SYNTHESES AND STUDIES ON ANTIMALARIAL ACTIVITY
OF SOME
NITROGEN HETEROCYCLIC COMPOUNDS

A Thesis
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by

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Canberra
July, 1993
To my beloved (late) Dad,

Mum,

and my Husband
Certificate of Originality

The work described in this thesis was carried out by the candidate at the Australian National University. Where the work of others was employed or quoted, appropriate references are given.

[Signature]
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ABSTRACT

A large number of heterocyclic compounds has been prepared and tested for antimalarial activity. Computer modelling and DNA intercalation studies for a selection of these compounds have also been described.

Several new series of Mannich base derivatives of 2(3 and 4)-[7-chloro(and trifluoromethyl)quinolin-4-ylamino]phenols have been prepared from the relevant 2(3 and 4)-nitrophenols through di(and mono)-Mannich derivatives thereof, which were reduced to the corresponding aminophenols and then condensed with 4,7-dichloro- or 4-chloro-7-trifluoromethyl-quinoline. Di(and mono)-Mannich bases derived from 2-[7-bromo(and trifluoromethyl)-1,5-napthyridin-4-ylamino]phenol were prepared similarly.

Mannich base derivatives of 2(and 4)-[2(and 8)-monotrifluoromethylquinolin-4-ylamino]phenol have been prepared for comparison with previously synthesised 7-trifluoromethyl analogues.

The mefloquine analogue, α-(7-bromo-1,5-naphthyridin-4-yl)-α-(piperidin-2-yl)methanol and some related compounds have been prepared.

Physical properties such as $^1$H n.m.r. and $^{13}$C n.m.r. have been determined and discussed in relation to the chemical structures.

All of the above compounds were evaluated (by others) for antimalarial activity in an in vitro screen against the chloroquine-sensitive strain of the human malaria Plasmodium falciparum (the FCQ-27 isolate) using both the morphological and visual tests. Some of the more active compounds were also tested using the $^3$H-hypoxanthine technique against both chloroquine-sensitive and chloroquine-resistant (K-1) isolates of P. falciparum. The results of the tests for each series of compounds are presented and discussed within each chapter. The most active compounds in the in vitro tests against the FCQ-27 isolate of P. falciparum were the di-Mannich bases derived from 2-(7-chloroquinolin-4-ylamino)-5-methylphenol and 4-(7-chloroquinolin-4-ylamino)phenol.
The more active compounds in the \textit{in vitro} tests described above were subjected to an \textit{in vivo} test against \textit{P. vinckei vinckei} in mice. These tests revealed that some of the di-Mannich bases derived from 2-(7-chloroquinolin-4-ylamino)phenol were very effective at suppressing parasitaemia when administered in a single dose of 200 mg/kg.

Molecular modelling studies using Silicon graphics were carried out on amodiaquine and some of the above compounds. The methods of conformational analysis and superimposition were applied and a common pharmacophore for malarial receptor binding was derived. Volume analysis of these compounds revealed information about the essential volume of the proposed receptor.

Finally, some selected compounds were investigated for intercalation with calf thymus DNA.
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CHAPTER I
CHAPTER I Introduction

I-1 Malaria - The Disease

Malaria remains one of the most deadly infectious diseases, affecting millions of people, and it is endemic in many parts of the world. In 1990, over 40% of the world's population remained exposed to malaria, distributed in 100 countries. It is estimated that 300 million people are infected with malaria and over 1 million die from it each year. Malaria presently occurs mostly in the tropical (and highly populated) areas of Africa, Asia (and the Pacific), and Central and South America.

Malaria is known as a disease caused by infection with parasites of the genus *Plasmodium*. There are at least one hundred species of plasmodia, of which only four species naturally infect humans, these are *Plasmodium falciparum*, *P. vivax*, *P. ovale* and *P. malariae*. *P. vivax* and *P. falciparum* are the most prevalent and account for more than 95% of the human malaria infections worldwide. The disease caused by *P. falciparum* is a malignant tertian malaria and is usually fatal in nonimmune persons if not treated promptly. *P. vivax*, the cause of benign tertian malaria, usually evokes milder clinical attack than *P. falciparum* and produces a lower mortality rate even in untreated adults. However, *P. vivax* is the human malaria most known for reoccurrence after treatment. *P. ovale* and *P. malariae* give milder infections. They have a rather restricted global distribution and they are readily susceptible to chemotherapy. Other species of plasmodia infect a variety of hosts including mammals, reptiles and birds. Those that occur in lower mammals such as rats, mice and bats are *P. berghei* and *P. vinckei*. Fortunately malaria parasites are quite host specific, they are only known to parasitise other species when they are closely related to the normal host.
I-2 Biological Characteristics

I-2.1 The Life Cycle of Human Malaria Parasites

After the all important discovery of Sir Ronald Ross\(^5\) around 1900, that the female *Anopheles* mosquito is the vector for malaria, it was not until 50 years later that the complete life cycle of the malaria parasite was elucidated.\(^6\)

In order to understand the rationale behind the chemotherapy, epidemiology, pathogenesis and immunology of malaria, it is necessary to understand the life cycle of the *Plasmodium* species. The various stages are presented in schematic form in Fig. I-17\(^a\) and consists of an asexual phase in a vertebrate host (man) and a sexual phase in the body of a mosquito. When an infected mosquito takes a blood meal from a vertebrate, it injects the infective form of the parasite, the sporozoites, into the blood stream. In less than an hour, these sporozoites invade the parenchymal cells of the liver, where they undergo asexual division to form hepatic schizonts containing numerous merozoites. The affected hepatic cells then burst, releasing merozoites into the blood stream. Within seconds, each merozoite invades a red blood cell. Again, the parasite undergoes growth and asexual division. A mature erythrocytic schizont is formed, which then ruptures, and releasing more merozoites. These merozoites quickly reinvade fresh erythrocytes and then can either begin a new cycle of blood schizogony or develop into sexual forms, called micro (male) and macro (female) gametocytes. These forms are the infective stage for the mosquito.

The sexual phase in the parasite's life cycle begins when the gametocytes are ingested by a suitable vector. In the mosquito's midgut the female gametocyte is fertilised by the male through a process of exflagellation, to form the zygote. The parasite then develops in the stomach wall to an oocyst containing thousand of sporozoites. Depending on temperature and species, the cyst ruptures within 20 days to release sporozoites which migrate to the salivary gland of the mosquito, where they are ready to infect a new vertebrate host. Various species of malaria parasites can have different life cycle characteristics, which lead to important chemotherapeutic consequences. For
example, *P. falciparum* and *P. malariae* have no extended development in liver cells, and therefore elimination of the erythrocytic form of the parasite produces a cure; the relapsing malarious, *P. vivax* and *P. ovale*, have a long-lasting or secondary tissue phase (i.e. hypnozoite stage, Figure I-1), thus elimination of the erythrocytic parasites will not produce a radical cure.

I-2.2 Control Measures and Immunology of Malaria

The use of chemotherapy to combat the disease is but one part of the total effort against Malaria. Other contributions include the control of the mosquito vector, progress in understanding of malaria immunology, and research aimed at the development of a vaccine.\(^8\) The mosquito vector may be controlled by using insecticides, by preventing breeding, interrupting man-vector contact\(^9\) with the use of house screening, pesticide-impregnated bed nets, specific mosquito pathogens\(^10\) and the release of genetically engineered insects.\(^11a\) Despite much effort to create vaccines using the powerful new tools of biotechnology during the 1980's, research on this technology still continues as before, making steady scientific progress, but no successful vaccine has yet been provided. The disappointing trials of candidate vaccines against sporozoite and merozoite have been reported.\(^11b\) This situation has been discussed to some extent by Cherfas\(^11b\) and Marshall.\(^3\) Vaccine targets currently envisaged are presented in Figure I-2.\(^12\)
Figure I-1  Schematic diagram of the life cycle of the malarial parasite.\textsuperscript{7a}
I-3 The Main Antimalarial Drugs

I-3.1 Terminology and Site of Drug Action

Antimalarial drugs may be classified according to the site at which they interact with the life cycle of the parasite. Treatment regimens for malaria also have different therapeutic goals (which depend on the species of infecting plasmodia, the chances of reinfection, and the severity of the infection). The following terms have been used in the classification of drugs, and their treatment aims.

(a) Causal prophylaxis: refers to the use of agents that exert a complete prevention of erythrocytic infection by destroying the pre-erythrocytic stages, i.e. the sporozoites and/or the tissue forms of the malaria parasite.

(b) Blood schizontocide: refers to a drug that destroys the erythrocytic asexual parasites.
(c) **Tissue schizontocide**: refers to a drug that destroys the tissue asexual parasites. If it acts upon the primary exoerythrocytic form, it is referred to as primary tissue schizontocide (and it is used for causal prophylaxis). If it acts against latent tissue stage hypnozoites of *P. vivax* and *P. ovale*, it is referred to as secondary tissue schizontocide and it is used as an anti-relapse drug, which can provide a radical cure for these infections. More specifically it is referred to as hypnozoitocide.

(d) **Gaemetocytocide**: drug that destroys the sexual forms of the parasites in the blood.

(e) **Sporontocide**: drug that when given to the malaria-infected vertebrate host, prevents or interrupts the formation of sporozoites (Fig. I-1) in mosquitoes feeding on that host.

(f) **Suppressive treatment**: treatment aimed at preventing or eliminating clinical symptoms and/or parasitemia by early destruction of erythrocytic parasites.

(g) **Clinical cure**: relief of symptoms of a malaria attack (e.g. by interrupting blood schizogony with blood schizontocides) without complete elimination of the infection.

(h) **Radical cure**: complete elimination of all asexual stages from the body so that relapses cannot occur.

The effectiveness of some major antimalarial drugs against different life cycle stages and strains of the malaria parasite are summarised in Table I-1.

### I-3.2 Currently Important Antimalarial Drugs

(a) **Blood Schizontocides**:

(i) **4-Aminoquinolines**

Chloroquine (I.1) and amodiaquine (I.2), an alternative, are the most widely used antimalarial drugs of this group. These drugs are effective against the asexual erythrocytic forms of human plasmodia but are inactive against the exoerythrocytic forms. They are used for both suppressive prophylaxis and for treatment of acute
Table I-1: Action of commonly used antimalarial drugs against different life cycle stages of human malaria parasites.\textsuperscript{13}

<table>
<thead>
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<th>Antimalarial</th>
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<td></td>
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<td>Gametocytes</td>
<td>P. vivax and</td>
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<td></td>
<td></td>
<td></td>
<td>P. malariae,</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>not active for</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P. falciparum</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>No action</td>
<td>Fast action</td>
<td>Active against P. falciparum</td>
</tr>
<tr>
<td>Mefloquine</td>
<td>Probably no action</td>
<td>Fast action</td>
<td>As chloroquine</td>
</tr>
<tr>
<td>Quinine</td>
<td>No action</td>
<td>Fast action</td>
<td>No action</td>
</tr>
<tr>
<td>Proguanil</td>
<td>Active, particularly on P. falciparum</td>
<td>Active, but relatively slow</td>
<td>No action</td>
</tr>
<tr>
<td>Pyrimethamine</td>
<td>Probably as proguanil</td>
<td>Active but slow acting</td>
<td>Uncertain</td>
</tr>
<tr>
<td>Sulfones and Sulfonamides</td>
<td>Possible action</td>
<td>Weak activity</td>
<td>Uncertain</td>
</tr>
<tr>
<td>Primaquine</td>
<td>Active but not used for prophylaxis</td>
<td>Weak activity</td>
<td>Direct and fast action on all P. falciparum</td>
</tr>
<tr>
<td>Tetracyclines</td>
<td>Active (only known for P. falciparum)</td>
<td>Active but slow acting (only known for P. falciparum)</td>
<td>No action (only known for P. falciparum)</td>
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attacks of malaria. As chloroquine is employed more commonly than the other 4-
aminoquinoline compounds, with sub-optimal doses, resistance to the drug by
*P. falciparum* has developed rapidly and extensively, making this drug less reliable.
Amodiaquine has the same mode of action and spectrum of activity as chloroquine, but
is more active against some strains of chloroquine-resistant *P. falciparum*.\(^{15}\) Despite
several reports of its adverse effects\(^{16-18}\) (such as agranulocytosis, neutropenia and
hepatitis; which only occur with the drug's extensive use in a large doses), amodiaquine
remains in use for the treatment of chloroquine-resistant infections.

**Mechanism of action:** The antimalarial mode of action of chloroquine has been in­
vestigated in great detail in recent years,\(^{19-21}\) but the overall mechanism is still
controversial. Two major hypotheses have been proposed and are mentioned below.
Fitch and his colleagues\(^{21}\) have shown that the drug has its effect by binding to
ferriprotoporphyrin IX (FP), a product of haemoglobin digestion within the parasite,
diverting it into a toxic drug-FP complex, and thus interfering with parasitic degradation
of haemoglobin, resulting in damage to critical parasite organelle membranes.
Alternatively, it has been suggested that the drug works by concentrating within
parasitic lysosomes, thus raising their pH and interfering with their activity. Full details
of these proposals have been reviewed by Howells.\(^{19}\)

Other considerations such as the effect of drugs on the biosynthesis and degrada­
tion of nucleic acids, and drug interaction with isolated nucleic acid have also been re­
ported.\(^{22,23}\)

(iii) **The 4-Quinolinemethanols (Amino Alcohols)**

The best known drugs of the amino alcohol type are quinine (I.3) and
mefloquine (I.4). Quinine and its optical isomer quinidine are the most important
alkaloids from the extract of cinchona bark. They are both effective blood
schizontocides for all human malarias and have gametocidal activity against *P. vivax* and
*P. malariae*. Although quinine is less effective and more toxic than other fast acting
blood schizontocides, both quinine and quinidine are extensively used as alternative
treatments for *P. falciparum* infections, especially those in areas with chloroquine-resistance. Mefloquine is a quinoline methanol derivative that is chemically related to quinine. It is an highly active blood schizontocide, generally well tolerated, safe and effective against certain chloroquine-resistant strains of *P. falciparum*. The use of mefloquine is being restricted by public health authorities in order to prevent the rapid development of mefloquine resistance.

**Mechanism of Action:** Quinine is thought to act by binding to plasmodial DNA, and thus interfering with protein biosynthesis. However, mefloquine has been shown to have no interaction or binds very weakly to DNA.\textsuperscript{24,25} The mode of action of mefloquine has not been fully elucidated. It may act by binding to FP and interfering with the parasite's digestion of haemoglobin like the 4-aminoquinolines, or by the impaired oxidation of parasite membrane NADH.\textsuperscript{26}

(iii) **Antifolates**

Both mammals and plasmodia require folic acid (FA) in its tetrahydro cofactor form (FAH\textsubscript{4}) for essential metabolic processes. Drugs that interrupt the biosynthesis of FAH\textsubscript{4} are known as antifolates, although they may interrupt the biosynthetic sequence at different places. Some antifolates are described below.

**Biguanides, Dihydrotriazines, and 2,4-Diaminopyrimidines**

The biguanides proguanil (I.5) and its analogue chlorproguanil (I.6) are effective against the asexual blood forms of all human malaria. Proguanil and chlorproguanil are prodrugs, being metabolised to the active dihydrotriazine metabolites cycloguanil (I.7) and chlorcycloguanil (I.8), respectively, which have themselves been made and used directly as antimalarials. Proguanil is active against the primary tissue form of *P. falciparum* and *P. ovale* but not against *P. vivax*; it does interfere with the development of the parasite within the mosquito and, therefore, with the transmission of the disease. Proguanil has been chiefly used for chemoprophylaxis.
Figure I-2  Chemical structures of some currently important antimalarial drugs.

(I.1) chloroquine

(I.2) amodiaquine

(I.3) quinine

(I.4) mefloquine

(I.5) proguanil (chloroguanide)

(I.6) chlorproguanil

(I.7) cycloguanil

(I.8) chlorcycloguanil
I.9 pyrimethamine

I.10 dapsone

I.11 sulfadoxine

I.12 halofantrine

I.13 qinghaosu

I.14 artesunate

I.15 artemether

I.16 primaquine
(iv) Halofantrine

Halofantrine (I.12), a phenanthrenemethanol, has a potent blood schizontocidal activity against chloroquine-resistant strains of *P. falciparum*. It has a mode of action similar to that of quinine and mefloquine. There has been observed a clear cross-resistance between halofantrine, mefloquine and quinine but not with chloroquine.

(v) Qinghaosu (Artemesinine)

Artemesinine (I.13) is a sesquiterpene lactone, derived from the herb *Artemisia annua*, and it has a structure completely different to all existing antimalarial drugs. Artemesinine is a highly potent blood schizontocide, with low toxicity, and low cross resistance to other antimalarials. Its derivatives artesunate (I.14) and artemether (I.15) are more soluble and more active. The Qinghaosu series of compounds act differently in mechanism from both chloroquine and the antifolate drugs. They mainly
interfere with membrane structures of the parasite and its mode of action may involve interruption of protein and nucleic acid syntheses by the parasites.30

(b) Tissue Schizontocides

8-Aminoquinolines (e.g. Primaquine)

Primaquine (I.16) remains the only 8-aminoquinoline drug in current use which has activity against the exoerythrocytic stages of infection. Other drugs in this group include pamaquine (I.17) and quinocide (I.18). Primaquine is also noted for its ability to destroy gametocytes of all human malaria, but it has only a weak effect on the asexual blood forms of the parasite. Because of its tissue schizontocidal activity, primaquine is used primarily to achieve radical cure of P. vivax and P. ovale malaria. The principle adverse effects of primaquine are methaemoglobinaemia and haemolysis, in which the latter is most common in patients who are genetically glucose-6-phosphate dehydrogenase deficient.

The mechanism of action of 8-aminoquinolines is not fully understood.

![Chemical structures of pamaquine and quinocide]

(I.17) pamaquine

(I.18) quinocide

Antibiotics

Some antibiotics such as tetracyclines and chloramphenicol are known to have antimalarial activity. They affect both the erythrocytic and tissue stages of the parasite. Due to their slow action, antibiotics are normally used in combination with fast-acting blood schizontocides, like a quinine, to treat drug-resistant strains of malaria.31
However, they should not be used on a long term basis. Clindamycin\textsuperscript{7c,31a} (Lincomycin) is another antibiotic to have been used in the treatment of chloroquine-resistant falciparum malaria. It also acts slowly and therefore, a rapidly acting antimalarial drug must be given prior to the antibiotics\textsuperscript{7c}.

**I-3.3 Resistance Phenomena - Mechanisms of Resistance**

Over recent years, the development of resistance by the malaria parasite to currently used antimalarial drugs has brought a major threat to the effective control of malaria. Many antimalarial drugs have become much less effective, resistance to new antimalarials has also rapidly developed, and yet the mechanisms of resistance are not fully understood. \textit{P. falciparum} has been the most prone to develop drug resistance, especially to chloroquine. A dramatic spread of chloroquine-resistant \textit{P. falciparum} strains has occurred since the early 1960\textsuperscript{s}, but until recently the biochemical basis of chloroquine resistance has been perplexing. Various theories to account for the mechanism of chloroquine resistance have been proposed. Fitch\textsuperscript{32,33} suggested that it may be due to the unavailability of FD IX to bind chloroquine by chloroquine-resistant \textit{P. falciparum} and he later proposed\textsuperscript{33} further explanation but no experimental evidence is available to support these hypotheses. The observation by Fitch\textsuperscript{32} and by others,\textsuperscript{34,35} that chloroquine-resistant \textit{P. falciparum} accumulates chloroquine to a lesser extent than in chloroquine-sensitive parasites, suggests another explanation of chloroquine resistance. However, Geary \textit{et al.}\textsuperscript{36} concluded from their examination of \textsuperscript{3H}chloroquine uptake in human erythrocytes infected with nine strains of \textit{P. falciparum} that the rate of drug accumulation or intracellular concentration at steady state could not differentiate sensitive from resistant strains adequately.

Other proposals for the mechanism(s) of resistance in malaria parasites include the following. An enhanced rate of chloroquine efflux from resistant cells thus preventing its accumulation to toxic levels (proposed by Martin \textit{et al.}\textsuperscript{37} who also demonstrated that verapamil, a calcium channel blocker was able to reverse chloroquine-resistance), an impairment in the mechanisms of chloroquine transport (reduction or loss,
of permease) into infected cells (postulated by Warhurst\textsuperscript{38}), and a change in microsomal enzymes (suggested by Salganik \textit{et al.}\textsuperscript{39} and by Rabinovitch \textit{et al.}\textsuperscript{40}). These proposals however remain speculative. More extensive reviews on the mechanism of resistance to chloroquine can be found in papers by Howells,\textsuperscript{19} and Cowman and Foote.\textsuperscript{41}

The mechanism of resistance to pyrimethamine and proguanil (DHFR inhibitors) has been recently identified as being due to point mutations within the DHFR gene that render the enzyme less susceptible to inhibition by the drug.\textsuperscript{41} Further details on this recent work have been described.\textsuperscript{41}

The use of drug combinations can be useful in the protection of each drug in the combination against possible resistance.\textsuperscript{42,43} The basis of combination drug therapy in overcoming drug resistance is that the mutations that give rise to resistance to each individual components are different and the mutations that give rise to cross resistance are less likely to occur.\textsuperscript{31b,41}

\section*{I-4 Mannich Bases as Potential Antimalarials}

Interest in the Mannich bases as a new class of antimalarial agents began in the mid 1940's when Burckhalter and his colleagues\textsuperscript{44} reported the synthesis of a series of Mannich base compounds such as compound \textbf{I.19} (5-\textit{t}-butyl-3-diethylaminomethyl-biphenyl-2-ol, SN 7,744) and compound \textbf{I.20} (bialamicol, SN 6,771), which possessed high antimalarial activity. Soon thereafter, the synthesis of various types of Mannich bases containing substituted heterocyclic nuclei were reported.\textsuperscript{45-48}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{mangnon_bases.png}
\caption{Mannich Bases (I.19 and I.20)}
\end{figure}
I-4.1 Di-(and mono) Mannich Base Derivatives of 2(3 and 4)-(Quinolin-4-ylamino)phenols

A method for the preparation of amodiaquine (I.2) was disclosed in 1948 by Burckhalter et al.,\textsuperscript{45} by the condensation of 4,7-dichloroquinoline (the 7-chloroquinoline nucleus is present in chloroquine and quinine) with a Mannich base of 4-aminophenol. Cycloquine\textsuperscript{49} (I.21), a di-Mannich compound, was synthesised and used in the USSR in 1965 as an antimalarial drug. Nabih et al.\textsuperscript{50} prepared compounds (I.22) and (I.23) in 1972, and compound (I.22, R=NEt\textsubscript{2}) was shown to have both curative and prophylactic effects against \textit{P. berghei} in mice.

In the mid 1980's, 2-(t-butylaminomethyl)-4-(7-trifluoromethylquinolin-4-ylamino)-5,6,7,8-tetrahydronaphthalen-1-ol (hydrochloride) (I.24) was reported by Shen et al.,\textsuperscript{51} and its antimalarial activity was also evaluated.

\begin{align*}
\text{I.21} & \quad \text{I.22} \\
\text{I.23} & \quad \text{I.24}
\end{align*}
Shen et al. 51 and Barlin et al. 52-54 reported the synthesis of Mannich base derivatives of 4-(7-trifluoromethylquinolin-4-ylamino)phenol and studies of their antimalarial activity. Compounds (I.25b) and (I.25c) were found to suppress *P. berghei* infections in mice at a low dosage,51 and (I.25b) was highly effective against *P. vinckei vinckei* in mice.52,53 These compounds were further investigated and shown to be highly effective against chloroquine-resistant strains in *in vivo* tests55 against the ANKA strain (EC123) of *P. berghei* in mice, and *in vitro* tests56,57 against the K-1 strain of *P. falciparum*.

Mannich base derivatives of 2(and 3)-(quinolin-4'-ylamino)phenols have not been examined extensively in the past; but some 2-(7-trifluoromethylquinolin-4'-ylamino)phenols were reported by Barlin and Yan58 recently.

### I-4.2 Mannich Base Derivatives of 2-(1,5-Naphthyridin-4-ylamino)phenols

The structural similarity of the ring system of 4-amino-1,5-naphthyridine to both 4- and 8-aminoquinoline has stimulated interest in such compounds as antimalarial agents. Significant antimalarial activity by compound (I.26a) was revealed in early literature.59,60 The synthesis and antimalarial activity studies of several 1,5-naphthyridine congeners (I.26 and I.27) of chloroquine were reported in 1970 by McCaustland and Cheng.61 Compound (I.26c) was found to be comparable to
chloroquine in activity against *P. berghei* in mice, and was much less toxic than chloroquine. No acute toxicity was noted even at high dose (640 mg/kg), whereas at 320 mg/kg, chloroquine was shown to be 100% lethal.

In 1982, Chen *et al.* explored several unsubstituted and 2,6-disubstituted 1,5-naphthyridines carrying di-Mannich base chains of *p*-aminophenol (e.g., compound I.27a). Some of these proved to be effective antimalarials.

![Chemical structures](image)

More recently, di-Mannich base derivatives of 4-[7-chloro-(7-bromo- and 7-trifluoromethyl) - 1,5-naphthyridin-4-ylamino]phenols (I.28) were synthesised. These compounds were shown to exhibit significant antimalarial activity.

![Chemical structure](image)

**I-5 Mefloquine and its Analogues as Potential Antimalarials**

Various α-dialkylaminomethyl-α-[2-phenyl-7(8)-trifluoromethylquinolin-4-yl]methanols were first prepared in 1968 for evaluation against *P. berghei* in mice by
Saggiomo et al. Compound (I.29) when administered to mice in a single dose was curative at 40 mg/kg. Some α-(piperidin-2-yl)-α-(trifluoromethylquinolin-4-yl) methanols with methoxy, methyl, or chloro substituents at position 6 or 8 were also found to have antimalarial activity; but they were moderately phototoxic. In a programme to prepare derivatives of α-(piperidin-2-yl)-α-(quinolin-4-yl)methanols (with the support from the U.S. Army Medical Research and Development Command), the synthesis of mefloquine (I.4) and its 2,6- and 2,7-bistrifluoromethyl isomers (I.30a and I.30b) soon followed in 1971. Mefloquine was 100% curative at 40 mg/kg against P. berghei in mice, twice as effective as the 2,7-isomer and 4 times as effective as the 2,6-isomer. Mefloquine is now available for clinical use as a drug for the suppression of parasitaemia.

I-6 Computerised Molecular Modelling (Computer-assisted Analysis of Bioactivity)

The increased abilities and availability of powerful computers have enabled scientists from all research areas to integrate their knowledge and expertise to design new ligands for receptor binding, to elucidate the molecular mechanism of action of drugs, to determine the structure of biologically active molecules and even to propose amino acid sequencing and folding for the proposed macromolecules.
Computer modelling is assuming an increasingly important role in understanding the basis of interaction of drugs with biological receptors, and assisting the medicinal chemist in the design of new effective therapeutic agents. The concept of receptor mapping and pharmacophores has been introduced to facilitate understanding of the forces and properties involved in drug-receptor interactions. A variety of computational methods are now well developed. Computer graphics, as a tool for handling models has been used for a relatively short time. The literature contains many reviews which include information on molecular graphics, conformational analysis, receptor mapping, and theoretical aspects of drug design.

I-7 Research Objectives

This study is divided into several fundamental sections: chemical syntheses of Mannich base derivatives of heterocyclic 2-, 3- and 4-aminophenols, the synthesis of a mefloquine analogue, and preliminary testing of these compounds for antimalarial activity; the use of u.v. spectroscopic techniques to examine some of the compounds prepared for drug-DNA interactions; and the use of a computer graphics program and conformational analysis studies to determine the set of pharmacophoric patterns consistent with the biological activity, and to map the areas adjacent to the pharmacophore to identify those which may be occupied by the receptor.
CHAPTER II
CHAPTER II Syntheses and Antimalarial Activity of Some Di-Mannich Base Derivatives of 2-(7-Chloroquinoline-4-ylamino)-phenol and 2-[7-Bromo(and trifluoromethyl)-1,5-naphthyridin-4-ylamino]phenol

II-1 Introduction

In view of the strong antimalarial activity against both the chloroquine-sensitive (FCQ-27) and chloroquine resistant (K-1) strains of *P. falciparum* observed for several di-Mannich bases of 2-(7-trifluoromethylquinolin-4-ylamino)phenol\(^5\) and the significant curative properties of some Mannich base derivatives of 4-(7-substituted-1,5-naphthyridin-4-ylamino)phenols,\(^6\) it was decided to examine Mannich base derivatives of 2-[7-bromo(and trifluoromethyl)-1,5-naphthyridin-4-ylamino]phenol and also 2-(7-chloroquinolin-4-ylamino)phenol (in which the heterocycle is the same as present in the antimalarials chloroquine and amodiaquine). The syntheses of such compounds are described in this chapter, and their structures were readily established from considerations of their \(^1\)H n.m.r. and analyses. Their ultraviolet (uv) spectra and mass spectra are also recorded and discussed. Finally the compounds were tested, and the results are reported, for antimalarial activity in *in vitro* tests against *P. falciparum* and by *in vivo* tests against *P. vinckei vinckei* in mice.
II-2 Syntheses

II-2.1 Preparation of Di(and mono)-Mannich Base Derivatives of 2-Nitrophenol

The di(and mono)-Mannich bases of 2-nitrophenol have been investigated previously, but methods for their syntheses, utilising the Mannich reaction, are briefly reviewed below for completeness.

The Mannich reaction consists of the condensation of formaldehyde (or paraformaldehyde) with ammonia or its salt (or primary or secondary amines) and a compound containing one (or more) active hydrogen atoms (such as methyl ketones or phenols). The reaction essentially involves the substitution of an active hydrogen atom by an aminomethyl or substituted aminomethyl group. The products of the condensation are referred to as 'Mannich bases'. For example, the reaction of phenol with formaldehyde and a secondary amine is illustrated by the following equation:

\[ C_6H_5OH + HCHO + R_2NH \rightarrow R_2NCH_2C_6H_4OH \]

Cumming and Shelton proposed base-catalysed and acid-catalysed reaction mechanisms for the Mannich reaction of cyclohexanone as follows.

(a) The base-catalysed reaction
Formaldehyde (II.1) combines with a secondary amine (II.2) to form the alkylaminomethylol (II.3) which reacts with the carbanion (II.4) of the cyclohexanone by an SN2 mechanism to yield the Mannich base (II.5).

(b) The acid-catalysed reaction

According to this mechanism, formaldehyde reacts with the amine (II.2) to gives compound (II.3) and thence (II.6) which reacts with active-hydrogen compound as the enol (II.7) to give (II.8) and then (II.5).74

Mannich75,76 was the first to recognise the general nature of the reaction although examples of the Mannich reaction were published earlier by Tollens77,78 and coworkers. Many reports and reviews of this reaction have been published.74,79-82

In the present work, the preformed Mannich base derivatives of 2-nitrophenol (II.11) were prepared by the condensation of the inexpensive 2-nitrophenol (II.9) with paraformaldehyde (II.10) and the appropriate amines (II.2) in ethanol under reflux, as shown in Scheme II-1. The readily purified nitro compound (II.11) was then catalytically reduced under hydrogen gas in the presence of Raney nickel to give the corresponding amine (II.12). The new 3-(or 5-)methyl-2-nitro-4,6-bis(piperidin-1-ylmethyl) phenols (II.13a or II.14a) prepared by procedures analogous to those
Scheme II-1

II.9

\[ \text{II.10} \quad \text{II.2} \]

1. EtOH/reflux

2. H\textsubscript{2}/Ni

\[ \text{R}_2\text{NCH}_2\text{OH} \]

\[ \text{R}_2\text{NCH}_2 \]

II.11; Z = NO\textsubscript{2}

II.12; Z = NH\textsubscript{2}

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for other di-Mannich bases as shown in Scheme II-1; the mono Mannich bases of 2-nitrophenol (II.15) were prepared similarly but with one equivalent only of paraformaldehyde and each amine.

\[
\begin{align*}
\text{II.15, } Z &= \text{NO}_2 \\
\text{II.16, } Z &= \text{NH}_2
\end{align*}
\]

II-2.2 Preparation of 7-Bromo(and trifluoromethyl)-4-chloro-1,5-naphthyridines

The two principal methods of preparation of 1,5-naphthyridines described in the literature involve either the Skraup reaction or the ethoxymethylenemalonic ester (EMME) method which are discussed briefly below.

(a) The Skraup Reaction

Various unsubstituted and substituted 1,5-naphthyridines (II.19) can be prepared by heating pyridin-3-amines (II.17) with glycerol (II.18) in concentrated sulfuric acid with arsenic pentoxide.\(^8\) Other modified Skraup reactions\(^8\) can also be applied by using different condensing agents such as methylacrolein and acetaldehyde. Differing results were observed when the 2-position of pyridine-3-amines was blocked by another group. For example, 2-halogenopyridin-3-amines gave 1,5-naphthyridines\(^8\) and 2-hydroxypyridin-3-amine gave 8-hydroxy-1,7-naphthyridine.\(^8\) Although 4-hydroxy-1,5-naphthyridines have been prepared by Skraup reaction,\(^8\) those required in the present work have not been prepared by this method.
(b) Ethoxymethylenemalonic ester (EMME) Method

The excellent quinoline synthesis developed by Adams et al.⁶⁰ and Price and Roberts⁸⁸ has been extended to the preparation of 1,5-naphthyridines.⁶⁰,⁸⁸,⁸⁹ In this reaction pyridin-3-amine (or a substituted pyridin-3-amine) is heated with ethyl ethoxymethylene malonate (EMME) in refluxing "Dowtherm A" (an eutectic mixture of diphenyl ether and biphenyl) and gave the ethyl 4-hydroxy-1,5-naphthyridine-3-carboxylate (II.20). This product was hydrolysed and decarboxylated to form the 1,5-naphthyridin-4-ol (II.21).

Other methods for the preparation of 1,5-naphthyridines have also been described in the literature.⁹⁰,⁹¹,⁹²
The 1,5-naphthyridines required in this work were prepared employing the EMME method as shown in Scheme II-2 and Scheme II-3 (using the literature methods\textsuperscript{64,65}).

The 4-chloro-7-trifluoromethyl-1,5-naphthyridine\textsuperscript{65} (II.28) was prepared from 3-chloro-5-trifluoromethylpyridine (II.22) (commercially available) by an initial treatment with 28\% aqueous ammonia in the presence of cuprous chloride at 170° in a high pressure vessel for 48 h, to provide good yields of 5-trifluoromethylpyridin-3-amine\textsuperscript{93} (II.23) which was subsequently condensed with EMME at 100° for 2 h to give diethyl 5-trifluoromethylpyridin-3-ylaminomethylenemalonate (II.24) and this was ring-closed in refluxing diphenyl ether to the known ethyl 4-hydroxy-7-trifluoromethyl-1,5-naphthyridine-3-carboxylate (II.25). The latter (II.25), in 10\% aqueous sodium hydroxide was hydrolysed to the corresponding acid (II.26) which in turn was decarboxylated in boiling quinoline and gave 7-trifluoromethyl-1,5-naphthyridin-4-ol (II.27). Chlorination\textsuperscript{94} of this hydroxy compound (II.27) gave 4-chloro-7-trifluoromethyl-1,5-naphthyridine (II.28) as shown in Scheme II-2.

7-Bromo-4-chloro-1,5-naphthyridine\textsuperscript{64} (II.36) was prepared by chlorination of 7-bromo-1,5-naphthyridin-4-ol (II.35) which was prepared by others from nicotinic acid (II.29) through 5-bromonicotinic acid\textsuperscript{95,96} (II.30), its amide (II.31),\textsuperscript{96} 5-bromopyridin-3-amine (II.32),\textsuperscript{96,97} ethyl 7-bromo-4-hydroxy-1,5-naphthyridine-3-carboxylate\textsuperscript{98} (II.33), and the acid (II.34) as shown in Scheme II-3.

II-2.3 Syntheses of Di(and mono)-Mannich Base Derivatives of 2-(7-Chloroquinolin-4-ylamino)phenol and 2-[7-Bromo(and trifluoromethyl)-1,5-naphthyridin-4-ylamino]phenol

These compounds may be prepared by the two routes shown in Scheme II-4 which are detailed below. The first route which was used in the present work, was generally employed when the required amino phenols could be obtained readily by reduction of the appropriate nitrophenols (as mentioned in Section II-2.1). Condensation of these compounds (II.38) with 4,7-dichloroquinoline (II.37) (or...
Scheme II-2

\[ \text{II.22} \xrightarrow{\text{NH}_3/\text{CuCl}} \text{II.23} \xrightarrow{\text{EMME}} \text{II.24} \]

\[ \text{1. } \text{Ph}_2\text{O} \]
\[ \text{2. } 10\% \text{ NaOH} \]

\[ \text{II.28} \xrightarrow{\text{POCl}_3/\text{PCl}_5} \text{II.27} \xrightarrow{\text{quinoline}} \text{II.25, } R=\text{Et} \]
\[ \text{II.26, } R=\text{H} \]

Scheme II-3

\[ \text{II.29} \xrightarrow{} \text{II.30} \xrightarrow{} \text{II.31} \]

\[ \text{II.33, } R=\text{Et} \]
\[ \text{II.34, } R=\text{H} \]

\[ \text{II.35} \]

\[ \text{II.36} \]
Scheme II-4

II.37

+ R₂NCH₂ OH

II.38

R₂NCH₂ R'

EtOH, H₂O reflux

II.39

Route 2

Route 1

II.40

H₂N

II.41 X = Br; II.42 X = CF₃

II.42

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</tbody>
</table>
with 7-bromo-4-chloro- or 4-chloro-7-trifluoromethyl-1,5-naphthyridine) in aqueous solution afforded the desired products (II.40) (or II.41, II.42). In these condensations it was beneficial to adjust the reaction mixtures (before refluxing) to pH 4.7 for reactions with 4,7-dichloroquinoline, and to pH 2.5 for those with the less basic 1,5-naphthyridines.

The second route (Route 2) has been used previously for the syntheses of 4-[7-bromo(and chloro)-1,5-naphthyridin-4-ylamino]phenols.53,63 The 4,7-dichloroquinoline (II.37)[or 7-bromo-4-chloronaphthyridine (II.36), or 4-chloro-7-trifluoromethyl-naphthyridine (II.28)] by reaction with o-aminophenol hydrochloride in aqueous methanol gave 2-(7-chloroquinolin-4-ylamino)phenol (II.39) {or 2-[7-bromo-(or trifluoromethyl)-1,5-naphthyridin-4-ylamino]phenol}. The product (II.39) with formalin in ethanol and a large excess of the appropriate amine at reflux afforded the di-Mannich bases (II.40) (or mono-Mannich bases if a moderate amount of amines was used).

The mono-Mannich base derivatives of 2-(7-chloroquinolin-4-ylamino)phenol (II.43) and 2-(7-bromo-1,5-naphthyridin-4-ylamino)phenol (II.44) in this study, were also prepared via route 1 Scheme II-4, from the preformed mono-Mannich bases (II.16).
II-3 Physical Properties

II-3.1 Nuclear Magnetic Resonance Spectra

(a) $^1$H Nuclear Magnetic Resonance Spectra

Examination of the $^1$H n.m.r. spectra of the Mannich base derivatives of 2-(7-chloroquinolin-4-ylamino)phenols (Table II-1) revealed the signals due to the methylene groups in all di-Mannich side chain appeared as two singlets, except in the case of compound (II.40f) (and its precursor (II.11f)) in which the CH$_2$ groups showed distinct geminal couplings. The methylene proton at C-6 was further downfield than that at C-4, and their positions were also found to be dependent on the amine present in the Mannich base. For those compounds containing the dialkylamino substituent, they occurred at δ 3.34-3.49 (4-CH$_2$) and δ 3.70-3.81 (6-CH$_2$), for the pyrrolidinyl substituent at δ 3.53 (4-CH$_2$) and δ 3.87 (6-CH$_2$), and for various piperidinyl derivatives at δ 3.38-3.40 (4-CH$_2$) and δ 3.7-3.73 (6-CH$_2$). The signal due to H 5 in all of these compounds was also upfield of that due to H 3 (see Experimental section)

Inspection of the $^1$H n.m.r. spectral data from Table II-1 and from the Experimental section revealed the protons attached to the benzene ring of 7-chloroquinoline nucleus appeared as an ABC system; with proton H 5' more deshielded than H 6'. H 2' was the most deshielded as it was adjacent to the ring nitrogen atom. The signals for the ring protons occurred for H 2' at δ 8.46-8.59, for H 3' at δ 7.05-7.14 [except in the case of compound (II.40k) where H 3' was further upfield at δ 6.11; presumably due to steric hindrance by the 3-methyl group], for H 5' at δ 7.94-7.99, for H 6' at δ 7.29-7.44, and for H 8' at δ 7.97-8.06; and the coupling constant applicable to each position was consistent, e.g. J$_{2',3'}$ was 5.5 Hz, J$_{5',6'}$ was 9 Hz, and J$_{6',8'}$ was 2 Hz.

The hydrogen atoms on the naphthyridine ring of the derivatives of 2-[7-bromo(or trifluoromethyl)-1,5-naphthyridin-4-ylamino]phenols differ enough in their electronic environment to allow accurate assignment of peaks. Inspection of their
Table II-1 $^1$H n.m.r. spectral data ($\delta$)\textsuperscript{A} for 2-(7-chloroquinolin-4-ylamino) phenols.

$$
\begin{array}{ccccccccc}
\text{Compound} & \text{NR}_2 & \text{H}^2' & \text{H}^3' & \text{H}^5' & \text{H}^6' & \text{H}^8' & 4-\text{CH}_2 & 6-\text{CH}_2 \\
\text{II.40a} & \text{NMe}_2 & 8.59 & 7.11 & 7.94 & 7.41 & 7.99 & 3.34 & 3.70 \\
\text{II.40b} & \text{NEt}_2 & 8.56 & 7.08 & 7.96 & 7.39 & 8.01 & 3.49 & 3.81 \\
\text{II.40c} & \text{NPr}_2 & 8.56 & 7.13 & 7.96 & 7.42 & 8.01 & 3.47 & 3.81 \\
\text{II.40d} & \text{N(\text{CH}_2)_4} & 8.58 & 7.10 & 7.95 & 7.39 & 8.01 & 3.53 & 3.87 \\
\text{II.40e} & \text{N(\text{CH}_2)_5} & 8.58 & 7.14 & 7.94 & 7.41 & 8.00 & 3.38 & 3.70 \\
\text{II.40f} & \text{NC}_5\text{H}_9\text{Me-}o\text{B} & 8.56 & 7.09 & 7.95 & 7.40 & 8.01 & \text{C} & \text{C} \\
\text{II.40g} & \text{NC}_5\text{H}_9\text{Me-}m\text{D} & 8.59 & 7.14 & 7.95 & 7.42 & 8.01 & 3.39 & 3.72 \\
\text{II.40h} & \text{NC}_5\text{H}_9\text{Me-}p\text{E} & 8.57 & 7.09 & 7.95 & 7.39 & 8.00 & 3.38 & 3.71 \\
\text{II.40i} & \text{NC}_5\text{H}_8\text{Me}_2-3,5\text{F} & 8.57 & 7.14 & 7.95 & 7.41 & 8.01 & 3.40 & 3.73 \\
\text{II.40j} & \text{N(C}_4\text{H}_8\text{O)}\text{G} & 8.59 & 7.08 & 7.97 & 7.44 & 8.02 & \text{H} & \text{H} \\
\text{II.40k} & \text{N(\text{CH}_2)_5} & 8.46 & 6.11 & 7.99 & 7.29 & 7.97 & 3.36 & 3.68 \\
\text{II.40l} & \text{N(\text{CH}_2)_5} & 8.55 & 7.05 & 7.95 & 7.40 & 8.06 & 3.35 & 3.79 \\
\text{II.40m} & \text{N(Et)C}_6\text{H}_{11} & 8.57 & 7.13 & 7.96 & 7.44 & 8.02 & 3.58 & 3.89 \\
\end{array}
$$

\text{A} Chemical shifts reported as part per million ($\delta$) down field from tetramethylsilane (T.M.S.) as internal standard in deuterochloroform (CDCl$_3$). \text{B} 2-Methylpiperidin-1-yl. \text{C} The signal appeared as a complex in the range $\delta$ 1.11-4.35. \text{D} 3-Methylpiperidin-1-yl. \text{E} 4-Methylpiperidin-1-yl. \text{F} 3,5-Dimethylpiperidin-1-yl. \text{G} Morpholin-4-yl. \text{H} The signal appeared as a complex in the range $\delta$ 2.40-3.80.

$^1$H n.m.r. spectra in Table II-2 revealed that H 6' was the most deshielded due to the adjacent ring nitrogen atom and the bromo (or trifluoromethyl) group; and the most shielded proton was H 3'. The peak for H 6' appears downfield at $\delta$ 8.78-8.80, the signal for H 8' at $\delta$ 8.42-8.44, that from H 2' occurs as a doublet at $\delta$ 8.55-8.60 and...
that due to H 3' is also a doublet occurring considerably upfield at 7.17-7.20; the
coupling constant was also consistent for each position, e.g. J2',3' was 5.5 Hz and J6',8'
2 Hz.

A comparison of the 1H m.n.r. spectral data (in Table II-2) for 2-[7'-bromo(and
trifluoromethyl)-1',5'-naphthyridin-4'-ylamino]-4,6-bis(pyrrolidin-1"-ylmethyl)phenols
(II.41 and II.42) revealed only small differences in the spectra of these two
compounds. For the protons of the side chain, this difference was only minor, 0.01-
0.07 ppm, and for the naphthyridine ring protons the differences were 0.07-0.2 ppm.
This implied that the bromo and trifluromethyl groups had similar electron-withdrawing
(inductive) effects which were slightly greater in the latter compounds.

Table II-2 1H m.m.r. spectral data (δ) a for 2-(7'-bromo(and 7' trifluoromethyl)-1',5'-naphthyridin-4'-ylamino)-4,6-bis(pyrrolidin-1"-ylmethyl)phenols.

<table>
<thead>
<tr>
<th>Compound</th>
<th>X</th>
<th>H2'</th>
<th>H3'</th>
<th>H6'</th>
<th>H8'</th>
<th>H3</th>
<th>H5</th>
<th>4-CH2</th>
<th>6-CH2</th>
<th>H2&quot;,5&quot;</th>
<th>H3&quot;,4&quot;</th>
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<tbody>
<tr>
<td>II.41d</td>
<td>Br</td>
<td>8.57</td>
<td>7.17</td>
<td>8.78</td>
<td>8.42</td>
<td>7.40</td>
<td>6.81</td>
<td>3.55</td>
<td>3.87</td>
<td>2.67</td>
<td>1.85</td>
</tr>
<tr>
<td>II.42a</td>
<td>CF3</td>
<td>8.65</td>
<td>7.24</td>
<td>8.98</td>
<td>8.53</td>
<td>7.42</td>
<td>6.82</td>
<td>3.56</td>
<td>3.88</td>
<td>2.60</td>
<td>1.83</td>
</tr>
</tbody>
</table>

a Reported as parts per million (δ) downfield form T.M.S. as internal standard in CDCl3 as solvent.

