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EXPLORING THE POTENTIAL OF CHLOROQUINE AND QUINACRINE DERIVATIVES AS NEW ANTIPROTOZOAL AND TUMOUR DRUG RESISTANCE REVERSAL AGENTS

By

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DECLARATION

I declare that " **Exploring the potential of chloroquine and quinoline derivatives as new antiprotozoal and tumour drug resistance reversal agents**" is my own work and that all sources that I have used or quoted have been indicated and acknowledged by means of complete reference.

Hayley Claire Haupt

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ABSTRACT

Compounds containing the quinoline and acridine moieties have been utilized extensively in the search for new antiprotozoal and multidrug resistance reversal agents. Hence, these moieties formed the basis for the synthesis of new compounds. New sulfonamides, ureas and amine analogues were synthesized and evaluated for inhibitory activity against trypanothione reductase (TryR), *in vitro* activity against the causative agents of trypanosomiasis, leishmaniasis as well as chloroquine-sensitive and resistant malaria. Some were also evaluated as potential tumour multidrug resistance reversal agents.

The best inhibitors of trypanothione reductase from each class are Naphthalne-2-sulfonic acid [3 -(6'-chloro-2'-methoxy-acridin-9'-ylamino)-propyl] -amide from the sulfonamides, Naphthalne-2-sulfonic acid [3-(6'-chloro-2'-methoxy-acridin-9'-ylamino)-propyl]-amide from the ureas and N-Benzyl-N'- (6"-chloro-2"-methoxy-acridin-9"-yl)-hexane-1, 6-diamine from the amine series. The bisquinoline ureas 1-[2-(7'-Chloro-quinolin-4'-ylamino)-ethyl]-3-[3-(7"-chloro-quinolin-4"-ylamino)-propyl]-urea and 1, 3 – Bis - [3 - (7' -chloroquinolin -4'-ylamino)-propyl]-urea as well as the quinoline-based urea 1-[3-(7'-Chloro-quinolin-4'-ylamino)-propyl]-3-(2-dimethylamino-propyl)-urea also showed inhibitory potency against trypanothione reductase. Those compounds with noteworthy antiparasitic activity were Naphthalne-2-sulfonic acid [3-(6'-chloro-2'-methoxy-acridin-9'-ylamino)-propyl]-amide which was active against *L. donovani* (ED_{50}=1.9μM), the urea 1-Benzyl-3- [3-(6'-chloro-2'-methoxy-acridin-9'-ylamino)-propyl]-urea active against both *L. donovani* (ED_{50}=1.9μM) and *P. falciparum* 3D7 (ED_{50}=0.0005μM) and K1 (ED_{50}=0.015μM) strains and 1-Benzyl-3- [4-(6'-chloro-2'-methoxy-acridin-9'-ylamino)-butyl] urea which showed activity against *L. donovani* (ED_{50}=1.9μM), *T. cruzi* (ED_{50}=<1μM) and *P. falciparum* 3D7 (ED_{50}=0.0013μM). The bisquinoline urea 1-[2-(7'-Chloro-quinolin-4'-ylamino)-ethyl]-3-[3-(7"-chloro-quinolin-4"-ylamino)-propyl]-urea and the urea 1-[3-(7'-Chloro-quinolin-4'-ylamino)-propyl]-3-(2-dimethylamino-propyl)-urea did not display any potency as antitrypanosomal and antileishmanial agents. This did not correlate with the inhibitory potency against TryR. The *in vivo* evaluation of the urea -[3-(7'-Chloro-quinolin-4'-ylamino)-propyl]-3-(2-dimethylamino-propyl) showed a reduction in parasitemia comparable to that of chloroquine.

Of the compounds tested as multidrug resistance (MDR) reversal agents, Naphthalene-2-sulfonic acid benzyl- [6-(7'-chloro-quinolin-4'-ylamino)-hexyl]-amide was the most successful. Paclitaxel recovered 99% of its activity against a resistant cancer cell line when co-administered with this compound at 1μM potential as a MDR reversal agent.

ABBREVIATIONS

4-AQ	4-aminoquinoline
9-AQ	9-aminoquinoline
Anal.	analytical
Ar	aryl
ATP	adenosine 5 'triphosphate
BBr_3	boron tribromide
BCl_3	boron trichloride
Bn	benzyl
br	broad (in NMR)
Bu	butyl
*t*Bu	tertiary butyl
°C	degrees Celsius
Calc.	calculated
CNS	central nervous system
CO_2	carbon dioxide
CQ	chloroquine
CVFF	constant valence force field
d	doublet (in NMR)
dd	doublet of doublets (in NMR)
DEAD	diethyl azodicarboxylate
DBU	1, 8-diazabicyclo [5.4.0] undec-7-ene
DIBAH	diisobutylaluminium hydride
DFMO	α-difluoromethylornithine
DMAP	4-dimethylaminopyridine
DMF	*N, N*- dimethylformamide
DMSO	dimethyl sulfoxide
δ	chemical shift in parts per million downfield from tetramethylsilane
EDTA	ethylenediaminetetraacetic acid
ED_{50}	effective dose required to inhibit 50% of parasite growth

EI	electron impact
Equiv.	equivalents
Et	ethyl
Et_3N	triethylamine
EtOAc	ethyl acetate
EtOH	ethanol
FAB	fast atomic bombardment
FAD	flavin adenine dinucleotide
g	gram(s)
Glu	glutamic acid
GSH	glutathione
GSSG	glutathione disulfide
h	hour(s)
HAT	Human African Trypanosomiasis
HEPES	[4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid]
HGR	human glutathione reductase
HRMS	high-resolution mass spectrometry
Hz	hertz
IC_{50}	inhibitory concentration to inhibit 50% of enzyme
IR	infrared
J	coupling constant (in NMR)
L.	*Leishmania*
LAH	lithium aluminium hydride
Leu	leucine
μ	micro (10^{-6})
M	moles per litre ($mol.dm^{-3}$)
m	multiplet (in NMR)
MDR	multidrug resistance
Mel B	melarsoprol
Met	methionine
MeOH	methanol
min	minutes

MHz	megahertz
ml	millilitre
mol	mole(s)
mp	melting point
m/z	mass to charge ratio (in mass spectra)
NADP	nicotinamide adenine dinucleotide phosphate
NADPH	reduced NADP
n-Bu_4NI	tetrabutyl ammonium iodide
$NH_2OH.HCl$	hydroxylamine hydrochloride
NMP	*N*-methyl-2-pyrrolidinone
NMR	nuclear magnetic resonance
Nu	nucleophile
p	*para*
P.	*Plasmodium*
Ph	phenyl
Pgp	P-glycoprotein
Phe	phenylalanine
PKDL	post-kala-azar leishmaniasis
PTBD	1, 5, 7-triazabicyclo [4.4.0.] dec-5-ene
Pro	proline
PS	polymer supported
Pyr.HCl	pyridine hydrochloride
q	quartet (in NMR)
R_f	retention factor (in chromatography)
RES	reticuloendothelial system
s	singlet (in NMR)
Ser	serine
t	triplet (in NMR)
T.	*Trypanosoma*
tert	tertiary
TG	thermogravimetric
THF	tetrahydrofuran

TLC	thin layer chromatography
TMSCl	trimethylsilylchloride
TMSI	trimethylsilyliodide
Trp	tryptophan
TryR	trypanothione reductase
$Try[S]_2$	trypanothione disulfide
$Try\ [SH]_2$	trypanothione
Ts	*p*-toluenesulfonyl, tosyl
Tyr	tyrosine
WHO	World Health Organisation

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CHAPTER 1

INTRODUCTION TO DISEASES

1.1 INTRODUCTION

The protozoa that are responsible for parasitic disease in both man and vertebrate animals are endoparasitic and live within the tissue of their hosts. They may be located in the epithelia or within the cavities surrounded by the epithelial membranes *e.g.* gut, associated with tissue cells or located in tissue fluids.

Heightened awareness of antiprotozoal resistance to the drugs available as well as the poor activity exhibited by available drugs in immunosuppressed hosts has led to the renewal of interest in antiprotozoal therapy. For many protozoal infections there is adequate therapy available in the form of drugs developed in the 1960s [1]. However, the value of a large number of these drugs has been lost as a result of the development of widespread drug resistance. Furthermore, for most parasitic infections there are few alternatives if resistance develops. An additional problem associated in current antiparasitic chemotherapy is that drugs available for a number of diseases are inadequate because of their variable efficacy and toxicity as well as the requirement for long courses of parenteral administration.

Trypanosoma and *Leishmania* belong to the kingdom Protozoa, phylum Sarcomastigophora, class Zoomastigophora and order Kinetoplastida. They are also known as *haemoflagellates,* referring to the location of the parasite in the host.

1.2 TRYPANOSOMIASIS

The genus *Trypanosoma* is responsible for a number of parasitic diseases that affect both man and other vertebrates. Fundamentally, trypanosomes are parasites of the bloodstream but some do adapt to intracellular existence. The trypanosome species is divivided into 2 major groups namely *Salivaria* and *Stecoraria* based on their development in the vector and the host. This results in two forms of trypanosomiasis. These are African trypanosomiasis, also called African sleeping sickness for which the Salivaria group is responsible and South American trypanosomiasis, also called Chagas'disease, caused by the Stecoraria group.

1.2.1 African trypanosomiasis:

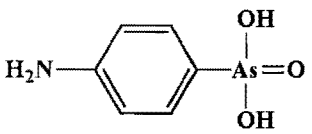
a) Introduction

Human African trypanosomiasis (HAT) is a systematic and central nervous system (CNS) infection. It is caused by two geographically distinct species of the group Salivaria: *Trypanosoma. brucei gambiense* (West and Central Africa) and *Trypanosoma. brucei rhodesiens*e (East Africa) [2]. The infection is transmitted by the tsetse fly and the parasites develop in the bloodstream. The two human species can be distinguished by the clinical pattern of the disease. *Trypanosoma brucei rhodiense* effects an acute infection with severe toxaemia (a condition caused by absorption of toxins formed by microorganisms into the tissues and blood) followed by involvement of the CNS resulting in death within a few months. The infection caused by *Trypanosoma brucei gambiense* may go undiagnosed for years with only less severe recurring fever spells. The disease may go undetected until involvement of the CNS. At this late stage, treatment is more difficult as not many drugs are capable of penetrating the CNS.

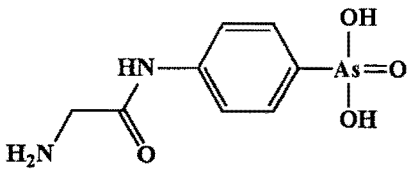
b) History of Chemotherapy

The first trypanocidal drugs were arsenical compounds. Atoxyl **1** (figure 1.1) was the first to be tried clinically. It was soon replaced by a less toxic derivative tryparsamide **2** (figure 1.1). Although tryparsamide was found to be useful in treating the later CNS stages of infection, it did have serious toxic side effects on the host. These include optic nerve damage, gastrointestinal upsets and severe skin reactions. Convulsions and reactive encephalopathy (a disease of the brain associated with toxic poisoning) occur less frequently. The main use of tryparsamide was in the treatment of Gambian sleeping sickness in the stages of CNS involvement. Resistant strains of the parasite have developed due to the long and continued use of the drug. Coupled with this, cross-resistance to certain related arsenicals has also emerged. As a result, it is rarely used today.

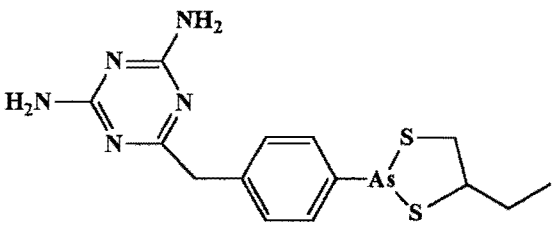
Melarsoprol (Mel B) **3** (figure 1.1) is a trivalent arsenical currently in use. Although it seemed to overcome drug resistance, use of this drug has been restricted by severe toxicity which can include reactive encephalopathy. It is estimated that up to 10% of the cases treated with melarsoprol react in this manner with a mortality of up to 5%. It is



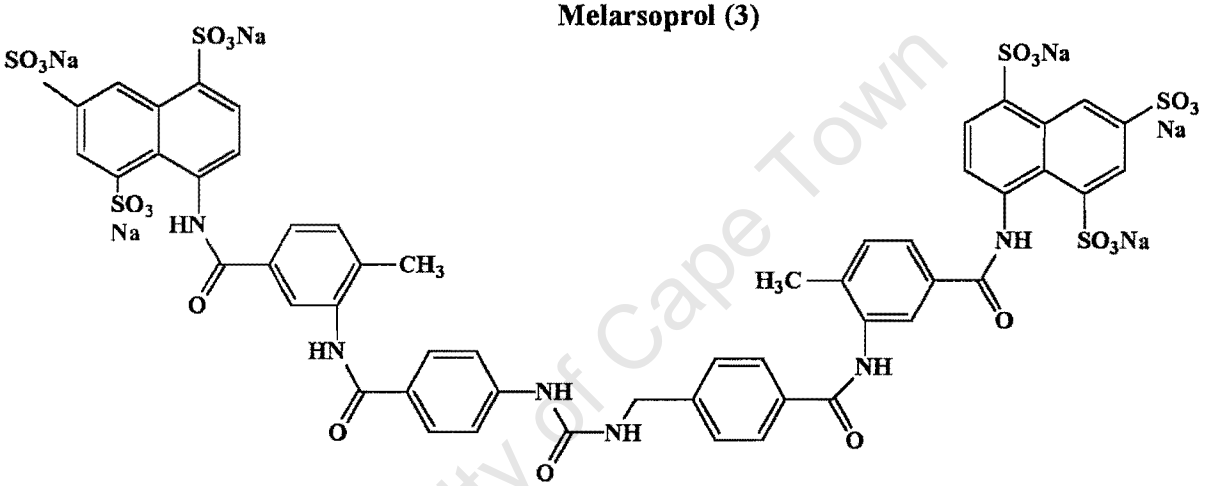
Atoxyl (1)



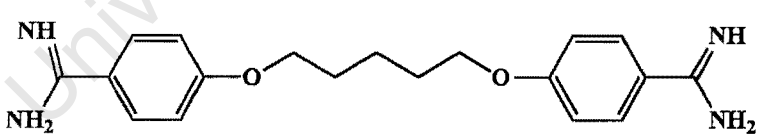
Tryparsamide (2)



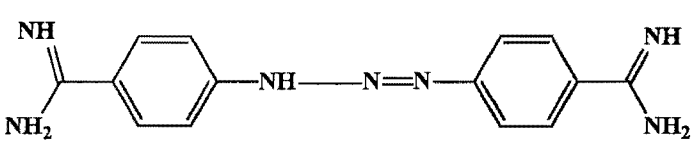
Melarsoprol (3)



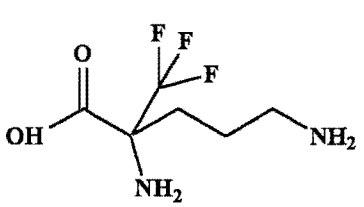
Suramin (4)



Pentamidine (5)



Diminazene (6)



DFMO (7)

Figure 1.1. Structures of drugs used in the treatment of African trypanosomiasis.

1.2.2 South American trypanosomiasis:

a) Introduction

This form of trypanosomiasis is transmitted by the stecorarian species *Trypanosoma cruzi*. The disease affects Central and South America and is widespread in areas where conditions favour multiplication of arthropod species of the family *Reduviidae*. The parasite is transmitted *via* the faeces of these blood-sucking bugs as they deposit infective faeces at the site of a bite or mucosal surface. Thus resulting in faecal contamination of the mucous membranes or abraded skin. The parasites then invade tissue cells of the intestine, oesophagus, peripheral nervous system and cardiac muscle cells. In adults, the acute phase of the disease shows only mild symptoms of fever and swelling of glands. However, in infants, quite severe reactions can occur, with death resulting from encephalitis or myocarditis. The chronic phase, which can last for decades leads to progressive disruptions in organs such as the heart, oesophagus and lower intestine. Death often follows heart failure due to wastage of this organ.

b) History of Chemotherapy

Current treatment is dependant on two drugs, the nitrofuran nifurtimox **8** (figure 1.2) and the 2-nitroimidazole benznidazole **9** (figure 1.2). Both have serious toxic side effects associated with them, including neuropathy and dermopathy.

Nifurtimox is effective in clearing *T. cruzi* parasitemia during acute stages of the disease. Nitrofurans are the so-called turncoat inhibitors of trypanothione reductase and act by inhibiting the reduction of trypanothione disulfide. It has also been used for treatment of late-stage disease but the efficacy of treatment for chronic infections is still uncertain. The drug exerts its' trypanocidal effect by inhibiting the enzyme trypanothione reductase resulting in the production of superoxide and peroxide. An intermediate nitro-aryl anion radical ($ArNO_2^{\cdot-}$) may be oxidised to generate superoxide anions ($O_2^{\cdot-}$). These, in turn, can be converted to hydrogen peroxide by the enzyme superoxide dismutase (SOD) (equ 1). The hydrogen peroxide will then react with more superoxide anion releasing highly toxic ionising OH radicals (equ 2).

$$O_2^{\cdot-} + O_2^{\cdot-} + 2H \longrightarrow 2H_2O_2 \qquad (1)$$

$$2H_2O_2 + O_2^{\cdot-} \longrightarrow OH^- + OH^{\cdot} + O_2 \qquad (2)$$

T.cruzi does not have the ability to release H_2O_2, as it does not possess the enzyme catalase. This leads to the accumulation of H_2O_2 and hence leads to more of the toxic OH radicals.

Nifurtimox only effects total parasite clearance in 50% of the patients. Toxic side effects include gastric upset, anorexia, weight loss, bone marrow depression and a high incidence of photosensitive skin rashes. There is also a marked variation in efficacy with regard to geographical region, suggesting that differences in parasite strains are compounding the problem of drug resistance.

Benznidazole is used to treat chronic *T. cruzi* infections and is partially effective in treating *T. cruzi* acute infection. Toxic effects are common including neuropathy and dermopathy in up to 40% of the cases treated. These effects are mainly reversible. As in the case with nifurtimox, a marked variation in drug sensitivity of different strains of *T.cruzi* has been observed.

Nifurtimox (8) **Benznidazole (9)**

Figure 1.2. Structures of drugs used in the treatment of Chagas disease.

1.3 LEISHMANIASIS

a) Introduction

Leishmaniasis comprises a group of widely spread diseases and is caused by at least 13 species and subspecies of the genus *Leishmania.* These 13 subspecies stem from 4 major species namely, *L. donovani*, *L. tropica*, *L. mexicana* and *L. braziliensis*. The parasite is transmitted to the mammalian host by the bite of over 50 species of female sandflies of the species phlebotomine. Infection in man gives rise to 3 forms of the disease based on their clinical patterns. These are cutaneous, mucocutaneous and

visceral leishmaniasis. The form of the disease depends mainly on the species of the causative agent.

Cutaneous Leishmaniasis is the most common form of the disease complex and the lesion is confined to the dermis. This lesion usually appears as a single eruption and includes local skin infections, subcutaneous tissue and regional lymph nodes. It may heal itself, present as a relapsing ulcer or it may spread to other cutaneous regions via the lymphatic vessels.

The causative agent of mucotaneous leishmania is the subspecies *L. braziliensis braziliensis*. A single skin lesion will spread to the cartilage and connective tissue of the nasopharynx. The result is extensive tissue damage and disfiguration, leading to death in severe cases.

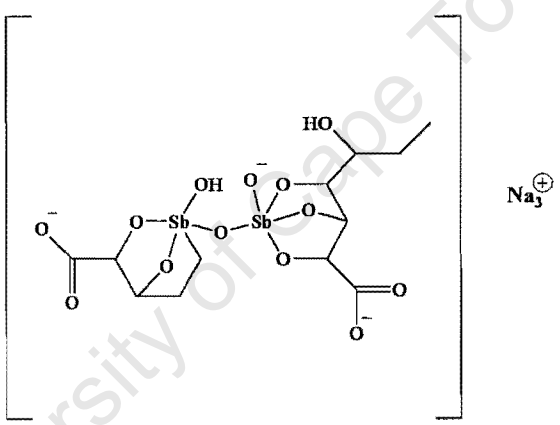
Visceral leishmaniasis (kala-azar) is the result of a systematic infection by the subspecies *L. donovani* and is considered the fatal form of the disease. It involves the reticuloendothelial system (RES) also known as the mononuclear phagocyte system, where parasites multiply within the macrophages causing fever and disfunction to organs such as the liver, spleen, bone marrow and lymph nodes. Relapses' resulting from incomplete or unsuccessful treatment of visceral leishmaniasis takes the form of post-kala-azar leishmaniasis (PKDL).

b) History of chemotherapy

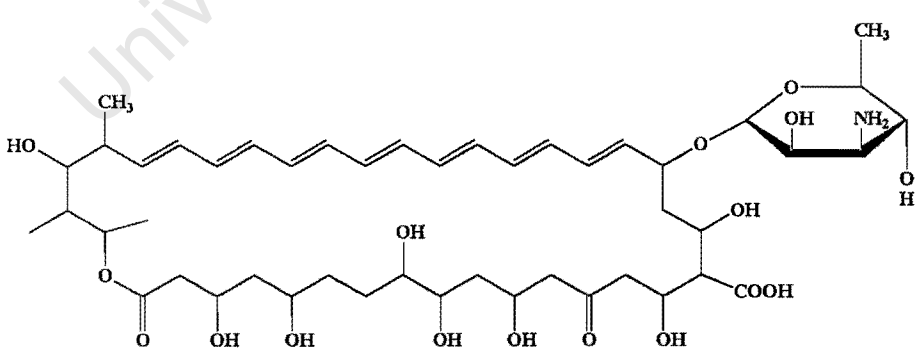
The two antimonic derivatives now in use for the treatment of leishmaniasis are sodium stibogluconate (Pentostam) **10** (figure 1.3) and meglumine antimonite (Glucantime). They remain the drugs of choice for treatment of all forms of leishmania. The main use of pentavalent antimonials is in visceral leishmaniasis as other forms of the disease are usually unresponsive. Disadvantages associated with these drugs are the lengthy treatment required and the fact that subsequent courses of treatment are needed to affect a clinical cure in some areas. The drugs have a low toxicity and tolerance is good. Exceptions may occur when the dosage is increased as in cases with renal insufficiency or when the response by the patient is poor. Hypersensitivity reactions may occur after several courses of treatment.

Pentamidine is also effective in the treatment of visceral leishmaniasis, particularly in East Africa and the Mediterranean where antimonials are ineffective. Side effects are common and toxic including hypoglycaemia and nephrotoxicity. The drug is not used to treat any other form of leishmaniasis.

The antibiotic Amphotericin B **11** is used in the treatment of advanced cases of mucotaneous leishmaniasis. Amphotericin B is the most toxic antibiotic in clinical use available and reactions to the drug are severe. Upon administration, immediate irritant effects, including vomiting, fever; thrombophlebitis (inflammation of the veins) and renal damage are observed. Hypersensitivity reactions observed are anaphylaxis, generalized pain and convulsions. As a result of these extremely toxic side effects, its use is deferred until severe mutilation is observed.



Pentostam (10)



Amphotericin B (11)

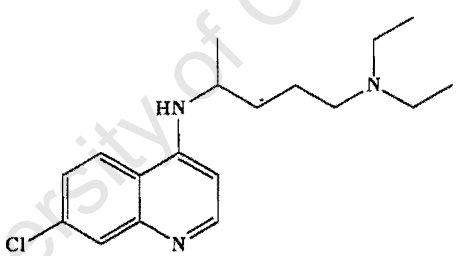
Figure 1.3. Chemical structures of compounds with antileishmanial activity.

1.4 MALARIA:

a) Introduction

Malaria is one of the most widespread and dangerous diseases in the world. It is widely spread in many Third World countries, including parts of Africa, Asia, Central America and South America. The World Health Organisation estimates that 300-500 million cases of clinical malaria occur each year and that this disease fatally affects 1.5-2.7 million people per annum. The disease is currently ranked fifth in terms of the number of deaths due to infectious diseases [4]. Human malaria is caused by four *Plasmodium* species: *P.falciparum, P. vivax, P. malariae and P. ovale*, with *P. falciparum* accounting for the majority of infections. The disease has resurfaced as a major public health problem over the past three decades primarily due to the development of worldwide resistance to many drugs, the most important being resistance by *P. falciparum* to chloroquine (CQ) **12** and other drug combinations.

Malaria is a protozoan infection transmitted by the female mosquito of the genus *Anopheles* during the blood meal.



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Protozoa divide either by binary or multiple fission. Multiple fission involves either a single cell, which is known as schizogony, or a zygote, which is known as *sporogony.* Schizogoy is an asexual form of reproduction in which the nucleus divides repeatedly to yield a multinucleate cell or a *schizont.* This then releases small cells with single nuclei known as *merozoites.* Sporogony on the other hand is sexual and involves the release of spores or *sporozoites* from a mature zygote which has been formed from the fusion of male and female cells or *gametes.*

b) History of Chemotherapy

The first synthetic drug for the treatment of malaria was quinacrine **13.** CQ later replaced quinacrine as the drug of choice for the prevention and treatment of malaria. Other antimalarial drugs include proguanil (Paludrine) **14**, amodiaquine **15,** primaquine **16,** tetracyclines and sulfonamides. The newest agents are mefloquine (Larium) **17**, halofantrine (Halfan) **18**, pyrimethamine **19** and artemisinin compounds **(20-23)** (figure 1.4).

Despite the widespread resistance to CQ, the drug is still in use. It is exclusively active against the blood stages of the parasite and is used to rapidly cure CQ sensitive malaria [5]. Prior to the onset of resistance, the drug was well suited for prophylactic and was a cheap and effective method of treatment. CQ has been the drug of choice as it is a cheap, oral drug with limited host toxicity, good tolerability, good absorption and distribution and is effective as treatment and for prophylaxis of this disease. Side effects may include gastrointestinal upset, mild headaches and visual disturbances.

Proguanil **14**, also known as chloroguanide, serves as a prodrug that is converted upon ingestion to a cyclic triazine metabolite. The use of proguanil is safe and well tolerated but has been compromised by the development of proguanil-resistant strains of *P. falciparum*. Side effects are few and include occasional nausea and diarrhoea. Large doses may result in abdominal pain, diarrhoea, hematuria and the transient appearance of epithelial cells and casts in the urine.

Amodiaquine **15** is also active against the blood stages of the parasite but is used much less extensively than CQ [6]. Significant cross resistance between CQ and amodiaquine has been shown. Amodiaquine exerts some serious toxic side effects such as agranulocytosis and hepatotoxicity and as a result is not recommended for chemoprophylactic use.

Primaquine **16** is the only drug available for the treatment and clearing of Plasmodia from the liver and is thus used in the cure of *P. vivax* and *P. ovale*. Toxic effects include mild anaemia, cyanosis and haemolysis [6], haemolysis being particularly prevalent in glucose-6-phosphate dehydrogenase deficient subjects. Rare symptoms include hypertension, arrhymias and symptoms referable to the CNS.

Mefloquine **17** is a readily tolerated antimalarial drug active against both the sensitive and resistant strains of *P. falciparum*. It is used in the treatment and prevention of CQ-

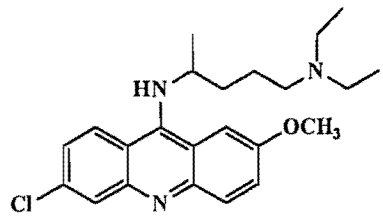
resistant falciparum malaria. Mefloquine is generally well tolerated and side effects are mild and transient and include nausea, vomiting, abdominal pain and dizziness. Symptoms of CNS toxicity present themselves rarely. However, widespread resistance has developed in Thailand [7] and Africa [8].

Pyrimethamine **19** is a 2, 4, -diaminopyrimidine whose main use is in prophylaxis, suppression and combined chemotherapy of CQ-resistant strains of falciparum malaria[9]. The antifolate pyrimethamine-sulphonamide combination and Fansidar (pyrimethamine-sulfadoxine) is important in the treatment of CQ-resistant malaria on the African continent. Pyrimethamine alone causes no significant toxicity with the occasional skin rashes and depression of haematopoiesis. The combination of pyrimethamine and sulfadoxine can cause severe cutaneous reactions such as Steven-Johnson syndrome and toxic epidermal necrolysis. Other reactions include serum-sickness type reactions, urticaria, exfoliative dermatitis and hepatitis. These adverse effects are attributed to the sulfonamide component of the mixture.

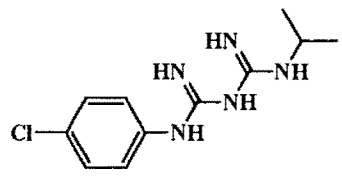
Both quinine **24** and quinidine are active only against the blood stages of the parasite[6]and are used to rapidly clear parasites form the blood. Artemisinin **20** is a sesquiterpene lactone with a peroxide bridge linkage, the peroxide moiety appearing to be responsible for the antimalarial activity. The poor solubility of the drug led to the synthesis of more soluble derivatives in the form of dihydroartemisinin **21**. Its esterification and etherification led to artesunate **22** and artemether **23** respectively. All of these derivatives have more potent antimalarial activity than artemisinin itself. Artemisinin and its derivatives have been proven to be effective against *P. falciparum* in sub-Saharan Africa while combinations of artemether or artesunate with mefloquine have proved to be effective in the treatment of multidrug resistant malaria in South East Asia. On a cautionary note, artemisinin resistance has been developed and demonstrated in the laboratory [10].

Quinine is more toxic and less effective than CQ as both a suppressive and therapeutic agent. Toxic effects resulting from repeated doses of quinine include a cluster of symptoms called cinchonism. In its mild form, this includes ringing in the ears, headaches, nausea and disturbed vision. Further reactions include hypotension, hypoglycaemia, electrocardiographic changes and vertigo [6]. CNS symptoms are noted in severe grades of poisoning, particularly headache, fever, vomiting, delirium and

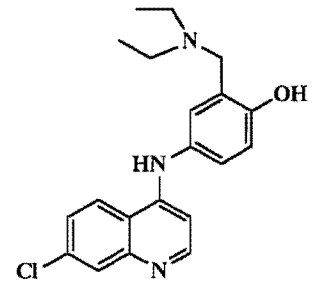
syncope. A decrease in sensitivity of P. falciparum has been reported in some areas although resistance is not widespread.



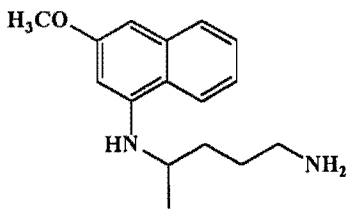
Quinacrine (13)



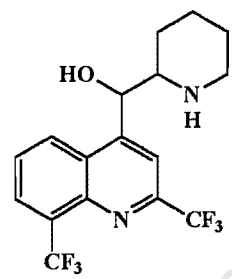
Proguanil (14)



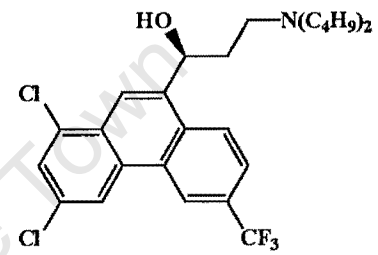
Amodiaquine (15)



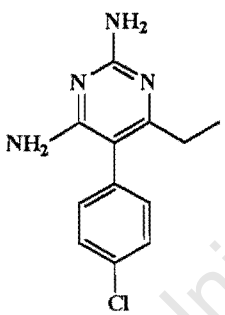
Primaquine (16)



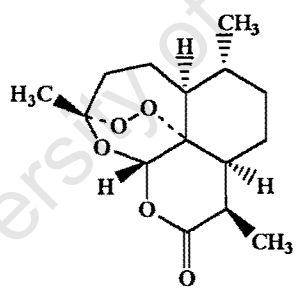
Mefloquine (17)



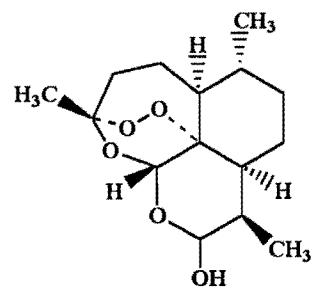
Halofantrine (18)



Pyrimethamine (19)



Artemisinin (20)



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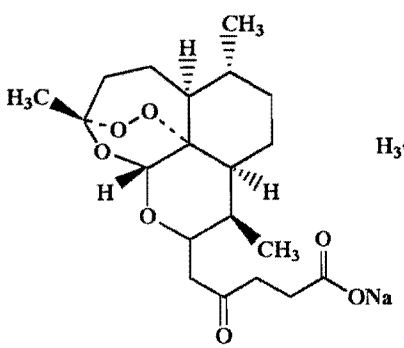

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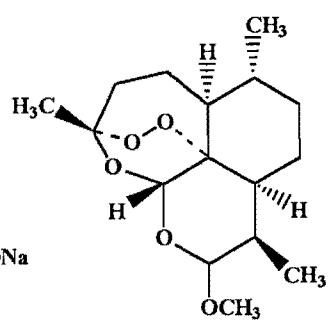

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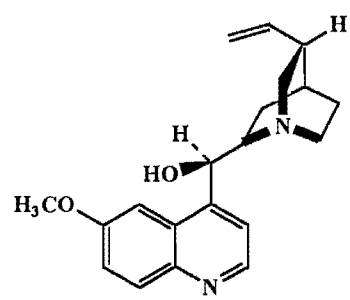
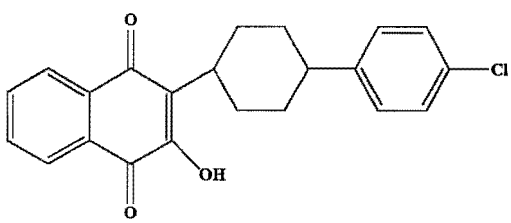


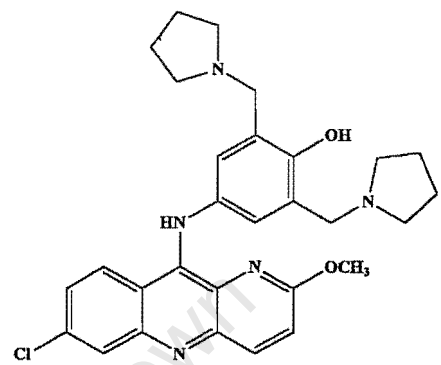
Quinine (24)

Figure 1.4. Chemical structures of some antimalarial agents.

Other alternative drugs take the form of atovaquone **25** (figure 1.5) which has broad-spectrum antiprotozoal activity, pyronaridine **26** (figure 1.5) which is still under development and sulfonamides and sulfones in combination with other available drugs e.g. pyrimethamine.



Atovaquone 25



Pyronaridine (26)

Figure 1.5. Structures of two alternate antimalarial drugs.

1.5 MDR CANCER AND ITS REVERSAL BY CHEMOSENSITIZERS

The incidence, geographic distribution and behaviour of specific types of cancers are related to a number of factors which include sex, age, race, genetic predisposition and exposure to environmental carcinogens. Cancer is a disease of cells which is characterised by a shift in the control mechanisms that govern cell proliferation and differentiation. Cells that have undergone this neoplastic transformation proliferate excessively and form local tumours that can constrict or invade adjacent structures.

Ideal cancer drugs would be able to eradicate cancer cells without harming normal tissues. However, none of the available agents meet this criteria and one has to weigh the benefits against the toxicity in the search for a favourable therapeutic agent. The drugs primarily used in cancer treatment are vinca alkaloids (vinbastine, vincristine), anthracyclines (doxorubicin), epipodophyllotoxins (etoposide), cisplatin and paclitaxel.

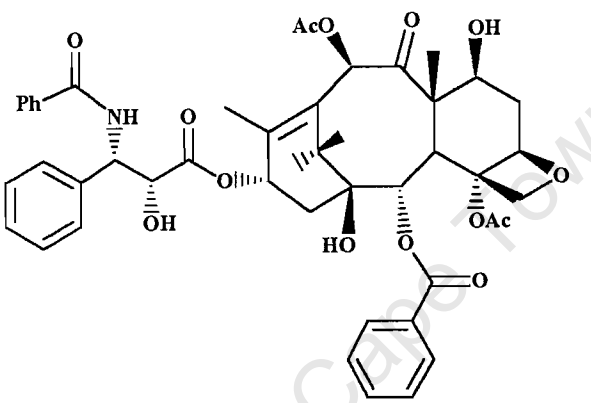
Paclitaxel (Taxol^{TM}) **27** is one of the best anticancer agents to be discovered in recent years[11]. In 1992 paclitaxel was approved by the US FDA for the treatment of advanced ovarian cancer and in 1994 for breast cancer. Its clinical use has been expanded to the treatment of lung, head, neck and gastrointestinal cancers. Paclitaxel has a novel mode

of action in promoting tubulin assembly and stabilizing the resulting microtubules[12, 13]. Unfortunately, like with many anticancer drugs, resistance to paclitaxel has presented new challenges. Thus restoring paclitaxel sensitivity to resistant tumours is of vital importance.

Only a few of the available drugs are effective for each specific tumour type because of this acquired or primary drug resistance. Primary resistance involves the absence of response on first exposure to the drug while acquired resistance develops in a number of drug-sensitive tumour types. This broad-spectrum resistance to anticancer agents is what constitutes the multidrug resistance (MDR) phenotype. This form of multidrug resistance is associated with an increased expression of an energy-dependent drug transport protein, P-glycoprotein (Pgp). This over expression results in less accumulation of the anticancer agent as the cancer cell can efficiently pump out the hydrophobic molecules [14, 15]. Pgp expels the drug as it enters the plasma membrane. Certain drugs have the ability to inhibit this MDR mechanism by binding preferentially to Pgp and blocking the efflux channel. This enables the anticancer agent to remain in the cancer cell and exert its effect. These inhibitors are called MDR reversal agents (chemosensitizers). Ideal chemosensitizers have been described as compounds without any intrinsic or inherent cytotoxicity which can reverse the resistance of the cells to the cytotoxic action of the drugs[16]. Since the discovery that verapamil, a calcium channel blocker, was able to effect the MDR mechanism, many other reversal agents have been identified. Included among these are antipsychotic agents (phenothiazine). In the presence of appropriate concentrations of chemosensitizers, drug toxicity is greatly potentiated in resistant cancer cells and, in many cases; the IC_{50} approaches that of susceptible cells [14]. The chemosensitization effect of verapamil is associated with an increase in drug accumulation. This process is thought to be the mechanistic basis of chemosensitization.

The widespread concept presumes inhibition of Pgp activity by competition with the cytotoxic agents for the same binding sites [17] It is well recognised that MDR chemosensitizers share common features needed for the reversal: they are amphipathic drugs and are mostly protonated [15]. These common features are related to the ability of the chemosensitizers to interact with the membrane phospholipids [18]. It has been shown that the parts of the protein that affect its transport specificity belong mainly to

amino acid sequences located in the membrane[15]. Pearce and co-workers concluded that the relative disposition of aromatic rings and basic nitrogen was important for their anti-MDR activity [19]. Further investigations have estimated several structural features of importance for their anti-MDR activity [19] and point to the role of hydrophobicity as a space-directed molecular property to explain differences in anti-MDR activity of the drugs studied [20].



Paclitaxel (27)

CHAPTER 2

BACKGROUND AND RATIONALE

2.1 TRYPANOTHIONE REDUCTASE AS A TARGET FOR ANTITRYPANOSOMAL AND ANTILEISHMANIAL AGENTS

2.1.1 Introduction

As described earlier, the current drugs available for the chemotherapy of trypanosomiasis and leishmanias are inadequate in terms of efficacy, toxic or both. Furthermore, resistance to a large number of these drugs has been reported. An additional problem is the high cost of the currently available drugs. Hence, there is a definite and urgent need for new treatments that are safe, effective and inexpensive.

Tyrpanothione reductase (TryR) is unique to the parasitic protozoa *Trypanosoma* and *Leishmania* and is an attractive and validated target for the rational design and development of new antiparasitic drugs. Studies have shown that there is a distinct metabolic difference between the trypanomastids and their mammalian host. The parasites and host utilize differing processes in the control of their intracellular reducing environment. Mammals utilize glutathione for this purpose whereas trypanomastids utilize the trypanothione system for redox defence [21]. TryR is the enzyme crucial to the management of oxidative stress in the aforementioned parasitic protozoa. The closest related human enzyme is glutathione reductase (hGR). TryR and hGR display mutual exclusive specificity for their substrates (trypanothione disulfide for TryR and glutathione disulfide for hGR) based on differences in their active sites. Simply put, this biochemical difference between host and parasite and the crucial role of TryR for thiol homeostasis suggests trypanothione metabolism as a possible target for antiparasitic drugs. The effective inhibition of TryR could disable the parasites' ability to cope with oxidative stress resulting in death or rendering it more susceptible to the host immune response or other drugs.

2.1.2 The glutathione redox cycle

Mammalian cells utilise hGR in the management of oxidative stress. When the mechanisms that defend the cell against various reactive oxygen species are compromised, the cell is said to be under oxidative stress. These reactive oxygen species include the superoxide anion ($O_2^{\cdot -}$), hydroxyl radicals ($HO^{\cdot}$), and hydrogen peroxide (H_2O_2). These reactive oxygen species are cytotoxic due to their ability to modify nucleic acids, thiol-containing proteins and membrane lipids. Reactive oxygen species of this nature are produced during normal aerobic respiration. The metabolic pathway for detoxification of these reactive oxygen intermediates is the glutathione redox cycle (figure 2.1). This is where hGR plays its crucial role. hGR catalyses the reduction of glutathione disulfide (GSSG) **28** to glutathione (GSH) **29** in a NADPH-dependent reaction. As a consequence, the GSH levels are maintained.

