# Totarol as a Privileged Natural Product Scaffold for Antimalarial Drug Discovery

**Mengisteab Bereketeab Gebregziabher**

**University of Cape Town**

**January 2007**

# Totarol as a Privileged Natural Product Scaffold for Antimalarial Drug Discovery

**A thesis submitted to the University of Cape Town for MSc Degree in Chemistry**

**By**

**Mengisteab B. Gebrezgiabher**

**Thesis supervisors:**
**Professor Kelly Chibale**
**Associate Professor Peter Smith**

Department of Chemistry
University of Cape Town
Rondebosch, 7701
Cape Town

## Acknowledgements

I would like to thank the Almighty God for his continuous blessings and for getting me out of numerous difficulties and leading me through to success.

I'd also like to extend my heartfelt thanks and appreciation to my supervisors, Professor Kelly Chibale and Associate Professor Peter Smith, for their continuous guidance, enthusiasm, and patience throughout the course of my MSc studies.

I'd like to thank my colleagues, Alex Chipeleme, Aloysius Nchinda, Chitalu Musonda, Claire Tacon, and the rest of medicinal chemistry research group for all their helpful discussions and advice. Many thanks are also extended to Dr. Cailean Clarkson, Division of Pharmacologyt University of Cape Town; my friends Dr. Henok Hadgu and Aklilu Asefaw for their continued help and advice. I thank Mr Pete Roberts, Mr Noel Hendricks and Dr. Philip Boshoff for their analytical services.

I would also like to thank my family; my mother Tsigeweini Tsehaye and my older brother, Haile Berekeateab, for all their sacrifices that have enabled me to continue with my studies and achieve my objectives. And many thanks to the rest of my family for all their support and encouragement through out my study.

I would like to thank Professor Ken Douglas, School of Pharmacy, University of Manchester UK, for all his unending support through out my writing up and beyond. Many thanks also go to Dr. Elena Bichenkova for her continuous encouragement and patience during my writing up. This thesis could not have been completed with out their support and advice.

**List of Abbreviations**

| | |
|---|---|
| AQ | Amodiaquine |
| CQ | Chloroquine |
| DHFR | Dihydrofolate reductase |
| DHPS | Dihydropteroate Synthase |
| µg | Microgram(s) |
| µM | Micromolar |
| Ar | Aromatic |
| br s | Broad singlet |
| Bn | Benzyl |
| $^{13}$C NMR | Carbon Nuclear Magnetic Resonance |
| d | Doublet (in 1H NMR) |
| DCM | Dichloromethane |
| dd | double doublet (in 1H NMR) |
| DMF | Dimethylformamide |
| DMSO | Dimethylsulfoxide |
| DMSO-*d*6 | Deuterodimethylsulfoxide |
| EI | Electron Impact |
| eq | Equivalent(s) |
| $Et_3N$ | Triethylamine |
| EtOAc | Ethyl acetate |
| EtOH | Ethanol |
| FAB | Fast Atomic Bombardment |
| 1H NMR | Proton Nuclear Magnetic Resonance |
| h | Hour(s) |
| Hex | Hexane |
| HRMS | High Resolution Mass Spectrometry |
| $IC_{50}$ | 50% Inhibitory Concentration |
| LRMS | Low Resolution Mass Spectrometry |
| m | Multiplet |

| | |
|---|---|
| MDR | Multi Drugt Resistant |
| m.p. | Melting point |
| m/z | Mass to charge ratio |
| MeOH | Methanol |
| min | Minute(s) |
| mM | Millimolar |
| mL | Millilitre |
| nM | Nanomolar |
| ppm | Parts per million |
| PFV | Parasitic food vacuole |
| Ph | Phenyl |
| *Rf* | Retention factor |
| RI | Resistant Index |
| s | Single (in 1H NMR) |
| SAR | Structural Activity Relationship |
| SI | Selectivity Index |
| t | Triplet (in 1H NMR) |
| THF | Tetrahydrofuran |
| TLC | Thin layer chromatography |

**Abstract**

Malaria is one of the major killer diseases in many countries of southern Asia, South America and Africa. Today over 40% of the world population is at risk from malaria[4]. It is the cause of 300 - 500 million infections and the death of more than 2 million people each year, most of whom are African children.[1, 5-6] Chloroquine has been the mainstream of malaria chemotherapy for nearly 60 years but widespread resistance now limits its usefulness. A continuous effort to find alternative antimalarials to this drug has led to the discovery of other effective antimalarials of different types, such as aminoquinolines, artemisinins and nucleic acid inhibitors. However, the emergence of multi-drug resistant strains of the malaria parasite has caused a marked increase in malaria related deaths, and there is a continuous need to develop other new antimalarials.[5]

Natural products play an important role in the antimalarial drug discovery process. Quinine and derivatives of artemisinin, the two most important drugs available to treat sever *falciparum* malaria, owe their origin to plants.[54] Furthermore, several effective antimalarials have been synthesized using quinine as a model compound (e.g. aminoquinoline antimalarials) or are results of relatively simple chemical modifications on the parent natural products (e.g. artemisinin). In this thesis, the natural product scaffold "totarol", which possesses inherent antiplasmodial activity, was used to design and investigate the antiplasmodial activity of three different compound classes namely: Mannich bases, *ß*-aminoalcohols and semicarbason derivatives. The aim was to incorporate important drug fragments into a natural product scaffold with intrinsic antiplasmodial activity, possibly leading to the discovery of new totarol based antimalarials.

In the first class of compounds, a series of novel Mannich base derivatives of totarol have been designed and synthesized to mimic the known Mannich base antimalarials, such as amodiaquine, amopyroquine and other functionally related antimalarials. These compounds differed from each other in the nature of their amino methyl side chain which was varied to include different structural requirements. It was found that the secondary amine Mannich bases possessed better antiplasmodial activity against chloroquine

sensitive strains of the parasite than the tertiary amine Mannich bases. However, none of the synthesized compounds were found to be as active as the parent compound (totarol). Based on the preliminary biological evaluations of the synthesized Mannich base derivatives, only selected primary amine derived *ß*-amino alcohols were synthesized in the second class of antimalarials. However, none of the synthesized compounds were found to possess significant antiplasmodial activity. This was consistent with previous findings whereby the presence of protonatable nitrogen at the beta position could be a necessary structural feature for high antiplasmodial activity with totarol-derived amino alcohols.[111] The semicarbazone derivative of totarol also did not show any antiplasmodial activity. Furthermore, none of the 2-isopropylphenol derived Mannich bases showed significant antiplasmodial activity; suggesting the importance of the diterpenoid backbone of totarol, in its inherent antiplasmodial activity.

# Table of Content

# CHAPTER ONE

## 1. INTRODUCTION

### 1.1. History of Malaria

Malaria is a tropical disease caused by minute parasitic protozoa of the genus Plasmodium and is transmitted by a female anopheles mosquito [1]. Malaria is a very old disease and is thought to have been one of the major killer diseases of prehistoric man. According to the literature, malaria possibly originated from Africa and spread to other parts of the world by way of human migration[2]. The earliest recorded description of what could have been malaria as a human disease was around 2700 BC. Hippocrates, the first malariologist, classified the fever now known to be malaria from fevers related to other infectious deseases in 460-370 BC; but was left unacknowledged for 2000 years. In the 14$^{th}$ century, Greeks related the fever to an exposure to swamps. However, swamp fevers were considered as diseases caused by inhalation or ingestion of swamp water or gases (poisonous gases). By the mid-16th century, Italians were using the term "Mal-aria" (spoiled air) to specify the cause of such a disease. In 1880s, Charles Louis Alphonse Laveran for the first time recognized the malaria parasite in human blood. Ronald Ross then established the life cycle of the malaria parasite in 1898, for which he received the Nobel Prize in 1902.[3]

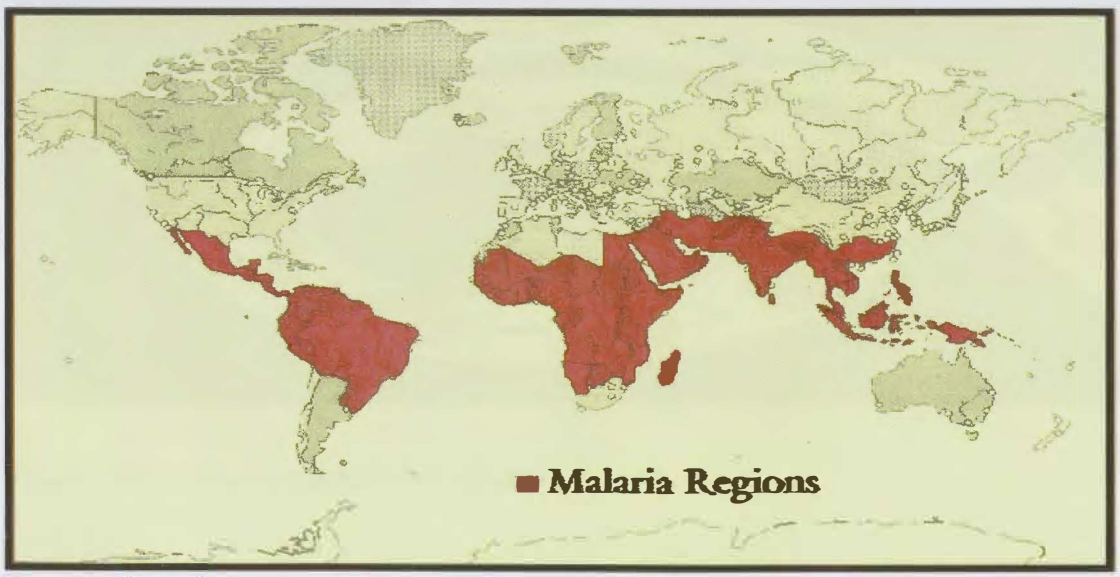



**Figure 1.1** World malaria distributions[2]

According to the literature, today over 40% of the worlds population is at risk from malaria[4]. It is the cause of 300 - 500 million infections and the death of more that 2 million people each year, most of whom are African children[1, 5, 6]. Although the extent of suffering caused by malaria epidemics is not adequately recorded, it is estimated that close to 110 million Africans are at risk of the epidemic.[7]

### 1.1.1. The Malaria parasite life cycle

Human malaria is caused by four species of the genus Plasmodium, namely *P. falciparum*, *P. vivax,* P. *ovale and P. malaria* [5]. Out of these four species, *Plasmodium falciparum* and to a much smaller extent, *Plasmodium vivax* are responsible for the majority of the cases and death from malaria, with *P. falciparum* accounting for 90% of cases in sub-Sahara Africa and 50% of the cases in Asia and Latin America. [6, 8]

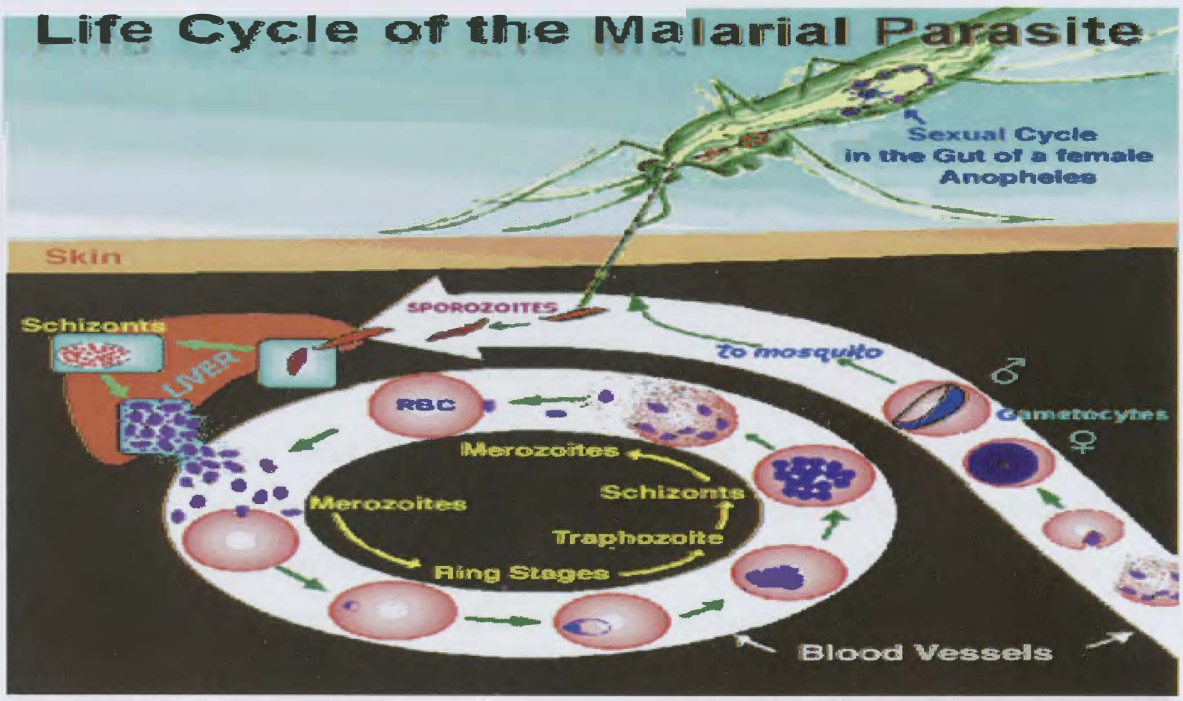



**Figure 1.1.1.** Malaria parasite life cycle[3]

The life cycle of the malaria parasite has two phases: a sexual reproductive stage which occurs in the midgut of the mosquito, and an asexual reproduction phase which takes place in the human host[1]. The human part of the cycle can be further subdivided into two important phases: the exoerythrocytic (hepatic) phase and the erythrocytic phase. Sexual reproduction of

gametocytes occurs in the gut of the mosquito and leads to the formation of zygotes that bury themselves in the gut lining of the mosquito. Within 18 to 24 hours, the zygote is transformed into a slowly motile ookinete which is eventually transformed into oocyts beneath the basement membrane of the midgut epithelium. After 7-15 days, depending on the plasmodium species and the ambient temperature, a single oocyst forms more than 10,000 sporozoites which will then migrate into the salivary glands of the mosquito.[1, 8]

A bite by an infected female mosquito transmits the sporozoites to humans. The sporozoites enter the blood stream and rapidly travel to the liver cells. They will then develop into exoerythrocytic schizonts during the next 5 to 15 days. At this stage (exoerythrocytic), the parasite multiplies asexually and hepatic schizonts contain 10,000 to 30,000 merozoites, which simultaneously burst the host cell and enter the blood stream. These merozoites then invade the red blood cells within 30 seconds. A further asexual reproduction of the merozoites at this stage, leads to the formation of erythrocytic schizonts through a blood cycle consisting ring, trophozoite and blood (erythrocytic) schizonts. The erythrocytes containing the mature schizont will then burst and release the merozoites, which will invade other red blood cells. Upon invading the red blood cell, some of the asexual parasites are converted to gametocytes (micro gametocytes and macro gametocytes), which may be taken up by an Anopheles mosquito to complete the cycle.[1, 8]

#### 1.1.1.1. Hemoglobin Proteolysis

The malaria parasite lacks the capacity to synthesize amino acids, which it needs for replication[6]. Its need for amino acids is mostly met by the hemoglobin catabolism of the host cell.[9] Hemoglobin comprises 95% of the cytoplasmic protein of the red blood cell, and the parasite degrades between 60-80 % of this hemoglobin during its intraerythrocytic stage of the life cycle.[6, 10]

Hemoglobin catabolism occurs within a specialized acidic lysosomal organelle called the digestive food vacuole (pH 4.5-5.2) [11]. This is catalysed by the combined action of cysteine[12] and aspartic proteases[13] and results in the release of protein fragments. This occurs through specific interactions with the substrate. That is, prior to the hydrolysis proteases bind the peptide substrates in their active site pockets. The carboxylic terminal from the scissile bond of the peptide and its coresponding side chain of the enzyme substrate are indicated as *prime*

sides (P1`, P2`, P3` P4`and S1`, S2`, S3`, S4`) and the amino terminal from the scissile bond and its corresponding side chain of the enzyme are termed as *non-prime* side (P1, P2, P3, P4 and S1, S2, S3, S4). This is illustrated in figures 1.1.1.1A and 1.1.1.1B.

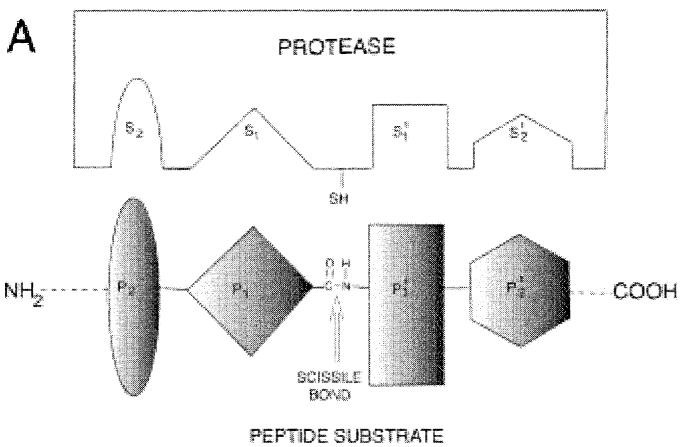



**Figure 1.1.1.1A** Schematic presentation of the peptide substrate interaction with the active site pockets of the cysteine protease. Amino acids from the peptide are denoted by "P" and the subsites within the cysteine protease are denoted by "S".[14]

Hemoglobin degradation begins with the initial cleavage between the α33Phe and α34Leu residues of the erythrocytic proteins by the aspartic proteases plasmepsin I and II. This cleavage is from the site responsible for holding the hemoglobin tetramer together when oxygen is bound. This exposes the protein for subsequent proteolysis of the remaining residue by other enzymes.[10, 15]

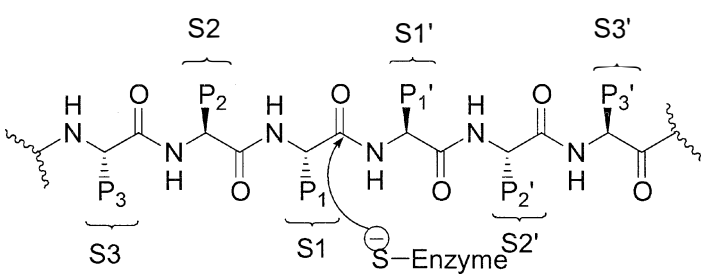



**Figure 1.1.1.1B** Standard nomenclature for protease substrate cleavage. Pn, P3, P2, P1, P1', P2', P3', Pn', designate the amino acid side chains of a peptide substrate. Cleavage occurs between the P1 and P1' residues of the amino acid side chains. The corresponding binding sites in the protease active-site are designated as Sn, S3, S2, S1, S1', S2', S3', Sn'.[16]

These two enzymes have specificities for these cleavages. Plasmepsin I prefers phenylalanine in the P1 position, while plasmepsin II prefers hydrophobic residues, leucine in the P1` position. Moreover, the aspartic proteases also maintain the differences in their sensitivity to various peptidomimetic inhibitors [6, 10]. The large fragments produced by the aspartic proteases are subsequently proteolysed into smaller fragments, by the action of cysteine protease called falcipains 2 and 3 [13]. These enzymes have substrate specificity similar to that of cathepsin L. The chemical nature of the protease S2 pocket, and in particular the amino acid residue 250 (papain numbering), is thought to be critical for substrate preferences.[10, 14]

The recent literature suggests that these smaller fragments are further degraded by a metalloprotease (falcilysin) found in the digestive vacuole and is proposed to be the last step of hemoglobin degradation before the small peptides are transported into the cytoplasm[17]. Finally, these fragments may further be degraded into individual amino acids in the cytoplasm. This mechanism was supported by the fact that no single amino acid was found in an extract of the digestive food vacuole, to suggest the vacuolar exopeptidase activity.[9, 17]

#### 1.1.1.2. Heme Detoxification

Hemoglobin proteolysis yields the required amino acids for the parasite. During the proteolysis a free heme ($F^{3+}$) is released into the vacuole. It is known that the heme concentration within the digestive vacuole could reach up to 400 mM [6]. At such concentration; heme is toxic to the parasite and is known to disrupt the metabolic function of the parasite *via* enhanced peroxidation of lipid membranes[18], inhibition of enzymes [19], and the generation of oxidative free radicals. [20]

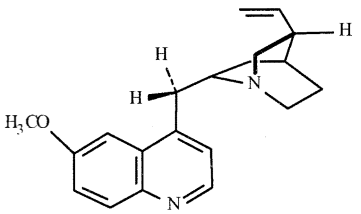
To counteract the effect of heme toxicity, the malaria parasite has developed a detoxification mechanism, by which the free heme is converted into a crystalline heme aggregate called hemozoin.[10] Hemozoin is a dimeric heme unit linked by reciprocating iron-carboxylate bonds to one of the propionic acid side chains of each porphyrin. Additionally, the Fe (III) PPIX dimers are hydrogen-bonded into chains, forming an extended crystalline network. Hemozoin formation is thought to be a lipid mediated process.[6]

### 1.1.2. Malaria Chemotherapy

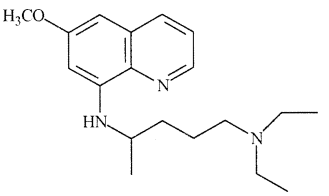
Anti-malarial drugs in use today target different stages of the malaria parasite, of which most of them act on the intra-erythrocytic phase of development.[3] According to their mode of action, the majority of antimalarial drugs fall into three classes:

Drugs Acting on Heme Detoxification (Quinoline containing drugs)

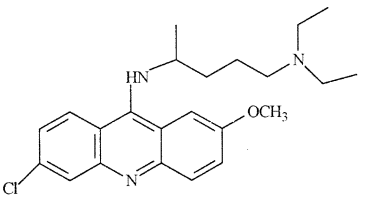
Malaria chemotherapy began in the 17th century after a patient took a remedy made from the bark of the cinchona tree. This led to the isolation of quinine from the Peruvian bark in 1820 [3]. Quinine **1** has been used for more than three centuries, and until the 1930`s it was the only effective agent for the treatment of malaria [2]. However, quinine has liabilities associated with toxicity (such as tinnitus) and requires long durations of administration and is now used only for treating severe falciparum malaria [22]. The strong demand for an effective quinine substitute led to the discovery of the first 8-aminoquinoline, pamaquine **2** in 1925 and quinacrine **3** (the first effective drug against falciparum malaria) in 1932. However, this drug was shown to have shortfalls as well as side effects. This subsequently led to the discovery of a fully synthetic 4-aminoquinoline, chloroquine (CQ) **4** in 1934.[23]



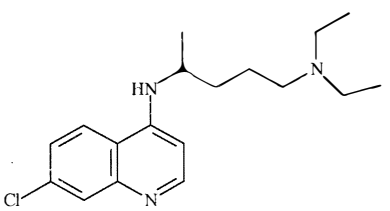
**Quinine (1)**



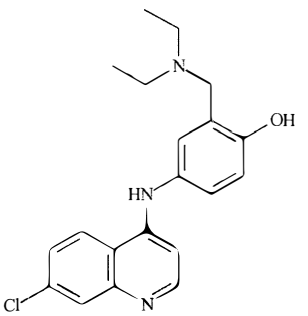
**Pamaquine (2)**



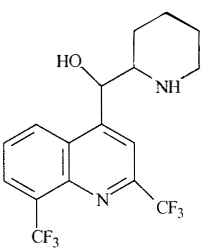
**Quinacrine (3)**



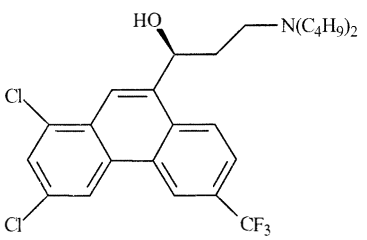
**Chloroquine (4)**



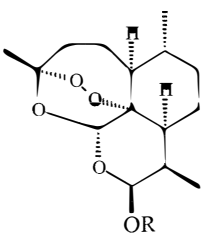
**Amodiaquine (5)**



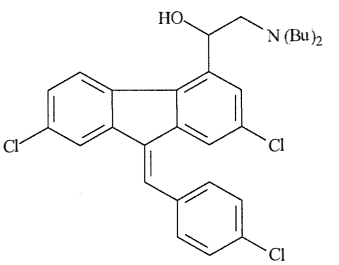
**Mefloquine (6)**



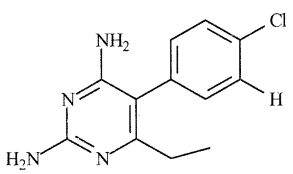
**Halofantrine (7)**



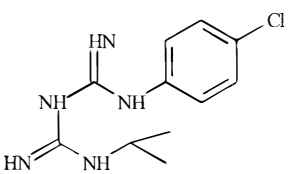
| | |
|---|---|
| R=H | **Dihydroartemisinine (8)** |
| R=Me | **Artemether (9)** |
| R=Et | **Arteether (10)** |
| R=$CO(CH_2)_2CO_2H$ | **Artesunate (11)** |



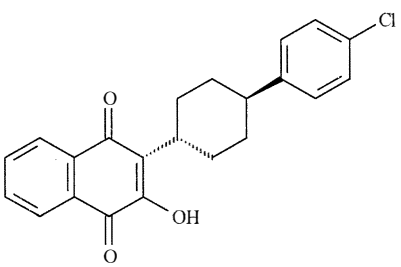
**Lumefantrine(12)**



**Pyrimethamine (13)**



**Proguanil (14)**



**Atovaquone (15)**

**Figure 1.1.2** Chemical structures of common antimalarial agents

Chloroquine has been a safe, cheap and effective antimalarial drug for several decades, and has been the agent of choice over the other drugs. However, parasite resistance to this drug was first observed in 1957 and spread to east Africa in the late 1970s and became a major problem in several areas of the continent by the mid-1980s [24]. Amodiaquine (AQ) **5** was introduced in the late 1950s and has been shown to be more effective than chloroquine in areas of high CQ resistance. The use of AQ declined in the 1990s, following its first report of agranulocytosis by WHO, when used for prophylaxis[25, 26]. However, an increase in its use is being reported recently due to its high degree of efficacy against all CQ-resistant strains[22].