(b) 13C Nuclear Magnetic Resonance Spectra

A 13C m.m.r. spectrum was run on 2-(7'-chloroquinolin-4'-ylamino)-5-methyl-
4,6-bis(piperidin-1"-ylmethyl)phenol (II.401) as a representative of this series (Figures
II-1 and II-2). The assignments of the 13C chemical shifts were carried out by running
a two dimensional heteronuclear one-bond correlation plot (HETCOR) and a long-range
n.m.r. spectrum of 2-(7'-chloroquinolin-4'-ylamino)-5-methyl-4,6-bis (piperidin-1'-ylmethyl)phenol (II-40)
Figure II.2: Expanded downfield region (110-155 ppm) of $^{13}$C n.m.r. spectrum of compound (II-400) shown in Figure II-1.
Figure II-3  2-D HETCOR spectrum of 2-(7'-chloroquinolin-4'-ylamino)-5-methyl-4, 6-bis(piperidin-1''-ylmethyl)phenol (II.401)
Figure II-4  2-D LRHETCOR spectrum of 2-(7'-chloroquinolin-4'-ylamino)-5-methyl-4,6-bis(piperidin-1''-ylmethyl)phenol (II.40I)
heteronuclear correlation (LRHETCOR) spectrum. The HETCOR spectrum was used to determine the carbon-hydrogen connectivities present in the molecule, and the LRHETCOR spectrum, to detect long-range carbon-hydrogen coupling connectivities while suppressing the one bond coupling. Figure II-3 shows the HETCOR spectrum of the methyl, methylene, methine and aromatic region of the hydrogen and carbon spectra making it possible to relate the assigned hydrogen spectrum to the carbon spectrum. The aromatic region of the LRHETCOR spectrum shown in Figure II-4 consisted of a number of cross peaks which are mostly due to three bond carbon-hydrogen coupling. These cross-peaks confirmed the assignment of the above tertiary carbons (made from the HETCOR spectrum) and allowed the assignment of the quarternary carbons (not connected with any protons). For example carbon C-1 was assigned on the basis of the cross peak between the resonance at δ 149.6 and the hydrogen atom peaks assigned to H 3 and 6-CH₂N. These cross peaks are due to three-bond coupling.

The ¹³C n.m.r. spectral data for compound II.401 are shown in Figure II-5, and further data in Table II-2a.

Figure II-5 The chemical shifts (ppm) of the carbon atoms in 2-(7'-chboroquinolin-4'-ylamino)-5-methyl-4,6-bis(piperidin-1'"-ylmethyl)phenol (II.401)
Table II-2a  The $^{13}$C n.m.r. spectral data for compound (II.40l): chemical shifts (ppm) of the carbon atoms and carbon-hydrogen connectivities in HETCOR and LRHETCOR spectra.

<table>
<thead>
<tr>
<th>Chemical shifts (ppm)</th>
<th>C-H connectivities</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HETCOR</td>
</tr>
<tr>
<td>C1 149.6</td>
<td>-</td>
</tr>
<tr>
<td>C2 124.2</td>
<td>-</td>
</tr>
<tr>
<td>C3 121.8</td>
<td>H3</td>
</tr>
<tr>
<td>C4 127.1</td>
<td>-</td>
</tr>
<tr>
<td>C5 131.2</td>
<td>-</td>
</tr>
<tr>
<td>C6 120.0</td>
<td>-</td>
</tr>
<tr>
<td>4-CH$_2$ 62.0</td>
<td>4-CH$_2$N</td>
</tr>
<tr>
<td>6-CH$_2$ 58.5</td>
<td>6-CH$_2$N</td>
</tr>
<tr>
<td>Me 14.5</td>
<td>Me</td>
</tr>
<tr>
<td>C2' 151.9</td>
<td>H2'</td>
</tr>
<tr>
<td>C3' 101.8</td>
<td>H3'</td>
</tr>
<tr>
<td>C4' 149.3</td>
<td>-</td>
</tr>
<tr>
<td>C4'a 118.6</td>
<td>-</td>
</tr>
<tr>
<td>C5' 121.7</td>
<td>H5'</td>
</tr>
<tr>
<td>C6' 125.6</td>
<td>H6'</td>
</tr>
<tr>
<td>C7' 134.8</td>
<td>-</td>
</tr>
<tr>
<td>C8' 128.7</td>
<td>H8'</td>
</tr>
<tr>
<td>C8'a 147.3</td>
<td>-</td>
</tr>
<tr>
<td>C2&quot;,6&quot; 53.8 - 54.5</td>
<td>H2&quot;,H6&quot;</td>
</tr>
<tr>
<td>C3&quot;,5&quot; 25.7 - 26.0</td>
<td>H3&quot;, H5&quot;</td>
</tr>
<tr>
<td>C4&quot; 23.7 - 24.5</td>
<td>H4&quot;</td>
</tr>
</tbody>
</table>
Scheme II-5  Fragmentation of methylquinolines$^{101}$

\[
\begin{align*}
\text{m/z 115} & \quad \text{m/z 142} & \quad \text{m/z 142} \\
\text{m/z 115} & \\
\end{align*}
\]
Scheme II-6  The major fragmentations in 2-(7'-chloroquinolin-4'-ylamino)-4,6-bis(piperidin-1''-ylmethyl)phenol (II.40e)

\[\text{m/z 464, 466}\]

\[\text{m/z 380, 382}\]

\[\text{or}\]

\[\text{m/z 380, 382}\]

\[\text{m/z 296, 298}\]
II-4 Antimalarial Activity

Various experimental models are available to access the activity of established and potential new antimalarial compounds. These methods include the use of avian, rodent and simian malarias; and the development of techniques for the continuous in vitro culture of *P. falciparum*, which has provided unprecedented opportunities to advance the search for effective new antimalarial drugs and for the elucidation of the mechanism of action of existing drugs.

In this work, the compounds prepared were examined for antimalarial activity first in an in vitro test against *P. falciparum* (which involved the application of a visual test and a microscopic test; and then a semi-automated microdilution technique for measurement of the inhibition of uptake of radiolabelled hypoxanthine). The more active compounds were then tested for in vivo activity against *P. vinckei vinckei* in mice.

II-4.1 In vitro Antimalarial Testing

The advent of a long-term (continuous) culture method for *P. falciparum* in human blood stages (by use of the "candle jar" technique), developed in 1976 by Träger and Jensen and by Haynes *et al.*, was an event of major importance; it provided the basis for several improved procedures for testing in vitro the response of the human malaria parasite, *P. falciparum* to drugs. Desjardins *et al.* utilised the methods of continuous culture to develop a rapid, semiautomated, computerised technique for massive screening of antimalarial drug activity. The basic method of Desjardins *et al.* has been used successfully by numerous workers. Träger recently reviewed the application of cultivation of *P. falciparum* in malaria research.

Other important in vitro techniques, which have been used in field-work include the original "macrotest" technique (this test was described by Rieckman *et al.* , and required a large quantity of parasitised blood (10-15 ml), the "microscopic" technique [microtest or morphological microtest, which is a further application of the
"candle jar" technique, and only requires a small amount of blood (50 µl) and a visual test (using capillary-blood specimens) also described by Rieckmann.\textsuperscript{104,112} This third test involved the determination by observation of the presence or absence of dark pigment precipitates in the wells containing various drug concentrations.

(a) Methods and Results

Compounds synthesised in this work were screened first for antimalarial activity in a preliminary test against the chloroquine-sensitive (FCQ-27) strain of \textit{P. falciparum} using both the microscopic test\textsuperscript{105} and the relatively inexpensive visual test;\textsuperscript{104,112} and the more active compounds therein (IC\textsubscript{50} < 50 nm) were then tested, by using the Desjardins\textsuperscript{107,113} radioisotopic technique with slight modification,\textsuperscript{57} for determination of the activity against both the FCQ-27 strain and the chloroquine-resistant (K-1) strain of \textit{P. falciparum}. The results for these \textit{in vitro} tests are listed in Table II-3 and II-4.

Table II-3 Results from a screen of Mannich base derivatives of 2-(7-chloroquinolin-4-ylamino)phenol and 2-[7-bromo(and trifluoromethyl)-1,5-naphthyridin-4-ylamino)phenol in \textit{in vitro} tests against the FCQ-27 isolate of \textit{P. falciparum} by using the visual test.

\begin{tabular}{|c|c|c|c|}
\hline
II.40a-j,m & Cl & CH & H & H \\
II.40k & Cl & CH & Me & H \\
II.40l & Cl & CH & H & Me \\
II.41a-j,m & Br & N & H & H \\
II.41k & Br & N & Me & H \\
II.41l & Br & N & H & Me \\
II.42a-d & CF\textsubscript{3} & N & H & H \\
II.42e & CF\textsubscript{3} & N & Me & H \\
\hline
\end{tabular}

III.43; X=Cl, Y=H \\
III.44; X=Br, Y=N
<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>NR$_2$</th>
<th>IC$_{50}^A$</th>
<th>IC$_{90}^B$</th>
<th>CH-factor$^C$</th>
</tr>
</thead>
<tbody>
<tr>
<td>II.40a</td>
<td>NMe$_2$</td>
<td>&lt;25</td>
<td>&lt;25</td>
<td>&gt;1</td>
</tr>
<tr>
<td>II.40b</td>
<td>NEt$_2$</td>
<td>&lt;25</td>
<td>&lt;25</td>
<td>&gt;1</td>
</tr>
<tr>
<td>II.40c</td>
<td>NPr$_2$</td>
<td>&lt;25</td>
<td>&lt;25</td>
<td>&gt;1</td>
</tr>
<tr>
<td>II.40d</td>
<td>N(CH$_2$)$_4$</td>
<td>&lt;25</td>
<td>&lt;25</td>
<td>&gt;1</td>
</tr>
<tr>
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<td>&lt;25</td>
<td>&gt;1</td>
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<td>&lt;25</td>
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<td>&lt;25</td>
<td>&gt;1</td>
</tr>
<tr>
<td>II.40h</td>
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<td>&lt;25</td>
<td>&gt;1</td>
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<tr>
<td>II.40i</td>
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<td>50-100</td>
<td>100</td>
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<tr>
<td>II.40j</td>
<td>N(C$_4$H$_8$O)$^H$</td>
<td>25-50</td>
<td>50</td>
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<td>N(CH$_2$)$_5$</td>
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<tr>
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<td>N(Et)C$<em>6$H$</em>{11}$</td>
<td>50-100</td>
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<tr>
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<td>II.41i</td>
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Table II-3 continued

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<th>CH-factor$^C$</th>
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<td>II.42a</td>
<td>N(CH$_2$)$_4$</td>
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<td>200</td>
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<tr>
<td>II.42b</td>
<td>N(CH$_2$)$_5$</td>
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<td>100</td>
</tr>
<tr>
<td>II.42c</td>
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<td>1</td>
</tr>
<tr>
<td>II.42d</td>
<td>N(C$_4$H$_8$O)$^H$</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>II.42e</td>
<td>N(CH$_2$)$_5$</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>II.43</td>
<td>N(CH$_2$)$_4$</td>
<td>50-100</td>
<td>100</td>
</tr>
<tr>
<td>II.44a</td>
<td>N(CH$_2$)$_4$</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>II.44b</td>
<td>NC$_5$H$_9$Me-$_m^E$</td>
<td>100-200</td>
<td>200</td>
</tr>
<tr>
<td>Chloroquine$^I$</td>
<td>20-40</td>
<td>40</td>
<td>1</td>
</tr>
</tbody>
</table>

$^A$ The IC$_{50}$ values (concentration of the inhibitor required to reduce parasite growth by 50%) are expressed as nmol l$^{-1}$(nM).

$^B$ The IC$_{90}$ values (the concentration of the inhibitor required to reduce parasite growth by 90%) are expressed as nmol l$^{-1}$.

$^C$ The CH-factor (chloroquine factor) is the comparative activity of the inhibitor under test compared to chloroquine; and it is the ratio of the IC$_{50}$ value for chloroquine over that for the inhibitor.

$^D$ 2-Methylpiperidin-1-yl.

$^E$ 3-Methylpiperidin-1-yl.

$^F$ 4-Methylpiperidin-1-yl.

$^G$ 3,5-Dimethylpiperidin-1-yl.

$^H$ Morpholin-4-yl.

$^I$ No significant activity at 200nM.

$^J$ As diphosphate salt.
Table II-4  Results from a screen of Mannich base derivatives of 2-(7-chloroquinolin-4-ylamino)phenol and 2-(7-bromo-1,5-naphthyridin-4-ylamino)phenol in the *in vitro* test against the FCQ-27 and K-1 isolates of *P. falciparum* by using the radioisotope test.

![Chemical Structure](image)

II.40; X=Cl, Y=CH  
II.41; X=Br, Y=N

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Substituents</th>
<th>FCQ-27</th>
<th>K-1</th>
<th>Resistance factor^B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IC₅₀^A</td>
<td>IC₉₀^A</td>
<td>IC₅₀^A</td>
</tr>
<tr>
<td>II.40a</td>
<td>H H NMe₂</td>
<td>12.5-25</td>
<td>25</td>
<td>12.5-25</td>
</tr>
<tr>
<td>II.40b</td>
<td>H H NEt₂</td>
<td>12.5-25</td>
<td>50</td>
<td>12.5-25</td>
</tr>
<tr>
<td>II.40c</td>
<td>H H NPr₂</td>
<td>12.5-25</td>
<td>25</td>
<td>50-100</td>
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<tr>
<td>II.40d</td>
<td>H H N(CH₂)₄</td>
<td>12.5-25</td>
<td>25</td>
<td>12.5-25</td>
</tr>
<tr>
<td>II.40e</td>
<td>H H N(CH₂)₅</td>
<td>12.5-25</td>
<td>25</td>
<td>12.5-25</td>
</tr>
<tr>
<td>II.40f</td>
<td>H H NC₅H₉Me-ο</td>
<td>25-50</td>
<td>50</td>
<td>25-50</td>
</tr>
<tr>
<td>II.40g</td>
<td>H H NC₅H₉Me-ᵐ</td>
<td>12.5-25</td>
<td>25</td>
<td>12.5-25</td>
</tr>
<tr>
<td>II.40h</td>
<td>H H NC₅H₉Me-ᵖ</td>
<td>12.5-25</td>
<td>25</td>
<td>12.5-25</td>
</tr>
<tr>
<td>II.40i</td>
<td>H H NC₅H₈Me₂-3,₅</td>
<td>25-50</td>
<td>50</td>
<td>50-100</td>
</tr>
<tr>
<td>II.40j</td>
<td>H H N(C₄H₈O)G</td>
<td>25-50</td>
<td>50</td>
<td>25-50</td>
</tr>
<tr>
<td>II.40k</td>
<td>Me H N(CH₂)₅</td>
<td>25-50</td>
<td>50</td>
<td>12.5-25</td>
</tr>
<tr>
<td>II.40l</td>
<td>H Me N(CH₂)₅</td>
<td>6.3-12.5</td>
<td>25</td>
<td>12.5-25</td>
</tr>
</tbody>
</table>
Table II-4 continued

| Inhibitor Substituents | FCQ-27 isolate | K-1 isolate | Resistance factor
<table>
<thead>
<tr>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IC$_{50}^A$</td>
<td>IC$_{90}^A$</td>
<td>IC$_{50}^A$</td>
</tr>
<tr>
<td>R' R'' NR$_2$</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>II.41a H H H NMe$_2$</td>
<td>25-50</td>
<td>50</td>
<td>25-50</td>
</tr>
<tr>
<td>II.41b H H H NEt$_2$</td>
<td>50-100</td>
<td>100</td>
<td>50-100</td>
</tr>
<tr>
<td>II.41d H H N(CH$_2$)$_4$</td>
<td>25-50</td>
<td>50</td>
<td>25-50</td>
</tr>
<tr>
<td>II.41f H H NC$_5$H$_9$Me-$o^C$</td>
<td>25-50</td>
<td>50</td>
<td>50-100</td>
</tr>
<tr>
<td>II.41g H H NC$_5$H$_9$Me-$m^D$</td>
<td>25-50</td>
<td>50</td>
<td>50-100</td>
</tr>
<tr>
<td>II.41k Me H N(CH$_2$)$_5$</td>
<td>25-50</td>
<td>50</td>
<td>25-50</td>
</tr>
<tr>
<td>II.41l H Me N(CH$_2$)$_5$</td>
<td>25-50</td>
<td>50</td>
<td>25-50</td>
</tr>
<tr>
<td>Chloroquine$^H$</td>
<td>20.3±4</td>
<td>30.5±3</td>
<td>396±96</td>
</tr>
<tr>
<td>Amodiaquine$^J$</td>
<td>12.2</td>
<td>20.8</td>
<td>26.6</td>
</tr>
</tbody>
</table>

- Results are expressed in nM concentrations
- Resistance factor is the IC$_{50}$ value against the K-1 isolate divided by that for the FCQ-27 isolate.
- $^A$ 2-Methylpiperidin-1-yl, $^B$ 3-Methylpiperidin-1-yl, $^C$ 4-Methylpiperidin-1-yl.
- $^D$ 3,5-Dimethylpiperidin-1-yl, $^E$ Morpholin-4-yl.
- $^F$ As diphosphate salt. $^G$ ±112.
- $^J$ As hydrochloride. $^K$ ±12.

(b) Discussion of Results

An examination of the results in Table II-3 and Table II-4 revealed in general a close correlation between IC$_{50}$ (and IC$_{90}$) values obtained against the FCQ-27 isolate in both the visual and radioisotope test. The IC$_{50}$ values obtained in the radioisotope test against the FCQ-27 isolate revealed that the majority of the di-Mannich compounds (II.40) had IC$_{50}$ values 12.5-25 nM. All these compounds were as active as, or more active than, chloroquine (IC$_{50}$ 20.3 ± 4 nM). The results in Table II-4 also revealed that these compounds were, in general, equally effective against the chloroquine-resistant K-1 isolate with resistance factor (RF) equal to 1 in most cases except for compounds (II.40c) and (II.40i); RF 2-4 and RF 1-2 respectively). Eight of these compounds in tests against the K-1 isolate had IC$_{50}$ values in the range 12.5-25 nM and should be
compared with that for chloroquine (IC$_{50}$ 396 ± 96 nM). The IC$_{90}$ values were only slightly higher than the IC$_{50}$ values.

The mono-Mannich compound (II.43) (Table II-3) was less active than the di-Mannich analogue (II.40d) and this is consistent with earlier observations.$^{63}$

Amongst, the 2-(7-bromo-1,5-naphthyridin-4-ylamino)phenols (II.41) [and (II.44)], none had biological activity superior to that of the 2-(7-chloroquinolin-4-ylamino)phenol analogous (II.40) [and (II.43)], and most were slightly less active. No significant differences in activity (as measured by IC$_{50}$ values) were observed in tests against the FCQ-27 and K-1 isolates. The most active of compounds (II.41) were compounds (II.41a,d,k,l) with IC$_{50}$ values 25-50 nM against both the FCQ-27 and K-1 isolates. The mono-Mannich compounds (II.44a,b) (Table II-3) were less active than their di-Mannich analogues (II.41d,g) and the 7-trifluoromethyl compounds (II.42) showed lower activity than their 7-bromo analogues (II.41).

II-4.2 In vivo Antimalarial Testing

The bergei group and the vinckei group, are two main series of rodent malarias. The former, containing P. bergei, P. yoelii, and other sub-species has been used in the majority of chemotherapeutic studies.$^{114,115}$ The vinckei group consists of several subspecies; of which P. vinckei has been used successfully in numerous malaria researches,$^{116,117}$ since it was first (isolated and) adapted to mice in 1952.

The rodent malaria models are often used as a primary and secondary screen before monkey malaria screens. The method of monitoring blood schizontocial activity in rodent malaria is usually performed in single-dose regimens (these comprise the Rane's test,$^{118}$ and Fink and Kretschmar's test,$^{119}$ or multiple-dose regimens (these include the Early Test Procedures, Four-Day Test, Drug-Diet methods and the Six-Day Test as summarised by Ager$^{120}$).
Table II-5 Antimalarial screening results for some compounds (II.40) and (II.41) against *P. vinckei vinckei* in mice.

Times given are those after injection of the chemical under test. Time: h, hours; d, days; 0 h denotes pretreatment. The symbol <1 indicates no visible parasitaemia observed.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mean percentage of parasite-infected red cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 h</td>
</tr>
<tr>
<td>(II.40a)</td>
<td>7</td>
</tr>
<tr>
<td>(II.40b)</td>
<td>4</td>
</tr>
<tr>
<td>(II.40c)</td>
<td>6</td>
</tr>
<tr>
<td>(II.40d)</td>
<td>7</td>
</tr>
<tr>
<td>(II.40e)</td>
<td>8</td>
</tr>
<tr>
<td>(II.40f)</td>
<td>7</td>
</tr>
<tr>
<td>(II.40g)</td>
<td>5</td>
</tr>
<tr>
<td>(II.40h)</td>
<td>2</td>
</tr>
<tr>
<td>(II.40i)</td>
<td>9</td>
</tr>
<tr>
<td>(II.40j)</td>
<td>4</td>
</tr>
<tr>
<td>(II.40k)</td>
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<td>(II.41a)</td>
<td>10</td>
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<td>(II.41b)</td>
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<td>(II.41d)</td>
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<td>(II.41e)</td>
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</tr>
<tr>
<td>(II.41f)</td>
<td>5</td>
</tr>
<tr>
<td>(II.41g)</td>
<td>5</td>
</tr>
<tr>
<td>(II.41k)</td>
<td>8</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>8</td>
</tr>
<tr>
<td>Peanut oil</td>
<td>2</td>
</tr>
<tr>
<td>Normal</td>
<td>9</td>
</tr>
<tr>
<td>Saline</td>
<td></td>
</tr>
</tbody>
</table>

*Chloroquine, Peanut oil, and Normal are for comparison.*
Footnotes to Table II-5
A One mouse dead.
B Second mouse dead.
C Two mice alive at day 41; parasitaemia levels < 1% at day 38.
D At day 11 one mouse dead and two destroyed because of high parasitaemia levels.
E One mouse alive at day 41; parasitaemia < 1 % at day 38.
F Three mice alive at day 41; parasitaemia levels < 1 % at day 38.
G One mouse dead at day 17, another dead at day 20.
H One mouse destroyed on each of days 14, 15, and 16 because of high parasitaemia levels.
I One mouse dead, other two mice destroyed because of high parasitaemia levels.
J Two mice dead.
K All mice dead.
L See Section II-5.3, p70, for experimental details. Dosage of compounds (II.40) and (II.41) was 200 mg/kg, that of chloroquine diphosphate 40 mg/kg.

(a) Methods and Results

In this study the potential antimalarial agents (which previously showed promise in the radioisotopic in vitro technique against both strains of P. falciparum (see II-4.1.a) were tested for in vivo activity against P. vinckei vinckei in mice by firstly carrying out a toxicity test, then a preliminary screen in mice infected with the parasite. The results are presented in Table II-5. Full details of the in vivo experimental procedure are given in Section II-5.3.

(b) Discussion of Results

The results of in vivo screening of compounds [(II.40) and (II.41)] against P. vinckei vinckei in mice recorded in Table II-5 revealed significant activity, particularly amongst those compounds (II.40) containing piperidine, methyipiperidine or morpholine. When the compounds (II.40-e-l) were each administered to three mice at a single dosage of 200 mg/kg of body weight, all except one of the 24 mice survived to day 41 with no visible parasitaemia revealed in thin blood smears taken at day 38. In all cases, parasites were not observed in blood samples taken 48 h after administration of the compounds (II.40-e-l), and no subsequent reoccurrence of infection was observed. Although compound (II.40b), when administered to three mice, was effective in removing parasites from blood samples, and two mice remained healthy at day 41, the
compounds (II.40a,c,d) were less effective. Such differences are not indicated in the \textit{in vitro} test results reported in Table II-3 and Table II-4.

In all cases the administration of the compound (II.40) at 200 mg/kg proved much more effective than chloroquine diphosphate at 40 mg/kg in removing (or suppressing) parasitaemia as shown clearly by the results at day 4.

The 7-bromo-1,5-naphthyridine compounds (II.41a,b,d,e,g,l) were also very effective at suppressing parasitaemia. The superior survival rate for mice treated with compounds (II.41a,d) relative to those treated with the 7-chloroquinoline compounds (II.40a,d) may be related to lower toxicities noted previously for 1,5-naphthyridines.\textsuperscript{61,64} The results obtained for mice treated with compounds (II.41f,k) were less encouraging.

In conclusion, the di-Mannich base derivatives of the 2-(7-chloroquinolin-4-yl)phenol are more effective than their 2-(7-bromo-1,5-naphthyridin-4-yl)phenol analogues, and the most effective against FCQ-27 isolate of \textit{P. falciparum} among this series was the di-Mannich base of 2-(7-chloroquinolin-3-ylamino)-5-methylphenol (II.40l, IC\textsubscript{50} 6.3-12.5 nM) which contained piperidine in the Mannich base side chain and a methyl group at C-5 of the phenol ring system. Further investigations of some derivatives of 2-aminophenols are reported in the following chapters, 2-[2(and 8)-trifluoromethylquinolin-4-ylamino]phenols are described in Chapter III, 4-chloro- and 4(and 6)-t-butyl-2-[7-chloro(and trifluoromethyl)quinolin-4-ylamino]phenols and 4-chloro-2[7-bromo(and trifluoromethyl)-1,5-naphthyridin-4-ylamino]phenols in Chapter IV.
II-5 Experimental

II-5.1 General Topics

1. Melting points (m.ps.) were taken in Pyrex capillaries with a Gallenkamp melting point apparatus and are uncorrected.

2. Analyses were performed by the Australian National University Analytical Service Unit, Canberra. All solids were dried for at least 4 hours under vacuum (0.2 mmHg) and at 50-100° prior to analysis, unless otherwise specified.

3. $^1$H Nuclear magnetic resonance spectra ($^1$H m.n.r.) were recorded either at 90 MHz and 30° on a Jeol FX90Q or at 300 MHz and 25° on a Gemini BB or Varian VXR300 fourier-transform spectrometer. Data were presented in the following order: chemical shift (δ) relative to tetramethylsilane in organic solvent; multiplicity; coupling constant (J) in Hz; and assignment (where possible). Samples were run in CDCl$_3$ unless specified otherwise. The following abbreviations were adopted: s (singlet); d (doublet); t (triplet); br s (broad singlet); m (multiplet); dd (doublet of doublets), and dt (doublet of triplets). Exchangeable protons were identified by their disappearance upon addition of deuterium oxide.

4. $^{13}$C Nuclear magnetic resonance techniques were performed on a Varian VXR 300 instrument at 25°. Samples were run in CDCl$_3$. Data were presented in the following order: chemical shift (ppm) relative to deuterated solvent peak and assignments where appropriate.

5. Two dimensional (2-D) nuclear magnetic resonance spectra such as the heteronuclear one-bond correlated (HETCOR) spectra, and the proton-proton correlation spectroscopy (COSY) spectra were run by Ms Tin Culnane, University Nuclear Magnetic Resonance Centre, the Australian National University, using a Varian VXR 300 spectrometer.

6. Low resolution mass spectra (MS) were recorded on an Incos data system attached to a VG-Micromass 7070F double focusing mass spectrometer using electron impact (EI) at 70 eV at the Research School of Chemistry under the supervision of
Dr J.K. MacLeod. Data are presented in the following order: m/z value; relative intensity as a percentage of the base peak. [The mass spectra are electron impact (EI) unless specified otherwise.]

7. Ultraviolet (u.v.) spectra were recorded on a CARY 219 u.v./visible spectrophotometer between 200-500 nm, in aqueous solution at 18-20° and at the pH specified.

8. Analytical thin layer chromatography (t.l.c.) was performed on glass plates or aluminium sheet with Merck kieselgel 60 F$_{254}$ or Merck aluminium oxide 60 F$_{254}$ neutral (type E) of 0.25 mm and 0.2 mm thickness, respectively. Preparative thin layer chromatography (PTLC) was performed on glass plates precoated with Merck aluminium oxide 60 F$_{254}$ (type E) of 2 mm thickness, or with Merck silica gel 60 F$_{254}$ of 2 mm thickness. Plates were visualised by both long (365 nm) and short (254 nm) wave ultraviolet light. Columns for chromatography were packed using Merck aluminium oxide 90 active neutral (0.063-0.200 mm, 70-230 mesh ASTM), aluminium oxide for chromatography (M&B Laboratory Reagent, MX6390), or silica gel (0.07-0.15 mm, 100-200 mesh, Ajax chemicals).

9. Reaction temperatures refer to external oil bath temperatures unless stated otherwise.

10. Where full experimental details are not recorded, percentage yields are given in brackets.

11. Starting materials, unless stated otherwise, were obtained commercially, usually from the Aldrich Chemical Company.

II-5.2 Synthetic Experimental

The products purified by t.l.c. in this chapter generally appeared as the major visible yellow band near R$_{f}$ 0.5. The preparation of some starting materials have been reported previously but some variations in preparative procedures are described below.
2-Nitro-4,6-bis(pyrrolidin-1'-ylmethyl)phenol (II.11d)

This compound was prepared\(^5^8\) from 2-nitrophenol, paraformaldehyde, and pyrrolidine in ethanol. The *title product* (m.p. 126-128°, lit.\(^5^8\) 127-129°) was obtained as yellow solid after column chromatography (alumina, 1% methanol in chloroform).

4,6-Bis(dipropylaminomethyl)-2-nitrophenol (II.11c) was obtained \(^5^8\) as an oil after column chromatography (alumina, chloroform; then alumina, 2% methanol in chloroform).

4,6-Bis(2-methylpiperidin-1-ylmethyl)-2-nitrophenol (II.11f)

The *title compound* \(^5^8\) was obtained as an oil after column chromatography (alumina, 2% methanol in chloroform; then alumina, methanol).

4,6-Bis(3-methylpiperidin-1-ylmethyl)-2-nitrophenol (II.11g) was a yellow solid (m.p. 74-75°, lit.\(^5^8\) oil) after recrystallisation from light petroleum (bp 60-80°).

4,6-Bis(4-methylpiperidin-1-ylmethyl)-2-nitrophenol (II.11h)

This compound was collected from the reaction mixture by filtration and washed with ethanol. It had m.p. 146-148° (lit.\(^5^8\) 147-149°).

4,6-Bis(3,5-dimethylpiperidin-1-ylmethyl)-2-nitrophenol (II.11i)

This compound crystallised from light petroleum (bp 60-80°) as a yellow solid, m.p. 134-135° (lit.\(^5^8\) 133-135°).

4,6-Bis(morpholin-4-ylmethyl)-2-nitrophenol (II.11j) as a yellow solid after column chromatography (alumina, chloroform/methanol, 5:1; then alumina, 1% methanol in chloroform). It had m.p. 113-115° (lit.\(^5^8\) 114-115°).
6-(3-Methylpiperidin-1-ylmethyl)-2-nitrophenol (II.15b)

This compound\(^5\) was obtained as an oil after column chromatography (silica, methanol).

2-Nitro-6-(pyrrolidin-1-ylmethyl)phenol (II.15a) was obtained as a yellow solid after column chromatography (silica, methanol). It had m.p. 162-163° (lit.\(^5\) 160-162°).

7-Bromo-4-chloro-1,5-naphthyridine (II.36)

This compound was prepared from 7-bromo-1,5-naphthyridin-4-ol\(^6\) with phosphoryl chloride at 120° and afforded 7-bromo-4-chloro-1,5-naphthyridine,\(^6\) m.p. 180-182° (lit.\(^6\) 181-183°).

4-Chloro-7-trifluoromethyl-1,5-naphthyridine (II.28)

This compound was prepared from 5-trifluoromethylpyridin-3-amine\(^5\) (m.p. 40-42°, lit.\(^5\) 40-41.5°) and diethyl ethoxymethylenemalonate through diethyl 5-trifluoromethylpyridin-3-ylaminomethylenemalonate\(^5\) (m.p. 64-66°, lit.\(^5\) 65-67°), ethyl 4-hydroxy-7-trifluoromethyl-1,5-naphthyridine-3-carboxylate\(^5\) (m.p. >300°, lit.\(^5\) >300°), 4-hydroxy-7-trifluoromethyl-1,5-naphthyridine-3-carboxylic acid\(^5\) (m.p. > 300°, lit\(^5\) >300°), and 7-trifluoromethyl-1,5-naphthyridin-4-ol\(^5\) (m.p. > 300°, lit\(^5\) >300°), which with a mixture of phosphorous pentachloride and phosphoryl chloride at 110° gave 4-chloro-7-trifluoromethyl-1,5-naphthyridine\(^5\) (m.p. 146-148°, lit.\(^5\) 147-149°).

2-(7'-Chloroquinolin-4'ylamino)-4,6-bis(piperidin-1'ylmethyl)phenol (II.40e) and Related Compounds

A solution of 2-amino-4,6-bis(piperidin-1'ylmethyl)phenol\(^5\) (0.2 g, 0.66 m mol), 4,7-dichloroquinoline (0.13 g, 0.66 m mol), ethanol (6.0 ml) and water (1.5 ml) was adjusted with concentrated hydrochloric acid to pH 4.7 and then refluxed in an oil

\(^*\) Kindly provided by Mr S. Ireland
bath at 100° for 8 h. The ethanol was evaporated under reduced pressure, the residue diluted with water (20 ml) and adjusted with aqueous ammonia to pH 7-8. The yellow precipitate was extracted into chloroform, the extract washed with water (2 x 5.0 ml), dried (Na₂SO₄) and the solvent evaporated to give the product (0.28 g). It was recrystallised from cyclohexane to give light brown crystals of the title compound (0.21 g; 69%), m.p. 165-167°. (Found, for a sample dried at 120°/0.1 mmHg for 5 h.: C, 69.8; H, 7.2; N, 11.8. C₂₇H₃₃C₁N₄O requires C, 69.7; H, 7.2; N, 12.0%).

¹H n.m.r. δ 1.55, m, H₃", 4", 5"; 2.38, m, H₂", 6"; 3.38, s, 4-CH₂; 3.70, s, 6-CH₂; 6.69, d, J₃,₅ 2Hz, H 5; 6.90, br, NH; 7.14, d, J₂,₃ 5.5 Hz, H 3'; 7.34, d, J₃,₅ 2 Hz, H 3; 7.41, dd, J₅',₆' 9, J₆',₈ 2 Hz, H 6'; 7.94, d, J₅',₆' 9 Hz, H 5'; 8.00, br s, H 8'; 8.58, d, J₂,₃ 6 Hz, H 2'; 8.55, br, OH. MS m/z 466, 464 (M) (1.8, 5.3%), 380 [M-(C₅H₉N)] (2.4%), 296 (25.1%), 298 (9.2%), 84 (C₅H₁₀N) (100%).

In a similar manner the following compounds were prepared.

2-(7'-Chloroquinolin-4'-ylamino)-4,6-bis(dimethylaminomethyl)phenol (II.40a)

This compound (53%) had m.p. 154-157°, after t.l.c. (alumina; chloroform) and recrystallisation from a mixture of chloroform and light petroleum (b.p. 60-80°) and also from a mixture of ethyl acetate and cyclohexane. (Found: C, 65.7; H, 6.5, N, 14.3. C₂₁H₂₅ClN₄O requires C, 65.5; H, 6.6; N, 14.6%). ¹H n.m.r. δ 2.25, s, 2.36, s, Me; 3.34, s, 4-CH₂; 3.70, s, 6-CH₂; 6.75, br s, H 5; 7.11, d, J 5.5 Hz, H 3'; 7.32, br s, H 3; 7.41, dd, J₅',₆' 9, J₆',₈ 2 Hz, H 6'; 7.94, d, J 9 Hz, H 5'; 7.99, br s, H 8'; 8.59, d, J 5.5 Hz, H 2'; 9.98, br, OH.

2-(7'-Chloroquinolin-4'-ylamino)-4,6-bis(diethylaminomethyl)phenol (II.40b)

This compound (45%), m.p. 141-144°, was obtained after t.l.c.(alumina; dichloromethane/ethyl acetate, 6:1) and recrystallisation from a mixture of cyclohexane and light petroleum (b.p. 40-60°) Found: C, 68.4; H, 7.2; N, 12.3. C₂₅H₃₃ClN₄O
requires C, 68.1; H, 7.6; N, 12.7%). \textsuperscript{1}H n.m.r. \( \delta 1.08, \text{q, J 7 Hz, Me}; 2.60, \text{complex, CH}_2\text{Me}; 3.49, \text{s, 4-CH}_2; 3.81, \text{s, 6-CH}_2; 6.78, \text{s, H 5}; 7.08, \text{d, J 5.5 Hz, H 3'}; 7.21, \text{br, NH}; 7.34, \text{br s, H 3}; 7.39, \text{dd, J}_5',_6' 9, J_6',_8' 2 \text{ Hz, H 6'}; 7.96, \text{d, J 9 Hz, H 5'}; 8.01, \text{br s, H 8'}; 8.56, \text{d, J 5.5 Hz, H 2'}; 9.54, \text{br, OH}.

2-(7'-Chloroquinolin-4'-ylamino)-4,6-bis(dipropylaminomethyl)phenol
\textit{(II.40c)}

This compound (41%) was obtained as an oil after t.l.c. (alumina; 4% ethyl acetate in dichloromethane then alumina; chloroform). (Found: C, 70.1; H, 8.4; N, 11.0. \( \text{C}_{29}\text{H}_{41}\text{ClN}_4\text{O} \) requires C, 70.1; H, 8.3; N, 11.3%). \textsuperscript{1}H n.m.r. \( \delta 0.82-2.61, \text{complex, Pr}; 3.47, \text{s, 4-CH}_2; 3.81, \text{s, 6-CH}_2; 6.74, \text{br s, H 5}; 7.07, \text{br, OH}; 7.13, \text{d, J 5.5 Hz, H 3'}; 7.36, \text{br s, H 3}; 7.42, \text{dd, J}_5',_6' 9, J_6',_8' 2 \text{ Hz, H 6'}; 7.96, \text{d, J 9 Hz, H 5'}; 8.01, \text{br s, H 8'}; 8.56, \text{d, J 5.5 Hz, H 2'}.

2-(7'-Chloroquinolin-4'-ylamino)-4,6-bis(pyrrolidin-1''-ylmethyl)phenol
\textit{(II.40d)}

Compound \textit{(II.40d)} (49%), m.p. 136-138\(^{0}\), was obtained after t.l.c. (alumina; chloroform) and recrystallisation from light petroleum (b.p. 60-80\(^{0}\)). (Found: C, 68.4; H, 6.7; N, 12.5. \( \text{C}_{25}\text{H}_{29}\text{ClN}_4\text{O} \) requires C, 68.7; H, 6.7; N, 12.8%). \textsuperscript{1}H n.m.r. \( \delta 1.81, \text{complex, H}_3''',_4'''; 2.64, \text{complex, H 2''', 5''}; 3.53, \text{s, 4-CH}_2; 3.87, \text{s, 6-CH}_2; 6.45, \text{br, OH}; 6.78, \text{br s, J 2 Hz, H 5}; 7.04, \text{br, NH}; 7.10, \text{d, J 5.5 Hz, H 3'}; 7.32, \text{d, J 2 Hz, H 3}; 7.39, \text{dd, J}_5',_6' 9, J_6',_8' 2 \text{ Hz, H 6'}; 7.95, \text{d, J 9 Hz, H 5'}; 8.01, \text{d, J}_6',_8' 2 \text{ Hz, H 8'}; 8.58, \text{d, J 5.5 Hz, H 2'}.

2-(7'-Chloroquinolin-4'-ylamino)-4,6-bis(2''-methylpiperidin-1''ylmethyl)phenol \textit{(II.40f)}

Compound \textit{(II.40f)} (25%), m.p. 166-168\(^{0}\), was obtained after t.l.c. (alumina; 1% methanol in chloroform then alumina; chloroform) and recrystallisation from light petroleum (b.p. 60-80\(^{0}\)). (Found: 70.5; H, 7.8; N, 11.2. \( \text{C}_{29}\text{H}_{37}\text{ClN}_4\text{O} \) requires C, 70.6; H, 7.6; N, 11.4%). \textsuperscript{1}H n.m.r. \( \delta 1.11-4.35, \text{complex, Me, H 2''', 3''', 4'', 5'', 6''
and 4 and 6-CH$_2$; 6.72, br s, H 5; 7.09, d, J 5.5 Hz, H 3'; 7.33, br s, H 3; 7.40, dd, J$_5',6'$ 9, J$_6',8'$ 2 Hz, H 6'; 7.95, d, J 9 Hz, H 5'; 8.01, br s, H 8'; 8.28, br, OH; 8.56, d, J 5.5 Hz, H 2'.

2-(7'-Chloroquinolin-4'-ylamino)-4,6-bis(3''-methylpiperidin-1''-ylmethyl)phenol (II.40g)

This title compound (23%) was obtained as an oil after t.l.c. (alumina; cyclohexane/ethyl acetate, 6:1 and alumina; chloroform). (Found: C, 70.4; H, 7.4; N, 11.1. C$_{29}$H$_{37}$ClN$_4$O requires C, 70.6; H, 7.6; N, 11.4%). $^1$H n.m.r. δ 0.88, complex, Me; 1.65-2.88, complex, H 2", 3", 4", 5", 6"; 3.39, s, 4-CH$_2$; 3.72, s, 6-CH$_2$; 6.72, br s, H 5; 7.05, br, OH; 7.14, d, J 5.5 Hz, H 3'; 7.36, br s, H 3; 7.42, dd, J$_5',6'$ 9, J$_6',8'$ 2 Hz, H 6'; 7.95, d, J 9 Hz, H 5'; 8.01, br s, H 8'; 8.59, d, J 5.5 Hz, H 2'.

2-(7'-Chloroquinolin-4'-ylamino)-4,6-bis(4''-methylpiperidin-1''-ylmethyl)phenol (II.40h)

Compound (II.40f) (32%) was obtained as an oil after t.l.c. (alumina; 1% methanol in chloroform then alumina, chloroform). (Found: C, 70.9; H, 7.8; N, 11.1. C$_{29}$H$_{37}$ClN$_4$O requires C, 70.6; H, 7.6; N, 11.4%). $^1$H n.m.r. δ 0.92, complex, Me; 1.20-3.06, complex, H 2", 3", 4", 5", 6"; 3.38, s, 4-CH$_2$; 3.71, s, 6-CH$_2$; 6.72, br s, H 5; 7.09, d, J 5.5 Hz, H 3'; 7.33, br s, H 3; 7.39, dd, J$_5',6'$ 9, J$_6',8'$ 2 Hz, H 6'; 7.95, d, J 9 Hz, H 5'; 8.00, br s, H 8'; 8.57, d, J 5.5 Hz, H 2'.

2-(7'-Chloroquinolin-4'-ylamino)-4,6-bis(3'',5''-dimethylpiperidin-1''-ylmethyl)phenol (II.40I)

This compound (30%), m.p. 128-130°, was obtained after t.l.c. (alumina; cyclohexane/ethyl acetate, 6:1 and alumina; 1% methanol in chloroform). (Found, for a sample dried at 20°/0.1 mmHg for 14 h: C, 71.3; H, 7.7; N, 10.5. C$_{31}$H$_{41}$ClN$_4$O requires C, 71.4; H, 7.9; N, 10.8%). $^1$H n.m.r. δ 0.88, complex, Me; 1.26-3.00, complex, H 2", 3", 4", 5", 6"; 3.40, s, 4-CH$_2$; 3.73, s, 6-CH$_2$; 6.72, br s, H 5; 7.06,
br, NH(?); 7.14, d, J 5.5 Hz, H 3'; 7.35, br s, H 3; 7.41, dd, J5',6' 9, J6',8' 2 Hz, H 6'; 7.95, d, J 9 Hz, H 5'; 8.01 br s, H 8'; 8.38, br, OH(?); 8.57, d, J 5.5 Hz, H 2'.

2-(7'-Chloroquinolin-4'-ylamino)-4,6-bis(morpholin-4''-ylmethyl)phenol (II.40j)

Compound (II.40j) (78%), m.p. 172-175°, was obtained after t.l.c. (alumina; chloroform). (Found: C, 64.0; H, 6.3, N, 11.7. C25H29CIN4O3 requires C, 64.0; H, 6.2; N, 11.9%). 1H n.m.r. δ 2.40-3.80, complex, H 2", 3", 5", 6" and 4- and 6-CH2; 6.10, br, OH; 6.78, br s, H 5; 7.08, d, J 5.5 Hz, H 3'; 7.38, br s, H 3; 7.44, dd, J5' 6.9, J6'. 8.2 Hz, H 6'; 7.97, d, J 9 Hz, H 5'; 8.02, br s, H 8'; 8.59, d, J 5.5 Hz, H 2'.

2-(7'-Chloroquinolin-4'-ylamino)-6-(pyrrolidin-1''-ylmethyl)phenol (II.43)

This title compound (22%) had m.p. 153-155° after t.l.c. (alumina; chloroform and alumina; 1% methanol in chloroform) and recrystallisation from light petroleum (b.p. 60-80°). (Found: C, 67.7; H, 5.9; N, 11.6. C20H20ClN30 requires C, 67.9; H, 5.7; N, 11.9%). 1H n.m.r. δ 1.88, complex, H 3", 4"; 2.69, complex, H 2", 5"; 3.90, s, CH2; 6.78-8.02, complex, H 3, 4, 5 and 2', 3', 5', 6', 8'; 8.56, br, NH.

4,6-Bis(N-cyclohexyl-N-ethylaminomethyl)-2-nitrophenol (II.11m)

N-cyclohexyl-N-ethylamine (3.66 g; 2.88 m mol) was added to a cold mixture of paraformaldehyde (0.86 g; 2.88 m mol) and 2-nitrophenol (1.0 g; 0.72 m mol) in ethanol (8.0 ml) and the mixture was refluxed in an oil bath at 100° for 66 h. The solvent was evaporated and the product subjected to column chromatography over alumina by elution with 2% methanol in chloroform. The material which was eluted first was again subjected to column chromatography (or t.l.c. on alumina with 1% methanol in chloroform) and gave as an oil the title compound (34%). (Found: C, 69.2; H, 9.5; N, 9.8. C24H39N303 requires C, 69.0; H, 9.4; N, 10.1%). 1H n.m.r. δ 0.89-2.73, complex, H 1', 2', 3', 4', 5', 6' and Et; 3.51, s, 4-CH2; 3.94, s, 6-CH2; 7.34, br s, H 5; 7.81, br s, H 3.
2-(7'-Chloroquinolin-4'-ylamino)-4,6-bis(N-cyclohexyl-N-ethylamino)phenol (II.40m)

4,6-Bis(N-cyclohexyl-N-ethylaminomethyl)-2-nitrophenol (0.61 g) was dissolved in a mixture of ethanol (30 ml) and ethanolic ammonia (30 ml) and shaken with hydrogen over Raney nickel until uptake ceased. The catalyst was filtered on celite and the solvent evaporated to leave an oil (0.53 g).