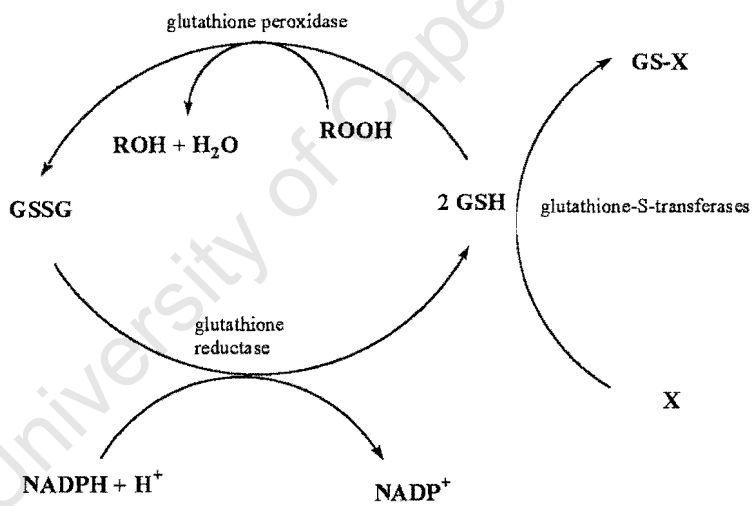
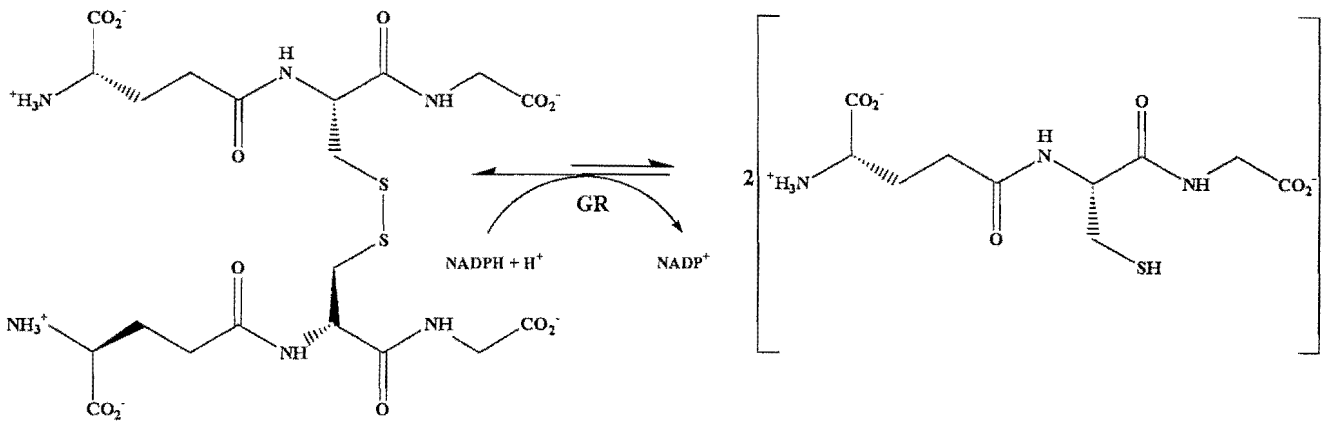


Figure 2.1. Reaction catalysed by glutathione reductase (hGR).



Glutathione disulfide (GSSG)
28

Glutathione (GSH)
29

Scheme 2.1. Diagram of the reduction of glutathione disulfide **28** to glutathione **29**, catalysed by glutathione reductase (hGR).

Glutathione (GSH) participates in the detoxification of organic hydrogen peroxides (ROOH). This process involves the use of the enzyme glutathione peroxidase. Two GSH molecules are oxidized to the disulfide (GSSG). GSH is regenerated by reduction with NADPH. GSH also binds various cytotoxic substances (X in figure 2.1), a process mediated by glutathione –S-transferases.

2.1.3 Trypanothione

As parasites, trypanomastids are exposed to various reactive oxygen species generated by the host redox system. Consequently, an analogous system has evolved in trypanosomes and leishmanias. They, however, have no glutathione reductase or glutathione redox cycle. They employ trypanothione (N^1, N^8-bis (glutathionyl) spermidine) **33** for this task rather than GSH. Trypanothione disulfide $Try[S]_2$ **34**, differs from GSSG only by the presence of a spermidine cross-link (figure 2.2). The parasite system is far less efficient than the mammalian redox defence system but has a much broader substrate specificity.

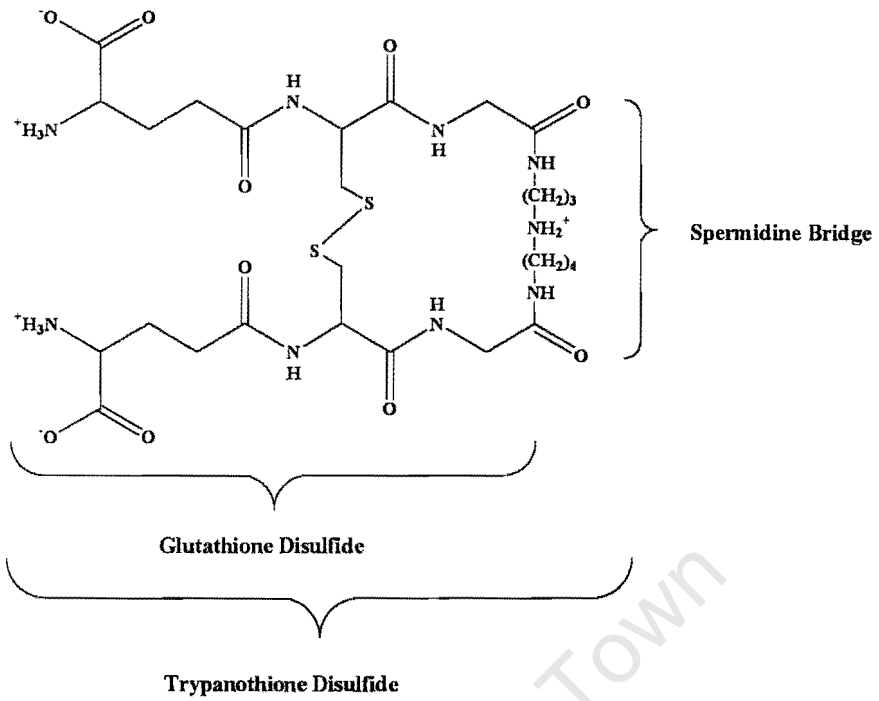


Figure 2.2. : Structures of Trypanothione disulfide **31**and Glutathione disulfide **28.**

Trypanothione **33** is synthesized from glutathione **29** and spermidine **30**. Spermidine is combined with glutathione by the enzyme glutathionylspermidine (Gsp) synthetase to yield monoglutathionylspermidine conjugates **31-32**. Trypanothione synthetase (TryS), an ATP-dependent enzyme, adds a second glutathione molecule to yield trypanothione **33**. Oxidation yields trypanothione disulfide **34**.

Trypanothione has other functions in the parasite. These include ascorbate homeostasis [22], thiol disulfide exchanges, conjugation of metals and drugs [23], synthesis of deoxyribonucleotides[24] and reduction of hydroperoxides [25].

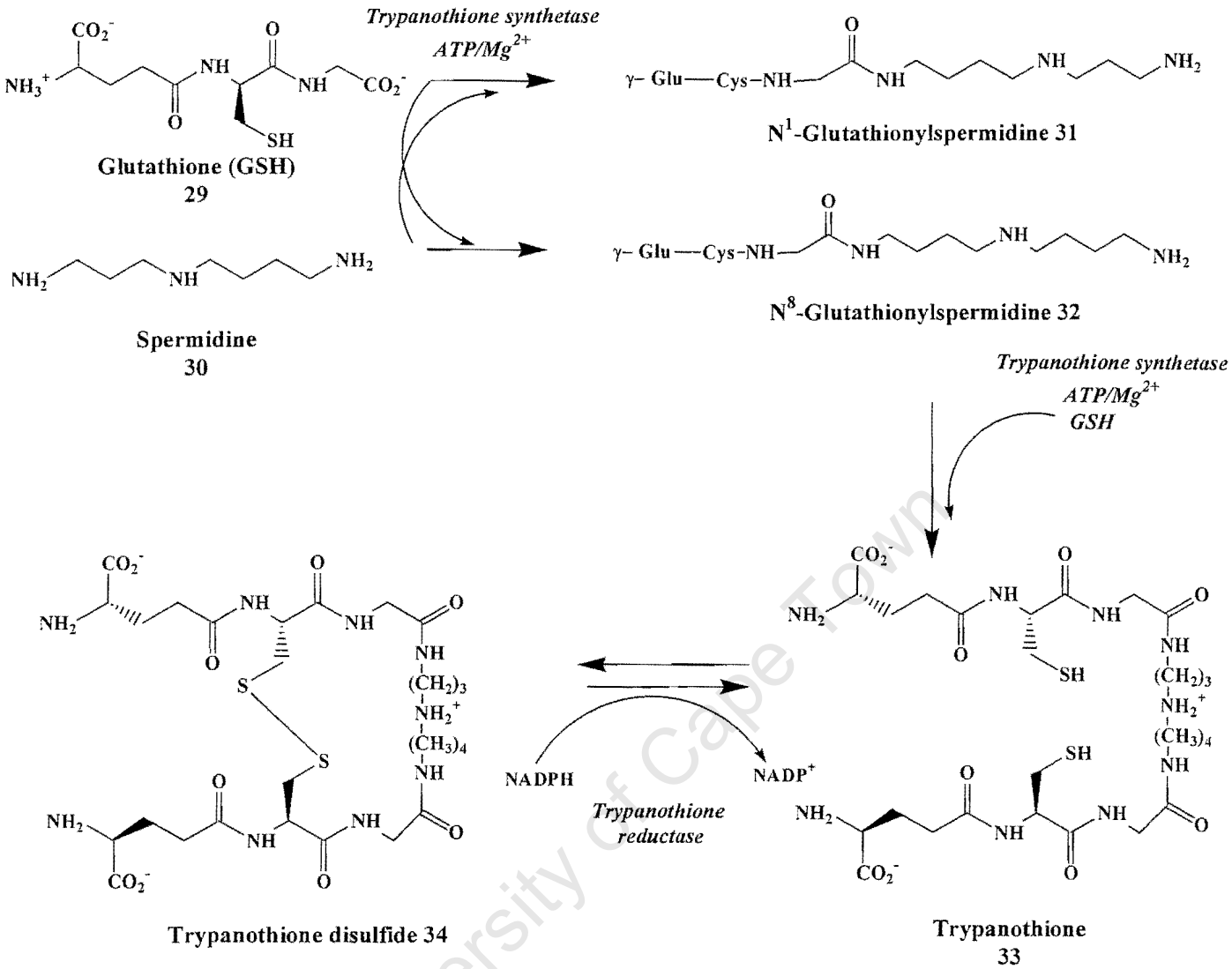


Figure 2.3. Metabolism of trypanothione in trypanosomes and leishmanias.

2.1.4 Structural and binding aspects of the target

TryR and hGR share close structural and mechanistic similarities. They are both dimeric proteins with a monomer molecular mass of 52 KDa. Each subunit is folded into four domains, the FAD binding, the NADPH binding, the central and the interface domains. There are two identical active sites which are formed by the residues of the FAD, NADPH and central domain of one of the monomers and the interface of the other. Both have a redox-active disulfide at their active sites.

Despite a 41% sequence homology between *T. Congolese* TryR and hGR, the two enzymes are mutually exclusive with regard to disulfide substrate specificity as described earlier [26, 27]. The different substrate specificities of the two enzymes can be

explained of the basis of the structure of the active site. The active site of TryR is large and negatively charged and contains a hydrophobic pocket for the recognition of the spermidine moiety of the substrate. On the other hand, the active site of hGR lacks the binding pocket and has an overall positive charge. These factors are responsible for the selectivity of inhibitors of either enzyme.

2.2 INHIBITORS OF TryR

2.2.1 Introduction:

Different classes of compounds have been revealed as selective inhibitors of TryR. These compounds react more weakly with the closest related human enzyme hGR. Three classes of these selective inhibitors are described below:

i) Competitive inhibitors.

They include crystal violet [28], a number of tricyclics based on acridine [29], phenothiazines e.g. **35-37** [30], benzoazepine [31], isoalloxazine [32] polyamine derivatives e.g. **38** [33], and pyridoquinoline [32] ring structures as well as 2-aminodiphenylsulfides e.g. **39-40** [34]. None of the aforementioned act as antiparasitic drugs but they could be used as leads in the synthesis of more specific inhibitors of TryR.

ii) Turncoat inhibitors (subversive substrates)

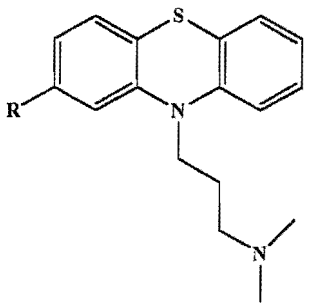
These include nitrofuran and naphthoquinone derivatives. They bind to the NADPH-reduced enzyme where they transfer electrons to molecular oxygen creating superoxide radicals which can trigger lipid peroxidation and other chain reactions. As such, they have a strong impact on redox metabolism of trypanosomes.

iii) Covalent inhibitors

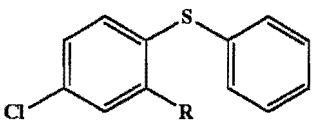
They include drugs such as carmustine that covalently inhibit TryR but also bind hGR [26]. Another example is ajoene [35].

Results to date suggest that a ligand composed of a moiety that is parasite enzyme specific, connected by a tailored spacer to a reactive group capable of covalent modification, would show promise as inhibitors of TryR. Peptide inhibitors have been tested as potential drug targets. The active site of the enzyme is composed of amino acids therefore peptides are conceptually easy to design. Their synthesis is straightforward although they do present problems as drugs (metabolism, immune recognition, charge, oral bioavailability). Several peptides have been found to be

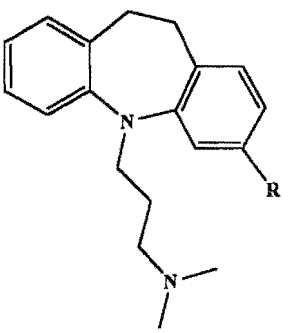
selective inhibitors of TryR over hGR [36]. Quarternization of a tertiary amine has lead to compounds which were found to be strong inhibitors of *T.cruzi* TryR [36].



Promazine (35): R=H
Trifluopromazine (36): R=CF_3
Chlorpromazine (37: R=Cl



2-amino-diphenylsulfide derivatives (38)



Imipramine (39): R=H
Chlomopramine (40): R

Figure 2.4. Representative structures of selective inhibitors of TryR.

2.2.2 Tricyclic Inhibitors of TryR

The utilization of rational drug design approaches against TryR [31] has led to the discovery of the tricyclic structures mentioned above as specific inhibitors of TryR over hGR. It has been suggested that such tricyclics may bind to TryR with the tricyclic group lodged against the hydrophobic wall (formed by Trp 21 and Met 113) (figure 2.5). An additional hydrophobic cavity, the Z site, lies nearby (figure 2.5) and may be accessed by hydrophobically functionalised molecules [37]. Phenothiazines and tricyclic antidepressants drugs could be accommodated at the hydrophobic wall site.

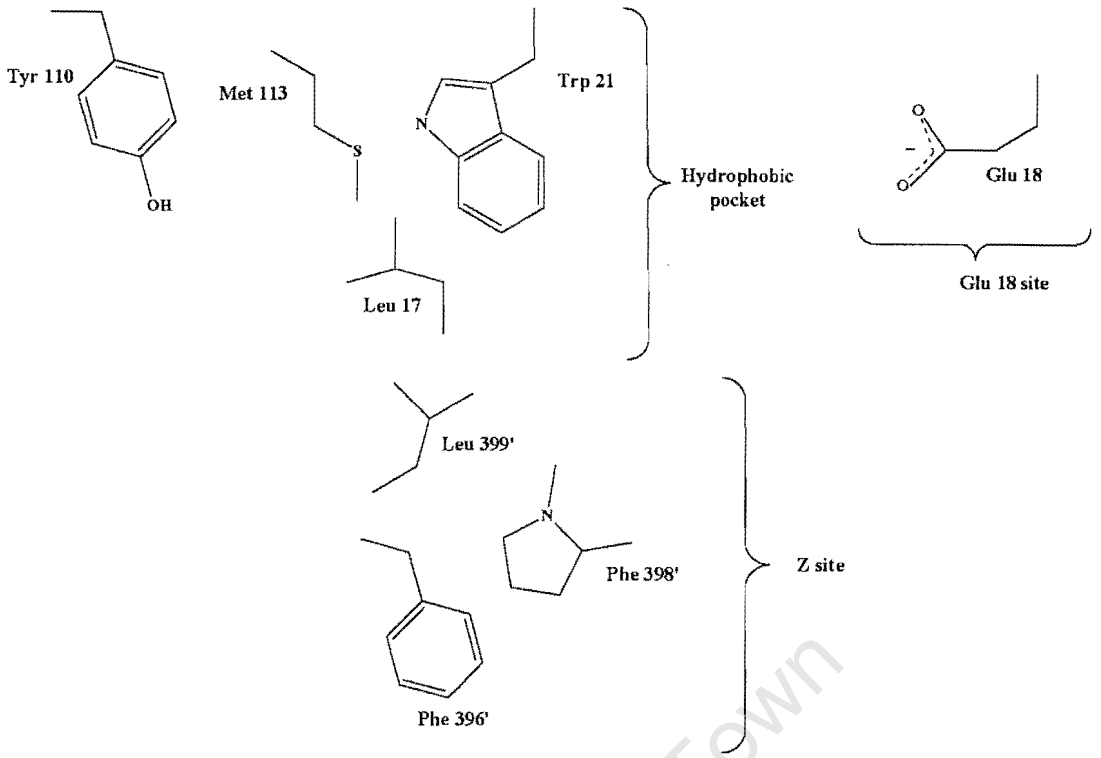
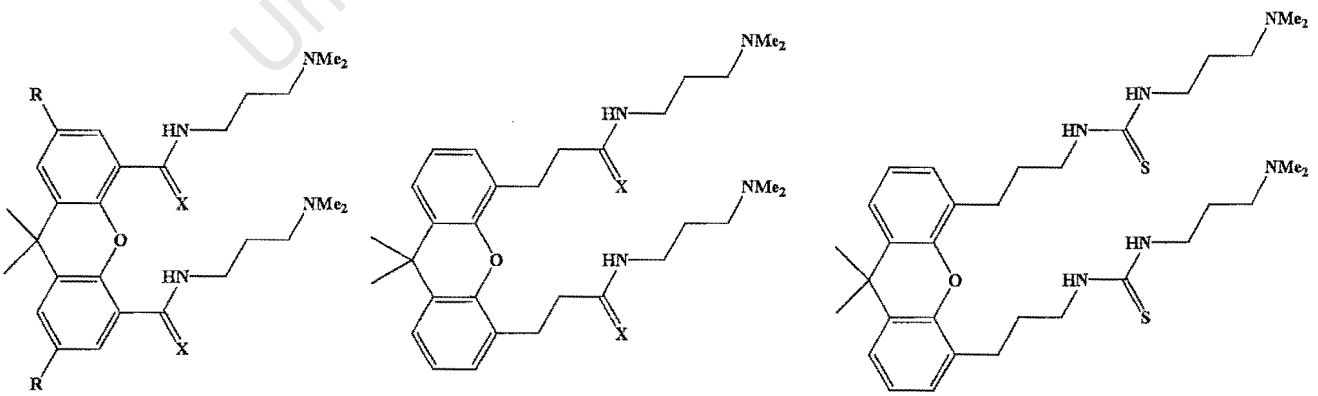


Figure 2.5. Schematic summary of the regions in the active site of TryR (not to scale). Adapted from Chan *et al* [37].

Tricyclics based on the 9, 9-dimethylxanthene moiety such as **41-46** have also been investigated as inhibitors of TryR [38]. The study showed that derivatives with a 2-3 carbon methylene spacer between the tricyclic moiety and the secondary nitrogen shows stronger TryR inhibition than those with 1 or no methylene spacer (figure 2.6). Nevertheless, these compounds were generally found to be weak inhibitors of TryR.



41: R=H, X=O
42: R=H, X=2H
43: R=*tert*-butyl, X=O

44: X=O
45: X=2H

46

Figure 2.6. Structures of, 9,9-dimethylxanthene tricyclics investigated as inhibitors of TryR.

2.2.3 Acridine Inhibitors

While the crystal structures of TryR [39, 40] and of complexes with its substrates have been solved [41, 42], only one structure of TR with an inhibitor has so far been elucidated: the TryR-quinacrine (mepacrine) complex [29]. It shows the atomic interactions between TryR and the ligand. Mepacrine (which inhibits TryR [43] but not hGR) is bound in the active site close to the hydrophobic wall (formed by Trp 21 and Met 113) with the ring nitrogen pairing with Met 113, the chlorine with Trp 21 and the methoxy group with Ser109. The positively charged alkylamino chain is directed towards the inner region of the active site and may form a water-mediated hydrogen bond with Glu18 (figure 2.5). This positively charged group has been shown to play a major role in selective binding to TryR [30]. As stated earlier, two binding modes for quinacrine have been predicted [36, 44]. These were evaluated via molecular modelling using a prediction algorithm used for the binding affinity of ligands to TryR. It utilizes the virtual screening of a database of 2D molecular sketches which it then converts to 3D ligand structures. The first binding mode places the tricyclic aromatic moiety in the hydrophobic pocket defined by Trp 21, Met 113 and Tyr 110 while the second places it at the Z site, which is approximately defined by Phe 396', Pro 398' and Leu 399'(figure 2.5).

The tricyclic acridine moiety has been investigated as an inhibitor of TryR. Experiments with a series of 9-aminoacridines **47-49** (figure 2.7) and acridine revealed that the 9-aminoacridines were weaker inhibitors of TryR than mepacrine. Kinetic studies showed that more than one inhibitor molecule can bind to the enzyme [43]. 9-Thioacridine **50-51** (figure 2.7) derivatives inhibit TryR with mixed type kinetics [43] but are still less efficient than mepacrine. Acridine itself had no demonstrable activity highlighting the importance of the alkylamino chain to binding [43]. An increase in one methylene within the alkyl chain of **48** to **49** led to improved TryR inhibition relative to mepacrine within this series.

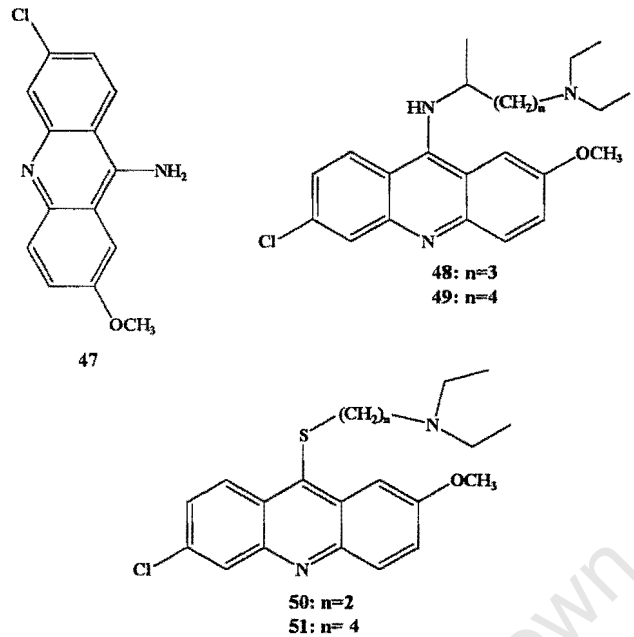


Figure 2.7. Representative structures of 9amino and 9-thioacridines targeted for investigation against TryR.

Members of the class of 9-anilinoacridine topoisomerase II inhibitors bearing electron donating 1'-anilino substituents have been shown to be active against both the promastigote and amastigote forms of the parasite *Leishmania major* [45] and 3, 6-diNMe$_2$ substitution on the acridine ring increases this toxicity to the leishmania parasite [46].

2.3 ACRIDINE AND QUINOLINE – BASED DRUGS:

2.3.1 Application in antiparasite chemotherapy:

Some of the very first known antimalarial drugs have been based on the acridine and quinoline moieties as a substructure. Historically the quinolines are the most important group of antimalarials. Quinine **24** was isolated from the bark of the cinchona tree and for centuries was the only treatment available. The first synthetic antimalarials yielded such drugs as quinacrine. Further work yielded such drugs as amodiaquine, primaquine and CQ. Although primaquine and amodiaquine displayed significant toxicity to the parasite, CQ became the drug of choice for almost five decades. With the development of parasite resistance to CQ, the search to find new antimalarials had begun. Currently under investigation and development by the WHO is another quinoline-based drug for

the treatment of malaria, pyronaridine. A series 4-aminoquinoline mannich base antimalarials have been shown to be active against both CQ-sensitive and CQ-resistant strains of *P. falciparum* [47].

Although quinoline-containing compounds have been used primarily for the treatment of malaria, their usefulness has been extended to other parasitic diseases. 8-Aminoquinoline (8-AQ) compounds have been extensively studied since the antileishmanial properties of primaquine were identified. An 8-alkoxypiperazinoalkylamino analogue, moxipraquine, showed promising antitrypanosomal properties as well as antileishmanial properties against cutaneous leishmaniasis [48]. CQ has also been investigated in terms of its antileishmanial properties and has been shown to have moderate *in vitro* activity against the amastigote cell line in *L. mexicana* [49].

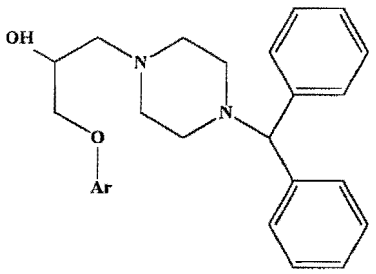
Acridines have immense utility in both the pharmaceutical and dye industries[50]. The discovery of acridines as antimalarial and antitumour agents has led to intensive interest and synthesis of several drugs based on acridine [50-52]. Of particular relevance to tropical diseases, quinacrine has been used in the treatment of cutaneous leishmaniasis and its *in vitro* activity against *T.cruzi* is known [53, 54]. As described before, quinacrine has also been used in the treatment of malaria. Recent investigations have revealed a number of 9-anilinooacridines and bis (9-amino-6-chloro-2-methoxyacridines) as having antimalarial, antileishmanial and antitrypanosomal activities [46, 55, 56].

2.3.2 Application to multidrug resistance (MDR) reversal in cancer:

As described earlier, chemosensitizers block the efflux of the anticancer agent allowing it to stay in the cell and exert its effect. Included among these chemosensitizers are quinine and chloroquine . The ability of the antimalarial drugs chloroquine and primaquine as well as other quinolines to act as MDR reversal agents for vinblastine-resistant (CEM/VLB100), and vincristine-resistant (K562/ADM) cancer cell lines has been previously reported [57-59].

Experiments were conducted to test the importance of the quinoline moiety. When the quinoline ring in **52** was replaced by a naphthyl **53** or phenyl **54** ring, the resulting derivatives were found to be significantly less active than verapamil whereas the MDR

reversing activity of **52** (figure 2.8) was 3-4 times higher than that of verapamil. Thus, it was concluded that the quinoline moiety seemed to play an important role in determining the drug activity.



52: Ar=5-quinolyl
53: Ar=1-naphthyl
54: Ar=C_6H_5

Figure 2.8. Representative structure of the aromatic derivatives investigated by Suzuki *et al*[59].

Within the context of paclitaxel chemosensitizing agents, some of the quinidine, quinine and quinacrine antimalarial drugs have recently been reported to overcome *in vitro* and *in vivo* paclitaxel resistance in vinblastine resistant (CEM/VLB100), non-Hodgkin's lymphoma and hormone-refractory prostate cancer cells [60-62].

2.4 RATIONALE

Chloroquine and primaquine are both drugs containing the biologically important quinoline moiety. Hence, this moiety has been utilized extensively in the drug discovery process towards the discovery of new, effective, potent and safe antiparasitic drugs. Since the discovery of the ability of CQ to act as a chemosensitizer in enhancing the cytotoxicity of vinca alkaloids e.g. vinblastine [57] in vinblastine resistant cancer cells, much research has gone into the investigation of the ability of other quinoline-containing drugs to effect the same result [58, 59].

Mepacrine contains the biologically active moiety acridine. Hence, it is known that the acridine moiety exhibits promising antiparasitic activity. It has also shown activity as MDR reversal agents. As mentioned earlier, studies have shown the importance of this moiety in the process of drug discovery. In terms of the inhibition of the enzyme trypanothione reductase, tricyclic moieties play an important part in binding of the

hydrophobic pocket and since the elucidation of the mepacrine TryR complex, there is much interest in further investigation into the optimum structure for binding to this enzyme *via* structure-activity relationships.

Consequently, both moieties are attractive targets on which to build scaffolds and diversify for the purpose of exemplifying structure activity relationships.

2.4.1 Acridine derivatives

We became interested in testing the binding model of quinacrine by incorporating methylene spacers between the tricyclic moiety of **13** and a second hydrophobic aromatic moiety. This led to the preliminary design of sulfonamides **55-58** ureas **59-62**, and amines **63-66** (figure 2.9). The amine, sulfonamide and urea moieties were incorporated with a view to improving solubility properties of the molecules. Substituted ureas have been shown to have important biological activities [63, 64] and are a common structural motif in biologically active molecules [65, 66]. The urea is considered a nonhydrolysable surrogate of an amide bond. We were also interested in the *in vitro* antiparasitic activities of these compounds in view of previous aforementioned literature reports on related compounds [46, 55, 56]. Series **67-69** further investigated this model. Work by W. N. Hunter on the X-ray crystal structure of mepacrine mustard bound to TryR (unpublished results, personal communication to Dr K. Chibale) together with the theory on binding modes, presented us with the opportunity to test the importance of the methoxy group in TryR inhibition by manipulating it to yield **70-73.**

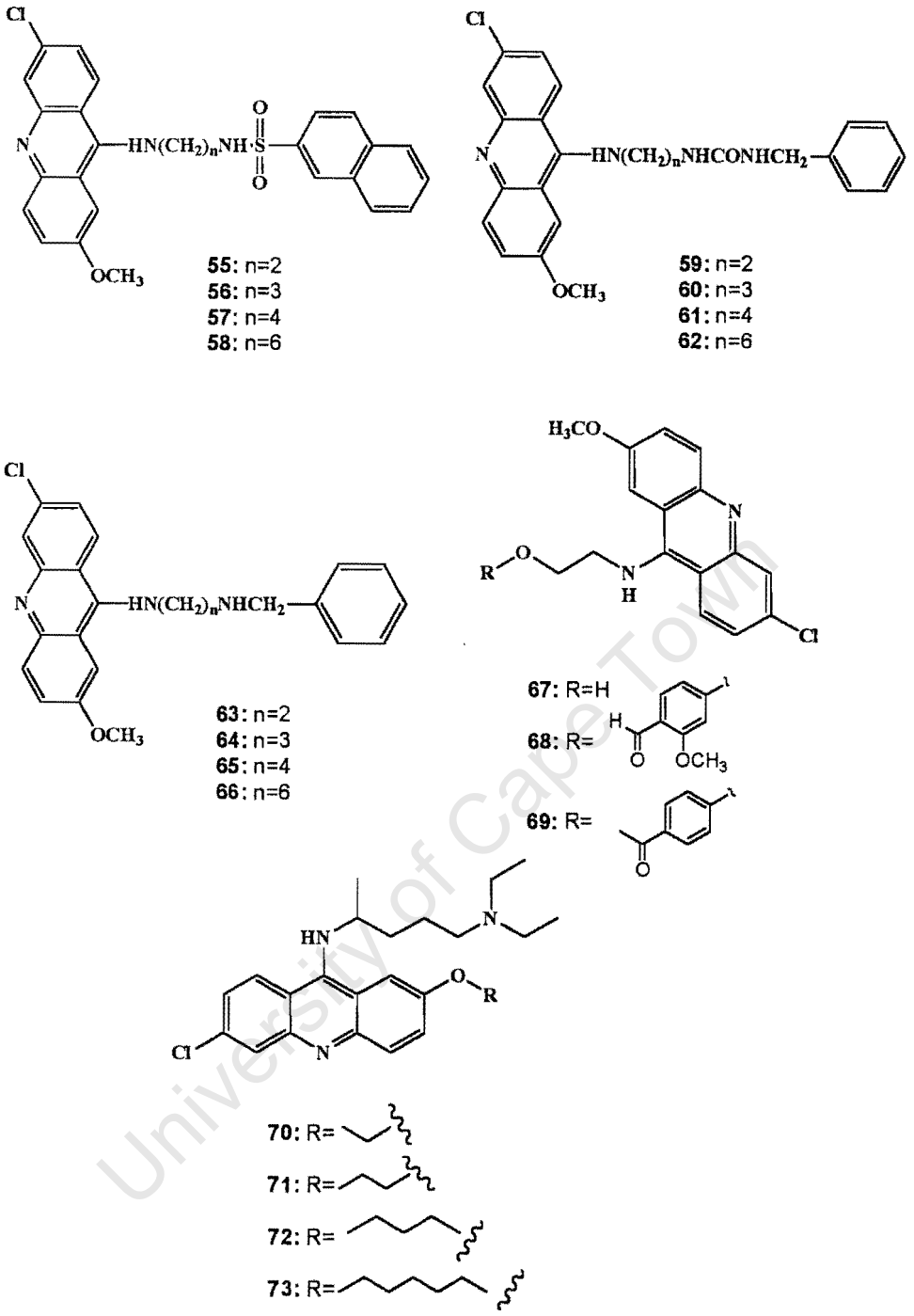


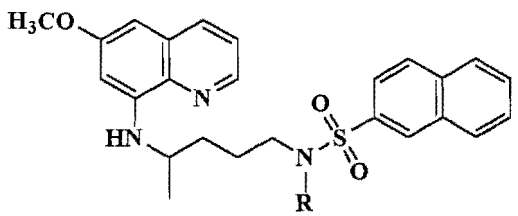
Figure 2.9. Structures of acridine compounds targeted for synthesis.

2.4.2 Quinoline derivatives

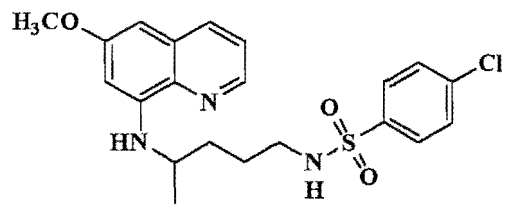
Since primaquine is the only structural class of compounds that shows activity against the tissue stages of the parasite, it is an area of intense interest. It has been shown that

the 6-methoxy-8-aminoquinoline drugs carrying a terminal tertiary amino group on the side chain, with 2 or 3 carbon atoms between the nitrogens on the side chain, display undesirable toxic reactions [67, 68]. Coupled with this, hydrophobicity is an important feature in MDR reversal agents. Consequently, our research has been aimed at the manipulation of this terminal tertiary amino group to form sulfonamides. Target compounds synthesized of that nature are **74**, **75**, and **76** (figure 2.10).

Following on from the previously described amide-based 5-alkoxyquinolines with MDRR activity [59], we targeted some aromatic quinoline sulfonamide derivatives with varying methylene spacer lengths as well as bisquinoline ureas and ureas for preliminary studies. This is in view of recent observations that CQ derivatives with appropriate alkyl chain spacer lengths appear to evade the mechanism(s) responsible for resistance to CQ in resistant malarial cells [69-71]. Since hydrophobicity is an important feature in MDR reversal agents, we reasoned that the presence of a sulfonamide proton should provide us with a diversity point for introducing various hydrophobic groups during the exploration of SAR. The urea moiety was incorporated with a view to improving solubility. Other quinoline compounds which were synthesized in this regard are **77-84** (figure 2.10) as well as the amines **85** and **86** (figure 2.11). Bisquinolines have been shown to inhibit the growth of CQ-sensitive and CQ-resistant parasites [72]. Consequently, several bisquinoline urea compounds **87-88** and a urea derivative **89** were synthesized *via* their respective carbamate derivatives, **90** and **91** (figure 2.11). Once again the urea moieties were incorporated with a view to improving the solubility of the compounds.



74: R= H
75: R=Bn



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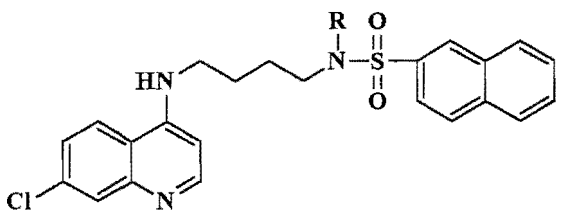
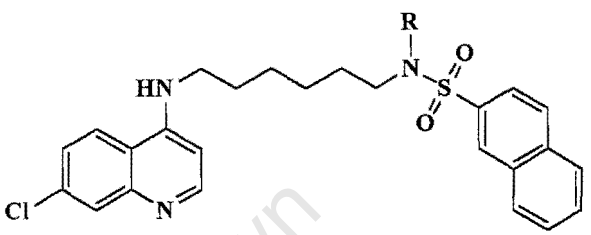
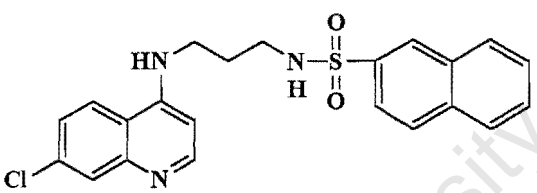


77: R=H
78: R=Bn



79: R=H
80: R=Bn



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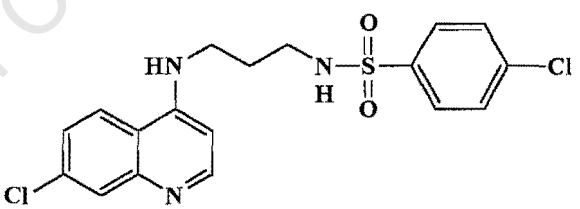

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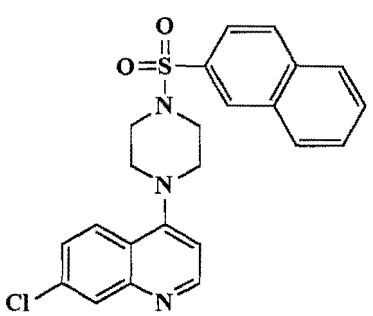

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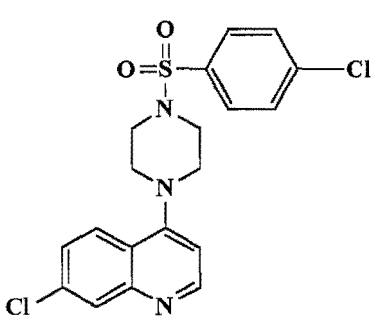

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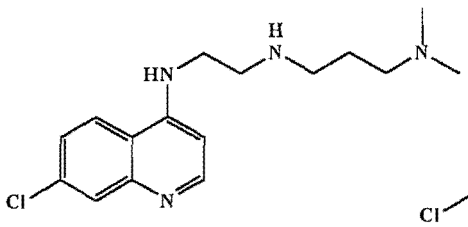
Figure 2.10. Structures of sulfonamide derivatives.



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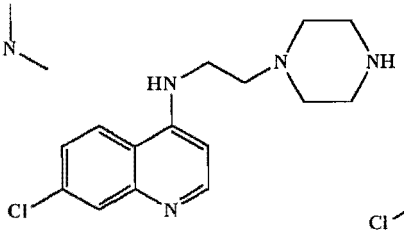

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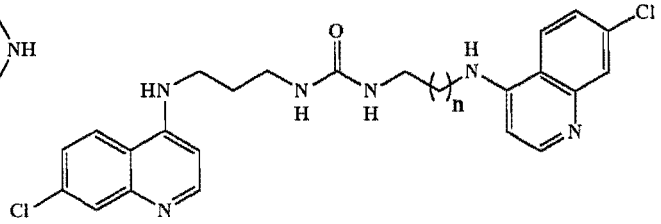
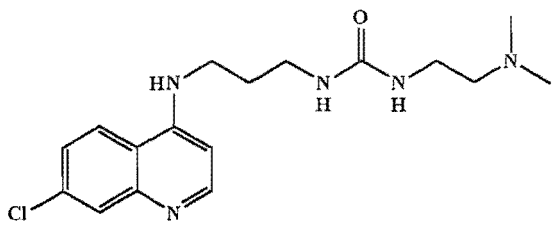


87: n=2
88: n=3



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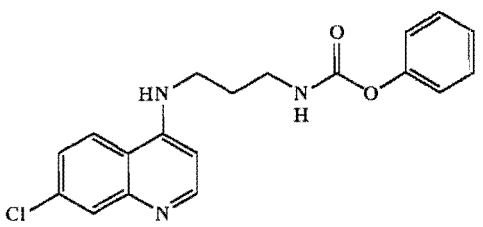

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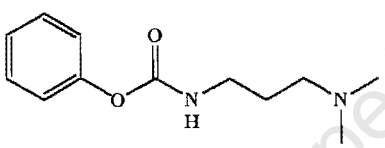


91

Figure 2.11. Structures of amine and urea derivatives.

