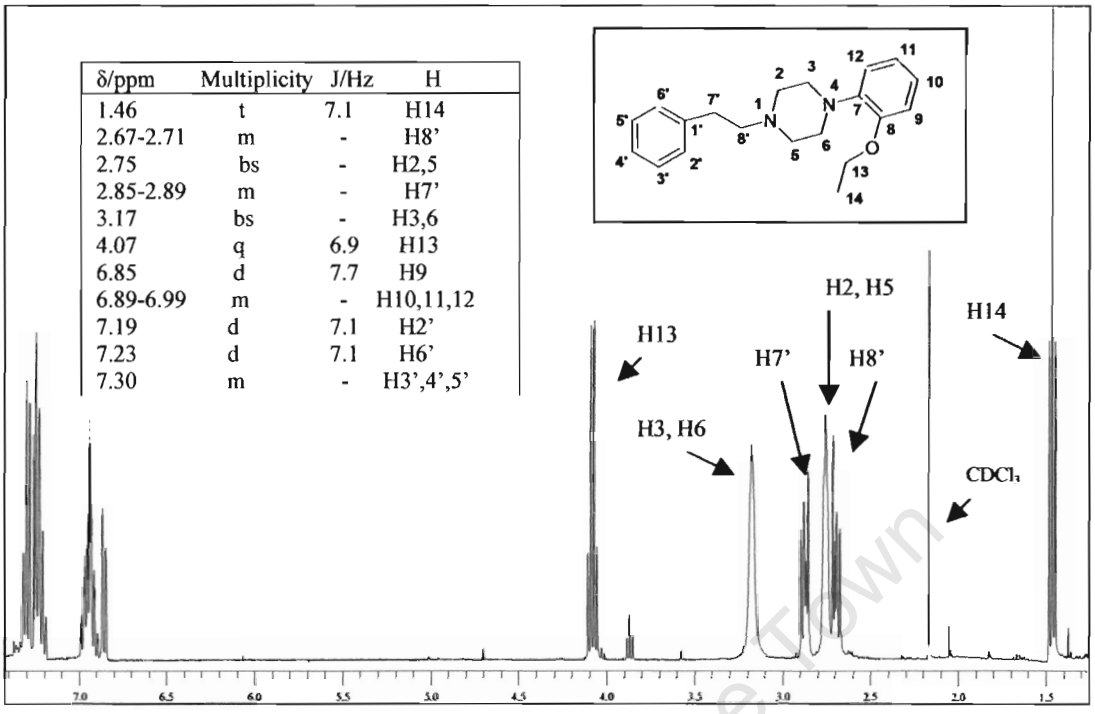

Fig.3.2: ^{1}H-NMR of CMP27 in $CDCl_3$ as the deuterated NMR solvent

3.3 Synthesis of arylpiperazine (CMP24) *via* hydroamination[73]

3.3.1 Retrosynthetic analysis

Due to poor yields generally obtained in the reductive amination reactions, an alternative synthetic approach to selected compounds exemplified by **CMP24** (35: n = 2, R = Ar = Ph) was explored with a view to improve yields. **Scheme 3.7** depicts the retrosynthetic analysis of target compound (**CMP24**) that identifies the addition of phenylpiperazine (**37**) (R = Ph) to styrene (**42**) in a classical hydroamination reaction.

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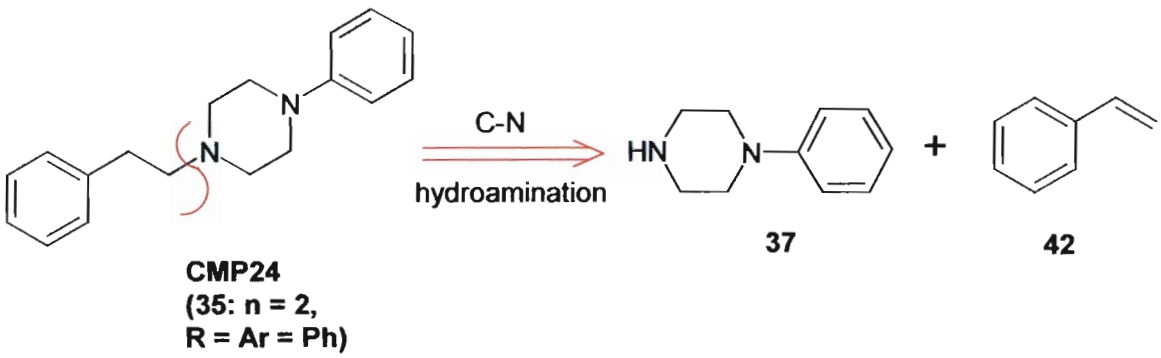
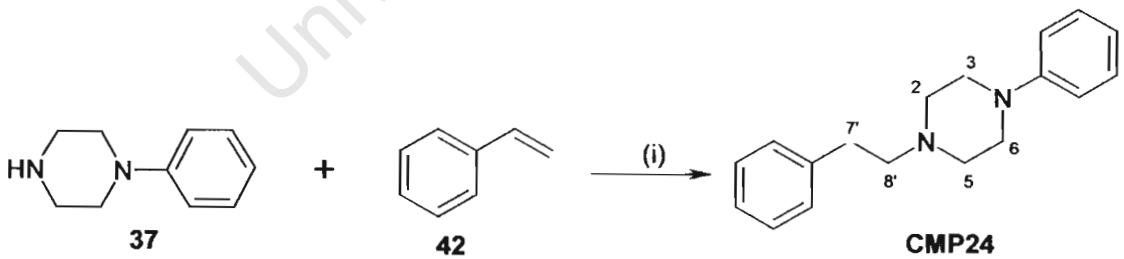


Scheme 3.7: Retrosynthetic analysis of CMP24 *via* a hydroamination reaction

3.3.2 Synthesis [73]

Target compound (**CMP24**) was synthesised by stirring styrene (**42**) and phenylpiperazine (**37**) (R = Ph) in the presence of a catalytic amount (20% mol) of *n*-butyllithium in dry tetrahydrofuran for 24 hours under nitrogen at 120°C, **Scheme 3.8**. Upon completion of the reaction two separable (column chromatography) products were obtained, **CMP24a** and **CMP24b**, in a ratio of 44:1 and yields of 88% and 2% respectively. Subsequent NMR (^{1}H and ^{13}C) analysis of the two respective products revealed that they were identical.



Scheme 3.8: *Reagents and conditions:* (i)n-BuLi, phenylpiperazine, THF, 25°C, 10 min (ii) styrene, 120°C, 24 hrs

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The presence of the piperazine methylene groups at 2.6 ppm (H2, H5) and 3.25 ppm (H3, H6), as well as the methylene groups at 2.6 ppm (H8') and 2.8 ppm (H7') in the ^{1}H NMR spectrum ($CDCl_3$) were indicative of the expected structure.

Gratifyingly, the hydroamination route to **CMP24** proceeded in a high yield of 88%, for the major product. This is in marked contrast to the reductive amination route that resulted in a poor (11%) yield of **CMP24**. However, despite the significant improvement in the isolated yield of **CMP24**, the presence of a minor (2%) product with essentially identical NMR (^{1}H and ^{13}C) spectra to the major product was rather puzzling and accordingly prompted further investigation. In view of the solid or crystalline nature of the major and minor hydroamination products, suitable crystals were grown for X-ray crystallographic analysis.

3.3.3 Crystal Analysis

Suitable crystals were grown in ethanol by first dissolving the compounds in hot ethanol and then allowing the solvent to evaporate very slowly. The crystal structures of the two chromatographically separable products (**CMP24a** and **CMP24b**) were solved by the Crystallography Section of the Department of Chemistry at the University of Cape Town, Fig. 3.3 and Fig. 3.4. It was found that the products were polymorphs of each other. A polymorph is the nature of a chemical substance; it is reflected in its ability to crystallise in different structural arrangements.[74] It is normal to have several polymorphs of the same compound under normal laboratory conditions. At a certain pressure and temperature only one of the polymorphs is stable thus making the other polymorphs metastable, with the transformation from the metastable to stable polymorph being slow.

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It has been observed that organic molecules tend to form different polymorphs due to weak and non-directional intermolecular interactions that occur in the solid state. The crystallisation process is affected by many physical parameters, i.e. nature of the solvent, cooling, stirring rates, temperatures, pressure and the presence of impurities. Even the smallest change in the preparative conditions can change the crystallisation of a polymorph to a non-thermodynamically stable form.[74]

Another reason for the two different polymorphs in the solid state is that it is possible for a compound to exist in two possible conformations if there is a sufficiently large energy barrier between them, this gives rise to different conformers in solution and thus to different polymorphs in the solid state.[74] This is the more likely explanation that accounts for the chromatographically separable **CMP24a** and **CMP24b**. The fact that these two compounds had different R_f values (**CMP24a** R_f 0.59, **CMP24b** R_f 0.31 in hexane:ethyl acetate 2:8) by TLC, and were ultimately separated by column chromatography on silica gel, implies that the two existed in two different conformations in solution and interacted differently with the silica gel stationary phase.

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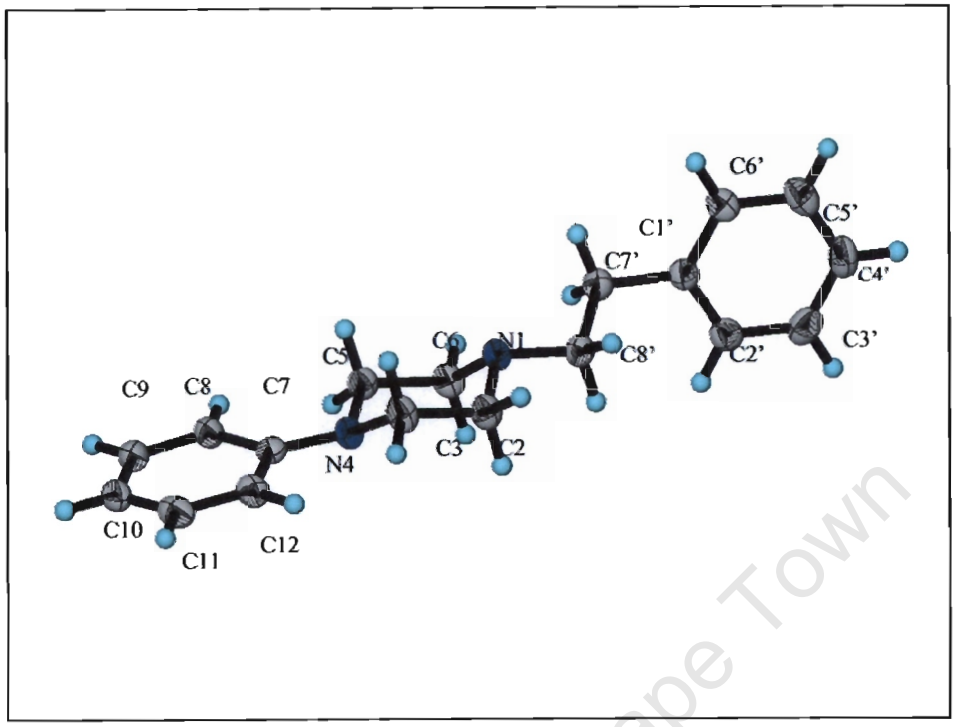

Fig. 3.3 Crystal structure of CMP24a

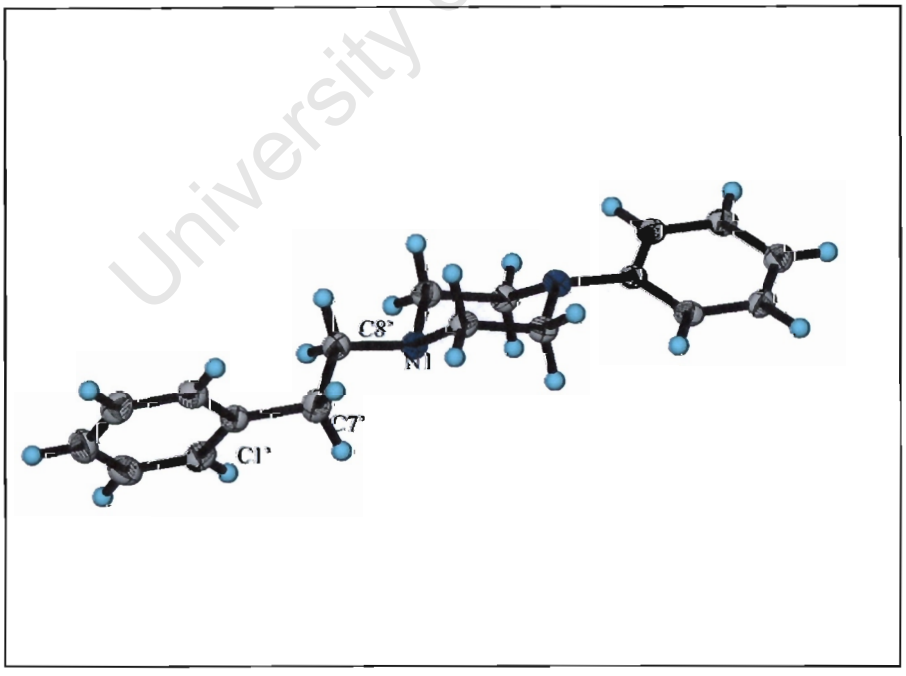

Fig. 3.4: Crystal structure of CMP24b

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The difference between these two crystal structures is the torsional angle between N1-C8'-C7'-C1'. For **CMP24a** the torsional angle is 163.7° and for **CMP24b** the angle is 173.9°. (Fig. 3.3 and Fig. 3.4)

The unit cell of a crystal is the smallest repeating unit in which one can construct a crystal by stacking the units in three dimension. There are seven basic shapes for a unit cell, which are called crystal systems. The crystal system is defined by the angles between the edges of the unit cell and the length of the edges. (Fig. 3.5 and Fig. 3.6)

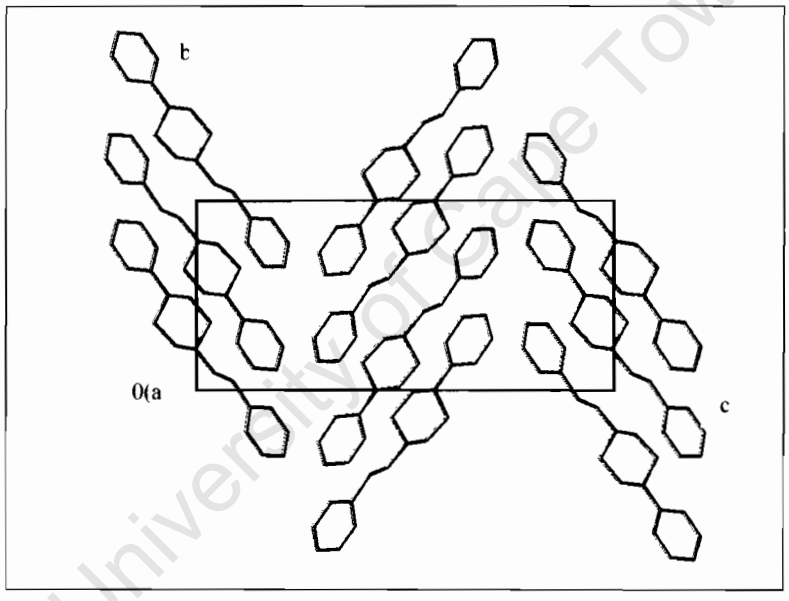

Fig. 3.5: Crystal packing of CMP24b

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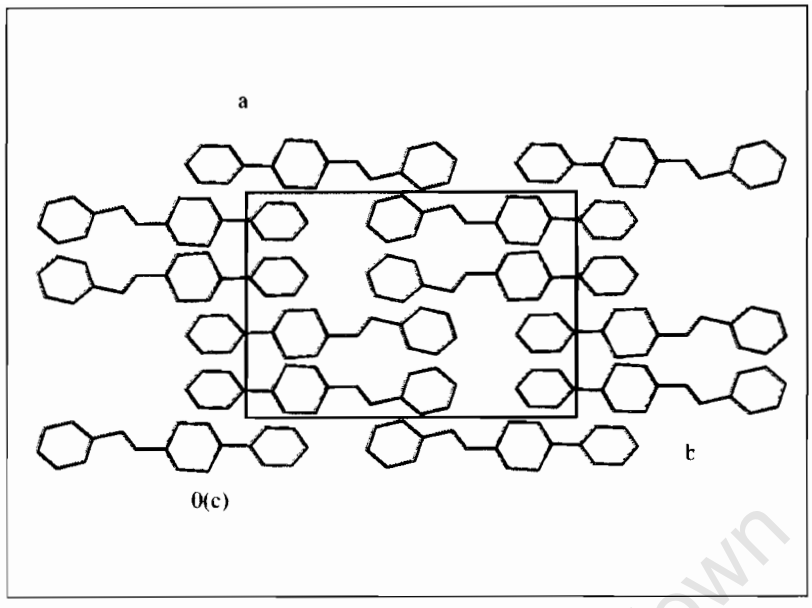

Fig. 3.6: Crystal packing of CMP24a

From the data in table 3.2 it is found that **CMP24a** has an orthorhombic crystal system, as $a \neq b \neq c$ and $\alpha = \beta = \gamma = 90^{\circ}$, and **CMP24b** has a monoclinic crystal system, as $a \neq b \neq c$ and $\alpha = \beta = 90^{\circ}$, $\gamma \neq 90^{\circ}$. Both unit cells have 4 atoms per unit making them face-centered cubic unit cells, this means that there are lattice points at the centre of each face and at each corner of the unit cell. The space group of the unit cell describes the arrangement of symmetry elements. For **CMP24a** which has an orthorhombic crystal system and a P*ca*2(1) space group, this means it is a primitive (P) space lattice with a glide plane (*ca*) and a simple rotation axis (2). An *a*-glide plane normal to *c* and through the origin involves a translation through *a* and a reflection in a plane normal to *c*. In other words a point at *x*, *y*, *z* translates and reflects to *x*, ½+*y*, -*z*.

For **CMP24b** the space group is $P2_1/n$. This means a primitive (P) space lattice with a two-fold screw axis (2_1) and a diagonal glide plane (*n*). A two-fold screw axis parallel to

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b and through the origin involves a two-fold rotation, ie *x,y,z* to *–x, y, -z*, and a translation of b/2, ie *–x, y, -z* to *–x, ½+y, -z*. A second screw operation converts *–x, ½+y, -z* to *x, 1+y, z* which is the same as *x, y, z*. The diagonal glide plane involves a translation from *x, y, z,* to *1+x, 1+y, z* then a reflection to ½(1+*x*), (1+*y*), ½ *z*.