A solution of the above product (0.28 g) and 4,7-dichloroquinoline (0.14 g) in a mixture of ethanol (8.0 ml) and water (2.0 ml) was adjusted with concentrated hydrochloric acid to pH 4.7 and then it was refluxed in an oil bath at 97° for 11 h. The ethanol was evaporated, the residue diluted with water and adjusted with aqueous ammonia to pH 9-10. The product was extracted into chloroform and purified by t.l.c. (alumina; chloroform twice then alumina, cyclohexane/ethyl acetate, 3:1). The title compound was extracted with chloroform and dried at 90°/0.1 mmHg for 14 h. (Found: C, 69.9; H, 8.0; N, 9.5. C_{33}H_{45}ClN_{4}O. 0.2 CHCl_{3} requires C, 69.6; H, 8.0; N, 9.8%). ¹H n.m.r. δ 0.95-2.80, complex, H 1", 2", 3", 4", 5", 6" and Et; 3.58, s, 4-CH_{2}; 3.89, s, 6-CH_{2}; 6.04, br, NH; 6.77, br s, H 5; 7.05, br, OH; 7.13, d, J 5.5 Hz, H 3'; 7.38, br s, H 3; 3.44, dd, J_{5',6} 9, J_{6',8} 2 Hz, H 6'; 7.96, d, J 9 Hz, H 5'; 8.02, br s, H 8'; 8.57, d, J 5.5 Hz, H 2'.

2-(7'-Bromo-1',5'-naphthyridin-4'-ylamino)-4,6-bis(dipropylaminomethyl)phenol (II.41c) and Related Compounds

A mixture of 2-amino-4,6-bis(dipropylaminomethyl)phenol_{58} (0.13 g, 0.038 m mole), 7-bromo-4-chloro-1,5-naphthyridine (0.094 g, 0.038 m mole), ethanol (6.0 ml) and water (2.0 ml) was adjusted with concentrated hydrochloric acid to pH 2.5 and the mixture refluxed in an oil bath at 100° for 8.5 h. The solvent was evaporated, the residue diluted with water (6 ml) and adjusted to pH 9-10, and extracted with chloroform. The product was purified by t.l.c. (alumina; 4% ethyl acetate in dichloromethane) and gave the title compound (0.16 g; 76%) as an oil. (Found: C, 62.1; H, 7.4, N, 13.1. C_{28}H_{40}BrN_{5}O requires C, 62.0; H, 7.45; N, 12.9%). ¹H n.m.r. δ 0.82-0.98, m, Me; 1.38-1.72, m. CH_{2}Me 2.33-2.61, m. CH_{2}CH_{2}Me; 3.49, s, 4-CH_{2};
3.81, s, 6-CH₂; 6.76, s, H 5; 7.19, d, J 5.5 Hz, H 3'; 7.44, s, H 3; 8.42, d, J 2 Hz, H 8'; 8.55, d, J 5.5 Hz, H 2'; 8.79, d, J 2 Hz, H 5'; 8.71, br, OH.

The following compounds were prepared in a similar manner.

**2-(7'-Bromo-1',5'-naphthyridin-4'-ylamino)-4,6-bis(dimethylamino-methyl)phenol (II.41a)**

Compound (II.41a) (46%) as yellow crystals, m.p. 111-113⁰, was obtained after t.l.c. (alumina; chloroform) and recrystallisation from light petroleum (b.p. 60-80⁰) containing a little chloroform. (Found: C, 56.1; H, 5.6; N, 16.0. C₂₀H₂₄BrN₅O requires C, 55.8; H, 5.6; N, 16.3%). ¹H n.m.r. δ 2.27, s, 2.37, s, Me; 3.37, s, 4-CH₂; 3.71, s, 6-CH₂; 6.60, br, OH; 6.77, br s, H 5; 7.20, d, J 5.5 Hz, H 3'; 7.41, br s, H 3; 8.44, br s, H 8'; 8.59, d, J 5.5 Hz, H 2'; 8.79, br s, H 6'.

**2-(7'-Bromo-1',5'-naphthyridin-4'-ylamino)-4,6-bis(diethylamino-methyl)phenol (II.41b) (50%)**, m.p. 59-61⁰, was obtained after t.l.c. (alumina; dichloromethane/ethyl acetate, 4:1 and alumina; chloroform). (Found, for a sample dried at 20⁰/0.1 mmHg for 14 h. C, 59.0; H, 6.6; N, 14.2. C₂₄H₃₂BrN₅O requires C, 59.2; H,6.6; N, 14.4%). ¹H n.m.r. δ 1.09, q, J 7 Hz, Me; 2.61, complex, CH₂Me; 3.51, s, 4-CH₂; 3.82, s, 6-CH₂; 6.79, d, J 2 Hz, H 5; 7.18, d, J 5.5 Hz, H 3'; 7.42, d, J 2 Hz, H 3; 8.42, d, J 2 Hz, H 8'; 8.57, d, J 5.5 Hz, H 2'; 8.73, br, OH; 8.79, d, J 2 Hz, H 6'; 9.41, br, NH.

**2-(7'-Bromo-1',5'-naphthyridin-4'-ylamino)-4,6-bis(pyrrolidin-1'-ylamino)phenol (II.41d)**

Compound (II.41d) (50%) was obtained as yellow crystals, m.p. 114-116⁰, after t.l.c. (alumina; chloroform) and recrystallisation from light petroleum (b.p. 60-80⁰) containing a little chloroform. (Found: C, 59.6; H, 5.9; N, 14.6. C₂₄H₂₈BrN₅O requires C, 59.7; H, 5.9; N, 14.5%). ¹H n.m.r. δ 1.85, complex, H 3", 4"; 2.67,
complex, H 2", 5"; 3.55, s, 4-CH₂; 3.87, s, 6-CH₂; 5.26, br, OH; 6.81, s, H 5; 7.17, d, J 5.5 Hz, H 3'; 7.40, br s, H 3; 8.42, d, J 2 Hz, H 8'; 8.57, d, J 5.5 Hz, H 2'; 8.74, br, NH; 8.78, d, J 2 Hz, H 6'.

2-(7'-Bromo-1',5'-naphthyridin-4'-ylamino)-4,6-bis(piperidin-1''-yl-amino)phenol (II.41e)

This title compound (68%) was obtained as a yellow solid, m.p. 189-191⁰, after recrystallisation from a mixture of cyclohexane and methanol. (Found, for a sample dried at 120⁰/0.1 mmHg for 5 h: C, 61.4; H, 6.5; N, 13.4. C₂₆H₃₂BrN₅O requires C, 61.2; H, 6.3; N, 13.7%). ¹H n.m.r. δ 1.59, complex, H 3", 4", 5"; 2.42, complex, H 2", 6"; 3.42, s, 4-CH₂; 3.71, s, 6-CH₂; 6.76, br s, H 5; 7.19, d, J 5.5 Hz, H 3'; 7.43, br s, H 3; 8.43, d, J 2 Hz, H 8'; 8.58, d, J 5.5 Hz, H 2'; 8.74, br, OH; 8.80, d, J 2 Hz, H 6'.

2-(7'-Bromo-1',5'-naphthyridin-4'-ylamino)-4,6-bis(2''-methylpiperidin-1''-ylmethyl)phenol (II.41f)

Compound (II.41f) (42%) was obtained as a yellow solid, m.p. 126-128⁰, after t.l.c. (alumina; 1% methanol in chloroform then alumina; chloroform). (Found: C, 62.1; H, 6.9; N, 12.8. C₂₈H₃₆BrN₅O requires C, 62.4; H, 6.8; N, 13.0%). ¹H n.m.r. δ 1.14-1.23, complex, Me; 1.30-4.37, complex, H 2", 3", 4", 5", 6" and 4- and 6-CH₂; 5.85, br s, NH; 6.74, br s, H 5; 7.17, d, J 5.5 Hz, H 3'; 7.40, br s, H 3; 8.42, d, J 2 Hz, H 8'; 8.55, d, J 5.5 Hz, H 2'; 8.70, br, OH; 8.79, d, J 2 Hz, H 6'.

2-(7'-Bromo-1',5'-naphthyridin-4'-ylamino)-4,6-bis(3''-methylpiperidin-1''-ylmethyl)phenol (II.41g)

The title compound (45%) was an oil after t.l.c. (alumina; chloroform, twice).(Found: C, 62.7; H, 7.0; N, 12.9. C₂₈H₃₆BrN₅O requires C, 62.4; H, 6.8; N, 13.0%). ¹H n.m.r. δ 0.88, complex, Me; 1.66-2.88, complex, H 2", 3", 4", 5", 6"; 3.40, s, 4-CH₂; 3.71, s, 6-CH₂; 6.74, br s, H 5; 7.19, d, J 5.5 Hz, H 3'; 7.43, br s,
H 3; 8.43, d, J 2 Hz, H 8'; 8.57, d, J 5.5 Hz, H 2'; 8.73, br, NH; 8.79, d, J 2 Hz, H 6'.

2-(7'-Bromo-1',5'-naphthyridin-4'-ylamino)-4,6-bis(4''-methylpiperidin-1''-ylmethyl)phenol (II.41h)

This compound (49%) was obtained as an oil after t.l.c. (alumina; 1% methanol in chloroform). (Found: C, 62.8; H, 7.0; N, 12.8. C_{28}H_{36}BrN_{5}O requires C, 62.4; H, 6.8; N, 13.0%). \(^1\)H n.m.r. \(\delta\) 0.96, complex, Me; 1.30-3.09, complex, H 2", 3", 4", 5", 6"; 3.45, s, 4-CH\(_2\); 3.74, s, 6-CH\(_2\); 4.60, br, NH; 6.77, br s, H 5; 7.19, d, J 5.5 Hz, H 3'; 7.40, br s, H 3; 8.44, d, J 2 Hz, H 8'; 8.59, d, J 5.5 Hz, H 2'; 8.72, br, OH; 8.81, d, J 2 Hz, H 6'.

2-(7'-Bromo-1',5'-naphthyridin-4'-ylamino)-4,6-bis(3'',5''-dimethylpiperidin-1''-ylmethyl)phenol (II.41i)

Compound (II.41i) (39%) as a yellow oil was obtained after t.l.c. (alumina; cyclohexane/ethyl acetate, 7:1). (Found: C, 63.3; H, 7.4; N, 12.3. C_{30}H_{40}BrN_{5}O requires C, 63.6; H, 7.1; N, 12.4%). \(^1\)H n.m.r. \(\delta\) 0.86, complex, Me; 1.26-3.00, complex, H 2", 3", 4", 5", 6"; 3.44, s, 4-CH\(_2\); 3.73, s, 6-CH\(_2\); 6.76, br s, H 5; 7.20, d, J 5.5 Hz, H 3'; 7.43, br s, H 3; 8.43, d, J 2 Hz, H 8'; 8.57, d, J 5.5 Hz, H 2'; 8.74, br, OH; 8.79, d, J 2 Hz, H 6'.

2-(7'-Bromo-1',5'-naphthyridin-4'-ylamino)-4,6-bis(morpholin-4''-ylmethyl)phenol (II.41j)

Compound (II.41j) (45%), m.p. 193-195°, was obtained after t.l.c. (alumina; chloroform). (Found, for a sample dried at 110°/0.1 mmHg for 14 h: C, 55.8; H, 5.5; N, 13.4. C_{24}H_{28}BrN_{5}O_{3} requires C, 56.0; H, 5.5; N, 13.6%). \(^1\)H n.m.r. \(\delta\) 2.40-3.84, complex, H 2", 3", 5", 6" and 4- and 6-CH\(_2\); 6.79, br s, H 5; 7.18, d, J 5.5 Hz, H 3'; 7.48, br s, H 3; 8.45, br s, H 8'; 8.60, d, J 5.5 Hz, H 2'; 8.78, d, J 2 Hz, H 6'.
2-(7'-Bromo-1',5'-naphthyridin-4'-ylamino)-4,6-bis(N-cyclohexyl-N-ethylaminomethyl)phenol (II.41m)

This compound (38%) was obtained as an oil after t.l.c. (alumina; chloroform, twice) and extraction with chloroform. (Found: C, 62.6; H, 6.9; N, 11.3. C_{32}H_{44}BrN_{5}O. 0.2 CHCl_{3} requires C, 62.5; H, 7.2; N, 11.3%). \textsuperscript{1}H n.m.r. δ 0.96-2.75, complex, H 1"", 2"", 3"", 4"", 5"", 6"" and Et; 3.60, s, 4-CH\textsubscript{2}; 3.89, s, 6-CH\textsubscript{2}; 5.91, br, NH; 6.79, br s, H 5; 7.20, d, J 5.5, H 3'; 7.46, br s, H 3; 7.42, d, J 2 Hz, H 8'; 8.56, d, J 5.5 Hz, H 2'; 8.72, br, OH; 8.79, d, J 2 Hz, H 6'.

4,6-Bis(pyrroldin-1'-ylmethyl)-2-(7''-trifluoromethyl-1',5',-naphthyridin-4''-ylamino)phenol (II.42a)

The title compound (60%) was an oil after t.l.c. (alumina; chloroform). (Found: C, 63.5; H, 6.2; N, 14.7. C_{25}H_{28}F_{3}N_{5}O requires C, 63.7; H, 6.0; N, 14.9%). \textsuperscript{1}H n.m.r. δ 1.83, complex, H 3', 4'; 2.60, complex, H 2', 5'; 3.56, s, 4-CH\textsubscript{2}; 3.88, s, 6-CH\textsubscript{2}; 6.82, br s, H 5; 7.24, d, J 5.5 Hz, H 3""; 7.42, br s, H 3; 8.53, br s, H 8""; 8.65, d, J 5.5 Hz, H 2""; 8.85, br, NH; 8.98, s, H 6""; 9.13, br, OH.

4,6-Bis(piperidin-1'-ylmethyl)-2-(7''-trifluoromethyl-1',5',-naphthyridin-4''-ylamino)phenol (II.42b)

Compound (II.42b) (61%) was obtained as a yellow solid m.p. 166-168\degree, after recrystallisation from cyclohexane (Found: C, 65.0; H, 6.7; N, 13.8. C_{27}H_{32}F_{3}N_{5}O requires C, 64.9; H, 6.5; N, 14.0%). \textsuperscript{1}H n.m.r. δ 1.58, complex, H 3', 4', 5'; 2.41, complex, H 2', 6'; 3.41, s, 4-CH\textsubscript{2}; 3.71, s, 6-CH\textsubscript{2}; 6.77, br s, H 5; 7.25, d, J 5.5 Hz, H 3""; 7.43, br s, H 3; 8.53, br s, H 8""; 8.68, d, J 5.5 Hz, H 2""; 8.84, br, OH; 8.99, br s, H 6"".

4,6-Bis(3',5'-dimethylpiperidin-1'-ylmethyl)-2-(7''-trifluoromethyl)-1',5',-naphthyridin-4''-ylamino)phenol (II.42c)

Compound (II.42c) (80%) was obtained as an oil after t.l.c. (alumina; cyclohexane/ethyl acetate, 6:1). (Found: C, 67.2; H, 7.4; N, 12.5. C_{31}H_{40}F_{3}N_{5}O
requires C, 67.0; H, 7.3; N, 12.6\%). $^1$H n.m.r. $\delta$ 0.82-0.89, complex, Me; 1.00-3.00, complex, H 2', 3', 4', 5', 6'; 3.43, s, 4-CH$_2$; 3.73, s, 6-CH$_2$; 6.76, br s, H 5; 7.28, d, J 5.5 Hz, H 3''; 7.43, br s, H 3; 8.54, br s, H 8''; 8.66, d, J 5.5 Hz, H 2''; 8.86, br, OH; 8.99, d, J 2 Hz, H 6''.

4,6-Bis(morpholin-4'-ylmethyl)-2-(7''-trifluoromethyl-1'';5''-naphthyridin-4''-ylamino)phenol (II.42d)

This compound (83%), m.p. 165-167$^0$, was obtained after t.l.c. (alumina; chloroform). (Found: C, 59.4; H, 5.7; N, 13.7. $C_{25}H_{28}F_{3}N_{5}O_{3}$ requires C, 59.6; H, 5.6; N, 13.9\%). $^1$H n.m.r. $\delta$ 2.44-3.84, complex, H 2', 3', 5', 6', 4-CH$_2$, 6-CH$_2$; 6.82, br s, H 5; 7.26, d, J 5.5 Hz, H 3''; 7.50, br s, H 3; 8.55, br s, H 8''; 8.71, d, J 5.5 Hz, H 2''; 8.86, br, OH; 9.01, d, J 2 Hz, H 6''.

2-(7'-Bromo-1',5'-naphthyridin-4'-ylamino)-6-(pyrrolidin-1''-ylmethyl)phenol (II.44a)

Compound (II.44a) (26%), as yellow crystals, m.p. 117-119$^0$, was obtained after t.l.c. (alumina; chloroform, twice) and recrystallisation from light petroleum (b.p. 60-80$^0$). (Found: C, 57.0; H, 4.8; N, 14.0. $C_{19}H_{19}BrN_{4}O$ requires C, 57.1; H, 4.8; N, 14.0\%). $^1$H n.m.r. $\delta$ 1.85, complex, H 3'', 4''; 2.68, complex, H 2'', 5''; 3.88, s, CH$_2$; 6.78-7.51, m, H 3, 4, 5; 7.19, d, J 5.5 Hz, H 3'; 7.80, br, NH, 8.42, d, J 2 Hz, H 8'; 8.56, d, J 5.5 Hz, H 2'; 8.78, d, J 2 Hz, H 6'.

2-(7'-Bromo-1',5'-naphthyridin-4'-ylamino)-6-(3''-methylpiperidin-1''-ylmethyl)phenol (II.44b)

Compound (II.44b) (36%), m.p. 186-189$^0$, was obtained after t.l.c. (alumina; 5% methanol in chloroform) and recrystallisation from chloroform. (Found, for a sample dried at 110$^0$/0.1 mmHg for 14 h.: C, 58.8; H, 5.4; N, 12.9. $C_{21}H_{23}BrN_{4}O$ requires C, 59.1; H, 5.4; N, 13.1\%). $^1$H n.m.r. $\delta$ 0.87, complex, Me; 1.60-2.90, complex, H 2'', 3'', 4'', 5'', 6''; 3.49, s, 6-CH$_2$; 4.24, br, OH; 6.88-7.59, complex, H 3, 4, 5; 8.30-8.63, complex, H 2', 3', 6', 8'.

3-Methyl-2-nitro-4,6-bis(piperidin-1'-ylmethyl)phenol (II.13a)

Piperidine (4.45 g) was added to a chilled mixture of 3-methyl-2-nitrophenol (2.0 g), paraformaldehyde (1.56 g) and ethanol (15 ml) and the mixture refluxed for 23 h. The solvent was evaporated and the product was subjected to column chromatography (alumina; chloroform, twice) to give, as an oil, the title compound (1.92 g). (Found: C, 65.4; H, 8.7; N, 11.9. C_{19}H_{29}N_{3}O_{3} requires C, 65.7; H, 8.4; N, 12.1%). \(^{1}\)H n.m.r.  \(\delta\) 1.53, complex, H 3', 4', 5'; 2.24, s, Me; 2.20-2.60, complex, H 2', 6'; 3.29, s, 4-CH\(_2\); 3.71, s, 6-CH\(_2\); 6.94, br s, H 5.

5-Methyl-2-nitro-4,6-bis(piperidin-1'-ylmethyl)phenol (II.13b)

Piperidine (4.45 g) was added to a chilled reaction mixture of 2-nitrophenol (2.0 g), paraformaldehyde (1.56 g) and ethanol (15 ml) and the mixture refluxed for 23 h. The solvent was evaporated and the product subjected twice to column chromatography (alumina; chloroform) to give the title compound (1.81 g) as a yellow solid, m.p. 54-56°. (Found, for a sample dried at 20°/0.2 mmHg for 24 h: C, 65.7; H, 8.5; N, 11.8. C_{19}H_{29}N_{3}O_{3} requires C, 65.7; H, 8.4; N, 12.1%). \(^{1}\)H n.m.r.  \(\delta\) 1.48-1.59, complex, H 3', 4', 5'; 2.30, s, Me; 2.30-2.65, complex, H 2', 6'; 3.32, s, 4-CH\(_2\); 3.85, s, 6-CH\(_2\); 7.74, s, H 3.

2-(7'-Chloroquinolin-4'-ylamino)-3-methyl-4,6-bis(piperidin-1''-ylmethyl)phenol (II.40k)

A mixture of 3-methyl-2-nitro-4,6-bis(piperidin-1'-ylmethyl)phenol (0.55 g), ethanol (30 ml), ethanolic ammonia (30 ml) and Raney nickel was shaken with hydrogen until uptake ceased. The catalyst was filtered on celite and the solvent was evaporated to give the crude amine (0.40 g) as an oil. \(^{1}\)H n.m.r.  \(\delta\) 1.55, complex, H3',4',5'; 2.15, s, Me; 2.40, complex, H 2', 6'; 3.37, s, 4-CH\(_2\); 3.60, s, 6-CH\(_2\); 6.37, s, H 5].

A mixture of this amine (0.27 g, 0.085 m mol), 4,7-dichloroquinoline (0.168 g, 0.085 m mol), ethanol (8.0 ml) and water (2.0 ml) was adjusted with concentrated hydrochloric acid to pH 4.7 and refluxed in an oil bath at 97° for 11.5 h. The solvent
was evaporated under reduced pressure and the residue diluted with water, adjusted to pH 9-10 and extracted with chloroform. The product obtained was purified by t.l.c. (alumina; chloroform) and gave as an oil the title compound (0.22 g; 54%). (Found: C, 70.2; H, 7.6; 11.4. C\textsubscript{28}H\textsubscript{35}ClN\textsubscript{4}O requires C, 70.2; H, 7.4; N, 11.7%). \textsuperscript{1}H n.m.r. δ 1.49, complex, H 3", 4", 5"; 2.16, s, Me; 2.43, complex, H 2", 6"; 3.36, s, 4-CH\textsubscript{2}; 3.68, s, 6-CH\textsubscript{2}; 6.11, d, J 5.5 Hz, H 3'; 6.88, br s, H 5; 7.29, dd, J\textsubscript{5',6} 9, J\textsubscript{6',8} 2 Hz, H 6'; 7.99, d, J 9 Hz, H 5'; 7.97, br s, H 8'; 8.46, d, J 5.5 Hz, H 2'.

2-(7'-Bromo-1',5'-naphthyridin-4'-ylamino)-3-methyl-4,6-bis(piperidin-1"-ylmethyl)phenol (II.41k)

A mixture of 2-amino-3-methyl-4,6-bis(piperidin-1'-ylmethyl)phenol (0.17 g, 0.05 m mol), 7-bromo-4-chloro-1,5-naphthyridine (0.13 g, 0.05 m mol), ethanol (8.0 ml) and water (2.0 ml) was adjusted with concentrated hydrochloric acid to pH 2.5 and then refluxed in an oil bath at 90° for 8.5 h. Work up was as above and the product was purified by t.l.c. (alumina; chloroform) and gave as an oil the title compound (0.1 g). (Found: C, 61.5; H, 6.5; N, 13.0. C\textsubscript{27}H\textsubscript{34}BrN\textsubscript{5}O requires C, 61.8; H, 6.5; N, 13.3). \textsuperscript{1}H n.m.r. δ 1.51, complex, H 3", 4", 5"; 2.22, s, Me; 2.39, complex, H 2", 6"; 3.37, s, 4-CH\textsubscript{2}; 3.69, s, 6-CH\textsubscript{2}; 6.25, d, J 5.5 Hz, H 3'; 6.89, br s, H 5; 7.74, br, NH; 7.99, br s, H 8'; 8.43, br, OH; 8.46, d, J 5.5 Hz, H 2'; 8.78, d, J 2 Hz, H 6'.

2-(7'-Chloroquinolin-4'-ylamino)-5-methyl-4,6-bis(piperidin-1"-ylmethyl)phenol (II.40l)

A mixture of 5-methyl-2-nitro-4,6-bis(piperidin-1'-ylmethyl)phenol (0.52 g), ethanol (30 ml), ethanolic ammonia (30 ml) and Raney nickel was shaken with hydrogen until uptake ceased. The catalyst was filtered on celite and the solvent evaporated to give the crude amine (0.35 g) [\textsuperscript{1}H n.m.r. δ 1.56, complex, H 3', 4', 5'; 2.13, s, Me; 2.38, complex, H 2', 6'; 3.31, s, 4-CH\textsubscript{2} 3.69, s, 6-CH\textsubscript{2}; 6.60, s, H 3].

A mixture of the above amine (0.22 g), 4,7-dichloroquinoline (0.138 g), ethanol (8 ml) and water (2 ml) was adjusted with concentrated hydrochloric acid to pH 4.7 and
the mixture refluxed in an oil bath at 95° for 10.5 h. Work up was as above and the product was subjected to t.l.c. (alumina; chloroform, twice) and crystallised from light petroleum (b.p. 60-80°) to give yellow crystals of the title compound (0.16 g; 48%), m.p. 190-193°. (Found: C, 70.0; H, 7.6; N, 11.5. C_{28}H_{35}ClN_{4}O requires C, 70.2; H, 7.4; N, 11.7%). \(^1\)H n.m.r. δ 1.49-1.60, complex, H 3", 4", 5"; 2.23, s, Me; 2.30-2.60, complex, H 2", 6"; 3.35, s, 4-CH_2; 3.79, s, 6-CH_2; 7.05, d, J 5.5 Hz, H 3'; 7.29, s, H 3; 7.40, dd, J_5'; J_6', 9, J_6', 8' 2 Hz, H 6'; 7.95, d, J 9 Hz, H 5'; 8.06, br s, H 8'; 8.55, d, J 5.5 Hz, H 2'; 9.96, br, NH.

In a similar manner to that described above for the 7-bromo analogue and 3-methyl isomer the following compounds were prepared.

3-Methyl-4,6-bis(piperidin-1'-ylmethyl)-2-(7"-trifluoromethyl-1",5"-naphthyridin-4"-ylamino)phenol (II.42e)

Compound (II.42e) (46%), m.p. 132-134° was obtained after t.l.c. (alumina; chloroform). (Found: C, 65.8; H, 6.7; N, 13.4. C_{28}H_{34}F_{3}N_{5}O requires C, 65.5; H, 6.7; N, 13.6%). \(^1\)H n.m.r. δ 1.53, complex, H 3', 4', 5'; 2.24, s, Me; 2.43, complex, H 2', 6'; 3.39, s, 4-CH_2; 3.71, s, 6-CH_2; 6.34, d, J 5.5 Hz, H 3"; 6.93, br s, H 5; 8.11, br s, H 8'; 8.57, d, J 5.5 Hz, H 2"; 8.54, br, OH; 9.02, d, J 2 Hz, H 6".

2-(7'-Bromo-1',5'-naphthyridin-4'-ylamino)-5-methyl-4,6-bis(piperidin-1"-ylmethyl)phenol (II.411)

This title compound (26%) had m.p. 198-200° after t.l.c. (alumina; chloroform, twice) and recrystallisation from light petroleum (b.p. 60-80°) and then a mixture of chloroform and light petroleum. (Found: C, 61.4; H, 6.8; N, 13.0.C_{27}H_{34}BrN_{5}O requires C, 61.8; H, 6.5; N, 13.3%). \(^1\)H n.m.r. δ 1.50-1.60, complex, H 3", 4", 5"; 2.24, s, Me; 2.35-2.65, complex, H 2", 6"; 3.38, s, 4-CH_2; 3.79, s, 6-CH_2; 7.12, d, J 5.5 Hz, H 3'; 7.37, s, H 3; 8.41, d, J 2 Hz, H 8'; 8.55, d, J 5.5 Hz, H 2'; 8.66, br, NH; 8.78, d, J 2 Hz, H 6'. MS m/z 526, 524 (M^+) (0.6, 0.6%), 356 (2.2%), 98 (40.9%), 84 (100%).
II-5.3 Measurement of Biological Activity

(a) *In vitro* testing against *P. falciparum*

The *in vitro* tests were carried out by the staff of the Army Malaria Research Unit (AMRU), Ingleburn, New South Wales, Australia.

Three different *in vitro* techniques were employed in this work for the determination of antimalarial activity against *P. falciparum*:

i. The modified microscopic test (morphological microtest) described by Rieckmann *et al.*105 In this test, the criterion of drug response was morphological (i.e. the failure of the asexual stages to develop to schizogony) when cultured for 24 h at 38-40°.

ii. The visual observation of pigment precipitation also described by Rieckmann.112

iii. The 3H-hypoxanthine uptake method modified from the semi-automated microdilution technique described by Desjardines107 and others.57,116 This technique involved the growing of cultures in 96-well microtiter plates with the addition of a radiolabelled substrate which would be incorporated into the nucleic acid of the parasites. The level of 3H-hypoxanthine incorporated into the parasite was determined using liquid scintillation counting and gave a measure of the development of parasite (after an incubation period).

The two isolates of *P. falciparum* used in the *in vitro* screen were maintained routinely by the culture technique of Träger and Jensen103 and are from the following sources:

FCQ-27 isolate: (Origin Madang, Papua New Guinea) was isolated at the Walter and Eliza Hall Institute of Medical Research, Melbourne.

K-1 isolate: (Origin Kanchanaburi, Thailand) was also isolated at the Walter and Eliza Hall Institute of Medical Research, Melbourne.

Both strains were routinely cultured at the AMRU.
(b) *In vivo* screen against *P. vinckei vinckei*

**Experimental animals**

Male CBA/CaH mice (6-8 weeks old) were used in all experiments. These were bred and maintained in the John Curtin School of Medical Research Animal Breeding Establishment (under specific pathogen free conditions) and fed normal laboratory diet pellets and tap water *ad libitum*. Average body weight was no greater than 20 g.

**Parasite**

*P. vinckei vinckei* (strain V52, originally from Professor F. Cox, Kings College, London) was stored frozen in liquid nitrogen and had been passaged several times before experimental use in the CBA/CaH mice.

(i) **Toxicity Testing**

Each compound was examined for acute toxicity in three mice by intraperitoneal injection, each with a single dose in peanut oil, at a dosage of 200 mg/kg of body weight.

No apparent ill effects were observed, and all mice survived to and beyond 6 days in these tests, and in a control experiment with peanut oil.

(ii) **Preliminary Antimalarial Screen**

**Infection of mice**

The infection of mice was initiated by intraperitoneal injection with $10^6$ erythrocytes infected with *P. vinckei vinckei* (obtained from an infected donor mouse*). After 5 days (and daily thereafter) each mouse was examined for suitable parasitaemia levels (generally 10-20%). The percentage parasitaemia was monitored by thin blood smears (taken from each mouse's tail vein), slides were fixed with methanol, stained (Diff-Quik stain) and then examined under an oil immersion microscope. The mean percentage of parasite-infected red cells was determined as the average of two or more counts on each slide which varied by no more than ±5% of the mean value.

---

* Kinkly supplied by Dr Kirk Rockett
Drug treatment

Each test compound at a dosage of 200 mg/kg of body weight in 0.4 ml of peanut oil was given intraperitoneally (except for reference tests run against chloroquine diphosphate, which was administered at a dosage of 40 mg/kg in normal saline) to three mice whose individual parasitaemia (infection levels of preferably 10-20%) had just previously been determined. Thereafter thin blood smears were taken from each mouse at time intervals indicated in Table (II-5) and the parasitaemia assessed as above.

Control tests were made against peanut oil and normal saline, in which there was a 100% mortality three days after treatment.
CHAPTER III
CHAPTER III  Syntheses and Antimalarial Activity of Some Di (and mono)-Mannich Base Derivatives of 2(and 4)-[2(and 8)-Trifluoromethylquinolin-4-ylamino]phenols

III-1  Introduction

The value of substituting a fluoro or fluorine-containing group for other halogens, hydrogen, hydroxyl or amino groups in prototype medicinals has been demonstrated in the past. Such an effect is shown by mono-(trifluoromethyl) substituents in the quinoline nucleus of antimalarials described in Chapter I. For example, various 7-trifluoromethylquinolinemethanols have been shown to possess higher antimalarial activity against \textit{P. berghei} in mice, than the chloro analogues. \textit{Di-Mannich} base derivatives of 4-(7-trifluoromethylquinolin-4-ylamino)phenol have also been revealed by Barlin \textit{et al.} to have high antimalarial activity against \textit{P. vinckei vinckei} in mice.

In this chapter, we examine various mono- and di-Mannich base derivatives of series of 2(and 4)-[2(and 8)-trifluoromethylquinolin-4-ylamino]phenols. The preparation of these compounds will be reported and their $^1$H n.m.r.. spectral data discussed in relation to the structures. The \textit{in vitro} antimalarial test results of these compounds, against the human malaria parasite \textit{P. falciprum} will then be reported and discussed.

III-2  Syntheses

III-2.1  Methods for the Preparation of Some 4-Chloroquinolines

(a) 4-Chloro-8-trifluoromethylquinoline

Probably the most generally useful method for the preparation of the precursor, 8-trifluoromethylquinolin-4-ol, is that due originally to Gould and Jacobs, and later
developed by Price and Roberts.\textsuperscript{88} In this method, the aromatic amine (III.1) was condensed with diethyl ethoxymethylenemalonate, yielding the ethyl \(\alpha\)-ethoxycarbonyl-\(\beta\)-arylaminoacrylate (III.2) which was converted to the quinolin-4-ol (III.4) by cyclisation, saponification, and decarboxylation.

In this way, 7-trifluoromethylquinolin-4-ol\textsuperscript{94} was prepared from 3-trifluoromethylaniline, and chlorination of the former with a mixture of phosphorus pentachloride and phosphoryl chloride afforded 4-chloro-7-trifluoromethylquinoline;\textsuperscript{94} 4,7-dichloroquinoline\textsuperscript{88} can be prepared similarly from 3-chloroaniline through 7-chloroquinolin-4-ol,\textsuperscript{88} by chlorination with phosphoryl chloride.

\[
\begin{align*}
\text{III.1} & \quad \text{X} - \text{NH}_2 \\
\text{III.2} & \quad \text{X} - \text{EtO}_2\text{C} - \text{C=O} - \text{C}_2\text{Et} \\
\text{III.3} & \quad \text{OH} - \text{CO}_2\text{Et} \\
\text{III.4} & \quad \text{X} - \text{N} - \text{CH} - \text{H} \\
\end{align*}
\]

8-Trifluoromethylquinolin-4-ol\textsuperscript{123} (III.10) was synthesised in a similar manner (Scheme III-1) from \(o\)-trifluoromethylaniline (III.5) and diethyl ethoxymethylenemalonate (III.6) through diethyl \(o\)-trifluoromethylanilinomethylenemalonate (III.7), ethyl 4-hydroxy-8-trifluoromethylquinoline-3-carboxylate (III.8), and 4-hydroxy-8-trifluoromethylquinoline-3-carboxylic acid (III.9), as reported by Allais and Meier,\textsuperscript{123} except that the decarboxylation step was effected by heating in a metal bath at 290\textdegree\ for \(ca\) 10 min. Chlorination of (III.10) was carried out with a mixture of phosphorus pentachloride and phosphoryl chloride.
Scheme III-1

III.5 + III.6

IIII.7

250°C (C₆H₅)₂O

III.8 → III.9

10% NaOH

III.11 → III.10

Cl

PCl₅/POCl₃
(b) 4-Chloro-2-trifluoromethylquinoline

β-Keto esters, such as ethyl acetoacetate, can react with an aromatic amine in either of two ways. Reaction of the amine at the ester group leads to the formation of an acetoacetanilide (III.12) whereas reaction of the amine at the β-keto group leads to the formation of the ethyl β-anilinocrotonate or the anil (III.13). On cyclisation, these products form the quinolin-2-ol (III.14) or the quinolin-4-ol (III.15), respectively. Hauser and Reynolds\textsuperscript{124} have clarified the factors governing the manner in which the condensation takes place in great detail.

\[
\begin{align*}
\text{III.1, } \text{X} &= \text{H} \\
+ & \\
\text{R} \text{COCH}_2\text{CO}_2\text{Et} \\
\text{OH}
\end{align*}
\]

Coffey \textit{et al.}\textsuperscript{126} observed in the Conrad-Limpach syntheses from \textit{m}-chloroaniline that when hydrochloric acid was used as a catalyst, the β-\textit{m}-chloroanilinocrotonate (III.16) was obtained in good yield. Cyclisation of this compound (III.16) was achieved in refluxing diphenyl ether to give 7-chloro-2-methylquinolin-4-ol (III.17) as a major product together with a small quantity of the isomeric 5-chloro-2-methylquinolin-4-ol\textsuperscript{127} (III.18).
The condensation of ethyl 4,4,4-trifluoroacetoacetate (III.19) with various aromatic amines (III.1) in the presence of polyphosphoric acid (PPA) gave only the quinolin-4-ols\textsuperscript{67,68,128,129} (III.20), whereas this reaction with ethyl acetoacetate gave a mixture of the quinolin-2(and 4)-ols. Apparently the electron-withdrawing power of the trifluoromethyl group leads to exclusive reaction of the electron-deficient β-keto group with the amine\textsuperscript{129} and the formation of (III.20).

Scheme III-2

\[
\text{CF}_3\text{COCH}_2\text{CO}_2\text{Et} + \text{III.1} \xrightarrow{\text{PPA}} \text{III.20}
\]
4-Chloro-2-trifluoromethylquinoline\textsuperscript{129} (III.21) required in this work was prepared from aniline (III.1; X=H) and ethyl 4,4,4-trifluoroacetoacetate (III.19) through 2-trifluoromethylquinolin-4-ol (III.20; X=H) (Scheme III-2). The intermediate (III.22) in the formation of (III.20; X=H) can be isolated using the Conrad-Limpach procedure\textsuperscript{129} and it is cyclised in boiling diphenyl ether to give (III.20; X=H).

\begin{center}
III.22
\end{center}

**III-2.2 Syntheses of Mannich Base Derivatives of 2(and 4)-[2(and 8)-Trifluoromethylquinolin-4-ylamino]phenols**

The required starting materials, the Mannich base derivatives of 4-aminophenol\textsuperscript{130} (III.23) and of 2-aminophenol\textsuperscript{58} have been prepared previously (by Mannich reactions on 2(and 4)-nitrophenol, followed by catalytic reduction with hydrogen over Raney nickel as described in Section II-2.1). The compounds (III.23) condensed readily with 4-chloro-2(and 8)-trifluoromethylquinoline (III.21 and III.11), at pH 3-3.5 in aqueous ethanol at reflux and gave the corresponding Mannich base derivatives of 4-[2(and 8)-trifluoromethylquinolin-4-ylamino]phenols (III.24) (as shown in Scheme III-3).

The Mannich base derivatives of 2-[2(and 8)-trifluoromethylquinolin-4-ylamino]phenols (III.25) were prepared similarly from Mannich base derivatives of 2-aminophenols (II.5) and 4-chloro-2(and 8)-trifluoromethylquinoline (III.21 and III.11).
Scheme III-3

III.11  X=CF₃, Y=H

\[ X = \text{CF}_3, \ Y = \text{H} \]

III.21  X=H, Y=CF₃

\[ X = \text{H}, \ Y = \text{CF}_3 \]

III.23  EtOH/H₂O⁺

reflux

EtOH/H₂O⁺

reflux

III.24

<table>
<thead>
<tr>
<th>X</th>
<th>Y</th>
<th>Z</th>
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<td>H</td>
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<tr>
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<td>H</td>
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<td>NEt₂</td>
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<td>H</td>
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<td>N(CH₂)₄</td>
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<tr>
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<td>H</td>
<td>CH₂N(CH₂)₅</td>
<td>N(CH₂)₅</td>
</tr>
<tr>
<td>CF₃</td>
<td>H</td>
<td>H</td>
<td>NEt₂</td>
</tr>
<tr>
<td>CF₃</td>
<td>H</td>
<td>H</td>
<td>N(CH₂)₄</td>
</tr>
<tr>
<td>CF₃</td>
<td>H</td>
<td>H</td>
<td>N(CH₂)₅</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>X</th>
<th>Y</th>
<th>Z</th>
<th>NR₂</th>
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<tr>
<td>H</td>
<td>CF₃</td>
<td>CH₂NMe₂</td>
<td>NMe₂</td>
</tr>
<tr>
<td>H</td>
<td>CF₃</td>
<td>CH₂NEt₂</td>
<td>NEt₂</td>
</tr>
<tr>
<td>H</td>
<td>CF₃</td>
<td>CH₂N(CH₂)₄</td>
<td>N(CH₂)₄</td>
</tr>
<tr>
<td>H</td>
<td>CF₃</td>
<td>CH₂N(CH₂)₅</td>
<td>N(CH₂)₅</td>
</tr>
<tr>
<td>H</td>
<td>CF₃</td>
<td>H</td>
<td>NEt₂</td>
</tr>
<tr>
<td>H</td>
<td>CF₃</td>
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</tr>
<tr>
<td>H</td>
<td>CF₃</td>
<td>H</td>
<td>N(CH₂)₅</td>
</tr>
</tbody>
</table>
### III-3 Physical Properties

#### III-3.1 Nuclear Magnetic Resonance Spectra

(a) **1H Nuclear Magnetic Resonance Spectra**

The **1H** n.m.r. spectra of 8-trifluoromethylquinolin-4-ols (III.8-III.10), 4-chloro-8-trifluoromethylquinoline (III.11) and 2( and 4)-(8-trifluoromethylquinolin-4-yl-amino)phenols (III.24a and III.25a) are listed in Table III-1. All spectra are reported in CDCl3 solution, except for compounds (III.9) and (III.10) which were in CD3SOCD3.

The spectral data of compounds (III.8 - III.11) are consistent with their structures. Compound (III.8) shows two signals due to the ethyl group; a triplet at δ 1.47 with J 7.5 Hz due to CH3 and a quartet at δ 4.52, J 7.5 Hz due to CH2, whereas in the acid (III.9) this signal is absent. Although a direct comparison of the spectrum of compound (III.8; in CDCl3) with compound (III.9; in CD3SOCD3) was not possible, small differences in spectra of the carboxylate and corresponding carboxylic acid compounds were apparent; the signals in the spectrum of the ester (III.8) were
downfield of those due to the acid (III.9). This is consistent with the $^1$H n.m.r. data published$^{131}$ for 6-methylyridin-2-carboxylic acid and its butyl ester in deuterated dimethyl sulfoxide.

An examination of the $^1$H n.m.r. spectra of 4-chloro-8-trifluoromethylquinoline (III.11; in CDCl$_3$) with that of the 4-hydroxy analogue (III.10; in CD$_3$SOCD$_3$), given in Table III-1, revealed that all protons of compound (III.11) are downfield of those in compound (III.10); with the effect being most pronounced at H 3. This is consistent with the strong inductive electron-withdrawal of the chloro substituent (relative to the hydroxy group).

Comparison of the $^1$H n.m.r. chemical shifts of the quinoline nucleus in compounds (III.24a) and (III.25a) with those of 4-chloro-7-trifluoromethylquinoline (III.11) (Table III-1) revealed that the protons of compounds (III.24a) and (III.25a) are further upfield of those in compound (III.11). The remaining spectral features for compound (III.25a) are consistent with the assignments made in Section II-3.
Table III-1. $^1$H n.m.r. spectral data ($\delta$)$^{a}$ for some 8-trifluoromethyl-quinolines.

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>X</th>
<th>Y</th>
<th>Solvent</th>
<th>H2</th>
<th>H3</th>
<th>H5$^b$</th>
<th>H6$^c$</th>
<th>H7$^b$</th>
<th>Other</th>
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<tr>
<td>III.8</td>
<td>OH</td>
<td>CO$_2$Et</td>
<td>CDCl$_3$</td>
<td>9.23</td>
<td>-</td>
<td>8.58</td>
<td>7.61</td>
<td>8.15</td>
<td>1.47(Me); 4.52(CH$_2$Me)</td>
</tr>
<tr>
<td>III.9</td>
<td>OH</td>
<td>CO$_2$H</td>
<td>CD$_3$SOCD$_3$</td>
<td>8.90</td>
<td>-</td>
<td>8.53</td>
<td>7.49</td>
<td>8.04</td>
<td></td>
</tr>
<tr>
<td>III.10</td>
<td>OH</td>
<td>H</td>
<td>CD$_3$SOCD$_3$</td>
<td>8.02</td>
<td>5.98</td>
<td>8.38</td>
<td>7.08</td>
<td>7.72</td>
<td></td>
</tr>
<tr>
<td>III.11</td>
<td>Cl</td>
<td>H</td>
<td>CDCl$_3$</td>
<td>8.93</td>
<td>7.59</td>
<td>8.43</td>
<td>7.68</td>
<td>8.14</td>
<td></td>
</tr>
<tr>
<td>III.24a</td>
<td>d</td>
<td>H</td>
<td>CDCl$_3$</td>
<td>8.60</td>
<td>6.70</td>
<td>8.18</td>
<td>7.45</td>
<td>7.97</td>
<td>6.99(H3,5,ArH); 3.54(2,6-(CH$_2$)$_2$); 2.31(Me)</td>
</tr>
<tr>
<td>III.25a</td>
<td>e</td>
<td>H</td>
<td>CDCl$_3$</td>
<td>8.72</td>
<td>7.16</td>
<td>8.21</td>
<td>7.47</td>
<td>8.01</td>
<td>6.75,7.29 (H5,H3,ArH); 3.32(4-CH$_2$); 3.68(6-CH$_2$); 2.23,2.34(2xMe)</td>
</tr>
</tbody>
</table>

$^a$ Reported as parts per million ($\delta$) downfield from T.M.S. as internal standard in CDCl$_3$ or CD$_3$SOCD$_3$ solutions. $^b$ Broad doublet. $^c$ Broad triplet.
(b) $^{13}$C Nuclear Magnetic Resonance Spectra

The $^{13}$C n.m.r. spectral assignments for 4,6-bis(pyrrolidin-1'-ylmethyl)-2-(7''-trifluoromethylquinolin-4''-ylamino)phenol (III.25c) are shown in Figure III-1 and Table III-1a.

The assignments of the $^{13}$C n.m.r. chemical shifts were carried out by running decoupled $^{13}$C n.m.r., 2D-HETCOR and LRHETCOR spectra.

The aromatic carbon atoms (C2', 3', 5', 6', 7', 3, 5); and methylene carbons were assigned using the HETCOR spectrum. The other carbon atoms, such as quarternary carbon atoms (not connected with any protons) were assigned from the LRHETCOR spectrum.

In the proton decoupled $^{13}$C-spectrum, the carbon atom of the trifluoromethyl group appeared as a quartet with a coupling constant ca. 30 Hz, due to C-F coupling. In contrast with the $^1$H n.m.r. spectrum of compound (III.25c), the signal in the $^{13}$C spectrum due to the carbon of the 4-CH$_2$ group (attached to the phenol) was down field of that due to the 6-CH$_2$ group.