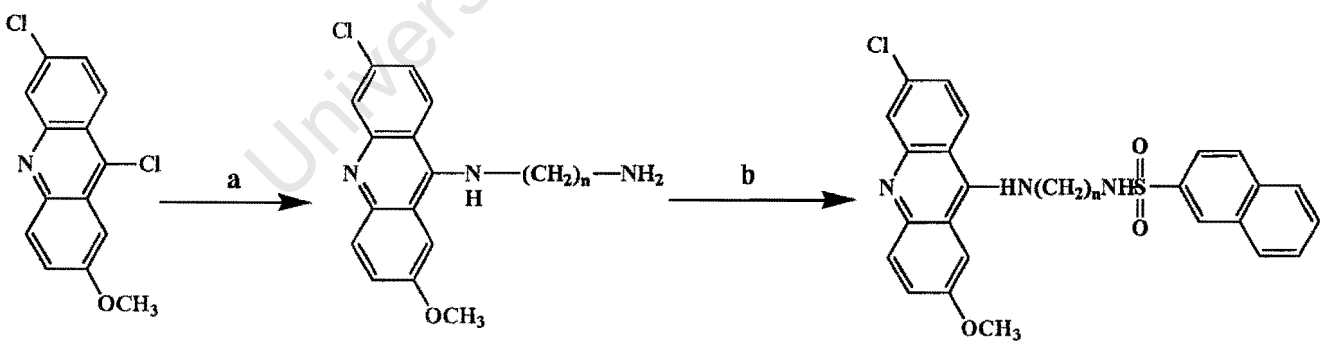
CHAPTER 3

ACRIDINE DERIVATIVES

3.1 CHEMICAL SYNTHESIS

3.1.1 Synthesis of sulfonamides 55-58

The aminoalkylacridine subunits **92-95** were prepared from the treatment of commercially available 6, 9-dichloro-2-methoxyacridine **96** with an excess of the appropriate diaminoalkane species (Scheme 3. 1)[73]. A large excess of the diaminoalkane species is required to avoid dimerisation. The four different amines **92-95** were placed in separate vials and dissolved in a small amount of DMF. The amines were treated with 1.4 equivalents of 2-naphthalene sulfonylchloride and 2 equivalents of triethylamine (scheme 3.1). After 14 hours the reaction was complete. Water was used to quench the reaction. **56** and **57** formed solid precipitates upon addition of the water and simple filtration yielded the desired products. On the other hand, compounds **55** and **58** separated out as oils and were extracted with ethyl acetate. The resulting crystals were recrystallized to yield the product.

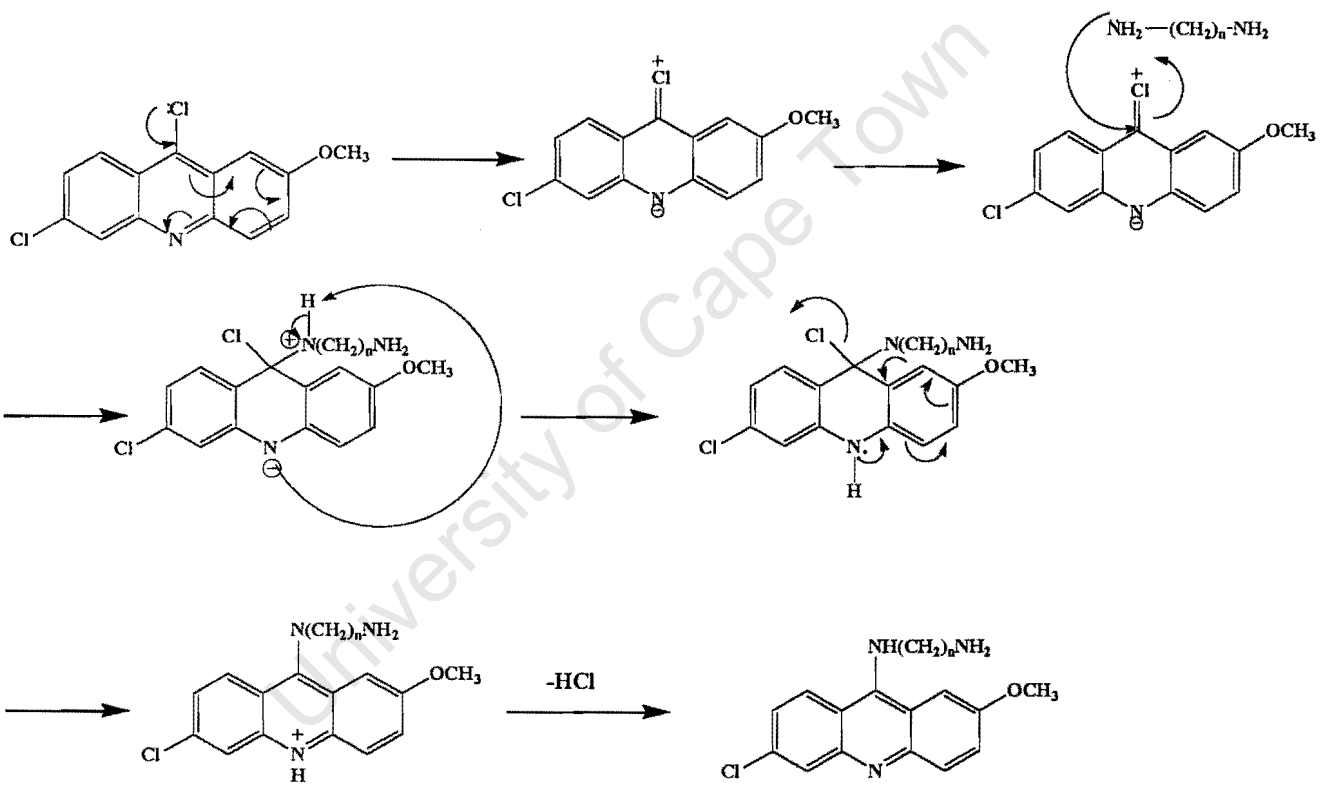


96 92-95 55-58

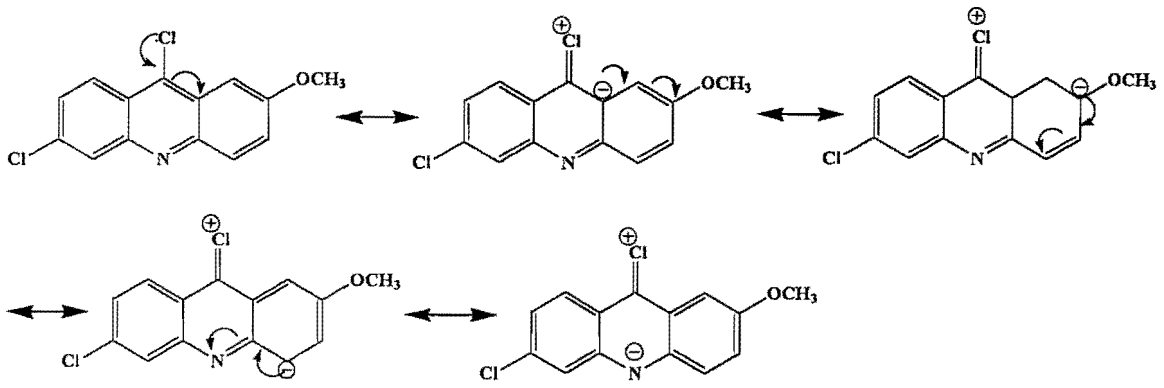
Scheme 3. 1. Reagents and conditions: (a) 32 equiv. of diaminoalkane, phenol, 90°C, 4h, 74-95%; (b) 1.4 equiv. of 2-naphthalene-sulfonyl chloride, 2 equiv. of Et_3N, DMF, 20°C, 14h, 43-94%.

Although direct amination of an aromatic system at the sp^2 hybridised carbon *via* nucleophilic substitution would be envisaged, amongst other methods, with the use of

palladium-catalysed chemistry, in this system amination occurs *via* nucleophilic substitution of the 9-chloro group (Scheme 3.2). This is presumably due to resonance stabilization facilitated by the acridine nitrogen. Mesomeric release of electrons on the chlorine atom into the ring system results in resonance stabilization with the negative charge residing on the acridine nitrogen (Scheme 3.3). Although resonance stabalization can also be achieved through the chlorine atom at position 6, the proximity of the acridine nitrogen to the chlorine at position 9 is the driving force for nucleophilic attack at position 9. This resonance release to the acridine nitrogen creates an electrophilic carbon centre bonded to the 9-chloro atom.



Scheme 3. 2. Mechanistic interpretation of amination of acridine moiety.



Scheme 3.3. Resonance stabilization facilitated by the acridine nitrogen.

Table 1. Table indicating the no. of methylene groups from amines and their synthetic yields

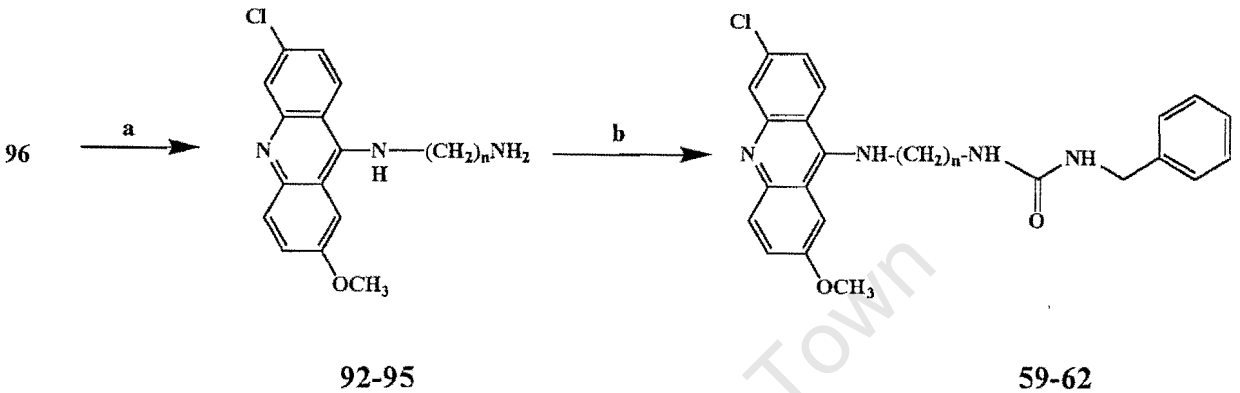
Compound no.	No. of methylene groups, n=	% yield
92	2	95
93	3	95
94	4	74
95	6	95

3.1.2 Synthesis of ureas 59-62

The classical synthesis of ureas involves the reaction of amines with isocyanates. Isocyanates themselves are prepared from amines by reaction with phosgene. This presents a problem if the desired isocyanates are not commercially available. A novel method of synthesis utilizes alkyl and arylamines and di-*tert*-butyldicarbonate and DMAP [74]. The isocyanate is generated from the amine and trapped *in situ* with excess amine. This yields symmetrical and unsymmetrical ureas. An alternative method for small library synthesis involves the use of nitrophenylcarbamates [75].

The amines **92-95** were placed in four different vials and dissolved in a small amount of dichloromethane. They were then treated with 1.1 equivalents of benzyl isocyante. TLC analysis showed that the reaction was complete within 4 hours (Scheme 3.4). The

precipitate that had formed during the reaction was isolated by filtration. Only **61** and **62** required further purification by column chromatography to remove side products that could not be removed by washing after filtration. Spectroscopic NMR evidence of the benzylic protons (δ 4.3-4.4) as well as those of the urea NH (δ 4.8-5.0) groups confirmed that the urea had been obtained.

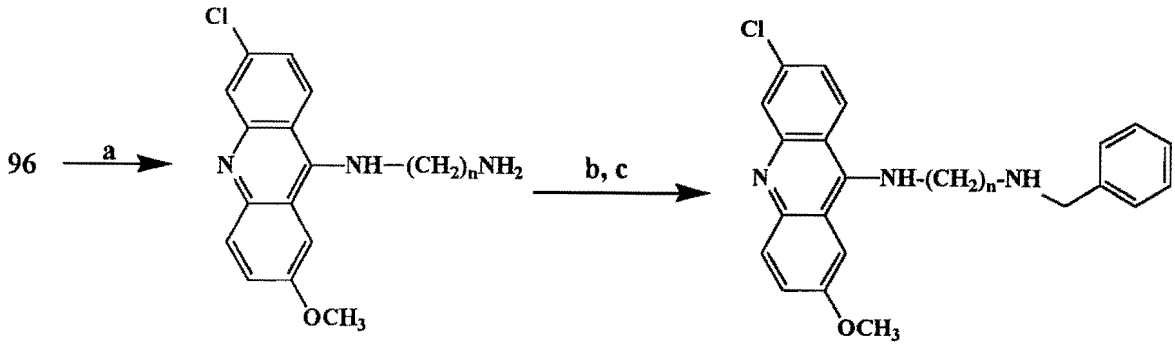


92-95 **59-62**

Scheme 3. 4. Reagents and conditions: (a) 32 equiv. of diaminoalkane, phenol, 90°C, 4h, 74-95%; (b) 1.1 equiv. of benzyl isocyanate, CH_2Cl_2, 20°C, 4h, 60-96%.

3.1.3 Synthesis of amines 63-66 *via* reductive amination of benzaldehyde

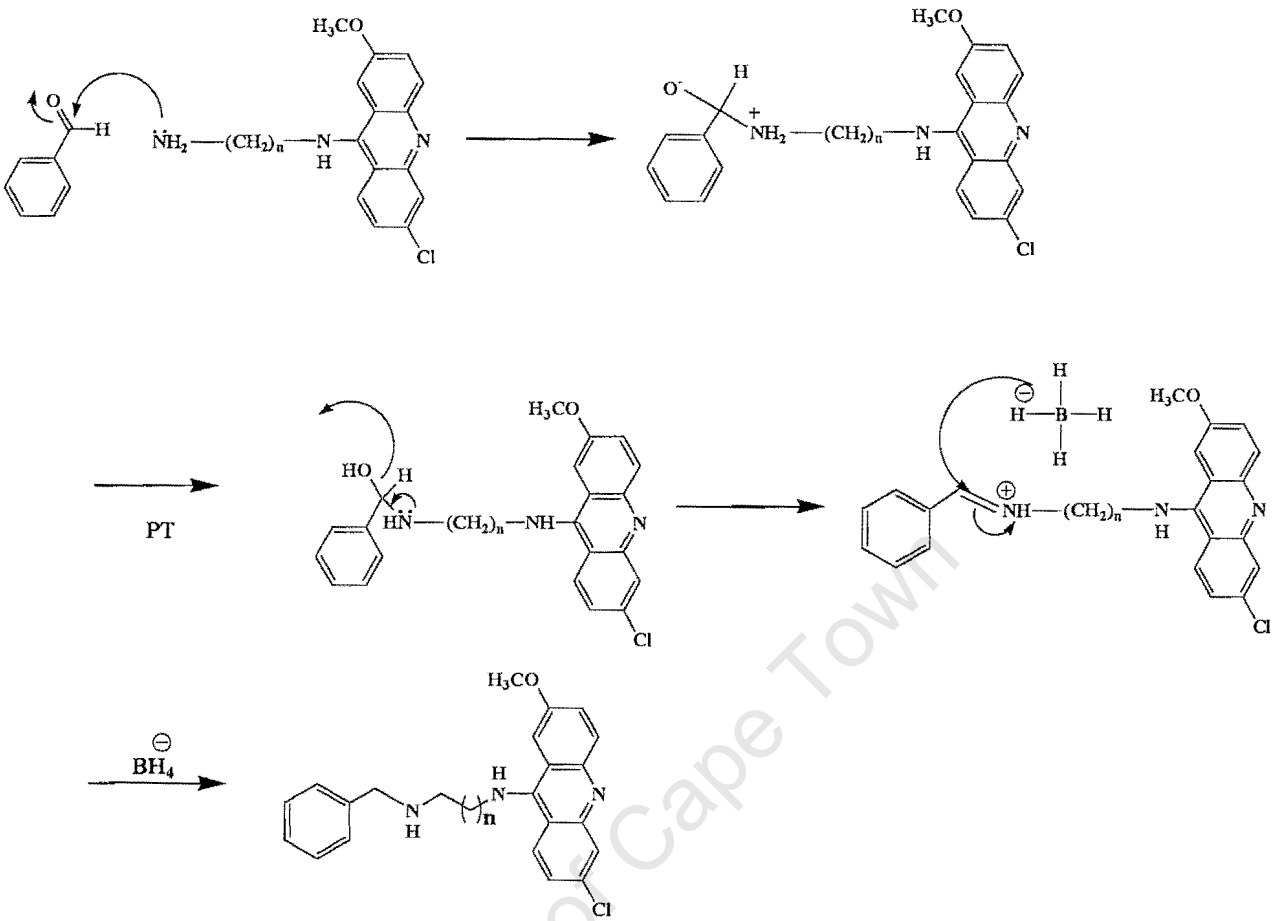
The amines **92-95** were placed into four separate vials and dissolved in a small amount of methanol. To these solutions 1.5 equivalents of benzaldehyde was added (Scheme 3.5). The vials were shaken for 2-3 hours and treated with amberlyst IRA-400 borohydride resin to reduce the imine formed *in situ.* The resulting slurry was then shaken for an additional 24 hours to effect complete reduction to the secondary amine. The resin beads were removed by filtration and washed with several aliquots of methanol. Excess benzaldehyde was removed *via* column chromatography to give the products in moderate yield. Alternatively, chromatography could have been avoided and excess benzaldehyde trapped using a resin-bound amine which could have been removed from the product solution by filtration. The mechanism for this reductive amination is outlined in scheme 3.6.



92-95 **63-66**

Scheme 3.5: Reagents and conditions: (a) 32 equiv. of diaminoalkane, phenol, 90°C, 4h, 74-95%; (b) 1.5.equiv. of benzaldehyde, MeOH, 20°C, 2-3 h; (c) 2 equiv. of Amberlite IRA 400 resin, MeOH, 20°C, 24 h, 48-70%.

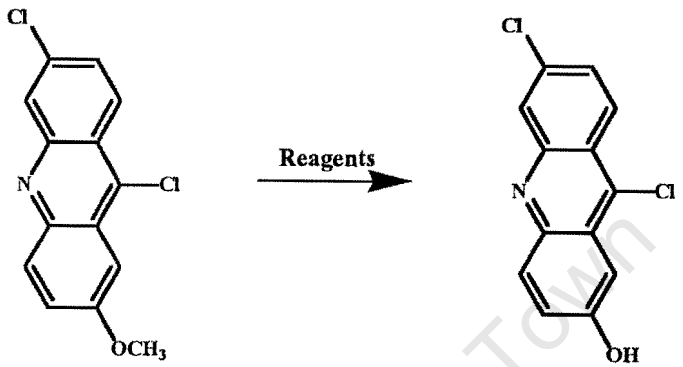
The first step involves nucleophilic addition of the amine to the aldehyde carbonyl to give a dipolar intermediate. Proton transfer from nitrogen to oxygen then yields a nonpolar amino alcohol intermediate. This is followed by dehydration of the amino alcohol intermediate to give the neutral imine and water. Reduction of the imine gives the secondary amine.



Scheme 3.6. Mechanism for reductive amination of benzaldehyde.

The sulfonamides **55-58**, ureas **59-62** and amines **63-66** were shown *via* thermogravimetric (TG) analysis to have water included within the crystal structure of the molecule. Temperature evaluation at which the water was released was the

dichloromethoxyacridine moiety as well as quinacrine, a number of methods were employed before finding success with the use of boron tribromide. Since the signal for the protons of the methoxy group resonates in a very distinctive position in the proton NMR spectrum, this was used as the primary indication of conversion of the methoxy ether to the phenol.



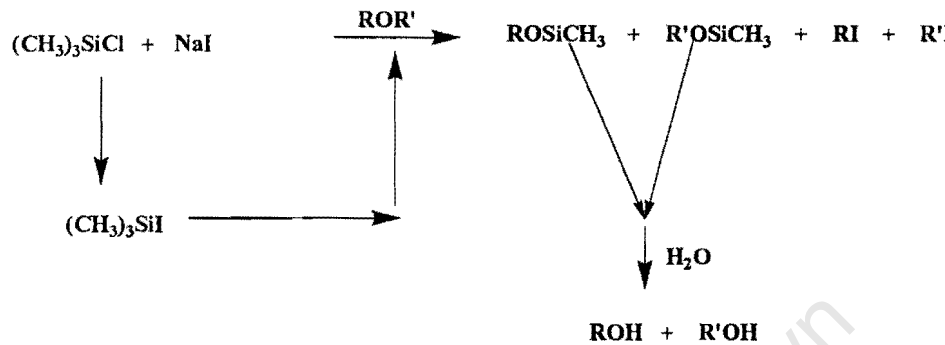
96 97

Scheme 3.7. General scheme for demethylation of quinacrine

a) Iodotrimethylsilane (TMSI)

The use of organosilicon reagents has gained ground in organic synthesis in recent years. The high bond energy of the silicon-oxygen bond makes it thermodynamically favourable to use with a reagent that possesses a weak Si-X bond and react it with an appropriate oxygen-containing molecule to form a silicon-oxygen bonded intermediate, which can then be transformed to another product in a subsequent step [76]. One such reagent is iodotrimethylsilane (TMSI) [76]. TMSI needs to be freshly prepared under strict anhydrous conditions from chlorotrimethylsilane and magnesium iodide, phenyltrimethylsilane and iodine or hexamethyldisiloxane/iodine/aluminium powder. The reagent needs to be isolated from the reaction mixture by distillation. Phenyltrimethylsilane/iodine reagent can be prepared *in situ* but requires a very high reaction temperature and iodine may cause side reactions. The discovery of the simple chlorotrimethylsilane (TMSCl) reaction with sodium iodide in acetonitrile has proved to be an inexpensive and effective alternative. The TMSI reagent is generated *in situ* and has been shown to readily cleave both dialkyl and aryl alkyl ethers, although dialkyl

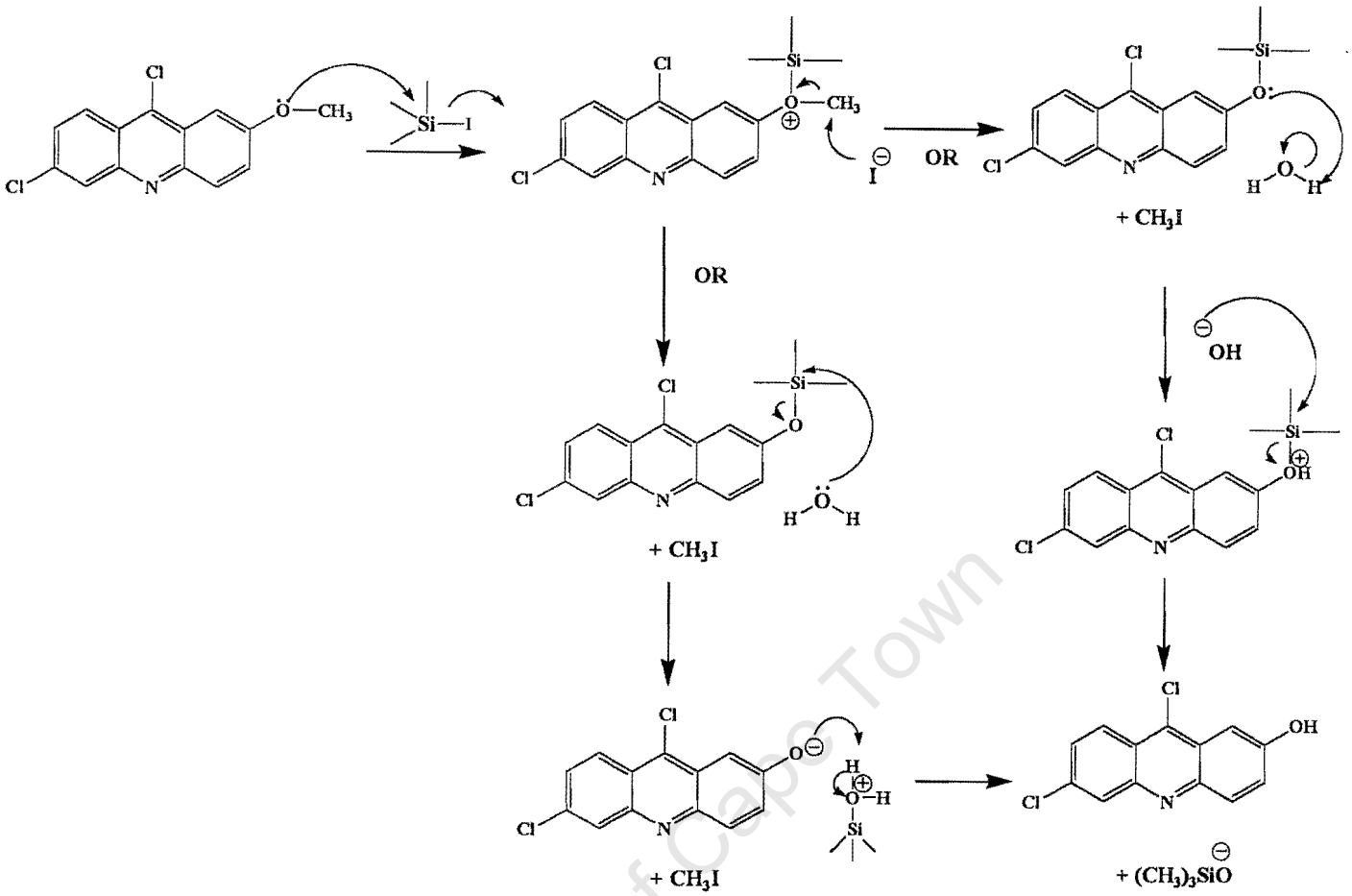
ethers cleave faster (Scheme 3.8). An excess of sodium iodideis crucial to the success of the process as it stabalizes the TMSI. If the reaction is allowed to proceed for longer periods, the intermediate silyl ethers are converted to alkyl iodides. Simple hydrolysis of silyl ether yields the alcoholic products.



Scheme 3.8. Proposed method of action of TMSI.

Our attempts to use this reagent in the demethylation of both quinacrine **13** and 6, 9 dichloro-2-methoxyacridine **96** were unsuccessful. **13** or **96** and 3.8 equivalents of sodium iodide were dissolved in acetonitrile. To this solution 3 equivalents of TMSCl was slowly added. The reaction mixture was stirred at room temperature before being brought to reflux.

A possible explanation may lie in understanding the mechanism (Scheme 3.9). Nucleophilic displacement of the iodide by the oxygen is the first step. This iodide then reacts to demethylate the resulting species. Base-mediated hydrolysis of the silyl ether with water yields the alcohol. A clear byproduct of the reaction is methyl iodide. Since TMSI is oxophilic, the acridine nitrogen may be free to react with the methyl iodide resulting in alkylation of this nitrogen yielding water-soluble quarternary salt. Addition of water upon workup may result in loss of the product. This general mechanism is the same for all of the Lewis acids described below.

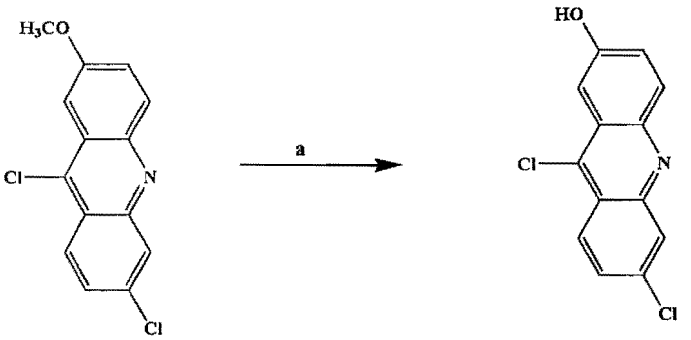


Scheme 3.9. Possible mechanism of TMSI-mediated demethylation.

b) Diisobutylaluminium Hydride (DIBAH)

The Lewis acid properties of this reagent allow it to form donor-acceptor complexes with ethers. The stability of these structures depends on the structure of the Lewis bases but this interaction does seem to be weak with aromatic ethers [77]. The reagent has proved useful in the demethylation of aromatic steroidal ethers [78].

Attempts at demethylation of 6, 9, dichloro-2-methoxy acridine **96** rendered the phenol **97** in 24% yield but demethylation of quinacrine proved to be unsuccessful. The dichloromethoxyacridine was dissolved in toluene and 6.2 eq of DIBAH was added and the solution refluxed for 10 hours. The limiting reagent had been consumed and purification yielded the product (Scheme 3.10).



96 97

Scheme 3.10. Reagents and conditions: (a) 6.2equiv DIBAH, toluene, reflux, 10h, 24%.

c) Pyridine Hydrochloride (pyr.HCl)

Pyridine hydrochloride has been used quite extensively in the cleavage of ether linkages [79-81]. The reagent was used in the absence of solvent as is the normal practice and the reaction mixture heated to 210°C for 40 minutes. The product **97**, after extensive, workup was clean and no further purification is needed. However, once again the yield obtained was very low at 25%. The low yield may be a result of decomposition during the reaction as a large amount of unidentifiable baseline material was isolated by chromatography.

d) Boron Trichloride (BCl_3)

Boron-based reagents are particularly versatile for the transformation of primary alkyl aryl ethers as the Lewis acidity of the boron and the nucleophilic nature of the ligands can be effectively manipulated. The mixture of boron trichloride and tetra-*n*- butyl ammonium iodide (*n*-Bu_4NI) has been shown to be a powerful reagent combination for the cleavage of primary alkyl aryl ethers at low ambient temperatures [82]. Although boron trichloride on its own does not remove isolated aryl methyl groups at low temperatures, the combination with BCl_3 with *n*-Bu_4NI greatly enhances its reactivity. *n*-Bu_4NI is a source of iodide. It has been shown that the reaction does not proceed in the absence of iodide [82].

Substrates that possess multiple Lewis base sites require additional BCl_3 to chelate the more basic spectator groups. As a general rule, 1.5 equivalents of BCl_3 is sufficient to

effect dealkylation provided that there is 1.0 equivalents of additional reagent for each additional basic group. Following this, we decided to use 4.5 equivalents in our reactions to attempt demethylation of the aryl ether. Using the procedure outlined [82], 1.5 equivalents of *n*-Bu_4NI and quinacrine **13** in dichloromethane are treated with 4.5 equivalents of BCl_3 (1M, CH_2Cl_2) at -78°C and them warmed to 0°C. The reaction was stirred for 30 min at 0°C before being warmed to room temperature and stirred for 24 hours without any change. Generally, this reagent provides mild, selective ether cleavage at low temperature in short reaction times. The reagent could, however, not affect ether cleavage on our system.

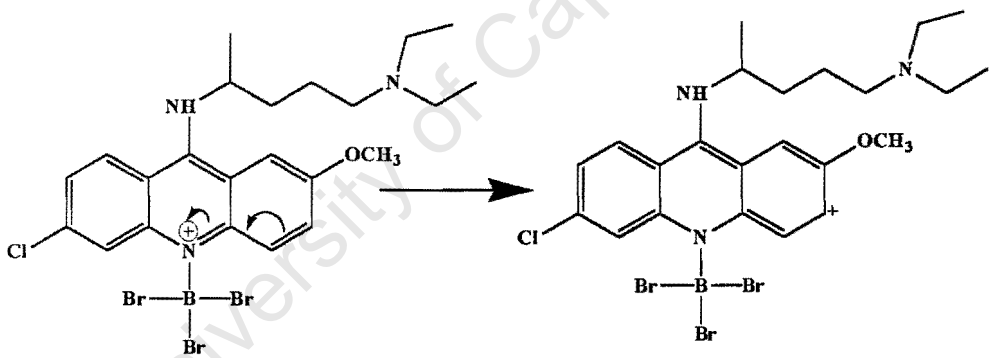
e) Boron tribromide (BBr_3)

Boron tribromide is regarded as one of the reagents of choice for dealkylation of ethers. BBr_3 effects cleavage of the ether linkage without affecting ester groups or double bonds. The general reaction procedure stipulates heating the reaction mixture on a water bath at approximately 40°C. As such, our first attempt with this reagent was carried out at this temperature.

6, 9 Dichloro-2-methoxyacridine **96** was dissolved in dichloromethane and cooled in an ice-bath. 3 equivalents of BBr_3 were added dropwise and the reaction was slowly allowed to warm to room temperature. The mixture was reacted at 24°C for 24 hours before being placed on a water bath and stirred for 12 hours. As there was still no change in the reaction, the mixture was brought to reflux for 10 hours. This still had no effect on the reaction and yielded only starting material.

Further investigation led to a procedure where the reaction temperature was kept at -89°C. The starting ether, 6,9 dichloro-2-methoxyacridine **96** or quinacrine **13**, was dissolved in dichloromethane and the solution cooled to -89°C in a dry-ice/propanol bath. BBr_3 (3eq, 1M in CH_2Cl_2) was slowly added to this solution and the mixture stirred at -89°C for 1 hour. The solution was then warmed to 17°C and stirred for a further 20 hours. Upon completion, the reaction was quenched with ice water and stirred for 30 minutes before the product was isolated, without further purification, by filtration with a yield of 70%.

From the result of this reaction, it seems as if temperature has a major role to play in the demethylation of this system. It is well known that boron has a high affinity for and forms strong bonds with nitrogen and as the results indicate that at temperatures of 0°C and above, there is competition between the ether oxygen and the acridine nitrogen for the reagent. If complexation does indeed occur at the acridine nitrogen, the Lewis basicity of the ether oxygen would be compromised *via* the inductive pull of electrons towards the positively charged acridine nitrogen. This would result in a diminished reaction between the ether oxygen and boron tribromide. However, at -89°C, it appears as if this competition is minimized and hence retrieval of the product is effected. The low temperature seems to affect the kinetics of the reaction perhaps allowing this reaction to occur but at a rate so slow that it is almost negligible *i.e.* complexation of the boron tribromide to the nitrogen is under thermodynamic control while complexation to the ether oxygen is under kinetic control.



Scheme 3.11. Inductive pull of electrons towards acridine nitrogen.

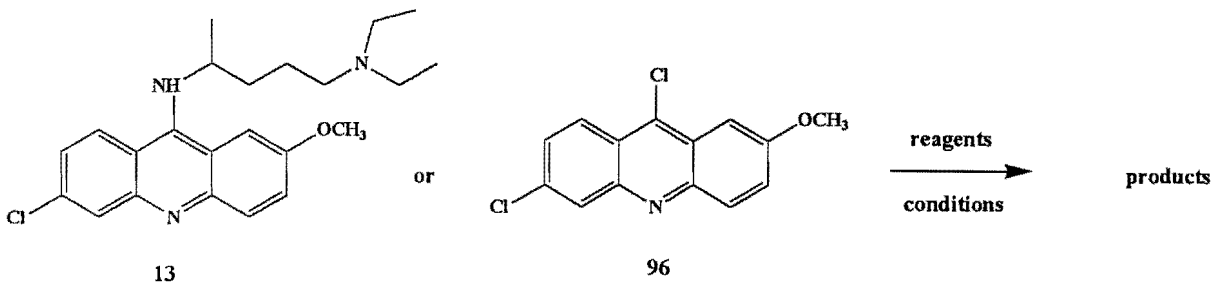


Table 2. Reagents and conditions for attempted demethylation procedures

Entry	Compound	Reagent	Conditions	Product, % yield
1	**13** and **96**	TMSI	TMSCl (3eq), NaI (3.8eq), CH_3CN, 24-reflux, N_2	nr[a]
2	**96**	DIBAH	DIBAH (6.2eq), toluene, reflux, 10h	24%
3	**13**	Pyridine hydrochloride	Pyr.HCl (5eq), 210°C, 40 min	25%
4	**97**	Boron trichloride	BCl_3 (4.5eq), n-Bu_4NI (1.5eq), CH_2Cl_2, -78°C -24°C, 24 h	nr
5	**13**	Boron tribromide	BBr_3 (3eq), CH_2Cl_2, 0-24°C	nr
	13 and **96**		BBr_3 (6eq), CH_2Cl_2, -89-24°C, 18 h	70%

[a] no reaction occurred

3.1.5 Synthesis of ether targets, 70-73

Combinatorial chemistry had emerged as an important tool for the rapid synthesis of a large number of structurally diverse small molecule libraries and has become an important methodology for expediting the drug discovery process. Alkylation of a phenol building block is one approach to the synthesis of large libraries of aryl ethers. The ether targets **99-102** were synthesized utilizing the polymer bound base 1, 5, 7-triazabicyclo [4.4.0.] dec-5-ene (PTBD).[83] (Figure 3.1). This polymer-supported base serves a dual purpose in its role as a base for deprotonation of phenols and also acts as a scavenger for trapping [84] the unreacted excess starting phenol. The excess phenol is

used to drive the reaction to completion and products are isolated by simple filtration and solvent evaporation.

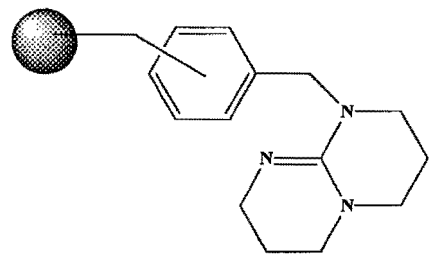
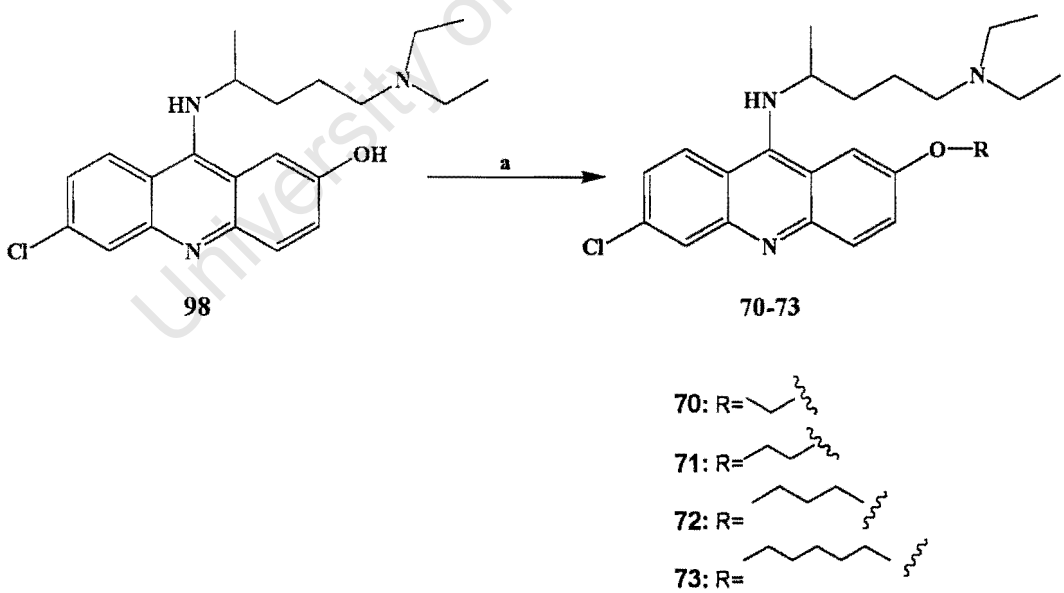


Fig 3.1. Structure of the polymer bound base PTBD.

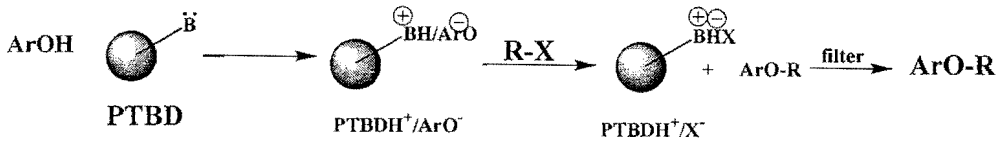
In a typical reaction procedure, phenol **98** and 0.83 equivalents of the alkyl halide were added to a reaction vessel containing the PTBD resin in a small amount of acetonitrile (Scheme 3.11). The reaction was shaken vigorously at 24°C until the alkyl halide was consumed. The polymeric material was filtered off, washed with acetonitrile and the solvent removed. The reaction proceeded well affording the chemically pure product which was verified by NMR.



Scheme 3.11. Reagents and conditions: (a) alkyl halide (R-X), 2 equiv. PTBD, CH_3CN, 24°C, 45-78%.

Mechanistically deprotonation of the phenol by the polymeric base is the first step. This forms the $PTBDH^+/ArO^-$ complex (Scheme 3.12). The phenoxide species undergoes an

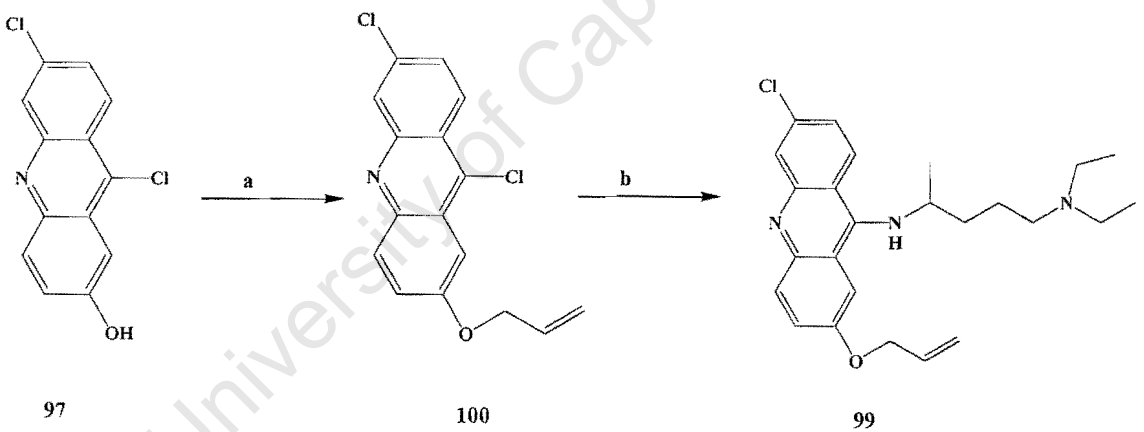
SN_2 reaction with the alkyl halide to generate the alkyl ether ArOR. The base also scavenges the resulting acid (H-X).