Parameter	CMP24a	CMP24b
Chemical formula	$C_{18}H_{22}N_2$	$C_{18}H_{22}N_2$
Molecular mass	266.17857	266.17857
Crystal system	Orthorhombic	Monoclinic
Space group	P*ca*2(1)	$P2_1/n$
a (Å)	11.1980	19.8925
b (Å)	16.234	8.5995
c (Å)	8.1306	8.8415
α (°)	90	90
β (°)	90	90
γ (°)	90	98.32
Volume ($Å^3$)	1477.88	1496.56
Formula Units (Z)	4	4

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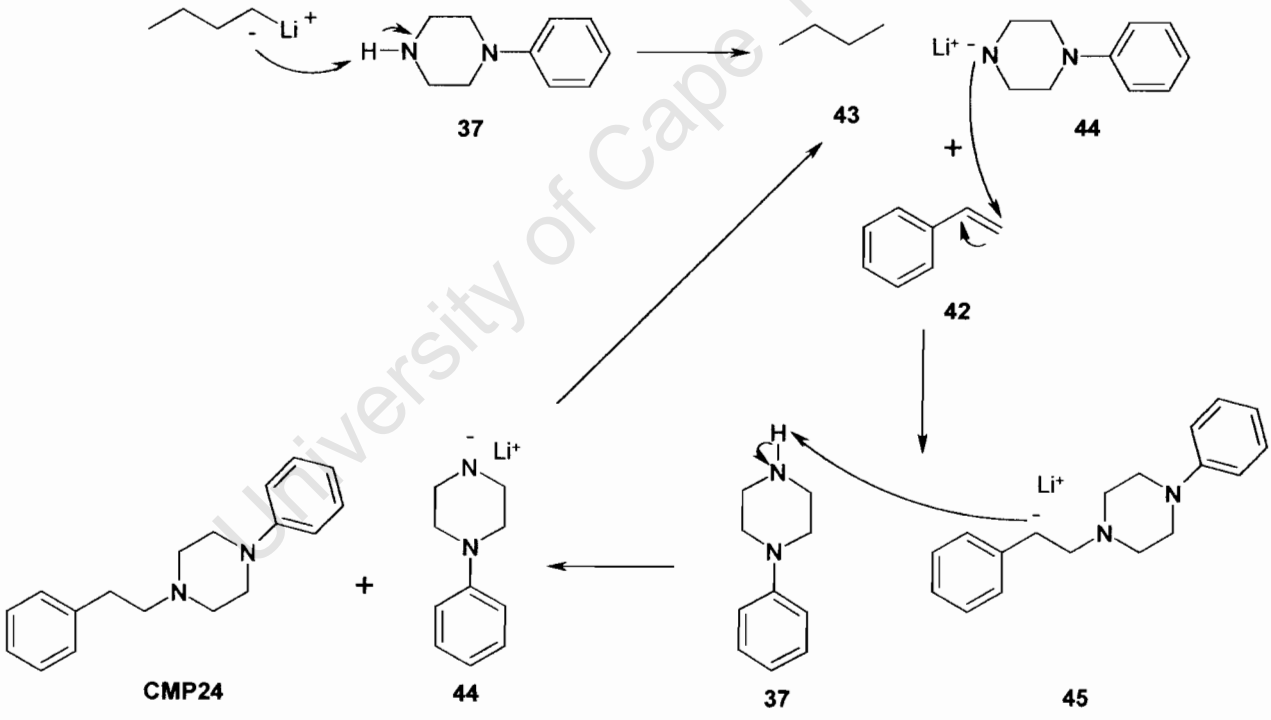
Table 3.2: Crystal Data and Structure Refinement for CMP24a and CMP24b

3.3.4 Mechanism of hydroamination

Deprotonation of the phenyl piperazine (**37**) (R = Ph) with *n*-butyllithium, which is a strong base, at the reaction temperature generates butane (**43**) and a stronger charged

nucleophile (**44**), which is required to attack a somewhat poorly electrophilic carbon of the styrene (**42**). Only a catalytic amount of *n*-butyllithium is required as the initial attack of the deprotonated piperazine (**44**) generates the intermediate carbanion (**45**), which can also act as a strong base like the *n*-butyllithium. The carbanion (**45**) takes up the proton to regenerate the deprotonated piperazine (**44**) and to form the product (**CMP24**). The deprotonated piperazine (**44**) then follows the same pathway by attacking the carbon on the styrene (**42**) and continuing to the target compound. This cycle continues until all the styrene has been reacted, **scheme 3.9**.



Scheme 3.9: Mechanism of hydroamination

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The deprotonated piperazine (**44**) preferentially attacks the electrophilic terminal carbon of the double bond of the styrene (**42**). The rationalisation of the nucleophilic attack on the styrene by the deprotonated piperazine is based on two factors:

1) extended conjugation of the external styrene double bond with the π-system of the aromatic ring renders the terminal (C1) carbon of the external double bond electrophilic.
2) Attack at the terminal (C1) carbon of the external double bond results in a resonance stabilised benzylic carbanion, on the other hand, attack at the C2 carbon of the styrene would result in an unstabilised carbanion, Fig. 3.5.

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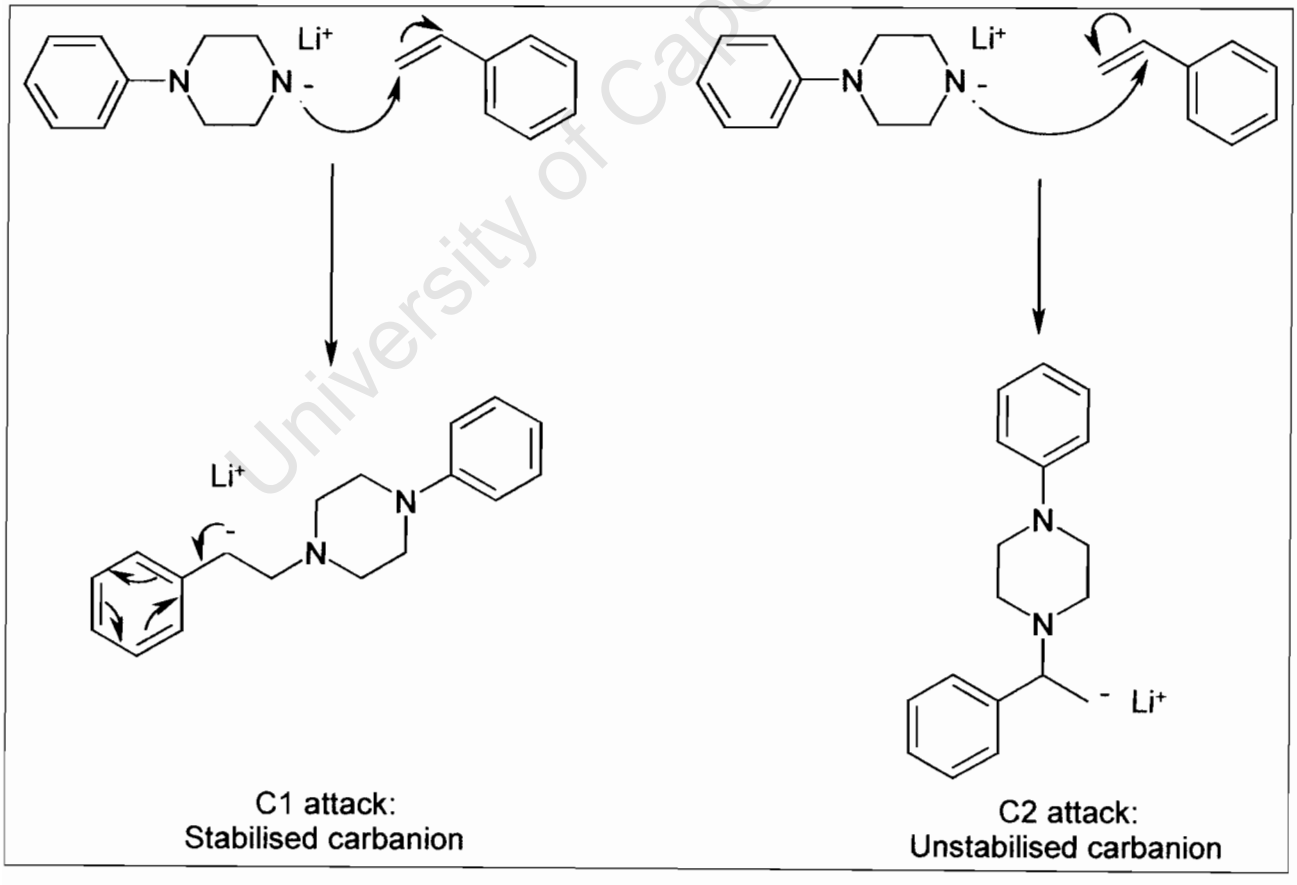
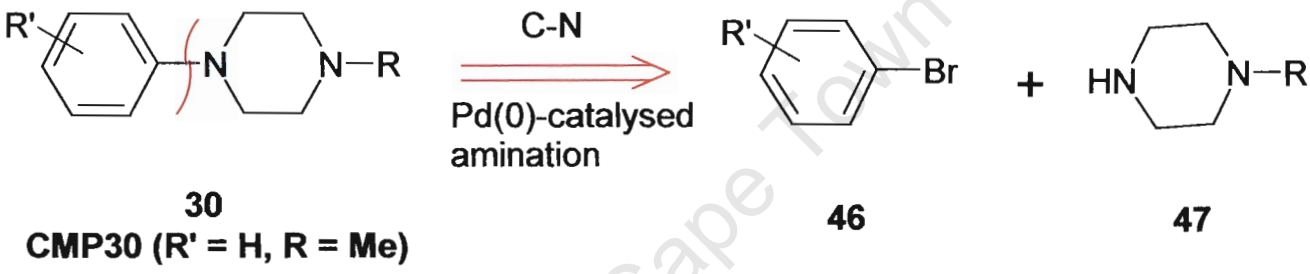


Fig. 3.5: Carbanion stability resulting from C1 and/or C2 attack

3.4 Synthesis of arylpiperazine (CMP30) *via* a palladium catalysed reaction[75]

3.4.1 Retrosynthetic analysis

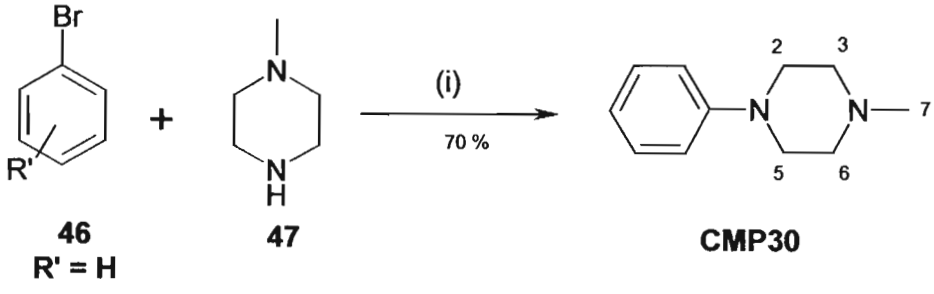
The retrosynthetic analysis of the target compound (**30**) (**CMP30**: R' = H, R = Me) identifies the reaction of phenyl bromide (**46**) and *N*-methylpiperazine (**47**) (R = Me) *via* a palladium (0) catalysed amination, **scheme 3.10**.



Scheme 3.10: Retrosynthetic analysis of target compound CMP30

3.4.2 Synthesis[75]

CMP30 was synthesised by refluxing the phenylbromide (**46**) and *N*-methyl piperazine (**47**) in the presence of tris(dibenzylideneacetone) palladium (0), sodium *tert*-butoxide and BINAP in dioxane for 18 hours under nitrogen, **scheme 3.11**. The target compound was obtained in 70% yield after column chromatography.



Scheme 3.11: *Reagents and conditions:* (i) Na-tOBu, $Pd(dba)_3$, BINAP, dioxane, 100°C, 18 hrs

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The structure of **CMP30** was confirmed using ^{1}H and ^{13}C NMR as well as infra-red spectroscopy, mass spectroscopy and micro-analysis. The spectroscopic indicators from the proton NMR spectra for **CMP30** in deuterated chloroform were aromatic protons in the region 6.8 – 7.2 ppm, the two triplets in the region 2.5 – 3.2 ppm (*J* 5.0 Hz) for the piperazine methylene protons (H2, 3, 5, 6) and a singlet at 2.3 ppm for the methyl group (H7).

3.4.3 Catalytic cycle: Palladium catalysed amination[76]

Scheme 3.12 describes the catalytic cycle for a palladium-catalysed amination. BINAP is used as a ligand with the palladium (II) intermediate to form a palladium (0) species (**48**). Oxidative addition is the first step to give intermediate (**49**). The amine is then coordinated to (**49**) to form a pentacoordinate intermediate (**50**). Deprotonation of the amine in (**50**) by sodium *tert* butoxide gives (**51**), which undergoes a reductive elimination reaction to regenerate the BINAP(Pd) catalyst and to give the product (**52**).

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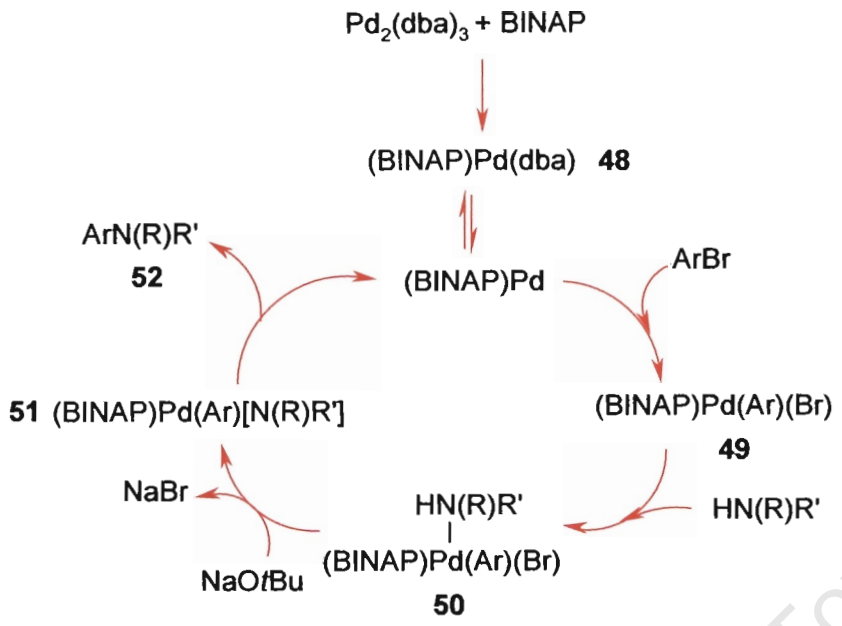

Scheme 3.12: Catalytic cycle for a palladium-catalysed amination

3.5 Synthesis of biaryl ether (CMP40 – CMP42) and arylamines (CMP43 – CMP45) *via* copper promoted arylation[77] and palladium catalysed amination[75]

3.5.1 Retrosynthetic analysis

Scheme 3.13 shows the retrosynthetic analysis of the target compounds (**30**), which identifies the addition of piperazine (**31**) and bromo-arylethers (**53**) in a palladium catalysed amination. The retrosynthetic analysis of the bromo-arylether intermediates (**53**) identifies the coupling of bromophenol (**54**) and phenyl boronic acid (**55**) *via* a copper mediated arylation reaction.

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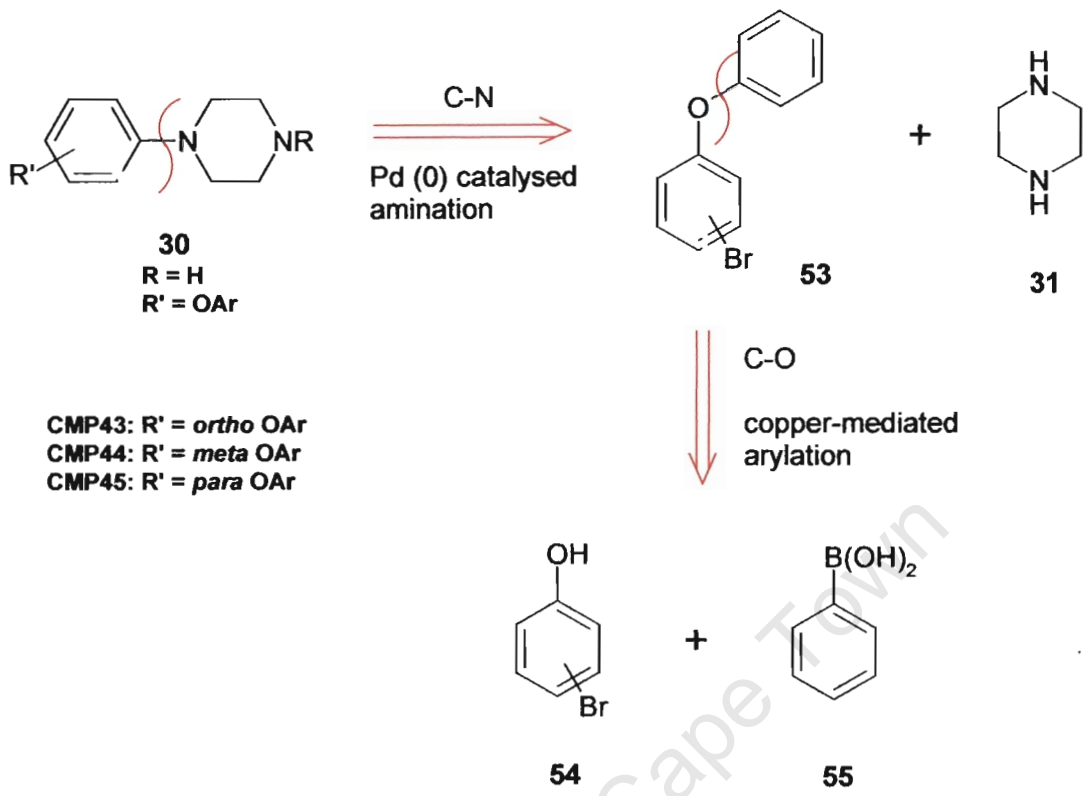

Scheme 3.13: Retrosynthetic analysis of CMP43, CMP44 and CMP45

3.5.2 Synthesis

Bromophenols (**54**) and phenyl boronic acid (**55**) were stirred in the presence of copper (II) acetate and pyridine in dry dichloromethane as the solvent for 48 hours under nitrogen at 25°C, to give intermediate bromo-arylethers (**53**). These then underwent a palladium-catalysed amination with piperazine (**31**) as previously described for **CMP30** on page 59. **CMP43, CMP44** and **CMP45** were obtained in a yield range of 31% - 50% after column chromatography, **scheme 3.14**.

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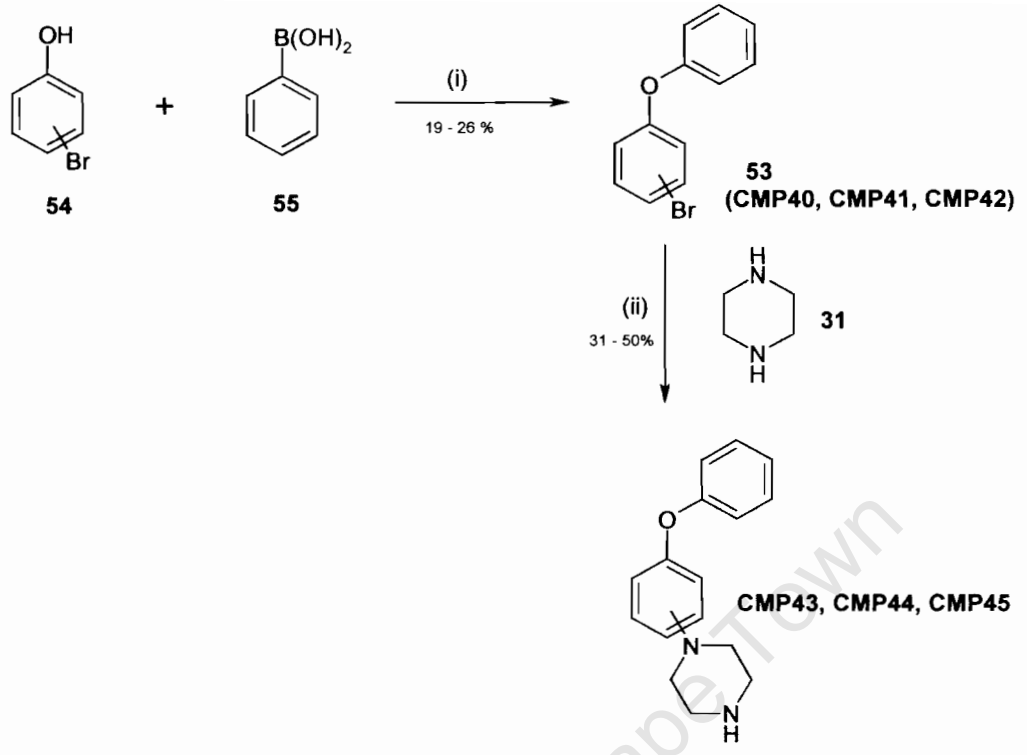

Scheme 3.14: *Reagents and conditions:* (i) copper acetate (II), pyridine, DCM, 25°C, 48 hrs

(ii) $Pd(dba)_3$, Na-*t*OBu, BINAP, dioxane, 100°C, 18 hrs

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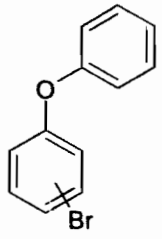
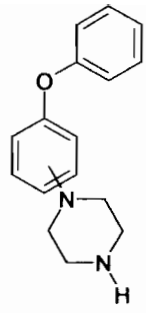
The structures of both intermediates (**CMP40, CMP41, CMP42**) and products (**CMP43, CMP44, CMP45**) were confirmed by spectroscopic and analytical means (NMR, MS, IR, EA). The increases in the number of aromatic protons in the ^{1}H NMR spectra of **CMP40 – CMP45**, relative to the starting bromophenols, were a key indication of the expected intermediates and/or products. The presence of the piperazine methylene protons in the region 2.8 – 3.2 ppm distinguished **CMP43 - CMP45** from intermediates **CMP40 – CMP42**, as shown in Fig. 3.7. The broad singlet due to the NH at 2.55 ppm was also a key indicator. The synthetic yields of the intermediates and the target compounds are shown in **table 3.3**.