![Chemical Structure of III.25c]

**III.25c**

**Figure III-1** The chemical shifts (ppm) of the carbon atoms in 4,6-bis(pyrrolidin-1'-ylmethyl)-2-(8''-trifluoromethylquinolin-4''-ylamino)phenol (III.25c)
Table III-1a  The $^{13}$C n.m.r. spectral data for compound (III.25c): chemical shifts (ppm) of the carbon atoms and carbon-hydrogen connectivities in HETCOR and LRHETCOR spectra.

<table>
<thead>
<tr>
<th>Chemical shifts (ppm)</th>
<th>C-H connectivities</th>
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<tr>
<td></td>
<td>HETCOR</td>
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<tr>
<td>C1</td>
<td>148.7</td>
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<tr>
<td>C2</td>
<td>129.4</td>
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<td>C4</td>
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<tr>
<td>C5</td>
<td>123.5</td>
</tr>
<tr>
<td>C6</td>
<td>120.1</td>
</tr>
<tr>
<td>4-CH$_2$</td>
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</tr>
<tr>
<td>6-CH$_2$</td>
<td>58.6</td>
</tr>
<tr>
<td>C2',5'</td>
<td>53.4 - 54.1</td>
</tr>
<tr>
<td>C3',4'</td>
<td>23.4 - 23.6</td>
</tr>
<tr>
<td>C2&quot;</td>
<td>151.7</td>
</tr>
<tr>
<td>C3&quot;</td>
<td>102.9</td>
</tr>
<tr>
<td>C4&quot;</td>
<td>147.4</td>
</tr>
<tr>
<td>C4&quot;a</td>
<td>120.9</td>
</tr>
<tr>
<td>C5&quot;</td>
<td>124.6</td>
</tr>
<tr>
<td>C6&quot;</td>
<td>123.4</td>
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<tr>
<td>C7&quot;</td>
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<td>146.0</td>
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<tr>
<td>CF$_3$</td>
<td>126.8</td>
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III-3.2 Mass Spectra

The mass spectra of di-Mannich bases (III.24d) and (III.25a), from 4-(2-trifluoromethylquinolin-4-ylamino)phenol and 2-(8-trifluoromethylquinolin-4-ylamino)phenol, respectively, were examined. Each spectrum showed a peak due to the molecular ion: for compound (III.24d) at m/z 498 (together with M+1 at 499) and for compound (III.25a) at m/z 418 (together with M+1 at 419). Initial cleavages appear to involve the N-C benzylic bonds of one or both Mannich side chains; compound (III.24d) through loss of piperidinyl groups (mass 84) gave fragments appearing at 414 (and 330) and compound (III.25a) through loss of dimethylamino groups (mass 44) gave fragments appearing at 374 (and 330).

III-4 Antimalarial Activity

The compounds prepared in this study were tested for antimalarial activity against *P. falciparum* (FC-27 isolate) in the relatively inexpensive *in vitro* 'visual test'\( ^{104,112} \) and the results checked in the microscopic test\(^ {105} \) ('microtest' or 'morphological test') as described in Section II-4.1.

III-4.1 Results of the *in vitro* Testing

The results obtained in the visual test are recorded in Table III-2 as IC\(_{50}\) and IC\(_{90}\) values for those compounds with IC\(_{50}\) values < 200 nM. [Compounds (III.24) and (III.25) described in this work, which do not appear in Table III-2, had IC\(_{50}\) values > 200 nM]. Generally the results from the microscopic test paralleled those for the visual test and indicate the suitability of the latter for preliminary antimalarial screening. The results for the microscopic test are not recorded in Table III-2 except some minor variations (from the visual test) which are recorded in footnotes.
Table III-2  Results for antimalarial testing of compounds (III.24) and (III.25) in *in vitro* visual test against the FC-27 isolate of *P. falciparum*

![Chemical structures](image)

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Mannich Substituent(s)</th>
<th>IC$_{50}^A$</th>
<th>IC$_{90}^B$</th>
<th>CH-factor$^C$</th>
</tr>
</thead>
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<tr>
<td>III.24c</td>
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<td>100-200</td>
<td>D</td>
<td>0.2</td>
</tr>
<tr>
<td>d</td>
<td>CH$_2$NR$_2$ = CH$_2$N(CH$_2$)$_5$</td>
<td>D$^E$</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>III.25a</td>
<td>R'='R''= CH$_2$NMe$_2$</td>
<td>100-200</td>
<td>D</td>
<td>0.2</td>
</tr>
<tr>
<td>c</td>
<td>= CH$_2$N(CH$_2$)$_4$</td>
<td>50-100</td>
<td>200</td>
<td>0.4</td>
</tr>
<tr>
<td>d</td>
<td>= CH$_2$N(CH$_2$)$_5$</td>
<td>50-100</td>
<td>100</td>
<td>0.4</td>
</tr>
<tr>
<td>f</td>
<td>R' = CH$_2$N(CH$_2$)$_5$; R''=H</td>
<td>D$^F$</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>i</td>
<td>R'='R''= CH$_2$N(CH$_2$)$_4$</td>
<td>50-100</td>
<td>100</td>
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<td>j</td>
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<td>50-100</td>
<td>100</td>
<td>0.4</td>
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<td>Chloroquine$^G$</td>
<td></td>
<td>20-40</td>
<td>40</td>
<td>1</td>
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</table>

$^A$ The IC$_{50}$ values (the concentration of the inhibitor required to reduce parasite growth by 50%) are expressed as nmol l$^{-1}$ (nM).

$^B$ The IC$_{90}$ value (the concentration of the inhibitor required to reduce parasite growth by 90%) are expressed as nmol l$^{-1}$ (nM).

$^C$ The C-H factor (chloroquine factor) is the comparative activity of the inhibitor under test compared to chloroquine; and is the ratio of the IC$_{50}$ value for chloroquine over that for the inhibitor.

$^D$ No significant activity at 200 nM.

$^E$ The microscopic test gave IC$_{50}$ 50-100 nM.

$^F$ The microscopic test gave IC$_{50}$ 100-200 nM.

$^G$ As diphosphate salt.
III-4.2 Discussion of Results

The results in Table III-2 for some of the comparable mono- and di-Mannich base derivatives (III.25a-f) and (III.25g-l) of 2-[8(and 2)-trifluoromethylquinolin-4-ylamino]phenol, reveal that more of the 8-trifluoromethyl compounds [namely (III.25a,c,d,f)] had IC\textsubscript{50} values <200 nM than the 2-trifluoromethyl isomers (III.25i,j). Likewise, comparison of the mono- and di-Mannich base derivatives of 4-[8(and 2)-trifluoromethylquinolin-4-ylamino]phenol (III.24) revealed that only two compounds, the 8-trifluoromethyl compounds (III.24c,d), had IC\textsubscript{50} values <200 nM. These observations support the views that the 8-trifluoromethyl compounds have higher \textit{in vitro} antimalarial activity than the 2-trifluoromethyl isomers, and that the Mannich base derivatives of 2-[8(and 2)-trifluoromethylquinolin-4-ylamino]phenol (III.25) have generally higher activity than analogous 4-[8(and 2)-trifluoromethylquinolin-4-ylamino]phenols (III.24). Also the di-Mannich base derivatives showed higher activity (i.e. lower IC\textsubscript{50} values) than the corresponding mono-Mannich derivatives. For example, the following orders of activity were observed: (III.25d) > (III.25f), (III.25j) > (III.25l), (III.24c) > (III.24f) and (III.24d) > (III.24g).

Generally in these series of compounds, superior activity was observed in Mannich bases derived from the amines pyrrolidine and piperidine.

The compounds described in this chapter were not further tested for \textit{in vivo} antimalarial activity because they did not meet our selection criteria of IC\textsubscript{50} < 50 nM in the \textit{in vitro} tests.

The results of our studies on this series of compounds revealed that compounds (III.24) and (III.25), here reported, were significantly less active than the analogous 2(or 4)-(7-trifluoromethylquinolin-3-ylamino)phenols,\textsuperscript{56-58} which had IC\textsubscript{50} values generally < 5 nM. Thus the order of activity among such 2-,7- or 8-trifluoromethylquinoline derivatives was 7 >> 8 > 2. Moreover, the present results do not parallel the observations that Mannich base derivatives of 4-[2,8-bis(trifluoromethyl)quinolin-4-ylamino]phenol were at least as active, or slightly more
active, than the corresponding derivatives of 4-[2,7-bis(trifluoromethyl)quinolin-4-ylamino]phenol.

Some mono-Mannich base derivatives of 2-aminophenols with different substituents at C-4 are investigated in the next chapter.
The general procedure and experimental details for the antimalarial testing are recorded in Chapter II-5.1 and 5.3.

### 4-Chloro-2-trifluoromethylquinoline (III.21)

This compound was prepared\textsuperscript{129} from aniline and ethyl 4,4,4-trifluorooctoacetate through ethyl 3-anilino-3-trifluoromethylacrylate\textsuperscript{129} and 2-trifluoromethylquinolin-4-ol\textsuperscript{129} which with a mixture of phosphorus pentachloride and phosphoryl chloride at 120\textdegree C afforded 4-chloro-2-trifluoromethylquinoline,\textsuperscript{129} m.p. 40-42\textdegree C (lit.,\textsuperscript{129} 38-40\textdegree C).

### 4-Chloro-8-trifluoromethylquinoline (III.11)

This compound was prepared from \(o\)-trifluoromethylaniline and diethyl ethoxymethylene malonate through diethyl \(o\)-trifluoromethylanilinomethylene malonate,\textsuperscript{123} \[^1\text{H n.m.r.} \delta 1.34, t, 1.38, t, J 7.5\text{Hz}, 2 \times \text{Me}; 4.27, q, 4.36, q, J 7.5\text{Hz}, \text{CH}_2\text{Me}; 7.17-7.72, \text{complex}, \text{H 3',4',5',6'}; 8.40, s, 8.54, s, \text{CH}_2=\text{N}], \text{ethyl 4-hydroxy-8-trifluoromethylquinoline-3-carboxylate}^\text{123} \[^1\text{H n.m.r.} \delta 1.47, t, J 7.5\text{Hz}, \text{Me}; 4.52, q, J 7.5\text{Hz}, \text{CH}_2\text{Me}; 7.61, \text{br t, J 8Hz, H 6}; 8.15, \text{br d, J 7Hz, 8.58, br d, J 8Hz, H 5,7}; 9.23, s, H 2], 4-hydroxy-8-trifluoromethylquinoline-3-carboxylic acid\textsuperscript{123} \[^1\text{H n.m.r.} (\text{CD}_3\text{SOCD}_3) \delta 7.49, \text{br t, J 8Hz, H6}; 8.04, \text{br d, J 7Hz, 8.53, br d, J 8Hz, H5,7}; 8.90, s, H 2], \text{and 8-trifluoromethylquinolin-4-ol}^\text{123} \[^1\text{H n.m.r.} (\text{CD}_3\text{SOCD}_3) \delta 5.98, d, J 5.5\text{Hz, H 3}; 7.08, \text{br t, J 8Hz, H 6}; 7.72. \text{br d, J 7Hz, 8.38, br d, J 9Hz, H 5,7}; 8.02, d, J 5.5\text{Hz, H 2}].\text{4-Chloro-8-trifluoromethylquinoline had m.p. 77-79\textdegree C (lit.}^{123} 78\textdegree C) \[^1\text{H n.m.r.} \delta 7.59, d, J 5.5\text{Hz, H 3}; 7.68, \text{br t, J 8Hz, H 6}; 8.14, \text{br d, J 7Hz, 8.43, br d, J 9Hz H5,7}; 8.93, d, J 5.5\text{Hz, H 2}].
4-Nitro-2-(pyrrolidin-1'-ylmethyl)phenol

Pyrrolidine (2.04 g; 28.75 mmol) was added to a chilled mixture of 4-nitrophenol (4.0 g; 28.75 mmol), paraformaldehyde (0.86 g, 28.75 mmol) and ethanol (10.0 ml) and the mixture refluxed at 100° for 2 h. The solvent was evaporated under reduced pressure and the oily residue was subjected to column chromatography (silica gel; methanol). It gave the title compound as a yellow solid (1.55 g), m.p. 124-126° (Found: C, 59.4; H, 6.5; N, 12.5. C_{11}H_{14}N_{2}O_{3} requires C, 59.4; H, 6.4; N, 12.6%). \(^1\)H n.m.r. \(\delta\) 1.91, m, H 3',4'; 2.72, m, H 2',5'; 3.93, s, CH₂; 6.83, d, J 8 Hz, H 6; 7.94, d, J 2 Hz, H 3; 8.09, J 5, 6 8 Hz, J 3, 5 2 Hz, H 5.

Mannich Base Derivatives of 2-[2(and 8)-Trifluoromethylquinolin-4-ylamino]phenols

4,6-Bis(piperidin-1'-ylmethyl)-2-(2''-trifluoromethylquinolin-4''-ylamino)phenol (III.25j)

A mixture of 2-amino-4,6-bis(piperidin-1'-ylmethyl)-phenol\(^ {58}\) (0.168 g; 0.55 mmol), 4-chloro-2-trifluoromethylquinoline (0.128 g, 0.55 mmol), ethanol (8 ml) and water (2 ml) was adjusted with hydrochloric acid to pH 2.5-3.0 and then refluxed in an oil bath at 100° for 38 h. The solvent was evaporated under reduced pressure and the residue diluted with water and adjusted with ammonium hydroxide to pH 9-10. The mixture was extracted into chloroform, extract dried (Na₂SO₄) and solvent evaporated and the oily product subjected to t.l.c. (alumina; 1% methanol in chloroform). It gave the title compound (0.080 g) as an oil (Found: C, 67.7; H, 6.6; N, 11.0. C₂₈H₃₃F₃N₄O requires C, 67.4; H, 6.7; N, 11.2%). \(^1\)H n.m.r. \(\delta\) 1.61, m, H 3',4',5'; 2.43, m, H 2',6'; 3.41, s, 4-CH₂; 3.74, s, 6-CH₂, 6.79, s, H 5; 7.22-8.21, m, H 3, 3'', 5'', 6'', 7'', 8''.
6-(Diethylaminomethyl)-2-(2'-trifluoromethylquinolin-4'-yl-amino)phenol (III.25k)

A mixture of 2-diethylaminomethyl-6-nitrophenol\textsuperscript{132} (0.61g; 2.78mmol) [\textsuperscript{1}H n.m.r. $\delta$ 1.17, t, J 7.5 Hz, Me; 2.77, q, J 7.5 Hz, CH\textsubscript{2}Me; 3.91, s, 2-CH\textsubscript{2}; 6.72, t, J 8Hz, H 4; 7.24, d, J 8 Hz, H 3; 7.87, d, J 8Hz, H 5], ethanol (30 ml), ethanolic ammonia (25 ml) and Raney nickel was shaken with hydrogen until uptake ceased. The catalyst was filtered on celite and the solvent was evaporated to leave the amine as an oil (0.46 g). [\textsuperscript{1}H n.m.r. $\delta$ 1.10, t, J 7.5 Hz, Me; 2.61, q, J 7.5 Hz, CH\textsubscript{2}Me; 3.71, s, 2-CH\textsubscript{2}; 6.33-6.63, m, H 3,4,5].

A mixture of the above amine (0.24 g, 1.23 mmol), 4-chloro-2-trifluoromethylquinoline (0.28 g, 1.23 mmol) ethanol (7 ml) and water (2 ml) was adjusted with hydrochloric acid to pH 3.5 and refluxed in an oil bath at 100° for 24 h. After evaporation of the solvent, adjustment to pH 9-10 and extraction with chloroform, the product was subjected to t.l.c. (alumina; hexane/ethyl acetate, 3:1 then alumina; chloroform). The title compound (0.10 g) was obtained as an oil (Found: C, 64.5; H, 5.8; N, 10.5. C\textsubscript{21}H\textsubscript{22}F\textsubscript{3}N\textsubscript{3}O requires C, 64.8; H, 5.7; N, 10.8%). $\textsuperscript{1}$H n.m.r. $\delta$ 1.14, t, J 7.5Hz, Me; 2.68, q, J 7.5Hz, CH\textsubscript{2}Me; 3.85, s, 6-CH\textsubscript{2}; 6.81-8.20, complex, H 3, 4, 5, 3', 5', 6', 7', 8'; 9.47, br, OH.

In a similar manner the following compounds were prepared.

4,6-Bis(dimethylaminomethyl)-2-(2'-trifluoromethylquinolin-4'-ylamino)phenol (III.25g)

Compound (III.25g) (43%) was obtained as an oil after t.l.c. (alumina; 1% methanol in chloroform) (Found: C, 63.0; H, 5.9; N, 13.1. C\textsubscript{22}H\textsubscript{25}F\textsubscript{3}N\textsubscript{4}O requires C, 63.1; H, 6.0; N, 13.4%). $\textsuperscript{1}$H n.m.r. $\delta$ 2.27, s, Me; 2.37, s, Me; 3.36, s, 4-CH\textsubscript{2}; 3.71, s, 6-CH\textsubscript{2}; 6.81, d, J 2Hz, H 5; 7.20-8.20, complex, H 3, 3', 5', 6', 7', 8'; 8.65, br, OH.
4,6-Bis(diethylaminomethyl)-2-(2'-trifluoromethylquinolin-4'-ylamino)phenol (III.25h)

This *title compound* (29%) was obtained as an oil after t.l.c. (alumina; 1% methanol in chloroform) (Found: C, 65.8; H, 7.0; C_{26}H_{33}F_{3}N_{4}O requires C, 65.8; H, 6.8%). $^1$H n.m.r. δ 1.10, m, Me; 2.63, m, CH$_2$Me; 3.52, s, 4-CH$_2$; 3.84, s, 6-CH$_2$; 6.82, br s, H 5; 7.21-8.20, complex, H 3, 3', 5', 6', 7', 8'; 8.97, br, OH (?).

4,6-Bis(pyrrrolidin-1'-ylmethyl)-2-(2'-trifluoromethylquinolin-4'-ylamino)phenol (III.25i)

Compound (III.25i) (30%) was obtained as an oil after t.l.c. (alumina; 1% methanol in chloroform) (Found: C, 66.4; H, 6.2; N, 11.7. C_{26}H_{29}F_{3}N_{4}O requires C, 66.4; H, 6.2; N, 11.9%). $^1$H n.m.r. δ 1.84, m, H 3',4'; 2.63, m, H 2',5'; 3.56, s, 4-CH$_2$; 3.90, s, 6-CH$_2$; 6.86, br s, H 5; 7.21-8.21, complex, H 3, 3'', 5'', 6'', 7'', 8''; 9.22, br, OH.

6-(Piperidin-1'-ylmethyl)-2-(2''-trifluoromethylquinolin-4''-ylamino)phenol (III.25r)

A mixture of 2-nitro-6-(piperidin-1'-ylmethyl)phenol$^{132}$ (0.59 g) [$^1$H n.m.r. δ 1.60, m, H 3',4',5'; 2.61, m, H 2',6'; 3.80, s, 6-CH$_2$; 6.76, m, J 8 Hz, H 4; 7.24, d, J 8 Hz, H 5; 7.86, d, J 9 Hz, H 3], ethanol (25 ml) and ethanolic ammonia (30 ml) was shaken with hydrogen over Raney nickel as for the diethylaminomethyl analogue above and gave the corresponding amine [$^1$H n.m.r. δ 1.55, m, H 3',4',5'; 2.50, m, H 2',6'; 3.62, s, 6-CH$_2$; 6.33-6.50, m, H 4,5; 6.62, d, J 6Hz, H 3].

This amine and 4-chloro-2-trifluoromethylquinoline as described above gave the *title compound* (52%) as an oil after t.l.c. (alumina; chloroform) (Found: C, 66.0; H, 5.8; N, 10.5. C$_{22}$H$_{22}$F$_{3}$N$_{3}$O requires C, 65.8; H, 5.5; N, 10.5%). $^1$H n.m.r. δ 1.57, m, H3',4',5'; 2.56, m, H 2',6'; 3.74, s, 6-CH$_2$; 6.76-6.95, m, H 4,5; 7.29-8.18, complex, H 3, 3'', 5'', 6'', 7'', 8''.
4.6-Bis(dimethylaminomethyl)-2-(8'-trifluoromethylquinolin-4'-ylamino)phenol (III.25a)

A mixture of 2-amino-4,6-bis(dimethylaminomethyl)phenol58 (0.266 g, 1.19 mmol), 4-chloro-8-trifluoromethylquinoline (0.276 g, 1.19 mmol), ethanol (7 ml) and water (2 ml) was adjusted with concentrated hydrochloric acid to pH 3.5-4.0 and then refluxed in an oil bath at 100° for 30 h. The solvent was evaporated under reduced pressure and the residue diluted with water and adjusted with ammonium hydroxide to pH 9. The precipitate was extracted into chloroform and purified by t.l.c. (alumina; 1% methanol in chloroform) to give the title compound (0.315 g; 63%) m.p. 171-173°. (Found: C, 63.1; H, 5.9; N, 13.2. C22H25F3N4O requires C, 63.1; H, 6.0; N, 13.4%). 1H n.m.r. δ 2.23, s, 2.34, s, Me; 3.32, s, 4-CH2; 3.68, s, 6-CH2; 6.75, d, J3,5 1.5 Hz, H 5; 7.10, br, NH; 7.16, d, J 6 Hz, H 3'; 7.29, d, J 2 Hz, H 3; 7.47, t, J 8 Hz, H 6; 8.01, d, J 7 Hz, 8.21, d, J 8 Hz, H 5', 7', 8.72, J 6 Hz, H 2'; 9.71, br, OH. MS m/z 419 (M + 1) (5.5%), 418 (M) (20.3%), 374 (4.3%), 330 (65.1%), 58 [(CH2N(CH3)2] (100%).

The following compounds were prepared by similar procedures.

4.6-Bis(diethylaminomethyl)-2-(8'-trifluoromethylquinolin-4'-ylamino)phenol (III.25b)

Compound (III.25b) (51%) was obtained as a yellow solid m.p. 154-156° after t.l.c. (alumina; chloroform) (Found: C, 65.9; H, 6.9; N, 11.5. C26H33F3N4O requires C, 65.8; H, 7.0; N, 11.8%). 1H n.m.r. δ 1.10, m, J 7.5 Hz, Me; 2.61, m, J 7.5 Hz, CH2Me; 3.50, s, 4-CH2; 3.83, s, 6-CH2; 6.79, br s, H 5, 7.10, br s, NH; 7.19, d, J 6 Hz, H 3'; 7.36, d, J 2 Hz, H 3; 7.50, t, J 9 Hz, H 6', 8.04, d, 8.25, d, J 8 Hz, H 5',7'; 8.74, d, J 6 Hz, H 2'; 10.32, br, OH.

4.6-Bis(pyrrolidin-1'-ylmethyl)-2-(8''-trifluoromethylquinolin-4''-ylamino)phenol (III.25c)

This compound (52%) as a white solid, m.p. 180-182° after t.l.c. (alumina; 1% methanol in chloroform) and recrystallisation from a mixture of cyclohexane and ethyl
acetate (Found: C, 66.5; H, 6.3; N, 11.7. C_{26}H_{29}F_{3}N_{4}O requires C, 66.4; H, 6.2; N, 11.9%). \textsuperscript{1}H n.m.r. δ 1.84, m, H 3',4'; 2.63, m, H 2',5'; 3.55, s, 4-CH\textsubscript{2}; 3.89, s, 6-CH\textsubscript{2}; 6.82, br s, H 5, 7.08, br s, NH; 7.19, d, J 5.5 Hz, H 3''; 7.34, br s, H 3; 7.52, t, J 7 Hz, H 6'', 7.82, br s, OH; 8.04, d, J 8 Hz, 8.24, d, J 9 Hz, H 5'',7''; 8.74, d, J 5.5 Hz, H 2''.

4,6-Bis(piperidin-1'-ylmethyl)-2-(8''-trifluoromethylquinolin-4'-ylamino)phenol (III.25d)

Compound (III.25d) (54%) was obtained as pale yellow crystals, m.p. 179-181° after t.l.c. (alumina; chloroform) and recrystallisation from a mixture of cyclohexane and ethyl acetate (Found: C, 67.2; H, 6.8; N, 11.0. C_{28}H_{33}F_{3}N_{4}O requires C, 67.4; H, 6.7; N, 11.2%). \textsuperscript{1}H n.m.r. δ 1.59, m, H 3',4',5'; 2.42, m, H 2',6'; 3.41, s, 4-CH\textsubscript{2}; 3.72, s, 6-CH\textsubscript{2}; 6.75, br s, H 5, 7.09, br s, OH; 7.20, d, J 5.5 Hz, H 3''; 7.34, d, J_{3,5} 2 Hz, H 3; 7.52, t, J 8 Hz, H 6''; 8.04, d, J 7 Hz, 8.25, d, J 9 Hz, H 5'',7''; 8.74, d, J 5.5 Hz, H 2''.

6-(Diethylaminomethyl)-2-(8'-trifluoromethylquinolin-4'-ylamino)phenol (III.25e) (43%) m.p. 149-150°, was obtained after t.l.c. (alumina; hexane/ethyl acetate, 3:1) and recrystallisation from a mixture of hexane and ethyl acetate (Found: C, 64.5; H, 5.8; N, 10.7. C_{21}H_{22}F_{3}N_{3}O requires C, 64.8; H, 5.7; N, 10.8%). \textsuperscript{1}H n.m.r. δ 1.14, t, J 7.5 Hz, Me; 2.67, q, J 7.5 Hz, CH\textsubscript{2}Me; 3.84, s, 6-CH\textsubscript{2}; 6.77-7.40, complex, H 3, 4, 5, 3'; 7.51, t, J 8 Hz, H 6'; 8.03, d, J 7 Hz, 8.24, d, J 9 Hz, H 5',7'; 8.74, d, J 5.5 Hz, H 2'.

6-(Piperidin-1'-ylmethyl)-2-(8''-trifluoromethylquinolin-4'-ylamino)phenol (III.25f)

The title compound (65%), m.p. 178-180°, was obtained after t.l.c. (alumina; chloroform) and recrystallisation from a mixture of hexane and ethyl acetate (Found: C, 66.0; H, 5.6; N, 10.4. C_{22}H_{22}F_{3}N_{3}O requires C, 65.8; H, 5.5; N, 10.5%).
\[ ^1H \text{ n.m.r.} \delta 1.57, m, H 3',4',5'; 2.58, m, H 2',6'; 3.71 s, 6-CH_2; 6.76-7.39, \text{complex}, H 3, 4, 5, 3''; 7.49, t, J 8 \text{ Hz}, H 6''; 8.03, d, J 8 \text{ Hz}, 8.24, d, J 9 \text{ Hz}, H 5'', 7''; 8.73, d, J 5.5 \text{ Hz}, H 2'', 10.35, \text{br}, \text{NH ?}. \]

**Mannich Base Derivatives of 4-[2(and 8)-Trifluoromethylquinolin-4-ylamino]phenols**

**2-(Pyrrolidin-1'-ylmethyl)-4-(8'-trifluoromethylquinolin-4'-ylamino)phenol (III.24f)**

A mixture of 4-nitro-2-(pyrrolidin-1'-ylmethyl)phenol (0.55 g), ethanol (30 ml), ethanolic ammonia (30 ml) and Raney nickel was shaken with hydrogen until uptake ceased. The catalyst was filtered on celite and the solvent evaporated to give the amine \[ ^1H \text{ n.m.r.} \delta 1.83, m, H 3',4'; 2.62, m, H 2',5'; 3.72, s, 2-CH_2; 4.94, \text{br}, \text{OH (?)}; 6.41-6.71, m, H 3,5,6].

A mixture of this amine (0.173 g, 0.89 mmol), 4-chloro-8-trifluoromethylquinoline (0.21 g, 0.89 mmol), ethanol (1 ml) and water (2 ml) was adjusted with concentrated hydrochloric acid to pH 3.2 and then refluxed for 41 h. The solvent was evaporated and the residue diluted with water and adjusted to pH 9-10. The product was extracted into chloroform, subjected to t.l.c. (alumina; 1% methanol in chloroform) and recrystallised from a mixture of hexane and ethyl acetate and gave the title compound (0.19 g), m.p. 210-212° (Found: C, 65.1; H, 5.0; N, 10.6. C_{21}H_{20}F_{3}N_{3}O requires C, 65.1; H, 5.2; N, 10.8%). \[ ^1H \text{ n.m.r.} \delta 1.87, m, H 3',4'; 2.68, m, H 2',5'; 3.84 s, 2-CH_2; 6.61, \text{br}, \text{OH (?)}; 6.72, d, J 5.5 \text{ Hz}, H 3''; 6.83-7.17, \text{complex}, H 3, 5, 6; 7.51, t, J 8 \text{ Hz}, H 6''; 8.08, m, H 5'', 7''; 8.63, d, J 5.5 \text{ Hz}, H 2''.

The following compounds were prepared in a similar manner.

**2,6-Bis(dimethylaminomethyl)-4-(2'-trifluoromethylquinolin-4'-ylamino)phenol (III.24h)** (46%), m.p. 163-165° was obtained after t.l.c. (alumina; 2% methanol in chloroform) and recrystallisation from a mixture of hexane
and ethyl acetate (Found: C, 63.0; H, 6.2; N, 13.1. C_{22}H_{25}F_{3}N_{4}O requires C, 63.1; H, 6.0; N, 13.4%). \(^1\)H n.m.r. δ 2.31, s, Me; 3.57 s, 2,6-(CH\(_2\))\(_2\); 7.02, s, H 3,5; 6.94-8.17, complex, H 3', 5', 6', 7', 8'.

**2,6-Bis(diethylaminomethyl)-4-(2'-trifluoromethylquinolin-4'-ylamino)phenol (III.24i)**

This compound (51%) was obtained as an oil after t.l.c. (alumina; 1% methanol in chloroform) (Found: C, 65.5; H, 7.1; N, 11.6. C\(_{26}\)H\(_{33}\)F\(_3\)N\(_4\)O requires C, 65.8; H, 7.0; N, 11.8%). \(^1\)H n.m.r. δ 1.10, t, J 7.5 Hz, Me; 2.63 q, J 7.5 Hz, CH\(_2\)Me; 3.72, s, 2,6-(CH\(_2\))\(_2\); 6.87, br, NH (?); 7.08, s, H 3,5; 6.98-8.18, complex, H 3', 5', 6', 7', 8'; 9.29, br, OH (?).

**2,6-Bis(pyrrolidin-1'-ylmethyl)-4-(2''-trifluoromethylquinolin-4''-ylamino)phenol (III.24j)**

Compound (III.24j) (25%), m.p. 154-156°, was obtained after t.l.c. (alumina; 1% methanol in chloroform) and recrystallisation from a mixture of hexane and ethyl acetate. (Found: C, 66.4; H, 6.0; N, 11.7. C\(_{26}\)H\(_{29}\)F\(_3\)N\(_4\)O requires C, 66.4; H, 6.2; N, 11.9%). \(^1\)H n.m.r. δ 1.82, m, H 3',4'; 2.63, m, H 2',5'; 3.77, s, 2,6-(CH\(_2\))\(_2\); 6.88, br, NH (?); 7.05, s, H 3,5; 6.97-8.16, complex, H 3", 5", 6", 7", 8"; 8.63, br, OH (?).

**2,6-Bis(piperidin-1'-ylmethyl)-4-(2''-trifluoromethylquinolin-4''-ylamino)phenol (III.24k)**

This title compound (40%) had m.p. 208-209° after t.l.c. (alumina; chloroform) and recrystallisation from a mixture of hexane and ethyl acetate. (Found: C, 67.4; H, 7.0; N, 11.2. C\(_{28}\)H\(_{33}\)F\(_3\)N\(_4\)O requires C, 67.4; H, 6.7; N, 11.2%). \(^1\)H n.m.r. δ 1.57, m, H 3',4',5'; 2.53, m, H 2',6'; 3.63, s, 2,6-(CH\(_2\))\(_2\); 6.90, br, NH (?); 7.05, s, H 3,5; 6.98-8.18, complex, H 3", 5", 6", 7", 8".
2-Diethylamino-4-(2'-trifluoromethylquinolin-4'-ylamino)phenol (III.24l)

2-Diethylaminomethyl-4-nitrophenol[^1] [^H n.m.r. δ 1.04, t, J 7.5 Hz, Me; 2.58, q, J 7.5 Hz, CH₂Me; 3.77, s, CH₂; 6.65, d, J 9 Hz, H 6; 7.81-7.98, m, H 3,5] was reduced with hydrogen over Raney nickel as described above to the amine [^H n.m.r. δ 1.09, t, J 7.5 Hz, Me; 2.61, q, J 7.5 Hz, CH₂Me; 3.66, s, CH₂; 5.28, br, OH (?), 6.39-6.69, m, H 3,5,6]

This amine and 4-chloro-2-trifluoromethylquinoline as described above gave the title compound (44%) as an oil after t.l.c. (alumina; chloroform) (Found: C, 65.0; H, 5.7; N, 10.4. C₂₁H₂₂F₃N₃O requires C, 64.8; H, 5.7; N, 10.8%).[^1] H n.m.r. δ 1.14, t, J 7.5 Hz, Me; 2.68, q, J 7.5 Hz, CH₂Me; 3.80, s, 2-CH₂; 6.84-8.18, complex, H 3,5,6,3',5',6',7',8'; 8.97, br, OH (?).

2-(Pyrrolidin-1'-ylmethyl)-4-(2''-trifluoromethylquinolin-4''-ylamino)phenol (III.24m)

Compound (III.24m) (30%) was obtained as an oil after t.l.c. (alumina; 1% methanol in chloroform) (Found: C, 65.0; H, 5.2; N, 10.5. C₂₁H₂₂F₃N₃O requires C, 65.1; H, 5.2; N, 10.8%).[^1] H n.m.r. δ 1.87, m, H 3',4'; 2.66, m, H 2',5'; 3.83, s, 2-CH₂; 6.84-8.17, complex, H 3,5,6,3'',5'',6'',7'',8''.

2-(Piperidin-1'-ylmethyl)-4-(2''-trifluoromethylquinolin-4''-ylamino)phenol (III.24n)

A solution of 4-nitro-2-(piperidin-1'-ylmethyl)phenol[^1] (0.6g, 2.54 mmol) [^1]H n.m.r. δ 1.59, m, H 3',4',5'; 2.58, m, H 2', 6'; 3.77, s, 2-CH₂; 6.85, d, J 9 Hz, H 6; 7.90-8.12, m, H 3,5] in ethanolic ammonia was reduced with hydrogen over Raney nickel to give the amine [^H n.m.r. δ 1.53, m, H 3',4',5'; 2.50, m, H 2', 6'; 3.55, s, 2-CH₂; 5.65, br, OH (?); 6.36-6.71, m, H 3,5,6].

This amine and 4-chloro-2-trifluoromethylquinoline as described above gave the title compound (38%) as an oil after t.l.c. (alumina; 1% methanol in chloroform) (Found: C, 66.1; H, 5.6; N, 10.3. C₂₂H₂₂F₃N₃O requires C, 65.8; H, 5.5; N,
10.5\%). $^{1}$H n.m.r. $\delta$ 1.58, m, H 3',4',5'; 2.55, m, H 2', 6'; 3.67, s, 2-CH$_2$; 6.83-8.17, complex, H 3, 5, 6, 3'', 5'', 6'', 8''.

2,6-Bis(dimethylaminomethyl)-4-(8'-trifluoromethylquinolin-4'-ylamino)phenol (III.24a)

The title compound (50%) had m.p. 153-155°, after t.l.c. (alumina; 2% methanol in chloroform) and recrystallisation from a mixture of hexane and ethyl acetate (Found: C, 62.9; H, 5.8; N, 13.1. C$_{22}$H$_{25}$F$_3$N$_4$O requires C, 63.1; H, 6.0; N, 13.4%). $^{1}$H n.m.r. $\delta$ 2.31, s, Me; 3.54, s, 2, 6-(CH$_2$)$_2$; 6.70 d, J 5.5 Hz, H 3'; 6.88, br, NH (?); 6.99, s, H 3,5; 7.45, t, J 8 Hz, H 6'; 7.97-8.18, complex, H 5', 7'; 8.60, d, J 5.5 Hz, H 2'.

2,6-Bis(diethylaminomethyl)-4-(8'-trifluoromethylquinolin-4'-ylamino)phenol (III.24b)

Compound (III.24b) (53%), m.p. 163-164°, was obtained after t.l.c. (alumina; 1% methanol in chloroform) and recrystallisation from a mixture of hexane and ethyl acetate (Found: C, 65.5; H, 7.2; N, 11.6. C$_{26}$H$_{33}$F$_3$N$_4$O requires C, 65.8; H, 7.0; N, 11.8%). $^{1}$H n.m.r. $\delta$ 1.09, t, J 7.5Hz, Me; 2.63, q, J 7.5 Hz, CH$_2$Me; 3.70, s, 2, 6-(CH$_2$)$_2$; 6.75 d, J 5.5 Hz, H 3'; 6.76, br s, NH (?); 7.05, s, H 3, 5; 7.46, t, J 8 Hz, H 6'; 7.98-8.17, m, H 5',7'; 8.61, d, J 5.5 Hz, H 2'; 10.22, br, OH (?).

2,6-Bis(pyrrolidin-1'-ylmethyl)-4-(8''-trifluoromethylquinolin-4''-ylamino)phenol (III.24c)

This title compound (36%) had m.p. 190-192°, after t.l.c. (alumina; 1% methanol in chloroform) and recrystallisation from a mixture of hexane and ethyl acetate (Found: C, 66.3; H, 6.1; N, 11.9. C$_{26}$H$_{29}$F$_3$N$_4$O requires C, 66.4; H, 6.2; N, 11.9%). $^{1}$H n.m.r. $\delta$ 1.82, m, H 3',4'; 2.63, m, H 2'; 5'; 3.75, s, 2, 6-(CH$_2$)$_2$; 6.73 d, J 5.5 Hz, H 3''; 7.02, br s, H 3, 5; 7.48, t, J 8 Hz, H 6''; 7.99-8.15, m, H 5'',7''; 8.63, d, J 5.5 Hz, H 2''; 9.42, br, OH (?).
2,6-Bis(piperidin-1'-ylmethyl)-4-(8''-trifluoromethylquinolin-4''-ylamino)phenol (III.24d)

Compound (III.24d) (60%), m.p. 200-201°, was obtained after t.l.c. (alumina; chloroform) and recrystallisation from a mixture of hexane and ethyl acetate (Found: C, 67.2; H, 7.0; N, 11.1. C_{28}H_{33}F_{3}N_{4}O requires C, 67.4; H, 6.7; N, 11.2%). 1H n.m.r. δ 1.54, m, H 3', 4', 5'; 2.50, m, H 2', 6'; 3.60, s, 2, 6-(CH$_2$)$_2$; 6.73, d, J 5.5 Hz, H 3''; 6.79, br, OH (?); 7.01, s, H 3, 5; 7.45, t, J 8 Hz, H 6''; 7.97-8.16, m, H 5'', 7''; 8.60, d, J 5.5 Hz, H 2''; 8.61, br, NH (?). MS m/z 499 (M + 1) (1.7%), 498 (M) (8.0%), 414 (3.7%), 330 (12.8%), 84 (100%).

2-Diethylaminomethyl-4-(8''-trifluoromethylquinolin-4'-ylamino)phenol (III.24e)

This compound (46%) had m.p. 184-186°, after t.l.c. (alumina; chloroform) and recrystallisation from a mixture of hexane and ethyl acetate (Found: C, 64.9; H, 5.8; N, 10.5. C$_{21}$H$_{22}$F$_3$N$_3$O requires C, 64.8; H, 5.7; N, 10.8%). 1H n.m.r. δ 1.14, t, J 7.5 Hz, Me; 2.67, q, J 7.5 Hz, CH$_2$Me; 3.78, s, 2-CH$_2$; 6.71, d, J 5.5 Hz, H 3''; 6.80-7.14, m, H 3, 5, 6; 7.48, t, J 8 Hz, H 6'; 7.98-8.17, m, H 5', 7'; 8.61, d, J 5.5 Hz, H 2'; 9.85, br, OH (?).

2-(Piperidin-1'-ylmethyl)-4-(8''-trifluoromethylquinolin-4''-ylamino)phenol (III.24g)

Compound (III.24g) (61%), m.p. 199-201°, was obtained after t.l.c. (alumina; 1% methanol in chloroform) and recrystallisation from a mixture of hexane and ethyl acetate (Found: C, 65.6; H, 5.2; N, 10.2. C$_{22}$H$_{22}$F$_3$N$_3$O requires C, 65.8; H, 5.5; N, 10.5%). 1H n.m.r. δ 1.57, m, H 3', 4', 5'; 2.52, m, H 2', 6'; 3.65, s, 2-CH$_2$; 6.69, d, J 5.5 Hz, H 3''; 6.80-7.13, m, H 3, 5, 6; 7.44, t, J 8 Hz, H 6''; 7.96-8.18, m, H 5'', 7''; 8.59, d, J 5.5 Hz, H 2''.
CHAPTER IV
IV-1 Introduction

In the work reported in this chapter, a series of Mannich base derivatives of 2-[7-substituted-quinolin(and 1,5-naphthyridin)-4-ylamino]phenols were selected for examination for antimalarial activity. In particular, I have investigated the effect of (a) substitution of a chloro group at C-4, and (b) substitution of a t-butyl group at C-4 (and C-6).

The preparation of these compounds will be reported and their $^1$H n.m.r. spectral data discussed in relation to their structures; and also compared with related compounds reported in the previous chapter. Other physical properties such as n.m.r. and mass spectra will also be presented.

The results of testing for the antimalarial activity of these analogues using the in vitro method will then be reported and discussed. Finally the experimental detail of the preparation of these compounds will be given.

IV-2 Syntheses

IV-2.1 Preparation of New Mono-Mannich Base Derivatives of 4-Chloro-2-nitrophenol

These compounds were prepared by standard procedures described by Barlin and coworkers to make Mannich base derivatives of 2-nitrophenol$^{58}$ and 4-nitrophenol$^{130}$ as discussed in Chapter II-2.1. 4-Chloro-2-nitrophenol with four
equivalents of paraformaldehyde and the appropriate amines in refluxing ethanol for a period of 22-24 h gave Mannich bases (IV.1), in which the mono-Mannich side chain is inserted at position 6. These mono-Mannich compounds were readily purified by column and then preparative thin layer chromatography (PTLC) over alumina.

$$\text{IV.1, } X = \text{NO}_2$$

$$\text{IV.2, } X = \text{NH}_2$$

$$\text{IV.3}$$

$$\text{IV.4, } X = \text{Br}$$

$$\text{IV.5, } X = \text{CF}_3$$

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<td>c</td>
<td>NPr₂</td>
<td>h</td>
</tr>
<tr>
<td>d</td>
<td>N(CH₂)₄</td>
<td>i</td>
</tr>
<tr>
<td>e</td>
<td>N(CH₂)₅</td>
<td>j</td>
</tr>
</tbody>
</table>

**IV-2.2 Preparation of New Mono-Mannich Base Derivatives of 4(and 6)-t-Butyl-2-nitrophenol**

The above compounds were prepared by a similar method to that mentioned in Section IV-2.1. 4(and 6)-t-Butyl-2-nitrophenol with one equivalent of
paraformaldehyde and piperidine in refluxing ethanol for 24 h gave the mono-Mannich bases (IV.6) and (IV.7), respectively. These nitro compounds (IV.6) and (IV.7) were readily purified by recrystallisation and were catalytically reduced with hydrogen in the presence of Raney nickel to afford the corresponding amines (IV.8) and (IV.9), which were sufficiently pure for condensation directly with 4,7-dichloro- and 4-chloro-7-trifluoromethyl-quinoline.

\[
\begin{align*}
\text{IV.6, } & \quad X = \text{NO}_2 \\
\text{IV.7, } & \quad X = \text{NO}_2 \\
\text{IV.8, } & \quad X = \text{NH}_2 \\
\text{IV.9, } & \quad X = \text{NH}_2
\end{align*}
\]

IV-2.3 Syntheses of Some Mannich Base Derivatives of 4-Chloro-2-(7-chloroquinolin-4-ylamino)phenol, 4-Chloro-2-[7-bromo(and trifluoromethyl)-1,5-naphthyridin-4-ylamino]phenols and 4(and 6)-t-Butyl-2-[7-chloro(and trifluoromethyl)quinolin-4-ylamino]phenols

The nitro compounds (IV.1) were subjected to catalytic reduction with hydrogen over Raney nickel in ethanolic ammonia at atmospheric pressure and room temperature to give the corresponding amines (IV.2). These were then condensed with the appropriate 4-chloroheterocycles, namely 4,7-dichloroquinoline, and 4-chloro-[7-bromo(and trifluoromethyl)-1,5-naphthyridine, in aqueous ethanol containing a few drops of concentrated hydrochloric acid (the reaction mixture was adjusted to pH 4.7 for reaction with the 4-chloroquinoline, and pH 2.5 for those with the 4-chloro-1,5-naphthyridines) at reflux for 11-12 h. Each product was precipitated by addition of dilute ammonium hydroxide and then purified by PTLC. In this way, Mannich bases of
4-chloro-2-(7-chloroquinolin-4-ylamino)phenol (IV.3) and 4-chloro-2-[7-bromo(and trifluoromethyl)-1,5-naphthyridin-4-ylamino]phenol (IV.4 and IV.5) were prepared.

The mono-Mannich bases of 4-t-butyl-2-[7-chloro(and trifluoromethyl)quinolin-4-ylamino]phenol (IV.10 and IV.11) and 6-t-butyl-2-[7-chloro(and trifluoromethyl)quinolin-4-ylamino]phenol (IV.12 and IV.13) were also synthesised from the preformed amines (IV.8 and IV.9) in a similar manner.

IV.10, X = Cl
IV.11, X = CF₃

IV.12, X = Cl
IV.13, X = CF₃

IV-3 Physical Properties

IV-3.1 Nuclear Magnetic Resonance Spectra

The ¹H n.m.r. spectra of nitro compounds (IV.1e and IV.6) and amino compounds (IV.2e and IV.8) are given in Table IV-1. Inspection of the data revealed that the protons H 3 and H 5 of the phenolic ring of the nitro compounds (IV.1e, and IV.6) appeared at lower field by 1.48 - 1.63 ppm relative to those of the corresponding amino compounds (IV.2e, and IV.8); furthermore, the signal due to H 3 (adjacent to the nitro group) was further down field of that due to H 5, whereas in the amino compounds, H 3 (which is adjacent to amino group) appeared upfield relative to that of H 5. This is due to the strong electron-withdrawing power of the nitro group compared to the electron-donating properties of the amino group. Similarly, the methylene protons of the Mannich side chain in these nitro compounds (IV.1e, and IV.6) were at lower field by 0.18 - 0.20 ppm.
As expected, due to their relatively remote position, the signal due to the protons of the amine of the Mannich base did not show large differences between the nitro compounds (e.g. IV.1e and IV.6) and the amino compounds (e.g. IV.2e and IV.8, respectively); the signal due to the protons of the piperidine ring revealed an AA'BB' pattern.