Scheme 3.12. Proposed mechanism of action of PTBD

3.1.6 Synthesis of target 99

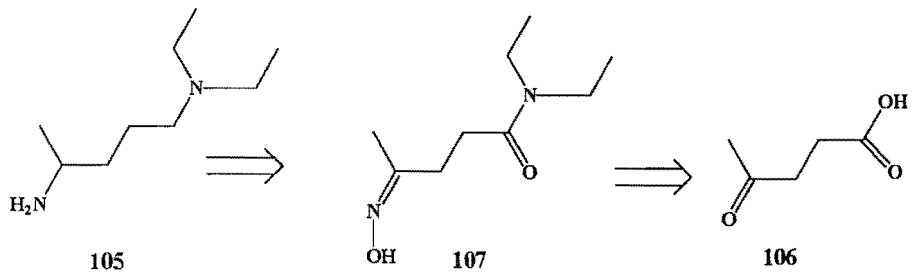
Isolation of phenol **97** *via* dealkylation with pyridine hydrochloride was among the first successful dealkylation procedures employed in this work. Attempts to alkylate with allyl bromide and PTBD as reagent proved unsuccessful and hence the use of potassium carbonate in DMF was investigated.



97 100 99

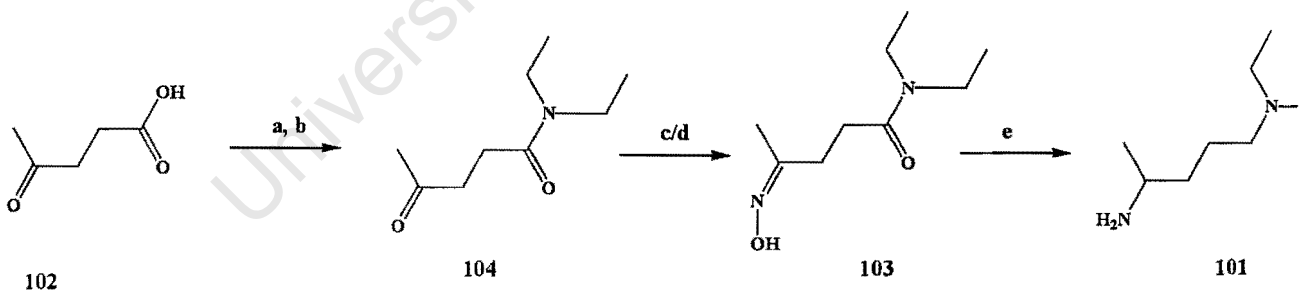
Scheme 3.13. Reagents and conditions: (a) 1.07 equiv. allyl bromide, 10 equiv. K_2CO_3, DMF, 20°C; (b) 32 equiv. of diaminoalkane, phenol, 90°C, 4h, 61%.

To a solution of the phenol **97** in DMF, 1.07 equivalents of allyl bromide was added, followed by 10 equivalents of potassium carbonate (Scheme 3.13). The reaction mixture was stirred at 18°C for 20 hours to effect alkylation of the phenol yielding **100**. Workup yielded the crude material which was carried through to the next step, involving addition of the side chain present in quinacrine, without further purification. The amine precursor for the synthesis of the side chain **101** was envisaged to come from levulinic acid **102** as shown in Scheme 3.14.



Scheme 3.14. Retrosynthetic analysis for the synthesis of amine **105**.

The oxime-amide intermediate **103** is obtained from the commercially available levulinic acid **102** in a two step procedure involving conversion to the acid chloride using oxalyl chloride followed by the reaction with diethylamine (Scheme 3.15). The ketone functionality of **104** was transformed to the oxime by two methods. The first method of refluxing **104** in ethanol with hydroxylamine hydrochloride and sodium hydroxide to give **103** in excellent yield. The second method utilized hydroxylamine hydrochloride and pyridine under reflux. Although the reaction time was much shorter for the latter method, the yield obtained was moderate. In the final step, the oxime and amide functionality of **103** were simultaneously reduced by lithium aluminium hydride to afford the amine **101** in moderate yield.

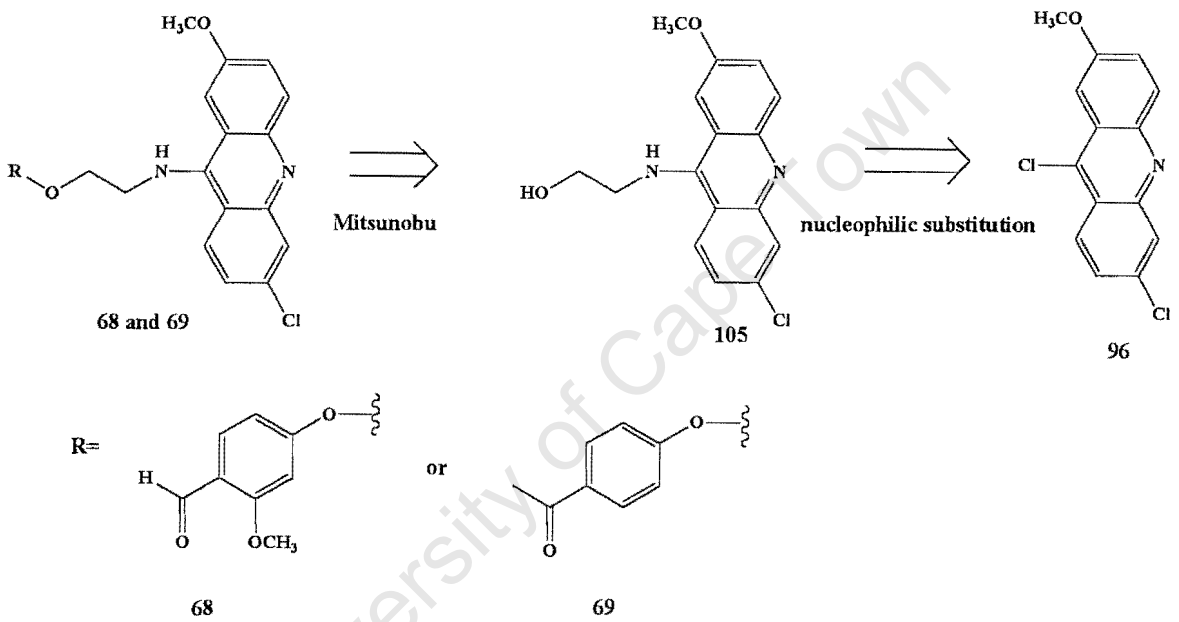


Scheme 3.15. Reagents and conditions: (a) 1.5 eqiv. $(COCl)_2$, 0°C, 2h; (b) 2.0 equiv. Et_2NH, CH_2Cl_2, 20°C, 1h, 65%; (c) 1.1. equiv. NH_2OH. HCl, 1.1 equiv. pyridine, EtOH, reflux, 30 min, 58%; (d) 1.6 equiv. NH_2OH.HCl, 3.3 equiv. NaOH, etOH, reflux, 2h, 86%; (e) 3.0 equiv. LAH, THF, 0°C-reflux, 6h, 67%.

3.1.7 Synthesis of 68-69

a) Retrosynthetic Analysis

The Mitsunobu reaction was invoked in the synthesis of ethers **68** and **69.** As outlined in the retrosynthetic scheme (Scheme 3.16). The alcohol **105** could be synthesized utilising the same chemistry as for the acridine amines **92-95.** This procedure is straightforward and high yielding. The Mitsunobu reaction of **105** with commercially available 4-hydroxy acetophenone or 4-hydroxy 2-methoxy benzaldehyde yielded **68** and **69** respectively.

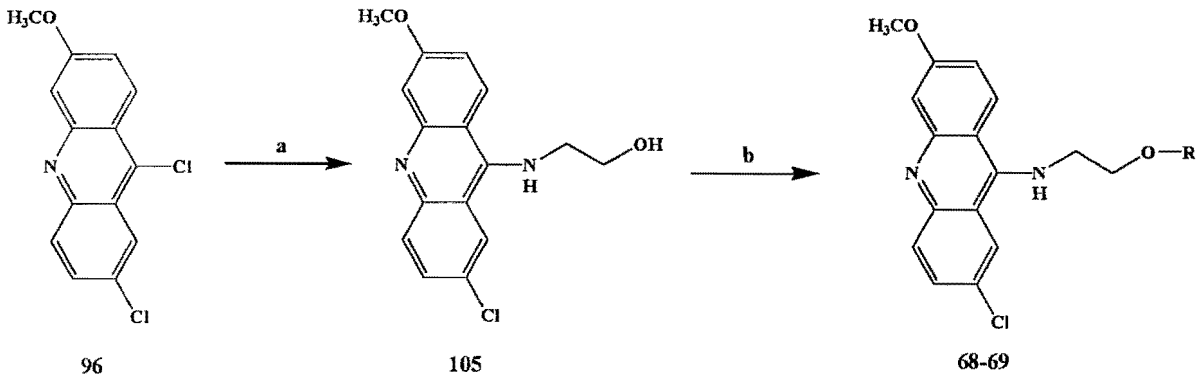


Scheme 3.16. Retrosynthetic scheme for 68 and 69.

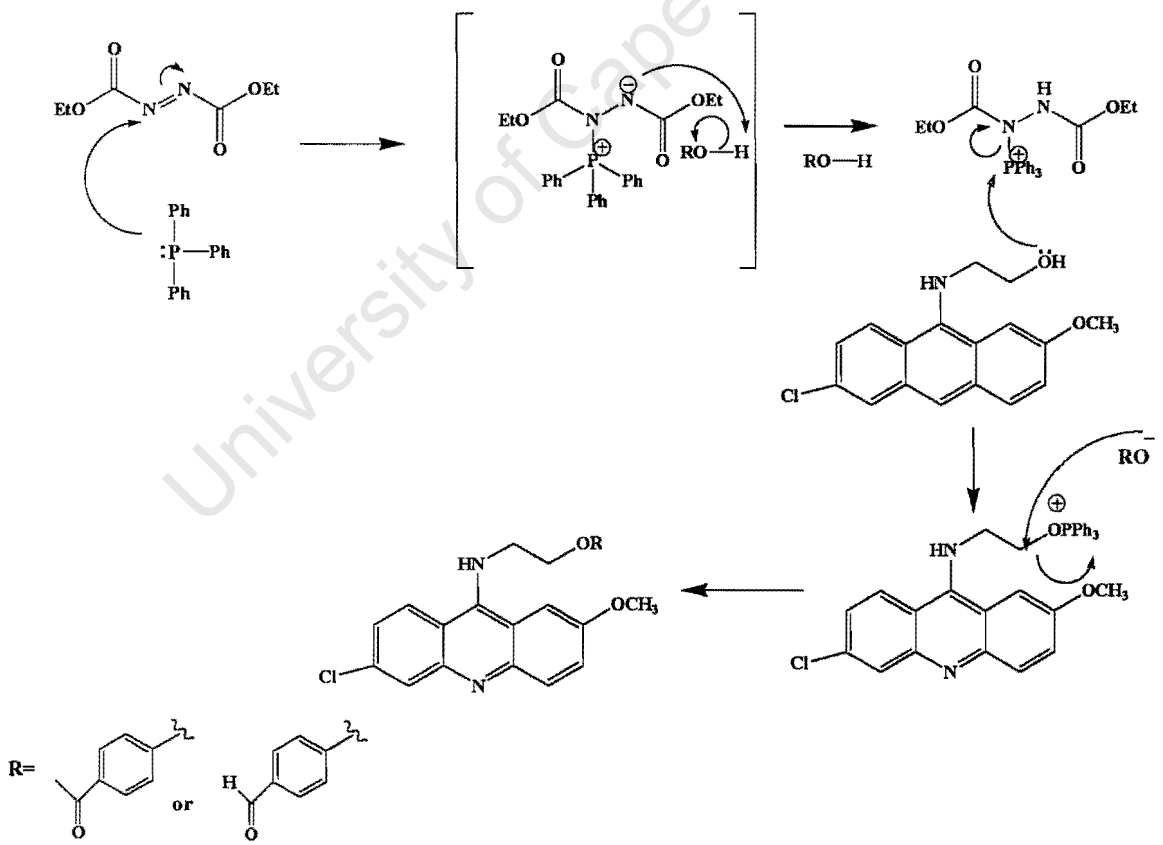
b) Synthesis

6, 9, Dichloro-2-methoxy acridine **96** was treated with ethanolamine in the presence of phenol in the manner described earlier to yield **105** in excellent yield. The alcohol was converted to the ether using standard Mitsunobu reaction conditions (Scheme 3.17). This reaction is believed to proceed through: (a) addition of triphenylphosphine to diethylazodicarboxylate (DEAD) to give a quarternary phosphonium salt, (b) the phosphonium salt is protonated in the presence of the R group, (c) the alcohol activation group involves the transfer of the phosphorous group to the alcohol to form the oxophosphonium salt and the reduced hydrazine by-product and (d) displacement of the

oxyphosphonium by the deprotonated R group to give **68** and **69** and triphenylphosphine oxide (Scheme 3.18).



Scheme 3.17. Reagents and conditions: (a) 32 equiv. diaminoalkane, phenol, 90°C, 4h, 80%; (b) 1.0 equiv. PPh_3, 1.0 equiv. DEAD, 1.0 equiv. 4-hydroxy-2-methoxy-benzaldehyde or 4-hydroxybenzaldehyde, 0-20°C, 24h, 26-58%.



Scheme 3.18. Mechanism for the Mitsunobu reaction.

CHAPTER 4

QUINOLINE DERIVATIVES

4. 1 CHEMICAL SYNTHESIS

4.1.1 Synthesis of sulfonamides

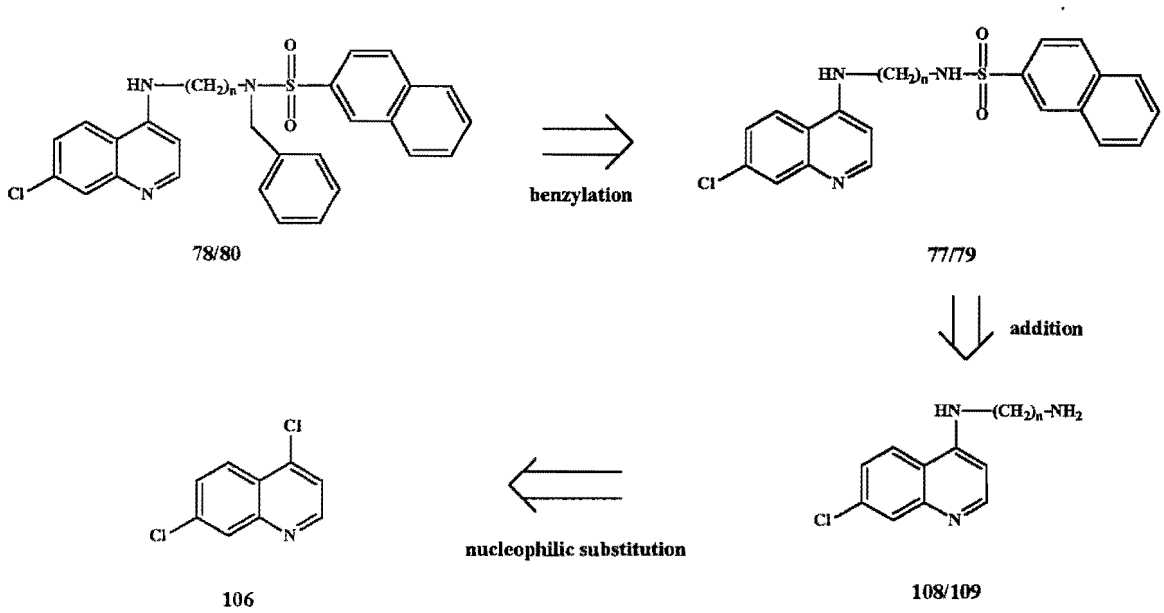
4.1.1.1 General method for sulfonamide synthesis

The amines were treated with 1.4 equivalents of 2-naphthalene sulfonylchloride and 2 equivalents of triethylamine. After 14 hours the reaction was complete. Water was used to quench the reaction. In the case of formation of a solid precipitate upon addition of the water, simple filtration yielded the desired products. However, in those cases where oils were obtained, these were extracted with ethyl acetate. In this case, recrystallization of the resulting material yielded the product. The reaction conditions are illustrated in Scheme 4.3.

4.1.1.2 Synthesis of 78 and 80

a) Retrosynthetic analysis

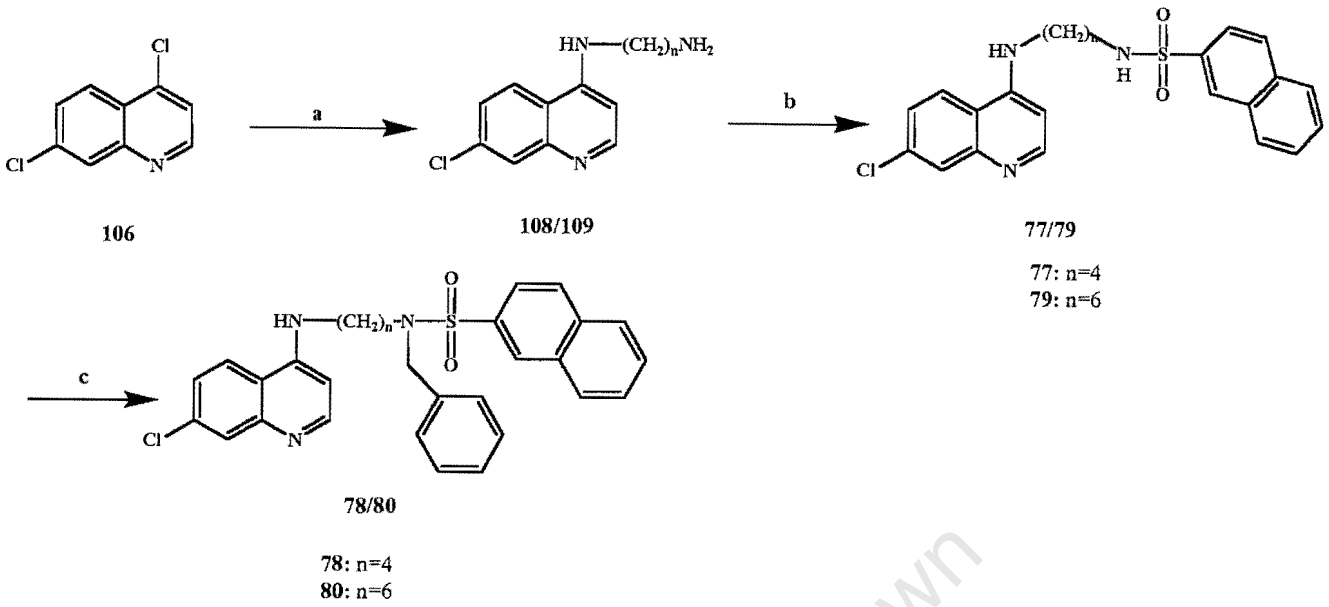
As outlined in the retrosynthetic scheme (Scheme 4.1), the benzylated products **78** and **80** could be synthesized by direct benzylation of the sulfonamide nitrogen. This is a simple procedure requiring a base in the form of potassium carbonate and the benzylating agent in solvent. The sulfonamides were synthesized as described above by addition of the sulfonyl chlorides to the respective amines. The amines were synthesized from the commercially available 4, 7 dichloroquinoline **106** in a nucleophilic substitution reaction as encountered earlier with acridines in chapter 3.



Scheme 4.1. Retrosynthetic scheme for **78** and **80.**

b) Synthesis

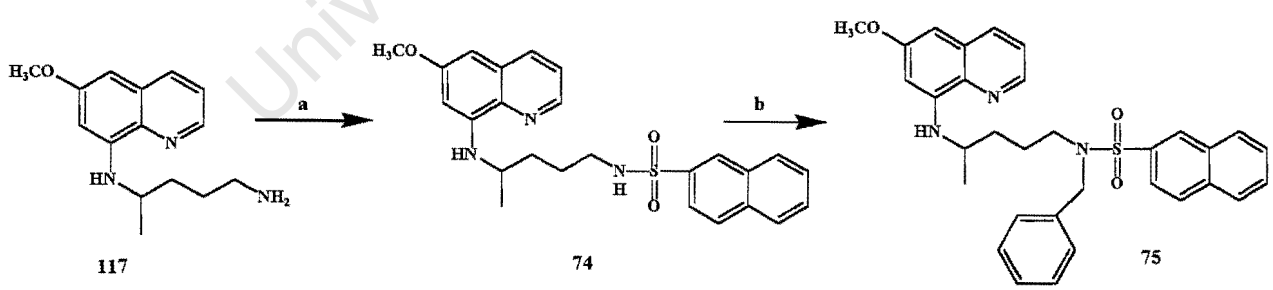
Amines **107-110** were synthesized directly from commercially available 4, 7 dichloroquinoline **106** (Scheme 4.2) and the appropriate diaminoalkane to yield quinolines of various methylene spacer lengths in the side chain. Amines **108** and **109** were utilized in these reactions. These were subjected to the conditions described above for the synthesis of the sulfonamides **77** and **79**. To a solution of the sulfonamides in DMF, 1.1 equivalents of benzyl bromide was added, followed by 10 equivalents of potassium carbonate. This yielded the benzylated targets required **78** and **80.**



Scheme 4.2. Reagents and conditions: (a) 4.5 equiv. of diaminoalkane, 80-130°C, 4h, 77-91%; (b) 1.4 equiv. of 2-naphthalene sulfonyl chloride, 2 equiv. of Et_3N, DMF, 20°C, 18h, 72-84%; (c) 1.1 equiv. of BnBr, 10 equiv. of K_2CO_3, DMF, 24h, 20°C, 48-60%

4.1.1.3 Synthesis of 75

The benzylated sulfonamide **75** was synthesized in precisely the same manner as **78** and **80.** The sulfonamide **74** was synthesized as above from primaquine **16** which is commercially available in the form of primaquine diphosphate.



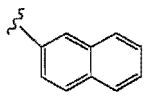
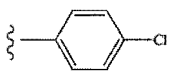
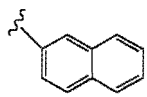
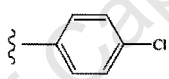
Scheme 4.3. Reagents and conditions: (a) 1.4. equiv. of 2-naphthalene sulfonyl chloride, 2 equiv. Et_3N, DMF, 20°C, 18h, 96%; (b) 1.1. equiv. of BnBr, 10 equiv. of K_2CO_3, DMF, 24h, 20°C, 60%.

4.1.1.4 Synthesis of 76, 81-84

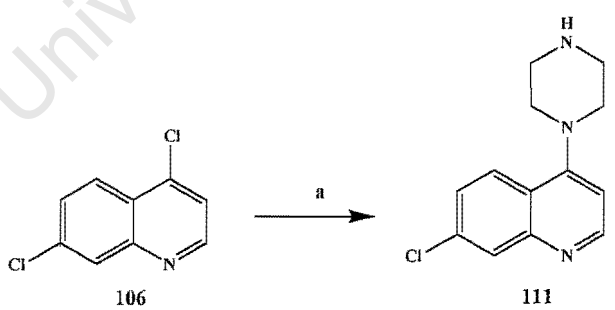
Again this series of sulfonamides was synthesized as outlined in the general method above. The 4-aminoquinoline (4-AQ) compounds **81** and **82** were synthesized from 4, 7,

dichloroquinoline **106** while **76** was synthesized from starting material primaquine. Yields are indicated below.

Table 3. Table showing substituents (R) from sulfonamides and their synthetic yields

Compound no.	R	% Yield
76		**37%**
81		**72**
82		**95**
83		**87**
84		**68**
		

The synthesis of the piperazine quinoline **117** from which compounds **83** and **84** were synthesized is indicated in scheme 4.4.

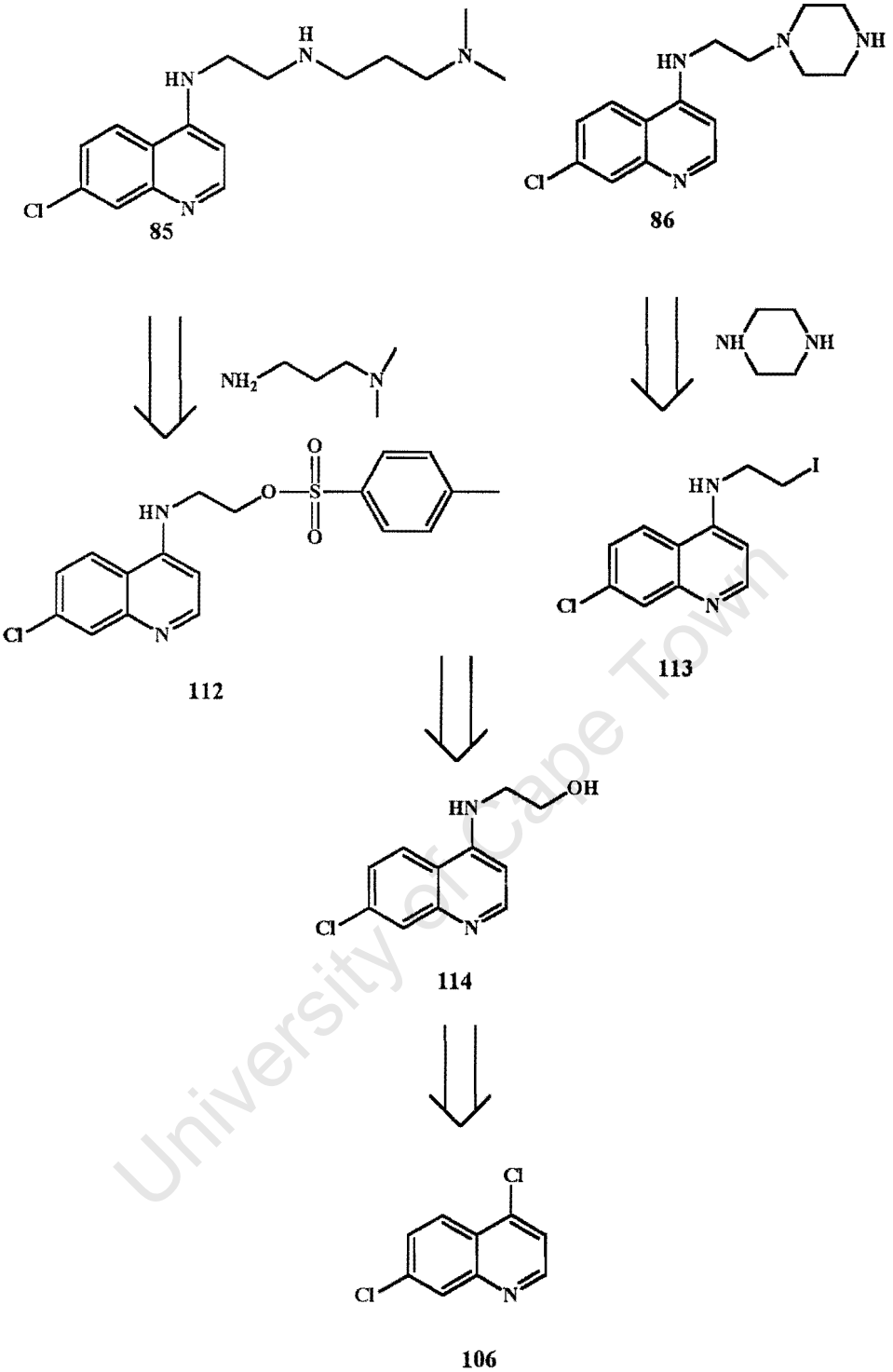


106 111

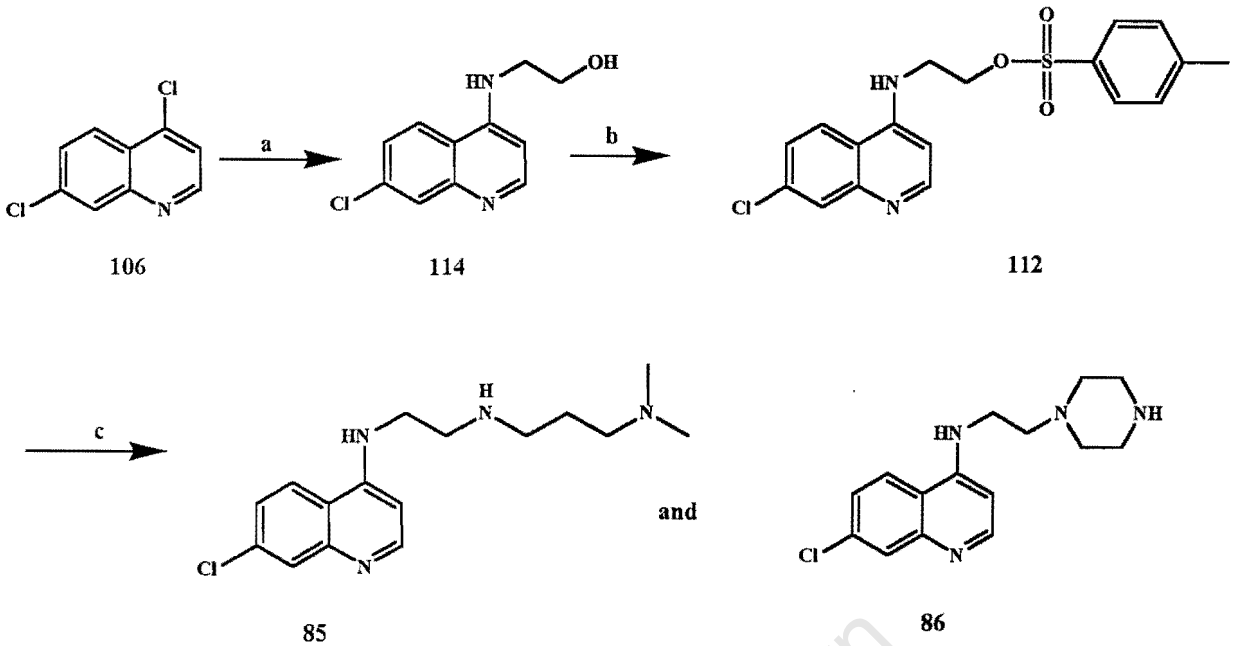
Scheme 4.4. Reagents and conditions: (a) 5 equiv. of piperazine, 1.5 equiv. of Et_3N, 0.3 equiv. K_2CO_3, NMP, 140°C, 18h, 85%.

4.1.1.5. Synthesis of 85 and 86

The synthesis was achieved through the tosylate intermediate **112** (Scheme 4.5) which was obtained from the 4, 7 dichloroquinoline **106** in a two-step procedure. The alcohol **120** was synthesized in the same manner as the amines **107-110** described above. In an attempt to synthesize **85** and **86** we attempted to ascertain whether the synthesis of a tosylate or iodide intermediate would be more feasible. The tosylate **112** was synthesized from tosyl chloride and triethylamine. The reaction proceeds from 0 to 20°C in 1 hour. The second procedure which was attempted involved the use of iodine, imidazole and triphenylphosphine to produce the iodide **113**. The reaction proceeded at 20°C for 5 hours with a very extensive workup involving filtration through celite. Since the former method gave a better yield and was much more convenient in terms of workup, that procedure was adopted. Reaction with either 3-dimethyl-1-propylamine or piperazine yielded the compounds **85** and **86** respectively (Scheme 4.6).



Scheme 4.5. Retrosynthetic analysis of **85** and **86.**

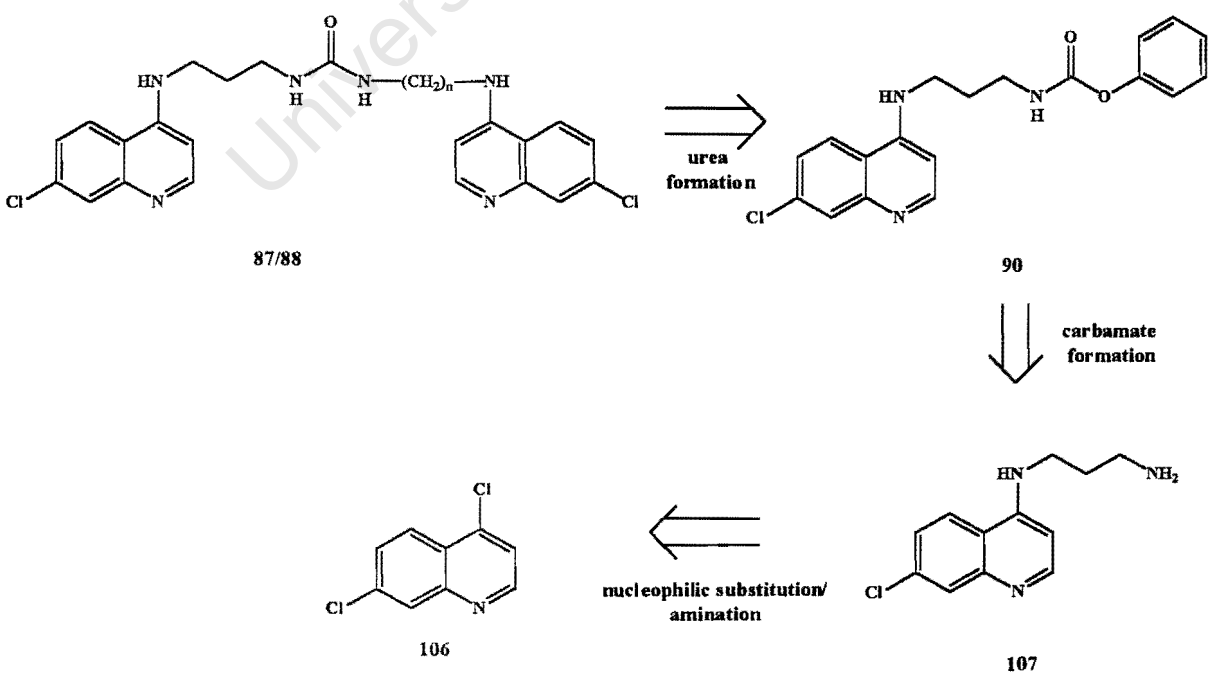


Scheme 4.6. Reagents and conditions: (a) 4.5. equiv. of ethanolamine, 90-140°C, 4h, 36%; (b) 2.2 equiv. of Et_3N, CH_2Cl_2, 0-20°C, 1h, 39%; (c) 2 equiv. of amine, CH_3CN, reflux, 24h, 37-66%

4.1.2 Synthesis of bisquinoline ureas 87 and 88

a) Retrosynthetic Analysis

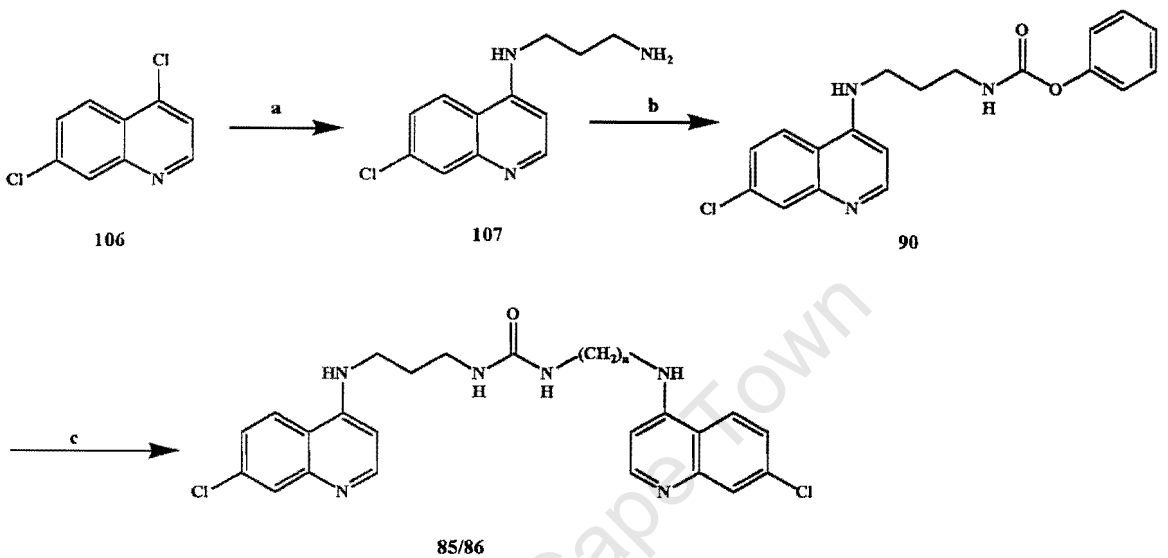
Synthesis of target compounds **87** and **88** was achieved through the carbamate **90** which was synthesized, in a two-step procedure, from 4, 7 dichloroquinoline **106. 107** was synthesized in the usual manner described for amines above.



Scheme 4.7. Retrosynthetic analysis of **87** and **88**

b) Synthesis

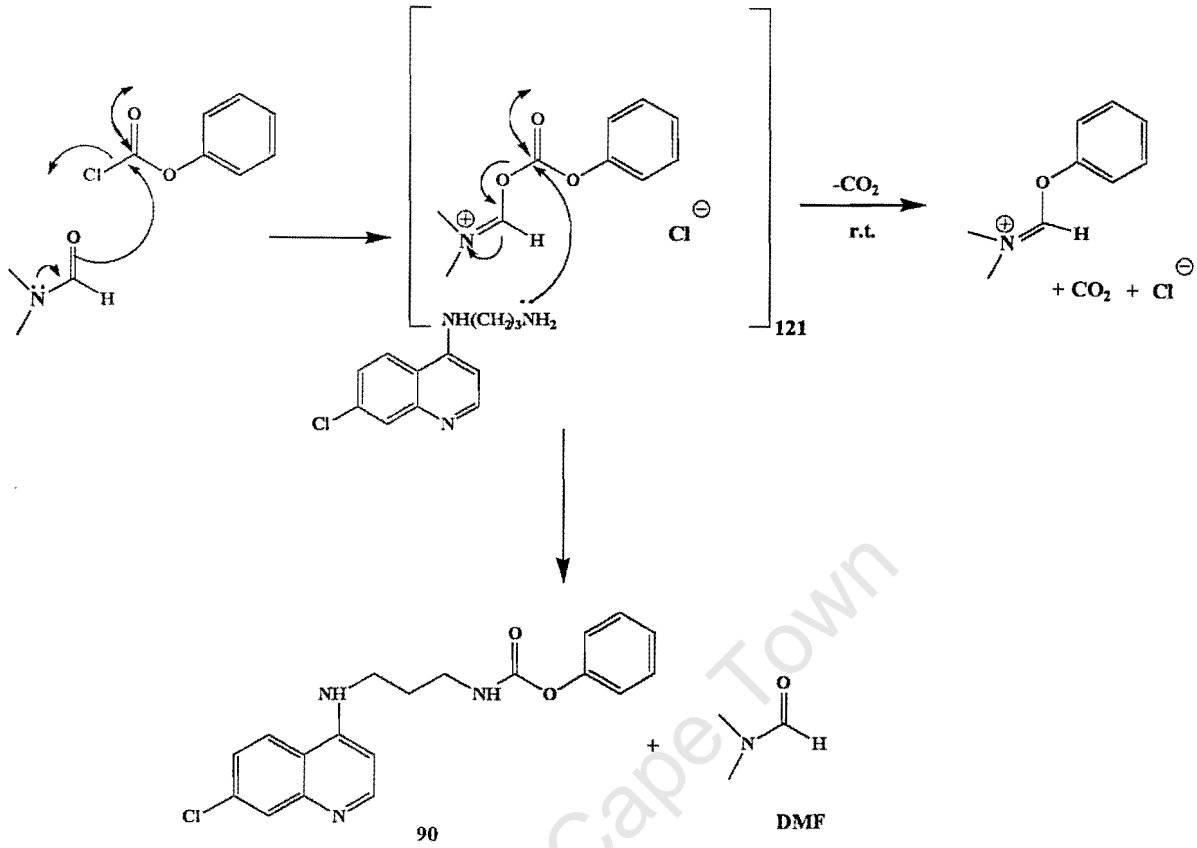
Compound **107** was transformed to the carbamate **90** by reaction with phenyl chloroformate at 0°C in the presence of DMF with dichloromethane as solvent. In the final step the carbamate was converted to the symmetrical urea (n=2), **88** and the unsymmetrical urea (n=3), **87** by reacting it with the amines **107** and **110** respectively.