CMP40 – CMP42



CMP43 – CMP45

INTERMEDIATE	POSITION	YIELD %	COMPOUND	YIELD %
CMP40	*ortho*	22%	**CMP43**	31%
CMP41	*meta*	26%	**CMP44**	42%
CMP42	*para*	19%	**CMP45**	50%

Table 3.3: Synthetic yields of intermediate bromo-arylethers (CMP40 – CMP42) and products (CMP43 – CMP45)

3.5.3 Catalytic cycle: Copper promoted arylation [77]

The first step in the copper promoted arylation is the transmetallation between (**55**) and the copper (II) acetate catalyst to give intermediate species (**56**). The phenoxide anion, from deprotonated phenol, displaces the acetate group from the species (**56**) to give arylcopper phenoxide intermediate (**57**). A reductive elimination then occurs to give the target compound (**58**), **scheme 3.15**. The pyridine has a dual role as both a base and a ligand (L) for the organocopper intermediates.

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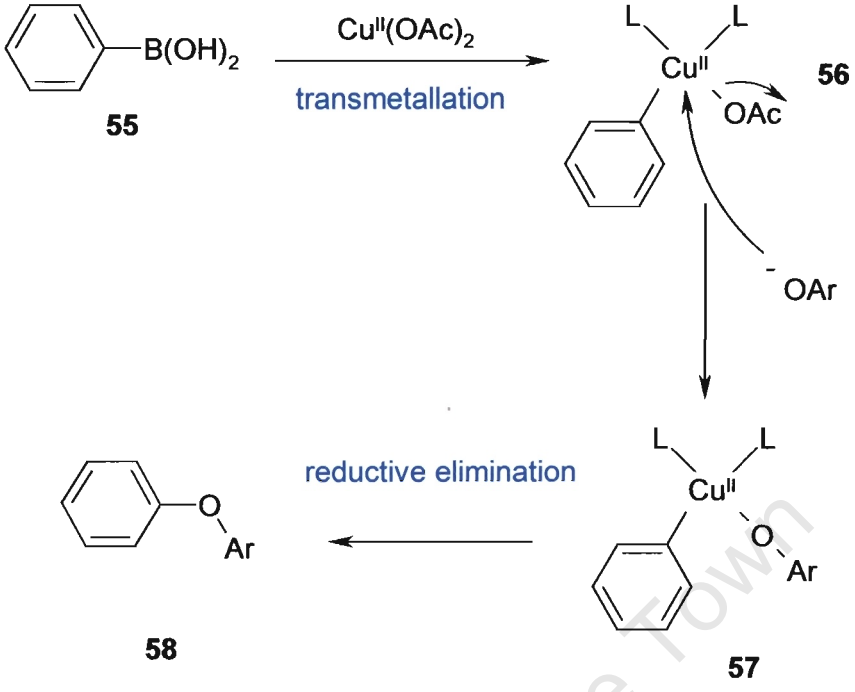

Scheme 3.15: Outline of copper (II) promoted arylation reaction

3.5.4 Spectral analysis

The ^{1}H-NMR of **CMP45** is shown in Fig.3.7. The piperazine methylene protons and aromatic protons are all easily assignable due to position and multiplicities.

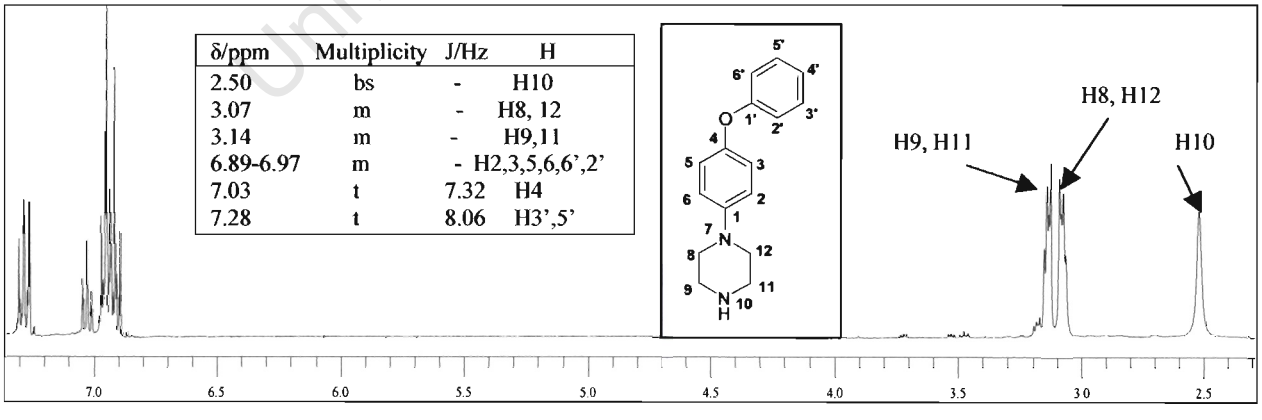

Fig.3.7: ^{1}H-NMR of CMP45 in $CDCl_3$ as the deuterated NMR solvent

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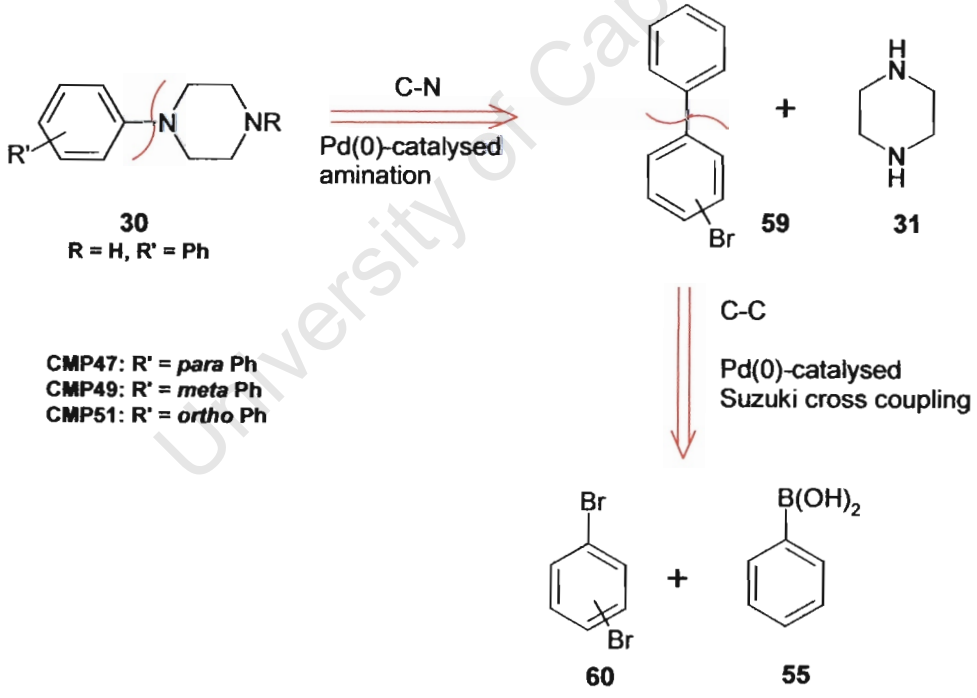
3.6 Synthesis of biaryl compounds (CMP46, CMP48 and CMP50) and arylamine (CMP47, CMP49 and CMP51) *via* a palladium catalysed Suzuki cross coupling reaction [78] and palladium catalysed amination [75]

3.6.1 Retrosynthetic analysis

Scheme 3.16 depicts the retrosynthetic analysis of compounds **CMP46, CMP48** and **CMP50**. It identifies the amination of bromides (**59**) with piperazine (**31**) *via* palladium catalysis. The retrosynthetic analysis of the bromides (**59**) identifies a palladium catalysed Suzuki cross coupling reaction, between phenylboronic acid (**55**) and dibromides (**60**).



Scheme 3.16: Retrosynthetic analysis of CMP47, CMP49 and CMP51

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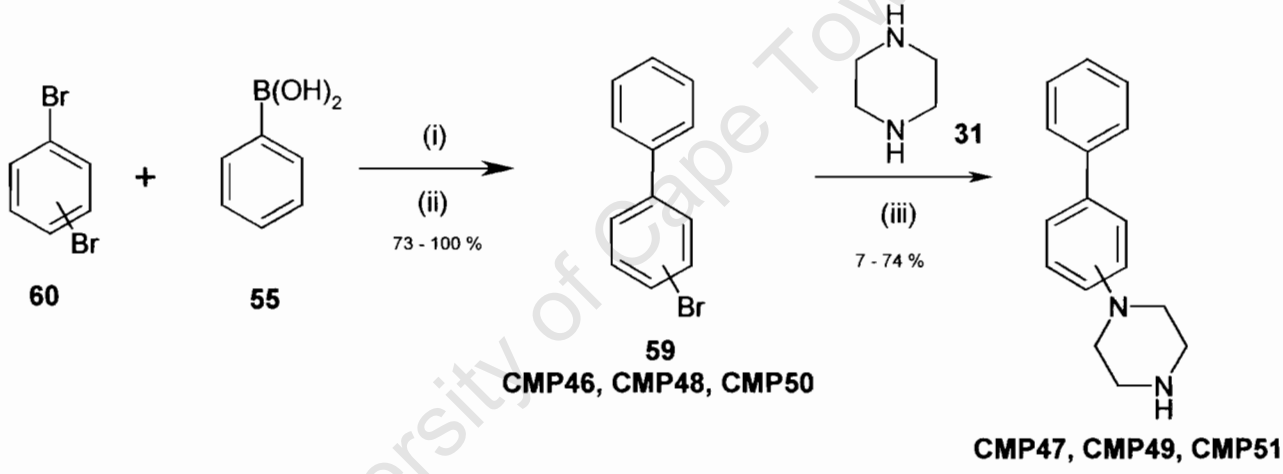
3.6.2 Synthesis

The intermediate bromides (**59**) were synthesised by refluxing a mixture of dibromides (**60**) and phenyl boronic acid (**55**) in the presence of a catalytic amount of palladium (II) acetate, triphenylphosphine, 2M Na_2CO_3 (aqueous) and deionised water in iso-propanol for 18 hours under nitrogen. The final target compounds (**CMP47, CMP49, CMP51**) were synthesized *via* palladium catalysed amination as described before on page 59, **scheme 3.17**. The yields for the suzuki reactions were generally higher than those for the amination reactions, **table 3.4.**



Scheme 3.17: *Reagents and conditions:* (i) n-propanol, 25°C, 15 min (ii) palladium acetate, PPh_3, Na_2CO_3, H_2O, 100°C, 18hrs (iii) $Pd(dba)_3$, Na-*t*OBu, BINAP, dioxane, 100°C, 18 hrs

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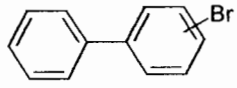
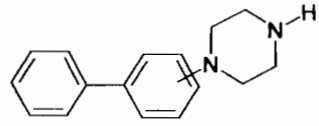


CMP46, CMP48, CMP50



CMP47, CMP49, CMP51

INTERMEDIATES	POSITION	YIELD %	COMPOUNDS	YIELD %
CMP46	*para*	73%	**CMP47**	74%
CMP48	*meta*	100%	**CMP49**	22%
CMP50	*ortho*	92%	**CMP51**	7%

Table 3.4: Synthetic yields of arylethers (CMP46, CMP48, CMP51) and products (CMP47, CMP49, CMP51)

As these compounds are structurally similar to the previously described arylether analogs (**CMP43 – CMP45**), confirmation of the structural identity of these compounds (**CMP47, CMP49, CMP51**) was easy as the NMR pattern was comparable in many respects.

3.6.3 Mechanism of the palladium catalysed Suzuki cross coupling cycle[79]

The first step in the Suzuki cross coupling reaction is an oxidative addition of, for example, phenylbromide (**61**) and the palladium (0) species. This forms a palladium (II) intermediate, such as (**62**). The substitution of the halide with an alkoxide (R'O) ligand gives the palladium aryl complex (**53**). The second step is a transmetallation between the palladium aryl complex (**63**) and the boronic acid, such as phenyl boronic acid (**55**), to give a palladium II species (**64**). The third and final step is a reductive elimination. This step regenerates the palladium (0) catalyst, by reduction with phosphine, and yields the product (**65**), **scheme 3.18**. If the base were not present in the catalytic cycle, the

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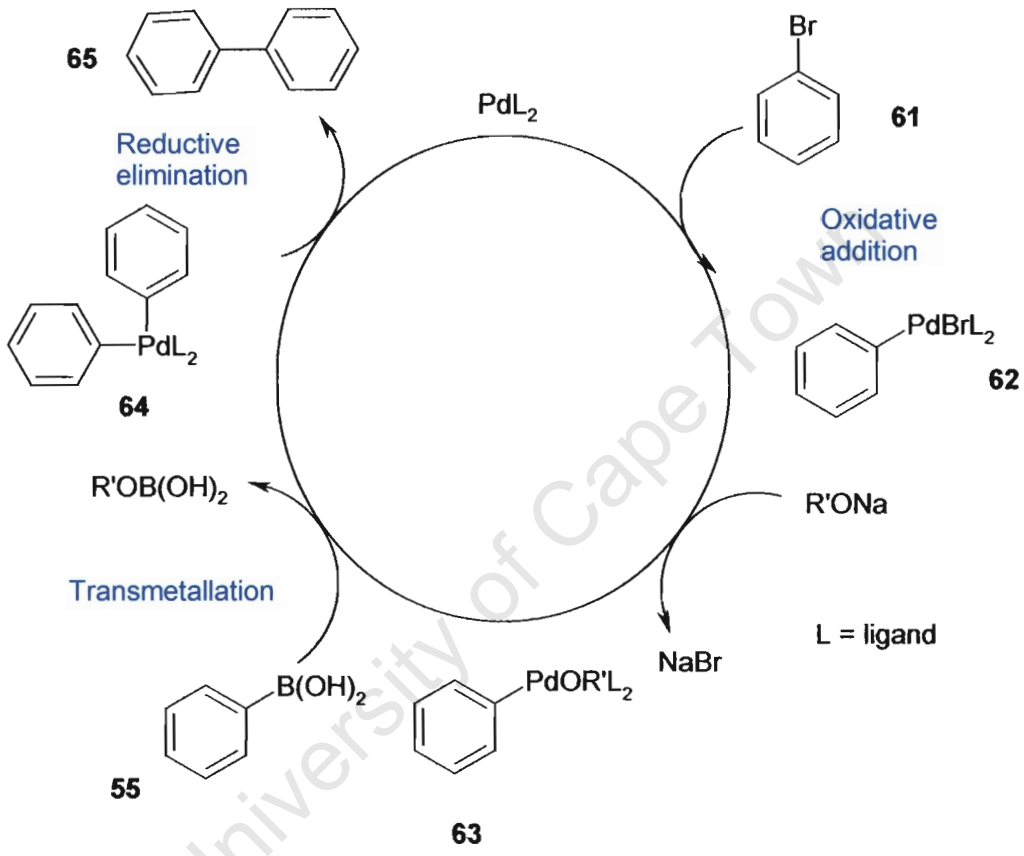
transmetallation step would not occur as readily due to the low nucleophilicity of the group on the boron atom. The nucleophilicity can be increased by quaternization of the boron with the base, which is anionic.[79]



Scheme 3.18: Palladium catalysed Suzuki cross-coupling cycle

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CHAPTER 4

BIOLOGICAL RESULTS AND DISCUSSION

4.1 Introduction

The effect of different substituents on the piperazine ring system on the antimalarial activity of the compounds in each strain will be discussed in this section. The compounds synthesised and the starting piperazines that were submitted for biological testing are shown in the tables below.

When comparing the structural relationships of the compounds it is evident that they can be grouped into 5 classes, Fig. 4.1.

1) The unsubstituted simple arylpiperazines, **CMP10 – CMP23**
2) The substituted benzyl arylpiperazines, **CMP1 – CMP9, CMP29**
3) The substituted phenylethyl arylpiperazines, **CMP24 – CMP28**
4) The substituted arylpiperazines, **CMP30, CMP31**
5) The unsubstituted biarylpiperazines, **CMP43 – CMP45, CMP47, CMP49** and **CMP51**

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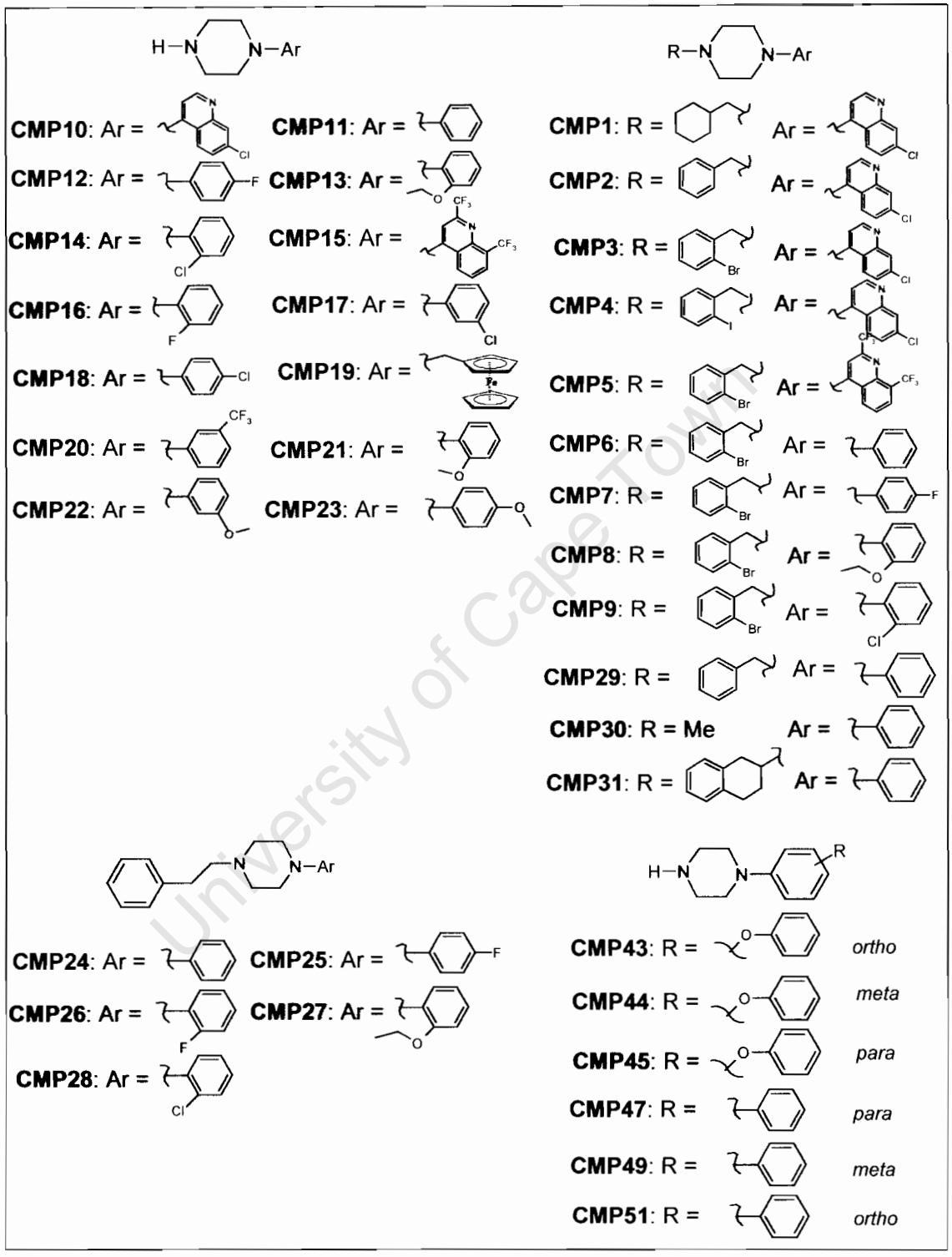

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Fig. 4.1: Structures of arylpiperazines

4.2 Antiplasmodial activity

4.2.1 Class 1: The unsubstituted simple arylpiperazines, CMP10 – CMP23

The abilities of a range of unsubstituted arylpiperazines to inhibit the growth of chloroquine-sensitive (D10, NF54) and chloroquine-resistant (W2, FCR3) *Plasmodium falciparum* were determined in the laboratories of Prof. Hagai Ginsburg, at the Department of Biological Chemistry, Institute of Life Sciences, The Hebrew University of Jerusalem (Israel), and data is shown in **Table 4.1**.