The signal due to the t-butyl group in the amino compound (IV.8) was slightly upfield of that in the nitro compound (IV.6)

Table IV-1. Chemical shift (δ)\textsuperscript{a} of some mono-Mannich base derivatives of 4-substituted-2-nitrophenols.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Y</th>
<th>Z</th>
<th>Bu\textsuperscript{t}</th>
<th>H3',4',5'</th>
<th>H2',6'</th>
<th>CH\textsubscript{2}N</th>
<th>H3</th>
<th>H5</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV.1e</td>
<td>Cl</td>
<td>NO\textsubscript{2}</td>
<td>-</td>
<td>1.63</td>
<td>2.63</td>
<td>3.78</td>
<td>7.91</td>
<td>7.24</td>
</tr>
<tr>
<td>IV.2e</td>
<td>Cl</td>
<td>NH\textsubscript{2}</td>
<td>-</td>
<td>1.59</td>
<td>2.53</td>
<td>3.58</td>
<td>6.37</td>
<td>6.61</td>
</tr>
<tr>
<td>IV.6</td>
<td>Bu\textsuperscript{t}</td>
<td>NO\textsubscript{2}</td>
<td>1.30, 3xMe</td>
<td>1.60</td>
<td>2.55</td>
<td>3.77</td>
<td>7.86</td>
<td>7.26</td>
</tr>
<tr>
<td>IV.8</td>
<td>Bu\textsuperscript{t}</td>
<td>NH\textsubscript{2}</td>
<td>1.24, 3xMe</td>
<td>1.55</td>
<td>2.51</td>
<td>3.59</td>
<td>6.38</td>
<td>6.68</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Reported as part per million (δ) downfield from tetramethylsilane (TMS) as internal standard in deuterchloroform (CDCl\textsubscript{3}) solution.

Inspection of the \textsuperscript{1}H n.m.r. spectra of the 4-chloro- or 4-t-butyl(or 6-t-butyl)-2-(7-chloroquinolin-4-ylamino)phenols (IV.3, IV.10, IV.12) reported in the Experimental section revealed that, like the Mannich base derivatives of 2-(7-chloroquinolin-4-ylamino) phenol described in Chapter II, the proton at position 3 of the quinoline ring was the most shielded (δ 6.31 - 7.23) whereas the most deshielded proton was that at position 2 (δ 8.59 - 8.67). The signal due to H 5 (δ 7.80 - 8.03) was also upfield of that due to H 6 (δ 7.35 - 7.51). The methylene protons of the Mannich
side chains appeared in the range δ 3.40 - 3.83. The assignments in the spectrum of compound (IV.10) were confirmed by running a 2-D COSY\textsuperscript{133} spectrum, in which coupling between H 5 and the methylene protons at C-6 was observed.

The \textsuperscript{1}H n.m.r. spectra of the compounds IV.4, IV.5, IV.13, and IV.14 are reported in the Experimental section.

\textbf{IV-3.2 Mass Spectra}

The mass spectra of compounds IV.3b and IV.4b revealed major fragmentations by cleavage at the N-C benzylic bond; loss of diethylamine gave m/z 318 and 364 respectively. Compound (IV.3b) showed three molecular ion peaks at 389, 391, and 393, in the ratio 1, 2/3, 1/9, which corresponded to the isotopic pattern for two chlorine atoms present in the compound [as calculated by the binomial expansion method\textsuperscript{102b, 134}].

The bromo compound (IV.4b) also showed three different molecular ion peaks values of 434, 436, 438 (corresponding to the two isotopes of chlorine, \textsuperscript{35}Cl and \textsuperscript{37}Cl; and two isotopes of bromine, \textsuperscript{79}Br and \textsuperscript{81}Br present in this compound) in the ratio 3/4, 1, 1/4.\textsuperscript{102b, 134}

The mass spectrum of compound (IV.11) showed high intensity peaks corresponding to the molecular ion (at m/z 457) and also M+1. The major fragmentation of this compound was similar to that for 2,6-bis(piperidinyl-1'-ylmethyl)-4-(8"-trifluoromethylquinolin-4"-ylamino)phenol (III.24d) in that the major cleavage was observed at the N-C benzylic bond. Fragmentation involving the loss of a methyl group (from the t-butyl-group) was also detected.
IV-4 Antimalarial Activity

The compounds prepared in this work were tested for antimalarial activity against the FC-27 isolate of *P. falciparum* in *in vitro* tests using the visual and microscopic procedures described in Chapter II-4.1 and 5.3.

IV-4.1 Results of the *in vitro* Testing

Generally both the visual and microscopic tests gave similar results. The results of the visual test are given in Table IV-2 together with some slight variations (recorded as footnotes in the Table) which were obtained in the microscopic test.

None of these compounds had an IC$_{50}$ value < 50 nM, and therefore they were not further tested by using the radioisotope technique nor subjected to *in vivo* testing.
Table IV-2 Results from a screen of some Mannich bases of 4-substituted-2-[7-chloro(and trifluoromethyl)quinolin-4-ylamino]phenols and 4-chloro-2-[7-bromo(and trifluoromethyl)-1,5-naphthyridin-4-ylamino]phenols in the in vitro visual test against the FCQ-27 isolate of *P. falciparum*.

![Chemical structure](image)

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Compounds IV.3</th>
<th>Compounds IV.4</th>
<th>Compounds IV.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV.3</td>
<td>IC50A</td>
<td>IC50A</td>
<td>IC50A</td>
</tr>
<tr>
<td>IV.4</td>
<td>NR2</td>
<td>50-100</td>
<td>100-200</td>
</tr>
<tr>
<td>IV.5;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a NMe2</td>
<td>50-100</td>
<td>100-200</td>
<td>B</td>
</tr>
<tr>
<td>c NPR2</td>
<td>B</td>
<td>B</td>
<td></td>
</tr>
<tr>
<td>d N(CH2)4</td>
<td>100-200</td>
<td>B</td>
<td>50-100</td>
</tr>
<tr>
<td>e N(CH2)5</td>
<td>100-200</td>
<td>500-100</td>
<td>50-100</td>
</tr>
<tr>
<td>f N(CHMe(CH2)4)</td>
<td>100-200</td>
<td>B</td>
<td>100-200</td>
</tr>
<tr>
<td>g N(CH2CHMe(CH2)3)</td>
<td>-</td>
<td>50-100</td>
<td>B</td>
</tr>
<tr>
<td>h N(CH2)2CHMe(CH2)2</td>
<td>100-200</td>
<td>100-200</td>
<td>100-200</td>
</tr>
<tr>
<td>i N(CH2)5</td>
<td>50-100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV.10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV.11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chloroquine</td>
<td>20-40</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A The IC50 values (the concentration of the inhibitor required to reduce parasite growth by 50%) are expressed as nmol l⁻¹ (nM). B No significant activity at 200 nM. C The microscopic test gave IC50 50 - 100 nM. D The microscopic test gave IC50 > 200 nM. E The microscopic test gave IC50 > 200 nM. F As diphosphate salt.
Table IV-3. Data for comparison of in vitro antimalarial activity of some selected compounds against the FCQ-27 isolate of P. falciparum determined by the visual method.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>IC$_{50}$ values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NR$_2$=</td>
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<tr>
<td>II.43</td>
<td>50-100</td>
</tr>
<tr>
<td>II.44a</td>
<td>&gt; 200</td>
</tr>
<tr>
<td>II.44b</td>
<td>-</td>
</tr>
<tr>
<td>IV.3d</td>
<td>100-200</td>
</tr>
<tr>
<td>IV.3e</td>
<td>-</td>
</tr>
<tr>
<td>IV.4d</td>
<td>&gt; 200</td>
</tr>
<tr>
<td>IV.4g</td>
<td>-</td>
</tr>
<tr>
<td>IV.10</td>
<td>-</td>
</tr>
<tr>
<td>Chloroquine$^B$</td>
<td>20-40</td>
</tr>
</tbody>
</table>

$^A$ Results are expressed as nmol l$^{-1}$

$^B$ As diphosphate salt

**IV-4.2 Discussion of Results**

The results of antimalarial testing for the compounds reported in this chapter and recorded in Table IV-2 revealed all had IC$_{50}$ values $\geq$ 50 nM. The activities of the corresponding Mannich base derivatives of 4-chloro-2-(7-chloroquinolin-4-ylamino)phenol (IV.3), 4-chloro-2-[7-bromo(and 7-trifluoromethyl)-1,5-naphthyridin-3-ylamino]phenol (IV.4 and IV.5) showed slight variations only. For example
compounds (IV.3a), (IV.4a) and (IV.5a) had IC\textsubscript{50} values of 50-100, 100-200 and > 200 nM, whereas compounds (IV.3h, IV.4h, and IV.5h) all had IC\textsubscript{50} 100 - 200 nM and compounds (IV.3e, IV.4e and IV.5e) had IC\textsubscript{50} values of 100-200, 50-100, and 50-100 nM respectively. Thus in this series of compounds there is little variation in activity between the different heterocyclic nuclei. The compounds (IV.3) containing the 7-chloroquinoline nucleus may be slightly the more active and compounds (IV.5) containing the 7-trifluoromethyl-1,5-naphthyridine nucleus may be slightly less active which would be in agreement with the results in Chapter II.

The compounds (IV.3, IV.4, and IV.10) have been tested to measure the effect of the 4-chloro- and 4-t-butyl substituents relative to the 4-unsubstituted mono Mannich derivatives (II.43 and II.44) of 2-(7-chloroquinolin-4-ylamino)phenol and 2-(7-bromo-1,5-naphthyridin-4-ylamino)phenol. Relevant data for this comparison are assembled in Table IV-3. 4-Chloro-2-(7-chloroquinolin-4-ylamino)-6-(pyrrolidin-1-ylmethyl)phenol (IV.3d; IC\textsubscript{50} 100 -200 nM) was less active than 2-(7-chloroquinolin-4-ylamino)-6-(pyrrolidin-1-ylmethyl)phenol (II.43, IC\textsubscript{50} 50-100 nM) but the reverse applied to the Mannich bases (IV.4g, IC\textsubscript{50} 50-100 nM and II.44b, IC\textsubscript{50} 100-200 nM) from 3-methylpiperidine. Thus the 4-chloro substituent did not significantly effect the antimalarial activity.

Compound (IV.10), 4-t-butyl-2-(7-chloroquinolin-4-ylamino)-6-(piperidin-1-ylmethyl)phenol (IC\textsubscript{50} 50-100 nM) with the t-butyl group in the 4-position was more active than its 4-chloro analogue (IV.3e, IC\textsubscript{50} 100-200 nM). Also compound (IV.11), 4-t-butyl-6-(piperidin-1-ylmethyl)-2-(7-trifluoromethylquinolin-3-ylamino)phenol was more active than 4-chloro-2-(7-chloroquinolin-4-ylamino)-6-(piperidin-1-ylmethyl)phenol (IV.3e, IC\textsubscript{50} 100 - 200 nM). [N.B. Mannich base derivatives of 2-(7-chloroquinolin-4-ylamino)phenol were generally more active than those from 2-(7-trifluoromethyl-1,5-naphthyridin-4-ylamino)phenol\textsuperscript{135}]

The results of our study on the mono-Mannich base derivatives of 2-aminophenol series, appear to suggest that the activity increased when the 4-substituent was changed from chloro (IV.3e) to t-butyl (IV.10). This indicated the effect of electron-withdrawing substituent, such as chloro group resulted in less favourable
activity, whereas a relatively weak electron-donating group, such as t-butyl generally had an beneficial effect. The Mannich base derivatives of 3-aminophenol are investigated in the next chapter.
The general procedure and experimental details for the antimalarial testing are recorded in Chapter II-5.1 and 5.3.

4-Chloro-2-nitro-6-(piperidin-1'-ylmethyl)phenol (IV.1e)

Piperidine (3.92 g, 46.09 mmol) was added to a chilled mixture of 4-chloro-2-nitrophenol (2.0 g, 11.52 mmol), paraformaldehyde (1.38 g, 46.09 mmol) and ethanol (6.0 ml) and the reaction mixture was refluxed in an oil bath at 97° for 17 h. After cooling the yellow precipitate was collected, washed with methanol, and recrystallised from methanol to give the title compound (1.52 g), m.p. 178-180°. (Found: C, 53.5; H, 5.6; N, 10.2. C_{12}H_{15}ClN_{2}O_{3} requires C, 53.2; H, 5.6; N, 10.3%). $^1$H n.m.r. δ 1.63, complex H 3', 4', 5'; 2.63, m, H 2', 6'; 3.78, s, CH$_2$; 7.23, d, J$_{3,5}$ 2.5 Hz, H 5; 7.89, d, J$_{3,5}$ 2.5 Hz, H 3.

4-Chloro-2-nitro-6-(pyrrolidin-1'-ylmethyl)phenol (IV.1d)

Pyrrolidine (3.28 g; 46.09 mmol) was added to a chilled mixture of paraformaldehyde (1.38 g, 46.09 mmol), and 4-chloro-2-nitrophenol (2.0 g, 11.52 mmol) in ethanol (6.0 ml) and the mixture was refluxed in an oil bath at 97° for 22 h. The solvent was then evaporated under reduced pressure and the oily residue was subjected to column chromatography (alumina; 2% methanol in chloroform) and gave a yellow solid (1.75 g). Portion of this product was recrystallised from a mixture of methanol and chloroform and gave the title compound, m.p. 214-216°. (Found: C, 51.7; H, 5.2; N, 10.6. C$_{11}$H$_{13}$ClN$_{2}$O$_{3}$ requires C, 51.5; H, 5.1; N, 10.9%). $^1$H n.m.r. δ 1.92, complex H 3', 4'; 2.79, m, H 2', 5'; 3.94, s, CH$_2$; 6.92, br s, OH; 7.29, d, J$_{3,5}$ 2.5 Hz, H 5; 7.93, d, J$_{3,5}$ 2.5 Hz, H 3.

In a similar manner the following compounds were prepared.
4-Chloro-6-dimethylaminomethyl-2-nitrophenol (IV.1a) (94%), m.p. 225-227°C (after recrystallisation from a mixture of ethyl acetate, acetone and methanol). (Found: C, 47.1; H, 4.8; N, 12.1. C_{19}H_{18}BrClN_{4}O requires C, 46.9; H, 4.8; N, 12.2%). ¹H n.m.r. δ 2.43, s, Me; 3.77, s, CH₂; 5.92, br s, OH; 7.34, d, J_{3,5} 2.5 Hz, H 5; 7.93, d, J_{3,5} 2.5 Hz, H 3.

4-Chloro-6-diethylaminomethyl-2-nitrophenol (IV.1b) (32%), m.p. 134-136°C after column chromatography (alumina; 8% methanol in chloroform then alumina; 4% methanol in chloroform) and recrystallisation from a mixture of methanol and cyclohexane. (Found: C, 51.0; H, 6.0; N, 10.6. C_{11}H_{15}ClN_{2}O_{3} requires C, 51.1; H, 5.9; N, 10.8%). ¹H n.m.r. δ 1.18, t, J 7 Hz, Me; 2.75, q, J 7 Hz, CH₂Me; 3.90, s, 6-CH₂; 7.19, d, J_{3,5} 2.5 Hz, H 5; 7.89, d, J_{3,5} 2.5 Hz, H 3.

4-Chloro-6-dipropylaminomethyl-2-nitrophenol (IV.1c) (27%), m.p. 123-125°C after column chromatography (alumina; chloroform). (Found: C, 54.6; H, 6.8; N, 9.5. C_{13}H_{19}ClN_{2}O_{3} requires C, 54.4; H, 6.7; N, 9.8%). ¹H n.m.r. δ 0.92, t, J 7 Hz, Me; 1.65, m, CH₂Me, 2.58, m, CH₂CH₂Me; 3.87, s, CH₂; 7.20, d, J_{3,5} 2.5 Hz, H 5; 7.88, d, J_{3,5} 2.5 Hz, H 3.

4-Chloro-6-(2'-methylpiperidin-1'-ylmethyl)-2-nitrophenol (IV.1f) (42%), m.p. 105-107°C after column chromatography (alumina; 2% methanol in chloroform). (Found: C, 55.2; H, 6.4; N, 9.7. C_{13}H_{17}ClN_{2}O_{3} requires C, 54.8; H, 6.0; N, 9.8%). ¹H n.m.r. δ 1.06-4.35, complex, H 2', 3', 4', 5', 6', Me and 6-CH₂; 7.17, d, J_{3,5} 2.5 Hz, H 5; 7.86, d, J_{3,5} 2.5 Hz, H 3.

4-Chloro-6-(3'-methylpiperidin-1'-ylmethyl)-2-nitrophenol (IV.1g) (97%), m.p. 95-97°C after column chromatography (alumina; chloroform). (Found: C, 54.5; H, 6.1; N, 9.5. C_{13}H_{17}ClN_{2}O_{3} requires C, 54.8; H, 6.0; N, 9.8%). ¹H n.m.r. δ 0.94, d, J 5.5 Hz, Me; 0.88-3.05, complex, H 2', 3', 4', 5', 6', 3.78, s, CH₂; 7.20, d, J_{3,5} 2.5 Hz, H 5; 7.88, d, J_{3,5} 2.5 Hz, H 3.
4-Chloro-6-(4'-methylpiperidin-1'-ylmethyl)-2-nitrophenol (IV.1h)
(71%), m.p. 171-173° after recrystallisation from a mixture of methanol and chloroform. (Found: C, 55.1; H, 6.3; N, 9.4. C_{13}H_{17}ClN_{2}O_{3} requires C, 54.8; H, 6.0; N, 9.8%). \(^1\)H n.m.r. \(\delta\) 0.99, d, J 4.5 Hz, Me; 1.38-3.1, complex, H 2', 3', 4', 5', 6', 3.81, s, 6-CH\(_2\); 7.26, d, J\(_{3,5}\) 2.5 Hz, H 5; 7.91, d, J 2.5 Hz, H 3.

4-Chloro-6-(3',5'-dimethylpiperidin-1'-ylmethyl)-2-nitrophenol (IV.1i)
(53%), m.p. 160-162° after column chromatography (alumina; 2% methanol in chloroform, twice, then alumina; chloroform). (Found: C, 56.5; H, 6.6; N, 9.4. C_{14}H_{19}ClN_{2}O_{3} requires C, 56.3; H, 6.4; N, 9.4%). \(^1\)H n.m.r. \(\delta\) 0.90, d, J 5.5 Hz, Me; 0.86-2.98, complex, H 2', 3', 4', 5', 6'; 3.77, s, CH\(_2\); 7.21, d, J\(_{3,5}\) 2.5 Hz, H 5; 7.88, d, J\(_{3,5}\) 2.5 Hz, H 3.

4-Chloro-6-(morpholin-1'-ylmethyl)-2-nitrophenol (IV.1j) (54%), m.p. 136-138° after recrystallisation from chloroform. (Found: C, 48.4; H, 4.9; N, 10.1. C_{11}H_{13}ClN_{2}O_{4} requires C, 48.4; H, 4.8; N, 10.3%). \(^1\)H n.m.r. \(\delta\) 2.56-3.82, complex, H 2', 3', 5', 6' and CH\(_2\); 7.45. br s, H 5; 7.94, s, H 3.

4-Chloro-2-(7'-chloroquinolin-4'-ylamino)-6-(piperidin-1''-ylmethyl)phenol (IV.3e)

A mixture of 4-chloro-2-nitro-6-(piperidin-1'-ylmethyl)phenol (0.6 g; 0.22 mmol), ethanolic ammonia (30 ml) and ethanol (30 ml) was shaken with Raney nickel and hydrogen until uptake ceased. The mixture was filtered through celite and the solvent evaporated to leave the oily amine (0.45 g) \(^1\)H n.m.r. \(\delta\) 1.59, m, H 3', 4', 5'; 2.52, m, H 2', 6'; 3.58, s, CH\(_2\); 6.37, s, H 5; 6.61, s, H 3].

A mixture of this amine (0.213 g; 0.088 mmol) and 4,7-dichloroquinoline (0.175 g; 0.088 mmol) with ethanol (8.0 ml) and water (2.0 ml) was adjusted with concentrated hydrochloric acid to pH 4.7 and then refluxed at 100° for 12 h. The solvent was evaporated under reduced pressure, the residue diluted with water, adjusted to pH 9-10, and extracted with chloroform to give an oil. This product was purified by
t.l.c. (alumina; chloroform) and recrystallised from a mixture of hexane and ethyl acetate to give yellow crystals of the title compound (0.17 g), m.p. 183-185°. (Found: C, 62.7; H, 5.4; N, 10.3. C_{21}H_{21}Cl_{2}N_{3}O requires C, 62.7; H, 5.3; N, 10.4%).

\[ ^1H \text{n.m.r. } \delta 1.60, \text{ complex, } H \text{ 3'', 4'', 5''}; 2.58, \text{ complex, } H \text{ 2'', 6''}; 3.69, s, CH_{2}; 6.73, d, J 2 Hz, H 5; 7.19, d, J 5.5 Hz, H 3'; 7.37, d, J 2 Hz, H 3; 7.46, dd, J_{5,6} 9, J_{6',8'} 2 Hz, H 6'; 7.97, d, J 9 Hz, H 5'; 8.05, d, J 2 Hz, H 8'; 8.64, d, J 5.5 Hz, H 2'.

2-(7'-Bromo-1',5'-naphthyridin-4'-ylamino)-4-chloro-6-(diethylaminomethyl)phenol (IV.4b)

A mixture of 4-chloro-6-diethylaminomethyl-2-nitrophenol (0.61 g; 0.24 mmol), ethanolic ammonia (30 ml) and ethanol (30 ml) was shaken with hydrogen over Raney nickel until uptake ceased. The catalyst was filtered on celite and the solvent evaporated to leave an oil (0.5 g) \[ ^1H \text{n.m.r. } \delta 1.18, t, J 7 Hz, Me; 2.73, q, J 7 Hz, CH_{2}Me; 3.74, s, CH_{2}; 6.39, d, J 3 = 2 Hz, H 5; 6.64, d, J_{3,5} 2 Hz, H 3]. This amine (0.25 g; 0.11 mmol) was mixed with 7-bromo-4-chloro-1,5-naphthyridine (0.266 g; 0.11 mmol), ethanol (8.0 ml) and water (2.0 ml) was adjusted with hydrochloric acid to pH 2.5 and then refluxed at 100° for 11.5 h. The solvent was evaporated and the residue diluted with water, adjusted to pH 9-10, and extracted with chloroform. The product was subjected to t.l.c. (alumina; chloroform) and gave, as a yellow solid, the title compound (0.28 g), m.p. 132-134°. (Found: C, 52.1; H, 4.6; N, 12.6. C_{19}H_{20}BrClN_{4}O requires C, 52.4; H, 4.6; N, 12.9%). \[ ^1H \text{n.m.r. } \delta 1.14, t, J 7 Hz, Me; 2.71, q, CH_{2}Me; 3.79, s, CH_{2}; 6.75, d, J_{3,5} 2 Hz, H 5; 7.24, d, J_{2,3} 5.5 Hz, H 3''; 7.45, d, J_{3,5} 2 Hz, H 3; 8.44, d, J 2 Hz, H 8'', 8.62, d, J_{2,3} 5.5 Hz, H 2'; 8.73, br s, OH(?); 8.80, d, J 2 Hz, H 6'. \] MS m/z 438,436 (M) (1.9, 8.8%), 434 (6.5%), 365 (4.8%), 364 (4.6%), 363 (14.5%), 362 (3.6%), 72 (NEt_{2}) (28.3%), 58 (100%).
4-Chloro-6-(pyrrolidin-1'-ylmethyl)-2-(7''-trifluoromethyl-1''',5'''-naphthyridin-4''-ylamino)phenol (IV.5d)

4-Chloro-2-nitro-6-(pyrrolidin-1-ylmethyl)phenol (0.4 g) was reduced catalytically as described above for similar compounds. The amino compound was obtained as an oil (0.25 g) \[^1H\text{n.m.r.} \delta 1.86, \text{br s}, \text{H} \text{3', 4'}; 2.68, \text{br s}, \text{H} 2', 5'; 3.75, \text{s}, \text{CH}_2; 6.41, \text{d}, J_{3,5} 2.5 \text{ Hz, H} 5; 6.63, \text{d}, J_{3,5} 2.5 \text{ Hz, H} 3].

A mixture of this amine (0.125 g, 0.055 mmol), 4-chloro-7-trifluoromethyl-1,5-naphthyridine (0.128 g, 0.055 mmol), ethanol (6.0 ml) and water (2.0 ml) was adjusted with concentrated hydrochloric acid to pH 2.5 and then refluxed at 100° for 11.5 h. The solvent was evaporated, the residue diluted with water, adjusted to pH 9-10, and the product extracted with chloroform. This product was purified by t.l.c. (alumina; chloroform) and recrystallised from a mixture of chloroform and light petroleum (b.p. 60-80°) to give the title compound (0.14 g), m.p. 158-160° (Found: C, 56.7; H, 4.4; N, 13.1. C\(_{20}\)H\(_{18}\)ClF\(_3\)N\(_4\)O requires C, 56.8; H, 4.3; N, 13.3%). \[^1H\text{n.m.r.} \delta 1.88, \text{m}, \text{H} \text{3', 4'}; 2.71, \text{m}, \text{H} 2', 5'; 3.86, \text{s}, \text{CH}_2; 6.79, \text{d}, J_{3,5} 2.5 \text{ Hz, H} 5; 7.30, \text{d}, J_{2'',3''} 5.5 \text{ Hz, H} 3''; 7.47, \text{d}, J_{3,5} 2.5 \text{ Hz, H} 3; 7.90, \text{br s, NH(?)}, 8.56, \text{d}, J 2 \text{ Hz, H} 8''; 8.73, \text{d}, J_{2'',3''} 5.5 \text{ Hz, H} 2''; 8.93, \text{br s, OH(?)}; 8.99, \text{s, H} 6''.

Mono-Mannich base derivatives of 2-amino-4-chlorophenol

Catalytic reduction of the corresponding 4-chloro-2-nitrophenols as described above gave the following mono-Mannich base derivatives of 2-amino-4-chlorophenol.

6-CH\(_2\)NMe\(_2\) (IV.2a): \[^1H\text{n.m.r.} \delta 2.30, \text{s, Me}; 3.53, \text{s, CH}_2; 6.36, \text{d}, J_{3,5} 2.5 \text{ Hz, H} 5; 6.61, \text{d}, J_{3,5} 2.5 \text{ Hz, H} 3.

6-CH\(_2\)NPr\(_2\) (IV.2c): \[^1H\text{n.m.r.} \delta 0.89, \text{t, J 7 Hz, Me}; 1.62, \text{m, J 7 Hz, CH}_2\text{Me}; 2.48, \text{m, J 8 Hz, CH}_2\text{CH}_2\text{Me}; 3.66, \text{s, CH}_2; 6.36, \text{d}, J_{3,5} 2.5 \text{ Hz, H} 5; 6.60, \text{d}, J_{3,5} 2.5 \text{ Hz, H} 3.
6-(2-methylpiperidin-1-yl)methyl (IV.2f): $^1$H n.m.r. $\delta$ 1.13-4.22, complex, H 2', 3', 4', 5', 6', Me and 6-CH$_2$; 6.36, d, J 2.5 Hz, H 5; 6.59, d, J 2.5 Hz, H 3.

6-(3-methylpiperidin-1-yl)methyl (IV.2g): $^1$H n.m.r. $\delta$ 0.91, d, J 5.5 Hz, Me; 0.85-2.85, complex, H 2', 3', 4', 5', 6'; 3.57, s, CH$_2$; 6.37, d, J$_{3,5}$ 2.5 Hz, H 5; 6.61, d, J$_{3,5}$ 2.5 Hz, H 3.

6-(4-methylpiperidin-1-yl)methyl (IV.2h): $^1$H n.m.r. $\delta$ 0.96, d, J 5 Hz, Me; 1.16-3.04, complex, H 2', 3', 4', 5', 6'; 3.59, s, 6-CH$_2$; 6.37, d, J$_{3,5}$ 2.5 Hz, H 5; 6.61, d, J$_{3,5}$ 2.5 Hz, H 3.

6-(3,5-dimethylpiperidin-1-yl)methyl (IV.2i): $^1$H n.m.r. $\delta$ 0.87, d, J 5.5 Hz, Me; 0.90-2.94, complex, H 2', 3', 4', 5', 6'; 3.56, s, 6-CH$_2$; 6.35, d, J 2.5 Hz, H 5; 6.59, d, J 2.5 Hz, H 3.

In a similar manner to the preparations described above the following compounds have been prepared.

4-Chloro-2-(7'-chloroquinolin-4'-ylamino)-6-(dimethylaminomethyl)phenol (IV.3a) (68%), m.p. 115-117° after t.l.c. (alumina; chloroform). (Found: C, 59.8; H, 4.8; N, 11.3. C$_{18}$H$_{17}$Cl$_2$N$_3$O requires C, 59.7; H, 4.7; N, 11.6%). $^1$H n.m.r. $\delta$ 2.37, s, Me; 3.67, s, CH$_2$; 6.51. br s, OH; 6.73, d, J$_{3,5}$ 2.5 Hz, H 5; 7.17, d, J$_{3,5}$ 5.5 Hz, H 3'; 7.37, d, J$_{3,5}$ 2.5 Hz, H 3; 7.44, dd, J$_{5',6'}$ 9, J$_{6',8'}$ 2 Hz, H 6'; 7.94, d, J$_{5',6'}$ 9 Hz, H 5'; 8.04, d, J 2 Hz, H 8'; 8.63, d, J$_{2',3'}$ 5.5 Hz, H 2'.

4-Chloro-2-(7'-chloroquinolin-4'-ylamino)-6-(diethylaminomethyl)phenol (IV.3b) (68%), m.p. 149-151° after t.l.c. (alumina; chloroform). (Found: C, 61.4; H, 5.5; N, 10.6. C$_{20}$H$_{21}$Cl$_2$N$_3$O requires C, 61.5; H, 5.4; N, 10.8%). $^1$H n.m.r. $\delta$ 1.15, t, J 7 Hz, Me; 2.68; q, J 7 Hz, CH$_2$Me; 3.80, s, CH$_2$; 6.72, d, J$_{3,5}$ 2.5 Hz, H 5; 7.18, d, J$_{2',3'}$ 5.5 Hz, H 3'; 7.36, d, J$_{3,5}$ 2.5 Hz, H 3; 7.44, dd, J$_{5',6'}$
9, J'6,8' 2 Hz, H 6'; 7.95, d, J5',6' 9 Hz, H 5'; 8.04, d, J 2 Hz, H 8'; 8.63, d, J2',3' 5.5 Hz, H 2'. MS m/z 393, 391 (M) (0.5, 4.4%), 389 (6.9%), 319 (3.2%), 318 (16.5%), 316 (24.9%), 72 (NEt2) (18.4%), 58 (Et2) (100%).

4-Chloro-2-(7'-chloroquinolin-4'-ylamino)-6-(dipropylaminomethyl)-phenol (IV.3c) (90%), m.p. 137-139° after t.l.c. (alumina; cyclohexane / ethyl acetate, 3:1) and recrystallisation from a mixture of chloroform and light petroleum (b.p. 60-80°). (Found: C, 63.2; H, 5.9; N, 9.8. C22H25Cl2N3O requires C, 63.2; H, 6.0; N, 10.0%). 1H n.m.r. δ 0.92, t, J 7 Hz, Me; 1.61; m, CH2Me; 2.54, m, CH2CH2Me; 3.78, s, CH2; 6.46, br s, NH(?); 6.72, d, J3,5 2 Hz, H 5; 7.10, br s, OH; 7.18, d, J2',3' 5.5 Hz, H 3'; 7.36, d, J3,5 2 Hz, H 3; 7.44, dd, J5',6' 9, J6',8' 2 Hz, H 6'; 7.95, d, J5',6' 9 Hz, H 5', 8.03, d, J 2 Hz, H 8'; 8.63, d, J2',3' 5.5 Hz, H 2'.

4-Chloro-2-(7'-chloroquinolin-4'-ylamino)-6-(pyrrolidin-1'-ylmethyl)phenol (IV.3d) (49%), m.p. 145-147° after t.l.c. (alumina; chloroform) and recrystallisation from a mixture of chloroform and cyclohexane. (Found: C, 61.5; H, 5.0; N, 10.5. C20H19Cl2N3O requires C, 61.9; H, 4.9; N, 10.8%). 1H n.m.r. δ 1.87, m, H 3", 4"; 2.68, m, H 2", 5"; 3.83, s, CH2; 6.73, d, J3,5 2 Hz, H 5; 7.15, d, J2',3' 5.5 Hz, H 3'; 7.35, d, J3,5 2 Hz, H 3; 7.42, dd, J5',6' 9, J6',8' 3 Hz, H 6'; 7.66, br s, OH; 7.94, d, J5',6' 9 Hz, H 5'; 8.03, d, J 3 Hz, H 8'; 8.62, d, J2',3' 5.5 Hz, H 2'.

4-Chloro-2-(7'-chloroquinolin-4'-ylamino)-6-(2''-methylpiperidin-1''-ylmethyl)phenol (IV.3f) (47%), as an oil after t.l.c. (alumina; chloroform). (Found: C, 63.2; H, 5.7; N, 9.9. C22H23ClN3O requires C, 63.5; H, 5.6; N, 10.1%). 1H n.m.r. δ 1.17-4.31, complex, H 2", 3", 4", 5", 6", Me and 6-CH2; 6.09, br s, NH; 6.72, d, J 2 Hz, H 5; 7.16, d, J2',3' 5.5 Hz, H 3'; 7.35, d, J3,5 2 Hz, H 3; 7.45, dd, J5',6' 9, J6',8' 2 Hz, H 6'; 7.97, d, J5',6' 9 Hz, H 5'; 8.04, d, J6',8' 2 Hz, H 8'; 8.62, d, J2',3' 5.5 Hz, H 2'.
4-Chloro-2-(7'-chloroquinolin-4'-ylamino)-6-(3''-methylpiperidin-1''-ylmethyl)phenol (IV.3g) (54%), m.p. 157-159° after t.l.c. (alumina; cyclohexane / ethyl acetate, 3:1) and recrystallisation from a mixture of chloroform and light petroleum (b.p. 60-80°). (Found: C, 63.2; H, 5.7; N, 9.8. C_{22}H_{23}Cl_{2}N_{3}O requires C, 63.5; H, 5.6; N, 10.1%). \(^1\)H n.m.r. δ 0.94, d, J 5.5 Hz, Me; 0.88-2.96, complex, H 2", 3", 4", 5", 6"; 3.69, s, CH₂; 6.72, d, J_{3,5} 2 Hz, H 5; 6.91, br s, OH(?); 7.18, d, J_{2',3'} 5.5 Hz, H 3'; 7.36, d, J_{3,5} 2 Hz, H 3; 7.44, dd, J_{5',6'} 9, J_{6',8'} 2 Hz, H 6'; 7.95, d, J_{5',6'} 9 Hz, H 5'; 8.04, d, J 2 Hz, H 8'; 8.63, d, J_{2',3'} 5.5 Hz, H 2'.

4-Chloro-2-(7'-chloroquinolin-4'-ylamino)-6-(4''-methylpiperidin-1''-ylmethyl)phenol (IV.3h) (76%), m.p. 121-123° after t.l.c. (alumina; chloroform) and recrystallisation from a mixture of ethyl acetate and cyclohexane. (Found: C, 63.5; H, 5.7; N, 9.93. C_{22}H_{23}Cl_{2}N_{3}O requires C, 63.46; H, 5.58; N, 10.1%). \(^1\)H n.m.r. δ 0.97, d, J 4.5 Hz, Me; 1.57-3.08, complex, H 2", 3", 4", 5", 6"; 3.71, s, 6-CH₂; 6.41, br, OH(?); 6.72, d, J 2 Hz, H 5; 7.18, d, J 5.5 Hz, H 3'; 7.36, d, J 2 Hz, H 3; 7.45, dd, J_{5',6'} 9, J_{6',8'} 2 Hz, H 6'; 7.96, d, J 9 Hz, H 5'; 8.04, d, J 2 Hz, H 8'; 8.63, d, J_{2',3'} 5.5 Hz, H 2'.

4-Chloro-2-(7'-chloroquinolin-4'-ylamino)-6-(3''',5'''-dimethylpiperidin-1''-ylmethyl)phenol (IV.3i) (48%), m.p. 101-103° after t.l.c. (alumina; chloroform) and recrystallisation from a mixture of chloroform and light petroleum (b.p. 60-80°). (Found: C, 64.5; H, 6.0; N, 9.6. C_{23}H_{25}Cl_{2}N_{3}O requires C, 64.2; H, 5.9; N, 9.8%). \(^1\)H n.m.r. δ 0.89, d, J 5.5 Hz, Me; 1.54-3.00, complex, H 2", 3", 4", 5", 6"; 3.71, s, 6-CH₂; 6.05, br s, NH(?); 6.74, s, H 5; 7.20, d, J_{2',3'} 5.5 Hz, H 3'; 7.38, s, H 3; 7.45, dd, J_{5',6'} 9, J_{6',8'} 2 Hz, H 6'; 7.95, d, J_{5',6'} H 5'; 8.05, br s; H 8'; 8.65, d, J_{2',3'} 5.5 Hz, H 2'.

2-(7'-Bromo-1',5'-naphthyridin-4'-ylamino)-4-chloro-6-dimethylaminoethylphenol (IV.4a) (91%), m.p. 174-176° after t.l.c. (alumina; 2% methanol in chloroform). (Found: C, 49.9; H, 4.2; N, 13.7. C_{17}H_{16}BrClN_{4}O
requires C, 50.1; H, 4.0; N, 13.7%). $^1$H n.m.r. δ 2.37, s, Me; 3.67, s, CH$_2$; 6.76, d, J$_{3,5}$ 2.5 Hz, H 5; 7.24, d, J$_{2,3'}$ 5.5 Hz, H 3'; 7.47, d, J$_{3,5}$ 2.5 Hz, H 3; 8.45, d, J 2 Hz, H 8'; 8.63, d, J$_{2,3'}$ 5.5 Hz, H 2'; 8.80, d, J 2 Hz, H 6'; 8.87, br s, NH(?); 9.32, br s, OH.

2-(7'-Bromo-1',5'-naphthyridin-4'-ylamino)-4-chloro-6-dipropylaminomethylphenol (IV.4c) (98%) as an oil after t.l.c. (alumina; cyclohexane / ethyl acetate, 3:1). (Found: C, 54.1; H, 4.9; N, 11.8. C$_{21}$H$_{24}$BrClN$_4$O requires C, 54.4; H, 5.2; N, 12.1%). $^1$H n.m.r. δ 0.91, t, J 7 Hz, Me; 1.56, m, J 7 Hz, CH$_2$Me; 2.54, t, J 7 Hz, CH$_2$CH$_2$Me; 3.79, s, 6-CH$_2$; 6.76, d, J 2 Hz, H 5; 7.24, d, J$_{2,3'}$ 5.5 Hz, H 3'; 7.45, d, J$_{3,5}$ 2 Hz, H 3; 8.45, d, J 2 Hz, H 8'; 8.63, d, J$_{2,3'}$ 5.5 Hz, H 2'; 8.81, d, J 2 Hz, H 6'.

2-(7'-Bromo-1',5'-naphthyridin-4'-ylamino)-4-chloro-6-(pyrrolidin-1''-ylmethyl)phenol (IV4d) (33%) m.p. 154-155° after t.l.c. (alumina; chloroform) and recrystallisation from a mixture of methanol and cyclohexane. (Found: C, 52.9; H, 4.2; N, 12.8. C$_{19}$H$_{18}$BrClN$_4$O requires C, 52.6; H, 4.2; N, 12.9%). $^1$H n.m.r. δ 1.88, m, H 3'', 4''; 2.69, m, H 2'', 5''; 3.85, s, 6-CH$_2$; 6.77, d, J$_{3,5}$ 2.5 Hz, H 5; 7.24, J$_{2,3'}$ 5.5 Hz, H 3'; 7.46, J$_{3,5}$ 2.5 Hz, H 3; 7.61, br s OH(?); 8.45, d, J 2 Hz, H 8'; 8.62, d, J$_{2,3'}$ 5.5 Hz, H 2'; 8.80, d, J 2 Hz, H 6'.

2-(7'-Bromo-1',5'-naphthyridin-4'-ylamino)-4-chloro-6-(piperidin-1''-ylmethyl)phenol (IV.4e) (55%) m.p. 168-170° after t.l.c. (alumina; chloroform) and recrystallisation from a mixture of chloroform, methanol and cyclohexane. (Found: C, 53.9; H, 4.3; N, 12.3. C$_{20}$H$_{20}$BrClN$_4$O requires C, 53.6; H, 4.5; N, 12.5%). $^1$H n.m.r. δ 1.59, m, H 3'', 4'', 5'', 2.59, m, H 2'', 6''; 3.69, s, CH$_2$; 6.76, d, J$_{3,5}$ 2 Hz, H 5; 7.23, J$_{2,3'}$ 5.5 Hz, H 3'; 7.45, d, J$_{3,5}$ 2 Hz, H 3; 8.46, d, J 2 Hz, H 8'; 8.63, d, J$_{2,3'}$ 5.5 Hz, H 2'; 8.81, d, J 2 Hz, H 6'; 9.91, br s, OH.
2-(7'-Bromo-1',5'-naphthyridin-4'-ylamino)-4-chloro-6-(2''-methyl-piperidin-1''-ylmethyl)phenol (IV.4f) (67%), m.p. 166-168° after t.l.c. (alumina; chloroform) and recrystallisation from a mixture of methanol, chloroform and light petroleum (b.p. 60-80°). (Found: C, 54.4; H, 5.1; N, 11.9. \(\text{C}_{21}\text{H}_{22}\text{BrClN}_4\text{O}\) requires C, 54.6; H, 4.8; N, 12.1%). \(^1\)H n.m.r. \(\delta\) 1.21, d, J 6.5 Hz, Me; 1.18-4.30, complex, H 2'', 3'', 4'', 5'', 6'' and 6-CH\(_2\); 6.75, d, J 2 Hz, H 5; 6.91, br s, OH(?); 7.24, d, J\(_{2,3'}\) 5.5 Hz, H 3'; 7.44, d, J\(_{3,5}\) 2 Hz, H 3; 8.45, d, J 2 Hz, H 8'; 8.63, d, J\(_{2,3'}\) 5.5 Hz, H 2'; 8.82, d, J 2 Hz, H 6'.

2-(7'-Bromo-1',5'-naphthyridin-4'-ylamino)-4-chloro-6-(3''-methyl-piperidin-1''-ylmethyl)phenol (IV.4g) (66%), m.p. 150-152° after t.l.c. (alumina; cyclohexane / ethyl acetate, 3:1). (Found: C, 54.7; H, 4.5; N, 11.8. \(\text{C}_{21}\text{H}_{22}\text{BrClN}_4\text{O}\) requires C, 54.6; H, 4.8; N, 12.1%). \(^1\)H n.m.r. \(\delta\) 0.91, d, J 5.5 Hz, Me; 0.88-2.96, complex, H 2'', 3'', 4'', 5'', 6''; 3.71, s, CH\(_2\); 6.77, d, J\(_{3,5}\) 2 Hz, H 5; 7.24, d, J\(_{2,3'}\) 5.5 Hz, H 3'; 7.47, d, J\(_{3,5}\) 2 Hz, H 3; 7.57, br s, OH(?); 8.47, d, J 2 Hz, H 8'; 8.64, d, J\(_{2,3'}\) 5.5 Hz, H 2'; 8.82, d, J 2 Hz, H 6'.

2-(7'-Bromo-1',5'-naphthyridin-4'-ylamino)-4-chloro-6-(4''-methyl-piperidin-1''-ylmethyl)phenol (IV.4h) (60%), m.p. 172-174° after t.l.c. (alumina; chloroform) and recrystallisation from a mixture of chloroform, ethyl acetate and light petroleum (b.p. 60-80°). (Found: C, 54.3; H, 4.6; N, 11.9. \(\text{C}_{21}\text{H}_{22}\text{BrClN}_4\text{O}\) requires C, 54.6; H, 4.8; N, 12.1%). \(^1\)H n.m.r. \(\delta\) 0.96, d, J 4.5 Hz, Me; 1.36-3.07, complex, H 2'', 3'', 4'', 5'', 6''; 3.71, s, 6-CH\(_2\); 6.76, d, J\(_{3,5}\) 2 Hz, H 5; 7.23, d, J\(_{2,3'}\) 5.5 Hz, H 3'; 7.45, d, J\(_{3,5}\) 2 Hz, H 3; 8.45, d, J 2 Hz, H 8'; 8.63, d, J\(_{2,3'}\) 5.5 Hz, H 2'; 8.81, d, J 2 Hz, H 6'.
2-(7'-Bromo-1',5'-naphthyridin-4'-ylamino)-4-chloro-6-(3'',5'')-dimethylpiperidin-1''-ylmethylphenol (IV.4i) (72%), m.p. 121-123° after t.l.c. (alumina; chloroform). (Found: C, 56.2; H, 5.3; N, 11.7. C_{21}H_{24}BrClN_{4}O requires C, 55.5; H, 5.1; N, 11.8%). *H n.m.r. δ 0.88, d, J 6 Hz, Me; 1.53-2.99, complex, H 2'', 3'', 4'', 5'', 6''; 3.71, s, 6-CH$_2$; 5.57, br s, OH(?); 6.77, d, J 2 Hz, H 5; 7.24, d, J$_{2,3}$: 5.5 Hz, H 3'; 7.46, d, J$_{3,5}$ 2 Hz, H 3; 8.46, d, J 2 Hz, H 8'; 8.62, d, J$_{2,3}$: 5.5 Hz, H 2'; 8.81, d, J 2 Hz, H 6'.

4-Chloro-6-dimethylaminomethyl-2-(7'-trifluoromethyl-1',5'-'naphthyridin-4'-ylamino)phenol (IV.5a) (41%), m.p. 161-163° after t.l.c. (alumina; chloroform) and recrystallisation from a mixture of chloroform and cyclohexane. (Found: C, 54.2; H, 4.3; N, 13.8. C_{18}H_{16}ClF$_3$N$_4$O requires C, 54.5; H, 4.1; N, 14.1%). *H n.m.r. δ 2.39, s, Me; 3.69, s, CH$_2$; 6.78, d, J$_{3,5}$ 2.5 Hz, H 5; 7.31, d, J$_{2,3}$: 5.5 Hz, H 3'; 7.48, d, J 2.5 Hz, H 3; 8.56, d, J 2 Hz, H 8'; 8.73, d, J$_{2,3}$: 5.5 Hz, H 2'; 8.91, br s, OH(?); 9.00, d, J 2 Hz, H 6'.