Scheme 4.8. Reagents and conditions: (a) 4.5 equiv. diethylamine, 80°C, 4h, 77%; (b) 1.0 equiv. of phenyl chloroformate, 1.0 equiv. DMF, CH_2Cl_2, 0°C, 5h, 58%; (c) 1.4 equiv. of amine, THF, 20-60°C, 26h, 65-74%.

c) Mechanism of reaction

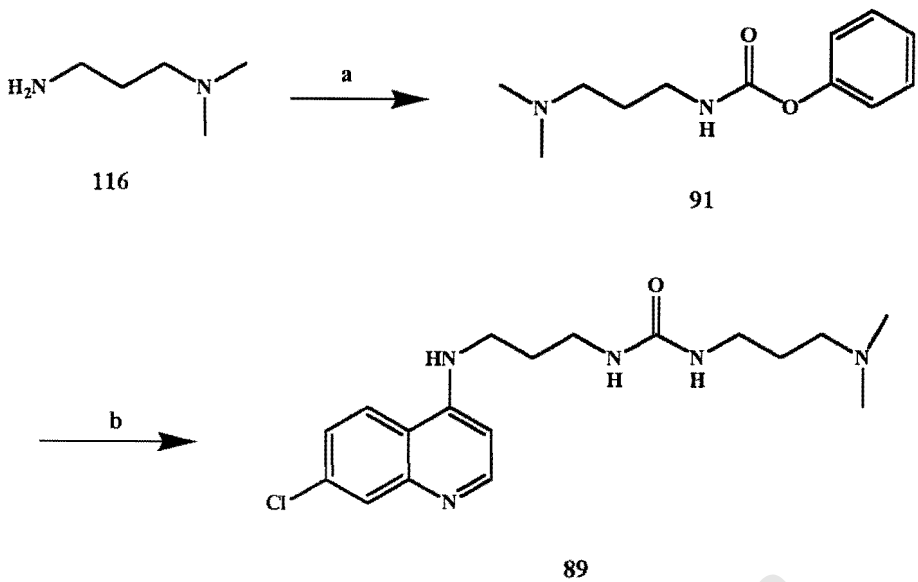
Nucleophilic attack of the DMF on the carbonyl centre of phenyl chloroformate produces the intermediate **115**. Both groups attached to the carbonyl group on **115** are good leaving groups but the regeneration of DMF is the driving force which favours expulsion of the iminium ion-enol ether group. This then produces DMF and the desired carbamate product (Scheme 4.9). The reaction proceeds at 0°C as the intermediate **115** is unstable above this temperature. Loss of carbon dioxide occurs easily at room temperature. [85] (Scheme 4.9)



Scheme 4.9. Mechanistic interpretation of carbamate formation and room temperature effects

4. 1.3 Synthesis of urea 89

Synthesis was carried out in precisely the same manner as described for **87** and **88.** The synthesis was carried out in a two-step procedure. The first step involved formation of the carbamate **91** from the commercially available 3-dimethylamino-1-propylamine **116**. Transformation of the carbamate to the urea took place in the presence of amine **109**. The synthesis of **89** was attempted with the use of two different solvents in an effort to improve the reaction yield. The method utilizing DMF resulted in a better yield than that of THF. An alternative synthesis *via* **90** and **116** could also have been employed.



Scheme 4.10. Reagents and conditions: 1.0 equiv. of phenyl chloroformate, 1.0 equiv. DMF, CH_2Cl_2, 0°C, 7h, 40%; (c) 1.4 equiv. of amine, THF, 20-60°C, 26h, 67%

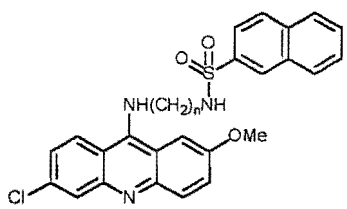
CHAPTER 5

BIOLOGICAL RESULTS AND DISCUSSION

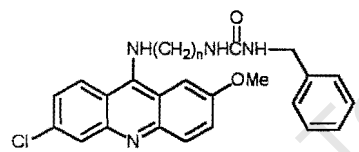
5.1 INHIBITORS OF TRYPANOTHIONE REDUCTASE

5.1.1 Inhibition activity of derivatives 55-57, 59-62, 63, 64, 66, 67-69 and 87-89

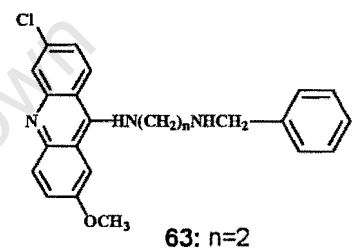
The data in Table 4 shows the activity of these derivatives with respect to inhibition of the enzyme TryR. The data for the standard control drugs is included for comparison purposes.



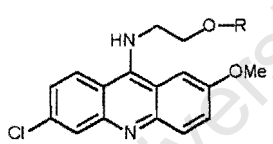
55: n=2
56: n=3
57: n=4



59: n=2
60: n=3
61: n=4
62: n=6

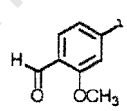


63: n=2
64: n=3
65: n=4
66: n=6

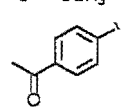


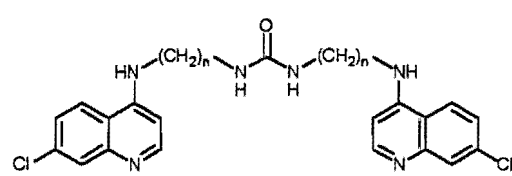
67: R=H

68: R=

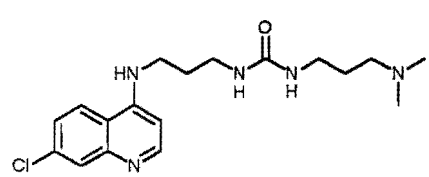


69: R=





87: n=2
88: n=3



89

Figure 5.1. Chemical structures of quinacrine and chloroquine derivatives.

Table 4: Inhibition of trypanothione reductase and glutathione reductase by quinacrine and analogues.

Compound	Number of Methylene Groups, n =	Trypanothione Reductase IC_{50}, μM	Glutathione Reductase IC_{50}, μM	Selectivity Index[a]
Quinacrine **13**	N/A	133 ± 11	> 1,000	> 7.5
55	2	5.9 ± 0.6	9.9 ± 0.4	1.7
56	3	3.3 ± 0.3	27.2 ± 0.6	8.2
57	4	5.0 ± 0.2	13.9 ± 1.3	2.8
59	2	19.3 ± 1.0	27.2 ± 0.6	1.4
60	3	13.1 ± 0.7	44.7 ± 4.4	3.4
61	4	15.5 ± 0.8	55.8 ± 2.6	3.6
62	6	11.4 ± 0.7	17.1 ± 3.1	1.5
63	2	19.6 ± 0.2	nd	nd
64	4	11.7 ± 0.5	nd	nd
66	6	9.5 ± 0.5	nd	nd
67	2	38.9 ± 1.2	nd	nd
68	2	173 ± 1	nd	nd
69	2	104 ± 9	nd	nd
87	2	7.6 ± 0.1	nd	nd
88	3	10.4 ± 0.3	nd	nd
89	-	3.2 ± 0.2	nd	

[a]ratio IC_{50} GR/IC_{50} TryR

The enzyme assays were performed essentially as described before [86]. Recombinant *T.cruzi* TryR (128 mU) was assayed using a Beckman DU640 spectrophotometer in 40 mM HEPES, pH 7.5, 1 mM EDTA and 200 μM NADPH at 25 °C followed by the addition of 100 μM $Try[SH]_2$. Human glutathione reductase, purified from human erythrocytes (42.3 mU), was analysed in a similar manner and under identical conditions followed by addition of glutathione disulfide (100 μM). Enzyme mixtures were preincubated with NADPH (10 min at 25 °C) before the addition of varying concentrations of inhibitor added in DMSO (1% v/v final concentration).

As can be seen from Table 4, the sulfonamides (**55-57**), ureas (**59-62**) and amines (**63, 64, 66**) were generally superior to quinacrine in inhibiting TryR. This has been demonstrated graphically for the sulfonamides **55-57** and ureas **69-62** (figure 5.2). The sulfonamide series was superior to the amine series which in turn was superior to the urea series. The superior nature of the sulfonamide series may be attributed to a more hydrophobic naphthalene moiety in **55-57** as compared to the benzyl moiety in both **59-62** and **63, 64, 66.** The amine series **63, 64, 66** is marginally better at inhibiting TryR than the urea series **59-62**. Despite the superior inhibitory activities of the sulfonamide and urea derivatives, selectivity against the human enzyme, glutathione reductase. The loss of the positive charge (from the terminal tertiary amino group) in moving from quinacrine to the new derivatives could account for this loss in selectivity [87]. The three and four-carbon methylene spacer length appears to be optimum for the inhibition of TryR within this series of compounds.

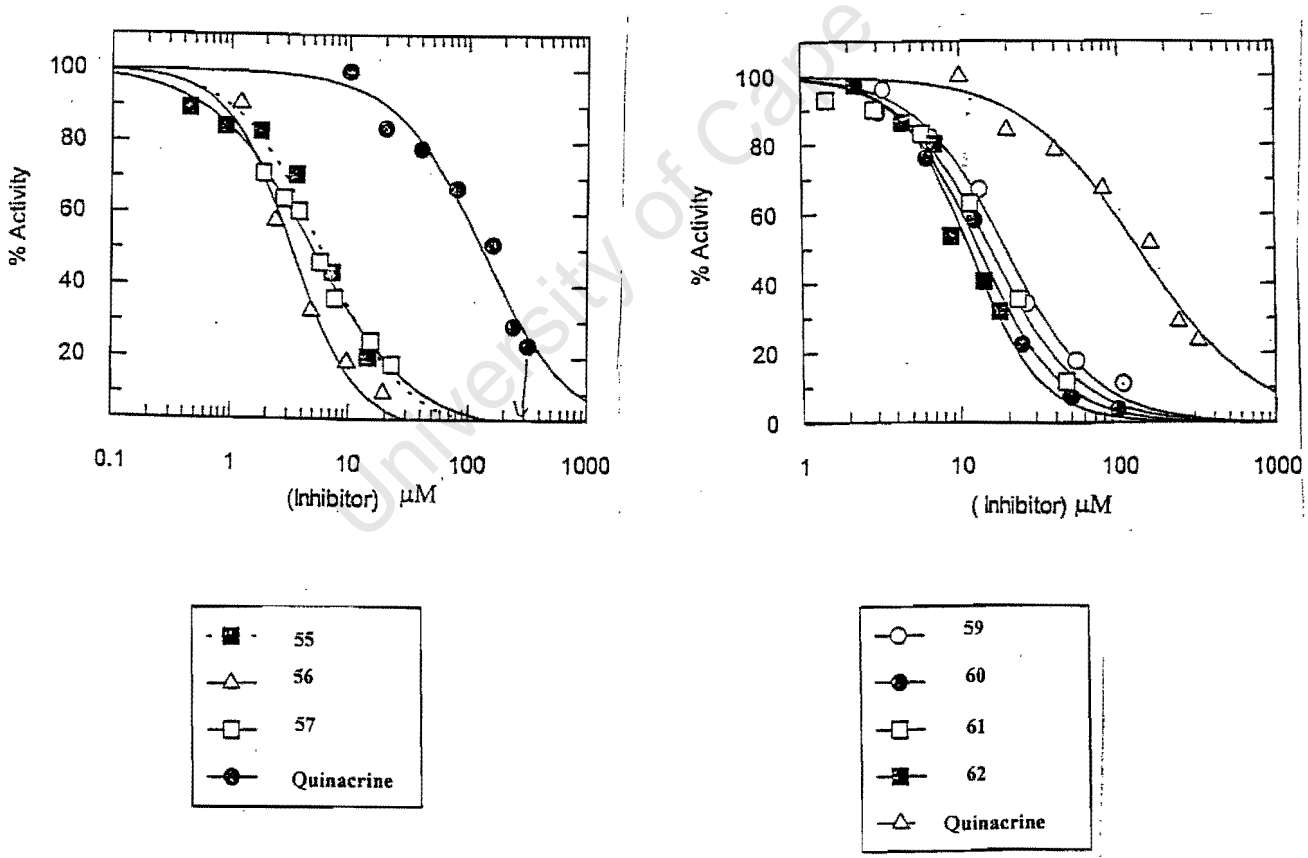


Figure 5.2. Graphical representation of the inhibition of TryR relative to quinacrine.

Compounds **67-69** show decreased inhibitory activity against TryR as compared to the other derivatives discussed. The methylene spacer in this series was kept constant at two carbons and the terminal group functionality manipulated. The most polar terminal group in the series is that of **68** which shows the least inhibitory activity. It is closely followed by **69**, whose only structural difference to **68** is the loss of a methoxy group. This suggests that an increase in the hydrophobic nature of the extended side chain has an effect on the inhibitory activity of the molecule. Compound **67** has the greatest inhibitory activity within the series. It is interesting to note that upon moving from the hydroxy group in **67** to the ethers of **68** and **69**, there is a marked decrease in the inhibition of TryR.

Compounds **87-89** showed general superiority to quinacrine in inhibiting TryR with **89** showing the greatest inhibition in the series. Comparing **88** and **89**, the only structural difference is the absence of the quinoline moiety in **89** where it has been replaced with a dimethylamine. This appears to have a marked effect on TryR inhibition.

5.1.2 Inhibition activity of ether derivatives 70-73

The model that quinacrine binds to two hydrophobic pockets in the active site of the enzyme TryR, was further investigated by substituting the methyl group of the methoxy group with longer alkyl chains **70-73**. As can be seen from Table 5, the effect of substituting this methyl group renders the derivatives more superior to quinacrine in inhibiting TryR. Effectively, increasing the number of carbons in the side chain results in a greater inhibitory activity of the molecule. Consequently, **73**, with five carbons in the side chain, is superior to the other compounds in the series. The three and four carbon analogues display similar activity in inhibiting TryR.

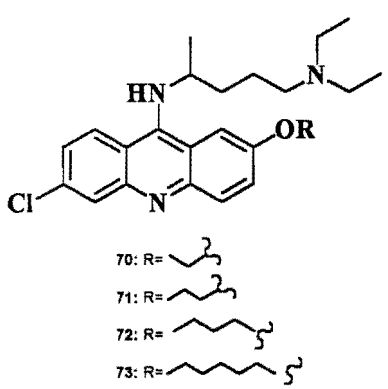


Figure 5.3. Structures of ether derivatives.

Table 5: Inhibition of trypanothione reductase and glutathione reductase by quinacrine analogues.

Compound	Number of Methylene Groups, n =	Trypanothione Reductase IC_{50}, μM
Quinacrine **1**	N/A	133 ± 11
70	2	50.7 ± 2.2
71	3	39.0 ± 0.9
72	4	41.4 ± 1.3
73	5	30.4 ± 0.7

5.2 ANTIPROTOZOAL ACTIVITY RESULTS OF SELECTED COMPOUNDS 55-57, 59-62 and 87-89

Due to factors beyond our control, some compounds were not tested within the time frame of the project. Although *P. falciparum* was not a target of the current project, literature reports on the antimalarial activity of quinacrine and chloroquine analogues prompted us to evaluate some compounds in this regard. Compounds were tested *in vitro* for activity against amastigote forms of *Leishmania donovani* (cultured in murine macrophages), amastigote forms of *Trypanosoma cruzi* (cultured in murine macrophages), and the bloodstream form trypomastigote *Trypanosoma brucei rhodesiense*. Experimental details have previously been described [38]. For *Plasmodium falciparum*, all compounds were tested against the chloroquine-sensitive strain (3D7) and the most potent also tested against a chloroquine-resistant strain (K1). The whole cell inhibition assay of *Plasmodium falciparum* growth in human red blood cells was carried out in a 48h [^{3}H]-hypoxanthine incorporation assay [88, 89]. Potential toxicity of the quinacrine derivatives was determined against the KB cell line in comparison to podophyllotoxin. The data are presented in Table 6. Data for standard control drugs are included for comparative purposes.

Considering the sulfonamides (**55-57**) and ureas (**59-62**): compounds generally showed significant activity against *L. donovani* with compounds **56**, **60**, and **61** being the most superior. This data correlates well with the data for inhibition of TryR in Table 4. With

the exception of urea compounds **61** and **62** which showed good activity against *T. cruzi*, the rest of compounds displayed weak to no activity at the test concentrations.

As with the inhibition of TryR and activity against *L. donovani*, compounds **56**, **60,** and **61** showed the best activity against *T. brucei*. The same picture is more or less true for *P. falciparum* where the compounds showed the greatest activity, compound **60** being most noteworthy. This compound was 4 and 10 times more active than chloroquine against 3D7 and K1 strains respectively. The fact that **60** is less active against the K1 than the 3D7 strain suggests haem polymerization as a target, like the 4-aminoquinolines [90]. This in turn suggests cross-resistance with chloroquine. The compounds that were active against *L. donovani* and *T. cruzi* were also toxic to KB cells at the same concentrations, indicating a lack of selectivity against these parasites.

Table 6. In vitro sensitivity of parasites to selected quinacrine and chloroquine analogues.

Compound	ED_{50}, µg/ml					Toxicity ED_{50}, µg/ml
	L. donovani	*T. cruzi*	*T. brucei*	*P. falciparum* 3D7[a]	*P. falciparum* K1[b]	
Podophyllotoxin						0.008
Pentostam	8.9					
Benznidazole		12.4				
Pentamidine			0.0002			
Chloroquine				0.002	0.15	
55	5.8	>30	0.47	0.025	nd[c]	2.7
56	1.9	23.9	0.078	0.010	nd	0.4
57	3.3	>30	0.12	0.033	nd	0.4
59	10.7	>30	0.42	0.069	nd	4.1
60	1.9	22.0	0.083	0.0005	0.015	0.7
61	1.9	<1	0.043	0.0013	nd	0.8
62	5.8	6.8	0.46	0.14	nd	4.3
87	>30	>30	14.72	<0.1	0.55	>300
88	>30	>30	13.37	<0.1	0.56	>300
89	>30	>30	>30	9.6	0.55	>300

[a]Chloroquine-sensitive strain; [b]Chloroquine-resistant strain; [c]Not determined

The bisquinoline urea series (**87-88**) and urea **89** generally did not show any significant activity against *L. donovani, T. cruzi. T. brucei* or *P. falciparum* K1. This data did not correlate with the data for inhibition of TryR in Table 4. Factors such as cell penetration and metabolism may play in explaining this lack of correlation. It is also important to note that for the intracellular *L. donovani* and *T. cruzi* amastigotes, the drug needs to pass through the macrophage to reach the amastigote. As a result, selective toxicity in *L. donovani* and *T. cruzi* is more difficult to achieve than for *T. brucei*. **87** and **88** showed the greatest inhibition against the *P. falciparum* 3D7 strain. Both are less active against the K1 strain than the 3D7 strain, once again suggesting haem polymerisation as a target.

5.3 ASSESMENT OF *IN VIVO* GROWTH INHIBITION ACTIVITY IN CQ-SUSCEPTIBLE *P. BERGHEI* ANKA RODENT MODEL

The *in vivo* activity of compounds **87-89** against malaria parasites was tested by determining their ability to protect mice from the lethal effects of *P. berghei* infection. These compounds were injected intraperitonealy at 25 mg/kg once a day every day.

Table 7:Response of *P. berghei* to selected quinoline derivatives in comparison with chloroquine.

Group	Dose (mg / kg)	Schedule	Mean Parasitemia	Mean Survival Time (days)
Control	-		16.99 ± 1.16	5.00 ± 0.00
Chloroquine	10	x 4 i. p.	0.00	28 ± 0.00
87	25	x 4 i. p.	12.59 ± 0.79	6.00 ± 0.45
88	25	x 4 i. p.	12.27 ± 0.55	9.0 ± 0.00
89	25	x 4 i. p.	0.00	26.40 ± 2.23

As can be seen in Table 7 these compounds reduced the level of parasitemia in mice infected with *P. berghei*. However, only **89** yielded a reduced parasitemia comparable to that of CQ.

The control mice died after 5 days post infection but the **87** treated mice lasted for 6 days, the **88** treated mice lasted for 9 days and the **89** treated mice lasted for 26.40 days. Again **89** is comparable to the survival time of the CQ treated mice at 28 days. The compounds **87** and **88** were able to slightly reduce parasitemia, however the parasites

were not completely cleared Presumably, another scheme of administration of the drugs could be required for the best results. A graphical representation of the data (figure 5.4) indicates how the tested compounds influenced the course of a well-established infection in mice.

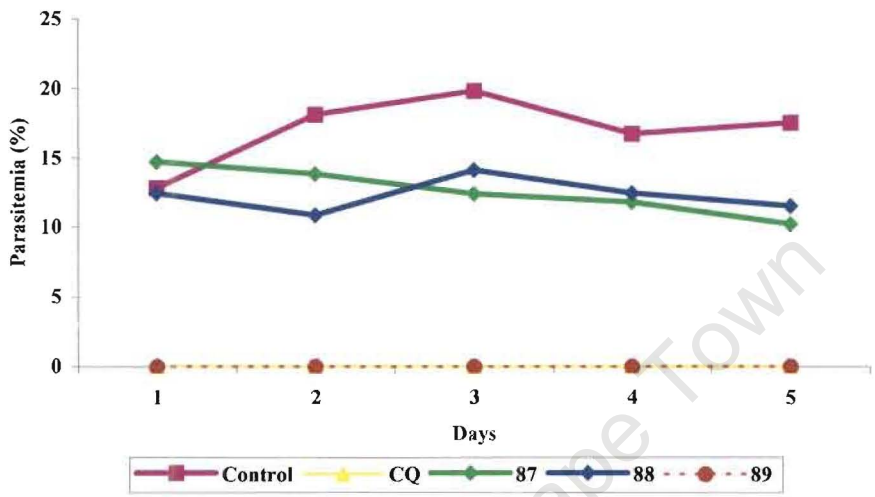
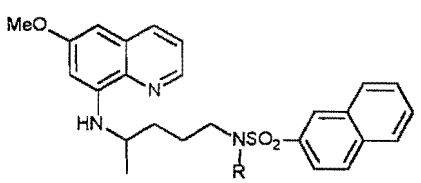


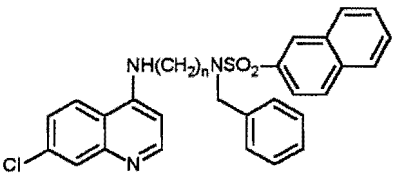
Figure 5.4. Effect of compounds **87-89** on the level of parasitemia in mice parasitized by *P. berghei*.

5.4 MODULATION OF HUMAN MAMMARY CELL SENSITIVITY TO PACLITAXEL

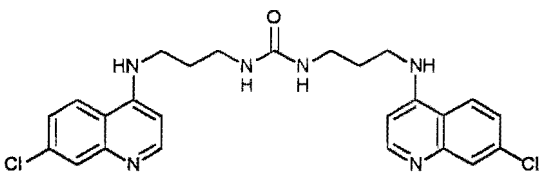
In order to establish the intrinsic anticancer activity, the derivatives were first tested against a paclitaxel-sensitive (LCC-WT-Human breast carcinoma) and paclitaxel-resistant (LCC6-MDR-MDR1 transfected) cell lines[91]. Cells were seeded at 2000 cells/well in 96-well plates in complete medium-RPMI-1640 containing 5% FCS, 5% Nuserum IV, 2 mM L-glutamine and 10 mM HEPES. Following overnight incubation, compounds were solubilized in 100% DMSO, diluted in complete medium and added to cell plates. After 72 hours, cells were fixed, stained and total protein/well was determined. Compound concentration that inhibited growth by 50% was determined and reported as IC_{50}. The data are summarised in Table 8. The compounds generally showed none or very weak cytotoxicity at a test concentration of 10 μM (1μM for **88** and **89**) in both cell lines. The only exception was compound **80** which was found to be cytotoxic in both cell lines.



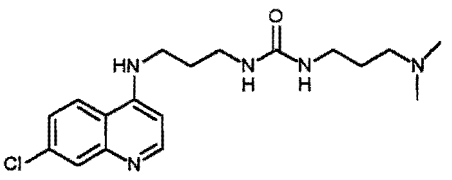
74: R=H
75: R=Bn



78: n = 4
80: n = 6



88



89

Figure 5.5. Structures of derivatives tested as MDR reversal agents

Table 8: Cytotoxicity of Quinolines **81, 82, 85** and **87** Against Sensitive and Resistant Human Breast Cancer Cells

Compound	**IC_{50} (μM) +/- S.D.**		**%Growth Inhibition at 10 μM**	
	LCC6-WT[a]	LCC6-MDR[a]	LCC6-WT	LCC6-MDR
Paclitaxel **27**	0.008 ± 0.001	0.612 ± 0.021		
81	>10	>10	16	8
82	>10	>10	0	8
85	>10	>10	6	3
87	1.7 ± 0.09	2.0 ± 0.24		
95	11 ± 90	>10		
96	>10	>10		

[a]LCC6-WT-human breast carcinoma; LCC-MDR-MDR1 transfected line

The compounds were then co-administered with paclitaxel at four different concentrations as shown in Table 9. Paclitaxel recovered 96-99% of its efficacy against the resistant human breast cancer cells when compounds **74**,**75**, **78** and **80** were co-administered at a concentration of 5 μM. However, when co-administered with paclitaxel at a concentration of 1 μM, only compound **80** exhibited 99% MDR reversal activity. Based on this, and the result with compound **78** in comparison with the results from compounds **74** and **75**, it is apparent that the chloroquine series **78** and **80** is

superior to the primaquine series **74** and **75**. It is noteworthy that primaquine compounds **74** and **75** are racemates and the biological activity may reside in one enantiomer only. As such higher potency may be expected from the appropriate single enantiomer. The bisquinoline urea **88** and the urea **89** display no significant activity.

Table 9: Modulation of Human Mammary Cell Sensitivity to Paclitaxel by Quinolines **74**, **75**, **78** and **80**

Compound	Concentration, (μM)[a]	IC_{50} (nM) LCC6-MDR	$\%IC_{50}$ Reduction
Paclitaxel		490	0
Paclitaxel + (74)	5	13	97
Paclitaxel + (75)	5	19	96
Paclitaxel + (78)	5	2.7	99
Paclitaxel + (80)	nd[b]	nd	nd
Paclitaxel + (74)	1	353	36
Paclitaxel + (75)	1	249	55
Paclitaxel + (78)	1	164	70
Paclitaxel + (80)	1	6.2	99
Paclitaxel + (80)	0.3	254	54
Paclitaxel + (80)	0.1	338	39
Paclitaxel + (88)	1	545 ± 15	17
Paclitaxel + (89)[b]	1	643 ± 29	2

[a]No growth inhibition was seen at this concentration of reversal agent; [b]Not determined due to cytotoxicity at 5 μM
[b]Paclitaxel IC_{50} 653 ± 18

5.5 COMMOM PHARMACOPHORE FOR 78, 80 TAXANE MDDR AGENTS, AND A PGP PHOTOAFFINITY LABELLING TAXOID

Since antimalarial drug analogs, **74, 75, 78** and **80** exhibited substantial MDRR activity against LCC6-ADR when co-administered with paclitaxel, it was reasoned that there might be a common pharmacophore for these sulfonamide based compounds and taxane-based MDR reversal agents that were developed earlier. Accordingly, compounds **78** and **80** as well as taxane MDR reversal agents (tRAs), SB-RA-30001 and SB-RA-31012, [92] and photoaffinity labeling taxoid {^{3}H}-SB-T-5111[93] for a molecular modelling study (Figure 5.6.), and investigated common structural features between these two totally different classes of compounds.

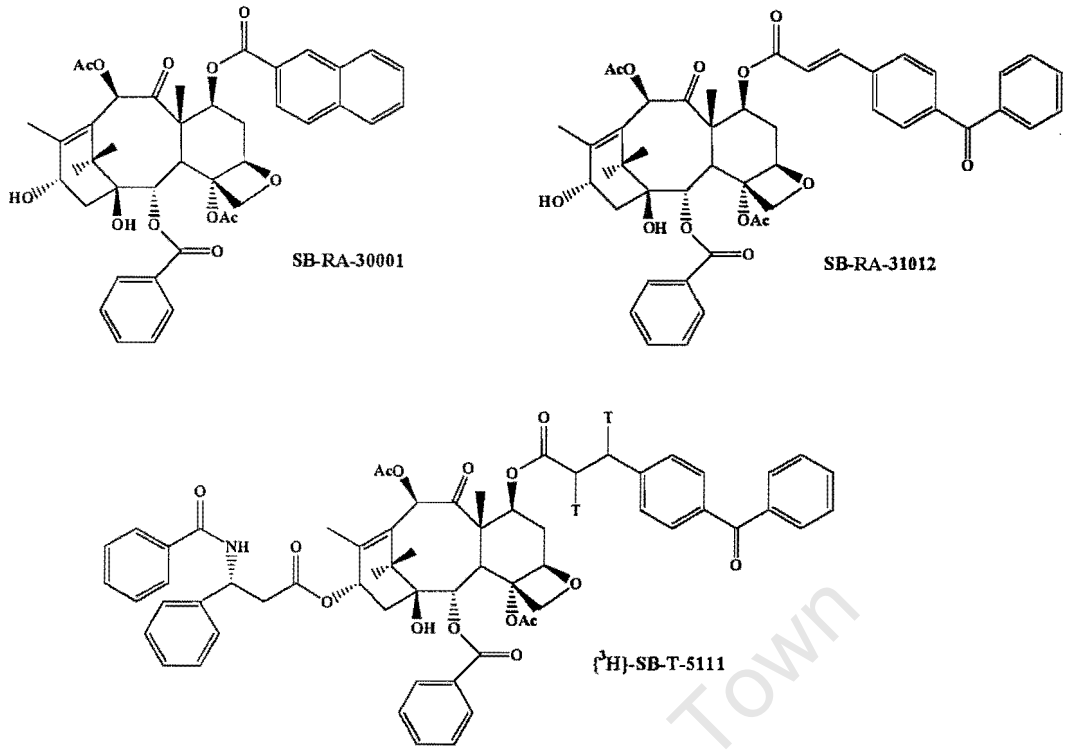


Figure 5.6. Structures of taxane MDR reversal agents (tRAs) SB-RA 31012 and photoaffinity labelling taxoid {^{3}H}-SB-T-5111.

The molecular modelling was performed on a Silicon Graphics O_2 workstation using two programs: SYBYL 6.4 and InsightII 2000. The quinolinesulfonamide structures, **78** and **80** were constructed in SYBYL 6.4 and energy-minimized (Tripos force field, charges calculated by the Gasteiger-Huckel method). The lowest energy conformations of SB-RA-30001 and SB-RA-31012, and photoaffinity taxoid {^{3}H}-SB-T-5111 were obtained using SYBYL 6.4 by modifying the X-ray structure of paclitaxel and minimizing the resultant conformers. The template forcing was performed using the InsightII 2000/Discover Module (tRAs or the taxoid as the template and **78** or **80** as the mover) using constant valence force field (CVFF). The resultant conformers of **78** and **80** were energy-minimized so that the energies of these conformers were within the 5-kcal/mol range from that of the lowest energy conformation. Then, these minimized conformers of **78** and **80** were overlaid with the tRAs and the taxoid. The overlay results are shown in Figure 5.7.

As Figure 5.7(a) shows, the two naphthyl moieties of **80** and the phenyl group of the C-2 benzoate as well as the naphthalene group of the C-7 acyl moiety of SB-RA-30001 have almost perfect fit. This is a rather surprising finding. SB-RA-30001 possesses a

high MDR reversal activity with paclitaxel (97.5% at 1 μM against human breast cancer cell line MCF7-ADR)[92]. As compared to **80**, the overlap of the naphthalene moiety of **78** with the naphthalene group of the C-7 acyl moiety of SB-RA-30001 is only partial. This observation is consistent with the difference in activity of **78** and **80**. Figure 5.7(b) shows the overlay of **78, 80**, and SB-RA-31012 that is a highly potent MDRR agent for paclitaxel (99% reversal activity at 1 μM and 92% at 0.1 μM against LCC6-ADR)[92]. In this case, one of the aromatic ring of the naphthyl moiety of **80** overlaps with one of the phenyl group of the benzophenone moiety of SB-RA-31012, but **78** does not have overlap in this part of the molecule. As Table 6 shows, **80** exhibits high potency (99% reversal activity) at 1 μM, but the potency drops to 33% at 0.1 μM. This might be due to the fact that the methylene chain of **80** is not long enough to reach more preferable hydrophobic binding site to which the benzophenone moiety of SB-RA-31012 binds. Figure 5.7(c) shows the overlay of **78**, **80**, and {^{3}H}-SB-T-5111 that has been used for the photoaffinity labeling of Pgp [93]. As anticipated, the overlaps in the C-2 benzoate moiety and the tethered benzophenone moiety at C-7 with **78** and **80** are essentially the same as the case of SB-RA-31012. It should be noted that the phenyl group of the benzylamino moiety of **78** and **80** is located in the "hydrophobic clustering" region of the taxoid. In addition, the phenyl and the naphthyl groups are apparently clustered through aromatic π-π interaction. As discussed above, the molecular modelling study has disclosed a probable common pharmacophore for these structurally very different classes of compounds, which provides a good rationale for the excellent MDR reversal activity of antimalarial drug related quinolinesulfonamides when used with paclitaxel.

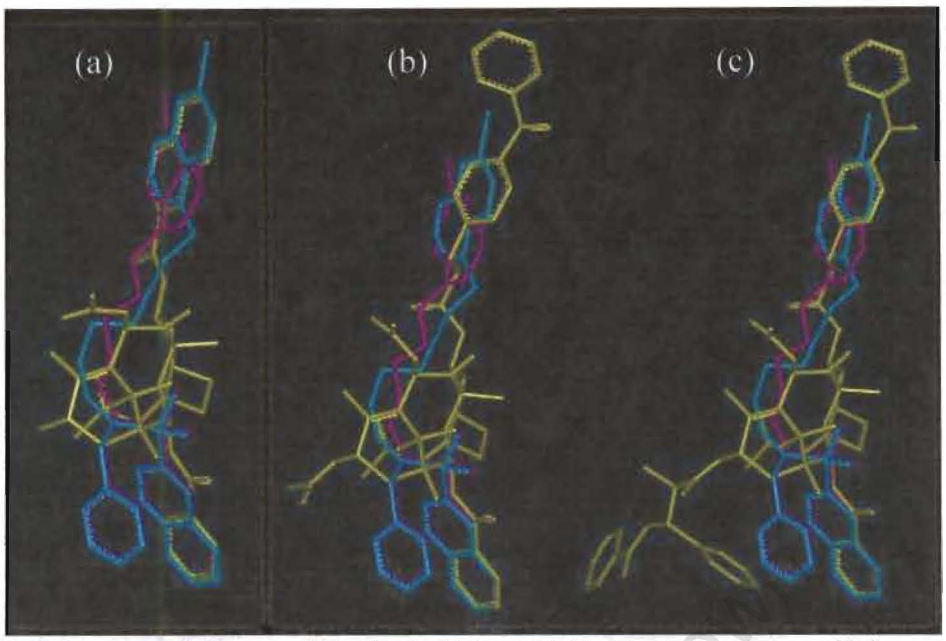


Fig 5.7: Molecular modelling graphics of quinolines overlaid with tRAs and photoaffinity labelling taxoid.

5.6 Summary of results

A series of sulfonamides **55-57**, ureas **59-62** and amines **63, 64, 66** analogues of quinacrine generally show superior activity (relative to quinacrine) against TryR. Although sulfonamide derivatives were the most active series inhibiting TryR, this trend did not correlate with the *in vitro* activities against *L. donovani*, *T. cruzi*, and *T. brucei*. Urea compounds in particular showed significant activity *in vitro* against all four parasites. Although *T.cruzi* appears to be the least sensitive compared to *L. donovani*, it is noteworthy that compounds display activity against these intracellular *T.cruzi* parasites where the drug must cross the macrophage to reach the amastigote (tissue form of the parasite placed near the cell nucleus). The mode of action of these compounds is not clear and merits further investigation. Although series **67-69** displayed activity better than that of quinacrine, the results were not comparable to the aforementioned series of compounds. The compounds **70-73** are a good indication that a longer alkyl group to replace the methyl of the methoxy group renders an increase in TryR inhibition as compared to quinacrine. This is an important piece of new information which could be exploited further in the use of such compounds as probes for the investigation of the mode of action of quinacrine.

The bisquinoline ureas **87-88** were superior to quinacrine at inhibiting TryR and the urea **89** showed potent inhibitory activity as well. Both series' however did not display any significant antiprotozoal activity apart from the activity exerted against the *P. falciparum* 3D7 strain. This result merits further investigation. These compounds were also ineffective as chemosensitizers in restoring the sensitivity of cancer cells to paclitaxel. Despite not having any significant *in vitro* activity, **89** has shown good *in vivo* activity on a level comparable to that of CQ. However, toxicology studies need to be carried out to determine the cytotoxicity of **89** to mammalian cells. As inhibitors of TryR, **87-89** were more 13-41 times more active than quinacrine.

Although the bisquinoline ureas did not show any real promise as chemosensitizers, we have shown the potential of quinoline-based compounds **78** and **80** to chemosensitize cancer cells to paclitaxel. These compounds are non-cytotoxic and, as such, they hold promise. The quinoline series was found to be superior to the primaquine series **74** and **75**. Once again, it is important to note that the biological activity of **74** and **75** may reside in one enantiomer. It needs to be further investigated whether a higher potency may be exerted by a single enantiomer as compared to the racemate.

CHAPTER 6

EXPERIMENTAL

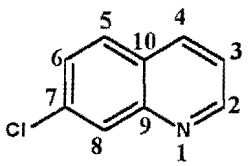
6. 1 General:

Melting points were determined on a Reichert-Jung Thermovar and a Fischer-Johns hot stage microscope and are uncorrected. Proton nuclear magnetic resonance spectra were recorded using trimethylsilane as an internal standard on a Varian VXR-200 (200MHz), Varian Mercury (300MHz) or a Varian Unity Spectrometer (400MHz). Carbon –13 nuclear magnetic resonance spectra were determined on the same instruments at 50, 75 or 100MHz (using trimethylsilane as an internal standard). Infrared spectra were recorded in solutions specified using a Perkin Elmer Paragon 1000 FT-IR or a Satellite FT-IR spectrometer. Elemental analyses were performed using a Fisons EA 1108 CHNS-O instrument. Mass spectra were recorded on a VG micromass 16F spectrometer operating at 70eV with an accelerating voltage of 4kV. Accurate masses were determined using a VG-70E spectrometer at the Cape Technikon.

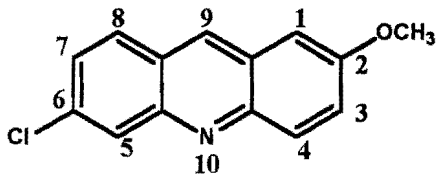
All reactions were monitored by thin layer chromatography using aluminium-backed silica gel $60F_{254}$ plates (Merck). The plates were visualised by a combination of ultraviolet light (254nm) and either anisaldehyde spray [prepared from a 2.5% solution of *p*-methoxybenzaldehyde ($20cm^3$) and 18 M sulphuric acid (1 cm^3)] or cerium (IV) ammonium sulfate in 8 M sulphuric acid and baking at 200°C. Column chromatography was carried out on silica gel (Merck Kieselgel 60: 70-230 mesh for gravity and 230-400 mesh for flash chromatography).

All solvents used were dried by the appropriate technique[94]. Tetrahydrofuran and diethyl ether were dried over sodium wire prior to use using benzophenone as indicator. Triethylamine was dried over and distilled from calcium hydride and it was stored over potassium hydroxide pellets. Dichloromethane was dried over phosphorous pentoxide and distilled. Solvents not mentioned which were used in reactions were anhydrous unless stated otherwise.

The following numbering system was used to assign aromatic protons in the proton nuclear magnetic resonance spectra of compounds based on quinoline and acridine moieties.

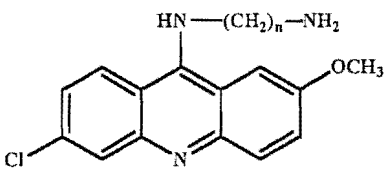


Quinoline ring system



Acridine ring system

6.2 PROCEDURES FOR ACRIDINE ANALOGUES



General procedure for preparation of acridine amines 92-95 - A mixture of 6,9-dichloro-2-methoxyacridine (10.3 mmol), diaminoalkane (330 mmol) and phenol (0.78 g/mmol) was heated at 90°C for 4 h[73]. The resulting yellow mixture was cooled to room temperature. Then water (400 cm^3) was added yielding a yellow precipitate. This was isolated by suction filtration and dried in a vacuum dessicator to yield the desired amine.

Diaminoalkanes used: 1, 3 diaminopropane, 1, 4 diaminobutane, 1, 6 diaminohexane and 1, 3 diaminoethane

N^1- (6'-chloro-2'-methoxyacridin-9'-yl) propane-1, 3-diamine (92). - (2.71 g, 95%) as yellow crystals, mp 89-92°C (from EtOAc/pet ether) (lit 98-102)[73]†; R_f 0.47 (NH_4OH: MeOH, 1:9) IR (Nujol): ν_{max}/cm^{-1} 3700 (NH_2), 3050 (C=C) and 1620 (C=N); δ_H (400 MHz, $CDCl_3$) 1.83 (2H, m, $ArNHCH_2CH_2$-) 3.07 (2H, t, *J* 6 Hz, $ArNH(CH_2)_2CH_2NH_2$), 3.91 (3H, s, OCH_3), 3.93 (2H, t, *J* 6.0 Hz, $ArNHCH_2$-), 7.19 (1H, dd, *J* 2.4 and 9.2 Hz, H3'), 7.34 (1H, d, *J* 2.8 Hz, H5'), 7.38 (1H, dd, *J* 2.8 and 9.2 Hz, H7'), 7.95 (1H, d, *J* 9.2 Hz, H4'), 8.01 (1H, d, *J* 2.4 Hz, H1') and 8.09 (1H, d, *J* 9.2 Hz, H8'); δ_C (100 MHz, $CDCl_3$) 32.5, 41.4, 50.8, 55. 5, 100.1, 114.7, 117.1, 123.4, 124.0, 125.1, 127.9, 131.2, 134.6, 146.6, 148.9, 150.4 and 155.5; HRMS (EI) *m/z* 315.11363 $(M)^+$; $C_{17}H_{18}ClN_3O$ requires 315.11384; Anal. Calc for $C_{17}H_{18}ClN_3.3/4\ H_2O$; C, 62.1%; H, 5.5%; N, 12.7%; Found C, 62.5%; H 5.7%, N 12.5%.