The different compounds (including chloroquine as a positive control) tested showed antiplasmodial activity in the IC_{50} range of 0.78 to 228 μM.

As previous observations[71] have shown the unsubstituted arylpiperazines (**CMP11, CMP16, CMP12, CMP14, CMP17, CMP18, CMP20, CMP21, CMP22, CMP23, CMP13**) were significantly more potent against the chloroquine-resistant strains than against the chloroquine-sensitive strains.

The unsubstituted 1-piperazinylmethyl ferrocene (**CMP19**) was also more active against the chloroquine-resistant strains. Even with the superior activity of **CMP19** relative to the above mentioned unsubstituted arylpiperazines, the difference in activity between the resistant and sensitive strains is much less pronounced in the case of **CMP19** compared to the other arylpiperazines.

The 4-aminoquinoline-based arylpiperazines (**CMP10** and **CMP15**) were equally active against the resistant and sensitive strains.

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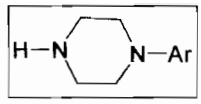


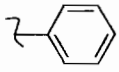
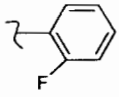
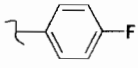
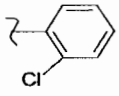
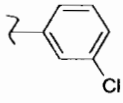
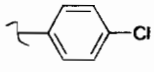
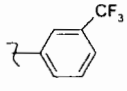
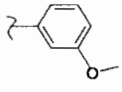
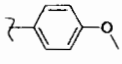
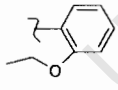
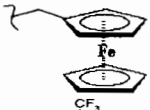
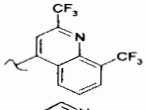
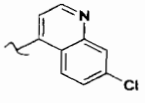
COMPOUND	Ar	W2 IC_{50} µM	FCR3 IC_{50} µM	NF54 IC_{50} µM	D10 IC_{50} µM
CMP 11		24.8±4.3	13.8±2.07	92.5±8.6	152.7±24.4
CMP 16		68.7±11.6	44.61±15.8	103.2±17.5	105.6±29
CMP 12		24.9±4.5	19.8±1.05	95.2±23.8	135.0±17.1
CMP 14		18.1±4.5	11.8±1.5	78.7±42.3	152.2±47.5
CMP 17		4.67±0.39	4.69±0.68	59.1±43.6	67.7±48
CMP 18		11.53±1.3	7.13±0.55	64.8±97	74±36
CMP 20		11.49±0.7	9.56±0.54	92.9±14.3	112.8±38
CMP 21		35.75±5.0	30.33±13.2	83.7±	151.2±
CMP 22		26.5±2.5	32.3±7.8	85.3±	228±
CMP 23		66.2±19.6	61.6±15	115.7±23	167±
CMP 13		16.8±3.5	10.96±0.91	93.1±17.8	143.3±17.0
CMP 19		1.06±0.03	0.78±0.08	15.2±2	1.9±0.5
CMP 15		11.2±4.1	2.03±0.97	15.0±0.1	7.8±1.9
CMP 10		1.21±0.03	1.36±0.037	1.02±0.05	2.02±0.1
CQ		0.33±0.044	0.40±0.058	0.034±0.002	0.044±0.006

Table 4.1: Antiplasmodial activity for CMP10 – CMP23

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Within this class, the unsubstituted arylpiperazines, the most favourable position for substitution appears to be the *meta* position, at least on the basis of the data obtained for the chloro (**CMP14, CMP17, CMP18**) series of compounds. As for the methoxy series of compounds (**CMP21, CMP22, CMP23**), in 3 of the 4 strains the *ortho* derivative is most active and in the fourth case the difference between *ortho* and *meta* is not statistically significant. Compound **CMP17**, which has a chloro substituent in the *meta* position of the aryl ring was the most active (IC_{50} = 4.68 μM) amongst all aryl substituents irrespective of parasite strain.

There have been a number of chloroquine resistance mechanisms proposed. These include essentially reduced accumulation of the drug[80] and higher levels of cellular glutathione.[81] Although some details on the precise mechanism of chloroquine resistance remains to be elucidated, and the mode of action of unsubstituted arylpiperazines is yet to be determined, based on this data it is quite clear that simple unsaturated arylpiperazines are displaying a mefloquine-type behaviour in being more active against the chloroquine-resistant strains than against the chloroquine-sensitive strains such as D10.[82] By implication, unsubstituted arylpiperazines appear not to be sufficiently well recognized by the chloroquine resistance mechanism. This may imply that the unsubstituted arylpiperazines mechanistically act differently from chloroquine. The data obtained for the two 4-aminoquinoline-based unsubstituted arylpiperazines (**CMP10** and **CMP15**), which were found to be equally active against the sensitive and resistant strains, suggests that chloroquine cross-resistance is not observed with these two 4-aminoquinolines. However, relative to the simple arylpiperazines, there is increased recognition of **CMP10** and **CMP15** by the chloroquine resistance mechanism. The lower antiplasmodial activity

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of **CMP10** and **CMP15** relative to chloroquine may be due to the absence of a more basic terminal nitrogen and lipophilic alkyl side chain in these two compounds. The more basic tertiary nitrogen in chloroquine, which is not present in **CMP10** and **CMP15**, is critical for accumulation in the acidic compartment of the parasite food vacuole via pH trapping.[82] The increased basicity and lipophilicity of the side chain in chloroquine may also be important for the uptake of the compound or increased toxicities of drug-ferriprotoporphrin IX complexes.[83]

Conversely, the weaker antiplasmodial activity displayed by the simple arylpiperazines compared to **CMP10** and **CMP15** may be due to the absence of the quinoline nitrogen in the arylpiperazines. The quinoline nitrogen in the 4-aminoquinolines is also important for the uptake and accumulation.[84] This fact may also account for the comparable activity of the ferrocenic benzylpiperazine (**CMP19**), which has a second protonable nitrogen, relative to **CMP10** and **CMP15**. With the exception of the NF54 strain, the data against W2, FCR3 and D10 in respect of **CMP19** are comparable to those of the 7-chloro-4-aminoquinoline piperazine (**CMP10**). In fact apart from chloroquine, **CMP19** and **CMP10** are the most active compounds in the series. The major structural difference between **CMP19** and **CMP10** is the presence of the 7-chloroquinolyl group in **CMP10** and the ferrocenyl moiety in **CMP19**. The 7-chloroquinolyl moiety is a well known haem binding template[32] while the ferrocenyl moiety is not. However, the ferrocenyl moiety is a well known hydrophobic and cytotoxic group.[85] The incorporation of a ferrocenyl moiety into the side chain of chloroquine has led to the discovery of ferroquine, which has excellent activity particularly against chloroquine-resistant parasites.[71] Ferroquine is

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currently under phase I clinical trials.[86] The precise mechanism of action of ferroquine is unknown, a probable mechanism has recently been shown to be in part similar to that of chloroquine in as far as haematin as the drug target and inhibition of haemozoin formation are concerned.[87] Since ferrocene itself does not inhibit β-hematin formation,[72] the activity of **CMP19** may in part be due to the lipophilic (log $P_{octanol/water}$ = 3.28) and /or cytotoxic nature of the ferrocene unit. The lipophilicity imparted by the ferrocenyl moiety presumably allows **CMP19** to traverse parasitic membranes.

4.2.2 Class 2: The substituted benzyl arylpiperazines, CMP1 – CMP9, CMP29

Compounds with *N*-substitution of the arylpiperazine (NH) nitrogen can be seen in **Table 4.2**. There are two noteworthy outcomes of this substitution:

1) There is a dramatic drop in activity across all strains.
2) The preferential and /or selective potency against resistant strains observed for all the unsubstituted compounds (Table 4.1) is completely abolished.

Some of the compounds such as **CMP1, CMP2, CMP4, CMP5** and **CMP8** were equally active against the sensitive and resistant strains. Table 4.3 gives a clearer picture of the reduction in the resistance index (RI) on moving from unsubstituted to the substituted arylpiperazine.

The dramatic drop in activity upon the *N*-substitution of the arylpiperazine (NH) nitrogen, as far as compounds depicted in Table 4.2 are concerned, may suggest that the free NH in the unsubstituted arylpiperazines is involved in binding to an (as yet) unknown target. The abolition of the significant potency against resistant strains (relative to sensitive strains) upon *N*-substitution may also suggest a change in the mechanism of

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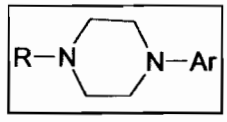
action and /or increased recognition of the substituted arylpiperazines by the chloroquine resistance mechanism.



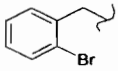
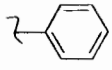
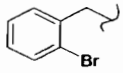
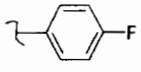
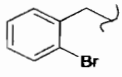
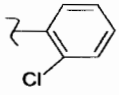
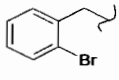
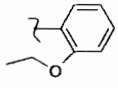
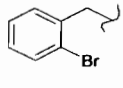
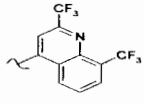
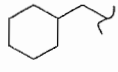
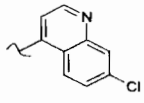
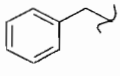
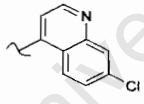
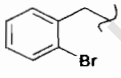
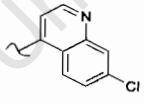
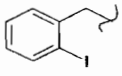
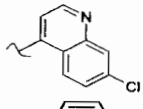
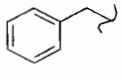
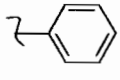
COMPOUND	R	Ar	W2 IC_{50} µM	FCR3 IC_{50} µM	NF54 IC_{50} µM	D10 IC_{50} µM	K1 IC_{50} µM
CMP 6			69.6±14.5	62.1±10.9	58±13	101.3±15.4	-
CMP 7			41.5±5.7	62.9±8.29	63.1±12.3	103.1±27.1	-
CMP 9			56.0±3.7	75.1±28.8	52.4±7.6	104.1±18.2	-
CMP 8			52.6±8.9	54.2±4.74	46.9±8.3	79.7±13.3	-
CMP 5			43.0±6.7	36.1±7.49	23.6±4.6	47.3±1.8	-
CMP 1			15.6±1.8	4.12±0.24	8.06±0.42	19.2±2.3	-
CMP 2			16.9±3.7	13.5±0.42	11.62±0.69	9.04±0.44	-
CMP 3			58.1±9.2	20.1±1.73	11.46±0.86	18.3±0.99	-
CMP 4			13.0±2.1	6.49±0.34	3.9±0.89	12.3±1.8	-
CMP 29			-	-	-	46.69±0.14	60.56±0.09
CQ			0.33±0.044	0.40±0.058	0.034±0.002	0.044±0.006	-

Table 4.2: Antiplasmodial activity CMP1-CMP9, CMP29

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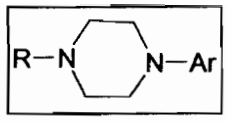


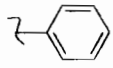
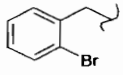
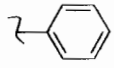
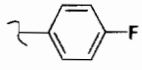
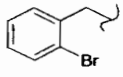
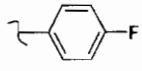
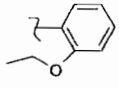
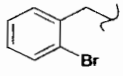
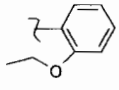
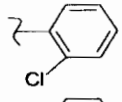
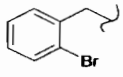
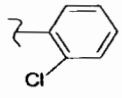
COMPOUND	R	Ar	Resistance Index W2 / NF54	W2 / D10	FCR3 / NF54	FCR3 / D10
CMP 11	H		0.27	0.09	0.15	0.09
CMP 6			1.20	0.69	1.07	0.61
CMP 12	H		0.26	0.18	0.21	0.15
CMP 7			0.66	0.40	0.997	0.61
CMP13	H		0.18	0.12	0.12	0.08
CMP 8			1.12	0.66	1.16	0.68
CMP14	H		0.23	0.12	0.15	0.08
CMP 9			1.07	0.54	1.43	0.72

Table 4.3: Resistance Indices of substituted and unsubstituted piperazines

4.2.3 Class 3: The substituted phenylethyl arylpiperazines, CMP24 – CMP28

The abilities of this class of substituted arylpiperazines to inhibit the growth of the chloroquine-sensitive (D10) and chloroquine-resistant (K1) strains of *Plasmodium falciparum*, were determined at the Division of Pharmacology (UCT) and the results are shown in **Table 4.4**. The antiplasmodial activity is in the IC_{50} range of 13 – 47 μM. Arylpiperazines, **CMP24a** and **CMP24b,** were more active against the chloroquine-resistant strain than against the chloroquine-sensitive strain. The comparison between the two polymorphs (**CMP24a, CMP24b**) shows that the results are comparable as would be

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expected *in vitro*. However, polymorphs are expected to show different activities *in vivo* due to differences in bioavailability.

On the other hand, arylpiperazines, **CMP25, CMP26, CMP27, CMP28** in which substituents were introduced in the aromatic ring, were more potent in the chloroquine-sensitive strain than the chloroquine-resistant strain. The slightly more favourable position for the fluoro group (**CMP25** and **CMP26**) is the *para* position. However, the change from the fluoro group to ethoxy and chloro groups makes the compounds slightly more active in both chloroquine-sensitive and chloroquine-resistant strains.

Increasing the chain length by one carbon, between the piperazine and side chain, can only be accurately compared between **CMP24** and **CMP29**, from Table 4.2, pg 77. The activity of **CMP29** is not statistically significantly different in the chloroquine-sensitive strain but there is a dramatic decrease in activity in the chloroquine-resistant strain. The resistance index shows that **CMP24** is 2.5 times more potent in the chloroquine-resistant strain than **CMP29**.

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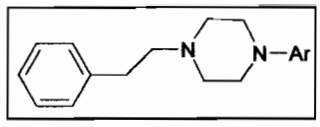


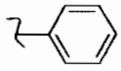
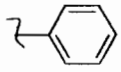
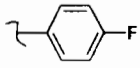
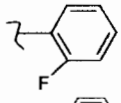
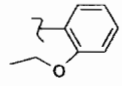
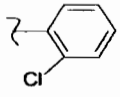
COMPOUND	Ar	D10 IC_{50} µM	K1 IC_{50} µM	RI (K1/D10)
CMP 24a		43.96 ± 0.18	29.35 ± 0.16	0.67
CMP 24b		47.45 ± 0.22	25.96 ± 0.16	0.55
CMP 25		18.38 ± 0.13	33.74 ± 0.13	1.84
CMP 26		19.45 ± 0.12	33.80 ± 0.13	1.74
CMP 27		14.40 ± 0.09	23.34 ± 0.08	1.62
CMP 28		13.18 ± 0.10	14.71 ± 0.14	1.12

Table 4.4: Antiplasmodial activity and Resistance Indices for CMP24-CMP28

4.2.4 Class 4: The substituted arylpiperazines, CMP30 and CMP31

These substituted arylpiperazines are more potent in the chloroquine-sensitive (D10) strain than the chloroquine-resistant (K1) strain, **Table 4.5**.

Comparing compound **CMP30** to **CMP11** (Table 4.1, pg 73), **CMP24** (Table 4.4, pg 80) and **CMP29** (Table 4.2, pg 77) it is observed that **CMP30** is significantly less potent than the other two substituted arylpiperazines (**CMP24, CMP29**) in both the chloroquine-sensitive and chloroquine-resistant strains. It is, however, more active in the chloroquine-sensitive strain than the phenyl-piperazine (**CMP11**) but less active in the chloroquine-resistant strain.

The activity of **CMP31** in the chloroquine-resistant (K1) strain was found to be >65.45 µM. The reason a specific value could not be determined was due to the compound's problems with solubility and lack of material. It would not completely dissolve in

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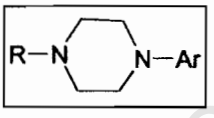
methanol, ethanol or DMSO and the activity of the compound was eventually determined in chloroform. On comparison of **CMP31** to the phenyl-piperazine (**CMP11**), the 1-phenylethyl-4-phenyl-piperazine (**CMP24**) and 1-benzyl-4-phenyl-piperazine (**CMP29**) it is observed that **CMP31** is less active, in both the chloroquine-sensitive and chloroquine-resistant strains, than both the substituted arylpiperazines (**CMP24, CMP29**) but it is more potent, in both strains, than the unsubstituted arylpiperazine (**CMP11**).

CMP31 is significantly more active than **CMP30** in both strains. The increase in the resistance indices also shows the reduction in potency.



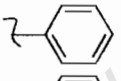
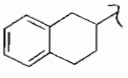
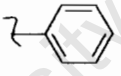
COMPOUND	R	Ar	D10 IC_{50} µM	K1 IC_{50} µM	RI (K1/D10)
CMP 30	Me		64.55 ± 0.26	100.83 ± 0.22	1.56
CMP 31			45.66 ± 0.11	> 65.45	> 1.43

Table 4.5: Antiplasmodial activity and Resistance Index for CMP30 and CMP31

4.2.5 Class 5: The unsubstituted biarylpiperazines, CMP43 – CMP45, CMP47, CMP49 and CMP51

As with compounds **CMP1 – CMP23, CMP25 – CMP31** all of these unsubstituted biarylpiperazines are more potent in the chloroquine-sensitive (D10) strain than the chloroquine-resisitant (K1) strain, as is observed in the data in **table 4.6**. This is contrary to the observations made previously[73] as well as the observations in class 1 (Table 4.1, pg 73).