4-Chloro-6-diethylaminomethyl-2-(7'-trifluoromethyl-1',5'-'naphthyridin-4'-ylamino)phenol (IV.5b) (45%), m.p. 138-139° after t.l.c. (alumina; cyclohexane / ethyl acetate, 3:1) and recrystallisation from a mixture of chloroform and light petroleum (b.p. 60-80°). (Found: C, 56.3; H, 4.8; N, 12.9. C$_{20}$H$_{20}$ClF$_3$N$_4$O requires C, 56.5; H, 4.8; N, 13.2%). *H n.m.r. δ 1.15, t, J 7 Hz, Me; 2.69, q, J 7 Hz, CH$_2$Me; 3.82, s, CH$_2$; 6.79, d, J$_{3,5}$ 2 Hz, H 5; 7.32, d, J$_{2,3}$: 5.5 Hz, H 3'; 7.47, d, J$_{3,5}$ 2 Hz, H 3; 8.55, s, H 8'; 8.73, d, J$_{2,3}$: 5.5 Hz, H 2'; 8.93, br s, NH(?); 9.02, d, J 2 Hz, H 6'; 9.91, br s, OH(?).

4-Chloro-6-dipropylaminomethyl-2-(7'-trifluoromethyl-1',5'-'naphthyridin-4'-ylamino)phenol (IV.5c) (48%), m.p. 102-104° after t.l.c. (alumina; cyclohexane / ethyl acetate, 3:1) and recrystallisation from a mixture of chloroform, methanol and light petroleum (b.p. 60-80°). (Found: C, 58.2; H, 5.4; N, 12.3. C$_{22}$H$_{24}$ClF$_3$N$_4$O requires C, 58.3; H, 5.4; N, 12.4%). *H n.m.r. δ 0.92, t, J 7
Hz, Me; 1.64, m, J 7 Hz, CH₂Me; 2.55, m, CH₂CH₂Me; 3.79, s, 6-CH₂; 6.77, d, J₃,₅ 2 Hz, H 5; 7.31, d, J₂,₃ 5.5 Hz, H 3'; 7.46, d, J₃,₅ 2 Hz, H 3; 8.56, s, H 8'; 8.72, d, J₂,₃ 5.5 Hz, H 2'; 8.90, br s, NH(?); 9.01, d, J 2 Hz, H 6'; 9.45, br s, OH(?).

4-Chloro-6-(piperidin-1'-ylmethyl)-2-(7''-trifluoromethyl-1''',5'''-naphthyridin-4''-ylamino)phenol (IV.5e) (54%), m.p. 163-165° after t.l.c. (alumina; chloroform, twice). (Found: C, 57.9; H, 4.8; N, 12.5. C₂₁H₂₉ClF₃N₄O requires C, 57.7; H, 4.6; N, 12.8%). ¹H n.m.r. δ 1.62, m, H 3', 4', 5'; 2.61, m, H 2', 6'; 3.71, s, 6-CH₂; 6.37, br s, NH(?); 6.78, d, J₃,₅ 2 Hz, H 5; 7.29, d, J 5.5 Hz, H 3''; 7.46, d, J₃,₅ 2 Hz, H 3; 8.56, br s, H 8''; 8.72, d, J₂,₃ 5.5 Hz, H 2''; 8.93, br s, OH(?); 9.00, d, J 2 Hz, H 6''.

4-Chloro-6-(2'-methylpiperidin-1'-ylmethyl)-2-(7''-trifluoromethyl-1''',5'''-naphthyridin-4''-ylamino)phenol (IV.5f) (68%), m.p. 144-146° after t.l.c. (alumina; chloroform) and recrystallisation from a mixture of chloroform, methanol and light petroleum (b.p. 60-80°). (Found: C, 58.3; H, 5.0; N, 12.1. C₂₂H₂₂ClF₃N₄O requires C, 58.6; H, 4.9; N, 12.4%). ¹H n.m.r. δ 1.23, d, J 6.5 Hz, Me; 1.19-4.36, complex, H 2', 3', 4', 5', 6' and 6-CH₂; 6.80, d, J₃,₅ 2 Hz, H 5; 6.90, br s, NH(?); 7.33, d, J₂,₃ 5.5 Hz, H 3''; 7.48, d, J₃,₅ 2 Hz, H 3; 8.58, br s, H 8''; 8.75, d, J₂,₃ 5.5 Hz, H 2''; 8.95, br s, OH(?); 9.04, d, J 2 Hz, H 6''.

4-Chloro-6-(3'-methylpiperidin-1'-ylmethyl)-2-(7''-trifluoromethyl-1''',5'''-naphthyridin-4''-ylamino)phenol (IV.5g) (90%), m.p. 134-136° after t.l.c. (alumina; cyclohexane / ethyl acetate, 3:1). (Found: C, 58.7; H, 4.9; N, 12.2. C₂₂H₂₂ClF₃N₄O requires C, 58.6; H, 4.9; N, 12.4%). ¹H n.m.r. δ 0.92, d, J 5.5 Hz, Me; 0.89-2.98, complex, H 2', 3', 4', 5', 6'; 3.72, s, CH₂; 6.80, d, J₃,₅ 2 Hz, H 5; 7.31, d, J₂,₃ 5.5 Hz, H 3''; 7.49, d, J₃,₅ 2 Hz, H 3; 8.07, br s, NH(?); 8.57, br s, H 8''; 8.74, d, J₂,₃ 5.5 Hz, H 2''; 8.93, s, H 6''; 9.02, s, OH(?).
4-Chloro-6-(4'-methylpiperidin-1'-ylmethyl)-2-(7''-trifluoromethyl-1'',5''-naphthyridin-4''-ylamino)phenol (IV.5h) (77%), m.p. 173-175° after t.l.c. (alumina; chloroform, then alumina; cyclohexane / ethyl acetate, 3:1) and recrystallisation from a mixture of ethyl acetate and cyclohexane. (Found: C, 58.3; H, 5.1; N, 12.1. C_{22}H_{22}ClF_{3}N_{4}O requires C, 58.6; H, 4.9; N, 12.4%). \( ^{1}H \) n.m.r. \( \delta \) 0.96, d, J 4.5 Hz, Me; 1.17-3.09, complex, H 2', 3', 4', 5', 6'; 3.73, s, CH\(_{2}\); 6.78, d, J 2 Hz, H 5; 7.05, br s, NH(?); 7.30, d, J 5.5 Hz, H 3''; 7.46, d, J 2 Hz, H 3; 8.57, s, H 8''; 8.72, d, J 5.5 Hz, H 2''; 8.92, br s, OH(?); 9.02, d, J 2 Hz, H 6''.

4-Chloro-6-(3',5'-dimethylpiperidin-1'-ylmethyl)-2-(7''-trifluoromethyl-1'',5''-naphthyridin-4''-ylamino)phenol (IV.5i) (74%), m.p. 156-157° after t.l.c. (alumina; chloroform). (Found: C, 59.7; H, 5.5; N, 11.7. C_{23}H_{24}ClF_{3}N_{4}O requires C, 59.4; H, 5.2; N, 12.0%). \( ^{1}H \) n.m.r. \( \delta \) 0.89, d, J 5.5 Hz, Me; 1.56-2.99, complex, H 2', 3', 4', 5', 6'; 3.72, s, 6-CH\(_{2}\); 5.71, br; NH(?); 6.80, s, H 5; 7.31, d, J_{2'',3''} 5.5 Hz, H 3''; 7.49, s, H 3; 8.57, H 8''; 8.73, d, J_{2'',3''} 5.5 Hz, H 2''; 9.01, s, H 6''; 8.94, br, OH(?).

4-t-Butyl-2-nitro-6-(piperidin-1'-ylmethyl)phenol (IV.6)

Piperidine (5.25 g, 61.5 mmol) was added to a chilled reaction mixture of 4-t-butyl-2-nitrophenol *_{136} (4 g, 20.5 mmol), paraformaldehyde (1.86 g, 61.5 mmol) and ethanol (12 ml), and the mixture was refluxed in an oil bath at 100° for 21 h. The solvent was evaporated and the product was recrystallised from a mixture of methanol, chloroform and ethyl acetate to give yellow crystals of the title compound (4.9 g), m.p. 168 - 170° (Found : C, 66.0; H, 8.3; N, 9.6. C_{16}H_{24}N_{2}O_{3} requires C, 65.7; H, 8.3; N, 9.6%). \( ^{1}H \) n.m.r. \( \delta \) 1.30, br s, Me\(_{3}C\); 1.60, m, H 3', 4', 5'; 2.55, m, H 2', 6'; 3.77, s, CH\(_{2}\); 7.26, d, J 2 Hz H 5; 7.86, d, J 2 Hz, H 3; 9.89, br, OH.

* Kindly provided by Mr S. Ireland
6-t-Butyl-2-nitro-4-(piperidin-1'-ylmethyl)phenol (IV.7)

Piperidine (2.62 g, 30.77 mmol) was added to a chilled mixture of 6-t-butyl-2-nitrophenol*, 137,138 (2 g, 10.25 mmol), paraformaldehyde (0.92 g, 30.77 mmol) and ethanol (12 ml), and the mixture was refluxed for 24 h. The solvent was evaporated and the product was recrystallised from a mixture of hexane, methanol and chloroform to give yellow crystals of the **title compound** (1.38 g), m.p. 97 - 99° (Found : C, 65.8; H, 8.5; N, 9.6. C16H24N2O3 requires C, 65.7; H, 8.3; N, 9.6 %). 1H n.m.r. δ 1.43, s, Me3C; 1.62, m, H 3', 4', 5'; 2.39, m, H 2', 6'; 3.43, s, CH2; 7.56, d, J 2 Hz, H 5; 7.96, d, J 2 Hz, H 3.

4-t-Butyl-2-(7'-chloroquinolin-4'-ylamino)-6-(piperidin-1"'-ylmethyl)phenol (IV.10)

A mixture of 4-t-butyl-2-nitro-6-(piperidin-1'-ylmethyl)phenol (0.75 g), ethanol (30 ml), ethanolic ammonia (30 ml) and Raney nickel was shaken with hydrogen until uptake ceased. The catalyst was filtered off on Celite and the solvent was evaporated to give the crude amine (0.56 g) as an oil [1H n.m.r. δ 1.22, s, Me3C; 1.55, m, H 3', 4', 5'; 2.51, m, H 2', 6'; 3.59, s, CH2; 6.38, s, H 3; 6.68, s, H 5].

A mixture of this amine (0.31 g, 1.17 mmol), 4,7-dichloroquinoline (0.23 g, 1.17 mmol), ethanol (8.0 ml) and water (2.0 ml) was adjusted with concentrated hydrochloric acid to pH 4.5 and refluxed in an oil bath at 100° for 22 h. The solvent was evaporated under reduced pressure and the residue diluted with water, adjusted to pH 9-10 and extracted with chloroform. The product obtained was subjected to t.l.c. (alumina, chloroform) and recrystallised from a mixture of methanol, chloroform, hexane, and ethyl acetate to give yellow crystals of the **title compound** (0.26 g), m.p. 236 - 238° (Found : C, 70.6; H, 7.3; N, 9.6. C25H30ClN3O requires C, 70.8; H, 7.2; N, 9.9 %). 1H n.m.r. δ 1.31, s, Me3C; 1.66, m, H 3", 4", 5"; 2.50, m, H 2", 6"; 3.74, s, CH2; 6.77, d, J 2 Hz, H 5; 6.97, br, NH; 7.09, d, J2,3' 5.5 Hz, H 3'; 7.40, d, J 2 Hz, H 3; 7.45, dd, J5',6' 9, J6',8' 2 Hz, H 6'; 7.96, d, J5',6' 5.5 Hz, H 5'; 8.02, d, J6',8' 2 Hz, H 8'; 8.59, d, J2,3' 5.5 Hz, H 2'.

* Kindly provided by Mr S. Ireland
6-\text{-}Butyl-4-(piperidin-1'\text{-}ylmethyl)-2-(7''-trifluoromethylquinolin-4'\text{-}ylamino)phenol (IV.13)

A mixture of 2-nitro-6-\text{-}butyl-4-(piperidin-1'\text{-}ylmethyl)phenol (0.75 g), ethanol (30 ml), ethanolic ammonia (30 ml) and Raney nickel was shaken with hydrogen until uptake ceased. The catalyst was filtered off on Celite and the solvent was evaporated to give the crude amine (0.62 g).

A solution of the above amine (0.29 g, 1.1 mmol), 4-chloro-7-trifluoromethylquinoline (0.26 g, 1.1 mmol), ethanol (8.0 ml) and water (2.0 ml) was adjusted with concentrated hydrochloric acid to pH 4.5 and then refluxed for 22 h. Work up was as above and the product was subjected to t.l.c. (alumina, chloroform) and recrystallised from a mixture of hexane and ethyl acetate to give crystals of the \textit{title compound} (0.02 g), m.p. 212-214° (Found : C, 67.8; H, 6.7; N, 9.1. \textit{C}_{26}\text{H}_{30}\text{F}_{3}\text{N}_{3}\text{O} requires C, 68.2; H, 6.6; N, 9.2%). $^1$H n.m.r. δ 1.50, br s, Me$_3$C; 1.55, m, H 3', 4', 5'; 2.35, m, H 2', 6'; 3.41, s, CH$_2$; 6.36, d, J$_{2',3''}$ 5.5 Hz, H 3''; 7.07, d, J 2 Hz, H 5; 7.19, d, J 2 Hz, H 3; 7.61, dd, J$_{5'',6''}$ 9, J$_{6'',8''}$ 2 Hz, H 6''; 7.91, d, J$_{5'',6''}$ 9 Hz, H5''; 8.26, br s, H 8''; 8.38, d, J$_{2'',3''}$ 5.5 Hz, H 2''.

In a similar manner to that described above, the following compounds were prepared.

\textit{4-\text{-}Butyl-6-(piperidin-1'\text{-}ylmethyl)-2-(7''-trifluoromethylquinolin-4'\text{-}ylamino)phenol (IV.11)} (55%) as a yellow solid, m.p. 224 - 226°, after t.l.c. (alumina, chloroform) and recrystallisation from a mixture of hexane, ethyl acetate and methanol (Found : C, 68.0; H, 6.8; N, 8.9. \textit{C}_{26}\text{H}_{30}\text{F}_{3}\text{N}_{3}\text{O} requires C, 68.2; H, 6.6; N, 9.2 %). $^1$H n.m.r. δ 1.31, s, Me$_3$C; 1.66, m, H 3', 4', 5'; 2.52, m, H 2', 6'; 3.74, s, CH$_2$; 6.79, d, J 2 Hz, H 5; 7.05, br, NH; 7.18, d, J$_{2'',3''}$ 5.5 Hz, H 3''; 7.41, d, J 2 Hz, H 3; 7.68, dd, J$_{5'',6''}$ 9, J$_{6'',8''}$ 2 Hz, H 6''; 8.15, d, J$_{5'',6''}$ 9 Hz, H 5''; 8.33, br s, H 8''; 8.68, d, J$_{2'',3''}$ 5.5 Hz, H 2''. MS m/z 458 (M+ 1) (12.6%), 457 (M) (25.0%), 372 (M - C$_5$H$_{10}$N) (100%), 373 (34.6%), 357 (20%), 84 (C$_5$H$_{10}$N) (29.3%).
6-\textit{t}-Butyl-2-(7-chloroquinolin-4'-ylamino)-4-(piperidin-1''-ylmethyl)phenol (IV.12) (6%) as a solid, m.p. 216 - 218° after t.l.c. (alumina, chloroform) and recrystallisation from a mixture of hexane and ethyl acetate (Found: C, 70.6; H, 7.2; N, 9.7. \( \text{C}_{25}\text{H}_{30}\text{ClN}_{3}\text{O} \) requires C, 70.8; H 7.2; N, 9.9%). \(^1\text{H} \) n.m.r. \( \delta \) 1.46, br s, Me3C; 1.58, m, H 3", 4", 5"; 2.36, m, H 2", 6"; 3.40, d, CH2; 6.31, d, J2',3' 5.5 Hz, H 3'; 7.07, br s, H 5; 7.18, br s, H 3; 7.47, dd, J5',6' 9, J6', 8' 2 Hz, H 6'; 7.80, d, J5',6' 9 Hz, H 5'; 8.02, d, J 2 Hz, H8'; 8.42, d, J2',3' 5.5 Hz, H 2'.
CHAPTER V
CHAPTER V Syntheses and Antimalarial Activity of Some
Mannich Base Derivatives of 3-[7-Chloro(and
trifluoromethyl)quinolin-4-ylamino]phenols

V-1 Introduction

In this chapter the syntheses are reported for some Mannich base derivatives of 3-[7-chloro(and trifluoromethyl)quinolin-4-ylamino]phenols and together with the results of testing for antimalarial activity. These compounds were chosen for comparison of the structural requirements for high antimalarial activity against chloroquine-resistant strains of malaria parasites with similar derivatives (II.40 and II.43; Chapter II-2.3) of 2-aminophenol.

In the first part of this chapter, the synthesis of these compounds are reported, including the synthesis of some new Mannich base derivatives of 3-nitrophenol. Then, some of their physical properties are discussed. This is followed by the results of in vitro testing, which are compared with the results for the corresponding 2-aminophenol analogues. Experimental details for the preparation of these compounds (including some physical data) are recorded at the end of this chapter.

V-2 Syntheses

V-2.1 Preparation of Some New Mannich Base Derivatives of 3-Nitrophenol

The synthesis of these compounds was generally undertaken in a similar manner to that for Mannich base derivatives of 2-nitrophenol58 (Chapter II-2.1). 3-Nitrophenol (V.1) with five equivalents of paraformaldehyde and piperidine at reflux in ethanol for 22 h afforded a mixture of the di- and tri-Mannich compounds, 3-nitro-2,4-bis(piperidin-1'-ylmethyl)phenol (V.2) and 3-nitro-2,4,6-tris(piperidin-1'-ylmethyl)phenol (V.3), but with one equivalent of the reagents only 3-nitro-2-(piperidin-1'-ylmethyl)phenol (V.4) was obtained (Scheme V-1). The structure of these compounds was established from the n.m.r. spectra and analyses. The compound
Scheme V-1

V.1

\[
\text{OH} \quad \text{NO}_2
\]

\[\text{V.1}\]

\[
\text{HN} \quad \text{/(CH}_2\text{O})\text{n}
\]

5 equiv.

\[
\text{V.2}
\]

\[
\text{V.3}
\]

\[
\text{V.4}
\]

1 equiv.

\[
\text{OH} \quad \text{CH}_2\text{N}
\]

\[\text{V.22}\]

\[
\text{V.10}
\]

\[
\text{V.17}, \quad x=\text{Cl}
\]

\[
\text{V.18}, \quad x=\text{CF}_3
\]
(V.4a) may also be present in low yield in the reaction mixture but could not be isolated from t.l.c. under a variety of conditions.

The corresponding reaction with five equivalents of pyrrolidine and paraformaldehyde in ethanol afforded 3-nitro-2,4,5-tris(pyrrolidin-1'-ylmethyl)phenol (V.5), but with one equivalent the two mono-Mannich isomers, 3-nitro-2-(pyrrolidin-1'-ylmethyl)phenol (V.6) and 5-nitro-2-(pyrrolidin-1'-ylmethyl)phenol (V.7), were produced (Scheme V-2). Their structures were established from consideration of their n.m.r. spectra.

The mono-Mannich base(s) 6-methoxy-3-nitro-2-[piperidin-1'-ylmethyl(or pyrrolidin-1'-ylmethyl)]phenol, compounds V.8 (and V.9), were also prepared from 2-methoxy-5-nitrophenol (commercially available) with one equivalent of paraformaldehyde and piperidine (or pyrrolidine). The structures of compounds (V.8 and V.9) were established form their \( ^1H \) n.m.r. spectra. However, reduction of these nitro compounds (V.8 and V.9) with hydrogen over Raney nickel gave products which decomposed rapidly in air.

\[ \begin{align*} 
V.4a & \quad \text{OH} \\
& \quad \text{Z} \\
& \quad \text{NR}_2 \\
V.8 & \quad \text{NO}_2 \quad \text{N(CH}_2)_5 \\
V.9 & \quad \text{NO}_2 \quad \text{N(CH}_2)_4 \\
V.15 & \quad \text{NH}_2 \quad \text{N(CH}_2)_5 \\
V.16 & \quad \text{NH}_2 \quad \text{N(CH}_2)_4 
\end{align*} \]

V-2.2 Syntheses of Some Mannich Base Derivatives of 3-[7'-Chloro(and trifluoromethyl)quinolin-4'-ylamino]phenol

Each of the above nitro compounds (V.2, V.4-V.9) was reduced by hydrogenation over Raney nickel to the corresponding aminophenols (V.10, V.11-V.16). The mono-Mannich compounds (V.11, V.13, V.14) condensed with 4,7-dichloro- or 4-chloro-7-trifluoromethyl-quinoline in aqueous ethanol (at pH ~ 4.5) at
reflux for 15-22 h, and gave the Mannich base derivatives of the 3-(substituted quinolin-4'-ylamino)phenols (V.17-V.21, Schemes V-1 and V-2).

Unfortunately the amines (V.10 and V.12) did not condense with 4,7-dichloro- or 4-chloro-7-trifluoromethyl-quinoline and, in my hands, compounds (V.22) and (V.23) could not be prepared by this method. Apparently steric hindrance by the two bulky Mannich side chains adjacent to the amino group of compounds V.10 and V.12 prevented them from condensing with the 4-chloroquinoline.

V-2 Physical Properties

V-2.1 Nuclear Magnetic Resonance Spectra

The structures of the products from the Mannich reaction were established from consideration of their n.m.r. spectra. 3-Nitro-2-(pyrrolidin-1'-ylmethyl)phenol (V.6) showed the presence of two doublets for H 4 and H 6 at δ 7.28 and 7.01 respectively: that due to H 4 adjacent to the nitro group was downfield, H 5 appeared as a triplet at δ 7.23, and the methylene protons at C-2 appeared at δ 4.11. A 2-D COSY spectrum as expected for structure V.6 did not show coupling between the methylene protons and the protons of the phenyl ring.

5-Nitro-2-(pyrrolidin-1'-ylmethyl)phenol (V.7) showed the presence of two multiplets; one at δ 7.07 - 7.12 due to H 3, and one at δ 7.58 -7.65 due to H 4 and H 6 (adjacent to the nitro group). The methylene group at C-2 appeared at δ 3.92, and the 2-D COSY spectrum showed the coupling between H 3 and the methylene protons at C-2.

The 1H n.m.r. spectra of compounds (V.19) and (V.21) are recorded in Table V-1. The assignments of the phenolic ring protons and H 3" of the quinoline ring of these compounds were based on proton-proton decoupling and 2-D COSY spectra. Compound (V.19) in its Homo-Decoupled 1H n.m.r. spectra, showed strong (coupling) effects between H 5 and H 6; H 4 and H 5 (phenolic ring); and H 2" and
Scheme V-2

\[
\begin{align*}
\text{V.1} & \quad \text{OH} \\
\text{V.2} & \quad \text{NO}_2 \\
\text{V.3} & \quad \text{H} \quad \text{O} \quad (\text{CH}_2\text{O})_n \\
\text{V.5} & \quad \text{5 equiv.} \\
\text{V.6} & \quad \text{1 equiv.} \\
\text{V.7} & \\
\text{V.13} & \\
\text{V.14} & \\
\text{V.12} & \\
\text{V.23} & \\
\text{a. X = Cl} \\
\text{b. X = CF}_3 \\
\text{V.19} & \\
\text{V.20, X = Cl} \\
\text{V.21, X = CF}_3
\end{align*}
\]
H 3° (quinoline ring) (Figures V-1, B-C and V-2, D-E). Spectrum B shows that irradiation of the proton at δ 7.25 (H 5) causes a change in the pattern of the peaks at δ 6.84 (H 4) and 6.75 (H 6). Spectrum C revealed that irradiation at δ 6.84 (H 4) causes the peak at δ 7.25 (H 5) to change. Spectrum D shows that on irradiation of the doublet of doublets at δ 6.75 (H 6), the peaks at δ 7.25 (H 5) was changed. Spectrum E shows that on irradiation of the doublet at δ 6.36 (H 3°) the pattern of the peak at δ 8.57 (H 2°) was changed to a singlet. These couplings were confirmed from the 2-D COSY spectrum.

The Homo-Decoupled ¹H n.m.r. (Figures V-3, B-C and V-4, D-E) and 2D COSY (Figures V-5 and V.6) spectra of compound (V.21) showed coupling between H 3 and H 4 (phenolic ring), and H 2° and H 3°. In the COSY spectrum, the coupling between H 3 and the methylene protons at C-2 was also observed.

Table V-1 ¹H n.m.r. spectral dataa,b for 2-(pyrrolidin-1'-ylmethyl)-3( and 5)-(7''-trifluoromethylquinolin-4''-ylamino)phenol

<table>
<thead>
<tr>
<th>Compound</th>
<th>H 3</th>
<th>H 4</th>
<th>H 5</th>
<th>H 6</th>
<th>H 2°</th>
<th>H 3°</th>
<th>H 5°</th>
<th>H 6°</th>
<th>H 8°</th>
<th>CH2</th>
<th>J3,4</th>
<th>J4,5</th>
<th>J4,6</th>
<th>J5,6</th>
</tr>
</thead>
<tbody>
<tr>
<td>V.19</td>
<td></td>
<td>6.84</td>
<td>7.25</td>
<td>6.75</td>
<td>8.57</td>
<td>6.36</td>
<td>8.08</td>
<td>7.70</td>
<td>8.34</td>
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<tr>
<td>V.21</td>
<td>7.02</td>
<td>6.71</td>
<td></td>
<td>6.78</td>
<td>8.64</td>
<td>7.14</td>
<td>8.04</td>
<td>7.67</td>
<td>8.33</td>
<td>3.86</td>
<td>8.0</td>
<td>2.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Chemical shift are reported as δ values downfield from tetramethylsilane (TMS) as internal standard in CDCl₃
b Coupling constants (J values) are reported in Hz
Figure V-1 $^1$H n.m.r. spectra of 2-(pyrrolidin-1'-ylmethyl)-3-(7''-trifluoromethylquinolin-4''-ylamino)phenol (V.19); (V.19) in CDCl$_3$ (A), irradiated at 7.25 ppm (B), and irradiated at 6.84 ppm (C).
Figure V-2 \(^1\)H n.m.r. spectra of 2-(pyrrolidin-1'-ylmethyl)-3-(7''-trifluoromethylquinolin-4''-ylamino)phenol (V.19); (V.19) in CDCl\(_3\) (A), irradiated at 6.75 ppm (D), and irradiated at 6.36 ppm (E).
Figure V-3 ¹H n.m.r. spectra of 2-(pyrrolidin-1'-ylmethyl)-5-(7''-trifluoromethylquinolin-4''-ylamino)phenol (V.21); (V.21) in CDCl₃ (A), irradiated at 7.14 ppm (B), and irradiated at 7.02 ppm (C).
Figure V-4 $^1$H n.m.r. spectra of 2-(pyrrolidin-1'-ylmethyl)-5-(7''-trifluoromethylquinolin-4''-ylamino)phenol (V.21); (V.21) in CDCl$_3$ (A), irradiated at 6.78 ppm (D), and irradiated at 6.71 ppm (E).
Figure V-5  2-D COSY spectrum of 2-(pyrrolidin-1'-ylmethyl)-5-(7''-trifluoromethylquinolin-4''-ylamino)phenol (V.21)
Figure V-6 Expansion of Figure V-5 at region A
V-3.2 Mass Spectra

The mass spectra of compounds \textbf{V.4}, \textbf{V.18} and \textbf{V.20} were recorded and each spectrum showed the molecular ion peak (at m/z 236, 401 and 353 respectively). Compound (\textbf{V.20}) gave peaks at m/z 353 and 355 (ratio 3:1) as expected for a chlorine containing compound.

Mass spectra for these compounds displayed similar fragmentation patterns as for compound (\textbf{IV.11}) (Chapter IV-3.2) in that the major cleavage involved the N-C benzylic bond. Fragmentation of compound \textbf{V.4} also involved loss of the NO$_2$ group from the molecular ion. The fragmentation pattern of this compound (\textbf{V.4}) is depicted in Scheme V-3

V-4 Antimalarial Activity

The antimalarial testing on the compounds prepared in this chapter was carried out in the \textit{in vitro} 'visual test' and the results were checked in the 'microscopic test' as outline in Chapter II-5.3.

V-4.1 Results of the \textit{in vitro} testing

The results for the visual test are shown in Table V-2 as IC$_{50}$ values. The results from the microscopic test paralleled those for the visual test and are not recorded in Table V-2 (except minor variation which are indicated in footnotes). For a complete and comparative study, some data obtained by others will be incorporated, with appropriate footnotes, in Table V-2.
Scheme V-3 Fragmentation of 3-nitro-2-(piperidin-1'-yl)phenol (V.4)

\[
\begin{align*}
\text{m/z} & \quad 236 \\
\text{m/z} & \quad 152 (6.4\%) \\
\text{m/z} & \quad 152 \\
\text{m/z} & \quad 219 (30.2\%) \\
\text{m/z} & \quad 189 (9.3\%) \\
\text{m/z} & \quad 135 (5\%) \\
\end{align*}
\]
Table V-2 Results of antimalarial testing of some Mannich base derivatives of 3-[7-chloro(and trifluoromethyl)quinolin-4-ylamino]phenols in *in vitro* tests against the FCQ-27 and K-1 isolates of *P. falciparum*.

![Chemical structures](image)

\(X, Y, \text{ and } Z\) are specified below.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Substituents</th>
<th>Visual test</th>
<th>Radioisotope test</th>
</tr>
</thead>
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<tr>
<td></td>
<td></td>
<td>FCQ-27 IC50(^A)</td>
<td>FCQ-27 IC50(^A)</td>
</tr>
<tr>
<td><strong>V.17-21</strong>, <strong>V.24-27</strong></td>
<td>Cl CH(_2)N(CH(_2))(_5) H</td>
<td>100-200</td>
<td>-</td>
</tr>
<tr>
<td><strong>V.18</strong></td>
<td>CF(_3) CH(_2)N(CH(_2))(_5) H</td>
<td>B</td>
<td>-</td>
</tr>
<tr>
<td><strong>V.19</strong></td>
<td>CF(_3) CH(_2)N(CH(_2))(_4) H</td>
<td>B</td>
<td>-</td>
</tr>
<tr>
<td><strong>V.20</strong></td>
<td>Cl H CH(_2)N(CH(_2))(_4)</td>
<td>(&lt;25) (C)</td>
<td>25-50</td>
</tr>
<tr>
<td><strong>V.21</strong></td>
<td>CF(_3) H CH(_2)N(CH(_2))(_4)</td>
<td>50-100</td>
<td>-</td>
</tr>
<tr>
<td><strong>V.24a</strong></td>
<td>Cl CH(_2)N(CH(_2))(_4) CH(_2)N(CH(_2))(_4) (D)</td>
<td>(\geq25)</td>
<td>12.5-25</td>
</tr>
<tr>
<td><strong>V.24b</strong></td>
<td>Cl CH(_2)N(CH(_2))(_5) CH(_2)N(CH(_2))(_5) (D)</td>
<td>25-50</td>
<td>25-50</td>
</tr>
<tr>
<td><strong>V.25a</strong></td>
<td>CF(_3) CH(_2)N(CH(_2))(_4) CH(_2)N(CH(_2))(_4) (D)</td>
<td>50-100</td>
<td>-</td>
</tr>
<tr>
<td><strong>V.25b</strong></td>
<td>CF(_3) CH(_2)N(CH(_2))(_5) CH(_2)N(CH(_2))(_5) (D)</td>
<td>50-100</td>
<td>-</td>
</tr>
<tr>
<td><strong>V.26</strong></td>
<td>Cl H H(D)</td>
<td>(&gt;800) (E)</td>
<td>-</td>
</tr>
<tr>
<td><strong>V.27</strong></td>
<td>CF(_3) H H(D)</td>
<td>(&gt;800) (E)</td>
<td>-</td>
</tr>
<tr>
<td><strong>II.40d</strong></td>
<td>Cl H CH(_2)N(CH(_2))(_4) CH(_2)N(CH(_2))(_4) (D)</td>
<td>(&lt;25)</td>
<td>12.5-25</td>
</tr>
<tr>
<td><strong>II.40e</strong></td>
<td>Cl H CH(_2)N(CH(_2))(_5) CH(_2)N(CH(_2))(_5) (D)</td>
<td>(&lt;25)</td>
<td>12.5-25</td>
</tr>
<tr>
<td>Chloroquine(^F)</td>
<td></td>
<td>20-25</td>
<td>20</td>
</tr>
</tbody>
</table>
V-4.2 Discussion of Results

The in vitro antimalarial activity observed by others* for compounds (V.24a,b and V.25a,b) showed that the di-Mannich compounds (V.24a and b), 3-(7'-chloroquinolin-1'-ylamino)-2,6-bis[pyrrolidin-1'-ylmethyl(and piperidin-1'-ylmethyl)]phenol respectively, had activity comparable to that of the corresponding 2-aminophenol analogues (compounds II.40d and e, Chapter II-4.1), against the FCQ-27 isolate of *P. falciparum*. However, they had lower activity than (II.40d) and (II.40e) against the chloroquine-resistant K-1 isolate as measured in the radioisotope test (Table V-2). The activities of compounds V.24a and V.24b were superior to their 7-trifluoromethyl analogues (V.25a) and (V.25b). The presence of Mannich side chains in such compounds appears to be essential for significant antimalarial activity because the 3-[7'-chloro(and trifluoromethyl)quinolin-4'-ylamino]phenols (V.26) and (V.27), which lacked such groups, did not show activity at 800 nM.

The results in Table V-2 revealed that mono-Mannich compounds (V.17, 20 and 21) exhibited antimalarial activity, which approached that of the corresponding di-Mannich compounds. Compound V.21, (IC$_{50}$ 50-100 nM) with the 6-(pyrrolidin-1'-ylmethyl) Mannich side chain remote from the quinoline ring as in the corresponding di-Mannich compound (V.25a), was appreciably more active than its 2-(pyrrolidin-1'-ylmethyl) isomer (V.19, IC$_{50}$ > 200 nM) in which the environment of the Mannich side chain was more crowded (and this group was closer to the quinoline ring). Compound V.20, IC$_{50}$ < 25 nM) showed higher activity than the corresponding mono-Mannich

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* Personal communication with Dr G.B. Barlin and Mr S. Ireland.
derivative of 2-aminophenol (compound II.43, IC\textsubscript{50} 50-100 nM) against the FCQ-27 isolate of \textit{P. falciparum}.

In conclusion, the results presented in Table V-2 for the \textit{in vitro} study suggest that there is a close correlation between the antimalarial activity of the di-Mannich bases of the 3-aminophenols and those of the 2-aminophenols; and amongst the mono-Mannich derivatives of the 3-aminophenols, those with the Mannich substituent at the 6-position were significantly more active than the 2-isomer.

Some Mannich derivatives of 4-aminophenols will be described in Chapter VI.
V-5 Experimental

The general procedure and experimental details for the antimalarial testing are recorded in Chapter II-5.1 and 5.3.

3-Nitro-2-(pyrrolidin-1'-ylmethyl)phenol (V.6) and 5-Nitro-2-(pyrrolidin-1'-ylmethyl)phenol (V.7)

Pyrrolidine (2.04 g, 28.75 mmol) was added to a chilled mixture of 3-nitrophenol (4.0 g, 28.75 mmol) and paraformaldehyde (0.86 g, 28.75 mmol) in ethanol (10 ml) and the mixture was refluxed at 100° for 2 h. The solvent was evaporated and the oily residue was subjected to column chromatography in methanol over silica (40 x 6 cm). Impurities and unchanged 3-nitrophenol were eluted first and then a mixture of two mono-Mannich products. This mixture was re-chromatographed in methanol over silica and gave as an oil in the first fractions 3-nitro-2-(pyrrolidin-1'-ylmethyl)phenol (0.57 g, 9%) (Found: C, 59.8; H, 6.6; N, 12.7. C₁₁H₁₄N₂O₃ requires C, 59.4; H, 6.4; N, 12.6%). *H n.m.r. δ 1.88, m, H 3', 4'; 2.70, m, H 2', 5'; 4.11, s, 2-CH₂; 7.01, d, J 4.5 Hz, H 6; 7.23, t, J 4.5 Hz, H 5; 7.28, d, J 4.5 Hz, H 4.

Later fractions from the column gave as an oil 5-nitro-2-(pyrrolidin-1'-ylmethyl)phenol (0.56 g, 8.8%) (Found: C, 59.3; H, 6.1; N, 12.4. C₁₁H₁₄N₂O₃ requires C, 59.4; H, 6.4; N, 12.6%). *H n.m.r. δ 1.89, m, H 3',4'; 2.68, m, H 2', 5'; 3.92, s, 2-CH₂; 7.07-7.12, m, H 3; 7.58 - 7.65, m, H 4, 6; 9.83, br, OH.

3-Nitro-2,4,6-tris(pyrrolidin-1'-ylmethyl)phenol (V.5)

To a chilled mixture of 3-nitrophenol (2 g, 14.78 mmol) and paraformaldehyde (2.16 g, 71.88 mmol, 5 equiv.) in ethanol (7 ml), was added pyrrolidine (5.11 g, 71.88 mmol, 5 equiv.) and the mixture was refluxed in an oil bath at 97° for 22 h. the solvent was evaporated and the oily residue was subjected to column chromatography in chloroform over alumina (40 x 4 cm). The majority of the product was obtained in the
early fractions and the later fractions when subjected to further column chromatography (alumina; chloroform, then 1% methanol in chloroform) and t.l.c. (alumina; 1% methanol in chloroform) gave additional product. The title compound was obtained as a yellow oil (4.02 g, 72%) (Found: C, 64.8; H, 8.6; N, 14.5. C_{21}H_{32}N_{4}O_{3} requires C, 64.9, H, 8.3; N, 14.4%). ^{1}H n.m.r. δ 1.81, m, H 3', 4'; 2.58, m, H 2', 5'; 3.53, s; 3.71, s; 3.75, s, 2-CH_{2}, 4-CH_{2} and 6-CH_{2}; 7.24, s, H 5.

3-Nitro-2-(piperidin-1'-ylmethyl)phenol (V.4)

Piperidine (2.45 g, 28.75 mmol) was added to a mixture of 3-nitrophenol (4 g, 28.75 mmol) and paraformaldehyde (0.86 g, 28.75 mmol) in ethanol (10.0 ml) and the mixture refluxed in an oil bath at 100° for 2 h. After cooling, the solvent was evaporated and the oily residue was subjected to column chromatography (silica; methanol). Unchanged 3-nitrophenol was eluted first and then impure mono-Mannich product(s) was obtained. The latter fractions were subjected to column (alumina; chloroform) and t.l.c. (alumina; chloroform) and in the latter the band at higher R_{F} gave 3-nitro-2-(piperidin-1'-yl-methyl)phenol (0.69 g, 10%) as a solid, m.p. 80-82° (Found: C, 60.6; H, 7.0; N, 11.6. C_{12}H_{16}N_{2}O_{3} requires C, 61.0; H, 6.8; N, 11.9%). ^{1}H n.m.r. δ 1.59, m, H 3', 4', 5'; 2.56, m, H 2', 6'; 3.93, s, 2-CH_{2}; 6.94-7.32 complex, H 4, 5, 6. MS m/z 236 (M) (4.0%), 219 (M - OH) (30.2%), 189[(M - OH)-NO] (9.3%), 152 (M - C_{5}H_{10}N) (6.4%), 136 (14.2%), 105 (17.9%), 84(C_{5}H_{10}N), (100%).

3-Nitro-2,4,6-tris(piperidin-1'-ylmethyl)phenol (V.3) and 3-Nitro-2,4-bis(piperidin-1'-ylmethyl)phenol (V.2)

To a chilled mixture of 3-nitrophenol (2 g, 14.38 mmol) and paraformaldehyde (2.16 g, 71.88 mmol) in ethanol (7.0 ml) was added piperidine (6.12 g, 71.88 mmol) and the mixture was refluxed in an oil bath at 100° for 22 h. The solvent was then evaporated and the oily residue was subjected to column chromatography over alumina in chloroform and then over alumina by elution with a mixture of hexane and ethyl acetate (2:1, then 1:1). The fractions which contained a mixture of tri- and di- Mannich
products were then subjected to t.l.c. (alumina; 10% ethyl acetate in hexane, developed twice) and gave at higher Rf the 3-nitro-2,4,6-tris(piperidin-1'-ylmethyl)phenol (1.22 g, 20%), m.p. 93-95° (Found: C, 67.2; H, 9.2; N, 12.9. C_{24}H_{38}N_{4}O_{3} requires C, 66.9; H 8.9; N, 13.0%). \textsuperscript{1}H n.m.r. \delta 1.52, m, H 3', 4', 5'; 2.22-2.45, m, H 2', 6'; 3.36, s, 6-CH\textsubscript{2}; 3.58, br s, 2,4-CH\textsubscript{2}; 7.18, s, H 5.

The band at lower Rf gave 3-nitro-2,4-bis(piperidin-1'-ylmethyl)phenol (1.29 g, 27%) as an oil (Found: C, 64.6; H, 8.5; N, 12.5. C_{18}H_{27}N_{3}O_{3} requires C, 64.8; H, 8.2; N, 12.6%). \textsuperscript{1}H n.m.r. \delta 1.53, m, H 3', 4', 5'; 2.48, m, H 2', 6'; 3.58, s, 2-CH\textsubscript{2}; 3.90, s, 4-CH\textsubscript{2}; 7.24, br s, H 5, 6.

6-Methoxy-3-nitro-2-(pyrrolidin-1'-ylmethyl)phenol  (V.9)

Pyrrolidine (0.42 g, 5.91 mmol) was added to a chilled mixture of 2-methoxy-5-nitrophenol (1.0 g, 5.91 mmol) and paraformaldehyde (0.18 g, 5.91 mmol) in ethanol (8.0 ml) and the mixture was refluxed for 2.5 h. The solvent was evaporated and the residual oil subjected to column chromatography (silica, methanol) and gave the title compound (0.75 g, 50.3%), m.p. 70-72° (Found, for a sample dried at 30°/0.1 mmHg for 14 h. C, 57.1; H 6.6; N, 11.0. C_{12}H_{16}N_{2}O_{4} requires C, 57.1; H, 6.4; N, 11.1%). \textsuperscript{1}H n.m.r. \delta 1.89, m, H 3', 4'; 2.74, m, H 2', 5'; 3.95, s, OMe; 4.26, s, 2-CH\textsubscript{2}; 6.80, d, J 9 Hz, H 5; 7.50, d, J 9 Hz, H 4.

6-Methoxy-3-nitro-2-(piperidin-1'-ylmethyl)phenol  (V.8)

Piperidine (0.5 g, 5.91 mmol) was added to a chilled mixture of 2-methoxy-5-nitrophenol (1.0 g, 5.91 mmol) and paraformaldehyde (0.18 g, 5.91 mmol) in ethanol (8.0 ml) and the mixture was refluxed for 2.5 h. The solvent was evaporated and the resulting oil was subjected to column chromatography (silica gel; methanol) and gave the title compound (0.8 g, 51%) (Found, for a sample dried at 30°/0.1 mmHg for 14 h: C, 58.4; H, 6.8; N, 10.4. C_{13}H_{18}N_{2}O_{4} requires C, 58.6; H, 6.8; N, 10.5%). \textsuperscript{1}H n.m.r. \delta 1.60, m, H 3',4', 5'; 2.63, m, H 2', 6'; 3.95, s, OMe; 4.09, s, 2-CH\textsubscript{2}; 6.79, d, J 9 Hz, H 5; 7.50, d, J 9 Hz, H 4; 8.70, br, OH(?).
When this nitro compound was reduced with hydrogen over Raney nickel the product darkened rapidly in air.

2-(Pyrrolidin-1'-ylmethyl)-3-(7'-trifluoromethylquinolin-4'-ylamino)phenol (V.19)

A mixture of 3-nitro-2-(pyrrolidin-1'-ylmethyl)phenol (0.46 g) in ethanol (30 ml) with ethanolic ammonia (30 ml) was shaken with Raney nickel and hydrogen until uptake ceased. The catalyst was filtered off on celite and the solvent evaporated to leave the amine (0.39 g, 98%).

This amine (0.165 g) and 4-chloro-7-trifluoromethylquinoline (0.199 g) in ethanol (7.0 ml) and water (1.5 ml) was adjusted with hydrochloric acid to pH 4.5 and the mixture refluxed for 22 h. The solvent was evaporated, the residue diluted with water and adjusted to pH 9-10, and the product extracted into chloroform. It was subjected to t.l.c. (alumina; chloroform) and gave the title compound (0.12 g, 36%) which after recrystallisation from a mixture of hexane/ethyl acetate/methanol was a yellow solid, m.p. 217-219° (Found: C, 63.9; H, 5.4; N, 10.7. C_{21}H_{20}F_{3}N_{3}O. 0.5 H_{2}O requires C, 63.6; H 5.4; N, 10.6%). ^1H n.m.r. δ 1.83, m, H 3', 4'; 2.58, m, H 2', 5'; 3.79, s, 2-CH_{2}; 6.36, d, J 2", 3" 5.5 Hz, H 3"; 6.75, d, J 5,6 8 H_{2}O; H 6; 6.84, d, J 4,5 8 H_{2}O; H 4; 7.25, t, J 4,5 8 Hz, H 5; 7.70, dd, J 5",6" 9 Hz, J 6",8" 2 Hz, H 6"; 8.08, d, J 5",6" 9 Hz, H 5"; 8.34, br s, H 8"; 8.57, d, J 2",3" 5.5 Hz, H 2".

5-(7'-Chloroquinolin-4'-ylamino)-2-(pyrrolidin-1'-ylmethyl)phenol (V.20)

5-Nitro-2-(pyrrolidin-1'-ylmethyl)phenol (0.56 g) was reduced as described above for the 3-nitro isomer and gave the corresponding amine (0.38 g).

This amine (0.17 g) and 4,7-dichloroquinoline (0.175 g) in a mixture of ethanol (7.0 ml) and water (1.5 ml) which was adjusted to pH 4.7 was refluxed for 15 h. The solvent was then evaporated and the residue diluted with water and adjusted to pH 9-10. The product was extracted into chloroform and subjected to t.l.c. (alumina; chloroform) and gave at low R_{f} the title compound (0.057 g, 18%) which after recrystallisation from
a mixture of hexane/ethyl acetate/methanol was a yellow solid, m.p. 136-138°
(Found: C, 66.5; H, 5.8; N, 11.4. \( \text{C}_{20}\text{H}_{20}\text{ClN}_3\text{O} \) requires C, 66.2; H, 5.8; N, 11.6%). \(^1\)H n.m.r. \( \delta \) 1.89, m, H 3", 4"; 2.69, m, H 2", 5"; 3.86, s, 2-CH\(_2\); 6.65-7.07, complex, H 3, 4, 5, 3'; 7.44, d, J\(_{5',6'}\) 9 Hz, H 6'; 7.89, d, J\(_{5',6'}\) 9 Hz, H 5'; 8.02, br s, H 8'; 8.55, d, J\(_{2',3'}\) 5.5 Hz, H 2'. MS m/z 355 (3.2%), 353 (8.5%) (M), 285 (10.7%), 283 (32.9%) (M-C\(_4\)H\(_8\)N), 91 (29%), 84 (8.5%), 70 (C\(_4\)H\(_8\)N) (100%).