Mefloquine **6** is a quinoline methanol derivative and was introduced in 1971. It is structurally related to quinine, and has been used against multi-drug resistant strains of the malaria parasite. It has long half-life, and been a good prophylactic drug. However, a widespread resistance, and undesirable side effects (occasional neuropsychiatric disturbances) led to a decline in its use.[2, 22] Halofantrine **7** is a very good antimalarial drug introduced in the 1980s. Unfortunately resistance and side effects of this drug have been reported. [2]

Drugs that induce Oxidative Stress (Artemisinins)

This class of compounds owes their origin from artemisinin, the active ingredient of a Chinese herbal remedy 'qinghao' (*Artemisia annua*) and was first isolated in the 1970s [24]. Artemisinin derivatives include artemether **9**, arteether **10** and artesunate **11**, which are all metabolized to dihydroartemisinin **8** (the main active agent in the body) [22]. These drugs target the gametocytes (the sexual stage of the parasite), which infect the mosquitoes. Their mode of action is known to be the generation of oxidative stress. They are fast-acting drugs, and are normally administered in combination with other effective long acting antimalarials, for reduced treatment time and increased individual compliance [1]. Mefloquine is a commonly recommended drug, for combination therapy with artemisinin [1, 22]. In recent times, a combination of artemether with lumefantrine **12** is increasingly being used and is thought to have the same activity and lesser toxicity than the mefloquine - artesunate association [22], the current standard treatment against multidrug-resistant malaria.[1]

Nucleic Acid Inhibitors

The third class of antimalarials falls under Nucleic acid inhibitors. They are a result of knowledge of cell biology and synthetic medicinal chemistry [22]. These drugs target the

enzymes of the folate pathway and act at all growing stages of the asexual erythrocytic cycle and early gametocytes [21]. Some of the common antifolate drugs include: (i) drugs which inhibit dihydrofolate reductase (DHFR) such as 2, 4-diaminopyrimidine (pyrimethamine) **13**, biguanide and triazin metabolites, quinazolines; and (ii) drugs which interfere with the action of the dihydropteroate synthase (DHPS) such as sulfonamides and sulfones. Folate antagonists were among the most widely used anti-malarials. However, the appearance of widespread resistance to these drugs, has limited their individual effectiveness.[21] At present they are being used in combination therapies and the most commonly used combination drug regimen is (pyrimethamine) /sulphadoxine known as Fansidar. Yet, there is still a growing concern of resistance and associated toxicity when used as prophylactic drug.[22, 24]

Another very important antifolate drug is Malarone; a combination of proguanil **14** and atovaquone **15,** used both for treatment and prophylaxis. It is a very effective antimalarial drug and has the advantage of being used during pregnancy [28]. This treatment has been reported to be 95% effective against chloroquine resistant *Plasmodium falciparum*[2]. However, it is a very expensive drug and is not easily accessible for use in developing countries [22]. Atovaquone is generally known to interfere with mitochondrial electron transport [1, 21, 24] but its synergic activity with proguanil is not clearly known, even though some literature reports relate to its interference with mitochondrial membrane potential. [21, 27]

## 1.2. Approaches to antimalarial chemotherapy

The emergence of multi-drug resistant strains of the malaria parasite has caused a huge increase in malaria related deaths. With the current available drugs falling short of combating the resistant strain of the parasite, there is a continuous need to develop new antimalarials [5]. However, malaria remains to be a disease of poverty [8] and irrespective of the increase in scientific knowledge, there is no sufficient market driven investment toward antimalarial drug research and development needed for developing new and inexpensive antimalarials [22, 29]. According to Rosenthal, "any approach to antimalarial drug discovery should take into account the specific concerns of malaria drug development. That is the requirement for very inexpensive, simple to use antimalarials as well as the need to limit the cost of drug discovery as the malaria markets are primarily in poor countries" [29]. Among the most important approaches used today include:

### 1.2.1. Optimization of therapy with existing drugs

This involves optimization of the activities of current drugs using new dosing regiments or formulations and combination therapies. The combination therapy may involve new agents or new combination of older drugs [29]. Use of combination therapy has two advantages:

I. It improves antimalarial efficiency through additive or synergistic antiparasitic activity.
II. It slows the development of parasite resistance to the new agents. It is more efficient if the combination involves new agents against which parasite resistance has not yet developed.

### 1.2.2. Development of analogues of existing agents

This approach does not need the knowledge of the mechanism of action or the biological target of the parent compound and simply involves improving the activity of existing antimalarials by chemical modifications[29]. This method has been extensively exploited and was responsible for the development of many effective antimalarials today. For example, chloroquine, primaquine and mefloquine were results of chemical modifications upon quinine [30]. More recently, chloroquine related 4-aminoquinolines have been shown to offer the antimalarial potency of the parent drug, even against chloroquine resistant parasites.[26, 31-33]

### 1.2.3. Compounds active against other diseases

The third approach involves identifying agents or drugs that are developed for treatments of other diseases and are also active against different targets in the malaria parasite. Considering funding problems associated with antimalarial drug discovery, this has an advantage over the other methods. That is, as they are already developed for human diseases and will not be expensive to develop as antimalarials; especially after the patent expires. However, the cost of production for drugs varies greatly and drugs that are developed for diseases of rich nations such as cancer will be too expensive to produce as antimalarials[29]. Antagonists, tetracycline and other antibiotics were developed for their antibacterial property and were later found to be active against malaria parasites.[34]

### 1.2.4. Drug resistance reversers

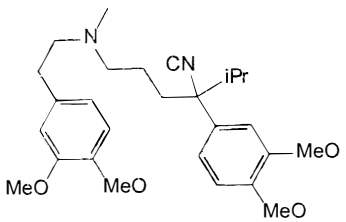
The continuous increase in malaria associated mortality & morbidity is primarily associated with the spread of chloroquine resistant strains of *P. falciparum* and the emergence of multi-drug resistant strains. Therefore, attacking the mechanism by which the resistance developed (resistance reversing) is an important approach toward antimalarial drug discovery and has attracted the attention of many researchers[35]. According to Batra, "the key point in designing any strategies toward developing resistance reversing agents is, the identification of the biochemical event, which may be correlated, with the development of the resistance".[36]

It has been found that the concentration of chloroquine within the acidic vacuole of the resistant strain of the parasite is much lower than that of the sensitive strain. It is therefore, understood that the parasite is surviving by reducing the accumulation of the drug within the acidic vacuole. However, the mechanism by which this happens is not clearly known [23]. There have been a variety of opinions regarding the mechanism of chloroquine resistance. One of the most controversial mechanisms being the P-glycoprotein-mediated efflux pump mechanism in *P. falciparum.* That is, if cells are over exposed to drugs they overexpress the protein involved in drug efflux (modulated by P-glycoprotein), which then increase pumping of the drug out of the acidic vacuole [37]. This was compared to the multi-drug resistance (MDR) phenotype observed in mammalian cancer cells [38].

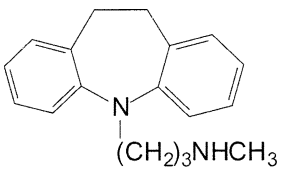
Another very important proposal is a mechanism involving the alteration of the pH of the digestive vacuole or change in the flux of chloroquine across the parasite's cytoplasmic or digestive vacuole membrane as a result of multiple mutations in the gene *PfCRT* (plasmodium falciparum chloroquine resistant transporter). The gene believed to be likely functioning as a transporter in the parasites digestive vacuole [23, 39, 40]. This has received wider acceptance over the first mechanism [37]. Some literature reports indicate the increase in $IC_{50}$ of chloroquine in a chloroquine-resistant strain in vitro with Pgh-1 [41]. However, this was related to fitness adaptation of Pgh-1 as a result of the physiological changes from *PfCRT* mutations rather that from chloroquine resistance [23].

Therefore, since it is mutation of this specific gene that has been associated with drug resistance in malaria, exploring this mechanism of resistance and targeting its components could lead to the development of chemosensitizers [37]. Chemosensitizers are drugs that could

be used in combination with other drugs for which resistance has already developed (e.g. Chloroquine) and make the resistant plasmodia susceptible to the combination therapy.[35]




**Verapamil (16)**




**Desipramine (17)**

**Figure 1.2.4** Examples of known chemosensitizers

A wide range of drugs have been shown to reverse the resistance of *P. falciparum* to chloroquine *in vitro*. Some of these drugs include: the antihypertensive drug verapamil **16** [42], the antidepressant desipramine **17** [43] and the calmodulin antagonists (trifluoperazine and phenothiazines) [44]. However, some of the resistance reversers are associated with unwanted side effects and others require unacceptable doses to be used clinically [29, 44]. Efforts to design new reversers of chloroquine resistance are underway [36, 45]. Thus, the identification of effective resistance reversers could lead toward re-introducing the once most effective antimalarials such as chloroquine as first-line therapy.

### 1.2.5. Compounds active against new targets

This is the most innovative approach to chemotherapy. It involves identification of new targets and subsequent discovery of compounds that act on these targets. Although this process is laborious and inefficient, there has been progress toward the characterisation of the biology of the malaria parasite. Thus, the availability of a parasite genome could lead to the discovery of a gene based therapy for malaria.[29]

### 1.2.6. Natural Products

Plants have been the source of important medicines for many centuries and are still continuing to form the bases for many traditional medicinal systems [46]. According to the WHO, the majority of the world inhabitants rely almost entirely on plant-derived medicines. It is estimated that about 200,000 species of higher plants are being used medicinally[47] and close to 60 million adults use herbal medicines each year [48]. Analysis on prescription drugs in the US and Canada shows that 25% of the drugs were found to contain compounds either isolated or derived from plant sources [49]. Therefore, screening plant products with specific clinical activity could result in better lead discovery as opposed to random screening. More importantly, they could be used as a model compounds for the development of fully synthetic or semi-synthetic drugs.[29]

According to recent literature reports, screening of natural product derivatives is even more effective than screening natural products alone [50]. Furthermore, the analysis of sources of new and approved drugs for the treatment of human diseases over the period 1981-2002 shows the significant role of natural products in the drug discovery and development process [51,52]. According to the reasearch, the utility of natural products as sources of novel structures, but not necessarily the final drug entity, is still alive and well. Based on the data presented, an average of 62% of the cancer drugs, 79% of the antibacterials owe their origin to a non-synthetic chemical entities.[53]

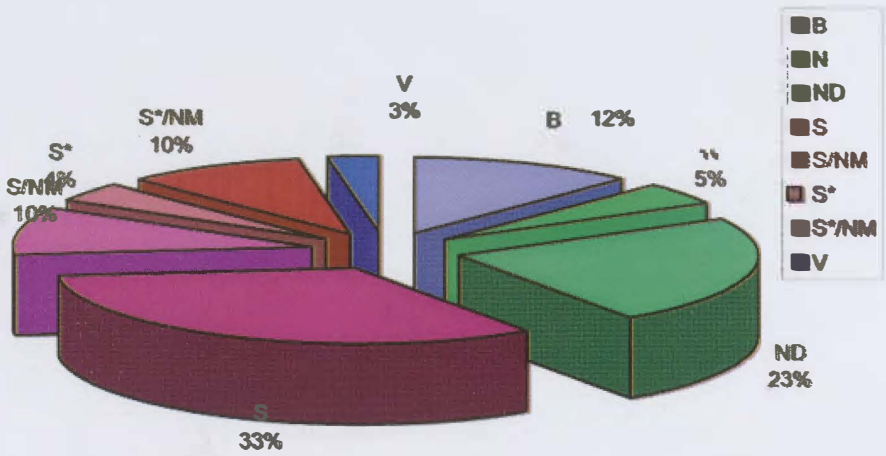



**Figure 1:** All new chemical entities, 1981-2002, by source (*N* - 1031)

The Major Categories of Sources used are as follows:

"**B**": Biological; usually a large (>45 residues) peptide or protein either isolated from an organism/cell line or produced by biotechnological means in a surrogate host.

"**N**": Natural product.

"**ND**": Derived from a natural product and is usually a semisynthetic modification.

"**S**": Totally synthetic drug, often found by random screening/modification of an existing agent.

"**S***": Made by total synthesis, but the pharmacophore is/was from a natural product.

"**V**": Vaccine.

"**NM**": Natural Product Mimic (see rationale and examples in ref. **52**).

Except for "**NM**" the amplification as to the rationales used for categorizing using the above subdivisions, could be consulted from ref. **51**.

Overall, of the 1031 new chemical entities (NCEs ) covering all diseases/countries/ sources in the years 1981-2002, 43% were synthetic in origin, but if one removes the S/NM category from this total, then the S category falls to 33%. Thus depending upon the categories, the gross figures for categories other than synthetic range from 57% to 67% over all diseases.[52]

## 1.3. Natural products as sources of new chemical scaffolds for antimalarial drug discovery

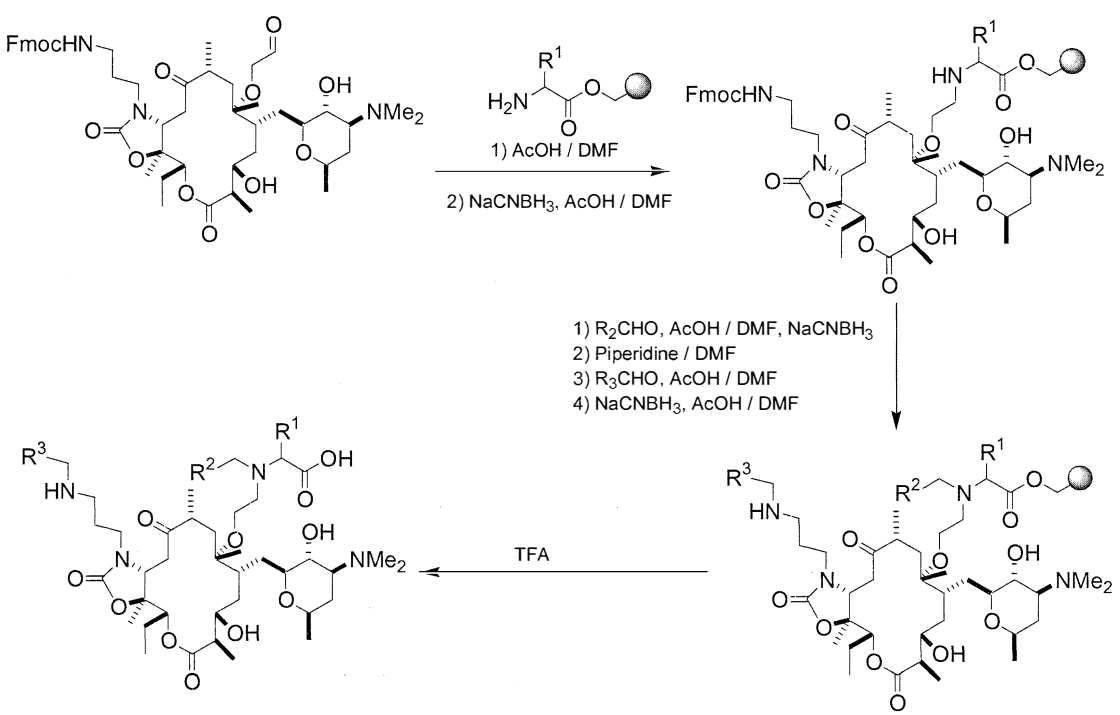
Natural products play an important role in the antimalarial drug discovery process. Quinine and derivatives of artemisinin, the two most important drugs available to treat sever falciparum malaria; owe their origin to plants [54]. A series of very effective 8-aminoquinolines and 4-aminoquinolines derivatives (including chloroquine) have been synthesisized using quinoline as a model compound. Relatively simple chemical modifications to artemisinin (parent compound), has led to a series of highly potent antimalarials such as artemether **9**, arteether **10** and artesunate **11** which are playing an important role in the treatment of malaria [55]. However, the cost of these compounds may be limiting, and so efforts to design fully synthetic endoperoxides that are less expensive to produce are an important priority.[56-57]

### 1.3.1. Strategies for generating libraries of natural products

Natural products are biologically validated starting materials for the design of combinatorial libraries and have received increasing attention during the last 5 years. The literature reveals that the hit rates in high throughput screens of natural product collections are often higher than the rate found for large classical libraries obtained from combinatorial chemistry efforts [50]. There are several strategies developed for generating libraries of natural product derivatives, which are briefly discussed in the following sections **1.3.1.1 – 1.3.1.3**.

#### 1.3.1.1. Modification of Natural Product Core Structure on Solid Phase

The use of a solid phase synthesis for the development of a combinatorial library is a powerful approach for rapid identification of new biologically relevant compounds. However, the efficiency of this library mainly depends on the choice of the original molecular scaffold. Therefore, the method requires a prior validation of the core structure against the biological target. [50, 58]




**Scheme 1.3.1.1** Library of erythromycin analogues [59]

This method has been employed in several reactions. Some of these include the preparation of libraries of sarcodictyin [53], the synthesis of the protein kinase C (PKC) activator [58], and more recently the combinatorial synthesis of macrolide analogues based on erythromycin A (Scheme **1.3.1.1**) [59]. Erythromycin A is an extremely important antibiotic with high efficacy and low toxicity and is used for the treatment of gram positive-bacteria. [59]

### 1.3.1.2. Total Synthesis of Molecular Skeleton on solid Phase

This involves building up the entire core structure on solid support. It is very challenging and may require a multi-step synthesis. However, it allows maximum diversity in the core structure (e.g. ring size, chain length), both by variation of stereochemistry as well as the introduction and derivatization of functional groups [50]. The application of this method is best exemplified by the successful synthesis of epothilone A by the Nicolaou group (Scheme **1.3.1.2**) .It was the first example to demonstrate the principal feasibility of multi step natural product synthesis on solid support. [60]

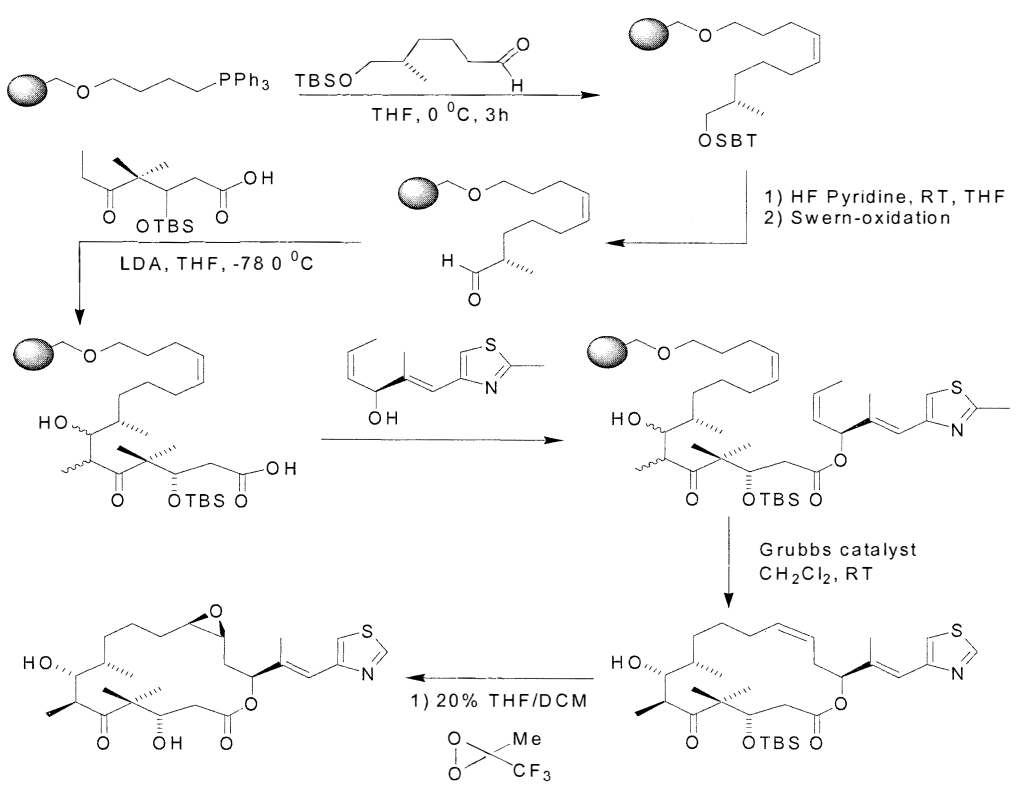
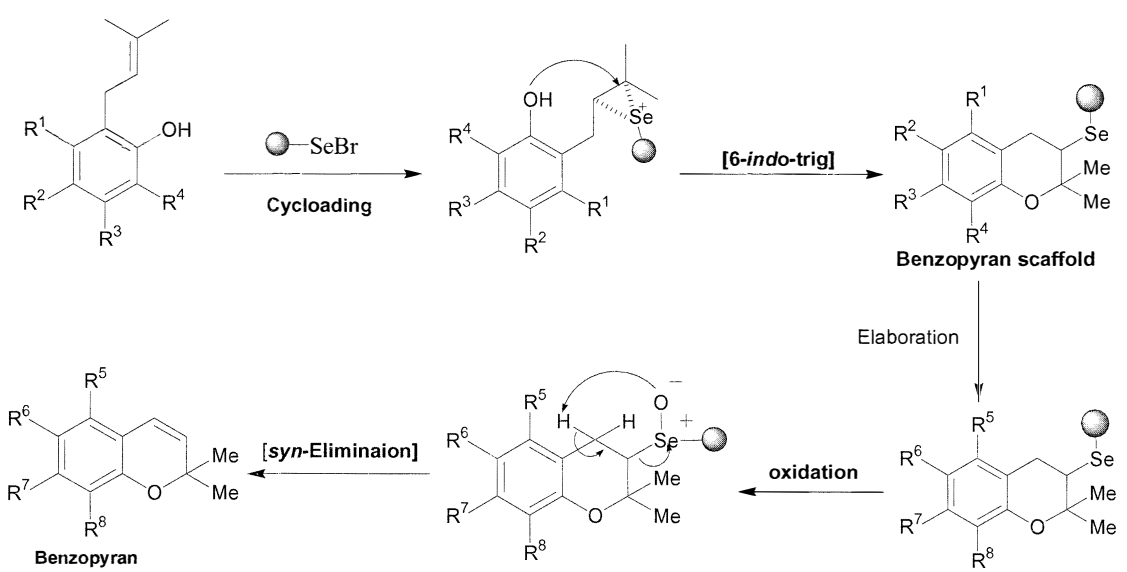



**Figure 1.3.1.2** Solid phase synthesis of epothilone A [60]

### 1.3.1.3. Production of Combinatorial Libraries around Privileged Structures Identified from Natural Products

The third approach for construction of libraries of natural product and natural product-like compounds, involves the development of combinatorial libraries around privileged structures identified from Natural products.[50] According to Evans, Privileged structures are compound classes; which can bind to various proteinous receptor surfaces [61]. Nicolaou and his coworkers, recently applied this approach to the construction of a 10,000-member natural product-like library, based on 2,2-dimethylbenzopyran moieties as shown in scheme 1.3.1.3 [62-63]. According to the group, the success of this approach depends on the proper choice of the structural motif to be used as a template for library development. That is, the structure should:

- Be present in a large number of natural products with diverse biological activities.
- Accommodate installation of a maximum degree of diversity.
- Have one or more rigid ring systems (for potential binding).
- Be sufficiently lipophilic (to ensure cell membrane penetration).




**Scheme 1.3.1.3** Synthesis of benzopyran scaffold for the production of natural product-like combinatorial library [62]

Some of the synthesized compounds include: libraries of chalcones, glycosides and pyranocoumarins. The group has summarized the strategy of developing a natural product-like combinatorial library as shown in the figure **1.3.1.3**.