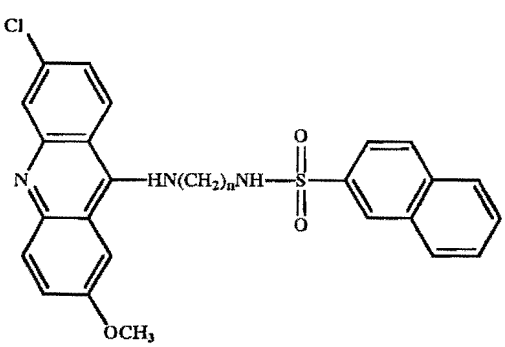
† This melting point was obtained after repeated recrystallization

N^1- (6'-chloro-2'-methoxy-acridin-9'-yl)-ethane-1, 2-diamine (93). - (1.43 g, 95%) as yellow crystals, mp 81-83°C (from EtOAc/pet ether); R_f 0.33 (NH_4OH: MeOH, 1:19); IR (Nujol): ν_{max} (CH_2Cl_2)/cm^{-1} 3700 (NH_2), 3050 (C=C) and 1620 (C=N); δ_H (400 MHz, $CDCl_3$) 3.01 (2H, t, *J* 5.6 Hz, $ArNHCH_2CH_2$-), 3.71 (2H, t, *J* 5.6 Hz, $ArNHCH_2$-), 3.95 (3H, s, OCH_3), 7.29 (H, dd, *J* 2.4 and 9.6 Hz, H3'), 7.38-7.42 (2H, m,), 7.99 (H, d, *J* 9.2 Hz, H4'), 8.05 (H, d, *J* 2 Hz, H1') and 8.13 (H, d, *J* 8.8 Hz, H8'); δ_C (50 MHz, $CDCl_3$) 42.0, 51.6, 55.5, 99.4, 105.0, 116.2, 121.3, 124.4, 124.5, 128.1, 131.3, 134.7,

144.4, 148.1, 150.3 and 155.9; HRMS (EI) *m/z* 301.09972 $(M)^+$, $C_{16}H_{16}ClN_3O$ requires 301.9819; Anal. Calc for $C_{16}H_{16}ClN_3O.3/4\ H_2O$; C, 61.0%; H, 5.1%; N, 13.4%; Found C, 59.7%; H, 5.4%, N, 13.1%.

N^1- (6'-chloro-2'-methoxy-acridin-9'-yl-butane-1, 4-diamine (94). - (1.11 g, 74%) as yellow crystals, mp 51-53°C (from EtOAc/pet ether); R_f 0.24 (NH_4OH: MeOH, 1:9), ν_{max} (CH_2Cl_2)/cm^{-1} 3700 (NH_2), 3050 (C=C) and 1620 (C=N); δ_H (400 MHz, $CDCl_3$) 1.61 (2H, quin, *J* 4 x 5.4 Hz ArNHCH$_2$*CH$_2$* -), 1.83 (2H, quin *J* 4 x 7.4 Hz, ArNH $(CH_2)_2$*CH$_2$*-), 2.79 (2H, t, *J* 6.8 Hz, ArNH*CH$_2$*-), 3.74 (2H, t, *J* 7.2 Hz, ArNH*CH$_2$*-), 3.95 (3H, s, O*CH$_3$*), 7.26-7.29 (2H, m), 7.42 (H, dd, *J* 2.8 and 6.4 Hz, H7'), 7.98-8.06 (3H, m);δ_C (100 MHz, $CDCl_3$) 29.0, 30.8, 41.5, 50.7, 55.5, 99.5, 114.4, 115.8, 117.9, 124.2, 124.3, 125.6, 128.2, 131.5, 134.7, 146.8, 149.9 and 155.9; HRMS (EI) *m/z* 329.13011$(M)^+$, $C_{18}H_{20}Cl\ N_3O$ requires 329.12949; Anal. Calc for $C_{18}H_{20}Cl\ N_3O$ .3/4 H_2O; C, 63.0%; H, 5.8%; N, 12.2%, Found C, 62.9% H 5.7%, N 11.9%.

N^1- (6'-chloro-2'-methoxy-acridin-9'-yl)-hexane-1, 6 diamine (95). - (1.43 g, 95%) as yellow crystals, mp 54-57°C (from EtOAc/pet ether); R_f 0.27 (NH_4OH: MeOH, 1:9); ν_{max} (CH_2Cl_2)/cm^{-1} 3700 (NH_2), 3050 (C=C) and 1620 (C=N); δ_H (400 MHz, $CDCl_3$) 1.35-1.46 (4H, m, 2 x CH_2), 1.75 (4H, m, 2 x CH_2), 2.66 (2H, t, *J* 6.4 ArNH$(CH_2)_5$*CH$_2$*NH_2), 3.68 (2H, t, *J* 7.2 Hz, ArNH*CH$_2$*-),3.95 (3H, s, O*CH$_3$*), 7.18 (H, d, *J* 2.6 Hz, H5'), 7.29 (1H, dd, *J* 2.0 and 9.2 Hz, H3'), 7.41 (1H, dd, *J* 2.6 and 9.2 Hz, H7'), 7.98 (H, d, *J* 9.2 Hz, H4'), 7.99 (H, d, *J* 9.2 Hz, H8') and 8.06 (1H, d, *J* 2.4 Hz, H1'); δ_C (100 MHz, $CDCl_3$) 26.7, 31.8, 33.5, 42.0, 50.7, 55.5, 99.2, 115.7, 115.9, 118, 124.0, 124.4, 124.6, 128.3, 129.5, 131.5, 134.8, 148.4, 149.7 and 156.1; %. HRMS (EI) *m/z* 357.16151 $(M)^+$, $C_{20}H_{24}ClN_3O$ requires 357.16079; Anal. Calc for .$C_{20}H_{24}ClN_3O2H_2O$; C, 60.9%; H, 6.1%; N 10.7%, Found C, 60.7%; H, 6.2%, N 10.7.



General procedure for preparation of sulfonamides 55-58 - 2-Naphthalene sulfonyl chloride (1.78mmol) was added to a solution of diamine (1.27mmol), triethylamine (2.54mmol) and anhydrous *N*, *N'*

dimethylformamide (1.97cm^3/mmol) in a vial. The resulting mixture was shaken for 14 h at 20°C[95]. Water (0.2 cm^3/mmol) was added to quench the reaction and the resultant mixture was extracted with ethyl acetate (0.1 cm^3/mmol). The organic residue was removed by pipette, dried with magnesium sulfate and the solvent removed *in vacuo*. Alternatively, the resultant yellow precipitate was isolated by filtration. The resulting yellow crystals were recrystallized from ethyl acetate/pet ether to yield the *sulfonamide*. Diamines used: **92-95**

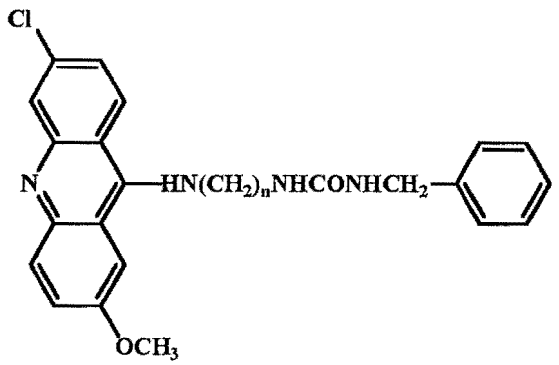
Naphthalene-2-sulfonic acid [2-(6'-chloro-2'-methoxy-acridin-9'-ylamino)-ethyl]-amide (55). - (0.35 g, 93 %), mp 129-131°C (from MeOH); R_f 0.39 (MeOH: CH_2Cl_2, 1:9); ν_{max} (DMSO)/cm^{-1} 3311 (N-H) and 1333 (SO_2NH); δ_H (300 MHz, DMSO-d_6) 3.45 (2H, t, *J* 2.7 Hz, $ArSO_2NHCH_2$-), 3.89 (3H, s, OCH_3), 4.11 (2H, t, *J* 5.7 Hz, $ArNHCH_2$-), 7.26 (H, dd, *J* 2.1 and 9.3 Hz, H3'), 7.46-8.37 (12H, m); δ_C (75 MHz, DMSO-d_6) 30.7, 35.7, 42.5, 48. 7, 55.7, 116.0, 121.9, 122.3, 122.8, 123.9, 124.0, 125.3, 126.2, 126.3, 127.1, 127.3, 127.4, 127.5, 127.7, 128.4, 128.9, 129.3, 129.5, 131.5, 133.9, 137.4, 152.5 and 155.3; HRMS (EI) *m/z* 491.10739 $(M)^+$, $C_{26}H_{22}ClN_3O_3$ requires 491.10704; Anal. Calc for $C_{26}H_{22}ClN_3O_3$. 0.75 H_2O; C, 61.8%; H, 4.4%; N, 8.3%, S, 6.3%, Found C, 61.5%; H, 4.7%; N, 7.9%; S, 6.6%.

Naphthalne-2-sulfonic acid [3-(6'-chloro-2'-methoxy-acridin-9'-ylamino)-propyl]-amide (56). - (0.33 g, 63%), mp 154-157°C(from MeOH); R_f 0.24 (MeOH: CH_2Cl_2, 1:9); ν_{max} (DMSO)/cm^{-1} 3361 (N-H) and 1319 (SO_2NH); δ_H (300 MHz, DMSO-d_6) 1.91 (2H, t, *J* 6 Hz, $ArNHCH_2CH_2$-), 3.85 (2H, t, *J* 6.6 Hz, $ArNH(CH_2)_2CH_2$-), 3.87 (3H, s, OCH_3), 4.05 (2H, br t, $ArNHCH_2$-), 7.08 (H, dd, *J* 2.2 and 9.3 Hz, H3'), 7.26-8.58 (12H, m); δ_C (75 MHz, DMSO-d_6) 30.1, 46.16, 55.6, 55.7, 122.0, 122.6, 123.9, 124.0, 125.0, 126.2, 126.3, 126.8, 127.1, 127.2, 127.3, 127.4, 127.7, 128.3, 128.5, 128.9, 129.2, 131.5, 132.6, 133.9, 137.2, 155.0 and 155.2; HRMS (EI) *m/z* 505.12192 $(M)^+$, $C_{27}H_{24}ClN_3O_3S$ requires 505.12269; Anal. Calc for $C_{27}H_{24}ClN_3O_3S.H_2O$; C, 61.9%; H, 4.6%; N, 8.0%; S, 6.1%, Found: C, 62.2%; H, 4.7%; N, 7.6%; S, 5.8%.

Naphthalene-2-sulfonic acid [4-(6'-chloro-2'-methoxy-acridin-9'-ylamino)-butyl]-amide (57). - (0.25 g, 94%), mp 156-159°C(from MeOH); R_f 0.32 (MeOH: CH_2Cl_2,

1:9); ν_{max} (DMSO)/cm^{-1} 3318 (N-H) and 1464 (SO_2NH); δ_H (300 MHz, DMSO-d_6) 1.47 (2H, quin, *J* 4 x 7.15 Hz, ArNH$(CH_2)_2CH_2$-), 1.73 (2H, m, ArNHCH$_2CH_2$), 2.78 (2H, q, *J* 3 x 6.5 Hz, ArNH$(CH_2)_3CH_2$-), 3.73 (2H, t, *J* 7.2 Hz, ArNHCH_2), 3.90 (3H, s, OCH_3), 7.29 (H, dd, *J* 2.2 and 9.3 Hz), 7.44 (H, dd, *J* 2.6 and 9.3 Hz), 7.61-7.84 (5H, m), 7.82 (H, dd, *J* 1.6 and 9.0 Hz), 8.06 (H, d, *J* 8.7 Hz), 8.1 (H, dd, *J* 2.0 and 8.0 Hz), 8.28 (H, d, *J* 9.3) and 8.38 (H, d, *J* 1.6 Hz); δ_C (75 MHz, DMSO-d_6) 26.5, 27.4, 48.8, 55.6, 101.4, 113.6, 116.4, 122.1, 122.6, 123.9, 123.9, 124.7, 126.2, 127.2, 127.4, 127.7, 128.2, 128.4, 128.5, 129.0, 129.2, 131.6, 133.9, 134.4, 137.4, 146.2, 151.2 and 155.1; HRMS (EI) *m/z* 519.14036 $(M)^+$, $C_{28}H_{26}ClN_3O_3S$ requires 519.13834; Anal. Calc for $C_{28}H_{26}ClN_3O_3S.0.5H_2O$; C, 63.6%; H, 4.9%; N, 8.0%; S, 6.1%, Found C, 63.4%; H, 5.1%; N, 7.6%; S, 6.3%.

Naphthalene-2-sulfonic acid [6-(6'-chloro-2'-methoxy-acridin-9'-ylamino)-hexyl]-amide (58). - (0.085g, 43%), mp 93-95°C (from MeOH); R_f 0.31 (MeOH: CH_2Cl_2, 1:9); ν_{max} (DMSO)/cm^{-1} 3318 (N-H) and 1334 (SO_2NH); δ_H (300 MHz, $CDCl_3$) 0.95 (2H, m ArNH $(CH_2)_4CH_2$-), 1.37 (4H, m, 2 x CH_2), 1.45 (2H, m, ANHCH$_2CH_2$-), 1.77 (2H, m, ArNH$(CH_2)CH_2$-), 3.61 (2H, m, ArNHCH_2-), 3.92 (3H, s, OCH_3), 7.35 (H, dd, *J* 2.0 and 9.0 Hz), 7.56 (H, dd, *J* 2.5 and 9.2 Hz), 7.76-7.86 (10H, m) and 8.37 (H, d, *J* 9.2 Hz); δ_C (75 MHz, $CDCl_3$) 25.6, 26.0, 26.2, 29.3, 29.4, 31.3, 31.43, 36.4, 42.9, 43.0, 50.3, 55.6, 99.5, 122.3, 124.2, 124.4, 126.6, 127.36, 127.5, 127.9, 128.3 128.7, 129.1, 129.5, 129.6, 132. 2, 134.7, 136.9, 156.0 and 162.6; HRMS (EI) *m/z* 547.16924 $(M)^+$, $C_{30}H_{30}ClN_3O_3S$ requires 547.16964; Anal. Calc for $C_{30}H_{30}ClN_3O_3S.0.5H_2O$; C, 64.7%; H, 5.4%; N, 7.6%; S, 5.8%, Found C, 65.0%; H, 5.5%; N, 7.2%; S, 6.1%.



General procedure for preparation of ureas 59-62 - Benzyl isocyanate (1.40 mmol) was added to a solution of diamine (1.27 mmol) in dichloromethane (7 cm^3/mmol) and allowed to shake for 4 h at 20°. The resulting suspension was filtered under suction to yield the *urea* In the case

of **61** and **62**; the precipitate was chromatographed (SiO_2, MeOH: CH_2Cl_2, 1:9) to yield the *urea.*

Diamines used: **95-95**

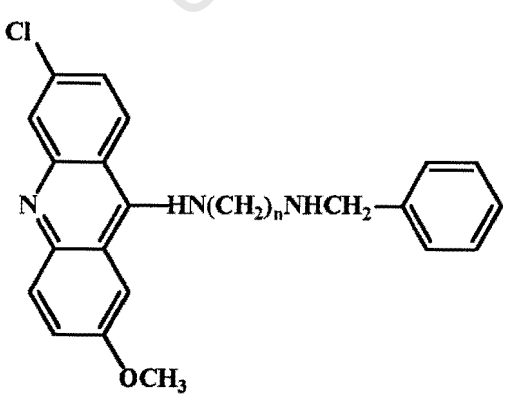
1-Benzyl-3- [2-(6'-chloro-2'-methoxy-acridin-9'-ylamino)-ethyl]-urea (59). - (0.33 g, 96%) as yellow crystals, mp 98-103°C (from MeOH); R_f 0.57 (MeOH: CH_2Cl_2, 1:9); ν_{max} (CH_2Cl_2)/cm^{-1} 3730(N-H), 3090 (N-H) and 1598 (C=O); δ_H (400 MHz, $CDCl_3$) 3.6-3.65 (2H, m, -NHCH$_2$*CH$_2$*CO-), 3.86 (2H, t, *J* 5.6 Hz, -NH*CH$_2$*CH$_2$NHCO), 3.98 (3H, s, O*CH$_3$*), 4.38 (2H, d, *J* 5.6 Hz, -NHCONHC*H$_2$*-), 7.09-7.38 (7H, m), 7.48 (1H, d, *J* 4 Hz), 7.90 (1H, d, *J* 9.6 Hz), 7.95 (1H, d, *J* 2.8 Hz) and 8.05 (1H, d, *J* 9.2 Hz); δ_C (100 MHz, $CDCl_3$) 38.9, 39.4, 39.8, 40.1, 43.4, 52.6, 99.6, 112.9, 115.7, 121.8, 122.5, 123.8, 124.0, 125.6, 126.4, 126.7, 127.8, 133.9, 139.2, 143.0, 148.0, 150.2, 154.9 and 159.9; HRMS (EI) *m/z* 434.15213 $(M)^+$, $C_{24}H_{23}ClN_4O_2$ requires 434.150953; Anal. Calc for $C_{24}H_{23}ClN_4O_2.1.5H_2O$; C, 62.5%; H, 5.0%; N, 12.2%, Found; C, 62.3%; H, 5.3%; N, 12.6%.

1-Benzyl-3- [3-(6'-chloro-2'-methoxy-acridin-9'-ylamino)-propyl]-urea (60). - (033 g, 96%) as yellow crystals, mp 162-166°C (from MeOH); R_f 0.47 (MeOH: CH_2Cl_2, 1:9); ν_{max} (CH_2Cl_2)/cm^{-1} 3730(N-H), 3090 (C=O) and 1598 (C=O); δ_H (400 MHz, $CDCl_3$) 1.80 (2H, m, ArNHCH$_2$*CH$_2$*CH$_2$NH-), 3.48 (2H, m, ArNHCH$_2$CH$_2$*CH$_2$*-), 3.73 (2H, t, *J* 6.4 Hz, ArNH*CH$_2$*(CH$_2$)$_2$NH-), 3.95 (3H, s, O*CH$_3$*), 4.42 (2H, d, *J* 6 Hz, ArNH(CH$_2$)$_3$NHCONH*CH$_2$*Ar), 4.85 (1H, t, *J* 6 Hz, -*NH*CONH-), 5.01 (1H, t, *J* 6.0, -NHCO*NH*-), 7.21-7.30 (6H, m), 7.35 (1H, dd, *J* 2.4 and 9.2 Hz, H7'), 7.56 (1H, d, *J* 2.8 Hz, H1'), 7.93 (1H, d, *J* 9.2 Hz, H4'), 8.01 (1H, d, *J* 2.4 Hz, H5') and 8.11 (1H, d, *J* 9.6 Hz, H8'); δ_C (100 MHz, $CDCl_3$) 31.8, 35.9, 43.5, 45.4, 55.1, 99.6, 117.3, 122.8, 123.4, 124.1, 124.6, 125.1, 126.15, 126.8, 126.8, 126.9, 126.95, 127.9, 129.9, 133.9, 139. 6, 147.85, 150.6, 155.3 and 159.5; HRMS (EI) *m/z* 448.16687 $(M)^+$, $C_{25}H_{25}ClN_4O_2$ requires 448.16660, Anal. Calc for $C_{25}H_{25}ClN_4O_2.1.5H_2O$; C, 63.1%; H, 5.3%; N, 11.8%, Found C, 63.5%; H, 5.2%; N, 11.6%.

1-Benzyl-3- [4-(6'-chloro-2'-methoxy-acridin-9'-ylamino)-butyl] urea (61). - (0.14 g, 53%) as yellow crystals, mp 142-144°C (from MeOH); R_f 0.43 (MeOH: CH_2Cl_2, 1:9);

ν_{max} (CH_2Cl_2)/cm^{-1} 3730(N-H), 3090 (CONH) and 1598 (CO); δ_H (400 MHz, $CDCl_3$) 1.57 (2H, t, *J* 6.4 Hz, ArNH$(CH_2)_2CH_2CH_2$NHCO-), 1.84 (2H, t, *J* 9.6 Hz, ArNHCH$_2CH_2$-), 3.16 (2H, t, *J* 7.2 Hz, ArNH$(CH_2)_3CH_2$NH-), 3.84 (2H, t, *J* 7.2 Hz, ArNH$CH_2(CH_2)_3$NH-), 3.92 (3H, s, OCH_3), 4.26 (2H, s, -NHCH_2Ar), 7.15-7.25 (6H, m), 7.36-7.48 (2H, m), 7.77 (2H, m) and 8.20 (H, d, *J* 9.2 Hz); δ_C (100 MHz, $CDCl_3$) 27.7, 28.0, 39.4, 43.5, 49.4, 55.2, 97.9 100.6, 114.0, 116.9, 123.2, 124.0, 125.4, 126.2, 126.7, 126.8, 126.9, 127.3, 128.2, 130.0, 138.7, 153.1, 140.2, 144.3, 156.2 and 160.0; HRMS (EI) *m/z* 462.18569 $(M)^+$, $C_{26}H_{27}ClN_4O_2$ requires 462.182254; Anal. Calc for $C_{26}H_{27}ClN_4O_2.H_2O$; C, 64.9%; H, 5.6%; N, 11.7%, Found: C, 64.7%; H, 5.5%; N, 11.7%.

Naphthalne-2-sulfonic acid [3-(6'-chloro-2'-methoxy-acridin-9'-ylamino)-propyl]-amide (62). - (0.146 g, 73%); mp 115-117°C (from MeOH); R_f 0.61 (MeOH: CH_2Cl_2, 1:9); ν_{max} (CH_2Cl_2)/cm^{-1} 3730 (N-H), 3090 (N-H) and 1598 (C=O); δ_H (400 MHz, $CDCl_3$) 1.40-1.70 (8H, m, 4 x CH_2), 3.13 (2H, m, ArNH$(CH_2)_5CH_2$NHCO-), 3.65 (2H, t, *J* 7.2 Hz, ArNHCH_2-), 3.93 (3H, s, OCH_3), 4.34 (2H, d, *J* 5.6 Hz, -NHCH_2Ar), 4.77 (br t, -*NH*CONH-), 5.14 (br t, -NHCO*NH*-), 7.23-7.38 (8H, m), 7.93-8.01 (3H, m); δ_C (100 MHz, $CDCl_3$) 26.1, 26.2, 29.7, 39.1, 31.4, 39.9, 44.5, 50.0, 55.6, 99.7, 115.3, 117.6, 124.3, 124.4, 124.6, 127.2, 127.3, 127.4, 128.6, 130.6, 130.9, 135.2, 139.4, 145.6, 145.9, 147.8, 150.2, 156.0 and 158.5; HRMS (EI) 490.21404 $(M)^+$, $C_{28}H_{31}ClN_4O_2$ requires 490.213554; Anal. Calc for $C_{28}H_{31}ClN_4O_2.3/4H_2O$; C, 66.7%; H, 6.2%; N, 11.1%, Found C, 66.7%; H, 6.4%; N, 10.9%.



General procedure for preparation of amines 63-66 - Diamine (0.67mmol) was added to a solution of benzaldehyde (1.01mmol) in methanol (3.5cm^3/mmol). The reaction vessel was sealed and then shaken for 2-3 h at 20°C[96]. The resultant solution was treated with Amberlite IRA 400 borohydride resin (2.5mml BH_4^-/g resin). This slurry was

then shaken at 20°C for an additional 24 h. The polymer beads were removed by filtration and washed with several aliquots of methanol. Column chromatography (SiO_2, CH_2Cl_2: MeOH: Et_3N, 8:2:0.1) yielded the desired product.

Diamines used: **92-95**

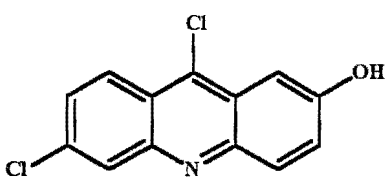
N-Benzyl-N'- (6''-chloro-2''-methoxy-acridin-9''-yl)-ethane-1, 2-diamine (63). - (0.122 g, 61%) as yellow crystals, mp 109-111°C; R_f 0.6 (CH_2Cl_2: MeOH: Et_3N, 8:2:0.1) 2; IR (Nujol): ν_{max} 3311 (N-H) and 1557 (C=C); δ_H (400 MHz, $CDCl_3$) 2.96 (2H, t, *J* 5.6 Hz, ArNHCH$_2$*CH$_2$*NH-), 3.74 (2H, t, *J* 6.0 Hz, ArNH*CH$_2$*-), 3.86 (5H, s, O*CH$_3$* and ArNH(CH$_2$)$_2$NH*CH$_2$*Ar), 7.21-7.41 (8H, m) and 7.98 (H, d, *J* 9.2 Hz, H8''), 8.05 (H, d, *J* 2.4 Hz, H1'') and 8.08 (H, d, *J* 9.2 Hz, H4''); δ_C (50 MHz, $CDCl_3$) 29.7, 48.9, 49.0, 53.4, 55.4, 99.4, 114.7, 116.0, 118.1, 124.2, 124.5, 124.5, 127.3, 128.1, 128.6, 130.7, 131.3, 134.7, 139.9, 146.5, 146.7, 148.4, 150.2 and 155.9; HRMS (EI) *m/z* 391.14523 $(M)^+$, $C_{23}H_{22}ClN_3O$ requires 391.14514; Anal. Calc for $C_{23}H_{22}ClN_3O.0.5H_2O$; C, 69.0%, H, 5.5%; N, 10.1%, Found: C, 69.4%; H, 5.2%; N, 10.4%.

N-Benzyl-N'- (6''-chloro-2''-methoxy-acridin-9''-yl)-propane-1, 3-diamine (64). - (0.131 g, 70%) as an orange gum; R_f 0.45 (CH_2Cl_2: MeOH: Et_3N, 8:2:0.1); IR (Nujol): ν_{max}/cm^{-1} 3310.6 (N-H) and 1556.8 (C=C); δ_H (200 MHz, $CDCl_3$) 1.90 (2H, quin, *J* 4 x 7.58 Hz, ArNHCH$_2$*CH$_2$*-), 2.98 (2H, t, *J* 5.4 Hz, ArNH(CH$_2$)$_2$*CH$_2$*-), 3.79 (2H, s, ArNH(CH$_2$)$_3$NH*CH$_2$*Ar), 3.88 (3H, s, O*CH$_3$*), 3.93 (2H, t, *J* 5.8 Hz, ArNH*CH$_2$*-), 7.18 (H, dd, *J* 2.2 and 11.4 Hz), 7.27-7.4 (7H, m), 7.93-8.02 (3H, m); δ_C(50 MHz, $CDCl_3$) 30.2, 48.9, 51.0, 54.5, 55.3, 100.4, 115.0, 116.9, 123.5, 124.0, 124.7, 126.8, 127.4, 127,9, 128.1, 128.2, 128.5, 128.7, 131.2, 134.6, 139.5, 146.8, 148.6, 150.5 and 155.3; Calc for $C_{24}H_{24}ClN_3O$; *m/z* 405.16079. Found HRMS (EI) 405.16214 $(M)^+$.

N-Benzyl-N'- (6''-chloro-2''-methoxy-acridin-9''-yl)-butane-1, 4-diamine (65). - (0.119 g, 60%) as an orange oil/gum; R_f 0.48 (8:2:0.1 CH_2Cl_2: MeOH: Et_3N, 0.48); IR (Nujol): ν_{max}/cm^{-1} 3312 (N-H) and 1557(C=C); δ_H (200 MHz, $CDCl_3$) 1.65 (2H, q, *J* 3 x 7.12 Hz, ArNHCH$_2$*CH$_2$*), 1.82 (2H, m, ArNH(CH$_2$)$_2$*CH$_2$*-), 2.69 (2H, t, *J* 6.8 Hz, ArNH*CH$_2$*(CH$_2$)$_3$), 3.77 (2H, s, ArNH(CH$_2$)$_4$NH*CH$_2$*Ar), 3.91 (3H, s, O*CH$_3$*), 7.20-7.43(8H, m), 7.96-8.1 (3H, m); δ_C (50 MHz, $CDCl_3$) 27.5, 29.4, 48.6, 49.2, 50.6, 54.0, 55.5, 99.5, 115.8, 117.8, 124.1, 124.3, 126.9, 127.1, 128.1, 128.2, 128.4, 128.5, 131.4,

134.7, 140.1, 146.8, 148.4, 149.9 and 155.8; Calc for $C_{25}H_{26}ClN_3O$; *m/z* 419.17644. Found HRMS (EI) *m/z* 419.17652 $(M)^+$.

N-Benzyl-N'- (6''-chloro-2''-methoxy-acridin-9''-yl)-hexane-1, 6-diamine (66). - (0.0951 g, 48%) as a yellow-orange oil/gum, R_f 0.49 (CH_2Cl_2: MeOH: Et_3N, 8:2:0.1); IR (Nujol): ν_{max} 3312 (N-H) and 1557 (C=C); δ_H (200 MHz, $CDCl_3$) 1.25-1.77 (8H, m, 4 x CH_2), 2.59 (2H, m, ArNH$(CH_2)_5CH_2$-), 3.67 (2H, t, *J* 7.2 Hz, ArNHCH_2-), 3.75 (2H, d, *J* 3 Hz, ArNH$(CH_2)_6$NHCH_2Ar), 3.94 (3H, s, OCH_3), 7.18-7.44 (8H, m), 7.96-8.1 (3H, m); δ_C (50 MHz, $CDCl_3$) 26.8, 27.0, 30.0, 31.7, 49.4, 54.1, 55.5, 99.2, 115.9, 117.9, 123.9, 124.0, 124.4, 124.5, 124.6, 126.8, 126.9, 128.1, 128.2, 128.4, 131.5, 134.8, 140.4, 146.7, 148.3, 149.7 and 156.0; Calc for $C_{27}H_{30}ClN_3O$; *m/z* 447.20774. Found HRMS (EI) *m/z* 447.20749 $(M)^+$.



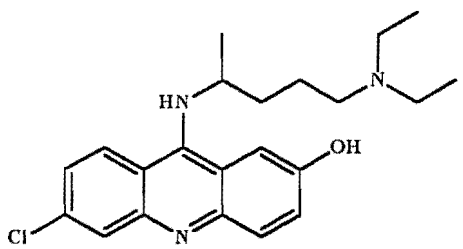
6, 9 Dichloro-acridin-2-ol (97).

Method (a)

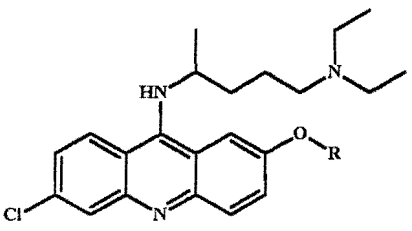
Pyridine hydrochloride (2.1 g. 18 mmol) and 6, 9-dichloro-2-methoxyacridine (1 g, 3.60 mmol) were heated at 210°C for 40 min[79]. The reaction mixture was allowed to cool to room temperature and then dissolved in ethyl acetate and diluted with water. The organic material was extracted with the ethyl acetate. (4 x 70 cm^3). The combined organic extracts were then washed with aqueous sodium chloride solution (1M, 75cm^3), dried over anhydrous sodium sulfate, filtered and concentrated. This yielded the *alcohol* **98** (0.24 g, 25 %) as lime green crystals; mp 142-145°C (from MeOH); R_f 0.48 (MeOH: CH_2Cl_2, 1:19); IR (MeOH): ν_{max}/cm^{-1} 3618 (OH), 3029 (C=C), 1642 (C=C) and 1297 (OH); δ_H (300 MHz, DMSO-d_6) 7.18 (H, dd, *J* 2.0 and 8.4 Hz, H3), 7.31 (H, dd, *J* 3.0 and 9.0 Hz, H7), 7.45 (H, d, *J* 9 Hz, H8), 7.52 (H, d, *J* 2.0 Hz, H1), 7.73 (H, d, *J* 3 Hz, H5) and 8.29 (H, d, *J* 8.7 Hz, H4); δ_H (75 MHz, DMSO-d_6) 101.2, 110.5, 117.6, 120.3, 122.4, 125.5, 130.1 138.6, 139.9,142.8, 143.0, 148.7 and 154.17; Anal. Calc for $C_{13}H_7Cl_2NO$; C, 59.1%; H, 2.7%; N, 5.3%; *m/z* 262.9905. Found C, 60.5%; H, 3.0%; N, 5.2%; HRMS (EI) *m/z* 245 $(M-H_2O)$.

Method (b)

Boron tribromide (1.5 cm^3, 1.53 mmol) is slowly added to a solution of 6, 9dichloro-2-methoxyacridine(0.15 g, 0.51 mmol) and dichloromethane (4cm^3) previously cooled to-89°C (dry ice/propan-2-ol bath)[97]. The mixture is stirred for 1 h, the cold bath is then removed and stirring continued at room temperature for 18 h. The mixture is poured into ice-water (12 cm^3) and stirred for 1 h resulting in the formation of a yellow precipitate. The mixture was filtered and the precipitate dried *in vacuo* affording the *alcohol* (0.27mg, 20%);



6-Chloro-9- (4'- diethylamino - 1' - methyl - butylamino) – acridin – 2 - ol (98). - Boron tribromide (6.3 cm^3, 6.25 mmol) is slowly added to a solution of quinacrine (0.5 g, 1.25 mmol) and dichloromethane (12 cm^3) previously cooled to-89°C (dry ice/propan-2-ol bath)[97]. The mixture is stirred for 1 h, the cold bath is then removed and stirring continued at room temperature for 18 h. The mixture is poured into ice-water (18 cm^3) and stirred for 1 h resulting in the formation of a yellow precipitate. The mixture was filtered and the precipitate dried *in vacuo* affording the *alcohol* **152** (0.35 g, 70%); as yellow crystals; mp 270-272°C (from CH_2Cl_2); R_f 0.28 (NH_4OH: MeOH, 1:4); IR ($CHCl_3$): ν_{max}/cm^{-1} 3562 (OH), 3256 (N-H), 3031 (C=C) and 1535 C=C); δ_H (300 MHz, $CDCl_3$) 097 (6H, t, *J* 6.9 Hz, -N(CH_2CH_3)$_2$), 1.02 (3H, d, *J* 6.0 Hz, ArNHCHCH_3), 1.54 (4H, m, 2 x CH_2), 2.34 (2H, br t, *J* 5.1 Hz -CH(CH_2)$_2CH_2$), 2.51 (4H, m, 2 x CH_2), 3.92 (H, q, *J* 6 Hz, *CH*), 7.24 (H, dd, *J* 1.8 and 9.3 Hz, H3'), 7.35 (H, d, *J* 2.1 Hz, H5'), 7.42 (H, dd, *J* 2.1 and 9.3 Hz, H7'), 7.91 (H, d, *J* 9.3 Hz, H4'), 7.99 (H, d, *J* 9.3 Hz, H8') and 8.08 (H, d, *J* 1.8 Hz, H1'); δ_H (75 MHz, $CDCl_3$) 10.7 (2 x CH_3), 22.0, 23.4, 30.8, 36.4, 46.4, 52.6, 55.4, 103.0, 116.7, 120.0, 124.2, 124.7, 126.1, 127.3, 130.5, 134.8, 145.4, 147.3, 149.1 and 155.0; Anal. Calc for $C_{22}H_{28}ClN_3O$; C, 68.5%; H, 7.3%; N, 10.9%; M 385.1921. Found C, 68.3%; H, 7.3%; N, 10.8%; M^+ 385 (M^+, 8%), 311 (3), 271 (2), 140 (4), 126 (22), 112 (7), 99 (9), 91 (14), 86 (100), 72 (3), 58 (14), 41 (7) and 30 (14).



General procedure for preparation of ethers 70-73 - The alcohol **98** (0.17 mmol) and alkyl bromide (0.14 mmol) were added to a reaction vessel containing 1, 5, 7-triazabicyclo [4.4.0] dec-5-ene (PTBD) resin (0.35mmol) in acetonitrile (8.4 cm^3/mmol) as solvent[83]. The reaction mixture was shaken vigorously at 24°C for 24 h. The polymer beads were removed by filtration and washed with several aliquots of methanol. The filtrate was concentrated under reduced pressure yielding the desired *ether.*

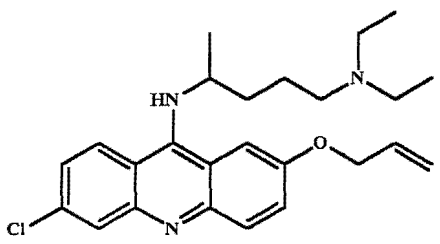
N^4- (6'-Chloro-2'-ethoxy-acridin-9'-yl)-N^1, N^1-diethyl-pentane-1, 4-diamine (70). - (0.056 g, 78%) as a brown gum; R_f 0.42 (NH_4OH: MeOH, 1:4); IR (MeOH): ν_{max}/cm^{-1} 3518 (N-H), 3031 (C=C), 1559 (C=C) and 1297 (C-O-C); δ_H (400 MHz, $CDCl_3$) 0.9 (6H, t, *J* 7.2 Hz, -N (CH_2CH_3)$_2$), 1.19 (3H, d, *J* 6.8 Hz, ArNHCHCH_3), 1.45 (3H, t, *J* 6.8 Hz, ArOCH_2CH_3), 1.57 (4H, m, 2 x CH_2), 2.31 (2H, t, *J* 7.6 Hz, -$CH(CH_2)_2CH_2$-), 2.39 (4H, q, *J* 5.4 Hz, -N(CH_2CH_3)$_2$), 3.95 (H, br m, *CH*), 4.11 (2H, q, *J* 6.9 Hz, ArOCH_2CH_3), 7.15 (H, d, *J* 2.8 Hz, H5'), 7.24 (H, dd, *J* 2.0 and 9.2 Hz, H3'), 7.35 (H, d, *J* 2.8 and 9.2 Hz, H7'), 7.92 (H, d, *J* 9.2 Hz, H4'), 7.93 (H, d, *J* 9.2 Hz, H8') and 8.01 (H, d, *J* 2 Hz, H1'); δ_H (100 MHz, $CDCl_3$) 11.5 (2 x CH_3), 14.74, 22.21, 23.90, 29.8, 36.93, 46.81, 52.76, 55.91, 63.82, 100.14, 117.8, 119.9, 123.89, 124.86, 124.92, 128.39, 131.54, 134.7, 146.8, 148.2, 149.5 and 158.00; Anal Calc for $C_{24}H_{32}ClN_3O$; M 413.2234; Found M^+ 413 (M^+, 11%), 384(2), (11) and 86 (100).

N^4- (6'-Chloro-2'-propoxy-acridin-9'-yl)-N^1, N^1-diethyl-pentane-1, 4-diamine (71). - (0.082 g, 74%) as a brown gum; R_f 0.39 (NH_4OH: MeOH, 1:4); IR (MeOH): ν_{max}/cm^{-1} 3518 (N-H), 3031 (C=C), 1559 (C=C) and 1297 (C-O-C); δ_H (300 MHz, $CDCl_3$) 0.96 (6H, t, *J* 7.2 Hz, -N (CH_2CH_3)$_2$), 1.13 (3H, t, *J* 7.2 Hz, Ar$O(CH_2)_2CH_3$), 1.45 (3H, d, *J* 6.3 Hz, ArNHCHCH_3), 1.57 (4H, m, 2 x CH_2), 2.39 (2H, t, *J* 7.6 Hz, -$CH(CH_2)_2CH_2$-), 2.47 (4H, m, -N(CH_2CH_3)$_2$), 4.07 (2H, t, *J* 6.3 Hz, ArOCH_2CH_3), 4.22 (H, br m, *CH*), 7.19 (H, d, *J* 2.8 Hz, H5'), 7.26-7.34 (2H, m), 7.42 (H, dd, *J* 2.58 and 9.4 Hz), 8.01 (H,

d, *J* 9.3 Hz) and 8.09 (H, d, *J* 2.1 Hz); δ_H (100 MHz, $CDCl_3$) 10.6, 11.4, 22.2, 22.6, 23.9, 36.9, 46.85, 51.8, 52.8, 55.9, 63.7, 69.86, 100.1, 117.2, 119.5, 124.0, 124.9, 128.4, 131.5, 131.6, 134.7, 146.9, 148.7, 149.2 and 155.7; Anal. Calc for $C_{25}H_{34}ClN_3O$; *m/z* 427.23904. Found HRMS (EI) *m/z* 427.24015 $(M)^+$.