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When comparing **CMP43, CMP44** and **CMP45** it is observed that the best position for the arylether side chain is the *para* position, for both strains. The potency of **CMP43**, with the arylether side chain in the *ortho* position, is significantly lower than that of **CMP44** and **CMP45**.

The most favourable position for the biphenyl side chain is the *meta* position. The activity of **CMP51,** which has the biphenyl side chain in the *ortho* position, is significantly lower than that of **CMP47** (*para*) and **CMP49** (*meta*).

From the data it is observed that the biphenyl system (**CMP47, CMP49, CMP51**) provides the compounds with better activity than the corresponding arylether system (**CMP43, CMP44, CMP45**). This data also shows that this class of compounds generally has better activity than classes 1, 2, 3 and 4 against D10, with the exception of the compounds with the aminoquinoline moieties (**CMP1, CMP2, CMP3, CMP4, CMP10, CMP15**) and the ferrocenic benzylpiperazine (**CMP19**). The resistance indices are however much higher and therefore suggest the biphenyl compounds are less likely to be more potent against the chloroquine-resistant strains than against the chloroquine-sensitive strains.

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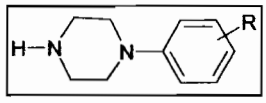


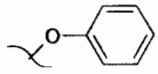
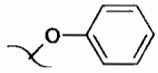
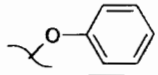
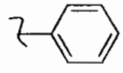
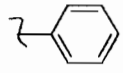
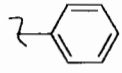
COMPOUND	R	position	D10 IC_{50} µM	K1 IC_{50} µM	RI (K1/D10)
CMP 43		*ortho*	34.16 ± 0.06	56.58 ± 0.09	1.66
CMP 44		*meta*	17.31 ± 0.11	35.34 ± 0.48	2.04
CMP 45		*para*	13.03 ± 0.06	20.12 ± 0.09	1.54
CMP 47		*para*	9.40 ± 0.13	11.97 ± 0.12	1.27
CMP 49		*meta*	5.50 ± 0.10	10.11 ± 0.24	1.84
CMP 51		*ortho*	30.61 ± 0.11	46.81 ± 0.27	1.53

Table 4.6: Antiplasmodial activity and Resistance Indices

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4.3 Conclusions

In conclusion, the results obtained have allowed partial confirmation of the earlier data obtained with the unsubstituted arylpiperazines and partially allowed the establishment of preliminary structure-activity relationships. Despite the modest antiplasmodial activity data obtained at this stage, the simple unsubstituted arylpiperazine nucleus shows promise as a scaffold for the assembly of novel antimalarial agents particularly active against chloroquine-resistant parasites and potentially with novel mechanisms of action. The unsubstituted biaryl piperazines however do not show the same potential as antimalarial agents against chloroquine-resistant parasites, although they are generally more potent than the substituted arylpiperazines in D10, except for the *ortho* positioned biaryl piperazines (**CMP43, CMP51**). Detailed meaningful structure-activity relationship studies need to be delineated clearly for these arylpiperazines to warrant mechanistic

studies and further development as novel antimalarials. The identified novel polymorphs, **CMP24a** and **CMP24b**, need to be tested *in vivo* in order to allow establishment of the more bioavailable form.

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CHAPTER 5

EXPERIMENTAL

5.1 General

All reactions were monitored by thin layer chromatography using aluminum-backed silica gel $60F_{254}$ plates (Merck). Ultraviolet light was used to visualise the plates. Column chromatography was carried out on silica gel (Merck Kieselgel 60: 70-230 mesh for gravity). ^{1}H NMR spectra were recorded on a Varian Mercury (300MHz) or a Varian Unity Spectrophotometer (400MHz) and were recorded in parts per million (ppm) with respect to tetramethylsilane (δ = 0.00). ^{13}C NMR spectra were recorded on the same machines but at 75 or 100 MHz. The Infrared Spectra were recorded on a Perkin Elmer Spectrum one FT-IR Spectrometer. Melting points were determined on a Reichert-Jung Thermovar and a Fischer-Johns hot stage microscope and are uncorrected. Low resolution masses were determined by the Department of Pharmacology (UCT) on an API 2000 from Applied Biosystems. The high resolution masses were determined at the University of the Witswatersrand on a VG70-SEQ micromass spectrometer. Elemental analysis was determined on a Fisons EA 110CHN elemental analyzer.

All solvents used were dried by the appropriate technique.[88] Tetrahydrofuran was dried over sodium wire prior to use in the presence of benzophenone as an indicator. Dichloromethane was dried over phosphorous pentoxide prior to use. Methanol, N-methyl-2-pyrrolidinone and dioxane were purchased as anhydrous solvents from Sigma-Aldrich and used as received under an inert atmosphere of nitrogen.

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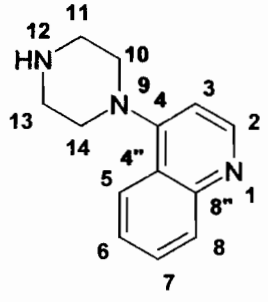
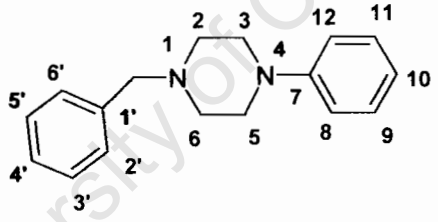
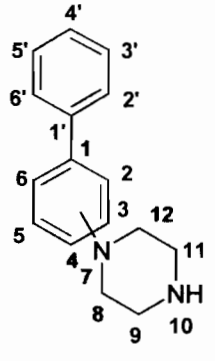
The following numbering system was used to assign the aromatic protons and methylene protons in the proton NMR spectra of the compounds based on the quinoline moiety:



The following numbering system was used to assign the protons in the proton NMR spectra of the compounds based on an aryl piperazine moiety:



The following numbering system was used to assign the protons in the proton NMR spectra of the compounds based on a biaryl piperazine moiety:



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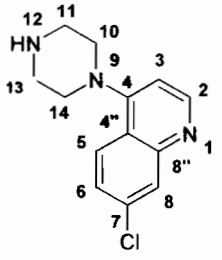
5.2 Synthesis of target compounds

5.2.1 Synthesis of compounds CMP10 and CMP15[71]



7-Chloro-4-piperazin-1-yl-quinoline **(CMP10)**

A mixture of 4,7-dichloroquinoline (1.00 g, 5.1 mmol), piperazine (2.17 g, 25 mmol), potassium carbonate (0.04 g, 0.28 mmol) and triethylamine (0.4 mL, 2.9 mmol) was refluxed at 135°C in N-methyl-2-pyrrolidinone (18 mL) under nitrogen for 4 hours. The mixture was diluted with dichloromethane (50 mL). The mixture was washed with water (50 mL) followed by brine (3 x 50 mL). The combined organic layers were then dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure to afford **CMP10** (0.86 g, 68%) as yellow-cream crystals; mp 109-112°C (from EtOH); R_f 0.40 (MeOH: DCM, 1:9); IR ($CHCl_3$): ν_{max}/cm^{-1} 3680 (NH), 3052 (C-H Ar), 1573 (C=C Ar and C=N), 1264 (C-N); ^{1}H-NMR δ_H (300 MHz, $CDCl_3$) 1.94 (1H, s, N*H*, H12), 3.17 (4H, broad s, N-CH_2, H10, H14), 3.18 (4H, broad s, N-CH_2, H11, H13), 6.83 (1H, d, *J* 5.12 Hz, Ar-*H*, H3), 7.41 (1H, dd, *J* 9.15, 2.11 Hz, Ar-*H*, H6), 7.96 (1H, d, *J* 9.15 Hz, Ar-*H*, H5), 8.03 (1H, d, *J* 2.01 Hz, Ar-*H*, H8), 8.72 (1H, d, *J* 4.94 Hz, Ar-*H*, H2); ^{13}C NMR δ_C (75 MHz, $CDCl_3$) 46.0 (2C), 53.5 (2C), 108.9, 121.9, 125.2, 126.1, 128.9, 134.9, 150.2, 151.9, 157.3; Found: M^+, 247.08934. $C_{13}H_{14}N_3Cl$ requires *M*, 247.08762; Found C: 62.21; H: 5.33, N: 16.25, $C_{13}H_{14}N_3Cl.0.2H_2O$ requires C: 62.13; H: 5.62; N: 16.71.

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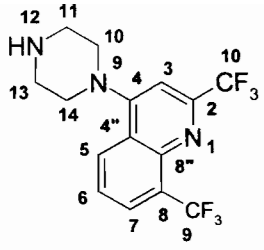

4-piperazin-1-yl-2,8-bis(trifluoromethyl)-quinoline **(CMP15)**

The title compound was prepared from 4-bromo-2,8-bis(trifluoromethyl)quinoline (0.50 g, 1.5 mmol), piperazine (0.63 g, 7.3 mmol), potassium carbonate (0.006 g, 0.04 mmol) and triethylamine (0.06 mL, 0.44 mmol) by the same method as **CMP10** to give **CMP15** (0.47 g, 92%) as yellow-cream crystals; mp 128-131°C (from EtOH); R_f 0.52 (MeOH: DCM 1:9); IR ($CHCl_3$): ν_{max}/cm^{-1} 3680 (NH), 3052 (C-H Ar), 1589 (C=C Ar and C=N), 1306 (CF), 1264 (CN); ^{1}H-NMR δ_H (300 MHz, $CDCl_3$) 1.77 (1H, broad s, N*H*, H12), 3.19 (4H, broad s, N-CH_2, H10, H14), 3.26 (4H, broad s, N-CH_2, H11, H13), 7.21 (1H, s, Ar-*H*, H3), 7.61 (1H, t, *J* 7.94 Hz, Ar-*H*, H6), 8.09 (1H, d, *J* 7.32 Hz, Ar-*H*, H5), 8.35 (1H, d, *J* 7.63 Hz, Ar-*H*, H7); δ_C (75 MHz, $CDCl_3$) 45.9 (2C), 53.6 (2C), 105.2, 125.4 (2C), 128.1 (2C), 128.6 (2C), 128.7, 148.0 (2C), 159.2; Found: M^+, 349.10042. $C_{15}H_{13}N_3F_6$ requires *M*, 349.10136; Found: C: 51.84; H: 3.97; N: 11.69. $C_{15}H_{13}N_3F_6$ requires C: 51.59; H: 3.75; N: 12.03.

5.2.2. General Procedure for the synthesis of CMP1 – CMP9, CMP19, CMP24 – CMP29 and CMP31[72]

A mixture of piperazine (1.0 eq) and aldehyde (1.5 eq) was stirred in anhydrous methanol (6 mL per 1.0 mmol piperazine) for 4 hours at room temperature under nitrogen. Sodium cyanoborohydride (2.1 eq) was added and the mixture stirred for a further 2 hours at

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room temperature under nitrogen. The solvent was removed under reduced pressure. The residue was dissolved in 1N HCl (aq), the mixture washed with diethyl ether (2 x) to remove any excess aldehyde. The organic fraction was discarded and the aqueous layer was neutralized with anhydrous sodium carbonate (white precipitate forms). To the neutralized aqueous layer dichloromethane was added. The organic layer was extracted with dichloromethane (3 x), dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure to afford the target compounds.

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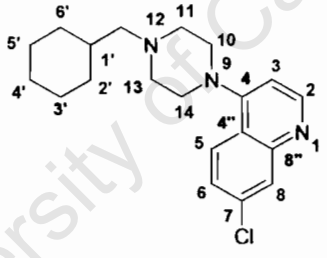
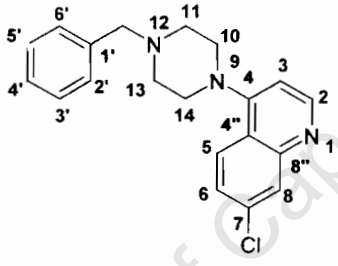


7-chloro-4-(4-cyclohexylmethyl-piperazin-1-yl)-quinoline **(CMP1)**

This compound was prepared from **CMP10** (0.50 g 1.46 mmol), cyclohexanecarboxaldehyde (0.28 g, 2.2 mmol) and sodium cyanoborohydride (0.19 g, 3.0 mmol) by the above method to give **CMP1** (0.27 g, 54%) as cream crystals; mp 91 - 92°C (from EtOH); R_f 0.46 (hexane:ethyl acetate 1:9); IR ($CHCl_3$): ν_{max}/cm^{-1} 3052 (C-H Ar), 1576 (C=C Ar and C=N), 1264 (CN); ^{1}H-NMR δ_H (300 MHz, $CDCl_3$) 0.93 (2H, q, *J* = 11.85 Hz, CH_2, H6'), 1.22 – 1.30 (3H, m, CH_2, H2', H3'a), 1.54 (1H, m, C*H*, H1'), 1.72 - 1.83 (5H, m, CH_2, H5', H4', H3'b), 2.26 (2H, d, *J* 7.15 Hz, CH_2, Hα), 2.69 (4H,

broad s, N-CH_2, H11, H13), 3.25 (4H, broad s, N-CH_2, H10, H14), 6.81 (1H, d, *J* 5.05 Hz, Ar-*H*, H3), 7.41 (1H, dd, *J* 8.89, 2.18 Hz, Ar-*H*, H6), 7.96 (1H, d, *J* 9.06 Hz, Ar-*H*, H5), 8.07 (1H, d, *J* 2.09 Hz, Ar-*H*, H8), 8.74 (1H, d, *J* 5.05 Hz, Ar-*H*, H2); ^{13}C NMR δ_C (75 MHz, $CDCl_3$) 26.1 (2C), 26.8, 31.9 (2C), 35.1, 52.2 (2C), 53.6 (2C), 65.6, 108.9, 121.9, 125.3, 125.9, 128.8, 134.8, 150.2, 151.9, 157.1; Found: M^+, 343.18214. $C_{20}H_{26}N_3Cl$ requires *M*, 343.18152. Found: C: 69.74; H: 7.44; N: 12.20. $C_{20}H_{26}N_3Cl$ requires C: 70.06; H: 7.35; N: 12.25; Cl: 10.34.



4-(4-Benzyl-piperazin-1-yl)-7-chloro-quinoline **(CMP2)**

The title compound was prepared from **CMP10** (0.50 g, 1.46 mmol), benzaldehyde (0.24 g, 2.2 mmol) and sodium cyanoborohydride (0.19 g, 3.1 mmol) by the above method to give **CMP2** (0.32 g, 64%) as cream crystals; mp 119 - 122°C (from EtOH); R_f 0.27 (hexane:ethyl acetate 1:9); IR ($CHCl_3$): ν_{max}/cm^{-1} 3052 (C-H Ar), 1576 (C=C Ar and C=N), 1264 (CN); ^{1}H-NMR δ_H (400 MHz, $CDCl_3$) 2.75 (4H, t, *J* 4.71 Hz, N-CH_2, H11, H13), 3.26 (4H, t, *J* 4.79 Hz, N-CH_2, H10, H14), 3.62 (2H, s, CH_2, Hα), 6.85 (1H, d, *J* 5.05 Hz, Ar-*H*, H3), 7.28 – 7.33 (3H, m, Ar-*H,* H3', H4', H5'), 7.35 (2H, d, *J* 6.80 Hz, Ar-*H*, H2', H6'), 7.41 (1H, dd, *J* 8.98, 2.18 Hz, Ar-*H*, H6), 7.95 (1H, d, *J* 8.89 Hz, Ar-*H*, H5), 8.05 (1H, d, *J* 2.09 Hz, Ar-*H*, H8), 8.73 (1H, d, *J* 5.05 Hz, Ar-*H*, H2); ^{13}C NMR δ_C (100 MHz, $CDCl_3$) 52.2 (2C), 52.9 (2C), 62.8, 108.6, 122.5, 124.9, 126.2, 127.3, 128.3

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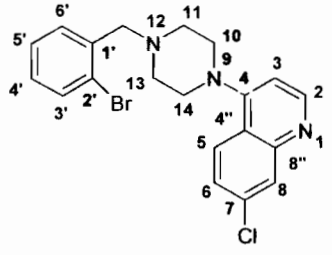
(2C), 129.1, 129.2 (2C), 135.6, 138.3, 150.3, 151.9, 157.1; Found: M^+, 337.13438. $C_{20}H_{20}ClN_3$ requires *M*, 337.13457. Found: C: 70.92; H: 5.34; N: 12.04. $C_{20}H_{20}ClN_3$ requires C: 71.10; H: 5.97; N: 12.44; Cl: 10.49.



4-[4-(2'-Bromo-benzyl)-piperazin-1-yl]-7-chloro-quinoline **(CMP3)**

This compound was prepared from **CMP10** (0.50 g, 1.46 mmol), 2-bromobenzaldehyde (0.29 g, 1.61 mmol) and sodium cyanoborohydride (0.19 g, 3.1 mmol) by the above method to give **CMP3** (1.16 g, 100%) as cream-yellow crystals; mp 90 - 93°C (from EtOH); R_f 0.52 (hexane:ethyl acetate 1:9); IR ($CHCl_3$): ν_{max}/cm^{-1} 3052 (C-H Ar), 1576 (C=C Ar and C=N), 1264 (CN); ^{1}H-NMR δ_H (300 MHz, $CDCl_3$) 2.83 (4H, t, *J* 4.79 Hz, N-C*H*$_2$, H11, H13), 3.25 (4H, t, *J* 4.71 Hz, N-C*H*$_2$, H10, H14), 3.77 (2H, s, C*H*$_2$, Hα), 6.84 (1H, d, *J* 5.05 Hz, Ar-*H*, H3), 7.16 (1H, t, *J* 7.67 Hz, Ar-*H*, H5'), 7.33 (1H, t, *J* 7.49 Hz, Ar-*H*, H4'), 7.43 (1H, dd, *J* 8.89, 2.18 Hz, Ar-*H*, H6), 7.50 (1H, d, *J* 7.67, Ar-*H*, H6'), 7.59 (1H, d, *J* 7.84 Hz, Ar-*H*, H3'), 7.97 (1H, d, *J* 9.06 Hz, Ar-*H*, H5), 8.07 (1H, d, *J* 2.27 Hz, Ar-*H*, H8), 8.73 (1H, d, *J* 5.05 Hz, Ar-*H*, H2); ^{13}C NMR δ_C (75 MHz, $CDCl_3$) 52.2 (2C), 52.9 (2C), 61.7, 108.9, 121.9, 124.8, 125.2, 126.1, 127.3, 128.6, 128.8, 130.9, 132.9, 134.9, 137.2, 150.1, 151.8, 157.0; Found: M^+, 415.04329. $C_{20}H_{19}BrClN_3$ requires

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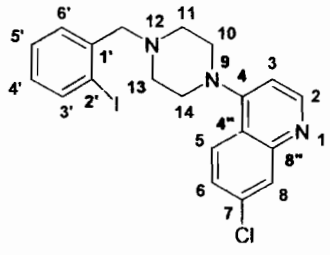
M, 415.04509. Found: C: 57.52; H: 4.59; N, 9.60. $C_{20}H_{19}BrClN_3$ requires C: 57.64; H: 4.60; N: 10.08; Br: 19.17; Cl: 8.51.