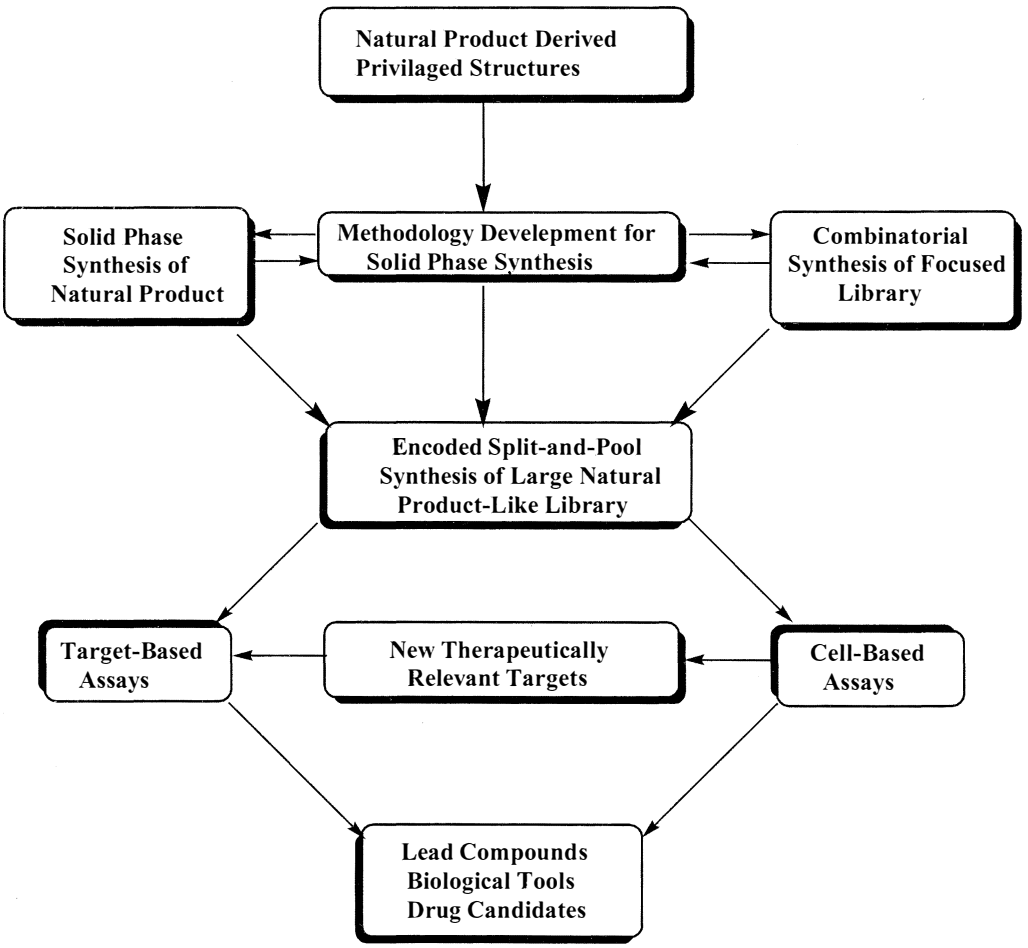



**Figure 1.3.1.3** Overall strategy for the design, synthesis and application of natural product-like combinatorial library based on privileged structures [62]

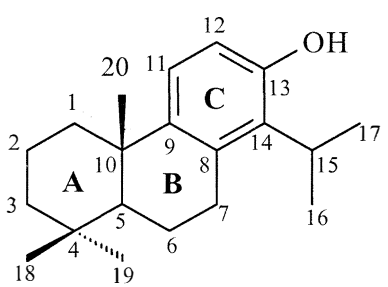
# CHAPTER TWO

## TOTAROL AS A PRIVILEGED NATURAL PRODUCT SCAFFOLD

### 2.1. Introduction

### 2.1.1. Totarol and its Chemistry

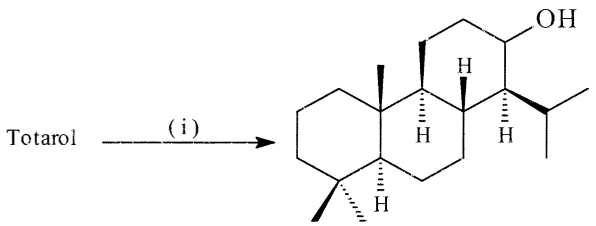
(+)-Totarol **18,** a naturally occurring phenolic diterpene with m.p. 132 $^{0}$C and $[\alpha]_D$ + 410, was first isolated from the hexane extract of the heartwood of the New Zealand native tree "*podocarpus totara*" in 1910 [64-65]. It was the first compound to be discovered with the chemical formula $C_{20}H_{30}O$. Due to its very slow acetylation, totarol was believed to have a tertiary alcohol and was named accordingly. In 1951, Short and Wang identified the structure of totarol (Figure 2.1.1) and realized that it does not obey the isoprene rule as shown in scheme 2.1.2B [66]. It was the first known exception among the diterpenoids and confirmation of this unusual structure came with the total synthesis of (±) totarol by Baltrop and Rogers[67], which showed a similar IR spectrum to that of the natural product. Chow and Erdtman[68] then established the absolute stereochemistry of totarol as a *trans* A/B ring junction of the conventional steroid type by optical rotary dispersion measurements and by direct correlation with dehydroabietic acid. The nomenclature of totarol as totara-8,11,13-trien-13-ol is based on the parent hydrocarbon totarane (**19**), which can be formed from catalytic hydrogenation of totarol shown in scheme 2.1.1A.[64]




**18**

**Figure 2.1.1** Structure of totarol

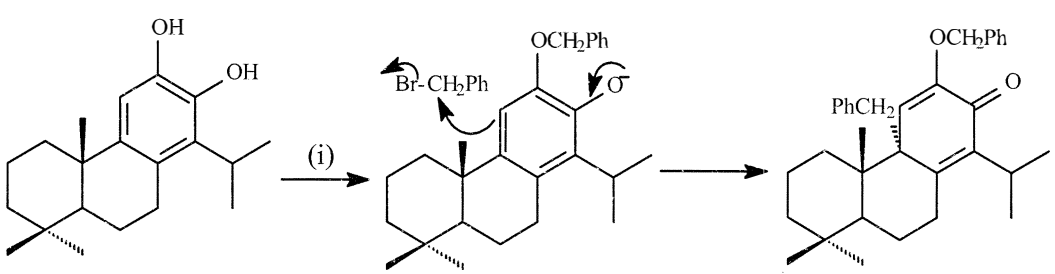
Totarol is found in a large number of plants, some of which are *Podocarpus totara*, *Podocarpus acutifolias*, *Dacrydium*, *Cupressus*, *Juniperas* etc [64, 65]. The most abundant source is the heartwood of *podocarpus totara*, in which totarol is the major constituent of the plant. A yield of up to 8% by weight has been reported from the timber of this plant.[65]




**19**

**Scheme 2.1.1A:** (i) $H_2$/Pd/C/HOAc/700/30 p.s.i.[64]

Unlike with the normal isoprenes, the isopropyl group of totarol is at the C14 position. This isopropyl group exerts steric hindrance upon the phenolic group at C13 and prevents the formation of intermolecular hydrogen bonding in liquid state. This is consistent with the sharp O-H absorption observed at 3630 $cm^{-1}$ in the IR spectrum, in contrast to the ordinary phenol's broad O-H absorption at 3300 $cm^{-1}$ [66]. This accounts for the slow rate of acetylation of totarol and a decrease in its apparent polarity [64]. In line with the above facts, an attempt to alkylate 12-hydroxytotarol (**20**) with benzyl bromide afforded alkylation at the less hindered hydroxy group C12 and elsewhere in the molecule, leaving the C13 phenolate unchanged to give the cross-conjugated dienone (**21**) as shown in scheme 2.1.1B. Furthermore the downfield (1.33 ppm) chemical shift of H16 and H17 in totarol (**18**) suggests that the methyl groups of the C14 isopropyl moiety scissor the phenolic group as the result of non-bonding interaction with the methyl hydrogens at C7. This unusual position of the isopropyl group on carbon 14 accounts for totarol's unique chemistry.[64]

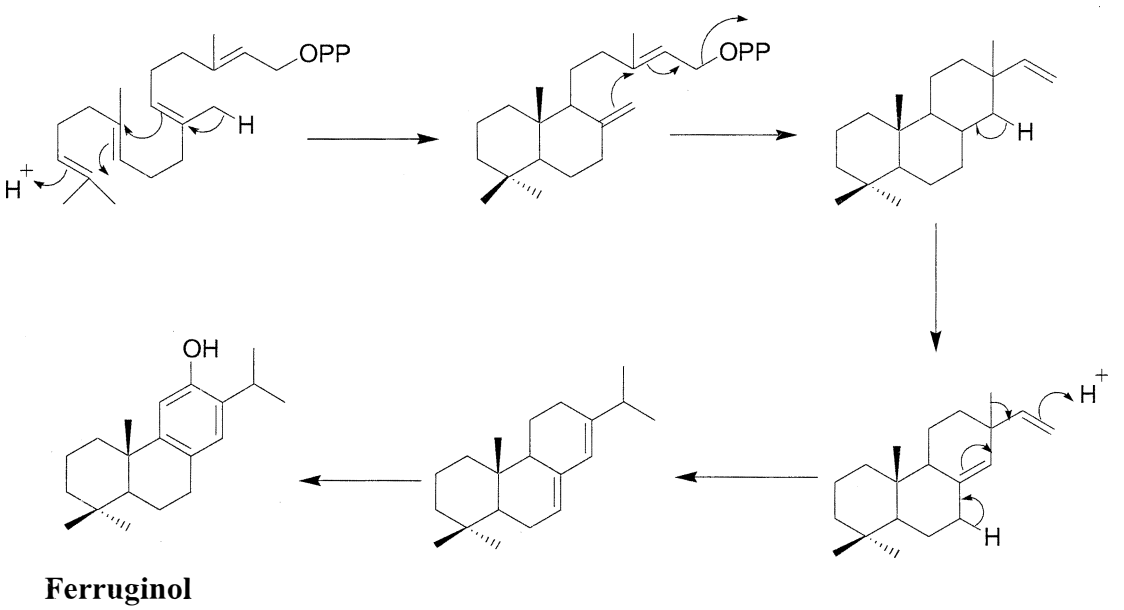



**20** **21**

**Scheme 2.1.1B**: (i) $PhCH_2Br$.[64]

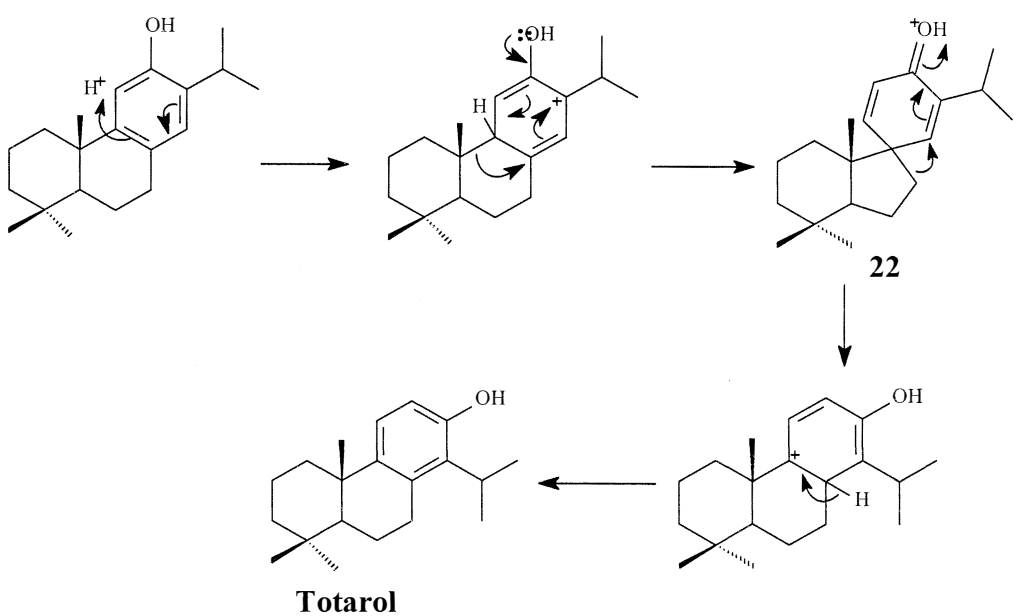
### 2.1.2. Biogenesis of Totarol

Totarol is not the result of diterpenoid biogenesis. A conventional diterpene biogenesis (scheme **2.1.2A**) occurs *via* ring closure of geranyl geranyl pyrophosphate to give a labdane skeleton, which then undergoes methyl group migration followed by oxidation, with accompanying aromatisation to give a normal diterpene, ferruginol, with the isopropyl group at the C 13 position. [64, 69]




**Ferruginol**

**Scheme 2.1.2A** Conventional diterpene biogenesis[64]



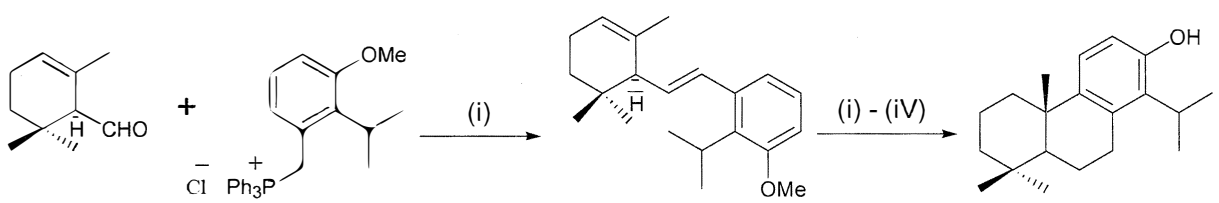

**Totarol**

**Scheme 2.1.2B** Non-Conventional biogenesis of totarol[64]

In case of totarol, the isopropyl group is placed at the C14 position, hence does not fit with the isoprene rule. The relocation of this isopropyl group is believed to proceed *via* rearrangement and the removal of the isopropyl group from C13 and its relocation to the C14 proceeds through a spiro intermediate **22**. This accounts for the non-conventional biogenesis of totarol (scheme2.1.2B).[64, 70]

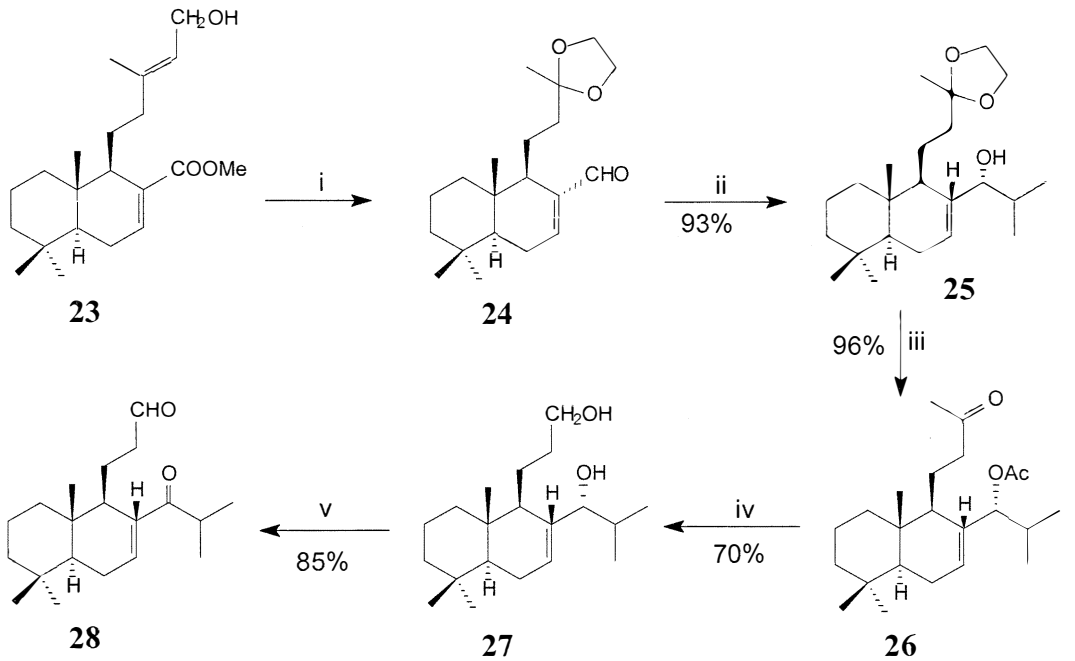
### 2.1.3. Total Synthesis of (+) totarol

Apart from the first synthesis of the racemate by Barltrop and Rogers[67], there have been five other different routes toward the synthesis of totarol, of which two are enantioselective syntheses. In 1979 Matsumoto and Suetsugùs[71] synthesized totarol employing a Wittig reaction between (R)-α-cyclocitral and (2-isopropyl-3-methoxybenzyl)triphenyl phosphonium chloride in the presence of *n*-butyllithium as the base to connect the A and C rings, followed by an intramolecular cyclization with aluminum chloride to give the B ring (scheme **2.1.3A**).

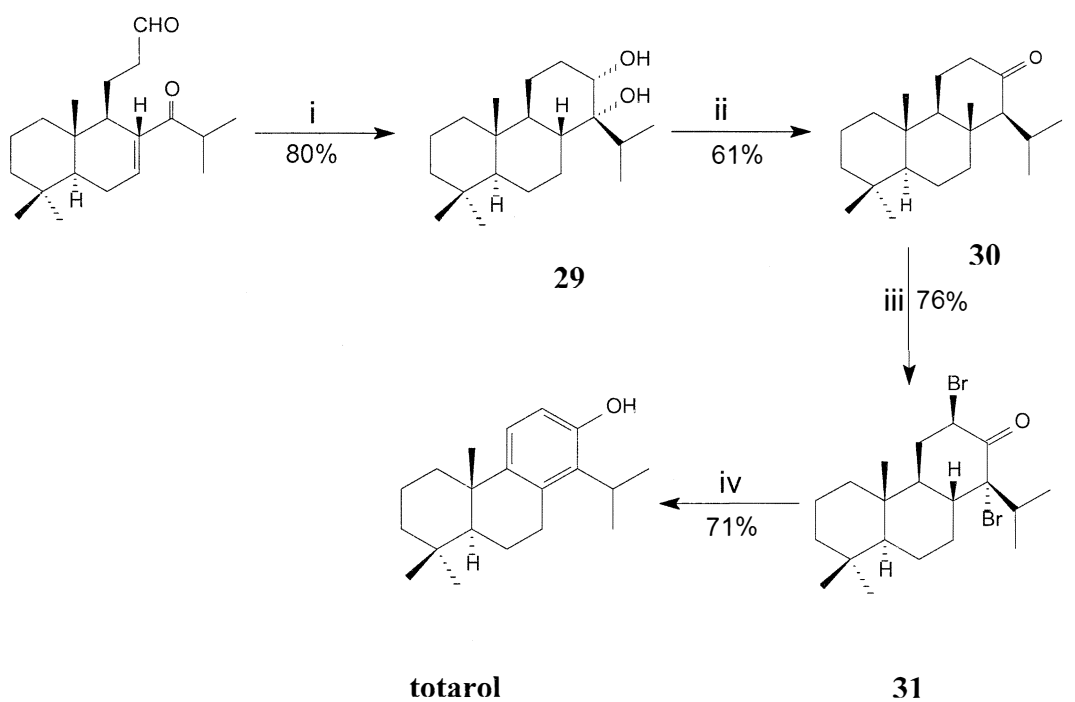



**Scheme 2.1.3A** (i) BuLi; (ii) $H_2$/Pd/C; (iii) $AlCl_3$; (iv) $BBr_3$ [71]

Recently, a different approach to (+)-totarol from zamoranic acid (scheme **2.1.3B** and scheme **2.1.3C**) was employed by Marcos [72], in which the methyl ester of zamoranic acid **23** was transformed into aldehyde **24**, followed by the addition of the isopropylmagnesium chloride to afford exclusively the 14R-hydroxy derivative **25**, in excellent yield. The transformation of **25** into secototarane **27** through the methyl ketone **26** needed acetylation at 70 $^0$C followed by deprotection. The authors found that the degradation of **26** by the haloform reaction was not possible. As an alternative, they employed a series of reactions. Thus, capture of the kinetic enolate of **26** as a trimethylsilyl enol ether and *cis* hydroxylation, followed by cleavage with $H_5IO_6$, esterification of the resulting acid and reduction with LAH gave the diol **27** in excellent overall yield from **25**.




**Scheme 2.1.3B**: *Reagents and conditions*: (i) as in ref 72b; (ii) *i*PrMgCl, THF, $0^0$C, 1 h; (iii) (a) $Ac_2O$, py, $70^0$C, 2 h, (b) p-TsOH, $Me_2CO$, rt, 40 min; (iv) (a) HMDSNa, TMSCL, $-78^0$C, 2 h, (b) $OsO_4$, NMO, rt, 48 h, (c) $H_5IO_6$, THF, $H_2O$, rt, 1 h, (d) $TMSCHN_2$, MeOH, $C_6H_6$, rt, 12 h, (e) $LiAlH_4$, $Et_2O$, rt, 1 h; (v) (a) $Ac_2O$, py, rt, 30 min, (b) TPAP, NMO, rt, 2 h, (c) $K_2CO_3$, MeOH, rt, 4 h, (d) $CrO_3$, py, rt, 1 h. [72]




**Scheme 2.1.3C**: *Reagents and conditions*: (i) $SmI_2$, THF, 0.1 M, MeOH, rt, 2 h; (ii) *p*-TsOH, $C_6H_6$, $60^0$C, 48 h; (iii) $CuBr_2$, MeCN, 72 h, $50^0$C; (iv) $Li_2CO_3$, BrLi, DMF, $140^0$C, 16 h. [72]

The oxidation of **27** to dicarbonyl system **28** was achieved by chemoselective acetylation of the primary hydroxyl group of **27** with $Ac_2O$; in pyridine over 30 min, oxidation of the secondary alcohol of the resulting monoacetate derivative, hydrolysis of the primary acetoxy group and subsequent oxidation gave compound **28**. Treatment of **28** with $SmI_2$ (0.1 M, prepared in situ) gave compound **29** in 80% yield as shown in scheme **2.1.3C**. Transformation of compound **29** into totarol requires the dehydration of the tertiary hydroxyl group and ring C aromatisation. However, the authors reported that attempts to dehydrate the oxidized form of compound **29** were not successful. Finally the aromatisation of ring C was achieved from ketone **30** obtained by pinacol rearrangement from **29,** using halogenation-dehydrohalogenation sequence *via* intermediate **31**, to give the target compound; totarol.[72]

### 2.1.4. Biological Activities of Totarol

The history of the antibacterial activity of totarol originated from the use of the timber of *podocarpus totara* as wharf piles, fence posts and foundation blocks on the early European settlers of New Zealand. This durability was linked to the potential antibacterial activity of totarol[64]. The antimicrobial activity of totarol has been investigated along with 5 other diterpenoids isolated from *Podocarpus nagi*. Totarol, the most abundant compound, was found to exhibit significant in vitro antibacterial activity against various Gram-positive bacteria but was not active against gram-negative bacteria or fungi.[73]

**Table 2.1.4**: *In vitro* antibacterial activity of totarol and penicillin[79]

| | MIC $\mu g ml^{-1}$ | |
|---|---|---|
| **Bacterium** | **Totarol** | **Penicillin G** |
| *Bacillus Subtilis* | 1.56 | 50 |
| *Brevibacterium ammoniagenes* | 0.78 | 0.39 |
| *Staphylococcus aureus ATCC 12598*$^S$ | 1.56 | 0.049 |
| *Staphylococcus aureus ATCC 29247*$^R$ | 0.78 | >800 |
| *Streptococcus mutans* | 0.78 | 0.049 |
| *Propionibacterium acnes* | 0.39 | 0.012 |

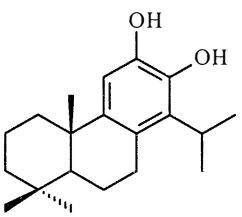
$^S$ Penicillin-Sensitive strain, $^R$ Penicillin-resistant strain

In a series of studies aimed at enhancing the antimicrobial activity of totarol, various derivatives of totarol have been synthesized. However, none of the synthesized compounds

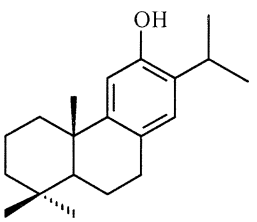
were found to be more potent than totarol itself. The results of this research were evaluated in terms of structure activity relationships (SAR) and revealed that; (i) a phenolic moiety was essential for antibacterial activity, (ii) derivatization at C-12 had an undesirable effect on the antibacterial activity, (iii) lipophilic substitution at C-12 diminished activity and (iv) all the most active compounds examined possessed primary or secondary alkyl substituents in the *ortho*-position to the hydroxyl group[74-76]. In addition to its antibacterial activity, totarol has also been shown to exert hypercholesterolemic activity in rats mainly by way of inhibition of cholesterol catabolism or excretion. It has also been reported to have mild antitumor activity[64]. Furthermore, Constantine and his co-workers have recently reported its antitubercular activity.[77]

## 2.2. Totarol as a Natural Product Scaffold for New Antimalarial Agents

In the course of research on the antiplasmodial and antituberculosis activity of selected South African medicinal plants, compounds **32** and **33** were isolated from *Harpagophytum procumbens*. However, the small quantities isolated did not permit possible chemical modification of the core structure of these compounds. On the other hand, the use of a commercially available similar compound, totarol, as a scaffold for novel natural product based antiplasmodial and antituberculosis agents, revealed the potential of ß-amino alcohol derivatives **34**, with enhanced activity against the intraerythrocytic stage of *P. falciparum* compared to the parent natural product totarol.[78]



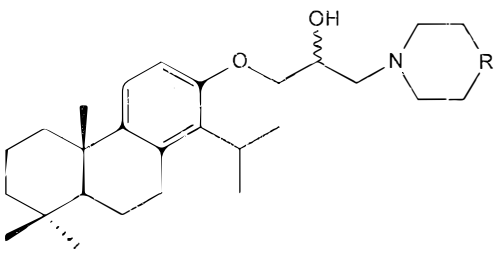

**32**




**33**

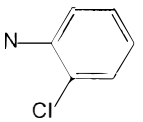
In the study, a range of secondary amine derivatives of totarol were synthesised and evaluated for their in vitro antiplasmodial activities both against drug sensitive and resistant strains. In comparison to totarol, all new compounds showed improved antiplasmodial activity and no cross resistance with CQ was observed. Out of all the compounds evaluated, compounds **35** and **36** were the most promising compounds, in terms of selectivity and antiplasmodial

activity. Both of these compounds exhibited significant antiplasmodial activity, high selective index and low resistance index as shown in table **2.2**.[78]




**34**

**35** If **R** =




**36** If **R** =

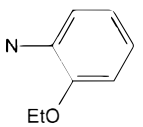



**Table 2.2** In *vitro* antiplasmodial activity and cytotoxicity[78]

| Compound | $IC_{50}$ D10 | $IC_{50}$ KI | $IC_{50}$CHO | SI[b] | RI[c] |
|---|---|---|---|---|---|
| Totarol | 7.51 | 4.29 | 1170.46 | 39.66 | 0.57 |
| **35** | 3.25 | 0.90 | >186 | >207 | 0.23 |
| **36** | 1.67 | 1.01 | >182 | >180 | 0.62 |

$IC_{50}$ (μM), SI = cytotoxicity $IC_{50}$/antiplasmodial $IC_{50}$, RI = KI $IC_{50}$/D10 $IC_{50}$

D10 = Chloroquin sensitive strain of *P. falsiparum,*

KI = Chloroquin resistant strain of *P. falsiparum*

CHO = Chinese Hamster Ovary(CHO) cells

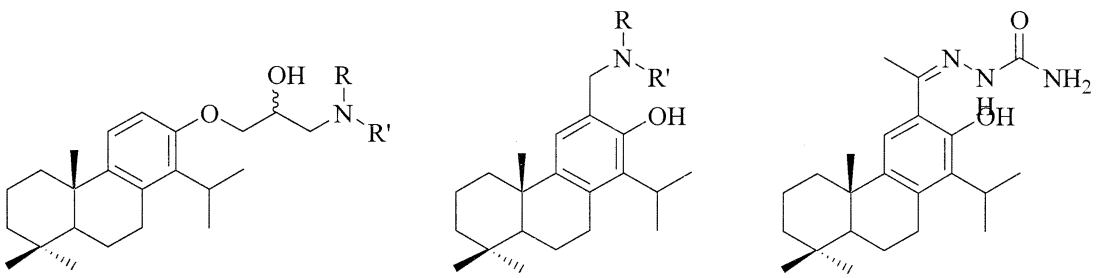
## 2.3. Project Objectives

As part of a continuing program to enhance the antimalarial activity of this potent natural product totarol; this project is aimed at the design and synthesis of a new series of derivatives of totarol in order to explore its structure-activity relationship studies for *in vitro* antimalarial activity. More specifically:

(i) to design, synthesize and characterize an exploratory new series of β-amino alcohol **37,** Mannich base **38** , and semicarbazone **39** derivatives of totarol,

(ii) to design, synthesize and characterize simplified analogs of the new series of totarol derivatives by replacing the hydrophobic diterpenoid-like fragment of totarol with simplified aromatic moieties (2-isopropyl phenol **40**);

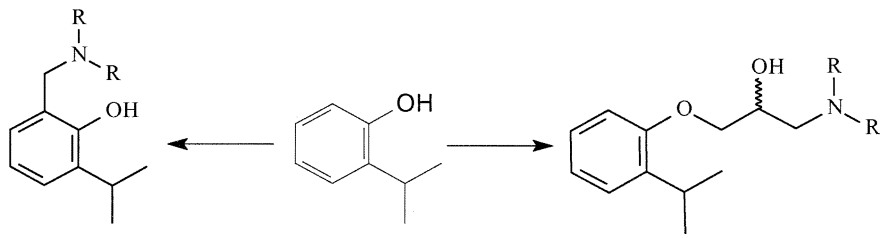
(iii) to evaluate the antimalarial properties of the synthesized compounds in vitro, with a view to delineating SAR trends.



**37** **38** **39**

**Figure 2.3A** General structures of the target compounds

A literature search revealed that the above modifications of totarol had not been previously investigated. The design of the simplified analogue (figure **2.3B**) was hypothesis-driven in that it was reasoned that the diterpenoid backbone of totarol was simply acting as a hydrophobic group in the antimalarial activity.