\( \text{2-(Pyrrolidin-1'-ylmethyl)-5-(7''-trifluoromethylquinolin-4''ylamino)phenol (V.21) } \) was obtained in a similar manner to its 7'-chloro analogue. After t.l.c. (alumina; 1% methanol in chloroform), the product (22%) was recrystallised from a mixture of hexane/ethyl acetate/methanol and gave the \textit{title compound}, m.p. 205-207° (Found: C, 64.8; H, 5.3; N, 10.8. \( \text{C}_{21}\text{H}_{20}\text{F}_3\text{N}_3\text{O} \) requires C, 65.1; H, 5.2; N, 10.9%). \(^1\)H n.m.r. \( \delta \) 1.87, m, H 3', 4'; 2.69, m, H 2', 5'; 3.86, s, 2-CH\(_2\); 6.67, br s, NH; 6.71, dd, J\(_{3,4}\) 8, J\(_{4,6}\) 2 Hz, H 4; 6.78, d, J\(_{4,6}\) 2 Hz, H 6; 7.02, d, J\(_{3,4}\) 8 Hz, H 3; 7.14, d, J 5.5 Hz, H 3"; 7.67, d, J 9 Hz, H 6"; 8.04, d, J 9 Hz, H 5"; 8.33, br s, H 8"; 8.64, d, J 5.5 Hz, H 2".

\( \text{3-(7'-Chloroquinolin-4'-ylamino)-2-(piperidin-1''-ylmethyl)phenol (V.17) } \)

A mixture of 3-nitro-2-(piperidin-1'-ylmethyl)phenol (0.48 g, 2.03 mmol) in ethanol (25 ml) and ethanolic ammonia (25 ml) was shaken with Raney nickel and hydrogen until uptake ceased. The catalyst was filtered on kieselguhr and the solvent evaporated to give the amine as an oil (0.33 g).

This amine (0.135 g) and 4,7-dichloroquinoline (0.13 g) with ethanol (6.0 ml) and water (1.5 ml) was adjusted with concentrated hydrochloric acid to pH 4.4 and refluxed at 98° for 18 h. The solvent was evaporated, the residue diluted with water and adjusted to pH 9-10. The product was extracted in chloroform and then subjected to t.l.c. (alumina; 1% methanol in chloroform and alumina; chloroform) and gave the \textit{title compound} (0.066 g, 27%), m.p. 240-241° (Found: C, 66.7; H, 6.1; N, 10.9. \( \text{C}_{21}\text{H}_{22}\text{ClN}_3\text{O} \). 0.5 H\(_2\)O requires C, 66.9; H, 6.1; N, 11.1%). \(^1\)H n.m.r. \( \delta \) 1.65, m,
H 3", 4", 5"; 2.52, m, H 2", 6"; 3.66, s, 2-CH2; 6.31, d, J 5.5 Hz, H 3'; 6.72-6.79, 
m, H 4, 6; 7.24, t, J 8 Hz, H 5; 7.50, d, J5',6' 9 Hz, H 6'; 8.00, d, J5',6' 9 Hz, H 5'; 
8.04, br s, H 8'; 8.47, d, J2',3' 5.5 Hz, H 2'.

2-(Piperidin-1'-ylmethyl)-3-(7'-trifluoromethylquinolin-4'-ylamino)phenol (V.18) was obtained in a similar manner to the 7'-chloro analogue. 
It was purified by t.l.c. (alumina; chloroform then alumina; 1% methanol in 
chloroform), and the product (12%) was recrystallised from a mixture of hexane and 
ethyl acetate and gave yellow crystals of the title compound, m.p. 219-221° (Found: C, 
65.5; H, 5.8; N, 10.45. C22H22F3N3O requires C, 65.8; H, 5.5; N, 10.5%).

1H n.m.r. δ 1.61, m, H 3', 4', 5'; 2.51, m, H 2', 6'; 3.67, s, 2-CH2; 6.37, d, J2", 3" 
5.5 Hz, H 3"; 6.77, d, J5,6 8 Hz, H 6; 6.84, d, J4,5 8 Hz, H 4; 7.24, t, J4,5 8 Hz, H 5; 
7.71, d, J5",6" 9 Hz, H 6"; 8.09, d, J5",6" 9 Hz, H 5"; 8.35, br s, H 8"; 8.58, d, J 5.5 
Hz, H 2". MS m/z 401 (M) (10.3%), 317 (M-C5H10N) (13.7%), 84 (C5H10N) 
(100%).
CHAPTER VI
CHAPTER VI  Syntheses and Antimalarial Activity of Some Mannich Base Derivatives of 3-Substituted(or 3-Unsubstituted)-[7-chloro(and trifluoromethyl)quinolin-4-ylamino]phenols and 2-t-Butyl-4-[7-chloro(and trifluoromethyl)quinolin-4-ylamino]phenols

VI-1  Introduction

In previous chapters, a series of Mannich base derivatives of 2(and 3)-aminophenols have been investigated. In this chapter some derivatives of 4-aminophenols, such as the di-Mannich bases derived from 3-unsubstituted or 3-fluoro [or 3-(piperidin-1-yl)]-4-[7-chloro(and trifluoromethyl)quinolin-4-ylamino]phenols, and 2-t-butyl-4-[7-chloro(and trifluoromethyl)quinolin-4-ylamino]-6-(piperidin-1-ylmethyl)phenols have been prepared and evaluated for antimalarial activity.

The synthetic methods for the preparation of these compounds are reported first and then some physical properties are discussed. Thereafter, the results of the in vitro testing studies against *P. falciparum* using the visual and microscopic methods, and the $^3$H-hypoxanthine uptake method are reported and discussed.

VI-2  Syntheses

VI-2.1  Preparation of Some New Mono(or di)-Mannich Base Derivatives of 2(or 3)-Substituted-4-nitrophenol

(a)  Di-Mannich Base Derivatives of 3-Substituted-4-nitrophenol

In this work, the required 3-fluoro-4-nitro-2,6-bis(piperidin-1-ylmethyl)phenol (VI.3) was prepared by the same procedure as for the Mannich base derivatives of 4-nitrophenol.$^{130}$ Interestingly, reaction of 3-fluoro-4-nitrophenol with excess
Scheme VI-1

\[
\begin{align*}
&\text{VI.1} \\
&\text{VI.2} \\
&\text{VI.3} \\
&\text{VI.4} \\
&\text{VI.5} \\
&\text{VI.6} \\
&\text{VI.9} \quad X = \text{Cl} \\
&\text{VI.10} \quad X = \text{CF}_3 \\
&\text{VI.11}
\end{align*}
\]
paraformaldehyde and piperidine (four equivalents) in refluxing ethanol for 20 h gave two products, one of which was the desired compound (VI.3), together with the unexpected compound (VI.4) which contained three piperidine residues (based on $^1$H n.m.r., analyses and mass spectroscopy) (Scheme VI-1). This latter compound was probably formed via the replacement of the 3-fluoro substituent with piperidine prior to the Mannich reaction. The $^1$H n.m.r. spectra of all of these compounds are consistent with these structures.

(b) 2-t-Butyl-4-nitro-6-(piperidin-1-ylmethyl)phenol

This nitro compound was prepared in a similar manner to that described above. 2-t-Butyl-4-nitrophenol, paraformaldehyde and piperidine at reflux gave the required mono-Mannich base (VI.7).
VI-2.2 Syntheses of Di-Mannich Base Derivatives of 3-Substituted(or 3-unsubstituted)-4-[7-chloro(and trifluoromethyl)quinolin-4-ylamino]phenols, and 2-t-Butyl-4-[7-chloro(and trifluoromethyl)quinolin-4-ylamino]-6-(piperidin-1-ylmethyl)phenols

The di-Mannich bases of 3-fluoro-4-[7-chloro(and trifluoromethyl)quinolin-4-ylamino]phenol were readily prepared by condensation of the preformed amine (VI.5) with 4,7-dichloro- or 4-chloro-7-trifluoromethylquinoline in the presence of a few drops of concentrated hydrochloric in aqueous ethanol (the reaction mixture had pH ~ 4.5) at reflux for 22 h to yield compounds (VI.9 or VI.10).

In a similar method, compounds (VI.11 and VI.14) were readily synthesised from the amines (VI.6) and (III.23, see Chapter III-2.2, Scheme III-3) respectively; and compound (VI.8) with the appropriate 4-chloro-7-substituted quinoline gave compounds (VI.12 and VI.13), respectively. The synthesis of compounds (VI.14a,b) was recently reported\textsuperscript{139} by Mannich reactions on 4-(7-chloroquinolin-4-yl)aminophenol.

VI-3 Physical Properties

VI-3.1 Nuclear Magnetic Resonance Spectra

(a) \textsuperscript{1}H Nuclear Magnetic Resonance Spectra

The structures of the di-Mannich nitro compounds (VI.3 and VI.4) were established from their \textsuperscript{1}H n.m.r. (see Experimental section). The spectral data of compound (VI.3) (Table VI-1) showed a doublet for H 5 at \(\delta\) 7.95 (due to the effect of a fluoro group); two multiplets, one for H 3', 4', 5' at \(\delta\) 1.57 and another one for H 2', 6' at \(\delta\) 2.51; and two singlets for the methylene protons at C-2 and C-6 at \(\delta\) 3.78 and 3.58, respectively. Structural assignments for compound (VI.4) were based on the
$^1$H n.m.r. which showed the presence of three multiplets (and appropriate integration) for H 3', 4', 5', 3", 4", 5"; H 2", 6" and H 2', 6' at $\delta$ 1.60, 2.49, and 2.93, respectively. The peak due to H 5 appeared as a singlet at $\delta$ 7.59, and two singlets for the methylene protons at C-2 ($\delta$ 3.82) and C-6 (3.48).

The $^1$H n.m.r. data of the mono-Mannich nitro compound (VI.7) (Table VI-1) gave signals for the methylene protons at C-6 ($\delta$ 3.74); and the two singlets for H 3 and H 5 (both ortho to the nitro group) appeared at $\delta$ 8.11 and 7.80, respectively. The signal due to H 3 (adjacent to the $t$-butyl group) was further downfield of that due to H 5 (adjacent to the piperidin-1-ylmethyl group). This is due to the $t$-butyl group which has a weaker electron-donating effect compared to the piperidin-1-ylmethyl group. The assignment of the structure of this compound was confirmed by running a 2-D COSY spectrum in which the coupling between H 5 and the methylene protons at C-6 was observed.

Table VI-1. Chemical shift ($\delta$)$^a$ of some Mannich base derivatives of 2(and 3)-substituted-4-nitrophenols.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Bu$^t$</th>
<th>H 2', 6</th>
<th>H 3', 4', 5'</th>
<th>2-CH$_2$</th>
<th>6-CH$_2$</th>
<th>H 3</th>
<th>H 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>VI.3</td>
<td>-</td>
<td>2.51 (m)</td>
<td>1.57 (m)</td>
<td>3.78 (s)</td>
<td>3.58 (s)</td>
<td></td>
<td>7.94 (d)</td>
</tr>
<tr>
<td>VI.7</td>
<td>1.42</td>
<td>2.50 (m)</td>
<td>1.68 (m)</td>
<td></td>
<td>3.74 (s)</td>
<td>8.11 (d)</td>
<td>7.80 (d)</td>
</tr>
</tbody>
</table>

$^a$ Reported as parts per million ($\delta$) downfield from tetramethylsilane (TMS) as internal standard in deuterchloroform (CDCl$_3$) solution.
Examination of the $^1$H n.m.r. spectra, in the Experimental section for the compounds (VI.14a - VI.14c), revealed that the methylene group in all Mannich side chains appeared as a singlet and that its position was dependent on the amine present in the Mannich base (a similar situation prevailed for the 2-aminophenols, see Chapter II-3.1). For compound (VI.14a) which contained the diethylamino substituent it occurred at $\delta$ 3.73, for the pyrrolidinyl substituent at $\delta$ 3.76 and for the piperidinyl substituent at $\delta$ 3.61. The signals due to H 3 and H 5 in all these compounds appeared as a singlet at $\delta$ 7.02 - 7.09. The signals due to the quinoline nucleus for compounds (VI.14a-c) are consistent with the assignments made in Chapters II-3.1 and IV-3.1.

The $^1$H n.m.r. spectra of compounds (VI.9-VI.13) are given in the Experimental section. Additional n.m.r. data relating to compound (VI.11) are given in Figures VI-1 - VI.6. The assignments in compound (VI.11) were confirmed by the 2-D COSY spectrum (Figures VI-4 - VI-6) which revealed coupling between H 5 and $H_2''', H_6'''$ and $H_3'', H_5'''$. the methylene protons at C-$\phi$. The assignments in the spectrum of compound (VI.9) were similarly confirmed.

(b) $^{13}$C Nuclear Magnetic Resonance Spectra

The $^{13}$C n.m.r. spectrum of 2-tert-butyl-6-(piperidin-1'-ylmethyl)-4-(7''-trifluoromethylquinolin-4''-ylamino)phenol (VI.13) has been determined and the results are shown in Figure VI-7.

The assignments of the $^{13}$C chemical shifts were based on a decoupled $^{13}$C n.m.r. spectrum, 2-D HETCOR and LRHETCOR spectra. The chemical shift of carbon atoms connecting with one or more protons were assigned from the HETCOR spectrum, other carbons such as quarternary carbons (not connected with any protons) were assigned from the LRHETCOR spectrum.

Comparison of the $^{13}$C chemical shifts of the quinoline nucleus in compound (VI.13) (Figure VI-7) with those of quinoline itself$^{140}$ (Figure VI-8) revealed that all carbon atoms in the benzene ring in VI.13 were upfield of the corresponding atoms by 0.16 - 8.0 ppm. The carbon C 4'' in VI.13 was downfield of that in quinoline by 13.8
Figure VI-1

$^{1}H$ n.m.r. spectrum of 4-(7'-chloroquinolin-4'-ylamino)-3-(piperidin-1'-yl)-2,6-bis(piperidin-1'-ylmethyl)phenol (VI.11) in CDCl$_3$
Figure VI-2: Expanded upfield region of $^1$H n.m.r. spectrum of compound (VI.11) shown in Figure VI-1.
Figure VI-3: Expanded aromatic region of $^1$H n.m.r. spectrum of compound (VI.11) shown in Figure VI-1.
Figure VI-4  2-D COSY spectrum of 4-(7'-chloroquinolin-4'-ylamino)-3- (piperidin-1'-yl)-2,6-bis(piperidin-1'''-ylmethyl)phenol (VI.11) in CDCl₃
Figure VI-5  Expansion of Figure VI-4 at region A
Figure VI-6  Expansion of Figure VI-4 at region B
ppm, but C 3" was upfield by 18.6 ppm, presumably due to the 4-(substituted amino) group. The carbon of the trifluoromethyl group appeared as a quartet at δ 130.8, J 32 Hz (due to the strong electron-withdrawing fluoro substituents).

Figure VI-7 The chemical shift of carbon atoms in 2-t-butyl-6-(piperidin-1'-ylmethyl)-4-(7'M-trifluoromethylquinolin-4'-ylamino)phenol (VI.13)

Figure VI-8 The chemical shifts (ppm) of the carbon atoms in quinoline.¹⁴⁰
VI-3.2 Mass Spectra

The mass spectra of compounds (VI.9, VI.11 and VI.14c) each showed two molecular ion peaks in the ratio 3:1. This is characteristic of molecules containing one chlorine atom and corresponds to the isotopic ratios. The major cleavage appeared at the N-C benzylic bond, due to the loss of one or two C₅H₁₀N (piperidinyl) group(s) from the molecular ion. This fragmentation pattern is consistent with that of 2-(7'-chloroquinolin-4'-ylamino)-4,6-bis(piperidin-1''ylmethyl)phenol (II.40e), 2,6-bis(piperidinyl-1'-ylmethyl)-4-(8''-trifluoromethylquinolin-4''-ylamino)phenol (III.24d) and 4-t-butyl-6-(piperidin-1'-ylmethyl)-2-(7''-trifluoromethylquinolin-4''-ylamino)phenol (IV.11) examined in previous chapters.

VI-4 Antimalarial Activity

The compounds prepared in this chapter were tested in vitro for antimalarial activity against *P. falciparum* (FCQ-27) by using both the microscopic and the visual tests. The more active compounds therein (IC₅₀ < 50 nM) were then tested in the ³H-hypoxanthine binding assay (radioisotope technique) against both strains of *P. falciparum* (FCQ-27 isolate and K-1 isolate) as described in Chapter II-4.1.a and 5.3.a.

VI-4.1 Results of the in vitro testing

The results for these in vitro tests are given in Table VI-2.
Table VI-2 Results from preliminary screening of Mannich base derivatives of 2(and 3)-substituted-4-[7-chloro(and trifluoromethyl)quinolin-4-ylamino]phenol and 3-unsubstituted-4-(7-chloroquinolin-4-ylamino)phenol in the in vitro test against the FCQ-27 and K-1 isolates of *P. falciparum* by using the visual test and the radioisotope test

![Chemical structures](image)

(Where X, Y, and Z are specified below)

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Substituents</th>
<th>Visual test</th>
<th>Radioisotope test</th>
<th>Resistance factor&lt;sup&gt;C&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>FCQ-27 IC&lt;sub&gt;50&lt;/sub&gt;&lt;sup&gt;A&lt;/sup&gt;</td>
<td>CH-factor&lt;sup&gt;B&lt;/sup&gt;</td>
<td>FCQ-27 IC&lt;sub&gt;50&lt;/sub&gt;&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>X Y Z or NR&lt;sub&gt;2&lt;/sub&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VI.9</td>
<td>Cl F</td>
<td>CH&lt;sub&gt;2&lt;/sub&gt;N(CH&lt;sub&gt;2&lt;/sub&gt;)&lt;sub&gt;5&lt;/sub&gt;</td>
<td>≤ 25</td>
<td>≥ 1</td>
</tr>
<tr>
<td>VI.10</td>
<td>CF&lt;sub&gt;3&lt;/sub&gt; F</td>
<td>CH&lt;sub&gt;2&lt;/sub&gt;N(CH&lt;sub&gt;2&lt;/sub&gt;)&lt;sub&gt;5&lt;/sub&gt;</td>
<td>25-50</td>
<td>0.5-1</td>
</tr>
<tr>
<td>VI.11</td>
<td>Cl N(CH&lt;sub&gt;2&lt;/sub&gt;)&lt;sub&gt;5&lt;/sub&gt;</td>
<td>CH&lt;sub&gt;2&lt;/sub&gt;N(CH&lt;sub&gt;2&lt;/sub&gt;)&lt;sub&gt;5&lt;/sub&gt;</td>
<td>25-50</td>
<td>0.5-1</td>
</tr>
<tr>
<td>VI.12</td>
<td>Cl H</td>
<td>Bu&lt;sup&gt;t&lt;/sup&gt;</td>
<td>D</td>
<td>-</td>
</tr>
<tr>
<td>VI.13</td>
<td>CF&lt;sub&gt;3&lt;/sub&gt; H</td>
<td>Bu&lt;sup&gt;t&lt;/sup&gt;</td>
<td>D</td>
<td>-</td>
</tr>
<tr>
<td>VI.14a</td>
<td>- -</td>
<td>CH&lt;sub&gt;2&lt;/sub&gt;N(Et)&lt;sub&gt;2&lt;/sub&gt;</td>
<td>&lt; 25</td>
<td>&gt;1</td>
</tr>
<tr>
<td>VI.14b</td>
<td>- -</td>
<td>CH&lt;sub&gt;2&lt;/sub&gt;N(CH&lt;sub&gt;2&lt;/sub&gt;)&lt;sub&gt;4&lt;/sub&gt;</td>
<td>&lt; 25</td>
<td>&gt;1</td>
</tr>
<tr>
<td>VI.14c</td>
<td>- -</td>
<td>CH&lt;sub&gt;2&lt;/sub&gt;N(CH&lt;sub&gt;2&lt;/sub&gt;)&lt;sub&gt;5&lt;/sub&gt;</td>
<td>&lt; 25</td>
<td>&gt;1</td>
</tr>
<tr>
<td>Chloroquine&lt;sup&gt;E&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amodiaquine&lt;sup&gt;E&lt;/sup&gt;</td>
<td></td>
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</table>

<sup>A</sup> The IC<sub>50</sub> values (the concentration of the inhibitor required to reduce parasite growth by 50%) are expressed as nmol l<sup>-1</sup> (nM).

<sup>B</sup> The CH-factor (Chloroquine factor) is the comparative activity of the inhibitor under test compared to chloroquine; and is the ratio of the IC<sub>50</sub> value for chloroquine over that for the inhibitor.

<sup>C</sup> Resistance factor is the IC<sub>50</sub> value against the K-1 isolate divided by that for the FCQ-27 isolate.

<sup>D</sup> No significant activity at 200 nM. <sup>E</sup> As diphosphate salt. <sup>F</sup> As hydrochloride.
VI-4.2 Discussion of Results

The results in Table VI-2 for compound VI.14a reveal that in \textit{in vitro} tests against the K-1 isolate of \textit{P. falciparum} it was more active than its mono-Mannich analogue, amodiaquine. The IC$_{50}$ values were 6.3 - 12.5 nM and 30 nM respectively (the differences were less for the FCQ-27 isolate). This is consistent with the results published by Scott, Tan and Barlin$^{57}$ for comparable tests with their 7-trifluoromethyl analogues in which the di-Mannich compound, 2,6-bis(diethylaminomethyl)-4-(7'-trifluoromethylquinolin-4'-ylamino)phenol had IC$_{50}$ 2.6 nM and the mono-Mannich compound, 2-diethylaminomethyl-4-(7'-trifluoromethylquinolin-4'-ylamino)phenol, had IC$_{50}$ 5.5 nM.

These results are qualitatively consistent with \textit{in vivo} data published since this work was commenced by Chen & Zheng$^{141}$ in which compound VI.14a and amodiaquine were tested against \textit{P. berghei} in mice. the ED$_{50}$ values were: 4.6 ± 1.2 and 45.0 ± 3.4 mg/kg respectively. Clearly the di-Mannich compound (VI.14a) was significantly more active. The ED$_{50}$ value obtained for compound (VI.14b) was 17.4 ± 1.4 mg/kg.

![Chemical Structure](image)

VI.15 $^{a}$ NR$_2$ = NET$_2$

$^{b}$ NR$_2$ = N(CH$_2$)$_4$

$^{c}$ NR$_2$ = N(CH$_2$)$_5$

Comparison of the \textit{in vitro} test results against \textit{P. falciparum} in Table VI-2 for the 7-chloro compounds (VI.14a-c) with those published$^{57}$ for their 7-trifluoromethyl analogues (VI.15a-c) [IC$_{50}$ values FCQ-27 isolate: 1.3, 0.9 and 0.7; K-1 isolate 2.8, 2.9 and 1.1 nM respectively] revealed that the trifluoromethyl compounds were more active. This indicated that replacement of the chloro-substituent by the similarly electron-withdrawing trifluoromethyl group, in this series, resulted in a beneficial effect.
Compound (VI.14c) (with piperidine in the Mannich side chain) was the most effective amongst the 4-(7-chloroquinolin-4-ylamino)phenols prepared in this work. In *in vitro* tests against the K-1 isolate of *P. falciparum* it had IC\textsubscript{50} 6.3 - 12.5 nM whereas that for chloroquine and amodiaquine were 584 and 30 nM respectively.

Amongst the compounds (VI.15) reported previously\textsuperscript{57} the di-Mannich compounds (VI.15c) was also the most active. Introduction of additional substituents into the phenol ring had differing effects. The small electron-withdrawing 3-fluoro-substituent in compound (VI.9) had little effect on antimalarial activity relative to compound (VI.14c), but comparison of the IC\textsubscript{50} values of the corresponding 7-trifluoromethyl compounds (VI.10) (in Table VI-2) and (VI.15c; values quoted above) revealed that the 3-fluoro derivative was appreciably less active. The large 3-(piperidin-1'-yl) group as in compound (VI.11) had a detrimental effect on activity relative to compound (VI.14c).

Replacement of one Mannich side chain in compounds (VI.14c) and (VI.15c) by the large t-butyl group [to give compounds VI.12 and VI.13] had a dramatic effect on activity; the IC\textsubscript{50} values in compounds (VI.12 and VI.13) increased (lower activity) to greater than 200 nM. This effect in compound (VI.12) [relative to compound (VI.14c)] is much greater than data in Table VI-2 shows between compound (VI.14a) and amodiaquine; and indicates that the t-butyl group has a significant detrimental effect. This observation is consistent with the lower activity of the 4-t-butyl compound (IV.10, IC\textsubscript{50} 50 - 100 nM, Table IV-2, Chapter IV-4.2) with that of its 4-(piperidin-1-ylmethyl) analogue (II.40e, IC\textsubscript{50} < 25 nM, Table II-3, Chapter II-4.1).

A comparison of the results on this series of compounds with the results obtained in previous chapters indicate generally that the di-Mannich base derivatives of 4-(7-chloroquinolin-4-ylamino)phenol exhibit higher antimalarial activity against *P. falciparum in vitro* than their 2(and 3)-aminophenol analogues.
VI-5 Experimental

The general procedure and experimental details for the antimalarial testing are recorded in Chapter II-5.1 and 5.3.

3-Fluoro-4-nitro-2,6-bis(piperidin-1'-ylmethyl)phenol (VI.3) and 4-Nitro-3-(piperidin-1'-yl)-2,6-bis(piperidin-1''-ylmethyl)phenol (VI.4)

To a chilled mixture of 3-fluoro-4-nitrophenol (2 g, 12.73 mmol) and paraformaldehyde (1.53 g, 50.92 mmol) in ethanol (8.0 ml) was added piperidine (4.34 g, 50.92 mmol) and the reaction mixture was refluxed in an oil bath at 100° for 20 h. The solvent was evaporated and the oily residue was subjected to column chromatography (alumina, 40 × 6 cm, 1% methanol in chloroform). Impurities and unchanged 3-fluoro-4-nitrophenol were eluted first and then a mixture of two di-Mannich products. This mixture was subjected to t.l.c. (alumina, 1% methanol in chloroform, twice) and gave as an oil in the band at higher RF a product which was identified as 4-nitro-3-(piperidin-1'-yl)-2,6-bis(piperidin-1''-ylmethyl)phenol (0.83 g, 15.6%). (Found: C 66.5; H, 8.7, N, 12.9. C_{23}H_{36}N_{4}O_{3} requires C, 66.3; H, 8.7; N, 13.4 %). \(^1\)H n.m.r. δ 1.60, m, H 3', 4', 5' and H 3'', 4'', 5''; 2.49, m, H 2'', 6''; 2.93, m, H 2', 6'; 3.48, s, 6-CH₂; 3.82, s, 2-CH₂; 7.59, s, H 5. The band at lower RF from the plate gave as a yellow solid 3-fluoro-4-nitro-2,6-bis(piperidin-1'-ylmethyl)phenol (1.43 g, 32%), m.p. 142-144° (Found: C, 61.6; H, 7.8; N, 11.7. C_{18}H_{26}FN_{3}O_{3} requires C,61.5; H, 7.5; N, 12.0%). \(^1\)H n.m.r. δ 1.57, m, H 3', 4', 5'; 2.51, m, H 2', 6'; 3.58, s, 6-CH₂; 3.78, s, 2-CH₂; 7.94, d, J 9 Hz, H 5.

4-(7'-Chloroquinolin-4'-ylamino)-3-fluoro-2,6-bis(piperidin-1''-ylmethyl)phenol (VI.9)

A mixture of 3-fluoro-4-nitro-2,6-bis(piperidin-1'-ylmethyl)phenol (0.63 g, 1.79 mmol), ethanolic ammonia (25 ml), ethanol (30 ml) and a catalytic amount of Raney nickel was shaken under hydrogen until uptake ceased. The catalyst was filtered off through Celite and the solvent was evaporated to leave the amine as an oil (0.59 g).
A mixture of the above amine (0.25 g, 0.77 mmol), 4,7-dichloroquinoline (0.154 g, 0.77 mmol), ethanol (8 ml) and water (2 ml) was adjusted with hydrochloric acid to pH 4.5 and then refluxed in an oil bath at 100° for 21 h. The solvent was evaporated under reduced pressure and the residue diluted with water and adjusted with ammonium hydroxide to pH 9-10. The mixture was extracted into chloroform, the extract dried (Na₂SO₄) and the solvent evaporated. The oily product was subjected to t.l.c. (alumina, 1% methanol in chloroform) and recrystallised from a mixture of hexane and ethyl acetate to give the title compound as a yellow solid (0.15 g, 40%), m.p. 144-146° (Found: C, 66.9; H 7.0; N, 11.3. C₂₇H₃₂ClFN₄O requires C, 67.1; H, 6.7; N, 11.6%). ¹H n.m.r. δ 1.58, m, H 3", 4", 5"; 2.53, m, H 2", 6"; 3.58, s, 6-CH₂; 3.75, s, 2-CH₂; 6.39, br, NH; 6.57, d, J₂",₃" 5.5 Hz, H 3'; 7.14, d, J 9 Hz, H 5; 7.45, dd, J₅',₆' 9 Hz, J₆',₇' 2 Hz, H 6'; 7.86, d, J₅',₆' 9 Hz, H 5'; 8.02, d, J 2 Hz, H 8'; 8.52, d, J₂',₃' 5.5 Hz, H 2'.

3-Fluoro-2,6-bis(piperidin-1'-ylmethyl)-4-(7'-trifluoromethylquinolin-4'"-ylamino)phenol (VI.10)

A mixture of 4-amino-3-fluoro-2,6-bis(piperidin-1'-ylmethyl)phenol (0.307 g, 0.95 mmol), 4-chloro-7-trifluoromethylquinoline (0.22 g, 0.95 mmol), ethanol (8.0 ml) and water (2.0 ml) was adjusted with concentrated hydrochloric acid to pH 4.5 and then refluxed in an oil bath at 100° for 21 h. Work up was as above and the product was subjected to t.l.c. (alumina, 1% methanol in chloroform) and crystallised from a mixture of hexane and ethyl acetate to give as a yellow solid the title compound (0.18 g, 36%), m.p. 174 - 176° (Found: C, 64.8; H, 6.5; N 10.5. C₂₈H₃₂F₄N₄O requires C, 65.1; H, 6.3; N, 10.8%). ¹H n.m.r. δ 1.49 - 1.69, m, H 3', 4', 5'; 2.52, m, H 2', 6'; 3.59, s, 6-CH₂; 3.75, s, 2-CH₂; 6.40, br, NH; 6.66, d, J₂",₃" 5.5 Hz, H 3'; 7.15, d, J 9 Hz, H 5; 7.68, dd, J₅',₆' 9 Hz, J₆',₇' 2 Hz, H 6'; 8.03, d, J₅',₆' 9 Hz, H 5'; 8.33, br s, H 8'; 8.6, d, J₂',₃' 5.5 Hz, H 2".
4-(7'-Chloroquinolin-4'-ylamino)-3-(piperidin-1''-yl)-2,6-bis(piperidin-1''''-ylmethyl)phenol (VI.11)

A mixture of 4-nitro-3-(piperidin-1'-yl)-2,6-bis(piperidin-1''-ylmethyl)phenol (0.25 g), ethanol (25 ml), ethanolic ammonia (25 ml) and Raney nickel was shaken with hydrogen until uptake ceased. The catalyst was filtered off on Celite and the solvent evaporated to give the crude amine (0.17 g). $^1$H n.m.r. δ 1.53, m, H 3', 4', 5' and H 3'', 4'', 5''; 2.49, m, H 2'', 6''; 2.98, m, H 2', 6'; 3.47, s, 6-CH$_2$; 3.71, s, 2-CH$_2$; 6.66, s, H 5.

A mixture of the above amine (0.17 g, 0.5 mmol), 4.7-dichloroquinoline (0.11 g, 0.5 mmol), ethanol (8.0 ml) and water (2.0 ml) was adjusted with concentrated hydrochloric acid to pH 4.5 and then refluxed in an oil bath at 100° for 24 h. Work up was as above and the product was subjected to t.l.c. (alumina, 1% methanol in chloroform, twice) and crystallised from a mixture of hexane and ethyl acetate to give yellow crysta$^5$of the title compound (0.09 g, 29%), m.p. 194 - 196° (Found: C, 69.6; H, 7.8, N, 12.3. C$_{32}$H$_{42}$ClN$_5$O requires C, 70.1; H, 7.7; N, 12.8%). $^1$H n.m.r. δ 1.58, m, H 3'', 4'', 5'' and H 3'': 2.48, m, H 2'', 6''; 2.98, m, H 2', 6'; 3.52, s, 6-CH$_2$; 3.77, s, 2-CH$_2$; 6.95, d, J$_{5',6'}$ 5.5 Hz, H 3': 7.31, s, H 5: 7.47, dd, J$_{5',6'}$ 9, J$_{6',8'}$ 2 Hz, H 6'; 7.61, br s, NH; 7.92, d, J$_{5',6'}$ 9 Hz, H 5'; 8.01, d, J$_{6',8'}$ 2 Hz, H 8'; 8.51, d, J$_{2',3'}$ 5.5 Hz, H 2'.

2-t-Butyl-4-nitro-6-(piperidin-1'-ylmethyl)phenol (VI.7)

Piperidine (2.61 g, 30.78 mmol) was added to a chilled mixture of 2-t-butyl-4-nitrophenol$^{137,138,*}$ (2 g, 10.26 mmol), paraformaldehyde (0.9 g, 30.78 mmol) and ethanol (12 ml), and the mixture was refluxed in an oil bath at 100° for 21 h. The solvent was evaporated and the product was recrystallised from a mixture of methanol, chloroform and ethyl acetate to give yellow solid of the title compound (2.6 g, 86%), m.p. 96 - 98° (Found: C, 65.5; H, 8.2; N, 9.4. C$_{16}$H$_{24}$N$_2$O$_3$ requires C, 65.7; H, 8.3; N, 9.6%). $^1$H n.m.r. δ 1.42, br s, Me$_3$C; 1.68, m, H 3', 4', 5'; 2.50, m, H 2', 6'; 3.74, s, CH$_2$; 7.80, d, J 3 Hz, H 5; 8.11, d, J 3 Hz, H 3.

* Kindly prepared by Mr S. Ireland
2-t-Butyl-6-(piperidin-1'-ylmethyl)-4-(7'-trifluoromethylquinolin-4'-ylamino)phenol (VI.13)

A mixture of 2-t-butyl-4-nitro-6-(piperidin-1'-ylmethyl)phenol (0.72 g), ethanol (30 ml), ethanolic ammonia (30 ml) and Raney nickel was shaken with hydrogen until uptake ceased. The catalyst was filtered off on Celite and the solvent evaporated to give the crude amine (0.62 g). $^1$H n.m.r. δ 1.40, br s, Me$_3$C; 1.62, m, H 3', 4', 5'; 2.49, m, H 2', 6'; 3.55, s, CH$_2$; 5.73, br, NH; 6.25, d, J 2.3 Hz, H 5; 6.60, d, J 2.7 Hz, H 3.

A solution of this amine (0.31 g, 1.18 mmol), 4-chloro-7-trifluoromethylquinoline (0.27 g, 1.18 mmol), ethanol (8 ml) and water (2.0 ml) was adjusted with concentrated hydrochloric acid to pH 4.5 and then refluxed in an oil bath at 98° for 22 h. Worked up was as above, the product was subjected to t.l.c. (alumina, chloroform) and recrystallised from a mixture of hexane and ethyl acetate to give the title compound (0.22 g, 40%) as a yellow solid, m.p. 185 - 187° (Found: C, 68.0; H, 6.7; N, 9.3. C$_{26}$H$_{30}$F$_3$N$_3$O requires C, 68.2; H, 6.6; N, 9.2%). $^1$H n.m.r. δ 1.41, br s, Me$_3$C; 1.65, m, H 3', 4', 5'; 2.46, m, H 2', 6'; 3.68, s, CH$_2$; 6.58, br, NH; 6.75, d, J$_{2'',3''}$ 5.5 Hz, H 3''; 6.83, d, J 2 Hz, H 5; 7.10, d, J 2 Hz, H 3; 7.65, dd J$_{5'',6''}$ 9, J$_6''$, 8'' 2 Hz, H 6 ''; 8.01, d, J$_{5'',6''}$ 9 Hz, H 5''; 8.31, br s, H 8''; 8.58, d, J$_{2'',3''}$ 5.5 Hz, H 2''.

4-(7'-Chloroquinolin-4'-ylamino)-2,6-bis(piperidin-1''-ylmethyl)phenol (VI.14c)

A mixture of 4-amino-2,6-bis(piperidin-1'-ylmethyl)phenol (0.31 g, 1.03 mmol), 4.7-dichloroquinoline (0.21 g, 1.03 mmol), ethanol (6 ml) and water (2 ml) was adjusted with hydrochloric acid to pH 4.7 and then refluxed at 98° for 24 hr. The solvent was evaporated under reduced pressure and the residue diluted with water and adjusted with sodium hydroxide to pH 9 - 10. The mixture was extracted into chloroform, the extract dried (sodium sulfate) and the solvent evaporated. The product was subjected to t.l.c. (alumina, 1.5% methanol in chloroform, twice) and recrystallisation from a mixture of hexane and ethyl acetate to give the title product as a
yellow solid (0.15 g, 30%), m.p. 141 - 143° (Found: C, 69.4; H 7.4; N, 12.0. C$_{27}$H$_{33}$ClN$_{4}$O requires C, 69.7; H, 7.2; N, 12.1%). $^1$H n.m.r. δ 1.56, m, H 3", 4", 5"; 2.46, m, H 2", 6"; 3.61, s, 2, 6-CH$_2$; 6.48, br s, NH(7); 6.63, d, J 5.5 Hz, H 3'; 7.02, s, H 3; 7.36, dd, H 6'; 7.76, d, J 10.5 Hz, H 5'; 7.99, d, J 2.4 Hz, H 8"; 8.45, d, J 5.5 Hz, H 2'.

In a similar manner to the preparations described above, the following compounds have been prepared.

2-t-Butyl-4-(7'-chloroquinolin-4'-ylamino)-6-(piperidin-1'-ylmethyl)phenol (VI.12)

This compound (47%) was obtained as a white solid (m.p. 216 - 218°), after t.l.c. (alumina, chloroform) and recrystallisation from a mixture of hexane, ethyl acetate and methanol. (Found: C, 70.9; H, 7.4; N, 9.5. C$_{25}$H$_{30}$ClN$_{3}$O requires C, 70.8; H, 7.2; N, 9.9%). $^1$H n.m.r. δ 1.44, br s, Me$_3$C; 1.69, m, H 3", 4", 5"; 2.55, m, H 2", 6"; 3.68, s, CH$_2$; 6.50, br, NH; 6.70, d, J$_{2',3'}$ 5.5 Hz, H 3'; 6.83, d, H 5; 7.09, d, H 3; 7.44, dd, J$_{5',6'}$ 9, J$_{6',8'}$ 2 Hz, H 6'; 7.85, d, J$_{5',6'}$ 9 Hz, H 5'; 8.01, br s, H 8'; 8.49, d, J$_{2',3'}$ 5.5 Hz, H 2'.

4-(7'-Chloroquinolin-4'-ylamino)-2,6-bis(pyrrolidin-1'-ylmethyl)phenol (VI.14b)

Compound (VI.14)$^{139}$ (34%) as a yellow solid, m.p. 183 - 185° (lit.$^{139}$ 183 - 184°), was obtained after t.l.c. (alumina, 2% methanol in chloroform; then alumina, 1% methanol in chloroform) and recrystallisation from a mixture of hexane and ethyl acetate. (Found: C, 68.7; H, 6.6; N, 12.5. Calc. for C$_{25}$H$_{29}$ClN$_{4}$O: C, 68.7; H, 6.7; N, 12.8%). $^1$H n.m.r. δ 1.83, m, H 3", 4"; 2.36, m, H 2", 5"; 3.76, s, 2, 6-CH$_2$; 6.50, br, NH (?); 6.61, d, J 5.5 Hz, H 3'; 7.02, s, H 3, 5; 7.36, dd, J 9 Hz, J 2 Hz, H 6'; 7.75, d, J 9 Hz, H 5'; 7.99, d, J 2 Hz, H 8'; 8.46, d, J$_{5,5}$ Hz, H 2'.

4-(7′-Chloroquinolin-4′-ylamino)-2,6-bis(diethylaminomethyl)phenol (VI.14a) (53%) was obtained as a yellow solid, m.p. 168 - 169° (lit.139 160 - 162°), after t.l.c. (alumina, 1% methanol in chloroform and recrystallisation from a mixture of hexane and ethyl acetate. (Found: C, 67.8; H, 7.3; N, 12.4. Calc. for C_{25}H_{33}ClN_{4}O: C, 68.1; H, 7.6; N, 12.7%). \textsuperscript{1}H n.m.r. δ 1.12, t, J 8 Hz, Me; 2.61, 9, J 7.5 Hz, CH\textsubscript{2}Me; 3.73, s, 2, 6-CH\textsubscript{2}; 6.54, br s, NH (?); 6.64, d, J\textsubscript{5.5} Hz, H 3′; 7.37, dd, J 9 Hz, J 2 Hz, H 6′; 7.77, d, J 9 Hz, H 5′; 7.99, d, J 2 Hz, H 8′; 8.45, d, J\textsubscript{5.5} Hz, H 2′.
CHAPTER VII
 CHAPTER VII  \(\alpha\)-(7-Bromo-1,5-naphthyridin-4-yl)-\(\alpha\)-(piperidin-2'-yl)\)methanol, its 1-Oxide and Related Compounds

VII-1 Introduction

Ohnmacht et al.\(^6\) in 1971, described the preparation and antimalarial activity of \(\alpha\)-(2,8-bistrifluoromethylquinolin-4-yl)-\(\alpha\)-(piperidin-2-yl)\)methanol (mefloquine) (VII.1; which is widely used as an antimalarial), and its 2,6 and 2,7-bistrifluoromethyl isomers.

Prior to the discovery of mefloquine, a series of 4-quinolinemethanols (VII.2)\(^{142-144}\) were shown to possess a high degree of antimalarial activity. Some reviews\(^{59,145}\) of this work indicated the notable changes in activity in this series were due to substituent variations in the aromatic ring.\(^6\) This data and the strong blood schizontocidal property, curative activity and low toxicity of 1,5-naphthyridine derivatives described in Chapter I-4.2 prompted the present work to synthesise the \(\alpha\)-(7-bromo-1,5-naphthyridin-4-yl)-\(\alpha\)-(piperidin-2-yl)\)methanol (VII.3) [and its 1'-oxide (VII.4)], which incorporated the 7-bromo-1,5-naphthyridine nucleus.
VII-2 Some Literature Preparations Relating to the Synthesis of 
\(\alpha\)-(Piperidin-2-yl)-\(\alpha\)-(quinolin-4-yl)methanols

VII-2.1 Some Background Literature

Ainley and King\textsuperscript{142} in 1938 described the syntheses of compounds (VII.5) 
\([R=R'=H; R=OMe, R'=H; R=H \text{ or } OMe, R'=\text{alkyl, allyl or crotenyl}]\) as shown in 
the two diastereoisomers of Scheme VII-1. In tests for antimalarial activity only compound (VII.5) 
\((R=OMe, R'=H)\) were found to be active against malaria parasites.

\(\alpha\)-(7-Chloroquinolin-4-yl)-\(\alpha\)-(piperidin-2-yl)methanol (VII.6) was synthesised 
by Senear \textit{et al.}\textsuperscript{146} using the improved\textsuperscript{147} procedure of Ainley and King.\textsuperscript{142}

A more convenient two-step synthesis of the \(\alpha\)-(2-arylquinolin-4-yl)-\(\alpha\)-(piperidin-2-yl)methanols (VII.9) from the corresponding quinoline-4-carboxylic acids (VII.7) was reported by Boykin \textit{et al.}\textsuperscript{144} in 1967. In this method, the second step 
involved a selective reduction of the intermediate compound (VII.8); catalytic 
hydrogenation reduced both the carbonyl and pyridinyl groups to afford compound 
(VII.9), whereas sodium borohydride reduced only the carbonyl group to give the 
\(\alpha\)-(pyridin-2-yl)methanols (VII.10) (Scheme VII-2).
Scheme VII-1

1. NaNH$_2$
2. 17% HCl

VII.5 [where R' = H]

VII.6
Patel and coworkers\textsuperscript{148} attempted the synthesis of \( \alpha-(7\text{-trifluoromethylquinolin-4-yl})\alpha-(\text{piperidin-2-yl})\text{methanol (VII.13)} \) (using the method described above by Boykin \textit{et al.}\textsuperscript{144}) but were unsuccessful. In this, the selective hydrogenation of the pyridin-2-yl ketone (VII.12) to compound (VII.13) did not proceed (Scheme VII-3).
VII-2.2 Syntheses of $\alpha$-(2,8-Bistrifluoromethylquinolin-4-yl)-$\alpha$-(piperidin-2-yl)methanol (Mefloquine)

Four different methods for the preparation of this compound have been described in the literature. The first method$^{68}$ (Scheme VII-4) involved the conversion of the quinolin-4-one (VII.14) by phosphoryl bromide into the 4-bromoquinoline (VII.15), and its conversion in the presence of butyllithium and carbon dioxide to the quinoline-4-carboxylic acid (VII.16). Addition of 2-pyridinyllithium to compound (VII.16) gave the pyridyl ketone (VII.17). Reduction of the latter compound (VII.17) with hydrogen over platinum oxide afforded a good yield of compound (VII.1).
The second method was reported by Adam-Molina\textsuperscript{149} (Hoffman-La Roche patent) in 1982 and involved the reaction of 6-bromohex-1-ene with potassium phthalimide in \(N,N\)-dimethylacetamide to give the \(N\)-(hex-5-en-1-yl)compound (VII.18). This compound (VII.18) was condensed with 4-bromo-2,8-bistrifluoromethylquinoline in the presence of tri-o-tolylphosphine and palladium acetate (a palladium-catalysed vinylation reaction) to afford compound (VII.19). Epoxidation of (VII.19) with \(m\)-chloroperoxybenzoic acid (\(m\)-CPBA) gave (VII.20) which on cyclisation, by treatment with hydrazine hydrate, gave mefloquine (VII.1) (Scheme VII-5)
The third method\textsuperscript{150} (Scheme VII-6) involved the application of the Wittig rearrangement of aryl ethers to arylcarbinols.\textsuperscript{151,152}
A fourth synthesis of mefloquine has also been reported recently by Adam,\textsuperscript{153} and involved the base-catalysed oxidative decyanation of a secondary nitrile to a ketone.\textsuperscript{154,155} The condensation of $\alpha$-(pyridin-2-yl)acetonitrile (VII.24) with the 4-chloroquinoline (VII.25) gave $\alpha$-(pyridin-2-yl)-$\alpha$-(quinolin-4-yl)acetonitrile (VII.26) in 91\% yield. This nitrile (VII.26) was converted to the ketone (VII.17) either by treatment with a combination of hydrogen peroxide and acetic acid, or through the cyanhydride (VII.27) which was subjected to base hydrolysis (Scheme VII-7). Catalytic hydrogenation of compound (VII.17) over platinum oxide (as reported above) gave the desired product (VII.1)
Various optically active isomers of mefloquine were tested for antimalarial activity, but the results show no significant differences between isomers.\textsuperscript{150}

\textbf{VII-2.3 Palladium-catalysed Vinylation of Organic Halides}

In the second synthesis of mefloquine described above, the method\textsuperscript{149} involved a palladium-catalysed vinylic substitution reaction with a heterocyclic halide. This reaction provides a very convenient method for carbon-carbon bond formation at unsubstituted vinylic positions. The general reaction\textsuperscript{157} is shown in Scheme VII-8.
A variety of organophosphine palladium complexes have been used as catalysts in this type of reaction,\textsuperscript{157} and the base required may be a secondary\textsuperscript{158} or tertiary amine\textsuperscript{159,160} or sodium bicarbonate.\textsuperscript{161,162}

A mechanism of the reaction has been proposed by Heck\textsuperscript{157} (Scheme VII-9) as follows.