7-Chloro-4-[4-(2'-iodo-benzyl)-piperzin-1-yl]-quinoline **(CMP4)**

This compound was prepared from **CMP10** (0.50 g, 1.46 mmol), 2-iodobenzaldehyde (0.38 g, 1.61 mmol) and sodium cyanoborohydride (0.19 g, 3.1 mmol) by the above method to give **CMP4** (0.18 g, 27%) as a yellow oil; R_f 0.57 (hexane:ethyl acetate 1:9); IR ($CHCl_3$): ν_{max}/cm^{-1} 3052 (C-H Ar), 1576 (C=C Ar and C=N), 1264 (C-N); ^{1}H-NMR δ_H (300 MHz, $CDCl_3$) 2.83 (4H, t, *J* 4.79 Hz, N-C*H*$_2$, H11, H13), 3.25 (4H, t, *J* 4.71 Hz, N-C*H*$_2$, H10, H14), 3.67 (2H, s, CH_2, Hα), 6.82 (1H, d, *J* 5.05 Hz, Ar-*H*, H3), 7.14 (1H, t, *J* 7.67 Hz, Ar-*H*, H5'), 7.34 (1H, t, *J* 7.49 Hz, Ar-*H*, H4'), 7.43 (1H, dd, *J* 8.98, 2.09 Hz, Ar-*H*, H6), 7.51 (1H, d, *J* 7.67 Hz, Ar-*H*, H6'), 7.86 (1H, d, *J* 6.71 Hz, Ar-*H*, H3'), 7.95 (1H, d, *J* 9.06 Hz, Ar-*H*, H5), 8.03 (1H, d, *J* 2.21 Hz, Ar-*H*, H8), 8.71 (1H, d, *J* 5.05 Hz, Ar-*H*, H2); ^{13}C NMR δ_C (75 MHz, $CDCl_3$) 52.2 (2C), 52.8 (2C), 66.3, 100.6, 108.9, 121.9, 125.2, 126.0, 128.0, 128.8, 128.9, 130.4, 134.9, 139.6, 140.2, 150.1, 151.8, 157.1; Found: M^+, 463.02897. $C_{20}H_{19}ClIN_3$ requires *M*, 463.03122. Found: C: 51.31; H: 4.05; N: 8.80. $C_{20}H_{19}ClIN_3 \cdot 0.1H_2O$ requires C: 51.68; H: 4.12; N: 9.04; Cl: 7.63; I: 27.30.

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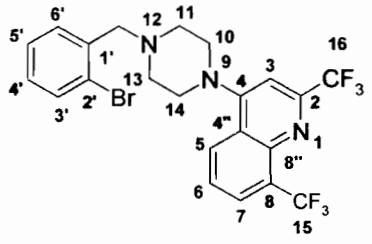

4-[4-(2'-Bromobenzyl)-piperazin-1-yl]-2,8-bis(trifluoromethyl)quinoline **(CMP5)**

The title compound was prepared from **CMP15** (0.50 g, 1.43 mmol), 2-bromobenzaldehyde (0.29 g, 1.61 mmol) and sodium cyanoborohydride (0.19 g, 3.1 mmol) by the above method to give **CMP5** (0.39 g, 59%) as cream crystals; mp 131 - 133°C (from EtOH); R_f 0.83 (hexane:ethyl acetate 1:9); IR ($CHCl_3$): ν_{max}/cm^{-1} 3052 (C-H Ar), 1589 (C=C Ar and C=N), 1309 (C-F), 1264 (C-N); ^{1}H-NMR δ_H (300 MHz, $CDCl_3$) 2.83 (4H, t, *J* 4.73 Hz, N-CH_2, H11, H13), 3.32 (4H, t, *J* 4.88 Hz, N-CH_2, H10, H14), 3.76 (2H, s, CH_2, Hα), 7.15 (1H, t, *J* 7.78 Hz, Ar-*H*, H5'), 7.22 (1H, s, Ar-*H*, H3), 7.32 (1H, t, *J* 7.48 Hz, Ar-*H*, H4'), 7.50 (1H, d, *J* 7.63 Hz, Ar-*H*, H6'), 7.64 – 7.57 (2H, m, Ar-*H*, H6, H3'), 8.07 (1H, d, *J* 7.32 Hz, Ar-*H*, H5), 8.26 (1H, d, *J* 7.63 Hz, Ar-*H*, H7); ^{13}C NMR δ_C (100 MHz, $CDCl_3$) 52.3 (2C), 52.6 (2C), 61.2, 105.1, 122.4, 124.1, 124.3, 125.1, 127.2, 127.9, 128.7 (2C), 130.9, 133.1, 134.1, 136.9, 144.8, 148.7, 148.9, 158.9; Found: M^+, 517.05931. $C_{22}H_{18}BrF_6N_3$ requires *M*, 517.05883. Found: C: 50.93; H: 3.52; N: 8.15. $C_{22}H_{18}BrF_6N_3$ requires C: 50.99; H: 3.50; N: 8.10; Br: 15.42; F: 21.99.

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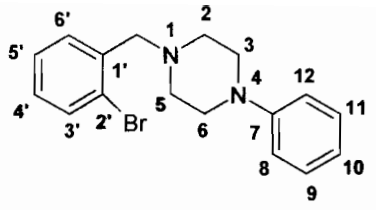

1-(2'-Bromobenzyl)-4-phenyl-piperazine **(CMP6)**

This compound was prepared from phenylpiperazine (0.50 g, 0.47 mL, 3.1 mmol), 2-bromobenzaldehyde (0.63 g, 3.4 mmol) and sodium cyanoborohydride (0.41 g, 6.5 mmol) by the above method to give **CMP6** (0.437 g, 42%) as cream crystals; mp 109 - 113°C (from EtOH); R_f 0.76 (hexane:ethyl acetate 1:9); IR ($CHCl_3$): ν_{max}/cm^{-1} 3052 (C-H Ar), 1599 (C=C Ar), 1264 (CN); ^{1}H-NMR δ_H (400 MHz, $CDCl_3$) 2.70 (4H, t, *J* 4.88 Hz, N-CH_2, H 2, H5), 3.23 (4H, t, *J* 4.88 Hz, N-CH_2, H3, H6), 3.68 (2H, s, CH_2, Hα), 6.85 (1H, t, *J* 7.32 Hz, Ar-*H*, H10), 6.92 (2H, d, *J* 7.94 Hz, Ar-*H*, H12, H8), 7.12 (1H, t, *J* 7.63 Hz, Ar-*H*, H5'), 7.26 (2H, t, *J* 8.09, Ar-*H,* H9, H11), 7.33 (1H, t, *J* 7.32 Hz, Ar-*H,* H4'), 7.55 (1H, d, *J* 7.94 Hz, Ar-*H*, H6'), 7.57 (1H, d, *J* 7.94 Hz, Ar-*H*, H3'); ^{13}C NMR δ_C (75 MHz, $CDCl_3$) 49.2 (2C), 53.1 (2C), 61.7, 116.0, 116.1 (2C), 116.4, 119.6, 127.2, 128.5, 129.1, 129.1, 130.8, 132.8, 151.4; Found: M^+, 330.07242. $C_{17}H_{19}BrN_2$, requires *M*, 330.07316. Found: C: 61.79; H: 5.84; N: 8.33. $C_{17}H_{19}BrN_2$ requires C: 61.64; H: 5.78; N: 8.45; Br: 24.12.

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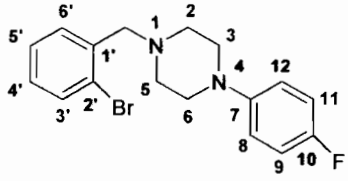
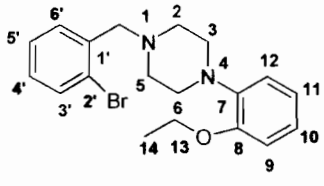


1-(2'-Bromobenzyl)-4-(4-fluoro-phenyl)-piperazine **(CMP7)**

The title compound was prepared from 4-fluorophenylpiperazine (0.50 g, 2.7 mmol), 2-bromobenzaldehyde (0.57 g, 3.4 mmol) and sodium cyanoborohydride (0.37 g, 5.8 mmol) by the above method to give **CMP7** (0.34 g, 35%) as cream crystals; mp 76 - 78°C (from EtOH); R_f 0.76 (hexane:ethyl acetate 1:9); IR ($CHCl_3$): ν_{max}/cm^{-1} 3052 (C-H Ar), 1508 (C=C Ar), 1420 (CF), 1264 (CN); ^{1}H-NMR δ_H (300 MHz, $CDCl_3$) 2.70 (4H, t, *J* 4.88 Hz, N-CH_2, H2, H5), 3.14 (4H, t, *J* 5.04 Hz, N-CH_2, H3, H6), 3.68 (2H, s, CH_2, Hα), 6.85 – 6.99 (4H, m, Ar-*H*, H8, H9, H11, H12), 7.12 (1H, t, *J* 7.63 Hz, Ar-*H*, H5'), 7.30 (1H, t, *J* 7.62 Hz, Ar-*H*, H4'), 7.51 (1H, d, *J* 7.63 Hz, Ar-*H*, H6'), 7.56 (1H, d, *J* 7.96 Hz, Ar-*H*, H3'); ^{13}C NMR δ_C (100 MHz, $CDCl_3$) 50.2 (2C), 53.0 (2C), 61.9, 115.2, 115.4, 117.6, 117.8, 124.7, 126.9, 128.3, 130.7, 132.9, 145.8, 155.6, 158.8; Found: M^+, 348.06045. $C_{17}H_{18}BrFN_2$ requires *M*, 348.06374. Found: C: 58.26; H: 5.11; N: 7.85. $C_{17}H_{18}BrFN_2 \cdot 0.1H_2O$ requires C: 58.17; H: 5.17; N: 7.98; Br: 22.88; F: 5.44.



1-(2'-Bromobenzyl)-4-(8-ethoxy-phenyl)-piperazine**(CMP8)**

1-(2-ethoxyphenyl)piperazine monohydrochloride (1.00 g, 4.12 mmol) and polymer-supported tetraalkylammonium carbonate macroporous triethylammonium

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methylpolystyrene carbonate (MP-carbonate) 2.74mmol/g loading (3.01 g, 8.24 mmol) were shaken in methanol for 3 hours at room temperature, the resin was removed by filtration and washed with methanol, the filtrate was concentrated under reduced pressure to give the free base (0.84 g, 98%).

This target compound was prepared from 1-(2-ethoxyphenyl)piperazine (0.50 g, 2.1 mmol), 2-bromobenzaldehyde (0.42 g, 2.3 mmol) and sodium cyanoborohydride (0.27 g, 4.3 mmol) by the above method to give **CMP8** (0.49 g, 64%) as a cream oil; R_f 0.84 (hexane:ethyl acetate 1:9); IR ($CHCl_3$): ν_{max}/cm^{-1} 3052 (C-H Ar), 1593 (C=C Ar), 1238 (CN), 1143 (C-O), 1042 (C-O); ^{1}H-NMR δ_H (300 MHz, $CDCl_3$) 1.45 (3H, t, *J* 7.02 Hz, C*H*$_3$, H14), 2.74 (4H, t, *J* 4.73 Hz, N-C*H*$_2$, H2, H5), 3.14 (4H, t, *J* 4.58 Hz, N-C*H*$_2$, H3, H6), 3.69 (2H, s, C*H*$_2$, Hα), 4.06 (2H, q, *J* 7.02 Hz, C*H*$_2$, H13), 6.93 – 6.98 (4H, m, Ar-*H*, H9, H10, H11, H12), 7.12 (1H, t, *J* 7.78 Hz, Ar-*H*, H5'), 7.30 (1H, t, *J* 7.63 Hz, Ar-*H*, H4'), 7.53 (1H, d, *J* 5.19 Hz, Ar-*H*, H6'), 7.57 (1H, d, *J* 7.94 Hz, Ar-*H*, H3'); ^{13}C NMR δ_C (100 MHz, $CDCl_3$) 15.3, 50.8 (2C), 53.4 (2C), 61.6, 63.0, 112.7, 117.9, 121.0, 122.5, 124.7, 127.6, 128.6, 131.0, 132.9, 137.6, 141.8, 151.7; Found: M^+, 374.10063. $C_{19}H_{23}BrN_2O$ requires *M*, 374.09937. Found: C: 60.65; H: 6.03; N: 7.89. $C_{19}H_{23}BrN_2O$ requires C: 60.81; H: 6.18; Br: 21.29; N: 7.46; O: 4.26.

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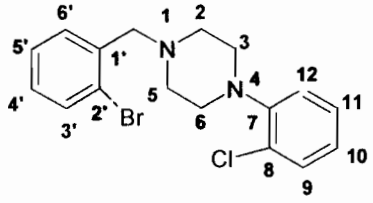

1-(2-Bromobenzyl)-4-(8-chloro-phenyl)-piperazine **(CMP9)**

1-(2-chlorophenyl)piperazine monohydrochloride (1.00 g, 4.29 mmol) and polymer-supported tetraalkylammonium carbonate macroporous triethylammonium methylpolystyrene carbonate (MP-carbonate) 2.74mmol/g loading (3.13 g, 8.58 mmol) were shaken in methanol at room temperature for 3 hours, the resin was removed by filtration and washed with methanol, the filtrate was concentrated under reduced pressure to give the free base (0.76 g, 90%).

This target compound was prepared from 1-(2-chlorophenyl)piperazine (0.50 g, 2.1 mmol), 2-bromobenzaldehyde (0.43 g, 2.4 mmol) and sodium cyanoborohydride (0.28 g, 4.5 mmol) by the above method to give **CMP9** (0.35 g, 38%) as cream crystals; mp 82 - 84°C (from EtOH); R_f 0.84 (hexane:ethyl acetate 1:9); IR ($CHCl_3$): ν_{max}/cm^{-1} 3059 (C-H Ar), 1586 (C=C Ar), 1264 (CN); ^{1}H-NMR δ_H (300 MHz, $CDCl_3$) 2.74 (4H, t, *J* 4.58 Hz, N-C*H*$_2$, H2, H5), 3.10 (4H, t, *J* 4.58 Hz, N-C*H*$_2$, H3, H6), 3.70 (2H, s, C*H*$_2$, Hα), 6.96 (1H, t, *J* 7.63 Hz, Ar-*H*, H11), 7.04 (1H, d, *J* 8.24 Hz, Ar-*H*, H12), 7.12 (1H, t, *J* 7.78 Hz, Ar-*H*, H5'), 7.21 (1H, t, *J* 7.63 Hz, Ar-*H*, H10), 7.30 (1H, t, *J* 7.58 Hz, Ar-*H*, H4'), 7.34 (1H, d, *J* 7.94 Hz, Ar-*H*, H9), 7.36 (1H, d, *J* 7.63 Hz, Ar-*H*, H6'), 7.57 (1H, d, *J* 7.94 Hz, Ar-*H*, H3'); ^{13}C NMR δ_C (100 MHz, $CDCl_3$) 51.3 (2C), 53.3 (2C), 61.8, 120.3, 122.9, 124.9, 127.2, 127.8, 128.3, 128.6, 130.5, 131.0, 133.2, 137.6, 149.0; Found: M^+, 364.0328. $C_{17}H_{18}BrClN_2$ requires *M*, 364.03419. Found: C: 55.83; H: 4.98; N: 7.75. $C_{17}H_{18}BrClN_2$ requires C: 55.83; H: 4.96; N: 7.66; Br: 21.85; Cl: 9.69.

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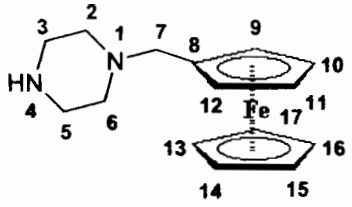
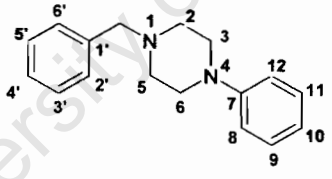


1-piperazinylmethyl ferrocene **(CMP19)**

(0.38 g, 53 %) as orange-yellow crystals; R_f 0.1 (methanol:dichloromethane 1:9); v_{max}/cm^{-1} 3688 (NH), 3015 (C-H Ar), 1603 (C=C Ar), 1216 (CN); ^{1}H-NMR δ_H (400 MHz, $CDCl_3$) 1.24 (1H, broad s, N-*H*, H4), 2.56 (4H, t, *J* 4.76 Hz, N-CH_2, H3, H5), 3.01 (4H, t, *J* 4.85 Hz, N-CH_2, H2, H6), 3.39 (2H, s, CH_2, H7), 4.09 (7H, s, C-*H*, H10, H11, H13, H14, H15, H16, H17), 4.14 (2H, s, C-*H*, H9, H12); ^{13}C NMR δ_C (100 MHz, $CDCl_3$) 44.3 (2C), 51.0 (2C), 58.3, 68.3 (2C), 68.5 (5C), 70.2 (2C), 81.7; Found: M^+, 284.09795. $C_{15}H_{20}N_2Fe$ requires *M*, 284.09759.



1-Benzyl-4-phenyl-piperazine **(CMP29)**

The title compound was prepared from phenylpiperazine (0.50 g, 0.48 mL, 3.1 mmol), benzaldehyde (0.36 g, 0.34 mL, 3.9 mmol) and sodium cyanoborohydride (0.41 g, 6.5 mmol) by the above method to give **CMP29** (0.14 g, 18%) was obtained as a yellow oil; R_f 0.65 (hexane:ethyl acetate 1:9); IR ($CHCl_3$): v_{max}/cm^{-1} 3007 (C-H Ar), 1599 (C=C Ar), 1296 (CN); ^{1}H-NMR δ_H (300 MHz, $CDCl_3$) 2.63 (4H, t, *J* 5.04 Hz, N-CH_2, H2, H5), 3.21 (4H, t, *J* 5.04 Hz, N-CH_2, H3, H6), 3.58 (2H, s, CH_2, Hα), 6.85 (1H, t, *J* 7.32 Hz, Ar-*H*, H10), 6.91 (2H, d, *J* 7.03 Hz, Ar-*H*, H12, H8), 7.23 (1H, d, *J* 7.94 Hz, Ar-*H*, H6'),

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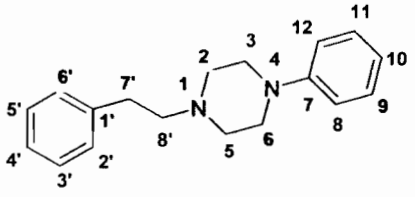
7.22 (1H, d, *J* 8.24 Hz, Ar-*H,* H2'), 7.36 (5H, m, Ar-*H*, H11, H9, H5', H4', H3'); ^{13}C NMR δ_C (100 MHz, $CDCl_3$) 49.3 (2C), 53.3 (2C), 63.3, 113.4, 116.3, 119.8, 127.2, 127.4, 127.9, 128.5, 128.8, 129.3 (2C), 129.5, 151.6; Found: M^+, 252.16456. $C_{17}H_{20}N_2$ requires *M*, 252.16265.



1-Phenylethyl-4-phenyl-piperazine **(CMP24)**

Method A: Reductive amination:

The title compound was prepared from phenylpiperazine (0.20 g, 0.19 mL, 1.2 mmol), phenylacetaldehyde (0.20 g, 0.2 mL, 1.8 mmol) and sodium cyanoborohydride (0.16 g, 2.6 mmol) by the above method to give **CMP24** (0.04 g, 11%) as cream crystals; mp 69 - 72°C (from EtOH); R_f 0.64 (hexane:ethyl acetate 2:8); IR ($CHCl_3$): ν_{max}/cm^{-1} 2934 (C-H Ar), 1599 (C=C Ar), 1234 (CN); ^{1}H-NMR δ_H (300 MHz, $CDCl_3$) 2.61 – 2.72 (6H, m, N-C*H*$_2$, C-*H*$_2$, H2, H5, H8'), 2.84 – 2.88 (2H, m, C-*H*$_2$, H7'), 3.25 (4H, t, *J* 5.06 Hz, N-C*H*$_2$, H3, H6), 6.86 (1H, t, *J* 7.32 Hz, Ar-*H*, H10), 6.94 (2H, d, *J* 7.84 Hz, Ar-*H*, H12, H8), 7.19 – 7.32 (7H, m, Ar-*H*, H11, H9, H2', H3', H4', H5', H6'); ^{13}C NMR δ_C (75 MHz, $CDCl_3$) 41.3, 54.1 (2C), 57.5 (2C), 63.4, 109.0 (2C), 112.0, 117.2, 119.1 (2C), 119.3 (2C), 119.7 (2C), 128.6, 137.8; Found: M^+, 266.17857. $C_{18}H_{22}N_2$ requires *M*, 266.17830. Found: C: 80.47; H: 8.32; N: 10.28. $C_{18}H_{22}N_2$:0.1H_2O requires C: 80.68; H: 8.28; N: 10.45.