**40**

**Figure 2.3B** 2-isopropyl phenol derived Mannich base and β-amino alcohol analogue

## 2.4. Rationale for the target compounds

### 2.4.1. Mannich bases

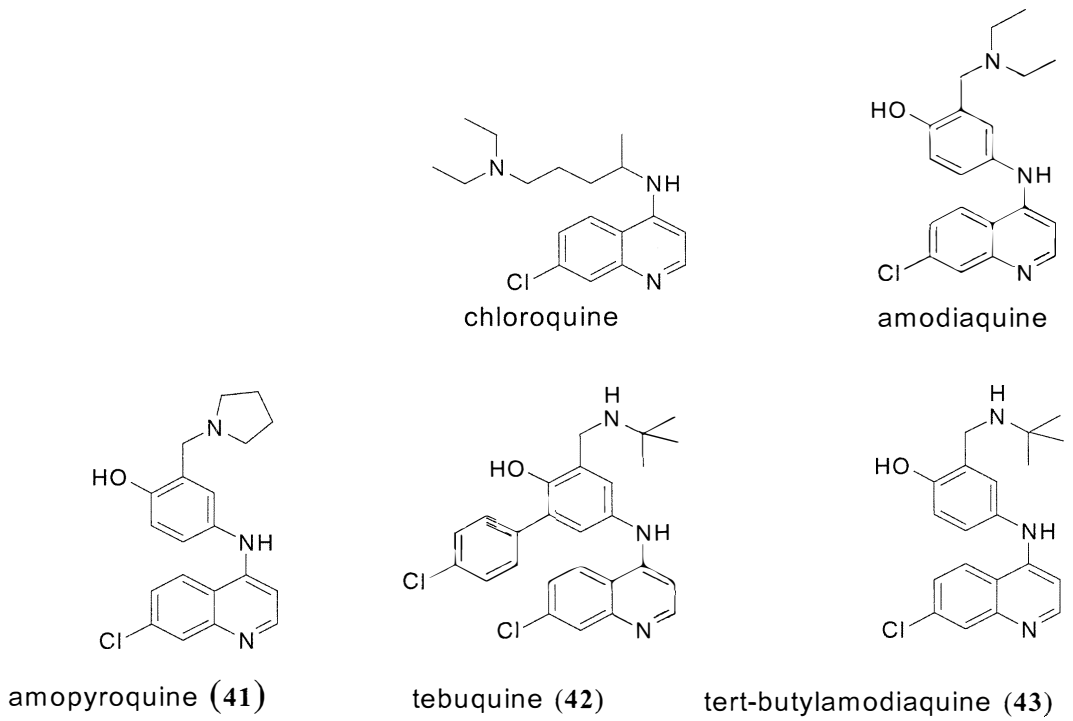



**Figure 2.4.1A** Chemical structures of some known Mannich base antimalarials[26]

The history of Mannich bases in the area of antimalarial chemotherapy dates back to 1950 with the introduction of amodiaquine (AQ) for treatment of malaria in the areas of high chloroquine (CQ) resistance. However, the use of AQ declined immediately following its initial report on granulosytosis and hepatitis in 1980 when used in prophylaxis [25, 26]. Finally, the drug was banned for use in malaria control program by the World Health Organization (WHO) in 1990.[26, 80]

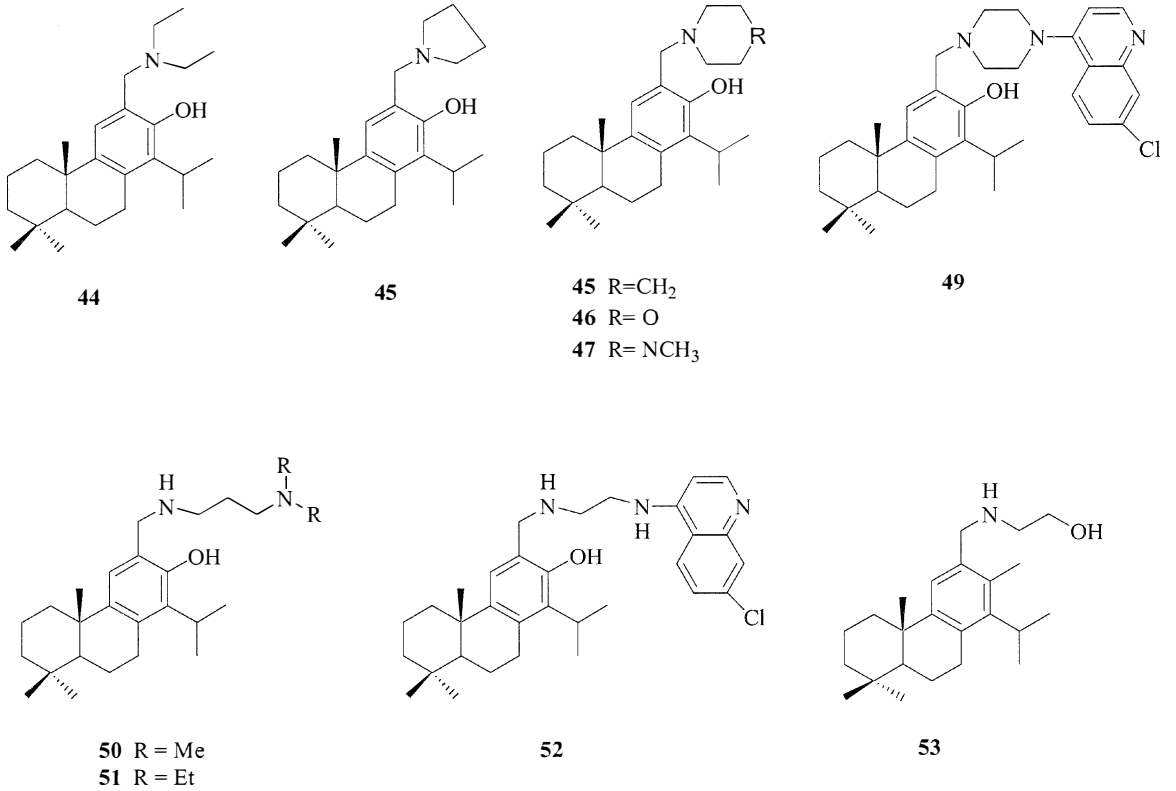
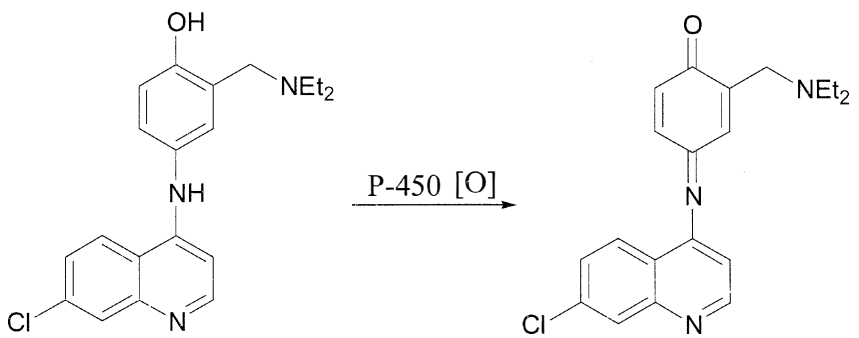



**Figure 2.4.1B** Chemical structures of the target Mannich bases

Amopyroquine **41**, a structural analogue of AQ, was introduced with the idea of reducing metabolism and increase bioavailability of the drug and was found to be more effective than both CQ and AQ against 11 CQ-resistant strains of the parasite[26]. Subsequently the need for Mannich bases containing side chains less susceptible to metabolism led to the synthesis of tebuquine **42** and *tert*-butyl amodiaquine **43** with increased antimalarial activity [26, 81]. However, tebuquine was shown to cause chronic toxicity in experimental animals [26].




**Scheme 2.4.1A:** Metabolic oxidation of amodiaquine to amodiaquine quinoneimine[83]

Despite the toxicity associated with many of the available Mannich base antimalarials, recent work on amodiaquine analogues by O'Neill and his group has revealed the potential of this group of compounds as a target for new effective antimalarials. They have reported several new amodiaquine derivatives with excellent in vitro antiplasmodial activity against both sensitive and resistant strains of the parasite [82, 83]. Moreover, some of the compounds were also shown to have improved in vivo bioactivation as compared to the parent drug amodiaquine. Metabolic oxidation of amodiaquine to amodiaquine quinoneimine (Scheme **2.4.1A**) is believed to be the cause for its toxicity.[83] Furthermore, the group has identified some essential features required when designing amodiaquine analogue antimalarials:

(i) The presence of a planar aminoquinoline ring system with a tertiary terminal nitrogen function that is charged at physiological pH, for haematin association and drug accumulation respectively. Molecular modelling studies also show an inter-nitrogen separation of approximately 8.3 Å between the quinoline nitrogen and the terminal tertiary alkylamino nitrogen; in both chloroquine and amodiaquine. This is dependent on the intra hydrogen bond between the oxygen of the hydroxyl group and the hydrogen of the protonated nitrogen in case of amodiaquine. [81, 82]

(ii) An electron withdrawing functional group at the 7-position of the aminoquinoline ring to confer beta-haematin inhibition; chloroquine being the substitute for optimal activity [82] as shown with chloroquine related 4-aminoquinolines. [84]

(iii) The presence of the Mannich base side chain is vital for the antimalarial activity. Diethyl, *t*-butyl, or pyrollidyl being preferred amine subsituents. The latter being the one with greater activity against resistant strains.[82]

(iv) The presence of 4' hydroxyl group within the aromatic ring, for inherent antimalarial activity against chloroquine resistant strains of the parasite [81-83]. Replacement of the hydroxyl group in tebuquine with fluorine results in reduction of its antimalarial activity [81]. Interchanging the position of the hydroxyl group and the Mannich base side chain however, improved its in vivo bioactivation while retaining the antimalarial activity. [83]

Even though the mechanism of action of these antimalarials is not clearly known, significant literature reports support the idea that haematin-quinoline association and subsequent inhibition of haemozoin formation could lead to the death of the parasite by haematin poisoning. According to the literature, ferriprotophyrin IX has a planar flat region of 30-40 Å to accommodate the flat quinoline ring, a central acceptor iron atom that can potentially bind to the quinoline nitrogen and two anionic carboxylates that can bind to the charged nitrogen of the aminoquinoline side chain as shown in figure **2.4.1C**. [81]

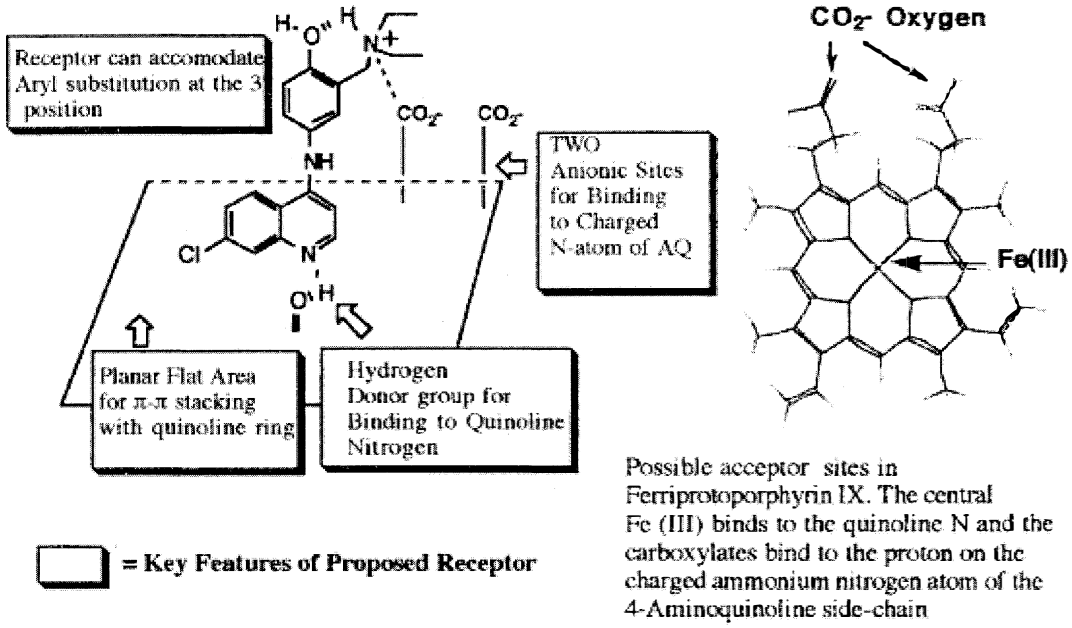
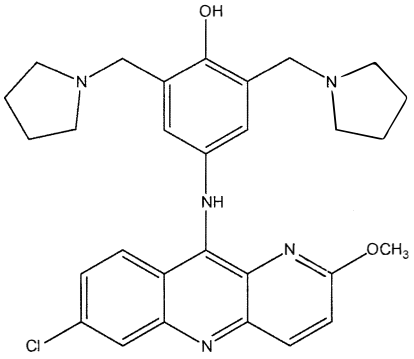



**Figure 2.4.1C**. Pharmacophoric groups of amodiaquine and proposed acceptor sites within ferriprotophyrin IX structure [85, 81]

The complex is believed to be predominantly *Π-Π* in nature [86, 87] which could enhance drug accumulation[88]. Therefore, it could be possible to design other novel Mannich base antimalarials with the help of the pharmacophoric groups of amodiaquine developed and essential features observed from the structure-activity relationship studies of related antimalarials.

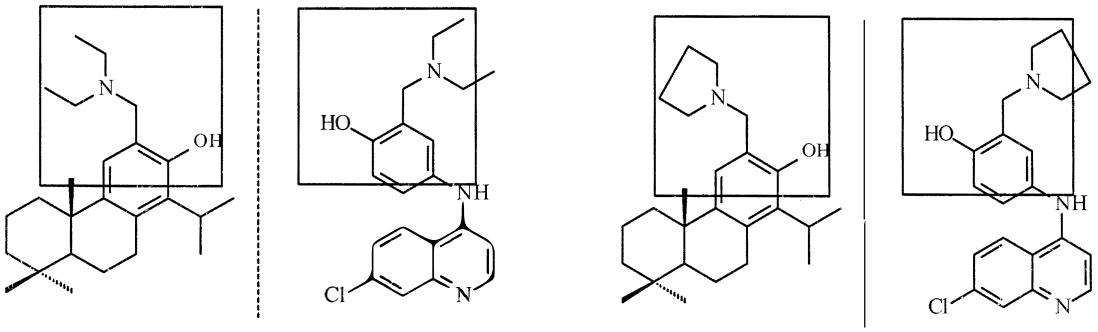
Cross-resistance to 4-aminoquinoline antimalarials is not consistent within different strains of the parasite. A strain that is resistant to one drug could be sensitive to another drug. This has drawn the attention of researchers to look for other drugs containing Mannich base side chains[82]. Pyronaridine, an acridine-type (benzonaphthyridine) is such a compound synthesized in 1970 in China and was shown to be well tolerated and highly effective in treating malaria-

infected patients in regions of chloroquine resistance[89]. A fixed drug combination of pyronaridine and artesunate known as pyramax; is being assessed as an oral treatment in both adult and child patients suffering from acute uncomplicated *P. falciparum* and *P. vivax* malaria and is in phase II safety and efficacy trial in Asia and Africa.[90]




**Pyronaridine**

The rational behind the design of totarol derived Mannich bases was based on the aforementioned history of the compounds in the area of malaria chemotherapy. The substitution of a Mannich base side chain at the C-12 position is aimed at mimicking the structure of the important drug amodiaquine. It is presumed that the aromatic moiety present in the totarol scaffold could compensate the requirement for aromatic ring system needed for binding with the planar ferriprotophyrin IX. Aromatic moieties play a major role in molecular recognition and could interact with polar substituents, such as amides or hydroxyl groups and even with positively charged moieties. Besides, incorporation of an important fragment into a natural product scaffold with intrinsic antiplasmodial activity could lead to the discovery of new totarol based antimalarials.




**44** **Amodiaquine** **45** **Amopyroquine**

**Figure 2.4.1D** Comparison of totarol Mannich bases with their antimalarial analogues

The rational behind introducing a terminal amino alkyl side chain in the compounds such as **44** is related to the weak base property. This was presumed to be important for increased accumulation in the parasite's acidic food vacuole via pH trapping [5, 88]. 4-Aminoquinolines such as chloroquine, amodiaquine and amopyroquine also contain terminal basic nitrogen, in addition to the quinoline nitrogen. Compound **45** was synthesized with the idea of potentially reducing the metabolic susceptibility of compound **44**. It is known that non-cyclic terminal amino alkyl side chains are susceptible to cytochrome P450-mediated dealkylation resulting in lower bioavailability. In this regard amopyroquine **41** has been shown to be more potent than amodiaquine (an open chain analogue) in vitro. Moreover, the pyrolidino-type side chain was related to structural features that confer activity against a resistant strain of the parasite [82]. Figure **2.4.1D** shows a comparison of the compound **44** and **45** on one hand with amodiaquine and amopyroquine on the other hand.

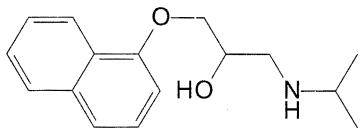
Compound **46** was introduced with the idea of exploring the steric tolerance of the terminal side chain amine. Compound **47** was synthesized with the idea of introducing a heteroatom into the amino alkyl side chain. This was envisaged to affect the antiplasmodial activity *via* a change in its electronic properties. Furthermore, the addition of the hydrogen bond acceptor (oxygen) could affect the nature of binding of the compound to its target. In case of **48**, the addition of a second protonatable nitrogen was predicted to increase the compound's passage across the cell membrane and accumulation in the parasite acidic food vacuole via pH trapping.[5, 88]. The aim of introducing a 7-chloro-4-aminoquinoline group in compound **49** was to explore the combined effect of the natural product totarol and the known antiplasmodial activity of 4-aminoquinolines. It is known that a 4-aminoquinoline moiety is required for the haematin association and antimalarial activity [5, 88, 91-92]. Furthermore, compounds **50** - **53** were synthesized in an effort to explore the effect of the nature of the amino group (secondary versus tertiary) and to improve the solubility and clogP properties of the compounds.

### 2.4.2. β-Amino alcohols

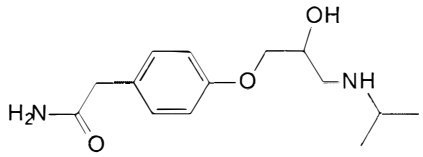
β-Blockers as antimalarials

One of the reasons for choosing amino alcohols was to mimc the β-blocker drugs (Figure 2.4.2B). "β-Blocker-drugs are drugs that act as competitive antagonists at the adrenergic β-

receptor and are commonly used to treat hypertension [93]. Recent studies by Hadar and co-workers showed the potential of such a drug, propranolol **54**, to inhibit intracelluar ring formation during erythrocytic infection by *P. falciparum* [94]. This stage of infection is responsible for all of the symptoms and pathologies associated with the disease. According to the research, there is involvement of the host β-adrenergic receptor and the heterotrimeric guanine nucleotide binding protein Gαs in the entry of the malaria parasite *P. falciparum* into erythrocytes [94]. Gαs is a cholestrol-rich protein associated with erythrocyte rafts that are incorporated into the paracitophorous vacuole.[93]




Propranolol (**54**)




Atenolol

**Figure 2.4.2B** Examples of known β-blocker-drugs

Erythrocytic infection by *P. falciparum* is initiated when the extracellular merozoite stage enters the red blood cell to form an intracellular ring. The Gαs peptide displayed a dose-dependent inhibition of new ring formation [93-94]. According to the literature, when infected erythrocytes lyse, antigene release causes an increase in catecholamines and these act through the β-adrenergic receptor to make uninfected erythrocytes more susceptible to attack by merozoites. Furthermore, treatment with agonists increased malaria infection while antagonists blocked the infection.Thus activation of Gαs via its receptor may influence malarial infection. [93-94]

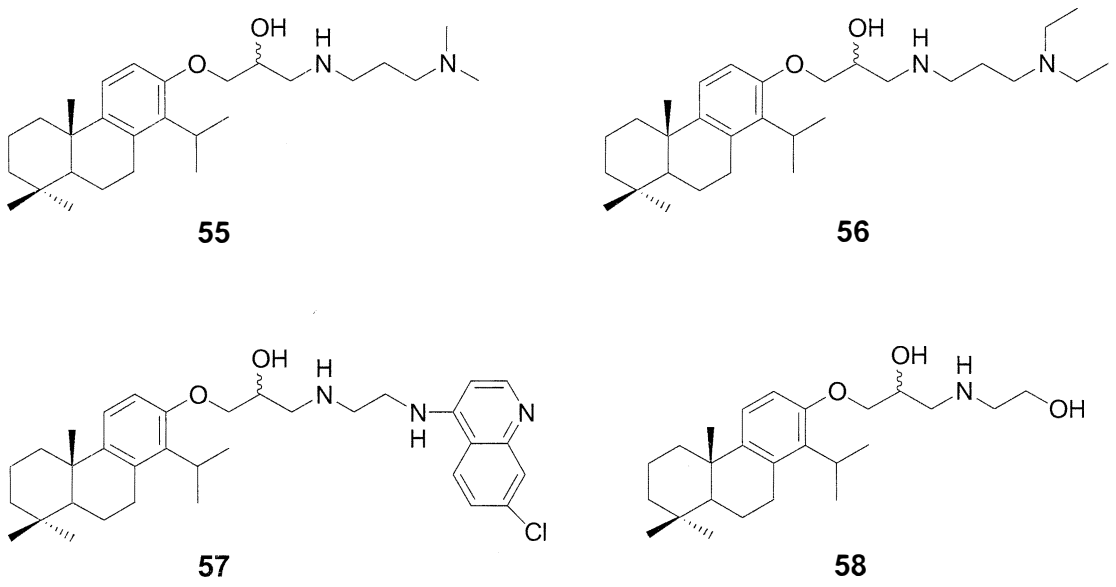
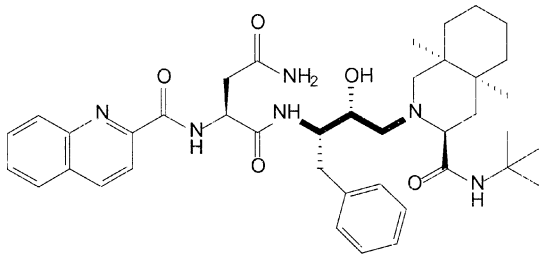
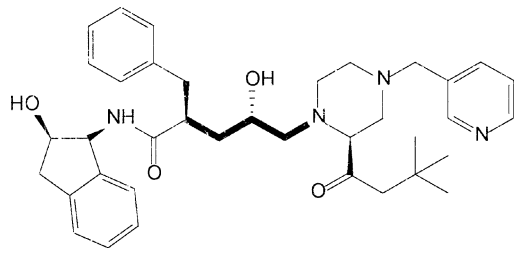



**Figure 2.4.2A** Chemical structures of the target amino alcohols

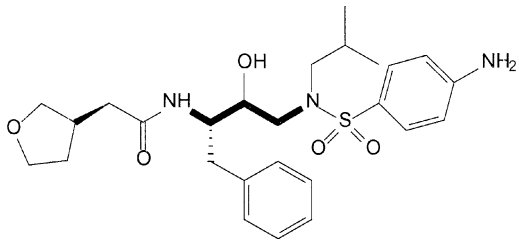
The rationale for the choice of the targeted β-amino alcohols could also be explained based on molecular recognition principles. One of the first and fundamental requirements for a drug to bind to its target is its molecular recognition by proteins [95]. β-Amino alcohols contain a hydroxyethylene unit in their structure and this moiety is specifically recognized by aspartyl proteases [96]. As explained in chapter one of this thesis, aspartic proteases namely plasmepsin I and Plasmepsin II are known to be involved in the initial stages of hemoglobin catabolism in which plasmepsin I prefers phenylalanine in the P1 position while plasmepsin II prefers hydrophobic residue, leucine in the P1` position of the erythrocytic proteins [6]. Hence these enzymes are important targets in the search for anti-malarials.



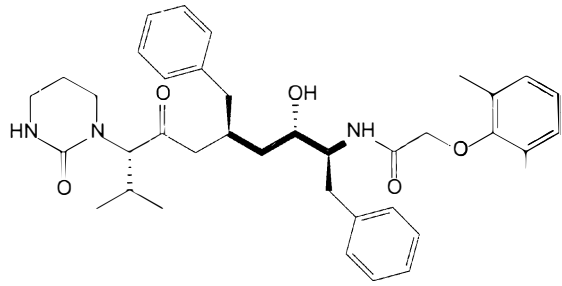
Saquinavir (HIV, Roche)



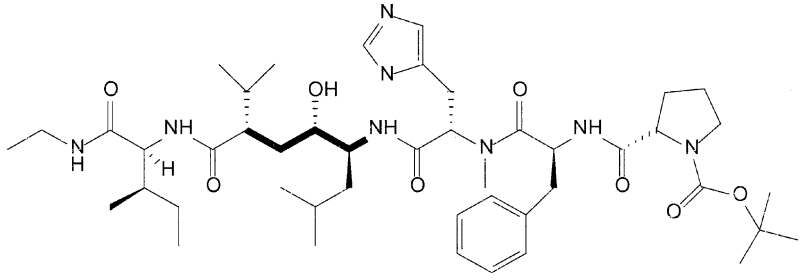
Indinavir(HIV, Merck)



Amprenavir (HIV, Vertex/GSK)



lopinavir (HIV, Abbott)

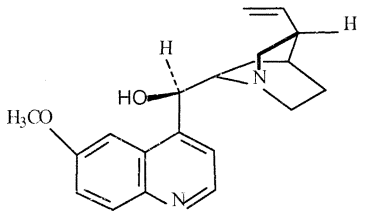


Ditekiren (renin, Phase II, Pharmacia)

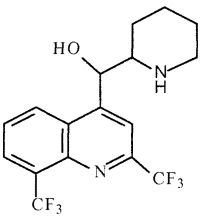
**Figure 2.4.2C** Commercially available HIV proteases and renin inhibitor[96]

More importantly, the hydroxylethylene unit present in the amino alcohols is an important fragment that can be used as a molecular anchor to build aspartic protease inhibitors. Fragments are small low molecular weight molecules that would typically comprise part of a drug compound. Once bound into the active site of a protein target, fragments can act as the starting point for medicinal chemistry, and can be developed into highly selective and potent drug candidates [95]. This hydroxylethylene unit is present in various HIV protease and renin inhibitors available on the market (Figure **2.4.2C**) [96]. Therefore the combination of this important fragment with the privileged natural product totarol may have an additive effect toward developing antimalarial lead compounds. Moreover, the β-amino alcohol moiety is present in the well-known antimalarial agents such as quinine and mefloquine. Based on the results of the preliminary biological evaluation of the synthesized Mannich bases, only the amines related to the relatively active compounds (**50-53**) were chosen for opening of the

epoxide, in the synthesis of amino alcohols. Therefore, only amino alcohols related to primary amines were synthesized.