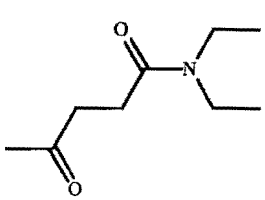
N^4- (2'-butoxy-6'-chloro-acridin-9'-yl)-N^1, N^1-diethyl-pentane-1, 4-diamine (72). - (0.09 g, 74%) as a brown gum; R_f 0.43 (NH_4OH: MeOH, 1:4); IR (MeOH): ν_{max}/cm^{-1} 3518 (N-H), 3031 (C=C), 1559 (C=C) and 1297 (C-O-C); δ_H (400 MHz, $CDCl_3$) 0.86 (6H, t, *J* 7.2 Hz, -N $(CH_2CH_3)_2$), 0.93 (3H, t, *J* 7.2 Hz, $ArO(CH_2)_3CH_3$), 1.17 (3H, d, *J* 6.4 Hz, $ArNHCHCH_3$), 1.57 (6H, m, 3 x CH_2), 1.78 (2H, m, $ArO(CH_2)_2CH_2CH_3$), 2.38 (2H, t, *J* 7.6 Hz, $-CH(CH_2)_2CH_2-$), 2.41 (4H, m, Hz, $-N(CH_2CH_3)_2$), 3.98 (H, br m, *CH*), 4.07 (2H, t, *J* 6.3 Hz, $ArOCH_2CH_3$), 7.15 (H, d, *J* 2.8 Hz, H5'), 7.26-7.35 (2H, m), 7.38 (H, dd, *J* 2.58 and 9.4 Hz), 7.98 (H, d, *J* 9.3 Hz) and 8.01 (H, d, *J* 2.1 Hz); δ_H (100 MHz, $CDCl_3$) 11.7, 11.8, 14.1, 19.6, 22.5, 24.2, 31.5, 37.2, 47.1, 50.9, 53.0, 56.2, 68.3, 100.3, 117.6, 119.7, 124.1, 125.2, 128.6, 131.7, 134.9, 146.9, 147.1, 148.4, 149.4 and 156.0; Anal. Calc for $C_{26}H_{36}ClN_3O$; *m/z* 441.25469. Found MS (FAB) *m/z* 441.3 $(M+1)^+$.

N^4- (6'-Chloro-2'-pentyloxy-acridin-9'-yl)-N^1, N^1-diethyl-pentane-1, 4-diamine (73). - (0.05 g, 45%) as a brown gum; R_f 0.44 (NH_4OH: MeOH, 1:4); IR (MeOH): ν_{max}/cm^{-1} 3518 (N-H), 3031 (C=C), 1559 (C=C) and 1297 (C-O-C); δ_H (300 MHz, $CDCl_3$) 0.96 (9H, superimposed t + t, -N $(CH_2CH_3)_2$ + $ArO(CH_2)_4CH_3$), 1.26 (3H, d, *J* 6.0 Hz, $ArNHCHCH_3$), 1.42 (8H, m, 4 x CH_2), 1.83 (2H, m, $ArO(CH_2)_2CH_2CH_3$), 2.41 (6H, m, $-CH(CH_2)_2CH_2-$ + $-N(CH_2CH_3)_2$), 4.01 (H, br m, *CH*), 4.10 (2H, t, *J* 6.3 Hz, $ArOCH_2CH_3$), 7.15 (H, d, *J* 2.6 Hz, H5'), 7.26-7.35 (2H, m), 7.38 (H, dd, *J* 2.3 and 9.3 Hz), 7.98 (H, d, *J* 9.3 Hz) and 8.01 (H, d, *J* 1.9 Hz); δ_H (100 MHz, $CDCl_3$) 11.5 (2 x CH_3), 14.1, 22.3, 22.5, 24.0, 28.3, 28.9, 36.9, 46.8, 50.8, 52.8, 55.9, 68.4, 100.1, 117.3, 119.5, 123.9, 124.9, 125.0, 128.4, 131.5, 134.7, 147.8, 148.2, 149.2 and 155.8; Calc for $C_{27}H_{38}ClN_3O$; *m/z* 455.27034. Found HRMS (EI) *m/z* 455.27059$(M)^+$.



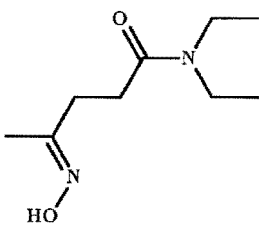
N^4- (2-Allyloxy-6-chloro-acrdin-9-yl)-N^1, N^1-diethyl-pentane-1, 4-diamine (99). - Allyl Bromide (0.035cm3, 0.41mmol) was added to a solution of alcohol **97** (0.1 g, 0.38 mmol) in *N, N'* Dimethylformamide (4.76 cm3), followed by potassium carbonate (0.52 g, 3.8 mmol). The reaction mixture was stirred at 18°C for 20 h. The reaction mixture was then diluted with water (20 cm^3) and the organic product extracted with ethyl acetate (3 x 30cm^3). The combined organic extracts were washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated to yield the ether. This was carried forward to the next step without purification. Ether **100** (0.04 g, 0.11 mmol) was reacted with amine **101** (0.09 g, 0.36 mmol) and stirred under nitrogen in the presence of *N*-methyl-2-pyrrolidinone (0.78 cm^3)[98], triethylamine (0.023 cm^3, 0.17 mmol) and potassium carbonate (0.005 g, 0.34 mmol) at 135-145°C. The reaction is stopped and cooled to room temperature. The product was washed with dichloromethane and brine before being separated. The organic extracts were dried over anhydrous sodium sulfate, filtered and concentrated yielding the desired *ether* **99** (0.029g, 61%) as a yellow gum; R_f (5% MeOH: CH_2Cl_2) 0.74;IR (MeOH): ν_{max}/cm^{-1}; δ_H (200MHz, $CDCl_3$) 0.85 (6H, superimposed t+t, 2 x CH_3), 1.26 (3H, d, *J* 8 Hz, ArNHCHCH_3), 1.57 (4H, m, 2 x CH_2), 2.36 (6H, m, 3 x -CH_2N(CH_2CH_3)$_2$), 4.67 (2H, dd, *J* 1.3 and 5.3 Hz, ArOCH_2-), 5.32 (H, d, *J* 10.7 Hz, ArOCH$_2$*CH*-), 5.47 (H, dd, *J* 1.6 and 18.8 Hz, ArOCH$_2$*CH*-), 5.95-6.02 (2H, m, ArOCH$_2$CHCH_2), 7.16 (2H, dd, *J* 1.6 and 8.4 Hz), 7.31 (2H, m), 7.88 (H, d, *J* 3.6 Hz) and 8.42 (H, d, *J* 8.8 Hz); δ_C (50 MHz, $CDCl_3$) 13.73, 21.60, 25.22, 30.85,, 49.45, 69.30, 108.01, 114.65, 116.99, 117.77, 118.04, 121.86, 125.08, 129.43, 131.01, 134.97, 148.12, 148.56 and 156.83; Calc for $C_{25}H_{32}ClN_3O$ 425.22339; HRMS (EI) *m/z* 125.22121 $(M)^+$.

4-Oxo-pentanoic acid diethylamide (104). - Levulinic acid (1.1 g, 9.47 mmol) **102**



was dissolved in dry CH_2Cl_2 (5.5 cm^3) under nitrogen and the solution cooled to 0°C. Oxalyl chloride (1.24 cm^3, 14.21 mmol) was added to the mixture which was stirred at 0°C for 3 h. The solvent was removed under reduced pressure and the residue dissolved in CH_2Cl_2 (3cm^3). The mixture was cooled to 0°C and a solution of

diethylamine (1.96cm^3, 1.9mmol) in CH_2Cl_2 (2 cm^3) was slowly added to the solution via a dropping funnel. The mixture was then allowed to warm to 20°C and was stirred for 1 h. Column chromatography (SiO_2, MeOH: CH_2Cl_2, 1:19) yielded the desired compound **104** (1.1 g, 65%) as a pale yellow oil. R_f 0.55 (MeOH: CH_2Cl_2, 1:19); IR ($CHCl_3$) ν_{max}/cm^{-1} 1710 (C=O of amide), 1624 (C=O of ketone); δ_H (400 MHz, $CDCl_3$) 1.09 (3H, t, *J* 7.1 Hz, CONH*CH*$_3$), 1.19 (3H, t, *J* 7.1 Hz, CONCH$_2$*CH*$_3$), 2.20 (3H, s, *CH*$_3$COC-), 2.58 (2H, t, *J* 6.4 Hz, -*CH*$_2$CH$_2$CON), 2.78 (2H, t, *J* 6.8 Hz, -CH$_2$*CH*$_2$CON), 3.34 (4H, m, CO (*CH*$_2$CH$_3$)$_2$), δ_C (100MHz, $CDCl_3$) 13.0, 14.1, 27.0, 30.1, 38.2, 40.3, 41.8, 170.6, 208.0; Calc for $C_9H_{17}NO_2$; *m/z* 171.1259. Found HRMS (EI) *m/z* 171.1245 $(M)^+$.



4-Hydroxyimino-pentanoic acid diethylamide (103).

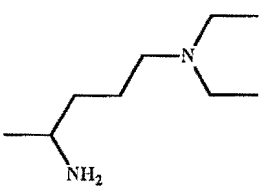
Method (a): The ketone **104** (1.1 g, 6.2 mmol) and hydroxylamine hydrochloride (0.69 g, 9.9 mmol) were dissolved in ethanol (30 cm^3)[99]. Sodium hydroxide (0.79 g, 20.32 mmol) in water (5.1 cm^3) was added dropwise to the ethanol solution with stirring. The solution was refluxed with stirring for 2 h and quenched with water (30 cm^3). The resulting solution was neutralized by treatment with CO_2 gas and extracted with dichloromethane (2 x 30 cm^3). The combined organic fractions were dried over sodium sulfate, filtered and concentrated. The compound was purified by column chromatography (SiO_2, MeOH: CH_2Cl_2, 1:19) to give the oxime **103** (0.98 g, 86%) as clear oil.

R_f 0.50 (MeOH: CH_2Cl_2, 1:19); IR ($CHCl_3$) ν_{max}/cm^{-1} 3665, 3589, 3294 (OH), 1728 (C=O); δ_H (400 MHz, $CDCl_3$) 1.24 (6H, t, *J* 6.8 Hz, -N(CH$_2$*CH*$_3$)$_2$), 1.86 (3H, s, *CH*$_3$-C=N-), 2.52 (2H, m, -*CH*$_2$-CON-), 4.13 (4H, quartet, *J* 7.2 Hz, -N(*CH*$_2$CH$_3$)$_2$); δ_C (100MHz, $CDCl_3$) 13.8, 14.1, 24.2, 30.9, 44.7, 156.5, 172.7; HRMS (EI) *m/z* 186.1373 $(M)^+$ ($C_9H_{18}N_2O_2$ requires 186.1368).

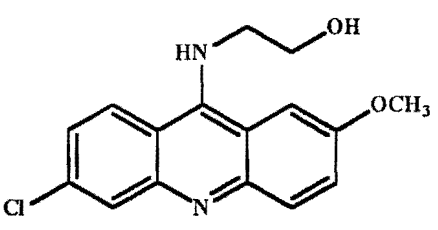
Method (b):

Pyridine (0.26 cm^3, 2.9 mmol) and hydroxylamine hydrochloride (0.22 g, 3.19 mmol) was added to a solution of ketone **104** (0.5 g, 2.9 mmol) in ethanol (14.5 cm^3). The

resulting solution was refluxed for 30 min with stirring. On completion of the reaction, the solvent was removed under reduced pressure. The residue was diluted with water and the organic product extracted with ethyl acetate (2 x 30 cm^3), dried over anhydrous sodium sulfate, filtered and concentrated to give the oxime **103** (0.29 g, 59%) as a clear oil.



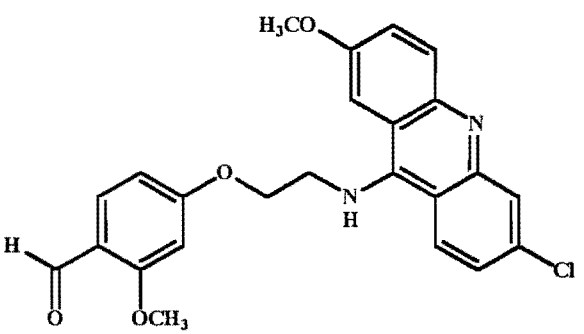
N^1, N^1- Diethyl-pentane-1, 4-diamine (101). - Lithium aluminium hydride (0.31 g, 8.06 mmol) was suspended in dry tetrahydrofuran (5cm^3) under nitrogen and cooled to 0°C[99]. Oxime **103** was dissolved in dry tetrahydrofuran (3 cm^3) and this was slowly added to the LAH slurry. The solution was allowed to warm to ambient temperature and then heated under reflux for 6 h. After cooling; the reaction was quenched with excess diethyl ether. The solution was transferred to a separating funnel and washed with brine (20 cm^3). The organic layer was dried over potassium carbonate and concentrated under reduced pressure to afford the *amine* **101** (0.28 g, 67%) as pale yellow oil; R_f 0.60 (NH_4OH: MeOH, 1:19); IR ($CHCl_3$) ν_{max}/cm^{-1} 3406 (NH stretch); δ_H (300 MHz, $CDCl_3$) 1.00 (9H, m, -*CH*$_3$-CH- and –N(CH_2*CH*$_3$)$_2$), 1.28 (2H, quin, *J* 4 x 7.7 Hz, -C-*CH*$_2$-C-), 1.44 (2H, q, *J* 3 x 7.1 Hz, -CH-*CH*$_2$-) 1.60 (2H, br s, NH_2- disappears in D_2O), 2.37 (2H, t, *J* 7.3 Hz, -*CH*$_2$N(CH_2CH_3)$_2$), 2.49 (4H, q, *J* 3 x 7.1 Hz, -N(*CH*$_2$$CH_3$)$_2$), 2.86 (H, sextet, *J* 6.2 Hz, -*CH*-); δ_C (75 MHz, $CDCl_3$) 11.5, 23.9, 30.7, 38.1, 46.9, 52.5 and 53.0; Anal. Calc for $C_9H_{22}N_2$; *m/z* 158.1783. Found HRMS (EI) *m/z* 158.1791 $(M)^+$.



2-(6'-Chloro-2'-methoxy-acridin-9'-ylamino)-ethanol (105).-

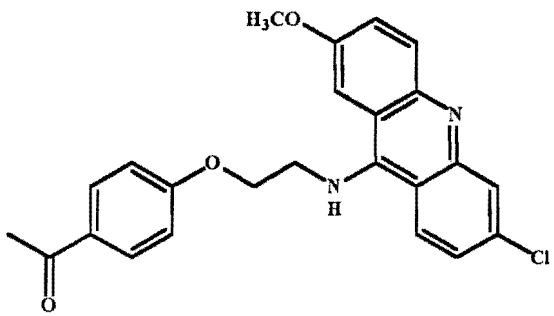
A mixture of 6,9-dichloro-2-methoxyacridine (1.5 g, 5.4 mmol), ethanolamine (10.4 cm^3, 173 mmol) and phenol (4.2 g) was heated at 90°C for 4 h. The resulting yellow mixture was cooled before 210 cm^3 of water was added yielding a yellow precipitate. This was isolated by suction filtration and dried in a vacuum dessicator to yield the desired alcohol **105** (1.31

g, 80%) as yellow crystals, mp 190-193°C (from MeOH); IR (MeOH): ν_{max}/cm^{-1}3563 (N-H), 3548 (OH), 3050 (C=C), 1620 (C=N) and 1390 (OH); δ_H (300 MHz, DMSO-d_6); 3.75 (4H, dd, *J* 4.8 and 24.9 Hz, 2 x *CH*$_2$), 3.93 (3H, s, O*CH*$_3$), 6.71 (H, br s, Ar*NH*), 7.37 (2H, m, H3' and H7'), 7.66 (H, d, *J* 2.7 Hz, H5'), 7.86 (2H, m, H1' and H4') and 8.39 (H, d, *J* 9.0 Hz, H8'); δ_C (75 MHz, DMSO-d_6) 51.8, 55.6, 60.7, 100.7, 114.9, 117.3, 122.8, 124.2, 126.3, 127.2, 130.8, 133.4, 146.2, 148.1, 150.6 and 155.1; Anal. Calc for $C_{16}H_{15}ClN_2O_2$; C, 63.5%; H, 5.0%; N, 9.3%; *m/z* 302.08220. Found C, 63.0%; H 4.8%, N 9.0%; HRMS (EI) *m/z* 302.0822 $(M)^+$.



4-[2-(6'-Chloro-2'-methoxy-acridin-9'-ylamino)-ethoxy]-2-methoxy-benzaldehyde (68)-

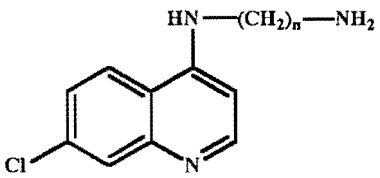
Diethylazodicarboxylate (0.26 cm^3, 1.65 mmol) was added dropwise to a stirred solution of triphenylphosphine (0.43 g, 1.65 mmol) in dichloromethane (7 cm^3) at 0°C. After 20 min, a solution of alcohol **67** (0.5 g, 1.65 mmol) and 4-hydroxy-2-methoxy-benzaldehyde (0.25g, 1.65mmol) in anhydrous dichloromethane (7 cm^3) was added slowly to the reaction mixture which was stirred at 0°C for 1 h. Thereafter, the solution was allowed to warm to room temperature and stirred for a further 23 h. Solvent was removed under reduced pressure and the residue chromatographed (SiO_2, MeOH: CH_2Cl_2. 1:19) to afford the *ether* **68** (0.36 g, 58%) as yellow crystalline material; mp 80-82°C (from EtOAC/pet ether); R_f 0.44 (MeOH: CH_2Cl_2, 1:19); IR (MeOH): ν_{max}/cm^{-1} 3517 (N-H), 3059 (C=C), 1712 (C=O), 1559 (C=C) and 1297 (C-O-C-); δ_H (300 MHz, $CDCl_3$) 3.75 (2H, t, *J* 9.0 Hz, ArNHCH$_2$*CH*$_2$-), 3.86 (6H, d, *J* 4.8 Hz, 2 x O*CH*$_3$), 4.27 (2H, m, ArNH*CH*$_2$CH$_2$) 6.39 (H, d, *J* 2.4 Hz), 6.54 (H, dd, *J* 1.8 and 8.7 Hz), 7.25-7.28 (2H, m), 7.33 (H, dd, *J* 1.8 and 7.2 Hz), 7.40 (H, dd, *J* 2.4 and 9.3 Hz), 7.80 (H, d, *J* 8.7 Hz), 8.00 (H, d, *J* 9.3 Hz), 8.10 (H, d, *J* 9.0 Hz) and 10.29 (H, s, CO*H*); δ_H (75 MHz, $CDCl_3$) 50.8, 55.7, 63.04, 67.7, 98.3, 98.9, 99.4, 99.92, 106.4, 107.3, 110.4, 124.2, 124.2, 125.3, 125.5, 127.7, 130.1, 130.9, 140.8, 141.0, 157.6, 159.5, 163.2 and 188.23; Anal. Calc for $C_{24}H_{21}ClN_2O_4$, C, 64.0%; H, 4.8%; N, 6.4%; *m/z* 436.1189 Found C, 64.3%; H, 5.1%; N 6.0%; HRMS (FAB) *m/z* 437.1 $(M+1)^+$.



1-{4-[2-(6'-Chloro-2'-methoxy-acridin-9'-ylamino)-ethoxy]-2-methoxyphenyl} ethanone (69).-

Diethylazodicarboxylate (0.26 cm^3, 1.65 mmol) was added dropwise to a stirred solution of triphenylphosphine (0.43 g, 1.65 mmol) in dichloromethane (7 cm^3) at 0°C. After 20 min, a solution of alcohol **67** (0.5 g, 1.65 mmol) and 4-Hydroxyacetophenone (0.22 g, 1.65 mmol) in anhydrous dichloromethane (7 cm^3) was added slowly to the reaction mixture which was stirred at 0°C for 1 h. Thereafter, the solution was allowed to warm to room temperature and stirred for a further 23 h. Solvent was removed under reduced pressure and the residue chromatographed (SiO_2, MeOH: CH_2Cl_2, 1:19) to afford the *ether* **69** (0.18 g, 26%) as a yellow gum; R_f 0.35 (MeOH: CH_2Cl_2, 1:19); IR (MeOH): ν_{max}/cm^{-1} 3517 (N-H), 3059 (C=C), 1712 (C=O), 1559 (C=C) and 1297 (C-O-C-); δ_H (300 MHz, $CDCl_3$) 2.55 (3H, s, *CH_3*), 4.05 (2H, t, *J* 5.1 Hz, ArNH*CH_2*CH_2), 4.12 (2H, t, *J* 4.8 Hz, ArNHCH$_2$*CH_2*), 6.92 (H, d, *J* 9 Hz), 7.25-7.69 (5H, m), 7.92 (H, d, *J* 9.0 Hz), 7.99 (H, d, *J* 9.3 Hz) and 8.6-8.09 (2H, m); δ_H (75 MHz, $CDCl_3$) 14.4, 26.3, 55.4, 67.4, 98.7, 114.2, 124.1, 125.2, 125.4, 127.7, 127.9, 128.4, 128.6, 130.7, 131.1, 131.7, 131.9, 131.98, 132.00, 132.1, 133.1, 156.7, 162.1 and 196.6; Anal. Calc for $C_{24}H_{21}ClN_2O_3$; *m/z* 420.12407; Found HRMS (EI) *m/z* 420.12424 $(M)^+$.

6.3 PROCEDURES FOR QUINOLINE ANALOGUES



General procedure for preparation of quinoline amines 107-110 - A mixture of 4,7-dichloroquinoline (2 g, 10.10 mmol) and the diaminoalkane (45.45 mmol) was heated at 80°C for 1 h with stirring [100]. Thereafter, at 135-145°C for 3 h. Sodium hydroxide (1M, 50 cm^3) was added and the organic product extracted with ethyl acetate (4 x 250 cm^3). The combined organic extracts were washed with water, dried over anhydrous sodium sulfate and concentrated to yield the amine.

Diaminoalkanes used: 1, 3 diaminopropane, 1, 4 diaminobutane, 1, 6 diaminohexane and 1, 3 diaminoethane

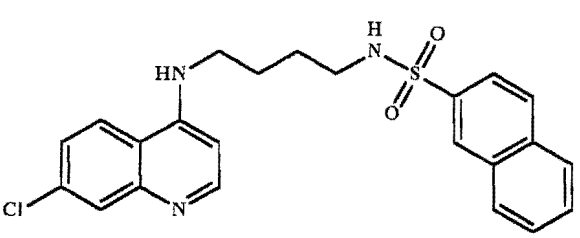
N^1- (7'-Chloro-quinolin-4'-yl)-propane-1, 3-diamine (107). - (1.8 g, 91%) as white crystals, mp 124-127°C (from MeOH), R_f 0.34 (NH_4OH: MeOH, 1:19); IR (Nujol): ν_{max}/cm^{-1} 3518 (NH_2), 3437 (NH_2), 3029 (C=C), 1612 (NH_2), 1582 (C=C and C=N); δ_H (300MHz, $CDCl_3$) 1.87 (2H, m, ArNHCH$_2$*CH$_2$*-), 3.02 (2H, t, *J* 5.7 Hz, ArNH(CH_2)$_2$*CH$_2$*NH_2), 3.39 (2H, m, ArNH*CH$_2$*-), 6.33 (H, d, *J* 5.4 Hz, H3'), 7.27(H, d, *J* 2.4 and 9.0 Hz, H5'), 7.69(H, d, *J* 9.0 Hz, H6'), 7.91 (H, d, *J* 2.4 Hz, H8'), 8.48 (H, d, *J* 5.4 Hz, H2'); δ_C ($CDCl_3$) 30.1, 41.4, 43.6, 98.3, 117.5, 121.9, 124.9, 128.6, 134.7, 149.2, 150.4 and 152.1; Anal. Calc for $C_{12}H_{14}ClN_3$; 61.15%; H, 6.0%; N, 17.8%; ***m/z*** 235.08763. Found C, 61.1%; H, 5.9%; N, 17.9%; HRMS (EI) ***m/z*** 235.08918 $(M)^+$.

N^1- (7'-Chloro-quinolin-4'-yl)-butane-1, 4-diamine (108). - (1.76 g, 89%) as a white powder; mp 45-48°C (from MeOH); R_f 0.88 (MeOH: CH_2Cl_2, 1:9); IR (Nujol): ν_{max}/cm^{-1} 3518 (NH_2), 3437 (NH_2), 3029 (C=C), 1612 (NH_2), 1582 (C=C and C=N); δ_H (300 MHz, $CDCl_3$) 1.62 (2H, m, ArNH(CH_2)$_2$*CH$_2$*-), 1.89 (2H, m, ArNHCH$_2$*CH$_2$*-), 3.04 (2H, t, *J* 6 Hz, ArNH(CH_2)$_3$*CH$_2$*-), 3.41 (2H, m, ArNH*CH$_2$*-), 6.32 (H d, *J* 5.7 Hz, H3'), 7.31 (H dd, *J* 2.4 and 9.0 Hz, H6'), 7.71 (H d, *J* 9.0 Hz, H5'), 7.92 (H, d, *J* 2.4 Hz, H8') and 8.49 (H, d, *J* 5.7 Hz, H2'); δ_C (75 MHz, $CDCl_3$) 30.0 (2 x CH_2), 41.4, 43.6, 98.3, 117.5, 121. 9, 124.9, 128.5, 134.6, 149.2, 150.4 and 152.1; Anal. Calc for $C_{13}H_{16}ClN_3$;

C, 62.5%; H, 6.4%; N, 16.8%; *m/z* 249.10327. Found C, 62.1%; H, 6.5%; N, 16.2%; HRMS (FAB) *m/z* 250.1 $(M+1)^+$.

N^1- (7'-Chloro-quinolin-4'-yl)-hexane-1, 6-diamine (109). - Column chromatography (SiO_2-NH_4OH: MeOH, 1:19) yielded the amine (1.73 g, 87%) as white crystals; mp 135-137°C (from MeOH) (lit[101] 135.8-136.8); R_f 0.88 (NH_4OH: MeOH, 1:9); IR (Nujol): ν_{max}/cm^{-1} 3518 (NH_2), 3437 (NH_2), 3029 (C=C), 1612 (NH_2), 15825 (C=C and C=N); δ_H (400 MHz, CD_3OD) 1.51 (6H, m, 3 x CH_2), 1.77 (2H, m, $ArNHCH_2CH_2$-), 2.67 (2H, t, *J* 7.2 Hz, $ArNH(CH_2)_5CH_2$-), 3.36 (2H, t, *J* 7.2 Hz, $ArNHCH_2$-), 6.50 (H, d, *J* 5.8 Hz, H3'), 7.38 (H, d, *J* 2.4 and 9.0 Hz, H6'), 7.77 (H, d, *J* 2.4 Hz, H8'), 8.01 (H, d, *J* 9.0 Hz, H5') and 8.34 (H d, *J* 5.8 Hz, H2'); δ_C (100 MHz, CD_3OD) 26.5, 26.7, 28.2, 31.8, 41.0, 42.7, 98.4, 117.6, 119.2, 123.1, 124.7, 126.4, 135.1, 148.6 and 151.3; Anal. Calc for $C_{15}H_{20}ClN_3$; C, 64.9%; H, 7.2%; N, 15.1%; *m/z* 277.13457. Found C, 64.7%; H, 7.5%; N, 15.6%; HRMS (FAB) *m/z* 278.2 $(M+1)^+$.

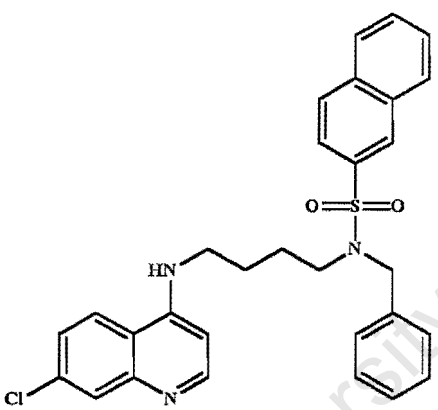
N^1- (7'-chloro-quinolin-4'-yl)-ethane-1, 2-diamine (110). - (1.71, 77%) as white crystals, mp 137-139°C (from MeOH)(lit[101]; 137-139°C) R_f 0.22 (MeOH-CH_2Cl_2, 1:9); IR (Nujol): ν_{max}/cm^{-1} 3518 (NH_2), 3437 (NH_2), 3029 (C=C), 1612 (NH_2), 1581.5 (C=C and C=N); δ_H (400 MHz, CD_3OD) 2.81 (2H, t, *J* 6.4 Hz, $ArNHCH_2CH_2NH_2$), 3.27 (2H, t, *J* 6.4 Hz, $ArNHCH_2CH_2NH_2$), 6.36 (H, d, *J* 5.6 Hz, H3'), 7.19 (H, dd, *J* 2.4 and 9.0 Hz, H6'), 7.57 (H, d, *J* 2.4 Hz, H8'), 7.91 (H, d, *J* 9.0, H5') and 8.12 (H, d, *J* 5.6 Hz, H2'); δ_C (100 MHz, CD_3OD) 39.5, 44. 6, 53.6, 98.6, 117. 7, 123.2, 124.9, 126.4, 135.3, 148.5 and 151.3; Anal. Calc for $C_{11}H_{12}ClN_3$; C, 59.6%; H, 5.5%; N, 17.9%; *m/z* 221.07198; Found: C, 59.5%; H, 5.2%; N, 17.8%; HRMS (EI) *m/z* 221.07194 $(M)^+$.



Naphthalene-2-sulfonic acid [4-(7'-chloro-quinolin-4'-ylamino)-butyl}-amide (77). –

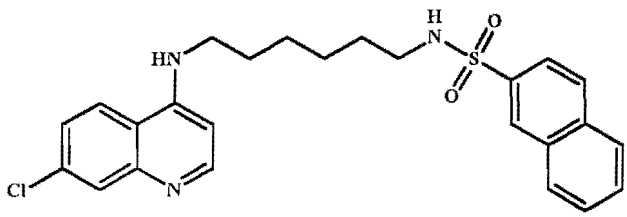
To a mixture of **108** (0.98 g, 3.93 mmol) and triethylamine (1.1 cm^3, 7.86 mmol) in *N, N'* dimethylformamide (7 cm^3). -2-Naphthalenesulfonyl chloride (1.25 g, 5.502 mmol) was added and the reaction mixture was allowed to shake for 18 h[95] at 20°C. Water (20 cm^3) was then added to quench the

reaction resulting in the formation of a precipitate. The crude product was chromatographed (SiO_2-MeOH-CH_2Cl_2, 1:9) to yield the *sulfonamide* **77** (0.83 g, 84%); R_f 0.42 (MeOH: CH_2Cl_2, 1:9); IR (Nujol): ν_{max}/cm^{-1} 3687 (N-H), 3646 (N-H), 1524 (C=C) and 1376 (S=O); δ_H (400 MHz, $CDCl_3$) 1.64 (2H, quin, *J* 4 x 7.4 Hz, CH_2), 1.81 (2H, quin, *J* 4 x 7.6 Hz, CH_2), 3.02 (2H, t, *J* 6.4 Hz, ArNHCH_2-), 3.28 (2H, br t, ArNH$(CH_2)_3CH_2$-), 6.25 (H, d, *J* 6.8 Hz, H3'), 6.85 (H br s, ArNH$(CH_2)_4NHSO_2$Ar), 7.16 (H, dd, *J* 2.0 and 8.8 Hz, H6'), 7.51-7.99 (9H, m) and, 8.25 (H, d, *J* 6.4 Hz, H_2'); δ_C (100 MHz, $CDCl_3$) 25.3, 27.0, 40.0, 42.8, 98.4, 122.3, 123.0, 124.0, 125.7, 126.3, 126.8, 127.5, 127.8, 127.9, 128.3, 128.8, 128.9, 129.2, 129.5, 132.0, 134.8, 149.2 and 151.7 ; Anal. Calc for $C_{23}H_{22}ClN_3O_2S$; C, 62.8%; H, 5.0%; N, 9.6%; S, 7.3%; *m/z* 439.11213. Found: C, 62.7%; H, 5.4%; N, 9.7%; S, 7.6%; HRMS (EI) *m/z* 439.11317 $(M)^+$.



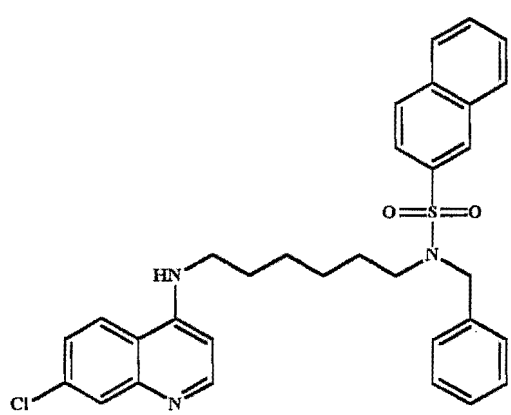
Naphthalene-2-sulfonic acid benzyl- [4-(7'-chloro-quinolin-4'-ylamino)-butyl]-amide (78). - Benzyl bromide (0.35 cm^3, 2.94 mmol) was added to a solution of **77** (1.2 g, 2.75 mmol) in *N*, *N'* Dimethylformamide (34cm^3), followed by potassium carbonate (3.80 g, 27.5 mmol). The reaction mixture was stirred at 20°C for 24 h, thereafter diluted with water and extracted with ethyl acetate (3 x 70cm^3). The combined organic extracts were washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The crude product was chromatographed (SiO_2, MeOH: CH_2Cl_2, 1:19) to give the desired product **78** (0.58 g, 48%); m.p. 99-102°C (from EtOAc/pet ether); R_f 0.38 (MeOH: CH_2Cl_2, 1:19); IR (Nujol)): ν_{max}/cm^{-1} 3377 (N-H), 1519 (C=C) and 1365 (S=O); δ_H (400 MHz, $CDCl_3$) 1.47 (2H, m, ArNH$CH_2(CH_2)_3$), 1.57 (2H, quin, *J* 4 x 7.2 Hz, ArNH$CH_2CH_2(CH_2)_2$), 3.11 (2H, q, *J* 3 x 5.6 Hz, ArNH$(CH_2)_2CH_2$-), 3.20 (2H, t, *J* 7.2 Hz, ArNH$(CH_2)_3CH_2$-), 4.33 (2H, s, NHCH_2Ph), 5.01 (H, br t, Ar*NH*), 6.22 (H, d, *J* 6.4 Hz), 7.19-7.24 (4H, m), 7.57-7.98 (10H, m), 8.36 (H, d, *J* 1.6 Hz) and 8.44 (H, d, *J* 1.6 Hz); δ_C (100 MHz, $CDCl_3$) 25.4, 26.1, 42.6, 48.2, 52.9, 98.9, 117.1, 121.1, 121.3, 122.37, 125.2, 127.7, 127.9, 128.0, 128.4, 128.5, 128.6, 128.7, 128.8, 129.2, 129.5, 131.6, 132.2, 134.8, 136.3, 136.4, 149.1, 149.7, 151.9 and 162.5; Anal Calc for

$C_{30}H_{28}ClN_3O_2S$; C, 67.8%; H, 5.3%; N, 7.9%; S, 6.1%; *m/z* 530.1; Found C, 67.7%; H, 5.3%; N, 7.8%; S, 5.7%; MS (FAB) *m/z* 530.3 $(M+1)^+$.



Naphthalene-2-sulfonic acid [6-(7'-chloro-quinolin-4'-ylamino)-hexyl]-amide (79). –

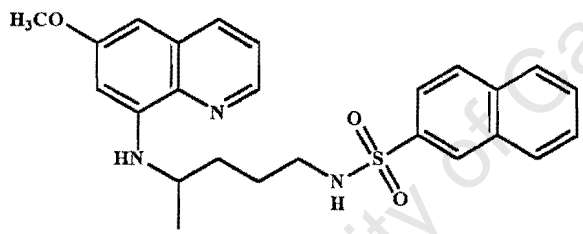
To a mixture of **110** (2.0 g, 7.21 mmol) and triethylamine (2 cm^3, 14.42 mmol) in *N, N'* dimethylformamide (13cm^3) was added 2- naphthalenesulfonyl chloride (2.29 g, 10.1 mmol) and the reaction mixture was allowed to shake for 18 h[95] at 20°C. Water (40 cm^3) was then added to quench the reaction resulting in the formation of a precipitate. The crude product was chromatographed (SiO_2, MeOH: CH_2Cl_2, 1:9) to yield the *sulfonamide* **79** (1.52 g, 72%); m.p. 132-135°C (from EtOAc/pet ether); Rf 0.36 (MeOH-CH_2Cl_2, 1:9); IR (Nujol): ν_{max}/cm^{-1} 3687 (N-H), 3646 (N-H), 1581 (C=C) and 1153 (S=O); δ_H (400 MHz, $CDCl_3$) 0.82 (2H, m *CH_2*), 1.37 (6H, m, 3 x *CH_2*), 3.00 (2H, m, *CH_2*), 3.23 (2H, m, *CH_2*), 6.34 (H, d, *J* 5.4 Hz, H3'), 7.33(H, dd, *J*, 2 and 8.8 Hz, H6'), 7.59-7.95 (8H, m), 8.41 (H, d, *J* 1.2 Hz) and 8.48 (H d, *J* 5.4 Hz, H2'); δ_C (100 MHz, $CDCl_3$) 26.0, 28.6, 29.7, 30.2, 43.0, 51.1, 83.7, 90.5, 99.0, 102.9, 107.6, 110.7, 115.4, 117.4, 121.0, 122.3, 127.9, 128.4, 128.8, 129.5, 134.8, 138.2, 141.1, 146.3 and 151.6; Anal. Calc for $C_{25}H_{26}ClN_3O_2S$; C, 64.2%; H, 5.6%; N, 8.9%; S, 6.9%; *m/z* 467.14343. Found: C, 64.4%; H, 5.6%; N, 8.5%; S, 7.2%; HRMS (EI) *m/z* 467.14205 $(M)^+$.



Naphthalene-2-sulfonic acid benzyl- [6-(7'-chloro-quinolin-4'-ylamino)-hexyl]-amide (80). –

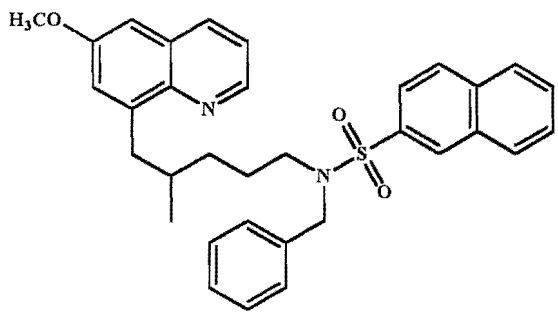
Benzyl bromide (0.40 cm^3, 3.33 mmol) was added to a solution of **79** (1.5 g, 3.11 mmol) in *N, N'* dimethylformamide (39 cm^3), followed by potassium carbonate (4.30 g, 31.1 mmol). The reaction mixture was stirred at 20°C for

24 h. Thereafter; it was diluted with water and extracted with ethyl acetate (3 x 70cm^3). The combined organic extracts were washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The crude product was chromatographed (SiO_2, MeOH: CH_2Cl_2, 1:19) to give the product **80** (0.88 g, 60 %) as a yellow oil; R_f 0.19 (MeOH: CH_2Cl_2, 1:19); IR (Nujol); ν_{max}/cm^{-1} 3252 (N-H), 1567 (C=C) and 1351. (S=O); δ_H (400 MHz, CD_3OD) 1.17 (4H, m, 2 x *CH*$_2$), 1.31 (2H, m, *CH*$_2$), 1.50 (2H, m, *CH*$_2$), 3.17 (4H, m, *CH*$_2$), 4.38 (2H, s, -NH*CH*$_2$Ph), 6.39 (H, d, *J* 5.6 Hz, H3'), 7.17-7.37 (5H, m), 7.59-8.05 (9H, m), 8.32(H, d, *J* 5.6 Hz, H2') and 8.4 (H, d, *J* 1.6 Hz); δ_C (100 MHz, $CDCl_3$) 27.2, 27.4, 29.0. 29.01, 43.8, 45.0, 53.2, 99.6, 119.1, 123.6, 124.3, 125.9 2 x H), 127.5 (2 x H), 128.7, 128.8, 129.0, 129.4, 129.5, 129.5, 129.9, 130.2, 130.6, 133.7, 136.3, 138.3, 138.4, 149.6, 152.3 and 152.7; Anal. Calc for $C_{32}H_{32}ClN_3O_2S$; *m/z* 557.190377. Found MS (FAB) *m/z* 558.1 $(M+1)^+$.