The reaction is catalysed by palladium in the presence of base because compound (VII.30) dissociates reversibly and the base shifts the equilibrium to the palladium (0) species (VII.28). Compound (VII.28) reacts with the organic halide (VII.29) and the cycle is repeated.\textsuperscript{157}

Many reports of palladium-catalysed vinylations of organic halides appear in the literature\textsuperscript{157,160-164} and a review of this reaction has been published.\textsuperscript{157}
Catalyst formation:

\[
PdX_2 + \begin{array}{c}
\text{C} \\
\text{=C} \\
\end{array} + 2L & \rightarrow PdL_2 + HX + \begin{array}{c}
\text{C} \\
\text{=C} \\
\end{array}
\]

(CVII.28)

Catalytic cycle:

\[
PdL_2 + RX \rightarrow \text{RPdL}_2X
\]

(CVII.28) (CVII.29)

\[
\text{RPdL}_2X + \begin{array}{c}
\text{C} \\
\text{=C} \\
\end{array} \rightarrow \begin{array}{c}
\text{R} \\
\text{C} \\
\text{=C} \end{array} \text{PdL}_2X
\]

\[
\begin{array}{c}
\text{H} \\
\end{array} \rightarrow \begin{array}{c}
\text{R} \\
\text{C} \\
\text{=C} \end{array} \text{PdL}_2X \rightarrow \begin{array}{c}
\text{C} \\
\text{=C} \\
\end{array} + \text{HPdL}_2X
\]

(CVII.30)

\[
\text{HPdL}_2X + \text{Base} \rightarrow \text{PdL}_2 + \text{Base} + H^+X^-
\]

(CVII.28)

VII-3 Syntheses of \(\alpha-(7\text{-Bromo-1,5-naphthyridin-4-yl})\)\(\alpha\)-(piperidin-2'-yl)methanol and Related Compounds

The above compounds required in this work were prepared by a method based, in part, on that described by Adam-Molina\textsuperscript{149} for the synthesis of mefloquine which is summarised in Section VII-2.2. The details of this procedure are illustrated in Scheme VII-10 and are discussed below.
**VII-3.1 Synthesis of α-(7-Bromo-1,5-naphthyridin-4-yl)-α-(piperidin-2'-yl)methanol 1-oxide**

6-Bromohex-1-ene with potassium phthalimide\(^{165}\) in dry \(N,N\)-dimethylacetamide at room temperature, gave the vinyl compound\(^{149}\) (VII.18). This compound (VII.18) with 4,7-dibromo-1,5-naphthyridine (VII.31) and a mixture of palladium acetate, tri-o-tolyphosphine, sodium iodide and tributylamine in DMF at 100° for 30 h gave compound (VII.32). When compound (VII.32) was treated with excess \(m\)-chloroperoxybenzoic acid (ca 3 equivalents) in refluxing chloroform, it gave compound (VII.33) (when the oxidation was carried out with 1 equivalent of peracid it gave \(N\)-oxidation without oxidation at the double bond). Hydrolysis and cyclisation of compound (VII.33) with hydrazine hydrate in methanol at reflux gave the α-(7-bromo-1,5-naphthyridin-4-yl)-α-(piperidin-2'-yl)methanol 1-oxide (VII.4)

The \(^1\)H n.m.r. spectra of the \(N\)-(hex-5-en-1-yl)phthalimide (VII.32), the oxiran-2'-ylbut-1-ylphthalimide (VII.33), and the α-(naphthyridin-4-yl)-α-(piperidin-2-yl)methanol 1-oxide (VII.4) are consistent with their structures. Compound (VII.32) showed \(H\) 5 as a doublet of triplets at \(\delta\) 6.78 with J 16 Hz indicating it existed as the \(trans\)-isomer; and \(H\) 6 was at lower field than \(H\) 5. (Cis-olefinic coupling constants have been reported to occur in the range 7-11 Hz and the \(trans\)-olefinic coupling constant in the range 12 - 18 Hz).\(^{166}\) As is to be expected, the \(^1\)H n.m.r. of compound (VII.33) did not show signals for olefinic protons as occur in the spectrum of (hex-5-en-1-yl)phthalimide (VII.32; \(H\) 5 at \(\delta\) 6.60 - 6.91 and \(H\) 6 at \(\delta\)7.50). However it did show the presence of two multiplets at \(\delta\) 1.76 and 3.74 integrating for six protons and two protons respectively, corresponding to the butyl linkage; two doublets at \(\delta\) 7.36 and 8.48, \(J\) 6 Hz (\(H\) 3" and \(H\) 2"), a multiplet at \(\delta\) 7.78, integrating for four protons (phthalimido), and two singlets at \(\delta\) 8.92 and 9.21, corresponding to \(H\) 8" and \(H\) 6" respectively. The oxiran protons \(H\) 2' and \(H\) 3' appeared as a multiplet and a singlet at \(\delta\) 2.94 and 4.62 respectively.

Comparison of the \(^1\)H n.m.r. spectra of compounds (VII.32 and VII.33) also revealed that, the signal for \(H\) 2" at \(\delta\) 8.48 in the 1-oxide VII.33 was upfield of that in
Scheme VII-10

VII.18 + VII.31

\[ \text{Pd(OAc)}_2 (o\text{-tolyl})_3P \]

VII.32 → m-CPBA

VII.33

VII.4 → VII.3
compound \(\text{VII.32}\) (\(\delta 8.87\)) whereas the signal for H 8" in the 1-oxide (\text{VII.33}) at \(\delta 8.92\) was significantly downfield of that in compound (\text{VII.32}) at \(\delta 8.54\).

The \(^1\text{H} \text{n.m.r.}\) spectrum of the methanol \(N\)-oxide \text{VII.4} clearly revealed the absence of the signals for phthalimide and oxiran protons as shown in compound \text{VII.33}, the appearance of signals for the piperidinyl protons, \(\delta 5.52\); H 2', 6', \(\delta 1.19\) - 1.74, H 3', 4', 5, and methine protons (CHOH, \(\delta 2.53\) - 3.16), distinguished it from the oxiranphthalimide (\text{VII.33}). (As expected, the spectra of these compounds indicated only small differences for the protons on the naphthyridine ring. These differences were 0.01 - 0.36 ppm)

\textbf{VII-3.2 Preparation of } \alpha-(7-Bromo-1,5-naphthyridin-4-yl)-\alpha-(piperidin-2'-yl)methanol

The removal of the \(N\)-oxide group from compound (\text{VII.4}) has been achieved, but in poor yield, by two reductive procedures. Compound (\text{VII.4}) with aqueous sodium dithionite in methanol at reflux for 45 min. gave a product which differed in physical properties from (\text{VII.4}). It has different Rf values on t.l.c. (alumina, 10% methanol in chloroform), different \(^1\text{H} \text{n.m.r.}\) spectrum and mass spectrum. After recrystallisation from cyclohexane, it analysed correctly for compound (\text{VII.3}).

When compound (\text{VII.4}) was shaken in methanol with Raney nickel and hydrogen,\(^{167}\) a mixture of products was obtained from which, after t.l.c., a solid was isolated with \(^1\text{H} \text{n.m.r.}\) identical to the compound obtained for sodium dithionite reduction.

The \(^1\text{H} \text{n.m.r.}\) spectrum of this compound (\text{VII.3}) revealed the signal for H 2 at \(\delta 8.95\) (was further downfield than H 6, \(\delta 8.90\)) and was also downfield of that in its \(N\)-oxide (\text{VII.4}) (\(\delta 8.50\)), whereas the signal for H 8 in compound (\text{VII.3}) at \(\delta 8.60\) was significantly upfield of that in the 1-oxide (\text{VII.4}) at 8.94.
VII-4 Antimalarial Activity

Compound (VII.3) reported in this chapter was tested \textit{in vitro} for antimalarial activity using the visual test as outlined in Chapter II-5. The result for this compound (VII.3) is shown in Table VII-1 together with those for its 7-trifluoromethylquinoline analogue (VII.13) and its \textit{N}-oxide (VII.34) (which were obtained by others), mefloquine, and chloroquine.

![Chemical structures of VII.13 and VII.34](image)

Table VII-1 \textit{In vitro} antimalarial activity of \(\alpha-(7\text{-bromo-1,5-naphthyridin-4-yl})\text{-}\alpha\text{-}(piperidin-2-yl)methanol\) (VII.3) and related compounds (VII.13 and VII.34) against \textit{P. falciparum}.

Results (IC\(_{50}\) values) are expressed as nmol l\(^{-1}\) (nM)

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>IC(_{50}) values Visual test</th>
<th>IC(_{50}) values (\text{(^3)H-hypoxanthine method})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FCQ-27</td>
<td>FCQ-27</td>
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</table>

<p>| | | |</p>
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<thead>
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<th></th>
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<tbody>
<tr>
<td>VII.3</td>
<td>A</td>
<td>-</td>
</tr>
<tr>
<td>VII.13(^B)</td>
<td>A</td>
<td>&gt;80(^C)</td>
</tr>
<tr>
<td>VII.34(^B)</td>
<td>A</td>
<td>&gt;80(^C)</td>
</tr>
<tr>
<td>Mefloquine</td>
<td>-</td>
<td>10.1(^D)</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>20-25</td>
<td>23</td>
</tr>
</tbody>
</table>

\(A\) No significant activity at 200 nM.

\(B\) G.B. Barlin and C. Jiravinyu, unpublished results.

\(C\) No activity at 80 nM.

The results in Table VII-1 revealed that compounds VII.3, VII.13 and VII.34, when subjected to the visual test against the chloroquine-sensitive (FCQ-27) isolate, all gave IC\(_{50}\) > 200 nM. In the \( ^3\)H-hypoxanthine test, compounds (VII.13 and VII.34) against the FCQ-27 isolate gave IC\(_{50}\) values > 80 nM but against the chloroquine-resistant K-1 isolate the IC\(_{50}\) values were 99 and 172 nM respectively. Compared to the 2,8-bis(trifluoromethylquinolin-4-yl) analogue (mefloquine, IC\(_{50}\) values of 10.1 and 4.9 nM against the FCQ-27 and K-1 isolates, respectively), compound (VII.13) was much less active. This is in contrast with the antimalarial activity of some analogous derivatives of 4-aminophenol, such as the Mannich base derivatives of 4-(7-monotrifluoromethylquinolin-4-yl)phenol,\(^{52,53,56,57}\) which were highly active and were much more active than the 2,8(or 2,7)-bis(trifluoromethyl)\(^{52}\) analogues.

Whereas the Mannich base derivatives of 2\(^{58,135}\)(and 4)\(^{52,53,56,57}\)-[7-chloro(and trifluoromethyl)quinolin-4-ylamino]phenol (discussed in Chapters II and VI) and of 2\(^{135}\)(and 4)\(^{53,56,57,65}\)-[7-chloro(bromo and trifluoromethyl)-1,5-naphthyridin-4-ylamino]phenol have been shown to possess high antimalarial activity, the compounds (VII.3 and VII.13) were much less active, and this may indicate a different mechanism of action.
N-(Hex-5-en-1-yl)phthalimide (VII.18)

Potassium phthalimide\(^{165}\) (7.5 g) and 6-bromohex-1-ene (5 ml) in dry \(N,N\)-dimethylacetamide (40 ml) were stirred at room temperature for 23 h. The mixture was extracted with ether and the crude product (9.4 g) was subjected to column chromatography [alumina; 5% ethyl acetate in light petroleum (b.p. 60 - 80°)] to give the title compound (9.2 g), m.p. 26 - 28° (lit.\(^{149}\) 17 - 20°) (Found, for a sample dried at 20°/0.2 mmHg for 18 h: C, 73.1; H, 6.9; N, 5.8. Calc. for \(\text{C}_{14}\text{H}_{15}\text{NO}_{2}\): C, 73.3; H, 6.6; N, 6.1%). \(^1\)H n.m.r. \(\delta\) 1.50, complex, H 2, 3; 2.06, complex, H 4; 3.70, t, \(J\) 6 Hz, H 1; 4.95, d, \(J_{6,5}\) 9 Hz, 4.99, \(J_{5,6}\) 16 Hz, H 6; 5.53 - 6.08, complex, H 5; 7.78, complex, ArH.

N-[6-(7'-Bromo-1',5'-naphthyridin-4'-yl)hex-5-en-1-yl]phthalimide (VII.32)

4,7-Dibromo-1,5-naphthyridine\(^*\) (2 g), N-(hex-5-en-1-yl)phthalimide (1.76 g), palladium acetate (0.031 g), tri-o-tolylphosphine (0.083 g), sodium iodide (0.055 g) and tributylamine (2 ml) in \(N,N\)-dimethylformamide (5 ml) were heated at 100° under nitrogen for 39 h. The mixture was diluted with water, extracted with ethyl acetate, the extract was washed with 0.1 N hydrochloric acid, neutralised with saturated sodium bicarbonate solution, washed with 10% sodium chloride solution, dried (\(\text{Na}_2\text{SO}_4\)) and evaporated. The crude product was purified by column chromatography (alumina, 20% ethyl acetate-hexane) and recrystallised from ethanol to give white crystals of the title compound (0.85 g), m.p. 116 - 118° (Found: C, 60.6; H, 4.5; Br, 18.5; N, 9.7. \(\text{C}_{22}\text{H}_{18}\text{BrN}_{3}\text{O}_{2}\) requires C, 60.7; H, 4.2; Br, 18.3; N, 9.6%). \(^1\)H n.m.r. \(\delta\) 1.71, complex, H 2, 3; 2.44, complex, H 4; 3.76, t, \(J\) 6 Hz, H 1; 6.78, dt, \(J\) 16 Hz, H 5; 7.50, d, \(J\) 16 Hz, H 6; 7.61 - 7.80, complex, phthalimido H and H 3'; 8.54, d, \(J\) 2 Hz, H 8'; 8.86, d, \(J\) 5.5 Hz, H 2'; 8.94, d, \(J\) 2 Hz, H 6'. MS m/z 437 (73%), 435 (75%) (\(\text{M}^+\)), 356 (18%), 290 (13%), 288 (15%), 277 (15%), 275

\(^*\) Prepared by Dr C. Jiravinyu.
(19%), 263 (33%), 261 (36%), 249 (92%), 247 (100%), 235 (56%), 233 (55%), 160 (46%).

*N-{4-[3'-(7''-Bromo-1''',5''-naphthyridin-4''-yl)oxiran-2'-yl]but-1-yl}phthalimide 1''-oxido (VII.33)*

*N-[6-(7'-Bromo-1',5'-naphthyridin-4'-yl)hex-5-en-1-yl]phthalimide (0.83 g, 1.9 mmol) and *m*-chloroper oxybenzoic acid (80%, 1.31 g, 6.09 mmol) in chloroform (6 ml) were refluxed at 70° for 6 h. The white precipitate was filtered off and the filtrate was washed with 10% sodium sulfite solution, saturated sodium bicarbonate solution, 10% sodium chloride solution, and the extract dried (Na$_2$SO$_4$) and the solvent evaporated. The crude product was recrystallised from a mixture of methanol and chloroform to give the **title compound** (0.48 g), m.p. 185 - 187° (Found: C, 56.5; H, 3.9; Br, 17.2; N, 8.8. C$_{22}$H$_{18}$BrN$_3$O$_4$ requires C, 56.4; H, 3.9; Br, 17.1; N, 9.0%).

$^1$H n.m.r. δ 1.76, complex, H 2,3,4; 2.94, complex, H 2'; 3.74, t, J 6 Hz, H 1; 4.62, d, J 2 Hz, H 3'; 7.36, d, J$_{2''}$,3'' 6 Hz, H 3''; 7.78, complex, phthalimido H; 8.48, d, J$_{2''}$,3'' 6 Hz, H 2''; 8.92, d, J$_6''$,8'' 2 Hz, H 8''; 9.21, d, J$_6''$,8'' 2 Hz, H 6''.

**α-(7-Bromo-1,5-naphthyridin-4-yl)-α-(piperidin-2'-yl)methanol 1-oxide (VII.4)**

*N-{4-[3'-(7''-Bromo-1''',5''-naphthyridin-4''-yl)oxiran-2'-yl]but-1-yl}phthalimide 1''-oxido (0.48 g) and hydrazine hydrate (0.25 ml) in methanol (4 ml) were heated at 100° for 8 h. The solvent was evaporated, water (8 ml) and concentrated hydrochloric acid (0.25 ml) were added and the mixture was refluxed for 6 h and cooled. The precipitate of phthalhydrazide was filtered off and the filtrate made alkaline with 1 M ammonium hydroxide, extracted with chloroform, extract washed with 10% aqueous sodium chloride, dried (Na$_2$SO$_4$) and evaporated. The residual product was subjected to t.l.c. (alumina, 6% methanol in chloroform) and recrystallised from ethyl acetate to give yellow crystal of the **title compound** (0.06 g), m.p. 150 - 151° (Found: C, 50.0; H, 4.8; N, 12.2. C$_{14}$H$_{16}$BrN$_3$O$_2$ requires C, 49.7; H, 4.8; N, 12.4%).
\[ \text{H n.m.r. } \delta 1.23, 1.56, \text{ complex, } H 3', 4', 5'; 2.76, \text{ complex, } H 2', 6'; 3.13, 3.26, \text{ br, OH, NH(?)}; 5.52, \text{ d, J 5 Hz, CHOH}; 7.71, \text{ d, J 6 Hz, H 3}; 8.50, \text{ d, J 6 Hz, H 2}; 8.94, \text{ d, J 2 Hz, H 8}; 9.22, \text{ d, J 2 Hz, H 6}. \text{ MS (Cl) m/z 340 (25%), 338 (25%)} (M+1), 324 (85%), 322 (100%), 305, 303, 239, 237; (El) 210, 208 (10%), 183, 181 (6%), 102 (10%), 84 (100%).

\( \alpha-(7\text{-Bromo-1,5-naphthyridin-4-yl})-\alpha-(\text{piperidin-2'-yl})\text{methanol (VII.3)} \)

**Method A**

To a solution of \( \alpha-(7\text{-bromo-1,5-naphthyridin-4-yl})-\alpha-(\text{piperidin-2'-yl})\text{methanol 1-oxide} \) (0.045 g) in methanol (3.0 ml) was added an aqueous solution of sodium dithionite (0.765 g) in water (5.0 ml) and the mixture heated with stirring in an oil bath at 60° for 45 min.

The mixture was cooled, diluted with water (2.0 ml) and adjusted with 10 M sodium hydroxide to pH 12. It was then extracted with chloroform (5 x 40 ml), extracted dried (Na\(_2\)SO\(_4\)), solvent evaporated, and the product subjected to t.l.c. (alumina; developed twice with chloroform and then with 10% methanol in chloroform). The major dark band at \( R_f \) ca 0.7 in 10% methanol in chloroform was extracted with methanol and the product recrystallised from cyclohexane with filtration (twice) and concentration to a small volume. The **title compound** separated as white crystals (0.004 g), m.p. 180 - 181° (Found, for a sample dried at 110°/710 mmHg for 5 h: C, 52.3; H, 5.0; N, 12.7. C\(_{14}\)H\(_{16}\)BrN\(_3\)O requires C, 52.2; H, 5.0; N, 13.0%).

\[ \text{H n.m.r. } \delta 1.25, 1.55, \text{ complex, } H 3', 4', 5'; 2.33, \text{ complex, } H 2', 6'; 3.49, 3.75, \text{ br, OH, NH (?)}; 5.41, \text{ d, J 5 Hz, CHOH}; 7.74, \text{ d, J 6 Hz, H 3}; 8.59, \text{ d, J 2 Hz, H 8}; 8.90, \text{ d, J 2 Hz, H 6}; 8.95, \text{ d, J 6 Hz, H 2}. \text{ MS (Cl) m/z 324 (100%), 322 (88%), 305 (25%), 303 (25%), 240 (55%), 238 (55%); (El) 305, 303 (1%), 240, 238 (5%), 84 (100%).} \]
Method B

A solution of α-(7-bromo-1,5-naphthyridin-4-yl)-α-(piperidin-2'-yl)methanol 1-oxide (0.01 g), methanol (7.0 ml) and a catalytic amount of Raney nickel was shaken under hydrogen for 3 h. The catalyst was filtered onto Celite (twice) and the solvent was evaporated. The residual product was subjected to t.l.c. (alumina, 10% methanol in chloroform) and gave a white solid which had $^1$H n.m.r. similar to the product obtained from (A).
CHAPTER VIII
CHAPTER VIII Computerised Molecular Modelling, and DNA

Intercalation Studies of Some Mannich Base Derivatives of 2(3 and 4)-(7-Chloroquinolin-4-ylamino)phenols and Their Analogues

VIII-1 Computerised Molecular Modelling

VIII-1.1 General Introduction

Computer modelling has recently become important as an aid to a more rational approach to drug design. The concepts of receptor mapping and pharmacophores have been introduced to facilitate the understanding of forces and properties involved in drug-receptor interactions. These concepts are especially important in cases where the receptor structure is not known.

There are two categories of structure-activity relationships based on how the chemical structure is represented: (1) the topological and (2) the geometric model method. The 'topological' method involves the use of a two-dimensional chemical structure of the molecule. The approach used in the current study is the second method known as geometric or 3-D modelling. This takes into account the fact that molecules are three-dimensional. Incorporated to this method is the 'lock and key' concept which defines drug molecules as keys that exert their effect by binding to the receptors (locks). In reality, drug molecules have a number of physiochemical properties which need to be considered whereas keys have a definite physical shape.

In this chapter a hypothetical model for a malaria receptor capable of binding 4-aminoquinolines was deduced by conformational analysis and receptor mapping. Receptor mapping is an indirect approach to determine the structure of a receptor binding site. This approach aims to evaluate the structure of a receptor binding site by regarding it as complementary to drugs which are known to fit the receptor. The

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\(^a\) This work was carried out under the supervision of Dr Margaret Wong in The Chemistry Department, Swinburne University, Hawthorn, Victoria.
Figure VIII-1  Structures of the eight antimalarial agents used as a basis for this study. Examples of some rotatable bonds used in conformational analysis are also shown.
analogues used in this study (amodiaquine and seven compounds which I have prepared and presented in previous chapters) are shown in Figure VIII-1, and their biological activities are listed in Table VIII-1 (For convenience the compounds selected for study in this chapter have been renumbered). These compounds were selected for these studies because compounds (VIII.1-VIII.4) had significant antimalarial activity whereas compounds (VIII.5-VIII.8) had lower activities, even though all compounds possessed some structural similarities. The latter four compounds will be regarded as relatively inactive for the purpose of this study. The investigation was carried out using a Silicon graphics computer with SYBYL171 software. Superimposition and volume analysis were employed with the analogues to deduce a model based on the concepts of receptor mapping.

Table VIII-1  *In vitro* antimalarial activity of compounds (VIII.1 - VIII.8)*a* against chloroquine-sensitive (FCQ-27) and chloroquine-resistant (K-1) strains of *P. falciparum*.

<table>
<thead>
<tr>
<th>Compound</th>
<th>FCQ-27 isolate</th>
<th>K-1 isolate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IC$_{50}$b</td>
<td>IC$_{50}$b</td>
</tr>
<tr>
<td>VIII.1</td>
<td>6.3 - 12.5</td>
<td>6.3 - 12.5</td>
</tr>
<tr>
<td>VIII.2c</td>
<td>12.2</td>
<td>26.6</td>
</tr>
<tr>
<td>VIII.3</td>
<td>12.5 - 25</td>
<td>12.5 - 25</td>
</tr>
<tr>
<td>VIII.4</td>
<td>6.3 - 12.5</td>
<td>12.5 - 25</td>
</tr>
<tr>
<td>VIII.5</td>
<td>50 - 100</td>
<td></td>
</tr>
<tr>
<td>VIII.6</td>
<td>50 - 100</td>
<td></td>
</tr>
<tr>
<td>VIII.7</td>
<td>&gt;200</td>
<td></td>
</tr>
<tr>
<td>VIII.8</td>
<td>&gt;200</td>
<td></td>
</tr>
</tbody>
</table>

*a* IC$_{50}$ values from Tables II-4, III-2, V-2, and VI-2. *b* Results are expressed in nM concentrations. *c* Amodiaquine.
VIII-1.2 Conformational Analysis

The essential element of the 'lock and key' concept for drug-receptor binding is the so-called 'pharmacophore hypothesis'. This assumes that in order for the receptor to be activated, the receptor must recognise a set arrangement of chemical groups, called the pharmacophore. With this assumption, a pharmacophore should be present in pharmacologically active molecules.

In deriving pharmacophores, it is necessary to manipulate the three dimensional structure of flexible drug molecules. Most molecules can change their conformation by rotation around bonds with an accompanying change in energy. The energetic study of various conformations of flexible molecules is called conformational analysis. Although the preferred conformations in solution have the lowest energy, it does not follow that drugs bind to their receptor in their lowest-energy conformation. In fact binding often occurs with slightly higher energy conformation. It also assumes that the receptor remains in a static conformation (although this is not always true since the binding forces alter the receptor surface to some extent).

VIII-1.3 Superimposition

The pharmacophore hypothesis assumes that the biologically active molecules bind at the same site via a common orientation of chemically similar groups. However the receptor and its binding groups are not yet experimentally known for antimalarial drugs. For the 4-aminoquinoline derivatives considered in this study, a pair of nitrogen atoms common to all molecules are conjectured to be important for receptor binding interactions. With this assumption, the two nitrogen atoms, separated by a definite distance, constitute a two point pharmacophore which is presented in Figure VIII-2. This, however, is not sufficient to define the pharmacophore. The spatial separation of these nitrogen atoms can be estimated by comparing this distance in low energy conformations of biologically active molecules. Only those conformations which are
both energetically feasible and have a similar N-N distances are considered as likely to fit the pharmacophore. This distance then forms a spatial and topological constraint for the conformation of the other related molecules in conformational analysis.

**Figure VIII-2 Initial two point pharmacophore assumption.**

The topography of the pharmacophore can be found by mapping the intersections of all relevant biologically active molecules. This is achieved by superimposing the molecules together, using the two spatially related nitrogen atoms as a frame for alignment. The molecules are finally oriented in three dimensional space by matching the centre of a common phenol ring.

**VIII-1.4 Volume Analysis**

Some of the 4-aminoquinoline compounds possess the same critical pair of nitrogen atoms and yet, they demonstrate low biological activities. This difference in activities may be due to steric factors. If this was the case, then the inactive molecules should present distinctive geometric differences when superimposed with the active ones. In fact these unique features can even provide new information about the unknown receptor.

With the assumption of the 'lock and key' hypothesis, the receptor must be 'physically' complementary to all the active molecules. The 'physical' volume displaced by each molecule is defined by a van der Waals surface which envelops it. The union of the volume of all active molecules when they are superimposed is called
the 'excluded volume'.\textsuperscript{177,178} For binding to occur, the receptor must be spatially complementary to the 'excluded volume'.

When inactive molecules are at the binding positions, portions of their volume might intrude into the physical space occupied by the receptor and inhibit binding. Such steric factors may be responsible for their biological inactivity,\textsuperscript{179} even though they might be structurally similar to the active analogs. On the other hand, these intruding portions of the inactive molecules may represent regions of the receptor. Hence they can be combined to form a rough model of the unknown receptor. To achieve this, the region common to the 'excluded volume' and the volume of the inactive molecule must be removed from the latter. The remaining volume of the inactive analogs then constituted a partial map of the receptor.\textsuperscript{177,179}

\section*{VIII-1.5 Procedure}

All molecules were initially built and optimised within the program SYBYL\textsuperscript{171} using their default molecular mechanic force field. All piperidine rings were built in a low energy chair form with the large substituent equatorial as this has been shown to be the energetically preferred conformation.\textsuperscript{180-182} The higher energy N-R axial and other forms were not considered. A comparison of several antimalarial compounds built and optimised within SYBYL with the crystal structures\textsuperscript{183} obtained from the Cambridge Crystallographic Data base showed a remarkably close geometric correlation confirming that the computation methods used were adequate.

The low energy conformations of the most biologically active compound (VIII.1) were used as a template for deducing the pharmacophore. Conformation studies were carried out to find all its local energy minima using the molecular modelling computer package SYBYL in a Silicon Graphics computer. Molecular mechanics techniques\textsuperscript{184} where each molecule was treated as a group of particles held together by elastic forces were employed using the Maximum 2 force field with SYBYL. The energy for each conformation was calculated using a potential function made up of a wide range of individual potentials describing different types of interactions such as, non-bonded repulsive and attractive interactions, bond
deformations, hydrogen bonding, torsional strain and electrostatic interactions.\textsuperscript{71} To obtain the global minimum energy conformation, the SEARCH\textsuperscript{185} option in the program SYBYL was used to generate a vast number of starting geometries, which were minimised to their local energy minima and duplicates discarded.

The lowest energy conformation of compound (\textbf{VIII.1}) was used to give the range of spatial distances between the critical nitrogen atoms. These distances were then employed as a constraint in the systematic search for the lowest energy conformation of the other molecules. The conformation of each molecule was then adjusted simultaneously with the constraints; this provided the best compromise between the quality of geometrical fit and the value of energy of each molecule.

For each molecule, conformations with energies within 10 kJ mol\textsuperscript{-1} of the global minimum were deemed feasible for binding to the receptor. Since all the molecules are structurally similar, a common pharmacophore is very possible. This was obtained by selecting from the pool of low energy conformations one conformer for each molecule which were topologically and electronically as similar as possible. This set of conformations was used for subsequent superimposition and volume analysis. The low energy conformation of compound (\textbf{VIII.1}) was then used as a template for superimposition with the other molecules. The center of a common phenol ring was chosen as the origin of each molecule. Each molecule was then superimposed onto the template using the component in SYBYL which provides a RMS (root mean square) fit of superimposed atoms to align the corresponding critical nitrogen atoms and the origin. All superimpositions with a RMS of greater than 1.0 Å were discarded.

Volume analysis\textsuperscript{177-179} was carried out using the program SYBYL to generate a van der Waals surface for each molecule. When all four active molecules were superimposed together, their combined volume was recorded. This 'excluded volume' was then superimposed with the volume of each inactive molecule individually. Any volume of the inactive molecules which lay inside the 'excluded volume' was subsequently removed, leaving behind the 'unique volume' of each inactive compound. These 'unique volumes' combine to form a possible map of the receptor,\textsuperscript{177,179} whereas their intersections define the so-called 'receptor essential volume'.\textsuperscript{177,178} All
surface volumes were orientated in the same direction as the individual molecules (Figures VIII-3 and VIII-4) and superimposition diagrams.

VIII-1.6 Results and Discussion

The global minimum energy conformations for each analogue was determined and they are shown in Table VIII-2. However, they proved not to match well in superimposition for the purpose of determining the pharmacophore. An alternative set of conformers which share a similar topology was chosen to be used instead. The local minimum energy values of this alternative set, the distances between the critical nitrogen atoms and their RMS fit are also recorded in Table VIII-2, and their conformations are shown in Figures VIII-3 and VIII-4. All molecules, except compound (VIII.4), have best fitting conformations which are more energetic than their respective global minimum conformation.

| TABLE VIII-2 Best fitting conformations for compounds (VIII.1-VIII.8) with their associated energies, N-N distances, and corresponding RMS fit to the proposed pharmacophoric points. |
|-----------------|-----------------|-----------------|-----------------|
| Compound Number | Global Minimum Energy (kJ mol$^{-1}$) | Local Minimum Energy (kJ mol$^{-1}$) | Distance Between N Atoms (Å) | RMS fit (Å) |
| VIII.1          | 0.291           | 3.14            | 8.1             |
| VIII.2          | 1.349           | 5.025           | 7.9             | 0.0306       |
| VIII.3          | 1.369           | 2.166           | 8.5             | 0.1233       |
| VIII.4          | 3.299           | 3.299           | 8.7             | 0.1180       |
| VIII.5          | 11.380          | 13.194          | 8.7             | 0.2232       |
| VIII.6          | 11.398          | 12.427          | 8.9             | 0.1326       |
| VIII.7          | 13.004          | 13.250          | 7.7             | 0.1787       |
| VIII.8          | 1.905           | 3.407           | 9.1             | 0.1077       |
Figure VIII-4
Best fitting conformations of the inactive compounds (VIII.5 - magenta, VIII.6 - purple, VIII.7 - yellow, VIII.8 - cerise).
The electrostatic potential energy for each molecule, which is important for receptor recognition, was calculated using SYBYL; and the results are presented in Figures VIII-5 and VIII-6.

The superimpositions reveal that all the active molecules have many overlaps and fit well with each other, as shown in Figure VIII-7. On the other hand, Figure VIII-8 shows that the low activity molecules do not match well with the active analogues. In particular their side-chains stray in many directions. The chlorine atom, which is common to all the active analogues, coincided very well under superimposition, and it was possible that it may constitute a part of the pharmacophore. The mean coordinates of the two critical nitrogen atoms and the chlorine atom are reported in Table VIII-3. Based on the eight analogs studied in this chapter these three atoms at their fixed separations constitute the proposed primary pharmacophore. This is presented in Figure VIII-9. The fixed distance between the nitrogen atoms is 8.2 Å.

Table VIII-3 Spatial coordinates of the proposed three point pharmacophore.

<table>
<thead>
<tr>
<th></th>
<th>Coordinates (Å)</th>
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<tbody>
<tr>
<td></td>
<td>X</td>
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<tr>
<td>N1</td>
<td>1.6859</td>
</tr>
<tr>
<td>N2</td>
<td>2.9802</td>
</tr>
<tr>
<td>Cl</td>
<td>-2.3065</td>
</tr>
</tbody>
</table>
Figure VIII-5  Electrostatic potential energy diagram for the four active compounds 
a) VIII.1  b) VIII.2  c) VIII.3  
d) VIII.4.
Figure VIII-6  Electrostatic potential energy diagram for the four inactive compounds a) VIII.5  b) VIII.6  c) VIII.7 d) VIII.8.
Figure VIII-7  Superimposition of the active compounds (VIII.1 - green, VIII.2 - white, VIII.3 - orange, VIII.4 - red).
Figure VIII-8  Superimposition of all eight antimalarial agents 
(compounds VIII.1 - green, VIII.2 - white, VIII.3 - orange, 
VIII.4 - red, VIII.5 - magenta, VIII.6 - purple, 
VIII.7 - yellow, VIII.8 - cerise).
The four active molecules were then superimposed together, and the program SYBYL was used to generate their total volume. This is shown in Figure VIII-10. This 'excluded volume' is the region of the receptor active site available for binding, and the receptor is assumed to envelope this volume. The volume of each inactive molecule was then superimposed onto the 'excluded volume' and each have significant regions lying outside of the latter. These 'unique volumes' (of the inactive molecules) are shown in Figure VIII-11, and could be responsible for negative interaction with the receptor. The union of these 'unique volumes' are pictured in Figure VIII-12, and together form a large covering of the space displaced by the active molecules. It is reasonable to assume that this combined 'unique volume' has common overlaps with the real receptor. In particular the region where these 'unique volumes' overlap could very well be the location of a section of the receptor. This intersection, known as the 'receptor essential volume', is shown in green colour in Figure VIII-12 and is 'isolated out' in Figure VIII-13.

Since the 'essential volume' is small compared to the combined 'unique volume', so other regions of the receptor might also be mapped out by the latter 'volume'. However it is not possible to propose further information about the receptor from these analogues alone. It is also important to note that the 'inactive' molecules studied in this chapter have actually only demonstrated low activity. Their potencies need to be further tested to determine more accurately the IC_{50} values. Nevertheless their low activity and the large 'unique volume' in Figure VIII-11 provide evidence for the existence of the malarial receptor. The knowledge of the different spatial regions involved can serve as a starting point for designing and predicting the chance of activity.
Figure VIII-10  Total volume of all active compounds known as excluded volume with the structures of the superimposed molecules included.
Figure VIII-11  Unique volume for each inactive compound with the structure of the molecule included (a) VIII.5, (b) VIII.6, (c) VIII.7, (d) VIII.8. The volume is orientated in the same direction as Figure VIII-8.
Figure VIII-12  Superimposition of unique volumes of each inactive compound. The intersection of these volumes is shown in green. The orientation is in the same direction as Figure VIII-8.
Figure VIII-13  The receptor essential volume and its spatial location relative to all the inactive molecules. The volume is orientated in the same direction as Figures VIII-8 and VIII-12.
for new analogues. 'Volume' studies with other molecules are needed to improve the receptor map and verify the proposed receptor 'essential volume'.

VIII-1.7 Conclusion

Superimposition of the four most active antimalarials allowed a pharmacophore to be proposed. The model pharmacophore requires two nitrogen atoms separated by 8.2 Å, and a chlorine atom as shown in Figure VIII-9.

'Volume' analysis of the active and inactive molecules revealed a large spatial region where the malarial receptor could be located. In particular, a small receptor 'essential volume' was obtained. Further studies of different and new analogues are needed to refine this model.
VIII-2 DNA Intercalation Studies

VIII-2.1 Introduction

A considerable amount of work by others on the binding of antimalarials to DNA has indicated that intercalation may be part of the mechanism of action. Some blood schizontocidal antimalarial drugs such as 4-aminoquinolines (chloroquine, amodiaquine), and 4-quinolinemethanols (quinine) are thought to act by binding to plasmodial DNA, and thus inhibiting protein biosynthesis. On the other hand, mefloquine was shown to have no interaction with DNA (see Chapter I-3.2).

In this study, the intercalation with DNA of the di(and mono)-Mannich bases of 2-(7-chloroquinolin-4-ylamino)phenols, (VIII.9 and VIII.10), and 2-(7-bromo-1,5-naphthyridin-4-ylamino)phenol, (VIII.11 and VIII.12), has been investigated.

VIII-2.2 Material and Methods

Calf thymus DNA (Sigma Chemical Co.) was used throughout this study. All of the binding experiments described in this section were carried out at room temperature (ca 22°C) and in 5mM Tris-HCl buffer unless otherwise indicated.

A stock solution of each compound (VIII.9 - VIII.12) was prepared at a concentration of 4mM in 4mM HCl. The interaction of each compound (VIII.9 - VIII.12) with DNA was determined by a direct spectrophotometric method using a

\[ \begin{array}{|c|c|c|c|}
\hline
\text{VIII.9} & \text{Cl} & \text{CH} & \text{CH}_2\text{N} (\text{CH}_2)_4 & \text{CH}_2\text{N} (\text{CH}_2)_4 \\
\hline
\text{VIII.10} & \text{Cl} & \text{CH} & \text{CH}_2\text{N} (\text{CH}_2)_4 & \text{H} \\
\hline
\text{VIII.11} & \text{Br} & \text{N} & \text{CH}_2\text{N} (\text{CH}_2)_4 & \text{CH}_2\text{N} (\text{CH}_2)_4 \\
\hline
\text{VIII.12} & \text{Br} & \text{N} & \text{CH}_2\text{N} (\text{CH}_2)_4 & \text{H} \\
\hline
\end{array} \]

\[a\] This work was carried out under the supervision of Dr W.L.F. Armarego.
double beam u.v./visible spectrophotometer (Cary model 219). The solution of each agent in a total volume of 1ml contained: 50mM Tris-HCl buffer (100μl), and H₂O to a volume of 1ml. The standard binding mixture in a total volume of 1ml contained: 50mM Tris-HCl buffer at pH 7.5 (100μl), 4mM of stock agent (100μl), calf thymus DNA [100μl (5.2mg/ml) in 5mM Tris buffer] and H₂O to a volume of 1ml. Reference cuvettes contained buffer alone. The absorbance of the agent (alone) and in the presence of DNA were recorded at 200-500nm using 1cm cuvettes (see Figures VIII-14 to VIII-17).

In order to compare the relative strength of interaction with DNA of each pair of structurally similar compounds (VIII.9 and VIII.10) (or compounds (VIII.11 and VIII.12)), the absorbances for each compound were measured in the presence of various amounts of calf thymus DNA (0-1.04mg) at the wavelength of its absorption maximum (analytical wavelengths). These absorbances at the fixed analytical wavelength were obtained on a Perkin-Elmer Lambda 1 single beam spectrophotometer for each compound (see Figures VIII-18 and VIII-19). The absorbance of the DNA was negligible at these wavelengths.

VIII-2.3 Results and Discussions

The solid lines in Figures VIII-14 and VIII-15 represent the absorption spectra of compounds (VIII.9 and VIII.10) respectively, at wavelengths above 300 nm (i.e the region at which DNA does not absorb light). The broken lines show the spectra of the test compounds in the presence of DNA. The DNA decreased the intensity of absorption throughout the absorption bands of both compounds. This result clearly indicates that the compounds interact with DNA. Similar effects were also observed with compounds (VIII.11 and VIII.120), and they are shown in Figures VIII-16 and VIII-17.
Figure VIII-14 Influence of calf thymus DNA upon the absorption spectrum of compound VIII.9

Figure VIII-15 Influence of calf thymus DNA upon the absorption spectrum of compound VIII.10
Figure VIII-16 Influence of calf thymus DNA upon the absorption spectrum of compound (VIII.11)

Figure VIII-17 Influence of calf thymus DNA upon the absorption spectrum of compound (VIII.12)
It is interesting to compare the relative strength of interactions of these compounds with DNA. However it is important to realise that different molecular structures might have different modes of interaction with the DNA. Thus it is sensible to compare only the interaction strengths of structurally similar compounds. The comparison between compounds (VIII.9 and VIII.10) is shown in Figure VIII-18. The absorbances decreased as more DNA was added, thus providing further evidence for DNA intercalation. To compare the compounds, these curves were treated as titration curves where absorbances reached constant values after 1.04 mg of DNA were added to the solution of the drug. From the plots, compound (VIII.9) showed a half maximum absorbance change (above its constant value at 1.04 mg of DNA) after about 364 µg of DNA was added, whereas compound (VIII.10) required about 432 µg of DNA to produce a similar effect. A similar comparison was made for compounds (VIII.11 and VIII.12) as shown in Figure VIII-19. The half maximum absorbance changes were attained after addition of 317µg of DNA for compound (VIII.11) and 416µg for compound (VIII.12). The data together with the antimalarial activities for the compounds are given in Table VIII-4.

Table VIII-4  Data for the interaction of compounds (VIII-9 - VIII.12) with DNA and their antimalarial activity.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration of compound (µM)</th>
<th>Analytical wavelength (nm)</th>
<th>Weight of DNA required for 50% absorbance changea (µg)</th>
<th>Antimalarial activity IC50b (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VIII.9</td>
<td>400</td>
<td>336</td>
<td>364</td>
<td>&lt;25a</td>
</tr>
<tr>
<td>VIII.10</td>
<td>400</td>
<td>340</td>
<td>432</td>
<td>100a</td>
</tr>
<tr>
<td>VIII.11</td>
<td>400</td>
<td>376</td>
<td>317</td>
<td>&lt;25a</td>
</tr>
<tr>
<td>VIII.12</td>
<td>400</td>
<td>352</td>
<td>416</td>
<td>&gt;200a</td>
</tr>
</tbody>
</table>

a The weights of DNA shown are those required to produce 50% of the total absorbance change.
b IC50 values from Tables II-3 and II-4.
Figure VIII-18  Plots of absorbance versus added DNA for compounds (VIII-9 and VIII-10) (at 400μM).

Figure VIII-19  Plots of absorbance versus added DNA for compounds (VIII-11 and VIII-12) (at 400μM).
The data in Table VIII-4 indicate that within each pair of compounds (VIII.9 and VIII.10, VIII.11 and VIII.12), there may be some relationship between DNA intercalation and antimalarial activity. However, a more detailed investigation is needed before any relationship is proposed.
Molecular modelling studies of the biologically active Mannich bases described in this thesis led to my proposal for the common pharmacophore of these antimalarials. The 'excluded volume' of the receptor active site was also determined in this study. From these results, new drugs may be designed which possess the pharmacophore, and also lay wholly within the 'excluded volume' to ensure active binding to the receptor.

Volume analysis of the less active antimalarial agents provided some new information about the possible location and shape of the malarial receptor. However, the potencies of these compounds must be further tested to determine relative activity. In addition, it must be noted that this work is based on the assumption that the IC$_{50}$ values are mainly due to the interaction of the drug with the malaria receptor, e.g. on the cell surface, and do not include other parameters, such as drug transport. Recent biological research$^{186}$ has found that chloroquine resistance of malaria parasites may be linked to the P glycoprotein, Pgh1, which is located on the surface of the cell membrane. Nevertheless, it would be interesting to further refine the proposed pharmacophore and malarial receptor model. This can be achieved by incorporating other agents (both biologically active and inactive) into the study.

In other studies, four compounds were shown to intercalate with DNA. However, the relationship, if any, between DNA intercalation and antimalarial activity remains undetermined. Further work to evaluate the interactions of these classes of compounds with DNA could prove fruitful.
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REFERENCES

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Mannich, C., and Krösche, W., Arch. Pharm., 1912, 250, 647.


SYBYL: Tripos Associates, St. Louis.


PUBLICATIONS
Publications
(Based on the work described in this thesis)

Potential Antimalarials. XV
Di-Mannich Bases of 2-(7'-Chloroquinolin-4'-yl-amino)phenol and
2-[7'-Bromo(and trifluoromethyl)-1',5'-napthyridin-4'-ylamino]phenol.

Potential Antimalarials. XVII
Di- and Mono-Mannich Bases of 2(and 4)-
[2(and 8)-Trifluoromethylquinolin-4-ylamino]phenol.

Accepted for Publication

3. Barlin, G.B., Ireland, S.J., Nguyen, T.M.T., Kotecka, B. and
Rieckmann, K.H.
Potential Antimalarials. XVIII
Some Mono- and Di-Mannich Bases of 3-[7-Chloro (and
7-trifluoromethyl)quinolin-4-ylamino]phenol.

4. Barlin, G.B., Ireland, S.J., Jiravinyu, C., Nguyen, T.M.T.,
Kotecka, B. and Rieckmann, K.H.
Potential Antimalarials. XIX
Syntheses and Testing of α-(Piperidin-2-yl)-α-(7'-trifluoro-
methylquinolin-4'-yl)methanol and α-(7-Bromo-1,5-napthyridin-4-yl)
-α(piperidin-2'-yl)methanol.