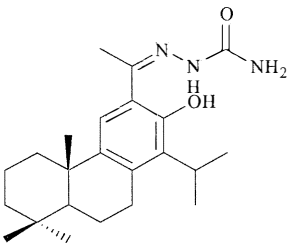


**Quinine**



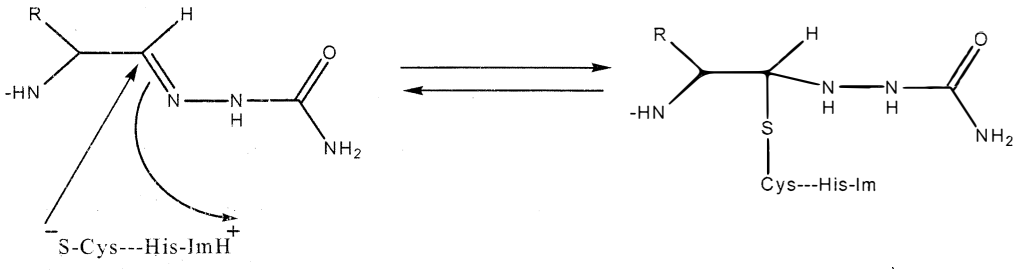
**Meflequine**

### 2.4.3. Semicarbazone Derivative



**59**

Cysteine proteases play a vital role in many different infectious diseases such as *Trypanosomiasis* and malaria, and are therefore promising targets for the development of anti-parasitic drugs. Peptidyl semicarbazones are known to inhibit cysteine proteases. The inhibition is believed to be the result of a nucleophilic attack by the thiolate group on the protected carbonyl carbon to form a tetrahedral adduct as shown in scheme 2.4.3[97]. Due to the reversible nature of their inhibition, they are also important ligands for purification of serine and cysteine proteases by affinity chromatography[98]. Moreover, they are important intermediates for the synthesis of peptidyl aldehydes which are important cysteine protease inhibitors.[97]



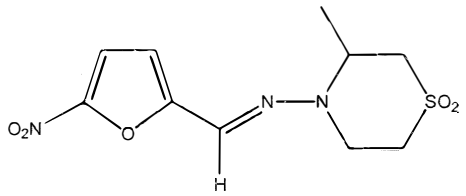
**Scheme 2.4.3.** Inhibition of cysteine proteases by peptidyl semicarbazones[97]

**Table 2.4.3:** Inhibitory activity of peptidyle semicarbazones

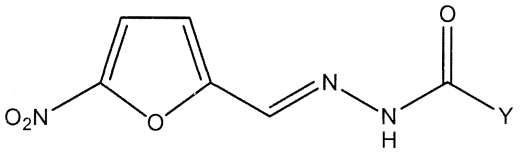
| Peptidyl semicarbasones | K1 Value in (nM) | |
|---|---|---|
| | Papain | Cathepsin |
| Z-Arg-Phe-Lle-Sc | 23 | 500 |
| Z-Phe-Lle-Sc | 2700 | 1200 |
| Z-Phe-Gly-Sc | ND | 20000 |
| Z-Gly-Phe-Gly-Sc | 9.2 | 1000 |

**ND**: not determined

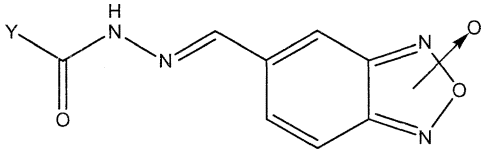
Nifurtimox [R] (Nfx) is the main drug in use against *Trypanosomiasis*. However, it is associated with undesirable side effects. The continuous search for other more selective and less toxic antitrypanosomal drugs revealed the potential of different semicarbazone derivatives such as 5-nitro-2-furaldehyde semicarbazone[99] and 1, 2, 5-oxadiazole N-oxide semicarbazone[100] as antitrypanosomal agents. Furthermore, other semicarbazone derivatives have also been reported for their anticonvulsant[101] and antihypertensive activity. [102-103]



**Nifurtimox [R]**



**5-nitro-2-furaldehyde semicarbazone**



**5-nitrothiophene-2-carboxaldehyde semicarbazone**

The rationale behind the design of the semicarbazone derivative of totarol is therefore, to explore this important class of compounds for antiplasmodial activity. Besides, they are cysteine protease inhibitors and cysteine proteases play an important role in malaria drug discovery.[97]

# CHAPTER THREE

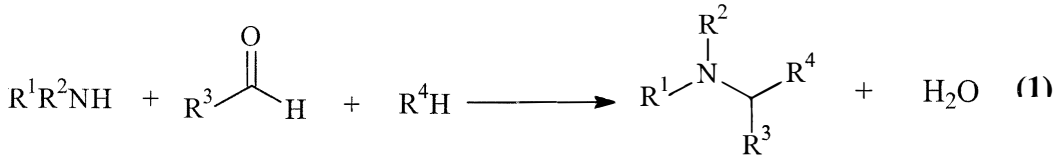
## SYNTHESIS AND CHARACTERIZATION OF THE TARGET COMPOUNDS

### 3.1 Chemical Modification on Totarol

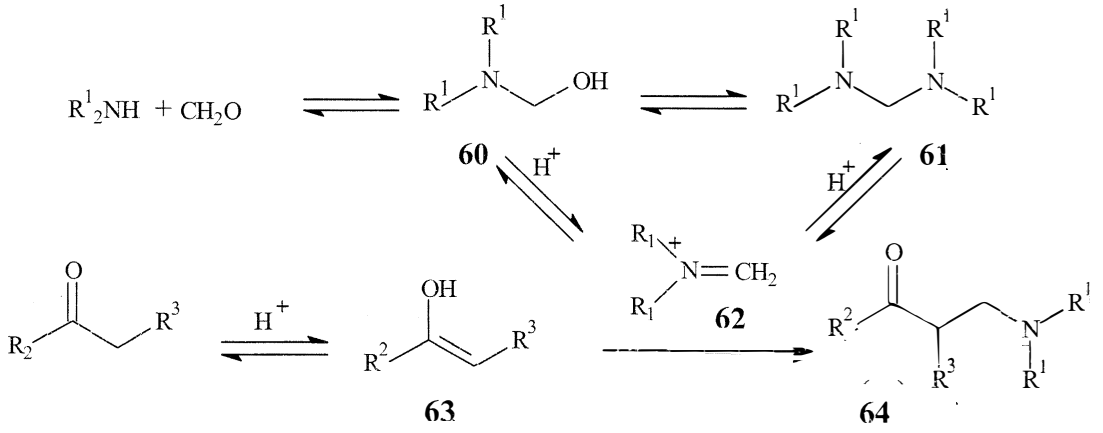
### 3.1.1 Synthesis of Mannich Bases

The Mannich Reaction

The Mannich reaction is a prototype of C-C bond forming reactions that involves the addition of resonance-stabilized carbon nucleophiles to iminium salts and imines. The reaction consists of three components: (i) ammonia or a primary or secondary amine; (ii) a non-enolizable aldehyde, usually formaldehyde; and (iii) an active methylene. These components condense with concomitant release of water to produce a new base, known as the '**Mannich base**', in which the active hydrogen is replaced by an amino methyl group as shown in equation 1.[104]

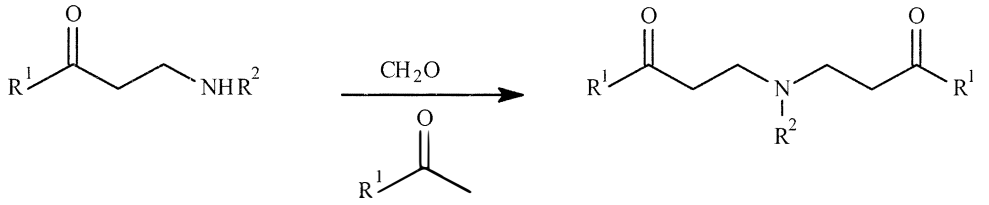



A Mannich reaction is most commonly performed in protic solvents and acid catalysis (usually employing the amine components as its hydrochloride salt). The reaction mechanism is thought to involve a reactive iminium intermediate **62**, which reacts with the active methylene component in its enol form **63** to produce the Mannich base **64** (See scheme 3.1.1A).[104]



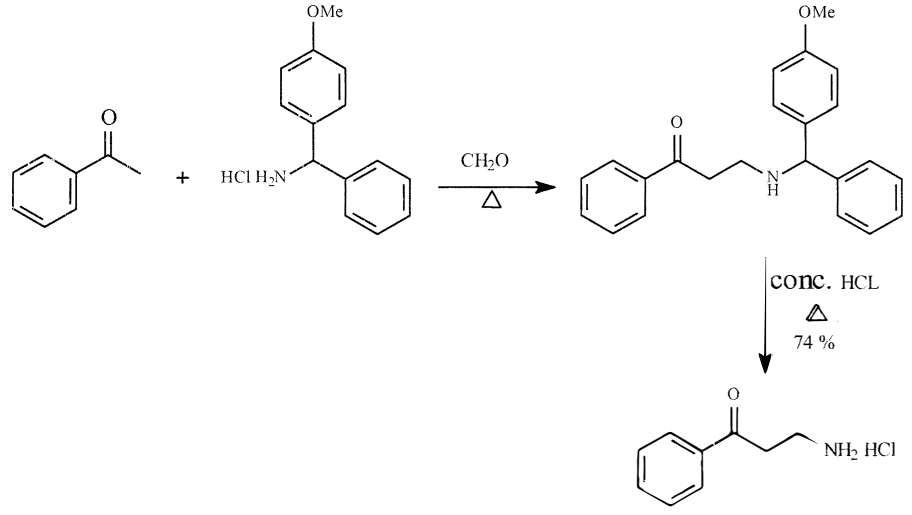

**Scheme 3.1.1A**: General Mannich reaction

Acid catalysis not only promotes the decomposition of **60** and **61** to the iminium salt **62**, but also the enolization of the active methylene component. Protic solvents support the formation of the charged iminium ion by virtue of their high dielectric constant. In addition, elevated temperatures are often necessary for generation of sufficient concentration of iminium ion[104]. With regard to the choice of the amine components that may be used, secondary amines react with most predictability and in relatively high yields. With primary amines the yield is usually unpredictable. This is because the initially formed secondary Mannich base can further react with the paraformaldehyde to give a tertiary amine (equation 2). Bulky primary amines and interestingly the use of amine oxalate salts instead of hydrochloride salts, are used to suppress these cross condensation reactions.[104]




(2)

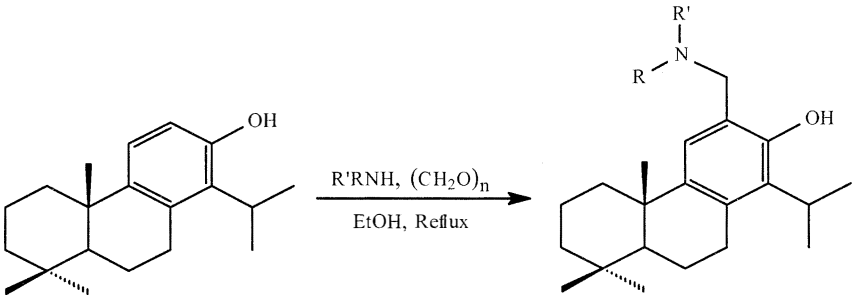
The use of ammonia for the synthesis of primary Mannich bases is generally complicated because of the greater likelihood of obtaining products derived from multiple substitutions. However, this can be achieved indirectly, by condensing a bulky primary amine containing a cleavable alkyl group as shown in scheme **3.1.1B**.




**Scheme 3.1.1B:**

### 3.1.1.1 The synthesis of Mannich bases of totarol

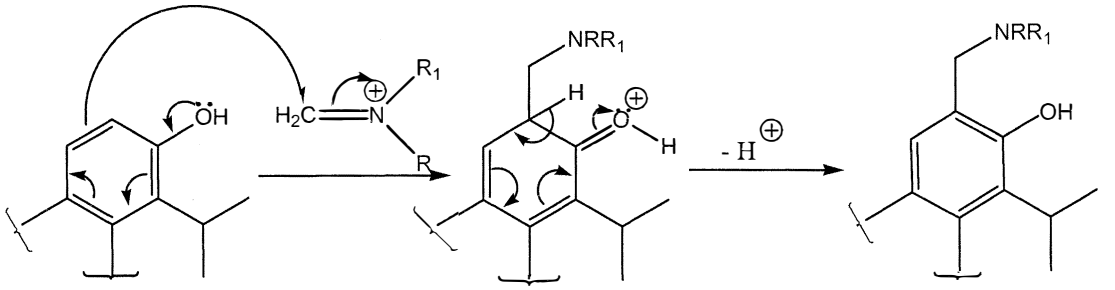
The general synthesis of the Mannich base derivatives of totarol was performed as shown in scheme **3.1.1.1A**. In all reactions, commercially available totarol was reacted with paraformaldehyde and a primary or secondary amine to a give Mannich base derivative, in which an amino-methyl group substitutes the acidic proton at C-12.




**Scheme 3.1.1.1A:** The synthesis of Mannich bases of totarol

Reaction mechanism

The mechanism involves the reaction of the resonance-stabilized carbon nucleophile of totarol with the preformed imine (iminium salt) to give the resulting Mannich bases (Scheme **3.1.1.1B**). In all the reactions, ethanol was acidic enough to catalyse the dehydration of the addition product to give the intermediate iminium ion. Therefore no addition of an external acid was required.



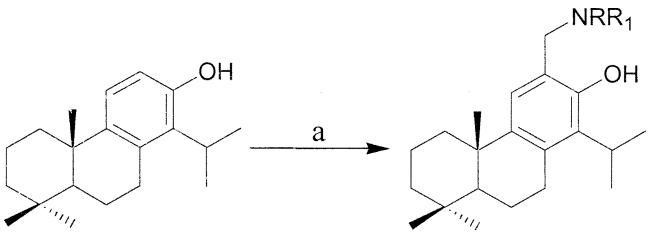

**Totarol** **Mannich base**

**Scheme 3.1.1.1B:** Reaction mechanism for totarol derived Mannich bases

Two different classes of Mannich bases were synthesized. The first class was based on secondary amines and comprises compounds **44-49**. The second class of Mannich bases were based on primary amine derivatives and comprise compounds **50–53**. In both classes, parallel solution phase synthesis was employed.

### 3.1.1.2 Synthesis of Class-I Mannich bases (Compounds 44 – 49)

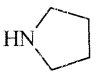
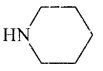
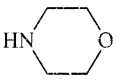
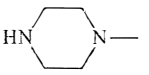
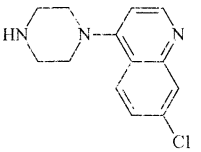
In this class of compounds, totarol **18** was divided into six carousel tubes. Each tube was then treated with 2.0 equivalents of the selected secondary amines (see table 3.1.1.2A), and 2.0 equivalents of paraformaldehyde in ethanol. The reaction mixture was then allowed to reflux for 48 h [26] to give the desired compounds **44 – 49** (as shown in scheme 3.1.1.2A).



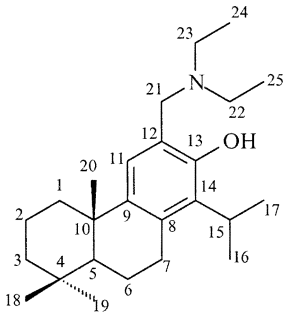

**44-49**

**Scheme 3.1.1.2A**: Reagents and Conditions: (a) 2.0 eq. of $(CH_2O)_n$, and 2.0 eq. of HNR'R in EtOH, reflux for 48 hr. R & R' ≠ H.

**Table 3.1.1.2A**: Synthesized class-I Mannich bases and their synthetic yields.

| Compound | Amine used | % Yield |
|---|---|---|
| **44** |  | 80 |
| **45** |  | 74 |
| **46** |  | 71 |
| **47** |  | 51 |
| **48** |  | 68 |
| **49** |  | 61 |

The $^{1}$H-NMR spectra of all the compounds closely resemble that of totarol. Noticeable similarities include the two singlets at 0.91 & 0.94 ppm due to the methyl ($CH_3$) protons at C-18 & 19 respectively, a singlet at 1.17 ppm due to the methyl ($CH_3$) protons at the C-20, two doublets between 1.35 – 1.36 ppm that belong to the six methyl protons at C-16 and 17, the multiplet at 3.25 ppm, due to the CH proton at C-15 and the proton resonances of the fused cyclic ring. To avoid repetition, only the main differences in the spectrum will be discussed in the following compounds **44-49**.




**44**

The molecular formula of compound **44** was determined by analysing its HRFABMS molecular ion at m/z 371.3185208, which was consistent with the theoretical value. $^{1}$H-NMR evidence for the structure of **44** was observed by the disappearance of one of the two aromatic protons, which with the other one normally appear as two doublets at 6.68 and 7.08 ppm, and appearance of a singlet at 6.73 ppm for the only aromatic proton. The other noticeable observation in the $^{1}$H-NMR spectra is the appearance of two-diastereotopic protons of the aminomethyl group (C-21) as two doublets with $J$=13.72 & $J$=13.81 Hz, at 3.62 and 3.75 ppm respectively, a multiplet at 2.59 ppm due to the 4 methylene protons of the diethylamino group and a triplet at 1.08 ppm for the corresponding methyl ($CH_3$) protons. Further evidence in support of the product was also observed from the $^{13}$C-NMR. The spectrum displayed distinctive signals at δ 57.8, 46.3 and 11.4 ppm due to C-21, C-22, C-23, and the two terminal methyl ($CH_3$) carbons (C-24, C-25) respectively. Furthermore the downfield shift of the signal for C-12 from 110.2 ppm in the case of totarol to 120.1 ppm confirmed substitution at the C-12 position of **44**. Due to the structural similarity of the substituted amines, the general structure (Figure **3.1.1.2B**), will be used to explain the key spectroscopic indicators as shown in tables **3.1.1.2B** and **3.1.1.2C**.

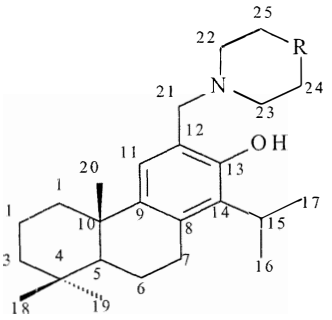
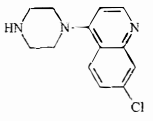


**Figure 3.1.1.2B**: General structure of compound **45-49**

**Table 3.1.1.2B**: Key $^{1}$H-NMR indicators of compounds **45-49**

| Compound # | R | H-21 | H-22 & 23 | H-24 &25 |
|---|---|---|---|---|
| **45** | - | 4.15, 4.08 ppm, 2d, *J*=12.24 & 12.25 Hz | 2.59 ppm, m | 2.18-2.05 ppm, m |
| **46** | -$CH_2$- | 3.53, 3.61 ppm, 2d, *J*=13.5 & 13.6 Hz | 2.46 ppm, m | 1.55-1.73 ppm, m |
| **47** | O | 3.60, 3.64 ppm, 2d, *J*=13.7 & 13.8 Hz | 2.54 ppm, br m | 3.73 ppm, t, *J*=4.5 Hz |
| **48** | N-$CH_3$ | 3.66, 3.58 ppm, 2d, *J*=13.4, 13.5 Hz | 2.52 ppm, br m | |
| **49** |  | 3.72, 3.80 ppm, 2d, *J*=13.72 & 13.81 Hz | 2.85 ppm, br s, | 3.29 ppm, br s, |

Compound **45** was synthesized with the idea of potentially reducing the metabolic susceptibility of compound **44**. The HRFABMS of compound **45** indicated an (M+H) ion peak at m/z 370.310739 that corresponds to the protonated form of the molecular formula of

$C_{25}H_{39}NO$. Due to the core structure similarity with compound **44-53** only the noticeable differences in the NMR spectra will be discussed in detail.

As can be seen from table 3.1.1.2B, the $^{1}$H-NMR of compound **45** revealed two doublets at 4.15 and 4.08 ppm with a coupling constant 12.24 / 12.25 Hz, confirming the formation of the aminomethyl group. There was a distinct shift of the doublets due to the diastereotopic protons at C-21 from around 3.60 ppm in the other compounds to 4.01 ppm, with a lower *J* value (12.60 Hz) in compound **45**. This could be due to the ring strain compared to the other compounds. Another characteristic peak is the appearance of the more deshielded multiplet around 2.05 - 2.18 ppm that is due to the methylene (CH2) protons at C-24 & 25 in the pyrolidine ring. Analysis of the $^{13}$C-NMR spectra also reveals the appearance of three distinct peaks. One at 55.7 ppm, and two other intense peaks at 53.1 and 23.2 ppm due to the C-21, C-22/23 and C-24/ 25 respectively. Furthermore the shift of C-12 from 110.0 ppm to a lower field 125.5 ppm confirmed substitution at the C-12 position.

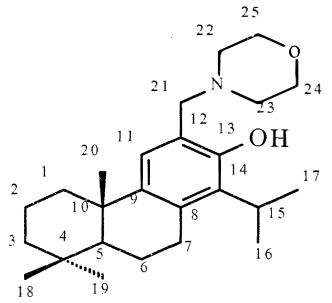
**Table 3.1.1.2C**: Key $^{13}$C-NMR indicators of the class-1 Mannich bases

| Compound # | C - 21 | C - 22 | C - 23 | C - 24 | C - 25 |
|---|---|---|---|---|---|
| **44** | 57.8 | 46.3 | 46.3 | 11.4 | 11.4 |
| **45** | 55.7 | 53.1 | 53.1 | 23.2 | 23.2 |
| **46** | 62.7 | 53.7 | 53.7 | 25.8 | 25.8 |
| **47** | 62.5 | 52.8 | 52.8 | 66.8 | 66.8 |
| **48** | 61.9 | 54.9 | 54.9 | 52.4 | 52.4 |
| **49** | 62.09 | 52.0 | 52.0 | 52.3 | 52.3 |

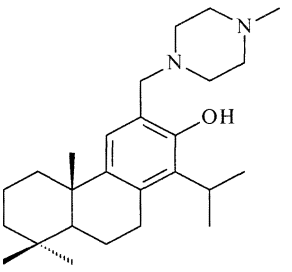
Compound **46** was synthesized using the same procedure described for compound **44** and was isolated from the workup as white needle shape crystals. The compound was sufficiently pure by $^{1}$H-NMR. The HRFABMS of compound **46** showed a molecular ion peak of m/z 383.3178958 and was found to be consistent with the molecular formula $C_{26}H_{41}NO$. In addition, the elemental analysis results were also within ± 0.4 of the theoretical value: Found C, 81.5, H, 10.2, N, 3.6 (Calc. C, 81.4, H, 10.7, N, 3.6).

The structure of compound **46** was also further confirmed from the $^{1}$H-NMR and $^{13}$C-NMR spectra, as shown in tables **3.1.1.2B** and **3.1.1.2C**. The integration for the multiplet observed at d 1.55--1.73 ppm revealed an additional two protons due to the two methylene ($CH_2$)

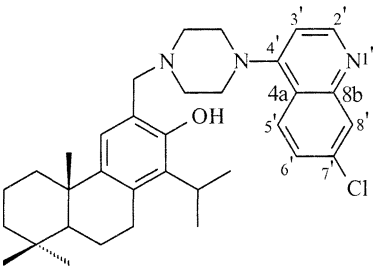
protons (H-26). Furthermore, the $^{13}$C-NMR spectra also displayed an additional distinctive peak at 24.1 ppm due to C – 26. As with compounds **44** and **45** a downfield shift of the C-12 resonance from 110.2 to 119.6 ppm was also observed due to the substitution of the amino methyl group at the C-12 position.




**47**




**48**




**49**

The structure of compound **47** was confirmed by different spectroscopic techniques such as NMR ($^1$H, $^{13}$C, COSY, HSQC, HMBC and DEPT), IR, MS and Microanalysis techniques. The HREIMS of compound **47** showed a molecular ion peak ($M^+$) at m/z 385.29876, which corresponds to the molecular formula $C_{25}H_{39}NO_2$. $^1$H-NMR analysis of compound **47** (table 3.1.1.2B), shows a relatively deshielded triplet at d 3.73 ($J$ = 4.5 Hz) for H-24 & 25. This is due to the presence of the morpholine oxygen. The $^{13}$C NMR also displayed distinct peaks at 66.8, 62.5, and 52.8, due to (C-24, 25), C-21, (C-22, 23) respectively. In a similar trend with compounds **44-46,** the C-12 peak was observed at a lower field (118.6 ppm), confirming the substitution at this position.

Compound **48** was synthesized using similar procedures described for compounds **44-47**. Its structure was confirmed using different spectroscopic techniques. The HREIMS of **48** showed a peak at m/z 398.32995, which corresponds to the protonated molecular ion peak for the formula $C_{26}H_{42}N_2O$. Elemental analysis of the compound reflected the correct molecular formula, C, 78.2, H, 10.63, N, 7.06 (Calc. C, 78.3, H, 10.6, N, 7.0). $^1$H-NMR spectrum displayed a broad singlet at d 2.52 due to the piperazine protons, and a singlet peak at 2.30 ppm due to the N- substituted methyl ($CH_3$) protons in addition to those outlined in the table **3.1.1.2B**. Further more, the $^{13}$C NMR spectrum also displayed a distinctive peak at 45.9 ppm due to the N-substituted methyl carbon (C-26), in addition to the characteristic $^{13}$C peaks outlined in table **3.1.1.2C**.

Compound **49** was synthesized with the aim of introducing a 7-chloro-4-aminoquinoline structure as a terminal amine in the torarol-derived Mannich base. This was to explore the combined effect of the natural product totarol and the known antiplasmodial activity of 4-aminoquinolines.