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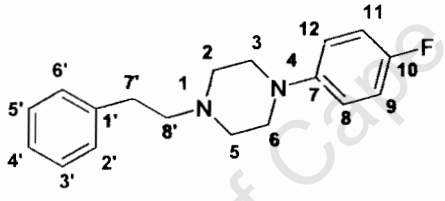
Method B: Hydroamination:[73]

This product was prepared from phenylpiperazine (0.20 g, 0.18 mL, 1.2 mmol), styrene (0.25 g, 0.16 mL, 2.46 mmol) and butyllithium (0.15 mL, 20 mol %) in dry tetrahydrofuran (10 mL) at 25°C for 10 min then 120°C for 24 hours under nitrogen. The mixture was quenched with methanol (10 mL) and the solvent removed under reduced pressure to give two products **CMP24a** and **CMP24b**. **CMP24a** as cream crystals (0.29g, 88%) R_f 0.59 (hexane:ethyl acetate 2:8); and **CMP24b** as white crystals (0.01g, 2%) R_f 0.31 (hexane:ethyl acetate 2:8);



1-(4-Fluoro-phenyl)-4-phenylethyl-piperazine **(CMP25)**

This compound was prepared from 1-(4-fluorophenyl)-piperazine (0.50 g, 2.8 mmol), phenylacetaldehyde (0.70 g, 0.68 mL, 6.1 mmol) and sodium cyanoborohydride (0.37 g, 8.8 mmol) by the above method to give **CMP25** (0.11 g, 13%) as cream crystals; mp 76 - 80°C (from EtOH); R_f 0.69 (hexane:ethyl acetate 2:8); IR ($CHCl_3$): ν_{max}/cm^{-1} 2949 (C-H Ar), 1509 (C=C Ar), 1234 (CN); ^{1}H-NMR δ_H (400 MHz, $CDCl_3$) 2.66 – 2.72 (6H, m, N-CH_2, C-H_2, H2, H5, H8'), 2.84 – 2.89 (2H, m, C-H_2, H7'), 3.17 (4H, t, *J* 4.94 Hz, N-CH_2, H3, H6), 6.87 (1H, d, *J* 4.58 Hz, Ar-*H*, H2'), 6.89 (1H, d, *J* 4.58 Hz, Ar-*H*, H6'), 6.97 (2H, t, *J* 8.61 Hz, Ar-*H*, H3', H5'), 7.19 – 7.37 (5H, m, Ar-*H*, H4', H8, H9, H11, H12); ^{13}C NMR δ_C (75 MHz, $CDCl_3$) 33.5, 50.1 (2C), 53.2 (2C), 60.4, 115.4, 115.7, 117.8, 117.9, 126.1, 126.5, 128.4 (2C), 128.6, 128.7, 128.7, 129.0; Found: M^+, 284.17.

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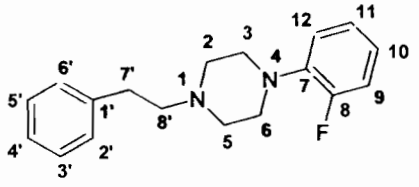
$C_{18}H_{21}N_2F$ requires *M*, 284.17. Found: C: 75.67; H: 7.51; N: 9.59. $C_{18}H_{21}N_2F$ requires C: 76.08; H: 7.45; N: 9.85.



***1-(2-Fluoro-phenyl)-4-phenylethyl-piperazine* (CMP26)**

This target compound was prepared from 1-(2-fluorophenyl)-piperazine (0.50 g, 0.44 mL, 2.8 mmol), phenylacetaldehyde (0.70 g, 0.68 mL, 6.1 mmol) and sodium cyanoborohydride (0.37 g, 8.8 mmol) by the above method to give **CMP26** (0.07 g, 9%) as a yellow oil; R_f 0.73 (hexane:ethyl acetate 2:8); IR ($CHCl_3$): ν_{max}/cm^{-1} 3007 (C-H Ar), 1498 (C=C Ar), 1238 (CN); ^{1}H-NMR δ_H (300 MHz, $CDCl_3$) 2.68 - 2.76 (4H, m, N-C*H*$_2$, H2, H5), 2.85 – 2.90 (4H, m, N-C*H*$_2$, H3, H6), 3.17 (2H, t, *J* 4.85 Hz, C*H*$_2$, H8'), 3.87 (2H, t, *J* 6.50 Hz, C*H*$_2$, H7'), 6.89 – 7.09 (2H, m, Ar-*H*, H9, H10), 7.22 (3H, d, *J* 7.14 Hz, Ar-*H*, H2', H6', H12), 7.30 (4H, t, *J* 7.23 Hz, Ar-*H*, H3', H4', H5', H11); ^{13}C NMR δ_C (100 MHz, $CDCl_3$) 33.2, 50.2 (2C), 53.3 (2C), 60.4, 116.2, 116.5, 119.3, 119.3, 122.9, 124.7, 126.5, 126.7, 128.7, 128.9 (2C), 129.2; Found: M^+, 284.16877. $C_{18}H_{21}N_2F$ requires *M*, 284.16888.

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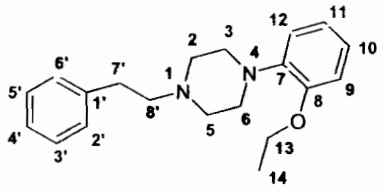

1-(2-Ethoxy-phenyl)-4-phenylethyl-piperazine **(CMP27)**

The title compound was prepared from 1-(2-ethoxyphenyl)-piperazine (0.20 g, 0.9 mmol), phenylacetaldehyde (0.18 g, 0.16 mL, 1.5 mmol), sodium cyanoborohydride (0.13 g, 2.0 mmol) and triethylamine (0.42 g, 0.57 mL, 4.1 mmol) by the above method to give **CMP27** (0.08 g, 26%) was obtained as a yellow oil; R_f 0.58 (hexane:ethyl acetate 1:9); IR ($CHCl_3$): ν_{max}/cm^{-1} 3007 (C-H Ar), 1595 (C=C Ar), 1238 (CN); ^{1}H-NMR δ_H (400 MHz, $CDCl_3$) 1.46 (3H, t, *J* 7.05 Hz, CH_3, H14), 2.67 – 2.71 (2H, m, CH_2, H8'), 2.75 (4H, broad s, N-CH_2, H2, H5), 2.85 – 2.89 (2H, m, CH_2, H7'), 3.17 (4H, broad s, N-CH_2, H3, H6), 4.07 (2H, q, *J* 6.96 Hz, O-CH_2, H13), 6.85 (1H, d, *J* 7.69 Hz, Ar-*H*, H9), 6.89 – 6.99 (3H, m, Ar-*H*, H10, H11, H12), 7.19 (1H, d, *J* 7.14 Hz, Ar-*H*, H2'), 7.23 (1H, d, *J* 7.14 Hz, Ar-*H*, H6'), 7.28 – 7.32 (3H, m, Ar-*H*, H3', H4', H5'); ^{13}C NMR δ_C (100 MHz, $CDCl_3$) 15.2, 33.8, 50.8 (2C), 53.7 (2C), 60.8, 63.8, 112.9, 118.4, 121.3, 122.9, 126.3, 128.6 (2C), 128.9 (2C), 140.6, 141.7, 151.8; Found: M^+, 310.20342. $C_{20}H_{26}N_2O$ requires *M*, 310.20451. Found: C: 75.88; H: 8.40; N: 8.76. $C_{20}H_{26}N_2O{\cdot}0.3H_2O$ requires C: 76.11; H: 8.30; N: 8.87.

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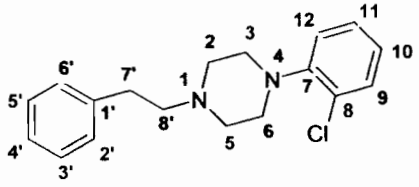

1-(2-Chloro-phenyl)-4-phenylethyl-piperazine **(CMP28)**

This target compound was prepared from 1-(2-chlorophenyl)-piperazine (0.50 g, 2.4 mmol), phenylacetaldehyde (0.60 g, 0.53 mL, 4.7 mmol), sodium cyanoborohydride (0.28 g, 4.5 mmol) and triethylamine (0.43 g, 0.60 mL, 4.3 mmol) by the above method to give **CMP28** (0.08 g, 13%) as cream crystals; mp 72 - 76°C (from EtOH); R_f 0.64 (hexane:ethyl acetate 1:9); IR ($CHCl_3$): ν_{max}/cm^{-1} 2942 (C-H Ar), 1588 (C=C Ar), 1230 (CN); ^{1}H-NMR δ_H (400 MHz, $CDCl_3$) 2.69 – 2.76 (6H, m, N-*CH*$_2$, *CH*$_2$, H3, H6, H8'), 2.88 (2H, m, C*H*$_2$, H7'), 3.14 (4H, t, *J* 4.30 Hz, N-C*H*$_2$, H2, H5), 6.97 (1H, t, *J* 7.60, Ar-*H*, H11), 7.06 (1H, d, *J* 8.16, Ar-*H*, H12), 7.20 – 7.24 (4H, m, Ar-*H*, H9, H10, H5', H6'), 7.28 – 7.32 (2H, m, Ar-*H*, H3', H4'), 7.35 (1H, d, *J* 7.87 Hz, Ar-*H*, H2'); ^{13}C NMR δ_C (100 MHz, $CDCl_3$) 33.8, 51.4 (2C), 53.6 (2C), 60.7, 120.6, 123.0, 126.3, 127.8, 128.6, 128.8, 128.9, 129.0, 129.2, 130.9, 140.4 , 149.5; Found: M^+, 300.13876. $C_{18}H_{21}N_2Cl$ requires *M*, 300.13932. Found: C: 71.75; H: 7.01; N: 9.24. $C_{18}H_{21}N_2Cl$ requires C: 71.87; H: 7.04; N: 9.31.

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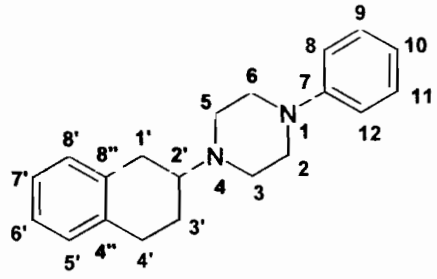

1-Phenyl-4-(1',2',3',4'-tetrahydronaphthalen-2'-yl)-piperazine **(CMP31)**

The title compound was prepared from phenylpiperazine (0.50 g, 3.1 mmol), ß-tetralone (0.99 g, 0.9 mL, 6.8 mmol) and sodium cyanoborohydride (0.41 g, 6.5 mmol) by the above method to give **CMP31** (0.26 g, 27%) as yellow crystals; mp 105 – 106 °C (from EtOH); R_f 0.63 (hexane:ethyl acetate 2:8); IR ($CHCl_3$): ν_{max}/cm^{-1} 3007 (C-H Ar), 1599 (C=C Ar), 1230 (C-N); ^{1}H-NMR δ_H (300 MHz, $CDCl_3$) 1.62 – 1.76 (1H, m, C-*H*, H2'), 2.98 – 3.03 (10H, m, N-C*H*$_2$, C*H*$_2$, H3, H5, H1', H3', H4'), 3.26 (4H, t, *J* 5.00 Hz, N-C*H*$_2$, H2, H6), 6.86 (1H, t, *J* 7.31 Hz, Ar-*H*, H10), 6.94 (2H, d, *J* 7.80 Hz, Ar-*H*, H8, H12), 7.11 (4H, m, Ar-*H*, H9, H11, H6', H7'), 7.25 (1H, d, *J* 7.31 Hz, Ar-*H*, H5'), 7.27 (1H, d, *J* 7.31 Hz, Ar-*H*, H8'); ^{13}C NMR δ_C (100 MHz, $CDCl_3$) 26.1, 29.3, 32.0, 49.2 (2C), 49.6 (2C), 60.4, 116.1 (2C), 119.7, 236.7, 125.8, 128.5, 129.1 (2C), 129.4, 135.8, 136.3, 151.4; Found: M^+, 292.19432. $C_{20}H_{24}N_2$ requires *M*, 292.19395. Found: C: 81.80; H: 8.30; N: 9.40. $C_{20}H_{24}N_2$ requires C: 82.15; H: 8.27; N: 9.58.

5.2.3. General procedure for the synthesis of CMP40 – CMP42[77]

A solution of bromophenol (0.50 g, 5.78 mmol), phenyl boronic acid (1.60 g, 12.72 mmol), copper (II) acetate (1.57 g, 8.67 mmol) and pyridine (1.16 mL, 14.45 mmol) were stirred in dry dichloromethane (15 mL) for 48 hours under nitrogen at 25°C. The reaction

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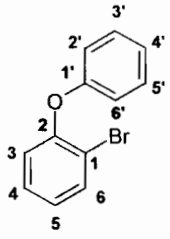
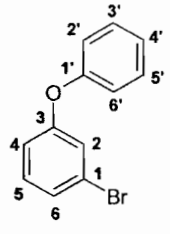
mixture was evaporated to dryness. The crude product was purified by column chromatography.

Starting bromophenols used: 2-bromophenol, 3-bromophenol and 4-bromophenol.



1-Bromo-2-phenoxy-benzene **(CMP40)**

(0.32 g, 22%) as a yellow-cream oil; R_f 0.78 (ethyl acetate:hexane 2:8); IR ($CHCl_3$): ν_{max}/cm^{-1} 3014 (CH Ar), 1599 (C=C Ar); ^{1}H-NMR δ_H (400 MHz, $CDCl_3$) 6.97 – 6.99 (3H, m, Ar-*H*, H4, H2', H6'), 7.01 (1H, d, *J* 7.87 Hz, Ar-*H*, H3), 7.12 (1H, t, *J* 7.42 Hz, Ar-*H*, H5), 7.26 (1H, t, *J* 8.61, Ar-*H*, H4'), 7.34 (2H, t, *J* 8.06 Hz, Ar-*H*, H3', H5'), 7.64 (1H, d, *J* 7.97 Hz, Ar-*H*, H6); ^{13}C NMR δ_C (100 MHz, $CDCl_3$) 114.9, 116.2, 118.1, 120.6, 123.4, 124.9, 128.6, 129.8, 132.0, 133.8, 153.7, 156.9; Found: C: 58.01; H: 3.41. $C_{12}H_9BrO$ requires C: 57.86; H: 3.64.



1-Bromo-3-phenoxy-benzene **(CMP41)**

(0.37 g, 26%) as a yellow-cream oil; R_f 0.81 (ethyl acetate:hexane 2:8); IR ($CHCl_3$): ν_{max}/cm^{-1} 3015 (CH Ar), 1581 (C=C Ar); ^{1}H-NMR δ_H (300 MHz, $CDCl_3$) 6.75 (1H, dt, *J*

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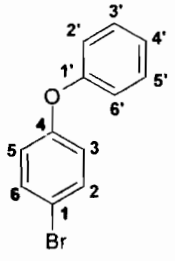
7.2 and 2.13 Hz, Ar-*H*, H5), 6.93 (1H, dt, *J* 7.2 and 1.95 Hz., Ar-*H*, H4'), 7.02 (1H, d, *J* 7.56 Hz, Ar-*H*, H6), 7.07 (1H, d, *J* 7.56 Hz, Ar-*H*, H4), 7.15 (2H, d, *J* 8.53 Hz, Ar-*H*, H2', H6'), 7.22 (1H, s, Ar-*H*, H2) 7.37 (2H, t, *J* 7.93 Hz, Ar-*H*, H3', H5'); ^{13}C NMR δ_C (75 MHz, $CDCl_3$) 114.2, 117.2, 118.9, 119.4, 121.7, 122.8, 123.9, 126.1, 129.9, 130.8, 156.3, 158.4; Found: C: 55.29; H: 3.65. $C_{12}H_9BrO{\cdot}0.7H_2O$ requires C: 55.31; H: 3.48; Br: 30.66; O: 6.14.



1-Bromo-4-phenoxy-benzene **(CMP42)**

(0.27 g, 19%) as a yellow-cream oil; R_f 0.81 (ethyl acetate:hexane 2:8); IR ($CHCl_3$): ν_{max}/cm^{-1} 3014 (CH Ar), 1577 (C=C Ar); ^{1}H-NMR δ_H (400 MHz, $CDCl_3$) 6.90 (2H, d, *J* 9.02 Hz, Ar-*H*, H3, H5), 7.02 (2H, d, *J* 7.56 Hz, Ar-*H*, H6, H2), 7.13 (1H, t, J 7.44 Hz, Ar-*H,* H4'), 7.35 (2H, t, *J* 8.04 Hz, Ar-*H,* H3', H5'), 7.44 (2H, d, *J* 9.02 Hz, Ar-*H,* H2', H6'); ^{13}C NMR δ_C (100 MHz, $CDCl_3$) 115.6, 119.0 (2C), 120.4 (2C), 123.7, 129.9 (2C), 132.6 (2C), 156.6, 156.7.

5.2.4. General procedure for the synthesis of CMP46, CMP48 and CMP50[78]

Dibromobenzene (7.17 g, 28 mmol) and phenyl boronic acid (1.00 g, 8.2 mmol) were combined in iso-propanol (10 mL) and stirred for 15 minutes allowing complete dissolution of all solids. Palladium acetate (6.40 mg, 0.03 mmol), triphenylphosphine

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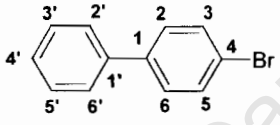
(0.02 g, 0.08 mmol), 2.0 M aqueous sodium carbonate (8.2 mL, 16.4 mmol) and deionised water (3 mL) were added. The mixture was stirred at 100 °C for 18 hours. The mixture was diluted with ethyl acetate (15 mL) and washed with 5% sodium carbonate (2 x 20 mL) and brine (2 x 20 mL). The organic layer was extracted, dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure to afford the crude products.

Starting dibromobenzenes used: *o*-dibromobenzene, *m*-dibromobenzene and *p*-dibromobenzene.



4-Bromobiphenyl **(CMP46)**

(1.39 g, 73%) was obtained as a yellow-cream oil; R_f 0.67 (hexane); IR ($CHCl_3$): ν_{max}/cm^{-1} 3007 (CH Ar), 1487 (C=C Ar); ^{1}H-NMR δ_H (400 MHz, $CDCl_3$) 6.99 (1H, d, *J* 8.42 Hz, Ar-*H*, H3), 7.05 (1H, d, *J* 8.61 Hz, Ar-*H*, H5), 7.15 (1H, d, *J* 6.78 Hz, Ar-*H*, H2), 7.21 (1H, t, *J* 7.32 Hz, Ar-*H*, H3'), 7.29 (1H, t, *J* 7.23 Hz, Ar-*H*, H4'), 7.39 (1H, d, *J* 6.59 Hz, Ar-*H*, H2'), 7.41 (1H, d, *J* 6.78 Hz, Ar-*H*, H6'), 7.45 (1H, t, *J* 7.51 Hz, Ar-*H*, H5'), 7.59 (1H, d, *J* 7.14 Hz, Ar-*H*, H6); ^{13}C NMR δ_C (100 MHz, $CDCl_3$) 113.2, 119.8, 126.7, 127.1, 128.5, 128.7, 128.8, 130.6, 131.4, 131.5, 140.1, 140.4.