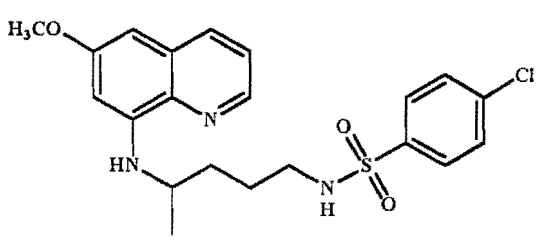


Naphthalene-2-sulfonic acid [4-(6'-methoxy-quinolin-8'-ylamino)-pentyl]-amide (74). –

To a mixture of primaquine (2.45 g, 9.49 mmol) and triethylamine (2.65 cm^3, 18.98 mmol) in *N, N'* dimethylformamide (16 cm^3) was added 2-naphthalenesulfonyl chloride (3.0g, 13.29 mmol) and the reaction mixture was shaken for 18 h at 20°C. Water (48 cm^3) was then added to quench the reaction resulting in the formation of a precipitate. The crude product was chromatographed (SiO_2, EtOAc: Hex, 1:2.3) to yield the *sulfonamide* **74** (2.36 g, 96%) as a dark oil; R_f 0.36 (EtOAc: Hex, 1:2.3); IR (Nujol): ν_{max}/cm^{-1} 3388 (N-H) and 1329 (S=O); δ_H (400 MHz, $CDCl_3$) 1.18 (2H, d, *J* 5.6 Hz, *CH*$_3$), 1.60 (4H, m, *CH*$_2$), 2.99 (2H, m, *CH*$_2$), 3.45 (H, br m, *CH)*, 3.85 (3H, s, O*CH*$_3$), 4.61 (H, t, *J* 6.0 Hz, *NH*SO_2Ar), 5.89(H, br t, Ar*NH*-), 6.19 (H, d, *J* 2.0 Hz,), 6.30 (H, d, *J* 2.4 Hz), 7.24-7.90 (8H, m), 8.38 (H, d, *J* 1.6 Hz) and 8.48 (H, dd, *J* 2.0 and 4.4 Hz); δ_C (100 MHz, $CDCl_3$) 20.6, 26.4, 33.6, 43.3, 47.7, 55,2, 91. 9, 96.9, 121.8, 122.3, 122.5, 127.5, 127.9, 128.4, 128.7, 129.2, 129. 5, 129.9, 132.2, 134.8, 135.3, 136.8, 144.3, 144.8 and 159.4; Anal. Calc for $C_{25}H_{27}N_3O_3S$; *m/z* 449.17731; Found HRMS (EI) *m/z* 449.17832 $(M)^+$.

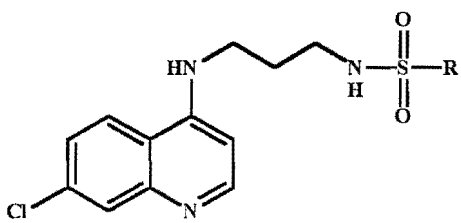


Naphthalene-2-sulfonicacid[5-(6'-methoxy-quinolin-8'-yl)-4-methyl-pentyl]-amide (75). – Benzyl bromide (0.67 cm^3, 5.64 mmol) was added to a solution of **74** (2. 4g, 5.27 mmol) in *N, N'* dimethylformamide (66 cm^3), followed by potassium carbonate (7.30 g, 31.1 mmol). The reaction mixture was stirred at 20°C for 24 h. Thereafter, it was diluted with water (100cm^3) and extracted with ethyl acetate (4 x 100 cm^3). The combined organic extracts were washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The crude material obtained was recrystallized from methanol to give the desired product **75** (0.88 g, 60%) as beige crystals; mp 152-155°C (from MeOH); R_f 0.77 (MeOH: CH_2Cl_2, 1:19); IR (Nujol): ν_{max}/cm^{-1} 3252 (N-H), 1568 (C=C) and 1351. (S=O); δ_H (400 MHz, $CDCl_3$) 1.07 (3H, d, *J* 6.4 Hz, CH_3), 1.45 (4H, m, 2 x CH_2), 3.17 (2H, m, CH_2), 3.36 (H, br m, *CH*), 3.86 (3H, s, OCH_3), 6.13 (H, d, *J* 2.4 Hz), 6.30 (H, d *J* 2.4 Hz), 7.18-7.29 (6H, m), 7.55-7.91 (7H, m), 8.36 (H, d, *J* 1.6 Hz) and 8.48 (H, dd, *J* 1.6 and 4.4 Hz); δ_C (100 MHz, $CDCl_3$) 20.4, 24.6, 33.5, 47.5, 48.0, 51.9, 55.2, 66.0, 91.7, 96.7, 121.5, 121.8, 122.5, 122.7, 127.4, 127.8, 128.0, 128.4, 128.5, 129.2, 129.4, 129.5, 130.0, 132.2, 134.7, 136.2, 136.3, 137.0, 142.0, 144.2 145.6 and 159.4; Anal. Calc for $C_{32}H_{33}N_2O_3S$; C, 71.2%; H, 6.2%; N, 7.8%; S, 5.9%; *m/z* 539.2243. Found C, 71.1%; H, 6.4%; N, 7.6%; S, 5.3%; MS (FAB) *m/z* 540.4 $(M+1)^+$.



4-Chloro-N- [4-(6'-methoxy-quinolin-8'-ylamino)-pentyl]-benzene sulfonamide (76). - Aryl sulfonyl chloride (7.49 mmol) was added to a mixture of primaquine (5.35 mmol) and triethylamine (1.49 cm^3, 10.7 mmol) in *N, N'* dimethylformamide (9 cm^3)[95]. The reaction mixture was shaken for 18 h at 20°C. Water (0.37 cm^3/mmol) was added resulting in the formation of a precipitate which was isolated by filtration. Chromatography (SiO_2, MeOH: CH_2Cl_2, 1:4) yielded the *sulfonamide* (0.51 g, 37%) as a dark oil, R_f 0.57 (MeOH: CH_2Cl_2, 1:4); IR: ν_{max}

(DMSO)/cm^{-1} 3343 (N-H) and1371 (S=O); δ_H (300 MHz, $CDCl_3$) 1.23 (3H, d, *J* 6.3 Hz, *CH*$_3$), 1.62 (4H, m, *CH*$_2$), 2.97 (2H,m, *CH*$_2$), 3.51 (H, br m, *CH*) 3.88 (3H, s. O*CH*$_3$), 4.7 (H, t, *J* 6.0-N*H*SO_2Ar), 5.59 (H, br s, -CH_2*NH*Ar), 6.2 (H, d, *J* 2.6 Hz), 6.34(H, d, *J* 2.57 Hz), 7.28-7.4(3H, m), 7.74-7.77(2H, m), 7.99(H, dd, *J* 6.54 and 8.13 Hz) and 8.5 (H, dd, *J* 2.6 and 4.2); δ_C (75 MHz, $CDCl_3$) 20.6, 26.3, 33.6, 43.2, 47.7, 55.2, 91.9, 96.9, 121.7, 121.9, 128.4, 129.3, 129.9, 134.8, 125.3, 138.6, 139.0, 144.4, 144.8, 144.9 and 159.4; Anal. Calc for $C_{21}H_{24}ClN_3O_3S$; *m/z* 433.12269. Found HRMS (EI) *m/z* 433.12398 (M^+).

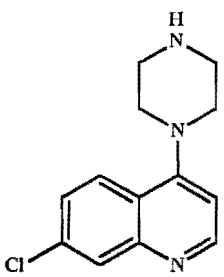


General procedure for preparation of sulfonamides 81-82 - Amine **107** (2.12 mmol) and triethylamine (4.24 mmol) were mixed in *N*, *N'* dimethylformamide (1.1 cm^3/mmol)[95]. Aryl sulfonyl chloride (2.97 mmol) was added and the mixture shaken for 18 h at 20°C. Water (4.7cm^3) was added and the resulting precipitate, which was isolated by filtration. Chromatography (SiO_2, MeOH: CH_2Cl_2, 1:9) yielded the *sulfonamide.*

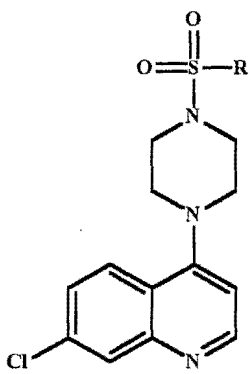
Aryl sulfonyl chlorides used: 4-Chlorosulfonyl chloride and 2-naphthalenesulfonyl chloride

Naphthalene-2-sulfonic acid [3-(7'-chloro-quinolin-4'-ylamino)-propyl]-amide (81) - (0.36g, 72%) as white crystals, mp 161-163°C (from MeOH); R_f 0.65 (MeOH: CH_2Cl_2, 1:9); IR: ν_{max} (DMSO)/cm^{-1} 3374 (N-H) and 1343 (S=O); δ_H (300 MHz, DMSO-d_6) 1.75 (2H, m -NHCH$_2$*CH*$_2$CH$_2$NHSO$_2$-), 2.92 (2H, t, *J* 6.85 Hz - *CH*$_2$(CH$_2$)$_2$NHSO$_2$Ar), 3.2 (2H, m,-(CH$_2$)$_2$*CH*$_2$NHSO$_2$Ar), 6.3 (H, d, *J* 5.4 Hz, H3'), 7.16 (H, t, *J* 6.0 Hz, -(CH$_2$)$_3$*NH*SO$_2$), 7.37 (H, dd, J 6.6 and 8.8 Hz), 7.61-8.4 (10H, m); δ_C (75MHz, $CDCl_3$) 27.8, 30.6, 38.1, 98.6, 117.4, 122.2, 123.9, 127.2, 127.4, 127.4, 127.7, 128.5, 129.0, 129.3, 131.7, 133.3, 134.0, 137.5, 149.0, 149.9 and 151.7; Anal. Calc for HRMS $C_{22}H_{20}ClN_3O_2S$; C, 61.0%; H, 4.7%; N, 9.9%; S, 7.5%; *m/z* 425.09648. Found C, 60.8%; H, 4.8%; N, 9.6%; S, 7.1%; MS (EI) *m/z* 425.09806 $(M)^+$.

4-Chloro-N- [3-(7'-chloro-quinolin-4'-ylamino)-propyl]-benzenesulfonamide (82) - (0.46 g, 95 %) as off white crystals, mp 174-177°C (from MeOH); R_f 0.36 (MeOH: CH_2Cl_2, 1:9); IR: ν_{max} (DMSO)/cm^{-1} 3345 (N-H) and 1343 (S=O); δ_H (300 MHz, DMSO-d_6) 1.75 (2H, quin, *J* 4 x 7 Hz, -NHCH$_2$*CH$_2$*CH$_2$NHSO$_2$-), 2.88 (2H, t, *J* 6.0 Hz ArNH*CH$_2$*(CH$_2$)$_2$NHSO$_2$Ar), 3.22 (2H, m, ArNH(CH$_2$)$_2$*CH$_2$*NHSO$_2$Ar), 6.37(H, d, *J* 5.4 Hz, H3'), 7.18 (H, t, *J* 5.4 Hz -(CH$_2$)$_3$*NH*SO$_2$-),7.42(H, dd, *J* 2.2 and 9.1 Hz, H6'), 7.58-7.77 (6H, m), 8.18 (H, d, *J* 9.1 Hz,H5'), 8.36 (H, d, *J* 5.4 Hz, H2'); δ_C (75 MHz, DMSO-d_6) 27.7, 30.6, 30.7, 98.6, 117.4, 123.9, 124.0, 127.4, 128.3, 129.0, 129.2, 133.3, 133.4, 137.2, 139.3, 148.9, 149.9 and 151.7; Anal. Calc for $C_{18}H_{17}Cl_2N_3O_2S$; C, 52.7%; H, 4.2%; N, 10.2%; S, 7.8%; *m/z* 409.04250. Found C, 53.1%; H, 4.4%; N, 9.9%; S, 7.7%; HRMS (EI) *m/z* 409.0422 $(M)^+$.



7-Chloro-4-piperazin-1-yl-quinoline (111). - 4,7-Dichloroquinoline (2.0 g, 10.1 mmol) was treated with piperazine (5.22 g, 60.6 mmol) **112** and stirred under nitrogen in the presence of *N*-methyl 2-pyrrolidinone (7 cm^3)[98], triethylamine (2.11 cm^3, 15.15 mmol) and potassium carbonate (0.42 g, 3.03 mmol) at 135-145° C for 18 h. After cooling to room temperature, the product was washed with dichloromethane and brine, the organic layer separated, dried over anhydrous sodium sulphate, filtered and concentrated. Column chromatography (SiO_2: NH_4OH: MeOH, 1:19) yielded the amine **111** (1.69 g, 85%) as orange/brown flakes; mp 109-113°C (from MeOH); R_f 0.29 (NH_4OH: MeOH, 1:19); IR (Nujol): ν_{max}/cm^{-1} 3518 (NH_2), 3437 (NH_2), 3029 (C=C), 1612 (NH_2), 1582 (C=C and C=N); δ_H (300 MHz, $CDCl_3$) 3.15 (8H, m, 4 x *CH$_2$*), 6.81 (H, d, *J* 5.1 Hz, H_3), 7.39 (1H, dd, *J* 1.5 and 6.6 Hz, H5), 7.93 (1H, dd, *J* 4.8 and 6.6 Hz, H6), 8.02 (H_{5} d, *J* 1.5 Hz, H_8) and 8.71 (H d, *J* 5.1 Hz, H_2); δ_C (75 MHz, $CDCl_3$) 46.3 (2 x CH_2), 53.7 (2 x CH_2), 109.2, 122.2, 125.4, 126.3, 129.1, 135.1, 150.4, 152.1 and 157.6; Anal Calc for $C_{13}H_{14}ClN_3$; C, 63.0%; H, 5.7%; N, 16.9%; *m/z* 247.08763. Found C, 63.5%; H, 5.8%; N, 16.5%; HRMS (EI) *m/z* 247.08970 $(M)^+$.

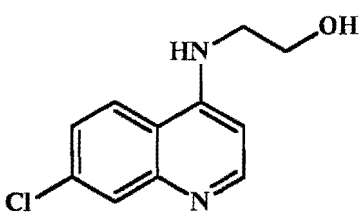


General procedure for preparation of piperazine sulfonamides 83-84 - 112 (4.04 mmol) and triethylamine (8.08 mmol) were mixed in *N, N'* dimethylformamide (1.7 cm^3/mmol)[95]. Aryl sulfonyl chloride (5.66 mmol) was added and the mixture shaken for 18 h at 20°C. Water (5 cm^3/mmol) was added resulting in the formation of a precipitate, which was isolated by filtration. Chromatography (SiO_2, MeOH: CH_2Cl_2, 1:4) yielded the desired *sulfonamide.*

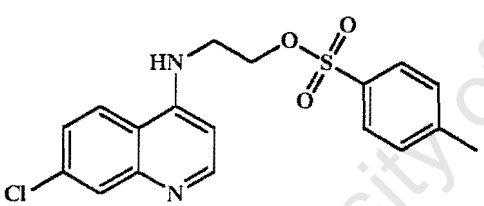
Aryl sulfonyl chlorides used: 4-Chlorosulfonyl chloride and 2-naphthalenesulfonyl chloride

7-Chloro-4- [4-(naphthalene-2'-sulfonyl)-piperazin-1'-yl]-quinoline (83). - (0.88 g, 88%) as off white crystals, mp 211-213°C (from MeOH); R_f 0.55 (MeOH: CH_2Cl_2, 1:9); IR: (DMSO)ν_{max} /cm^{-1} 3349 (N-H) and 1368 (S=O); δ_H (300 MHz, $CDCl_3$) 3.32 (8H, dd, *J* 4.8 and 29.7,4 x *CH_2*), 6,81 (H, d, *J* 5.1 Hz, H3), 7.29-8.02(9H, m,), 8.41(H, d, *J* 1.8 Hz), 8.7 (H, d, *J* 5.1 Hz, H2); δ_C (75 MHz, $CDCl_3$) 46.3 (2 x CH_2), 51.8(2 x CH_2), 109.7, 121.9, 123.2, 124.8, 126.8, 128.0, 128.3, 129.25, 129.3, 129.5, 129.5, 129.7, 132.5, 133.0, 135.3, 135.33, 150.3, 152.2 and 156.3; Anal. Calc for $C_{23}H_{20}ClN_3O_2S$; C, 63.1%; H, 4.6%; N, 9.6%; S, 7.3%; *m/z* 437.09647. Found C, 62.8% H, 4.6%; N, 9.3%; S, 7.2%; HRMS (EI) *m/z* 437.0974 $(M)^+$.

7-Chloro-4- [4-(4'-chloro-benzenesulfonyl)-piperidin-1-yl] quinoline (84). - (0.76g, 76%) as pale brown crystals, m.p. 159-162°C (from MeOH); R_f 0.69 (MeOH: CH_2Cl_2, 1:9); IR: ν_{max} (DMSO): /cm^{-1} 1347 (S=O); δ_H (300 MHz, $CDCl_3$) 3.39 (8H, d, *J* 5.7,4 x *CH_2*), 6,83 (H, d, *J* 5.0 Hz, H3), 7.37 (H, dd, *J* 2.2 and 9.1 Hz), 7.56-7.79 (6H, m), 8.03 (H, d, *J* 2.0 Hz), 8.73 (H, d, *J* 5.0 Hz, H2); δ_C (75 MHz, $CDCl_3$) 46.0 (2 x CH_2), 51.5 (2 x CH_2), 109.5, 121.7, 124.5, 126.6, 129.1, 129.2, 129.6, 129.8,134.2, 135.2, 135.3, 139.9, 150.1, 151.9 and 156.0; Anal. Calc for $C_{19}H_{17}Cl_2N_3O_2S$; C, 54.0%; H, 4.1%; N, 10.0%; S, 7.6%; *m/z* 421.04185. Found C, 54.2%; H, 4.1%; N, 10.1%; S, 7.6%; HRMS (EI) *m/z* 421.04282 $(M)^+$.

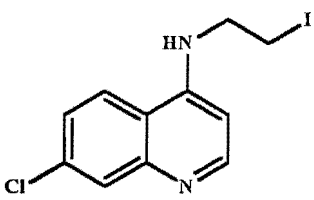


2-(7'-Chloro-quinolin-4'-ylamino)-ethanol (114). - A mixture of 4,7-dichloroquinoline (5 g, 25.2 mmol) and ethanolamine (6.8 cm^3, 113.4 mmol) was heated at 80°C for 1 h with stirring. Thereafter, at 135-145°C for 3h. Sodium hydroxide (1M, 75 cm^3) was added and the organic product extracted with ethyl acetate (6 x 250cm^3). The combined organic extracts were washed with water (3 x 50cm^3), dried over anhydrous sodium sulfate and concentrated to yield the alcohol **114** (1.78 g, 36%), mp 206-208°C (from MeOH); R_f 0.34 (MeOH: CH_2Cl_2, 1:19); IR (Nujol): ν_{max}/cm^{-1} 3618 (OH), 3564 (N-H), 3029 (C=C), 1534 (C=C) and 1392 (OH); δ_H (300 MHz, CD_3OD) 3.5 (2H, t, *J* 5.7 Hz, ArNH*CH*$_2$-), 3.84 (2H, t, *J* 5.7 Hz, ArNHCH$_2$*CH*$_2$-), 6.6 (H, d, *J* 5.7 Hz, H3'), 7.4 (H, dd, *J* 2.1 and 9 Hz, H6'), 7.8 (H, d, *J* 2.1 Hz, H8'), 8.1 (H, d, *J* 9.0, H5') and 8.35 (H, d, *J* 5.7 Hz, H2'); δ_C (75 MHz, CD_3OD) 30.1, 60.8, 118.8, 124.1, 124.4, 125,9, 127.5, 127.8, 136.36, 149.7 and 152.6; Anal. Calc for $C_{11}H_{11}ClN_2O$; M 222.5; Found M^+ (222, M^+) and (164, M^+-NH(CH$_2$)$_2$OH).

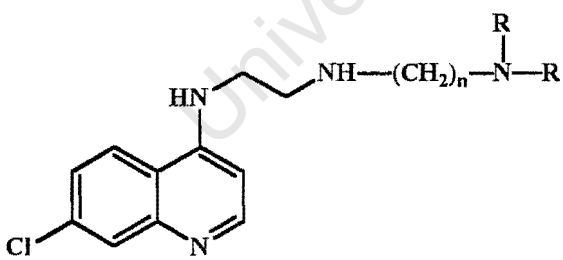


Toluene-4-sulfonic acid 2-(7'-chloro-quinolin-4'-ylamino) ethyl ester (112). - Triethylamine (3.3cm^3, 23.97mmol) was added to a solution of **114** (1.78 g, 7.99 mmol)[102] in CH_2Cl_2 (14 cm^3). The solution was cooled to 0°C in an ice-water bath. *p* - Toluenesulfonyl chloride (3.7 g, 17.58 mmol) dissolved in CH_2Cl_2 (3 cm^3) was slowly added to this cooled solution and stirred for 20 min at 0°C. The reaction mixture is allowed to warm to 20°C and stirred for a further 40 min. The solution is transferred to a separating funnel and washed with hydrochloric acid (2M, 2 x 20 cm^3) and saturated sodium carbonate. The organic phase is dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was chromatographed (SiO_2, MeOH:CH_2Cl_2, 1:19) to give the *tosylate* **112** (0.68g, 39%); mp 141-143°C (from EtOAc/pet ether; R_f 0.5 (MeOH-CH_2Cl_2. 1:19); IR (Nujol): ν_{max}/cm^{-1} 3225 (N-H), 1557 (C=C), 1416 (S=O) and 1147 (S=O); δ_H (400 MHz, acetone-d_6) 2.26 (3H, s, *CH*$_3$) 3.74 (2H, m, ArNH*CH*$_2$-), 4.34 (2H, t, *J* 5.2 Hz, ArNHCH$_2$*CH*$_2$-), 6.45 (H, d, *J* 5.4 Hz, H3'), 6.70 (H br t, ArNH(CH$_2$)$_2$*NH*SO$_2$Ar), 7.15 (2H, dd, *J* 2.0 and 6.2 Hz,), 7.40 (H, dd, *J* 2.0 and 8.8 Hz, H6'), 7.65 (H, dd, *J* 2.0 and 6.2 Hz,), 7.85 (H, d, *J* 2.0 Hz, H8'), 8.04 (H, d, *J* 8.8 Hz, H5') and 8.38 (H, d, *J* 5.4 Hz,

H2'); δ_C (100 MHz, acetone-d_6) 22.1, 42.9, 69.1, 100. 5, 119.6, 124.5, 126.1, 129.2, 129.8, 130.9, 131.3, 134.0, 134.4, 135. 6, 146.4, 151.0, 151.1, and 153.4; Anal. Calc for $C_{18}H_{17}ClN_2O_3S$; C, 57.4%, H, 4.55%; N, 7.4%; *m/z* 376.06484; Found: C, 57.3%; H, 4.4%; N, 7.2%; HRMS (EI) 376.06506 $(M)^+$.



(7'-Chloro-quinolin-4'-yl)-(2-iodo-ethyl)-amine (113). - Iodine (0.55 g, 2.19 mmol) was added to a stirred solution of alcohol **114** (0.23 g, 0.73 mmol), imidazole (0.15 g, 2.19 mmol) and triphenylphosphine (0.57 g, 2.19 mmol) dissolved in a mixture of ether: acetonitrile (3:1) under nitrogen. The mixture was stirred at 20°C for 5 h. The resulting yellow suspension was filtered through celite and the filtrate concentrated. Column chromatography (SiO_2, MeOH: CH_2Cl_2, 1:19) afforded the product **113** (0.026 g, 11%) as a gum; R_f 0.32 (MeOH: CH_2Cl_2, 1:19); IR (Nujol): ν_{max}/cm^{-1} 3563 (N-H), 3029 (C=C) and 1534 (C=C); δ_H (300 MHz, $CDCl_3$) 3.37 (2H, t, *J* 6.6 Hz, ArNHCH$_2$*CH$_2$*-), 3.7 (2H, br t, ArNH*CH$_2$*-), 6.38 (H, d, *J* 5.7 Hz, H3'), 7.36 (H, dd, *J* 2.0 and 9.0 Hz, H6'), 7.83 (2H, m, H5' and H8') and 8.42 (H, d, *J* 5.7 Hz, H2'); δ_c (75 MHz, $CDCl_3$) 8.4, 55.7, 104.5, 116.0, 121.2, 125.8, 128.8, 134.7, 149.4, 149.5 and 151.4; Anal. Calc for $C_{11}H_{10}ClIN_2$; *m/z* 331.9577. Found HRMS (FAB) *m/z* 332.9 $(M+1)^+$.



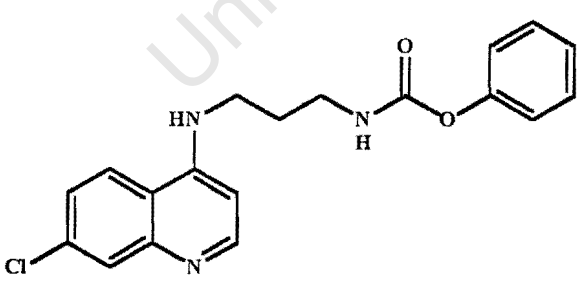
General procedure for preparation of compounds 85-86 - 112 (0.322 mmol) was added to a solution of amine (0.644 mmol) in anhydrous acetonitrile (0.2 cm^3/mmol) were mixed in a round-bottomed flask [103] and refluxed under nitrogen for 24 h. The crude mixture was chromatographed (SiO_2, MeOH: CH_2Cl_2, 1:19, then NH_4OH: MeOH, 1:19) yielded the *amine*.

Amines used: 3-dimethylamino-1-propylamine and piperazine

N- [2-(7''-chloro-quinolin-4''-ylamino)-ethyl]-N', N'-dimethyl-propane-1, 3-diamine (85). - (0.075 g, 66%); mp 61-67°C (from EtOAc/pet ether); R_f 0.25 (NH_4OH-MeOH, 1:19); IR (Nujol): ν_{max}/cm^{-1} 3183 (N-H) and 1362 (C=C); δ_H (400 MHz, $CDCl_3$)

1.66 (2H, quin, *J* 7 Hz, ArNH $(CH_2)_2NHCH_2CH_2$-), 2.17 (6H, s, 2 x CH_3), 2.32 (2H, t, *J* 7.2 Hz, ArNH $(CH_2)_2NH(CH_2)_2CH_2N(CH_3)_2$), 2.70 (2H, t, *J* 6.8 Hz, $ArNH(CH_2)_2NHCH_2$-), 3.00 (2H, t, *J* 6.0 Hz, $ArNHCH_2CH_2$-), 3.31 (2H, m, $ArNHCH_2$-), 5.92 (H, br s, Ar*NH*), 6.37 (H, d, *J* 5.4 Hz, H3''), 7.32 (H, dd, *J* 2.0 and 8.8 Hz, H6''), 7.71 (H, d, *J* 8.8 Hz, H5''), 7.92 (H, d, *J* 2.0, H8'') and 8.49 (H, d, *J* 5.4 Hz, H2''); δ_C (100 MHz, $CDCl_3$) 27.9, 41.9 (2 x CH_3), 45.5, 47.5, 47.8, 58.0, 99.2, 117.2, 121.3, 125.2, 128.7, 134.7, 149.0, 149.9 and 152.1; Anal. Calc for $C_{16}H_{23}ClN_4$; C, 62.6%; H, 7.6%; N, 18.3%; *m/z* 306.16112. Found: C, 62.5%; H, 7.5%; N, 18.2%; HRMS (EI) 306.14589 $(M)^+$.

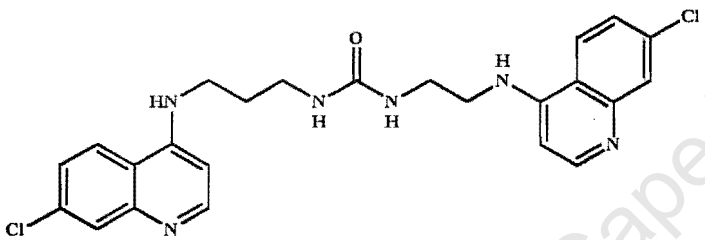
(7'-Chloro-quinolin-4'-yl)-(2-piperazine-1-yl-ethyl)-amine (86). -(0.1258 g, 37%); mp 124-127°C (from ETOAc/pet ether); R_f 0.41 (NH_4OH: MeOH, 1:19); IR (Nujol): ν_{max}/cm^{-1} 2972 (N-H) and 1553 (C=C and C=N); δ_H (400 MHz, $CDCl_3$) 2.49 (4H, br t, 2 x CH_2), 2.74 (2H, t, *J* 6.4 Hz, $ArNHCH_2CH_2$-), 2.91(4H, t, 4.8 Hz, 2 x CH_2), 3.29 (2H, q, *J* 3 x 6.0 Hz $ArNHCH_2CH_2$), 5.96 (H, br t, Ar*NH*), 6.35 (H, d, *J* 5.6 Hz, H3'), 7.36 (H, dd, *J* 2.2 and 8.8 Hz, H6'), 7.66 (H, d, *J* 8.8 Hz, H5'), 7.94 (H, d, *J* 2.2 Hz, H8') and 8.50 (H, d, *J* 5.6, H2'); δ_C (100 MHz, $CDCl_3$) 38.8, 46.3 (2 x CH_2), 54.0 (2 x CH_2), 56.1, 99.3, 117.2, 121.0, 125.3, 128.9, 134.8, 148.2, 149.6 and 152.1; Anal. Calc for $C_{15}H_{19}ClN_4$; C, 61.9%; H, 6.6; N, 19.3%; *m/z* 290.12982; Found: C, 61.8%; H, 6.5%; N, 19.0%; HRMS (EI) *m/z* 290.12953 $(M)^+$.



[3-(7'-Chloro-quinolin-4'-ylamino)-propyl]-carbamic acid phenylester (90). –

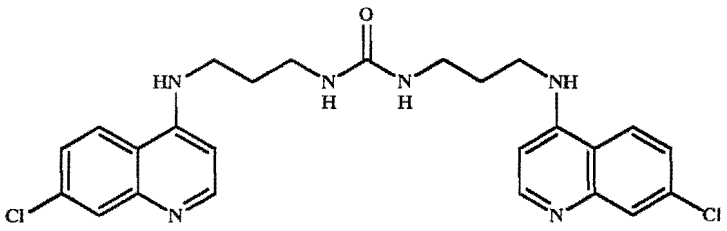
A mixture of *N*, *N'* dimethylformamide (0.30 cm^3, 3.82 mmol) and dichloromethane (15.3 cm^3) was cooled to 0°C and then added to phenyl chloroformate (0.48 cm^3, 3.8 mmol) at 0°C. This mixture was stirred at 0°C for 30 min, then amine **107** (0.9 g, 3.8 mmol) was added and the resulting heterogeneous mixture was stirred at 0°C for 5 h. The solvent was removed under reduced pressure and column chromatography (SiO_2, MeOH: CH_2Cl_2, 1:19) yielded the *carbamate* **90** (0.52 g, 58%) as pale yellow crystals; mp 115-118°C (from

EtOAC/pet ether); R_f 0.45 (MeOH: CH_2Cl_2, 1:9); IR ($CHCl_3$): ν_{max}/cm^{-1} 3434 (CONH), 3034 (C=C), 1714 (CO-O-C=C) and 1531 (C=C); δ_H (400 MHz, $CDCl_3$) 1.94 (2H, m, Hz, ArNHCH$_2$*CH$_2$*-), 3.41-3.50 (4H, m, ArNH *CH$_2$*-and ArNH CH$_2$CH$_2$*CH$_2$*-), 5.59 (H, br t, *NH*), 6.21 (H, br t, *NH*), 6.39 (H, d, *J* 5.6 Hz, H3'), 7.12 (H, dd, *J* 2 and 8.8 Hz, H6'), 7.20-7.39 (5H, m), 7.81 (H, d, *J* 8.8 Hz, H5'), 7.93 (H, d, *J* 2 Hz, H8') and 8.46 (H, d, *J* 5.6 Hz, H2'); δ_C (100 MHz, $CDCl_3$) 28.6, 38.1, 39.5, 98.4, 98.5, 117.2, 121.5 (2 x H), 121.8, 125.5, 127.8, 129.4 (2 x H), 135.3, 148.4, 150.1, 150.9, 151.0 and 155.8; Anal. Calc for $C_{19}H_{18}ClN_3O_2$; C, 64.1%; H, 5.1%; N, 11.8%; *m/z* 355.11. Found C, 64.0%; H, 5.1%; N, 11.7%; HRMS (FAB) *m/z* 356.2 $(M+1)^+$.

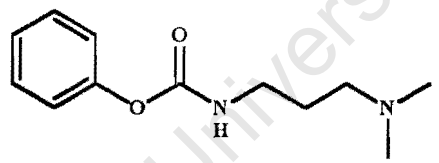


1-[2-(7'-Chloro-quinolin-4'-ylamino)-ethyl]-3-[3-(7''-chloro-quinolin-4''-ylamino)-propyl]-urea (87). –

The amine **110** (0.09 g, 0.39 mmol) was added to a stirred solution of carbamate **90** (0.1 g, 0.28 mmol) in anhydrous tetrahydrofuran (3.36 cm^3). The resulting heterogeneous solution was stirred at 20°C for 6h and subsequently at 50-60°C for 20 h. Column chromatography (SiO_2, NH_4OH: MeOH, 1:9) afforded the *urea* **87** (0.87 g, 65%) as beige crystals; mp 166-168°C (from MeOH); R_f 0.6 (NH_4OH: MeOH, 1:9); IR (MeOH): ν_{max}/cm^{-1} 3470 (N-H), 3423 (N-H), 1643 (C=O) and 1559 (C=C); δ_H (400 MHz, DMSO-d_6) 1.85 (2H, quin, *J* 4 x 6.8 Hz, ArNH CH$_2$*CH$_2$*-), 3.30 (4H, m, 2 x *CH$_2$*), 3.40 (2H, t, *J* 5.6 Hz, *CH$_2$*), 3.51 (2H, t, *J* 5.6 Hz, *CH$_2$*), 6.40 (H, d, *J* 5.6 Hz, H3'), 6.5(H, d, *J* 5.6 Hz, H3''),7.24 (H, dd, *J* 2.4 and 9.0 Hz, H6'), 7.34 (H, dd, *J* 2.2 and 8.8 Hz, H6''), 7.69 (H, d, *J* 2.4 Hz, H8'), 7.72 (H, d, *J* 2.2 Hz, H8''), 7.95 (H, d, *J* 9.0 Hz, H8'), 8.01 (H, d, *J* 8.8 Hz, H8''), 8.24 (H, d, *J* 5.6 Hz, H2'), 8.31 (H, d, *J* 5.6 Hz, H2''); δ_c (100 MHz, DMSO-d_6) 29.4, 38.0, 38.8, 40.1, 44.6, 99.3, 118.1, 118.2, 122.9, 123.0, 124.4, 124.7, 124.7, 124.8, 128.2, 128.23, 134.1,149.7, 149.8, 150.7, 150.8, 152.6, 152.6 and 159.8; Anal. Calc for $C_{24}H_{24}Cl_2N_6O$; C, 59.6%; H, 5.0%; N, 17.4%; *m/z* 483.39. Found C, 59.2%; H, 5.2%; N, 17.3%; MS (FAB) *m/z* 483.39 $(M)^+$.

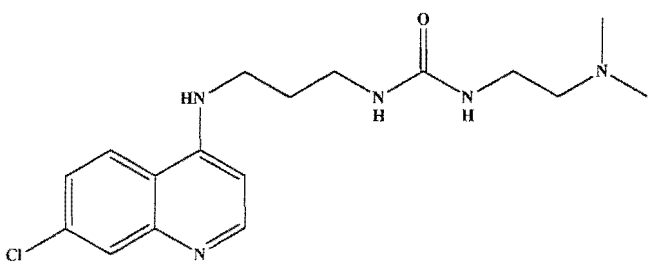


1, 3 – Bis - [3 - (7' - chloroquinolin -4'-ylamino)-propyl]-urea (88). – To a stirred solution of carbamate **90** (0.4 g, 1.13 mmol) in anhydrous tetrahydrofuran (14 cm^3), amine **107** (0.37 g, 1.58 mmol) was added. The resulting solution is stirred at 50-60°C for 20 h. The precipitate that formed was removed by filtration and the mother liquor was concentrated. Column chromatography (SiO_2, NH_4OH: MeOH, 1:9) afforded the *urea* **88** (0.41 g, 74%) as white crystals; mp126-129°C (from MeOH); R_f 0.34 (NH_4OH: MeOH, 1:9); IR (MeOH): ν_{max}/cm^{-1} 3470(N-H), 3423 (N-H), 1643 (C=O) and 1559 (C=C); δ_H (400 MHz, DMSO-d_6): 1.75 (4H, quin, *J* 4 x 6.8 Hz, 2 x CH_2), 3.12 (4H, q, *J* 3 x 6.5 Hz, 2 x CH_2), 3.26 (4H, q, *J* 3 x 6.4 Hz, 2 x CH_2), 6.50 (2H, d, *J* 5.6 Hz, H3'), 7.37 (2H, dd, *J* 2.0 and 8.8 Hz, H6'), 7.77 (2H, d, *J* 2.0 Hz, H8'), 8.46 (2H, d, *J*, 9.2, H5'), 8.33 (2H, d, *J* 5.6, H2'); δ_C (75 MHz, DMSO-d_6) 29.5, 37.8, 54.81, 99.3, 117.3, 124.7, 124.8, 127.9, 134.2, 149.5, 150.9, 152.3 and 159.3; Anal. Calc for $C_{24}H_{24}Cl_2N_6O$; C, 60.4%; H, 5.3%; N, 16.9%; ***m/z*** 496.15. Found C, 60.2%; H, 5.4%; N, 16.5%; MS (FAB) ***m/z*** 497.2 $(M+1)^+$.



(3-Dimethylamino-propyl-carbamic acid phenylester (91). - A mixture of *N, N'* dimethylformamide (1.51 cm^3, 19.5 mmol) and dichloromethane (78 cm^3) was cooled to 0° and then to phenyl chloroformate (2.4 cm^3, 19. 5mmol) at 0°C.This mixture was stirred at 0°C for 30 min then 3-Dimethylamino-1-propylamine (2.0 g, 19.5 mmol) was then added and the resulting solution was stirred at 0°C for 7 h. The solvent was removed under reduced pressure and column chromatography (SiO_2, MeOH: CH_2Cl_2, 1:9) yielded the *carbamate* **91** (1.7 g. 40%) as an off white a white gelatinous material; R_f 0.25 (MeOH: CH_2Cl_2, 1:9); IR ($CHCl_3$): ν_{max}/cm^{-1} 34238 (N-H), 3056 (C=C), 1714.42 (CO-O-C=C) and 1511 (C=C); δ_H (300 MHz, $CDCl_3$) 2.03 (2H, m, -$NHCH_2CH_2CH_2N(CH_3)_2$), 2.69 (6H, br d, 2 x CH_3), 2.99 (2H, m, -$NHCH_2CH_2CH_2N(CH_3)_2$), 3.36 (2H, m, -$NHCH_2CH_2CH_2N(CH_3)_2$), 5.93 (H, br t, ArOCO*NH*), 6.81-6.91 (2H, m) and 7.01-7.35 (3H, m) δ_C (100 MHz, $CDCl_3$) 25.1,

38.21, 42.9, 43.3, 55.68, 115.5, 120.0, 121.6, 125.3, 129.2, 129.45 and 156.52; Calc for $C_{12}H_{18}N_2O_2$; *m/z* 222.1368. Found HRMS (FAB) *m/z* 223.2 $(M+1)^+$.



1-[3-(7'-Chloro-quinolin-4'-ylamino)-propyl]-3-(2-dimethylamino-propyl)-urea (89). - Method (a): To a stirred solution of carbamate **91**(0.2 g, 0.90 mmol) in anhydrous tetrahydrofuran (11 cm^3), amine **107** (0.17 g, 0.72 mmol) is added. The resulting solution was stirred at 50-60°C for 20 h. Column chromatography (SiO_2, NH_4OH: MeOH, 1:9) afforded the *urea* **89** (0.14 g, 54%) as a beige gum.

R_f 0.1 (NH_4OH: MeOH, 1:9); IR (MeOH): ν_{max}/cm^{-1} 3467 (N-H), 3423 (N-H), 3028 (C=C), 1616 (C=O) and 1559 (C=C); δ_H (400 MHz, DMSO-d_6) 1.74 (4H, m, 2 x *CH_2*), 2.27 (6H, s, 2 x CH_3), 2.68 (4H, t, *J* 6.4 Hz, 2 x *CH_2*), 3.3 (4H, t, *J* 6.8 Hz; *CH_2*), 6.45 (H, d, *J* 5.4 Hz, H3'), 7.41 (H, dd, *J* 2.0 and 9.0 Hz, H6'), 7.75 (H, d, *J* 2.0 Hz, H8'), 8.21 (H, d, *J* 9.0 Hz, H5') and 8.37 (H, d, J 5.4 Hz, H2'), δ_C (100 MHz, DMSO-d_6) 31.8, 32.7, 415.5 (2 x CH_3), 42.9, 55.3, 58.7, 99.3, 118.2, 124.7, 124.7, 128.2, 134.0, 149.5, 149.8, 150.9, 152.6 and 159.2; Anal. Calc for $C_{18}H_{26}ClN_5O$; *m/z* 363.1826. Found MS (FAB) *m/z* 364.3 $(M+1)^+$.

Method (b): To a stirred solution of carbamate **91** (0.2 g, 0.90 mmol) in anhydrous *N, N'* dimethylformamide (11 cm^3), amine **107** (0.17 g, 0.99 mmol) is added. The resulting solution is stirred at 50-60°C for 20 h. The suspension was then transferred to a separating funnel and washed with water (2 x 25 cm^3) and extracted with ethyl acetate (3 x 50 cm^3). The combined organic extracts were dried over anhydrous sodium sulfate and concentrated. Column chromatography (SiO_2, $CH2Cl_2$: MeOH: Et_3N, 8:2:0.1) afforded the *urea* **89** (0.22 g, 67%).

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