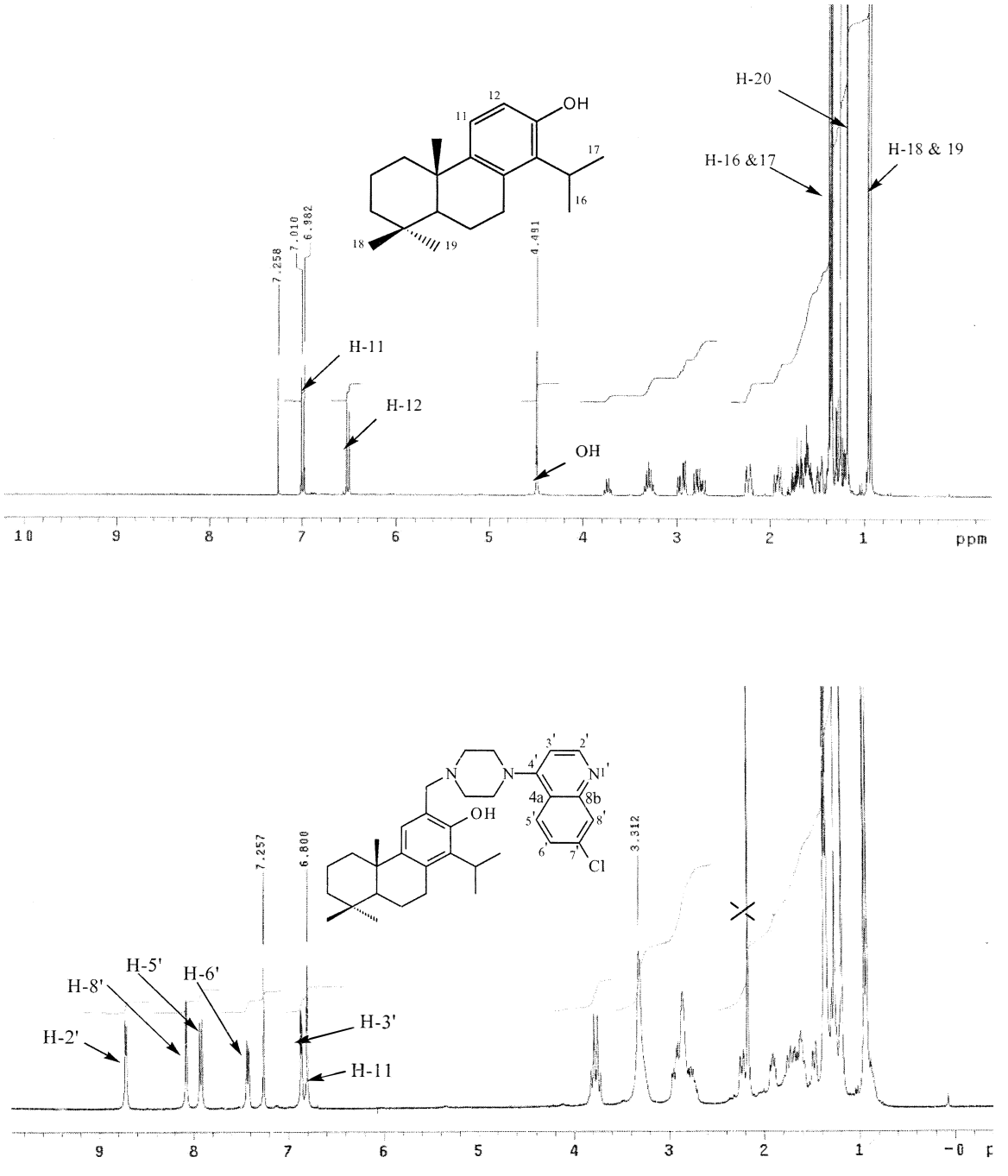
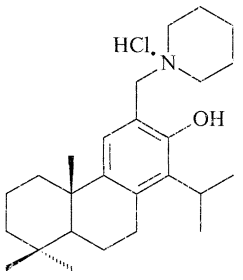


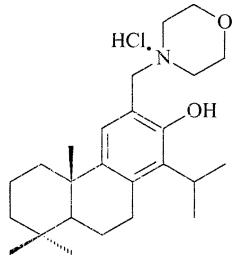

**Figure 3.1.1.2C**: 1H-NMR comparison of compound **49** with the starting material totarol

The HREIMS of compound **49** showed peaks at m/z 546.32538 corresponding to $[M(^{35}Cl) + H]^+$ and at m/z 548.3 corresponding to $[M(^{37}Cl) + H]^+$ (relative ratio3:1 respectively), which were consistent with the molecular formula $C_{34}H_{44}ClN_3O$. The $^1$H-NMR spectrum of compound **49** shows a split of the piperazinyl protons into two different resonances at 2.85 & 3.29 ppm, unlike in the case of compound **48** which were observed as one broad singlet at 2.52 ppm. This could be due to the conjugation of the lone pair electrons of the N atom with the aromatic aminoquinoline in **49**, thus affecting the two-methylene protons directly attached to that N atom of the piperazine. Other most striking signals were the five additional aromatic resonances due to the aromatic quinoline. The $^{13}$C-NMR evidence in support of the structure of **49** includes the appearance of the 9 carbon signals in the aromatic region of the spectra.

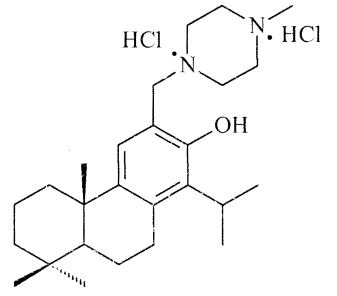
To improve the solubility of compounds **46**, **47** and **48**, hydrochloride salts of these compounds (**46b**, **47b**, and **48b**) were synthesized by dissolving 30 mmol of the Mannich bases in 2 - 3 ml of diethyl ether, cooled to 0 $^0$C and treated drop wise with stoichiometric amount of cold methanolic hydrochloric acid (2 M). The resulting precipitate was then filtered and dried [105]. The structure of the compound **46b** was confirmed by the apparent downfield shift to 3.15 ppm in the resonance of protons 22 and 23. The analogous resonances in parent compound **46** appeared at 2.46 ppm. Similar to compound **46b**, the $^1$H-NMR of compound **47b** also showed a downfield shift to 3.90 ppm in the resonance of protons 22 and 23 (2.54 ppm for the parent compound **47**). Comparing the $^1$H-NMR of compound **48b** with **48**, similar downfield shifts were seen for protons 24 and 25, and additionally for protons 22 and 23. This is due to the additional basic nitrogen present in **48**. The $^{13}$C-NMR of the compounds (**46b**-**48b**) did not show any significant difference from their coresponding parent compound.




**46b**



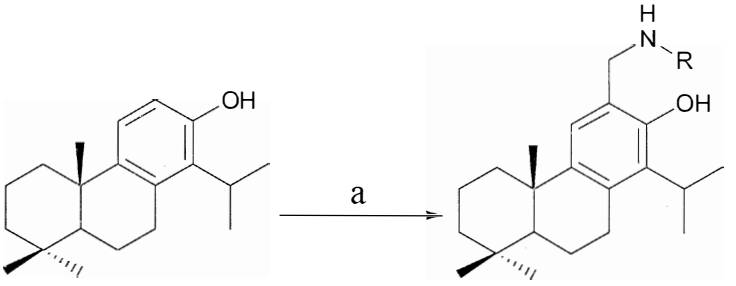

**47b**




**48b**

### 3.1.1.3 Synthesis of Class-II Mannich bases (Compounds 50 – 53)

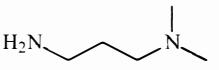
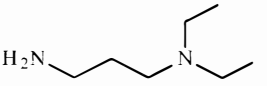
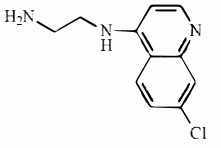
In an effort to explore the effect of the nature of the amino group (secondary verses tertiary) and to improve the solubility and clogP properties of the compounds, a series of primary amine-derived Mannich bases (**50-53**) were synthesized as shown in scheme **3.1.1.3**. To avoid the cross condensation reaction of the secondary Mannich base products, explained in section **3.1.1,** the number of equivalents of the primary amine and paraformaldehyde were increased by 2 fold. Increasing the amounts of primary amine and paraformaldehyde led moderate to good yields of secondary Mannich bases. Moreover, no side reactions were observed from the TLC.



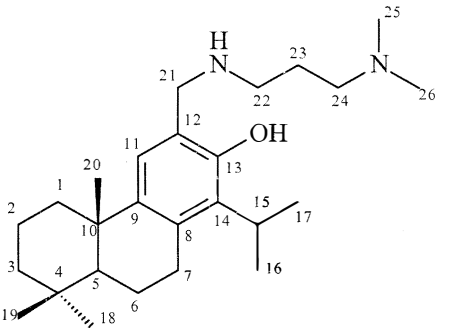

**50-53**

**Scheme 3.1.1.3**: *Reagents and Conditions*: (a) 4.0 eq. of $(CH_2O)n$, and 4.0 eq. of $NH_2R$ in EtOH, reflux for 48 hr.

**Table 3.1.1.3**: Synthesized primary amine derived Mannich bases and their synthetic yields

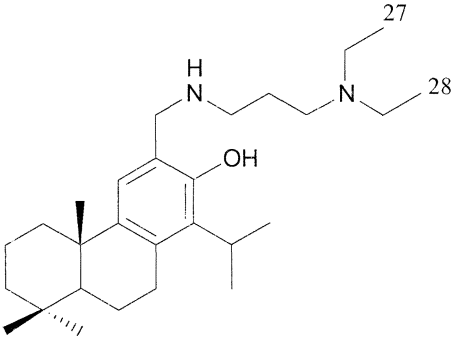
| Compound # | Amine used | Percentage yield |
|---|---|---|
| **50** |  | 72 |
| **51** |  | 80 |
| **52** |  | 86 |
| **53** |  | 65 |

The LREIMS of **50** indicated a molecular ion at m/z 401.4 (calculated $M^+$ 400.34536) that corresponded to the $(M+H)^+$ peak of a molecular formula of $C_{26}H_{44}N_2O$. Results from the elemental analysis of **50** were also within the ± 0.4% limit from the calculated values. The NMR data of the compound closely resembled that of totarol and only the main differences will be highlighted.




**50**

The key $^1$H-NMR evidence in support of the structure **50** was the appearance of a singlet for the aromatic proton resonating at 6.76 ppm, two doublets due to the amino methyl protons resonating at 3.82 ppm ($J$=13.5 Hz) and 3.88 ppm ($J$=13.4 Hz) two singlets at 2.20 and 2.16 ppm due to the two terminal methyl protons of the side chain amine. Further evidence for the structure of **50** was provided by the presence of two distinct signals in the $^{13}$C-NMR at δ 53.6 and 45.7 ppm due to the C-21 and (C-25/26) respectively. Another characteristic indicator is the shift of the C-12 signal from 109.9 in case of totarol to 120.9 ppm, due to the substitution.

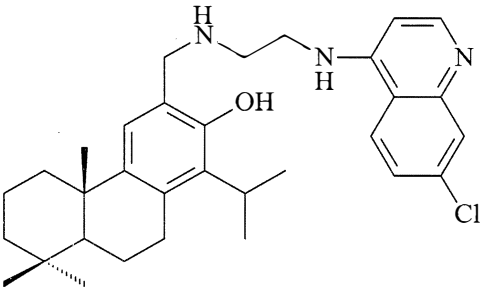



**51**

Consistent with the molecular formula of compound **51**, the LRFABMS analysis gave a peak at m/z 429.6 (calculated $M^+$ 428.37666), which corresponds to the $(M+H)^+$ peak of the

compound. Elemental analysis results of the compound were also found to be within the ± 0.4% limit from the calculated values. As can be seen from table **3.1.1.3**, the structure of **51** closely resembled that of **50**. Therefore to avoid repetitions, only the main differences in the spectrum will be discussed.

The most striking signals include the presence of a multiplet at δ 2.74 ppm which was assigned to H-7α & H-22, a multiplet at δ 2.49 ppm due to the resonance overlap of H-24, H-25 and H-26, another multiplet at 1.67 due to H-23, which overlapped with the multiplets of the fused ring protons corresponding to H-2 and H-6β. The most noticeable difference in the $^{1}$H- NMR was the appearance of a triplet at 1.02 ($J$=7.2 Hz) due to the terminal methyl ($CH_3$) protons of the side chain amine. Furthermore, the $^{13}$C-NMR spectrum also displayed 6 distinct peaks at 53.4, 51.3, 47.8, 46.9, 27.5 and 11.7 ppm, due to C-21, C-22, C-24, (C-25/ 26), C-23, and (C-27/ 28) respectively.

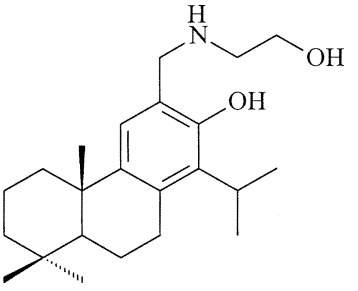



**52**

The LREIMS of compound **52** indicated peaks at m/z 520.4 (calculated 519.30164) and 522.4 (relative ratio of 3:1 respectively), corresponding to $[M(^{35}Cl) + H]^+$ and $[M(^{37}Cl) + H]^+$ ions respectively and were consistent with the formula $C_{26}H_{44}N_2O$. Results from the elemental analysis of the compound were also consistent with the structure **52** and were within ± 0.4% of the calculated value. To avoid the repetition of the totarol signals, only the main differences in the spectrum will be highlighted.

The key $^{1}$H-NMR evidences in support of the structure **52** were, the appearance of the additional five noticeable proton resonances in the aromatic region due to the protons from the quinoline ring; the two doublets resonating at 4.03 ($J$=16.8 Hz) and 3.97 ($J$=16.8 Hz) ppm, corresponding to the two diasterotopic protons of the amino methyl group and the two triplets

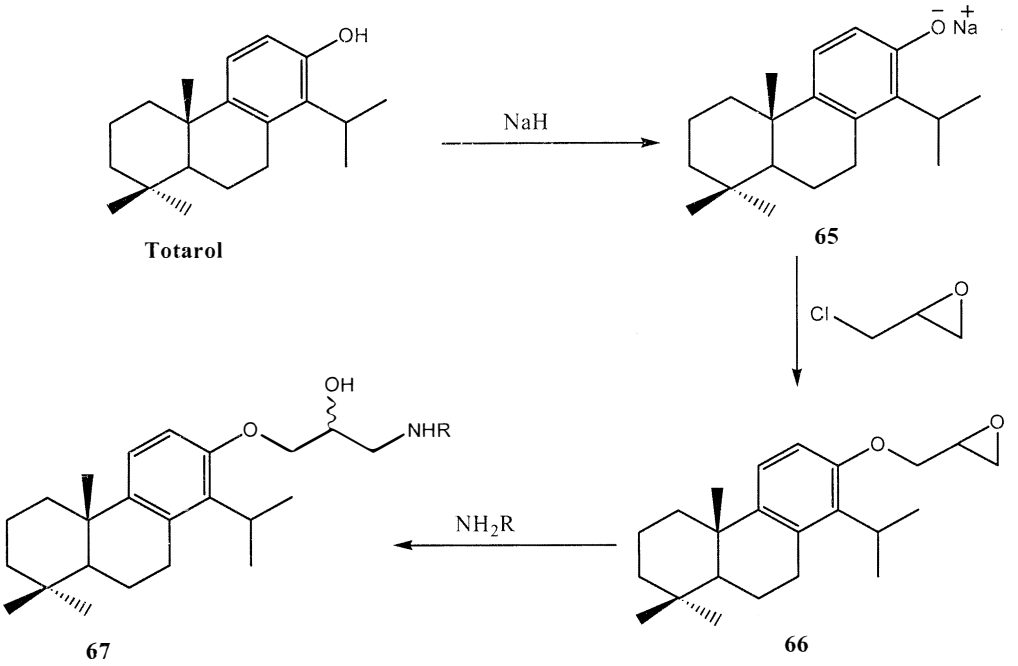
resonating at 3.42 and 3.17 ppm due to the two methylene protons of the side chain amine. Other characteristic indicators include disappearance of the two doublets corresponding to the aromatic proton of totarol due to substitution at the C-12 position and the appearance of a singlet at 6.77 ppm due to the unsubstituted aromatic proton of the totarol backbone. Further evidence from $^{13}$C-NMR indicate the presence 9 additional carbon resonances in the aromatic region corresponding to the quinoline carbons and other three distinct signals at 50.2, 49.5 & 40.1 ppm due to C-21, C-22 & 23 respectively.




**53**

The same method used to synthesise other compounds was employed to synthesize compound **53**. However, the reaction was relatively slow and didn't go to completion over 52 hrs. Evidence in support of the structure of **53** came from the $^{1}$H-NMR. In addition to the singlet, which appeared in all the Mannich bases at around 6.77 ppm due to the aromatic proton, the two doublets at 3.93 ppm ($J$=13.70 Hz) and 3.90 ppm ($J$=13.81 Hz) due to the two-diastereotopic protons of the aminomethyl group (H-21). Other noticeable signals include the appearance of a triplet signal ($J$=5.1 Hz) at δ3.77 due to the methylene protons at the carbon directly attached to the terminal hydroxyl group (H-23) and a triplet signal at 2.84 ($J$=5.1 Hz) due to the methylene protons at C-22. Other supporting evidence for the structure of **53** is the appearance of the three distinct signals in the $^{13}$C-NMR spectra at δ 61.4, 53.0 and 50.3 ppm due to C-23, C-21 and C-22 respectively. Furthermore a shift to a lower field (120.4) in the resonance of C-12 was also observed, signalling the substitution at the C-12 position.

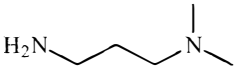
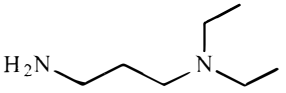
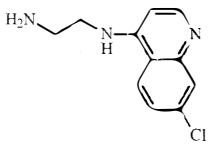
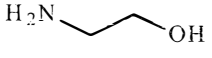
### 3.1.2 The synthesis of β-Amino alcohol analogues of Totarol

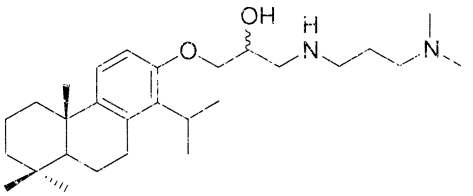



**Scheme 3.1.2**: Synthesis of totarol derived β-amino alcohols (**55-58**)

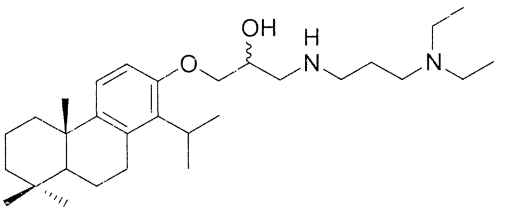
The method used for the synthesis of the target β-amino alcohols involved opening of an epoxide with primary amines (Scheme 3.12). As already mentioned, based upon the results of preliminary biological evaluation of synthesized Mannich base derivatives, only selected primary amines (See table **3.12A**) were used in the epoxide ring opening reaction.

**Table 3.1.2A**: Synthesized primary amine derived Mannich bases and their synthetic yield.

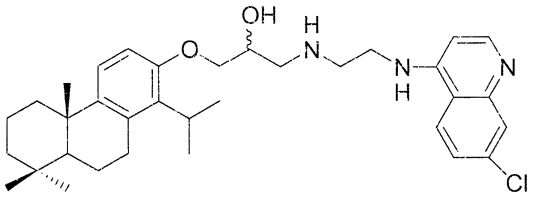
| Compound No. | Amine used | % Yield |
|---|---|---|
| **55** |  | 68 |
| **56** |  | 75 |
| **57** |  | 65 |
| **58** |  | 54 |



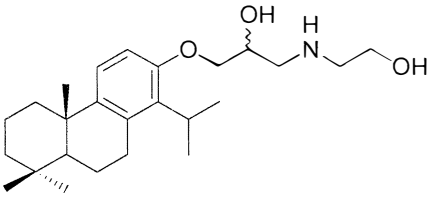
**55**



**56**



**57**



**58**

Compound **55** was synthesized in a relatively good yield. The HREIMS of the compound shows (M+H) ion peak at m/z 445.38028, which was consistent with the structure **55**. Characteristic peaks in $^1$H NMR spectrum are outlined in table 3.12B, in addition to the totarol core structure protons.

**Table 3.1.2B**: Key 1H-NMR indicators of compounds **55-58**

| Type of Proton | Compounds | | | |
|---|---|---|---|---|
| | **55** | **56** | **57** | **58** |
| H-21a | 4.01, dd, *J*= 5.23, 9.34 | 4.01, ddd, *J*=2.4, 5.22, 9.24 | 3.97, m | 4.01, dd, *J*=5.68, 2.3 |
| H-21b | 3.94, dd, *J*=5.42, 9.34 | 3.90, dd, *J*=5.30, 9.25 | | |
| H-22 | 4.10, m | 4.08, m | 4.16, m | 4.21, m |
| H-23a | 2.91, dd, *J*=3.80, 12.31 | 2.92, m | 2.91, m | 2.89-3.02, m |
| H-23b | 2.75, m, | 2.72, m | | |

The molecular formula $C_{30}H_{52}N_2O_2$ of Compound **56** was confirmed by its HREIMS, which displayed a molecular ($M^+$) ion peak at m/z 472.39678. The $^1$H-NMR spectra revealed the

appearance of a multiplet at 4.08 ppm due to H-22, a multiplet at 2.92 ppm due to H-23a, and a triplet ( $J$=7.15 Hz) due to the methyl ($CH_3$) protons of the terminal amine. Furthermore, the $^{13}$C-NMR also displayed the characteristic peaks outlined in table **3.1.2C**.

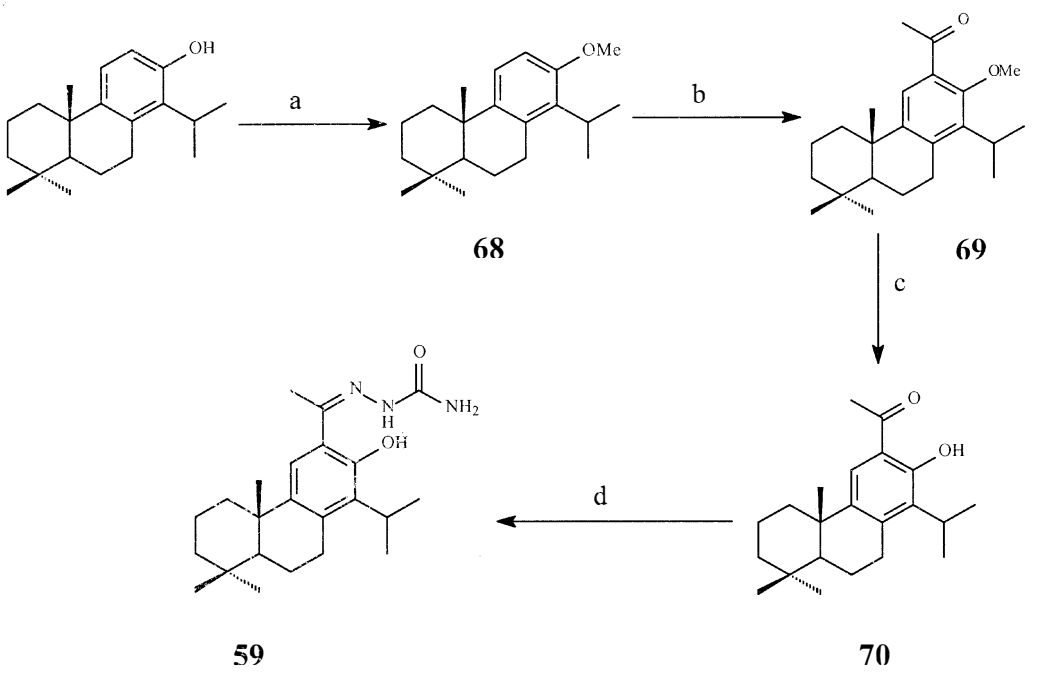
**Table 3.1.2C**: Key $^{13}$C-NMR indicators of compounds **55-58**

| Type of carbon | **55** | **56** | **57** | **58** |
|---|---|---|---|---|
| C-21 | 70.5 | 70.3 | 70.1 | 70.4 |
| C-22 | 68.1 | 68.5 | 68.7 | 69.5 |
| C-23 | 52.9 | 52.3 | 51.9 | 51.9 |

The HREIMS of compound **57** gave peaks at m/z 564.96513 and 566.9, for both $[M(^{35}Cl)]^+$ and $[M(^{37}Cl)]^+$ which reflects the molecular ion peaks for the formula $C_{34}H_{46}ClN_3O_2$. In addition to the key indicators in the $^1$H-NMR spectral data outlined above, other noticeable proton resonances include the five additional peaks in the aromatic region due to the protons of the quinoline ring. Furthermore, the $^{13}$C-NMR also reflects the aromatic carbons, in addition to the key $^{13}$C signals outlined in table **3.12C**. The HREIMS of compound **58** showed a molecular ion peak at m/z 403.30164 and was consistent with the calculated values. As with the other compounds, $^1$H and $^{13}$C-NMR also reflected the characteristic peaks outlined in table **3.12B** and **3.12C**.

### 3.1.3 Synthesis of the semicarbazone derivative of totarol

The semicarbazone derivative of totarol was one of the targeted compounds synthesized. As explained in chapter II, section **2.4.3**, this class of compounds has a rich history in the area of chemotherapy of different infectious diseases.

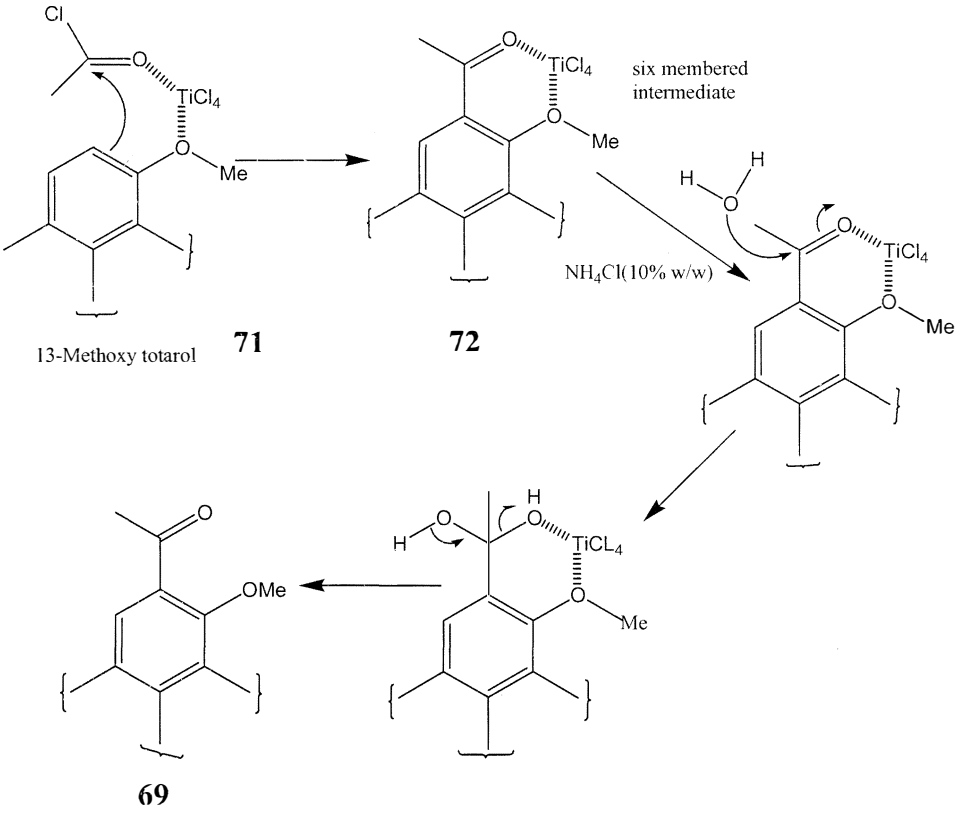



**Scheme 3.1.3A:** *Reagents and conditions*: (a) 1.5 equiv. of NaH, DMF, stirred at 0 $^0$C, 1.0 equiv. MeI, r.t., $N_2$, 14 hr (b) 2.0 equiv. of $CH_3COCl$, 4.0 equiv. of $TiCl_4$, $CH_2Cl_2$, 0 $^0$C, 30 min. (c) 5.0 equiv. of $BBr_3$, DCM, 30 min, r.t. (d) 2.0 equiv. of $NH_2CONHNH_2$, 2.0 equiv. of $NaOOCCH_3$, EtOH, reflux, 50 hr.