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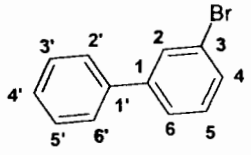
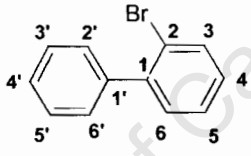


***3-Bromobiphenyl* (CMP48)**

(2.11 g, 100%) was obtained as a yellow-cream oil; R_f 0.47 (hexane); IR ($CHCl_3$): ν_{max}/cm^{-1} 3015 (CH Ar), 1566 (C=C Ar); ^{1}H-NMR δ_H (400 MHz, $CDCl_3$) 7.12 – 7.24 (4H, m, Ar-*H*, H2, H4, H5, H6), 7.30 – 7.34 (3H, m, Ar-*H*, H3', H5', H6'), 7.42 (1H, d, *J* 8.06 Hz, Ar-*H*, H2'), 7.55 (1H, t, *J* 2.01 Hz, Ar-*H*, H4'); ^{13}C NMR δ_C (100 MHz, $CDCl_3$) 122.6, 126.4, 127.6, 128.6, 128.9, 129.2, 129.9, 130.3, 131.5, 132.0, 139.9, 143.4.



***2-Bromobiphenyl* (CMP50)**

(1.76 g, 92%) as a cream-yellow oil; R_f 0.48 (hexane); IR ($CHCl_3$): ν_{max}/cm^{-1} 3015 (CH Ar), 1566 (C=C Ar); ^{1}H-NMR δ_H (400 MHz, $CDCl_3$) 7.15 – 7.19 (2H, m, Ar-*H*, H4, H5), 7.28 – 7.32 (3H, m, Ar-*H*, H3', H4', H5'), 7.45 – 7.47 (2H, m, Ar-*H*, H3, H6), 7.57 (2H, d, *J* 7.96 Hz, Ar-*H*, H2', H6'); ^{13}C NMR δ_C (100 MHz, $CDCl_3$) 124.5, 126.2, 127.4, 127.9, 128.5, 128.9, 130.1, 131.1, 131.3, 132.8, 133.4, 137.1.

5.2.5. General procedure for CMP43 – CMP45, CMP47, CMP49, CMP51 and CMP30[75]

The phenylbromide starting material (1.0 eq), piperazine (3.0 eq), sodium *tert*-butoxide (2.0 eq), (R)-(+)-2,2'-bis(diphenylphosphino)1,1'-binaphthyl (6 mol %) and

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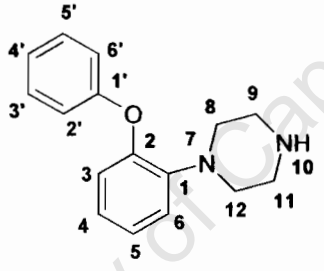
tris(dibenzylideneacetone)dipalladium (II) (4 mol%) were combined in dry dioxane (12 mL per 1 mmol phenylbromide starting material) and refluxed at 110°C for 18 hours under nitrogen. The reaction mixture was cooled to room temperature and evaporated to dryness. The residue was dissolved in dichloromethane (30 mL) and washed with water. The organic phase was dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure. The crude material was purified by column chromatography.

Starting phenylbromide material used: **CMP40**, **CMP41**, **CMP42**, **CMP46**, **CMP48**, **CMP50** and bromobenzene.



1-(2-Phenoxy-phenyl)-piperazine **(CMP43)**

This compound was prepared from **CMP40** (0.17 g, 0.69 mmol), piperazine (0.18 g, 2.08 mmol), sodium *tert*-butoxide (0.13 g, 1.39 mmol), BINAP (0.03 g, 0.04 mmol) and tris(dibenzylideneacetone)dipalladium (II) (0.03 g, 0.03 mmol) to give **CMP43** (0.06 g, 31%) as a yellow oil; R_f 0.37 (methanol:dichloromethane 2:8); IR ($CHCl_3$): ν_{max}/cm^{-1} 3015 (CH Ar), 1487 (C=C Ar), 1220 (CN); ^{1}H-NMR δ_H (300 MHz, $CDCl_3$) 1.89 (1H, broad s, N-*H*, H10), 2.83 (4H, t, *J* 4.75 Hz, N-C*H*$_2$, H8, H12), 3.05 (4H, t, *J* 4.75 Hz, N-C*H*$_2$, H9, H11), 6.89 – 7.14 (6H, m, Ar-*H*, H6, H5, H4, H3, H2', H6'), 7.25 – 7.28 (3H, m, Ar-*H*, H5', H4', H3'); ^{13}C NMR δ_C (75 MHz, $CDCl_3$) 46.1 (2C), 51.6 (2C), 117.1

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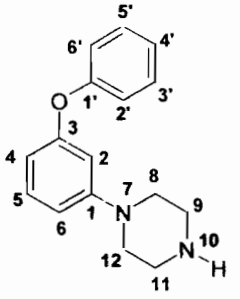
(2C), 119.2 (2C), 121.4, 122.6 (2C), 122.7, 124.7, 129.3 (2C), 157.5; Found: M^-, 254.14176. $C_{16}H_{18}N_2O$ requires *M*, 254.14191.



1-(3-Phenoxy-phenyl)-piperazine **(CMP44)**

This target compound was prepared from **CMP41** (0.20 g, 0.8 mmol), piperazine (0.21 g, 2.4 mmol), sodium *tert*-butoxide (0.16 g, 1.6 mmol), BINAP (0.03 g, 0.05 mmol) and tris(dibenzylideneacetone)dipalladium (II) (0.03 g, 0.03 mmol) to give **CMP44** (0.09 g, 42%) as a yellow oil; R_f 0.25 (methanol:dichloromethane 2:8); IR ($CHCl_3$): ν_{max}/cm^{-1} 3015 (CH Ar), 1577 (C=C Ar), 1216 (CN); ^{1}H-NMR δ_H (300 MHz, $CDCl_3$) 2.19 (1H, broad s, N-*H*, H10), 3.45 (4H, t, *J* 4.88 Hz, N-C*H*$_2$, H8, H12), 3.18 (4H, t, *J* 4.88 Hz, N-C*H*$_2$, H9, H11), 6.46 (1H, d, *J* 8.04 Hz, Ar-*H*, H6), 6.59 (1H, s, Ar-*H*, H2) 6.65 (1H, d, *J* 7.56 Hz, Ar-*H*, H4), 6.99 (2H, d, *J* 7.56 Hz, Ar-*H*, H2', H6'), 7.08 (1H, t, *J* 7.31 Hz, Ar-*H*, H5), 7.20 (1H, t, *J* 8.17 Hz, Ar-*H*, H4'), 7.32 (2H, t, *J* 8.04 Hz, Ar-*H*, H5', H3'); ^{13}C NMR δ_C (75 MHz, $CDCl_3$) 45.7 (2C), 49.6 (2C), 106.9 (2C), 110.1, 110.9, 118.8 (2C), 123.0, 129.6 (2C), 130.0, 153.1, 158.1; Found: M^+, 254.14063. $C_{16}H_{18}N_2O$ requires *M*, 254.14191.

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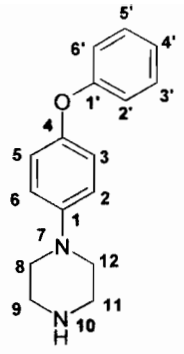
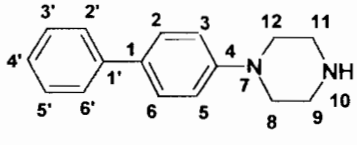


1-(4-Phenoxy-phenyl)-piperazine **(CMP45)**

The title compound was prepared from **CMP42** (0.20 g, 0.8 mmol), piperazine (0.21 g, 2.4 mmol), sodium *tert*-butoxide (0.16 g, 1.6 mmol), BINAP (0.03 g, 0.05 mmol) and tris(dibenzylideneacetone)dipalladium (II) (0.03 g, 0.03 mmol) to give **CMP45** (0.10 g, 50%) as a yellow oil; R_f 0.36 (methanol:dichloromethane 2:8); IR ($CHCl_3$): ν_{max}/cm^{-1} 3015 (CH Ar), 1505 (C=C Ar), 1227 (CN); ^{1}H-NMR δ_H (400 MHz, $CDCl_3$) 2.5 (1H, broad s, N-*H*, H10), 3.07 (4H, m, N-C*H*$_2$, H12, H8), 3.14 (4H, m, N-C*H*$_2$, H9, H11), 6.89 – 6.97 (6H, m, Ar-*H*, H2, H3, H5, H6, H6'H2'), 7.03 (1H, t, *J* 7.32 Hz, Ar-*H*, H4'), 7.28 (2H, t, *J* 8.06 Hz, Ar-*H*, H3', H5'); ^{13}C NMR δ_C (100 MHz, $CDCl_3$) 45.8 (2C), 50.7 (2C), 117.7 (2C), 117.9 (2C), 120.4 (2C), 122.4, 129.6 (2C), 148.1, 150.1, 158.4; Found: M^+, 254.14132. $C_{16}H_{18}N_2O$ requires *M*, 254.14191. Found: C: 71.99; H: 7.00; N: 10.79. $C_{16}H_{18}N_2O{\cdot}0.7H_2O$ requires C: 72.04; H: 6.80; N: 10.50; O: 6.29.



1-Biphenyl-4-yl-piperazine **(CMP47)**

This compound was prepared from **CMP46** (0.50 g, 2.2 mmol), piperazine (0.55 g, 6.4 mmol), sodium *tert*-butoxide (0.41 g, 4.3 mmol), BINAP (0.08 g, 0.13 mmol) and

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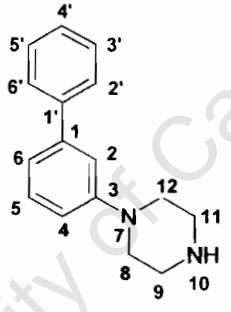
tris(dibenzylideneacetone)dipalladium (II) (0.08 g, 0.08 mmol) to give **CMP47** (0.38 g, 74%) as a cream-yellow oil; R_f 0.38 (methanol:dichloromethane 2:8); IR ($CHCl_3$): ν_{max}/cm^{-1} 3015 (CH Ar), 1508 (C=C Ar), 1241 (CN); ^{1}H-NMR δ_H (400 MHz, $CDCl_3$) 2.38 (1H, broad s, N-*H*, H10), 3.07 (4H, broad s, N-C*H*$_2$, H8, H12), 3.17 (4H, broad s, N-C*H*$_2$, H9, H11), 6.84 (2H, d, *J* 8.61 Hz, Ar-*H*, H5, H3), 7.08 (2H, d, *J* 8.42 Hz, Ar-*H*, H2, H6), 7.16 – 7.20 (3H, m, Ar-*H*, H3', H4', H5'), 7.25 (2H, d, *J* 6.96 Hz, Ar-*H*, H2', H6'); ^{13}C NMR δ_C (100 MHz, $CDCl_3$) 45.6 (2C), 49.8 (2C), 116.6 (2C), 125.9, 126.9, 128.4, 128.8, 129.1, 129.6 (2C), 132.9, 141.6, 149.7; Found: M^+, 238.8. $C_{16}H_{18}N_2$ requires *M*, 238.4.



***1-Biphenyl-3-yl-piperazine* (CMP49)**

This compound was prepared from **CMP48** (0.77 g, 3.3 mmol), piperazine (0.85 g, 9.9 mmol), sodium *tert*-butoxide (0.63 g, 6.5 mmol), BINAP (0.12 g, 0.19 mmol) and tris(dibenzylideneacetone)dipalladium (II) (0.12 g, 0.13 mmol) to give **CMP49** (0.20 g, 22%) as a yellow oil; R_f 0.29 (methanol:dichloromethane 2:8); IR ($CHCl_3$): ν_{max}/cm^{-1} 2949 (CH Ar), 1599 (C=C Ar), 1241 (CN); ^{1}H-NMR δ_H (400 MHz, $CDCl_3$) 2.61 (1H, broad s, N-*H*, H10), 3.07 (4H, broad s, N-C*H*$_2$, H8, H12), 3.16 (4H, t, *J* 4.70 Hz, N-C*H*$_2$, H9, H11), 6.68 (1H, d, *J* 7.32 Hz, Ar-*H*, H4), 6.74 – 6.76 (1H, m, Ar-*H*, H6), 7.14 – 7.20 (4H, m, Ar-*H*, H2, H5, H2', H6'), 7.24 – 7.28 (3H, m, Ar-*H*, H3', H4', H5'); ^{13}C NMR

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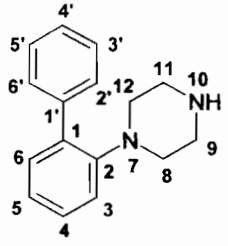
δ_C (100 MHz, $CDCl_3$) 45.6 (2C), 49.8 (2C), 113.9, 117.1, 120.9, 125.9, 128.4 (2C), 128.1 (2C), 129.1, 141.1, 141.9, 151.6; Found: M^+, 238.8. $C_{16}H_{18}N_2$ requires *M*, 238.4.



1-Biphenyl-2-yl-piperazine **(CMP51)**

The title compound was prepared from **CMP50** (0.97 g, 4.2 mmol), piperazine (1.10 g, 12.6 mmol), sodium *tert*-butoxide (0.81 g, 8.4 mmol), BINAP (0.16 g, 0.26 mmol) and tris(dibenzylideneacetone)dipalladium (II) (0.15 g, 0.16 mmol) to give **CMP51** (0.08 g, 7%) as a yellow oil; R_f 0.4 (methanol:dichloromethane 2:8); IR ($CHCl_3$): ν_{max}/cm^{-1} 2949 (CH Ar), 1595 (C=C Ar), 1223 (CN); ^{1}H-NMR δ_H (300 MHz, $CDCl_3$) 2.16 (1H, s, N-*H*, H10), 2.53 (4H, broad s, N-C*H*$_2$, H8, H12), 2.94 (4H, broad s, N-C*H*$_2$, H9, H11), 7.6 – 7.13 (3H, m, Ar-*H*, H3', H4', H5'), 7.25 – 7.30 (2H, m, Ar-*H*, H4, H5), 7.45 – 7.47 (2H, m, Ar-H, H3, H6), 7.52 (2H, d, *J* 7.80 Hz, Ar-*H*, H2', H6'); ^{13}C NMR δ_C (75 MHz, $CDCl_3$) 45.7 (2C), 53.9 (2C), 127.2 (3C), 128.4 (3C), 130.7 (3C), 132.8 (2C), 137.4; Found: M^+, 238.8. $C_{16}H_{18}N_2$, requires *M*, 238.4.

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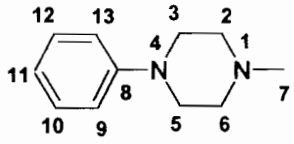

1-Methyl-4-phenyl-piperazine **(CMP30)**

The title compound was prepared from Bromobenzene (1.00 g, 0.6 mL, 6.4 mmol), N-methyl-piperazine (1.90 g, 2.1 mL, 19.1 mmol), sodium *tert*-butoxide (1.20 g, 12.7 mmol), BINAP (0.24 g, 0.38 mmol) and tris(dibenzylideneacetone)dipalladium (II) (0.23 g, 0.26 mmol) to give **CMP30** (0.79 g, 70%) as a yellow oil; R_f 0.48 (methanol: dichloromethane 2:8); IR ($CHCl_3$): ν_{max}/cm^{-1} 2935 (CH Ar), 1599 (C=C Ar), 1288 (CN); ^{1}H-NMR δ_H (300 MHz, $CDCl_3$) 2.33 (3H, s, C*H*$_3$, H7) 2.56 (4H, t, *J* 5.00 Hz, N-C*H*$_2$, H3, H5) 3.20 (4H, t, *J* 5.00 Hz, N-C*H*$_2$, H2, H6) 6.83 (1H, t, *J* 7.31 Hz, Ar-*H*, H11) 6.90 (2H, d, *J* 8.29 Hz, Ar-*H*, H9, H13) 7.23 (2H, t, *J* 8.05 Hz, Ar-*H*, H10, H12); ^{13}C NMR δ_C (75 MHz, $CDCl_3$) 31.1, 49.3 (2C), 55.4 (2C), 116.3 (2C), 119.9, 129.3 (2C), 151.5; Found: M^+, 176.13166. $C_{11}H_{16}N_2$ requires *M*, 176.13135.

5.3 Crystal Structure Determinations

A single crystal of **CMP24a** and **CMP24b** was covered in a small amount of paratone oil on a glass fibre. X-ray intensity data were collected at 113 K on a Nonius Kappa CCD with 1.5 kW graphite monochromated Mo radiation. The diffraction patterns were indexed with a primitive monoclinic cell. The strategy for the data collection was evaluated using the *Collect* Software.[89] The detector to crystal distance was 40 mm. Data was collected by a phi scan and several omega scans. The data was scaled and reduced using *Denzo*-SMN.[90] Unit cell dimensions were refined on all data. The space groups P*ca*2(1) and P2$_1$/*n* were chosen on the basis of systematic absences and intensity

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statistics. The structure was solved and refined using SHELX97.[91] Hydrogen atoms were placed in calculated positions and included in the model during later stages of the refinement. Plots of the molecular structure were obtained with ORTEP[92] and PLATON.[93] The program X-SEED[94], an interface to SHELX was used during the structure solution and refinements.

Table 5.1: Crystal Data and Structure Refinement for **CMP24a** and **CMP24b**.

Parameter	**CMP24a**	**CMP24b**
Chemical formula	$C_{18}H_{22}N_2$	$C_{18}H_{22}N_2$
Molecular mass	266.17857	266.17857
Crystal system	Orthorhombic	Monoclinic
Space group	P*ca*2(1)	$P2_1/n$
a (Å)	11.1980	19.8925
b (Å)	16.234	8.5995
c (Å)	8.1306	8.8415
α (°)	90	90
β (°)	90	90
γ (°)	90	98.32
Volume ($Å^3$)	1477.88	1496.56
Formula Units (Z)	4	4

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Table 5.2 Bond Lengths (Å) for CMP24b

BOND	LENGTH (Å)	BOND	LENGTH (Å)
N1-C2	1.465	C2-C3	1.509
C3-N4	1.468	N4-C6	1.454
C6-C5	1.519	C5-N1	1.460
N4-C7	1.403	C7-C8	1.402
C8-C9	1.391	C9-C10	1.373
C10-C11	1.378	C11-C12	1.383
C12-C7	1.403	N1-C8'	1.476
C8'-C7'	1.517	C7'-C1'	1.510
C1'-C2'	1.392	C2'-C3'	1.388
C3'-C4'	1.383	C4'-C5'	1.371
C5'-C6'	1.390	C6'-C1'	1.390

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Table 5.3 Bond Lengths (Å) for CMP24a

BOND	LENGTH (Å)	BOND	LENGTH (Å)
N1-C2	1.460	C2-C3	1.508
C3-N4	1.470	N4-C6	1.462
C6-C5	1.514	N1-C5	1.455
N4-C7	1.420	C7-C8	1.402
C8-C9	1.391	C9-C10	1.373
C10-C11	1.378	C11-C12	1.383
C12-C7	1.403	N1-C8'	1.464
C8'-C7'	1.524	C7'-C1'	1.508
C1'-C2'	1.392	C2'-C3'	1.388
C3'-C4'	1.383	C4'-C5'	1.371
C5'-C6'	1.390	C6'-C1'	1.390

Table 5.4 Selected Torsional Angles

	CMP24a	**CMP24b**
BOND	N1-C8'-C7'-C1'	N1-C8'-C7'-C1'
ANGLE (°)	163.7	173.9

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