The synthesis of the semicarbazone derivative **59** was achieved *via* an intermediate ketone **70** obtained through an acid catalyzed acylation of the 13-methoxy totarol **68** (scheme **3.1.3A**), followed by demethylation of **69** with $BBr_3$ and subsequent treatment of the resulting ketone (12-Acetyltotara-8,11,13-trien-13-ol) with semicarbazide hydrochloride in the presence of sodium acetate, in ethanol.[75]

The mechanism for the acid catalysed acylation of **68** could be explained as follows (Scheme **3.1.3B**). The Lewis acid ($TiCl_4$) chelates with lone pair of electrons of the oxygen atom in the methoxy group and that of the carbonyl oxygen to form a tetrahedral complex **71** containing the methoxy totarol and the acetyl chloride as part of its ligands. This creates a more positive centre at the carbonyl carbon and initiates a nucleophilic attack by the aromatic electrons in to

the carbonyl carbon to give a six membered intermediate **72,** which upon hydrolysis with aqueous ammonium chloride gives rise to the methylated ketone **69**.




**Scheme 3.1.3B**: Mechanism for the formation of the intermediate ketone

The HREIMS analysis of compound **59** showed a molecular ion peak at m/z 385.27294 and was consistent with the molecular formula $C_{23}H_{35}N_3O_2$. The $^1$H-NMR spectrum of the compound closely resembled that of the totarol, where noticeable similarities include the isopropyl group signals, the five methyl signals and the proton resonances of the fused cyclic rings. To avoid repetitions, only the main differences in the spectrum will be discussed.

The most noticeable differences in the $^1$H-NMR spectrum include the singlet peak resonating at δ 11.10 ppm due to the H-bonded proton of the hydroxyl group, a singlet at 8.53 ppm due to the proton of the NH at position 23, a singlet at 7.22 ppm due to the aromatic proton, a broad singlet at 5.35 due to the two terminal NH protons and a singlet at 2.31 ppm due to the substituted methyl protons at C-21.

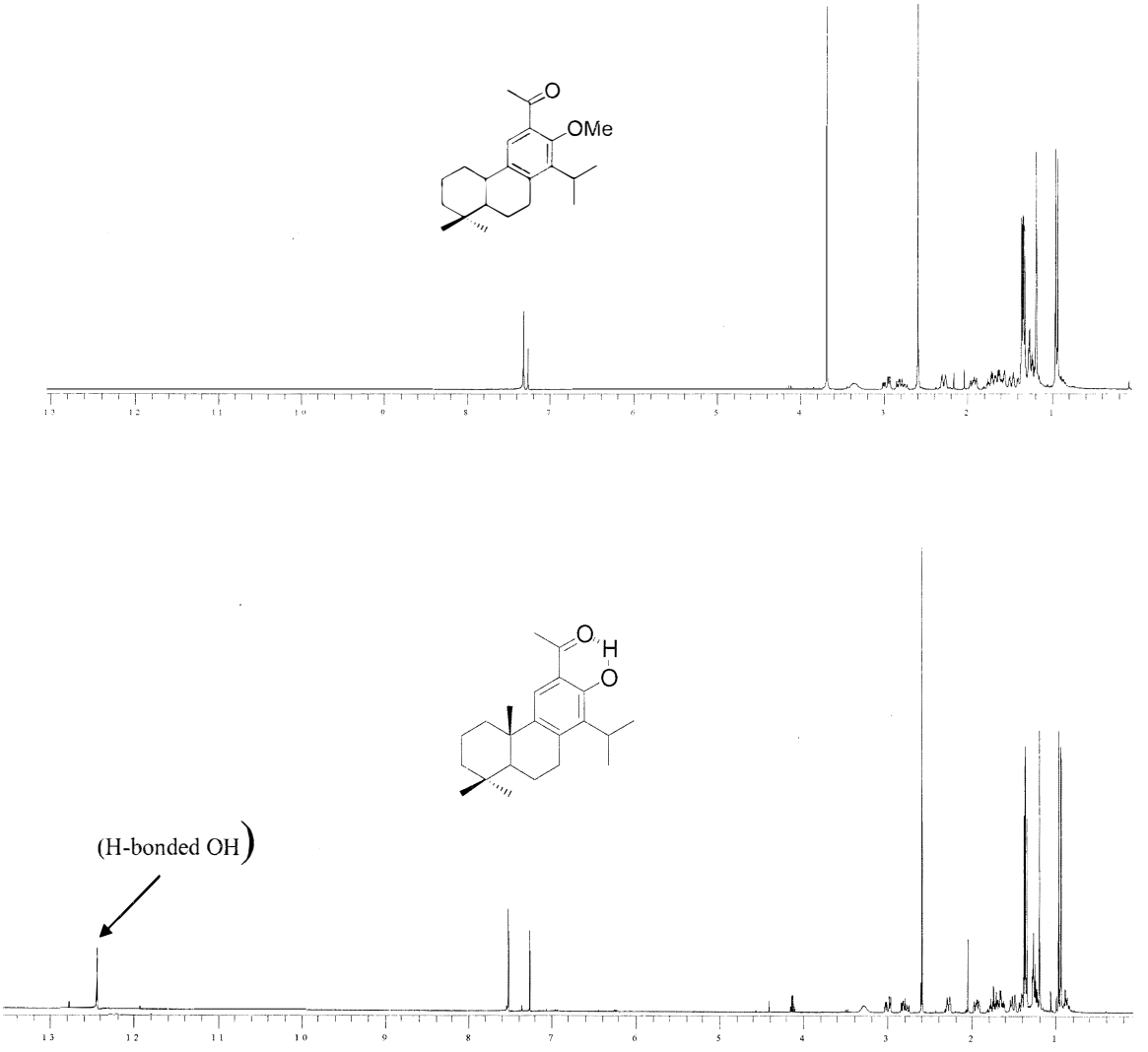
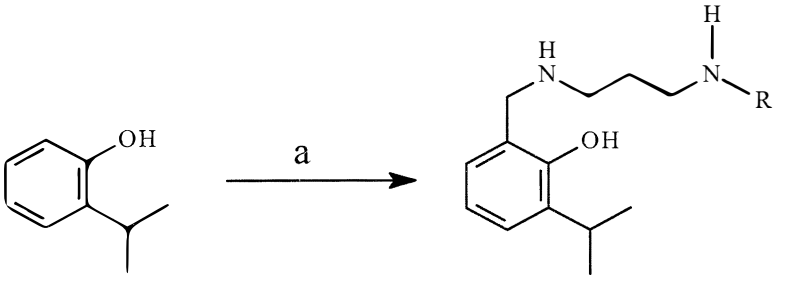



**Figure 3.1.3**: Comparison of $^{1}$H-NMR spectrum of the intermediate compounds **69** and **70**

### 3.2.1 Synthesis and characterization of 2-isopropyl phenol derivatives

In order to explore preliminary structural-activity relationship (SAR) studies of totarol with respect to the role of the diterpenoid backbone and the isopropyl group, a series of simplified Mannich base and β-amino alcohol analogues of 2-isopropyl phenol **40** were synthesized. In this group of compounds only primary amine derivatives were synthesized.

#### 3.2.1.1. Mannich base derivatives

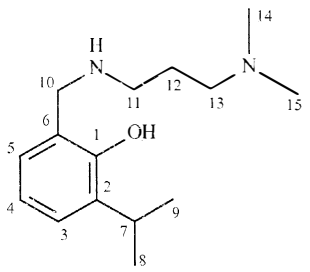



**40** **73**

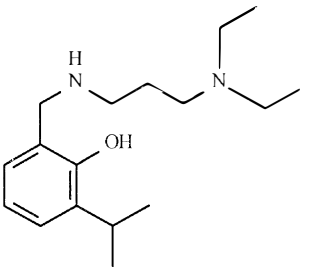
**Scheme 3.2.1.1:** General procedure for the synthesis of the Mannich base derivatives of 2-isopropyl phenol. *Reagents and conditions* (a) 4.0 equiv. of $(CH_2O)n$, 4.0 equiv. of $NH_2R$, EtOH, reflux,65 $^0C$, 13 hrs, $R \neq H$.

These simplified Mannich bases were synthesized using the standard Mannich reaction employed for synthesizing their totarol counterparts; as shown in scheme **3.2.1.1**. The rate of the reaction was temperature dependent and reactions conducted in methanol (refluxed at 65 $^0C$) were relatively slower than reactions done in ethanol (85 $^0C$). This is could be due to the increased concentration of the intermediate species (iminium ion **62**) with the increase in temperature.

The structure of compound **74** was confirmed by using various spectroscopic methods such as MS, NMR, IR and elemental analysis. The HREIMS analysis of compound **74** showed a molecular ion peak at m/z 250.20122 and was consistent with the molecular formula $C_{15}H_{26}N_2O$. The $^1H$-NMR evidence in support of the structure of **74** include the appearance of a broad singlet at δ 3.97 ppm due to the methylene protons (H-10) and a multiplet signal at 3.33 ppm due to H-7, and a singlet resonating at 2.22 ppm due to H-14/15. Results from the $^{13}C$-NMR also revealed two distinct signals at δ 57.9 and 27.1 ppm, due to C-10 and (C-15/14) respectively. Furthermore, the C-6 signal was relatively deshielded due to the substitution of the amino methyl group at the C-6 position.



74



75

The HREIMS analysis of compound **75** showed a molecular ion peak ($M^+$) at m/z 278.23897 and was consistent with the molecular formula of the molecule $C_{17}H_{30}N_2O$. Since the proton NMR of compound **75** closely resembled that of compound **74**, only the main differences in the spectrum will be discussed.

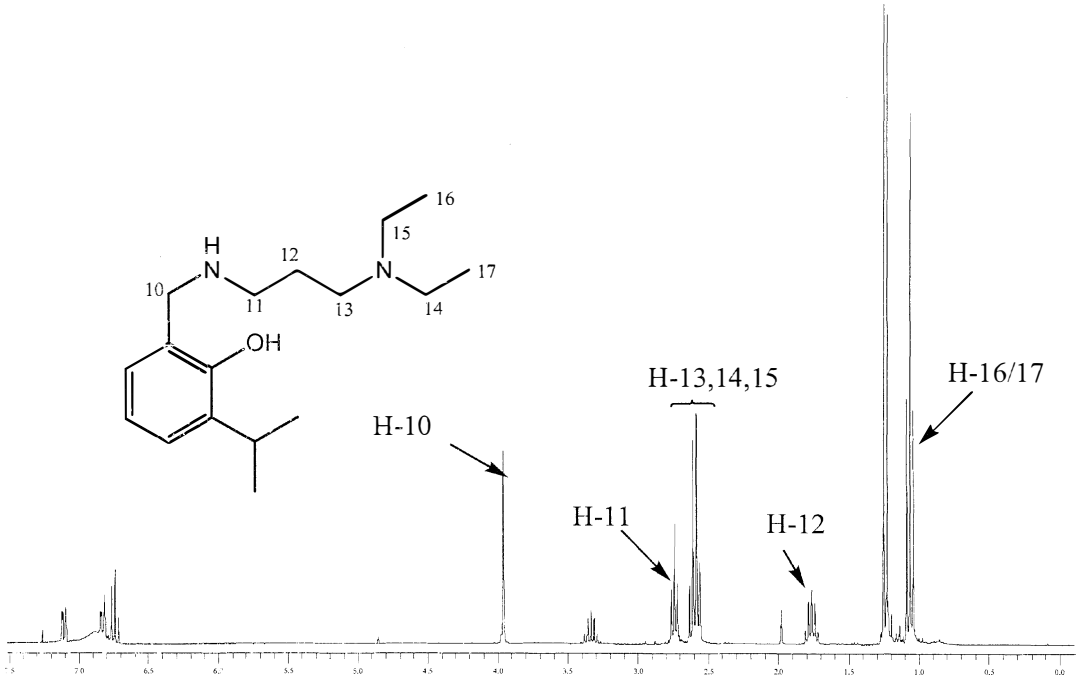
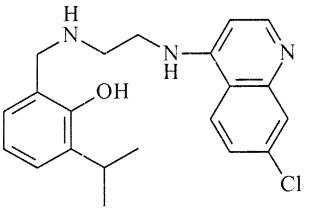


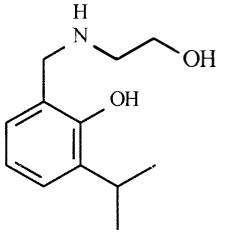

**Figure 3.2.1.1**: 1H-NMR spectrum of compound **75**

In addition to the characteristic peaks indicated above for compound **74**, other important proton resonances (Figure 3.2.1.1) of compound **75** include a triplet ($J$=6.57 Hz) at 2.73 ppm due to H-11, a quartet ($J$=7.20 Hz) at 2.59 ppm due to the four protons at H-14 and 15 and a triplet ($J$=7.25 Hz) at 1.05 ppm due to the terminal methyl protons (H-16 and 17) of the amine. This was also supported by the appearance of two additional peaks at 11.0 ppm due to C-16 and 17, in the $^{13}$C-NMR spectrum.



**76**

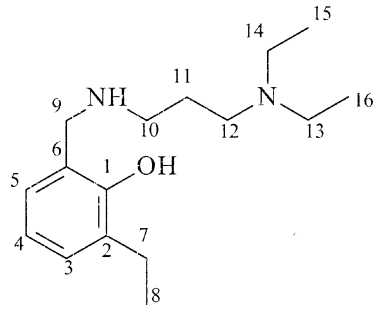
The formation of compound **76** was confirmed by the analysis of HREIMS results, which showed a peak at 383.15363 and at 385.1 at a relative ratio 3:1, coresponding to the two molecular ion peaks belonging to $[M(^{35}Cl)]^+$ and $[M(^{37}Cl)]^+$ of the compound $C_{21}H_{24}ClN_3O$. Other supporting evidence for the structure **76**, comes from the $^1$H-NMR, in addition to the characteristic broad singlet at 4.07 ppm due to the amino methyl protons (H-10), it also showed five other distinct aromatic proton resonances: d (J=5.7 Hz) at δ 8.35 ppm due to the H-2', d (J=8.96 Hz) at 8.05 ppm due to H-5', d (J=2.2 Hz) at 7.78 ppm due to H-8', a dd (J=9.01, 2.20 Hz) at 7.40 ppm due to the H-6' and a d (J=5.7 Hz) at 6.5 ppm due to H-5'. Furthermore, the $^{13}$C-NMR also reflected additional 9 distinct peaks at the aromatic region.



**77**

Unlike compounds **74-76**, the rate of the reaction was relatively slow, as observed with the synthesis of the totarol derived Mannich base analogue (**53**). However, compound **77** was obtained in a moderate yield of 60 %.

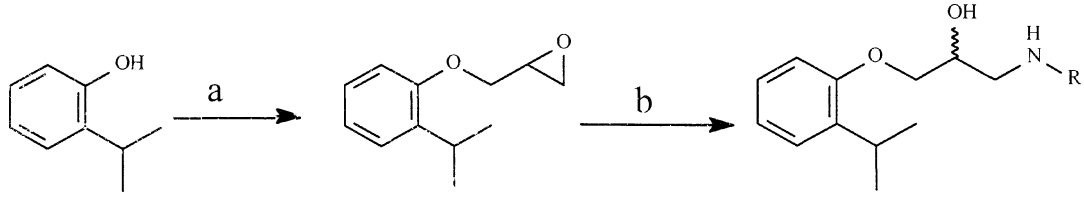
The HREIMS results for compound **77** show a molecular ion peak at m/z 209.13771, in support of the molecular formula $C_{12}H_{19}NO_2$. The key proton NMR evidence include a singlet peak at 4.92 ppm due to the two hydroxyl protons, a singlet at 4.34 ppm due to NH proton, a characteristic broad singlet at 4.10 ppm due to H-10, and two triplets at 3.73 and 2.87 ppm due to the methylene protons at H-12 and 11 respectively.




**78**

To explore the effect of the isopropyl group of totarol, a 2-ethyl-phenol derived Mannich base **78** was synthesized. Compound **78** was obtained in a moderate yield (65 %). The HREIMS indicated a molecular ion peak at 264.22045 ppm, which is in agreement with the molecular formula $C_{16}H_{28}N_2O$. Due to the similarity with compound **75**, only the key differences in the $^1$H-NMR spectrum will be discussed in detail. Key proton signals for the formation of compound **78**, were the appearance of a broad singlet at 3.96 ppm due to the amino methyl protons (H-9), and the absence of the multiplet signal of the isopropyl proton that is generally present in all the other compounds around 3.33 ppm. Furthermore the $^{13}$C-NMR spectrum also displayed a decrease in the intensity of the peak at 22.87 relative to that of compound **75** due to the removal of one carbon from the overlapped resonance in case of the other compounds.

### 3.2.1.2 β-Amino alcohol analogue




**79** **80-83**

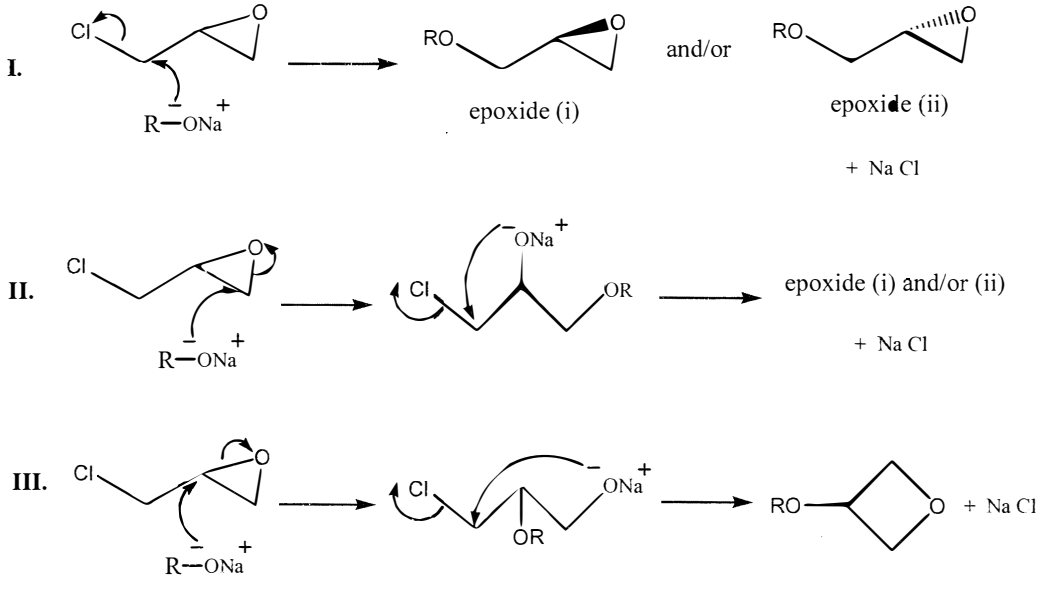
**Scheme 3.2.1.2A**: Reagents and conditions: (a) 3.0 equiv. of NaH, DMF, 0 $^0$C and 5 equiv. of 1-Cl-2,3-epoxy propane, r.t., 3hr. (b) 4.0 equiv. of $NH_2R$, EtOH, reflux, 3hr.

The β-amino alcohol derivatives were synthesized *via* an intermediate epoxide **79** obtained by treatment of the 2-isopropyl phenol with NaH in DMF at 0 $^0$C and subsequent reaction of the resulting salt with epichlorohydrin at room temperature (Scheme **3.2.1.2A**) [78]. The reactions were relatively faster than those of totarol, as they were completed within 3 hrs. Epichlorohydrin has three electrophilic centres (indicated as 1, 2 & 3 in scheme **3.2.1.2B**). Therefore a nucleophilic substitution could potentially occur at different sites depending on the reaction conditions.

I. An attack of the phenoxide anion at C-1 to generate epoxides (i) and or (ii) in a substitution nucleophilic bimolecular ($S_N2$) fashion.

II. An attack of the phenoxide anion at C-3 to produce an alkoxide intermediate followed by intra molecular $S_N2$ displacement of Cl to effect cyclization to the same epoxide [(i) or (ii)].

III. An attack of the phenoxide anion at C-2, to produce another alkoxide intermediate followed by an intramolecular displacement of Cl to give oxetane (iii) derivatives.




**Scheme 3.2.1.2B:** General structure of the epoxides (i) and (ii) and the oxetane (iii) [79]

The regiochemistry of epoxy alkyl halide reactions depend upon solvents used. Aprotic solvents lead to type I nucleophilic attack. Alcoholic solvents lead to a type II attack due to the weakening of the C-O bond as a result of H-bonding. In the presence of an acid the oxygen of the epoxide is protonated (or complexed to Lewis acids), resulting in preferential attack at the secondary C-2, where the developing positive charge is better stabilized. [106-107] The structure of the intermediate epoxide **79** was confirmed using $^{1}$H-NMR, $^{13}$C NMR as shown in figure **3.2.1.2A** and **3.2.1.2B** respectively.

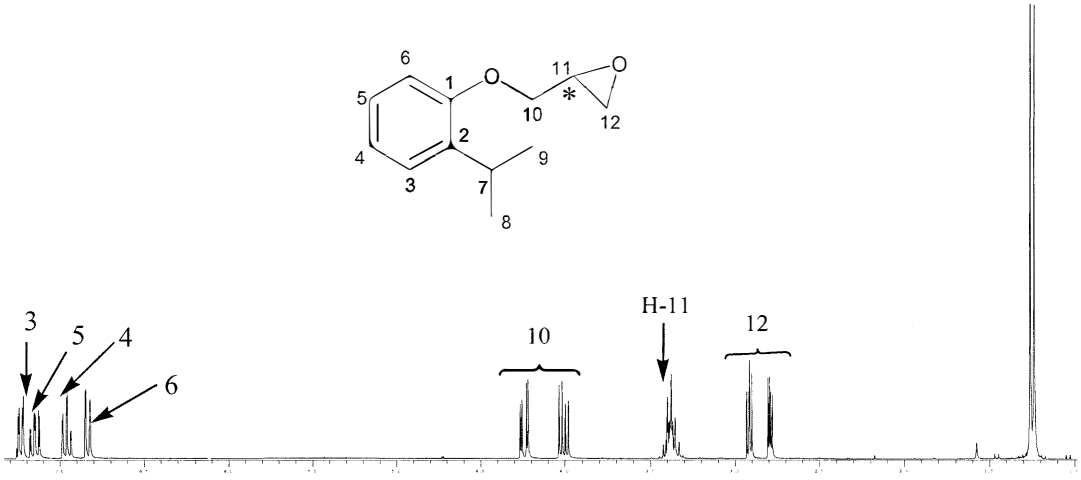



**Figure 3.2.1.2A** 1H-NMR spectrum of the intermediate epoxide **79**

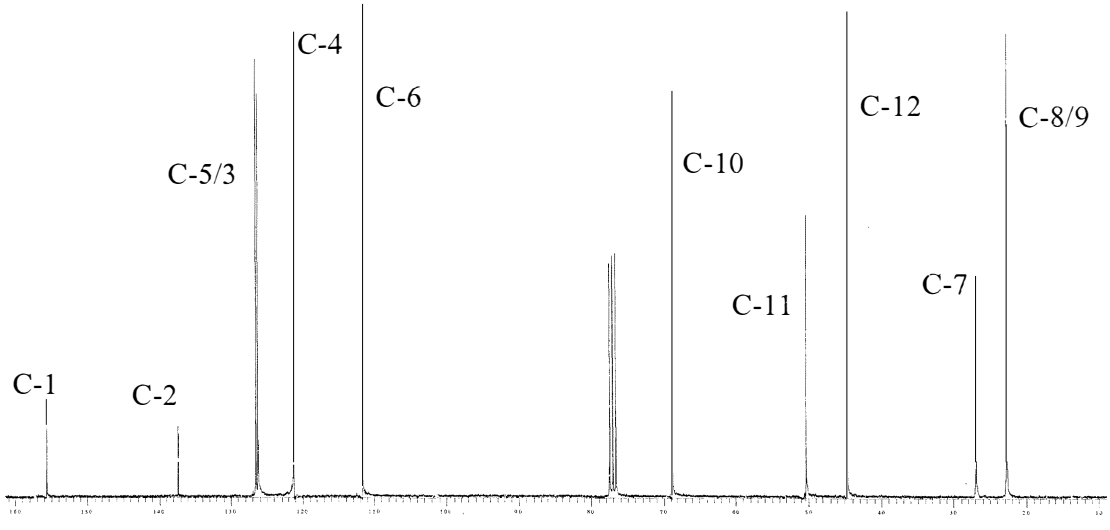
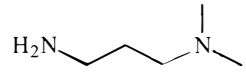
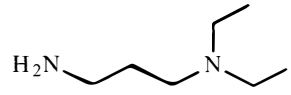
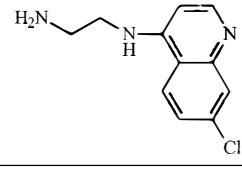
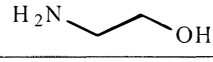



**Figure 3.2.1.2B**: $^{13}$C-NMR spectrum of the intermediate epoxide (**79**)

The procedure for the synthesis of compounds **80 - 83** from the epoxide intermediate was similar to that described for the synthesis of the β-amino alcohol derivatives of totarol. However, the rate of the reactions was faster relative to that observed for totarol derivatives. This could be due to the steric hindrance of the hydroxy group by the neighbouring isopropyl group in the case of totarol. It is well known that the hydroxy group of totarol is structurally hindered by the isopropyl group as a result of a non-bonding interaction between the methyl protons of the isopropyl group and the methylene protons at position C-7 in totarol [64]. All the compounds synthesized were extremely polar and were purified using the solvent system DCM:MeOH:$Et_3N$ in a ratio of 80:20:1 respectively.

**Table 3.2.1.2A**: Synthesized primary amine derived amino alcohols and their synthetic yield

| Compound # | Amine used | % Yield |
|---|---|---|
| **80** |  | 63 |
| **81** |  | 70 |
| **82** |  | 62 |
| **83** |  | 60 |

Compound **80** was synthesized in a moderate yield (63 %), and its structure was confirmed by spectroscopic means. The HRFABMS result showed $(M+H)^+$ peak at m/z 295.21323 which is consistent with the molecular formula $C_{17}H_{30}N_2O_2$. The $^1$H-NMR evidence for structure **80** include the appearance of a multiplet at δ 4.10 ppm due to the proton at the chiral centre (C-11), a double doublet ($J$=5.23 and 9.34) at 4.01 ppm due to H-10a, a double doublet ($J$=5.42, 9.34) at 3.94 ppm due to H-10b, a double doublet ($J$=12.31, 3.80 Hz) at 2.91 due to H-12a, and a multiplet at 2.75 ppm due to overlapped resonances of H-12a and H-13 protons. Furthermore $^{13}$C-NMR also shows three distinct peaks at 70.5, 68.1, 52.9 ppm due to C-10, C-11 and C-12 respectively.

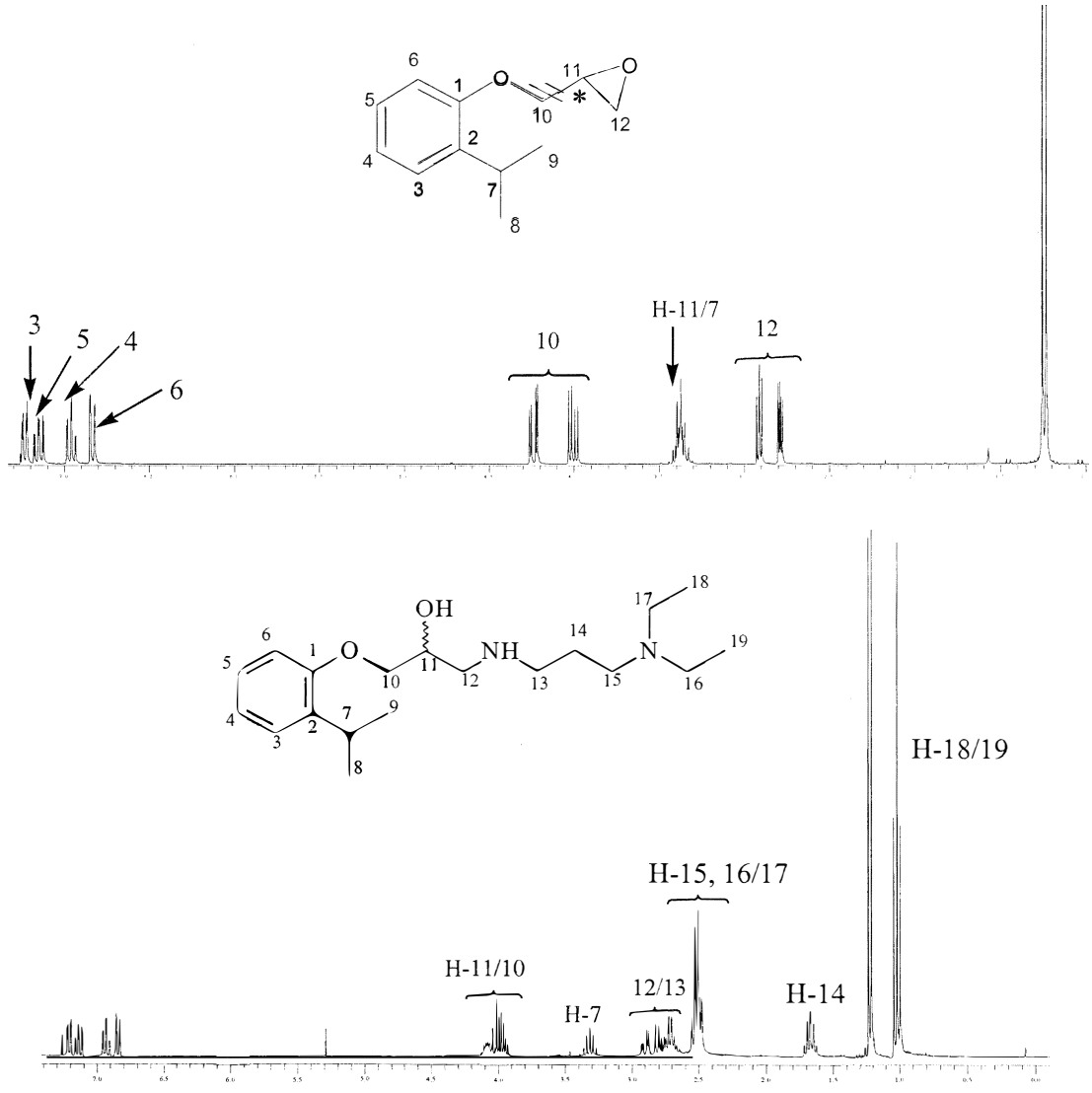



**Figure 3.2.1.2C**: Comparison of $^{1}$H-NMR spectrum of compound **81** and the epoxide intermediate

The HREIMS analysis of compound **81** revealed a peak at m/z 323.27541 which was consistent with the $(M+H)^{+}$ ion of the molecular formula $C_{19}H_{34}N_2O_2$. The $^{1}$H-NMR spectrum of the compound resembles that of compound **80** and its structure was confirmed by the characteristic $^{1}$H NMR resonances given in table **3.2.1.2B**. Furthermore the $^{13}$C-NMR also displays the key $^{13}$C resonances outlined in table **3.2.1.2C**.

**Table 3.2.1.2B:** Key $^{1}$H-NMR indicators of compounds **80-83**

| Type of Proton | **80** | **81** | **82** | **83** |
|---|---|---|---|---|
| H-10a | 4.01, dd, J=5.23, 9.34 | 4.01, dd, J=5.22, 9.26 | 4.01, m | 4.00, dd, J= 5.30, 9.44 |
| H-10b | 3.94, dd, J=5.42, 9.34 | 3.95, dd, J=5.22, 9.24 | | 3.94, m, J=5.30, 9.44 |
| H-11 | 4.10, m | 4.09, m | 4.24, m | 4.01, m |
| H-12a | 2.91, dd, J=3.80, 12.31 | 2.90, dd, J=3.78, 12.19, | 3.00, dd, J=3.76, 12.29, | 2.93-2.75, m |
| H-12b | 2.75, m, | 2.79, dd, J=7.53, 9.24 | 2.93, dd, J=7.51, 12.17 | |

The HREIMS of compound **82** showed a molecular ion peak ($M^{+}$) at m/z 413.18776 which is consistent with the molecular formula $C_{23}H_{28}ClN_3O_2$. In addition to the $^{1}$H-NMR indicators outlined in table 3.21B2, other important features include the five additional peaks in the aromatic region due to the protons of the aromatic quinoline in the amine side chain. Furthermore, the $^{13}$C-NMR also displayed eight additional peaks in the aromatic region besides the three characteristic peaks given in table **3.2.1.2C**.

**Table 3.2.1.2C**: Key $^{13}$C-NMR indicators of compounds **80-83**

| Type of carbon | **80** | **81** | **82** | **83** |
|---|---|---|---|---|
| C-10 | 70.5 | 70.5 | 70.3 | 70.5 |
| C-11 | 68.1 | 68.3 | 68.9 | 68.6 |
| C-12 | 52.9 | 52.0 | 51.8 | 52.0 |

As with the first three compounds, compound **83** was obtained in a moderate yield (62 %). The HREIMS of this compound showed a molecular ion peak $M^{+}$ at m/z 253.15273, which was consistent with the molecular formula $C_{14}H_{23}NO_3$. The structure of the compound was further confirmed using the $^{1}$H and $^{13}$C-NMR spectra, as outlined in table **3.2.1.2B** and table **3.2.1.2C**. Unlike all the other β-amino alcohols synthesized, each signal in the $^{1}$H NMR

integrated for two protons, which could be due to the existence of an intramolecular H-bonded conformation.

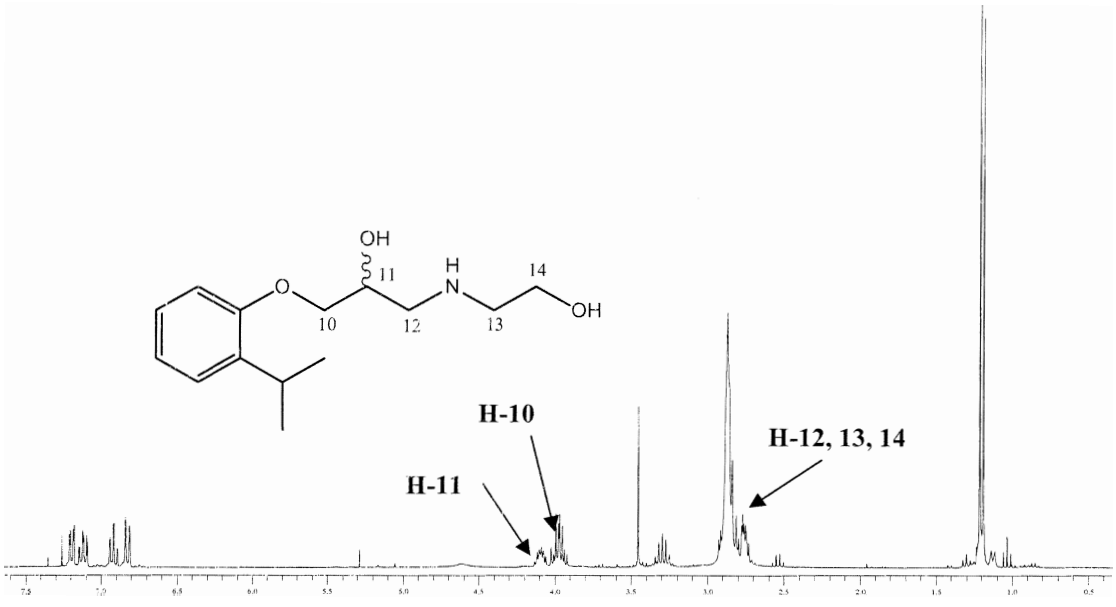



**Figure 3.2.1.2D** $^{1}$H-NMR spectrum of compound **83**

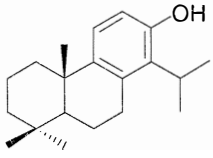
# CHAPTER FOUR

# BIOLOGICAL RESULTS AND DISCUSSION

## 4.1 *In vitro* antiplasmodial activity and cytotoxicity of totarol

Totarol has been reported to possess a marked antiplasmodial activity against both CQ-sensitive (D10) and CQ-resistant (K1) strain of *P. falciparum*. It has also been shown that the K1 strain is almost twice more sensitive to totarol than D10 (table 4.1). Moreover, its cytotoxic assays revealed its significant selectivity towards parasite cells [78]. The *in vitro* cytotoxicity of totarol has also been previously reported [74].

**Table 4.1**: *In vitro* antiplasmodial activity and cytotoxicity of totarol [78]

| Compound | $IC_{50}$ $\mu gml^{-1}$ ($\mu M$) | | | SI | RI |
|---|---|---|---|---|---|
| | D10 | K1 | CHO | | |
|   | 2.15 ± 0.18 (7.50 ±0.20) | 1.23 ± 0.15 (4.29 ± 0.25) | 48.79±0.96 (170.46±25.78) | 39.66 | 0.57 |

Selectivity Index (**SI**) = $IC_{50}$ (CHO)/ $IC_{50}$ (K1)

Resistance index (**RI**) = $IC_{50}$ (K1)/ $IC_{50}$ (D10)

(CHO) a mammalian Chinese hamster ovarian cell used for the cytotoxic assay.

## 4.2 *In vitro* antiplasmodial activity of Mannich base derivatives of totarol

### Methodology

All *in vitro* biological tests of the synthesized compounds were performed by the pharmacology department, University of Cape Town and the preparation of the parasite cultures (D10 and K1) were conducted using a modified method of Trager and Jensen [108]. All samples were tested in duplicate on a single occasion against a CQ-sensitive strain (D10) of the parasite. Compounds more active against the D10 strain were also tested against the K1 strain of the parasite. The parasites were continuously cultured, and parasite Lactate Dehydrogenase (pLDH) assay was then used to determining their in *vitro* antiplasmodial activity as described by Makler [109]. The pLDH assay is one of the commonly used assays for *in vitro* determination of antiplasmodial activity [110]. The average percentage parasite viability was then determined as three different concentrations.

All compounds were dissolved in 10% methanol (MeOH). The initial stocks were then diluted with water to reach a concentration of 20µl/ml. The samples were kept at -20$^0$C until use. All samples were diluted to 2000ng/ml in complete medium on the day of the experiment which was further diluted to give 10 concentrations; with the lowest concentration (highest concentration of solvents) being 1.953ng/ml in which the parasites were exposed to had no measurable effect on the parasite viability. In all the experiments Chloroquine was used as a positive control and a full dose-response was done on the most active compounds. The 50% inhibitory concentration ($IC_{50}$) values were obtained from the dose-response curves, using a non-linear dose-response curve fitting analyses via Graph Pad Prism v.2.01 software.

**Table 4.2A:** *In vitro* antiplasmodial activity of the Mannich base compounds against D10

| Compound | % Parasite Survival | | | $IC_{50}$ ng/ml |
|---|---|---|---|---|
| | 2000 ng/ml | 1000 ng/ml | 500 ng/ml | |
| **50** | 9.53 | 10.64 | 18.69 | 191.43 |
| **51** | 5.00 | 11.69 | 13.34 | 136.14 |
| **52** | 9.27 | 16.44 | 31.06 | 33.57 |
| **53** | 9.92 | 14.05 | 21.08 | 131.83 |
| **45** | 2.92 | 12.21 | 16.24 | 138.04 |
| **46** | 88.00 | 99.74 | 99.36 | - |
| **47** | 104.53 | 113.23 | 109.27 | - |
| **48** | 41.33 | 118.18 | 119.42 | - |

Out of the 14 compounds (Mannich bases) tested against *P. falciparum* D10 ($CQ^S$), five compounds showed promising antiplasmodial activity and were tested against *P.falciparum* K1 ($CQ^R$). However, all of the compounds appear to be slightly less active against K1 than against D10. The same initial stock were used both for the D10 and K1 experiment, and the K1 experiment were performed 2 weeks after the initial stock was made up. Hence, the reduced activity could be due to the compounds being unstable in solution. The difference in activity against K1 and D10 is only about two fold for most of the compounds. This could be due to experimental error, as the percentage parasite viability would differ slightly from day to day. The difference between K1 and D10 CQ sensitivity is about tenfold, so the compounds do not appear to follow a CQ-resistant pattern. Alternatively, the compounds may just be less active against a CQ-resistant strain. Nevertheless, a trend in activity can still be observed

within the different groups of compounds. Out of the compounds tested, the most active were **50**, **51**, **52**, **53** and **45.**

**Table 4. 2C:** *In vitro* antiplasmodial activity of D1● active compounds against K1

| Compound | % Parasite Survival | | | |
|---|---|---|---|---|
| | 2000 ng/ml | 1000 ng/ml | 500 ng/ml | 250 ng/ml |
| **50** | 16.10 | 21.70 | 41.47 | - |
| **51** | 23.87 | 32.75 | 59.59 | - |
| **52** | 24.97 | 62.64 | 59.63 | - |
| **53** | 27.68 | 39.62 | 56.45 | - |
| **45** | 23.48 | 45.55 | 61.64 | - |

**Table 4.2B:** *In vitro* antiplasmodial activity of chloroquine (positive control) against D10

| Compound | % Parasite Survival | | |
|---|---|---|---|
| | 30 ng/ml | 15 ng/ml | 7.5 ng/ml |
| Chloroquine | 34.96 | 86.69 | 90.41 |

**Table 4. 2D** *In vitro* antiplasmodial activity of chloroquine (positive control) against K1

| Compound | % Parasite Survival | | |
|---|---|---|---|
| | 200 ng/ml | 100 ng/ml | 50 ng/ml |
| Chloroquine | 39.75 | 74.14 | 79.46 |

### 4.2.1 Structure Activity Relationship

Replacement of the diethyl amine side chain in compound **44** with pyrolidine in compound **45** resulted in a drastic change in its antiplasmodial activity. This could be due to the side chain pyrrolidine being less susceptible to metabolism than the diethyl side chain as shown in the case between amopyroquine and amodiaquine [25]. The replacement of the terminal diethyl amine in compound **44** by both 3-diethyl-propylamine and 3-dimethyl-propylamine in compounds **51** and **50** respectively, enhanced its antiplasmodial activity against the chloroquine sensitive strain of the parasite.

With respect to the study of the role of the diterpenoid backbone of totarol in its intrinsic antiplasmodial activity, with the exception of compound **76**, an aminoquinoline containing compound, none of the 2-isopropyl-phenol derived Mannich bases were shown to possess any significant antiplasmodial activity. This demonstrates that, the diterpenoid backbone of totarol is not merely acting as a hydrophobic group in its intrinsic antimalarial activity. Moreover, the compounds have shown a similar trend in their relative activity as observed for their totarol analogues. The compound **76** has shown a significant antiplasmodial activity against the D10 strain of the parasite. This could be associated with the activity of the aminoquinoline moiety in the structure. However, the absence of this observed activity in its totarol analogues suggests that it could also be the resultof a combined effect of the 2-isopropyl phenol and the aminoquinoline moiety. Further evaluation is required to give any generalization on this specific compound.

### 4.3 In *vitro* antiplasmodial activity of ß-amino alcohol derivatives of totarol

Previous work by Clarkson [78] and Tacon[111] on *ß*-amino alcohols has revealed a range of potent antiplasmodial compounds. To expand the library of the potent amino alcohols, we have synthesized various *ß*-amino alcohol derivatives of totarol. Based on the result of preliminary biological evaluations of the synthesized Mannich base derivatives, only selected primary amines derived amino alcohols were synthesized.

A similar methodology was employed for testing the compounds against a chloroquine sensitive strain of *plasmodium falciparum* (D10), but Compound **56** and **57** were first dissolved in 100µl of 100% dimethyl sulfoxide (DMSO)/MeOH. As can be seen from the percentage parasite viability (table: 4.3), none of the synthesiszed amino alcohols except compound **82** (aminoquinoline containing derivative) from the structural activity relatioship, were found to be active against the D10 strain of the parasite.

**Table 4.3**: *In vitro* antiplasmodial activity of the β-amino alcohol compounds against D10

| Compound | % Parasite Survival | | |
|---|---|---|---|
| | 1000 ng/ml | 500 ng/ml | 250 ng/ml |
| **55** | 91.69 | 115.58 | 101.82 |
| **56** | 105.83 | 107.07 | 105.85 |
| **57** | 18.38 | 24.48 | 95.41 |
| **58** | 42.98 | 91.89 | 82.79 |
| **80** | 57.84 | 63.74 | 83.98 |
| **81** | 49.38 | 76.24 | 81.39 |
| **82** | 2.51 | 0.00 | 0.69 |
| **83** | 67.00 | 77.47 | 84.37 |

## 4.4 Compliance to Lipinski's Rule

The factors that have been considered as a primary guide to correlating physical properties with successful drug development by previous researchers are; lipophilicity, molecular weight, and the number of hydrogen bond donors and acceptors [112-114]. Lipinski's "Rule of 5" for oral drugs states that poor adsorption and permeation through membranes and cellular structures is more likely when:

i. There are more than 5H-bond donors.
ii. More than 10 H-bond acceptors,
iii. The molecular weight is over 500 and
iv. The clogP is greater than 5.

According to the Lipinski rule, any drug-like compound which meets any three of the four criteria is likely to have good absorption and permeation. Most of the synthesised compounds (Mannich bases, Aminoalcohols and Semicarbazon) meet the minimum criteria (Table **4.1.4)** and have potential to be developed as oral drugs. However, none of them were found to be potent against the resistant strain of the parasite.

**Table 4.4:** Compounds **44-59** and their compliance to Lipinski's rule of 5

| Compound # | Molecular formula | Molecular weight | c Log P | H-bond donors | H-bond acceptors | No. of Criteria met |
|---|---|---|---|---|---|---|
| | | <500 | <5 | <5 | <10 | At least 3 |
| **44** | $C_{25}H_{41}ON$ | 371.31852 | 6.688 | 1 | 1 | 3 |
| **45** | $C_{25}H_{39}ON$ | 371.31097 | 6.339 | 1 | 1 | 3 |
| **47** | $C_{25}H_{39}O_2N$ | 383.29806 | 5.782 | 1 | 2 | 3 |
| **46** | $C_{26}H_{41}ON$ | 383.31879 | 6.844 | 1 | 1 | 3 |
| **48** | $C_{26}H_{42}N_2O$ | 398.32929 | 5.827 | 1 | 2 | 3 |
| **49** | $C_{34}H_{44}ClN_3O$ | 546.32538 | 8.415 | 1 | 3 | 2 |
| **52** | $C_{32}H_{42}ClN_3O$ | 520.15110 | 8.051 | 3 | 1 | 2 |
| **53** | $C_{23}H_{37}NO_2$ | 359.5541 | 5.059 | 3 | 0 | 3 |
| **50** | $C_{26}H_{44}N_2O$ | 400.6511 | 5.99 | 2 | 1 | 3 |
| **51** | $C_{28}H_{48}N_2O$ | 428.7051 | 6.744 | 2 | 1 | 3 |
| **55** | $C_{28}H_{48}N_2O_2$ | 444.37158 | 5.57 | 2 | 2 | 3 |
| **56** | $C_{30}H_{52}N_2O_2$ | 472.40288 | 6.322 | 2 | 2 | 3 |
| **57** | $C_{25}H_{41}NO_3$ | 403.59798 | 4.638 | 3 | 1 | 4 |
| **58** | $C_{34}H_{46}ClN_3O_2$ | 563.32786 | 7.642 | 3 | 2 | 2 |
| **59** | $C_{23}H_{35}N_3O_2$ | 385.5521 | 5.45 | 3 | 2 | 4 |

## 4.5 Conclusion

Generally, the secondary amine Mannich bases were shown to possess better antiplasmodial activity than the tertiary amine Mannich bases. This could be associated with the improved solubility and clogP values of the compounds. Even though, none of the synthesized Mannich bases seems to be as active as the starting material totarol, an important conclusion could be drawn from their structural activity relationship.

With regard to the amino alcohol derivatives of totarol, none of the compounds synthesiszed possesses significant antiplasmodial activity. As can be seen from the structure of the compounds (**56-58**), none of the synthesized amino alcohols possess protonatable nitrogen

(tertiary amine) at the beta position of the hydroxyl group. This was consistent with previous findings in our group [111] that the presence of protonatable nitrogen at the beta position could be a necessary structural feature for high antiplasmodial activity with totarol-derived amino alcohols. Besides, weakly basic compounds are likely to become traped in acidic compartments due to the existing transmembrane proton gradiant. The semicarbazone derivative of totarol also did not show any antiplasmodial activity.

The difference in the preference of the amines with respect to the Mannich bases and aminoalcohols could suggest differences in the mechanism of action of the respective compounds.

# CHAPTER 5
# EXPERIMENTAL RESULTS

## 5.1 General

Reactions were monitored by thin layer chromatography on aluminium backed silica gel 60 $F_{254}$ plates (Merck) and visualized with a combination of ultraviolet light (254 nm) and either anisaldehyde spray [freshly prepared from a 2.5 % solution of p-methoxybenzaldhyde (20 $cm^3$) and 18 M sulphuric acid (1 $cm^3$)] or ceric ammonium sulphate in 8 M sulphuric acid, followed by heating at 200 $^0$C. Column chromatography and preparative layer chromatography (p.l.c) was carried out using Merck Kieselgel 60: 70-230 mesh.

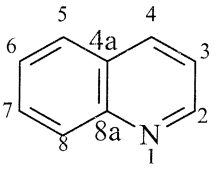
$^1$H NMR spectra where recorded on either a Varian Mercury 300 MHz or a Varian Unity Spectrometer at 400 MHz and are recorded as parts per million (ppm) relative to tetramethylsilane (TMS). $^{13}$C NMR was recorded on the same instrument operating at 75 MHz or 100 MHz with the same internal standard. The following abbreviations were used in the $^1$H NMR spectra: s, singlet; d, doublet; dd, doublet of doublet; ddd, doublet doublet doublet, t, triplet; q, quartet; m, multiplet; *J* coupling constant and is measured in Hz.

Infrared spectra were measured in solution form using chloroform or as solids (KBr Pellets) or Nujol mulls on a satellite FT-IR spectrophotometer. Melting points were determined on a Reichert-Jung Thermovar (temperature range 0-350 $^0$C) on cover slips and are uncorrected.

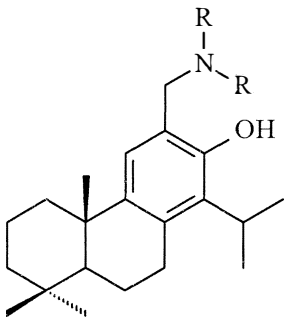
Mass spectra were recorded on a VG (micromass) 70-SE magnetic sector mass spectrometer (at Kent, UK). Other mass spectra were recorded in a VG 70SEQ using field regulation Electron impact ionisation (EI) mass spectrometry (at Wits University, South Africa). Micro (elemental) analysis for C, H, N were performed using a Fison`s Instruments Elemental Analyser EA 1108.

Commonly used solvents were purified and dried accordingly as described in the literature. Removal of solvents from the products was achieved by using a Buchi Rotary Evaporator under reduced pressure and anhydrous sodium sulphate and anhydrous magnesium sulphate were used in the drying of organic solvents after extraction.

The following numbering system was used to assign aromatic protons in the quinoline ring system.

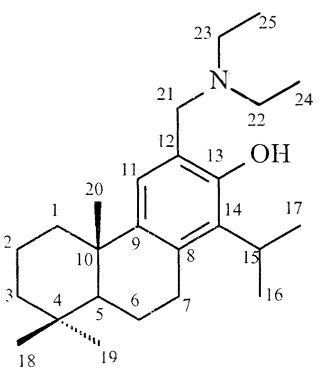


## 5.2 General procedure for the preparation of 44-49




A 0.35 mmol of totarol was added in to a stirred solution of aqueous paraformaldehyde (0.70 mmol) and a primary amine (1.4 mmol) or secondary amine (0.70 mmol) in 2 ml ethanol. The reaction mixture was then allowed to reflux for 48 hours at 80 $^0$C. After refluxing for 48 hr the solvent was removed under reduced pressure and the residue was dissolved in dichloromethane (5 ml). The organic solution was extracted with dilute hydrochloric acid (0.1 M, 2 x 10 ml). The acidic aqueous phase was basified to 9-10 pH and extracted with dichloromethane (3 x 10 ml). The combined organic fractions were washed with water (1 x 10 ml), dried over $MgSO_4$, filtered and evaporated under reduced pressure.[8] Compounds 12-14 were isolated as crystals by filtration and was not required to do the workup.

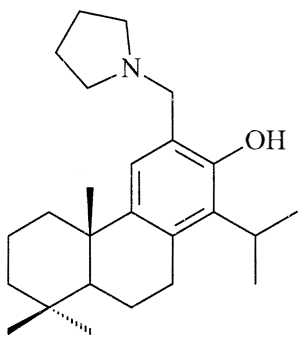
**3-Diethylaminomethyl-1-isopropyl-4b,8,8-trimethyl-4b,5,6,7,8,8a,9,10-octahydro-phenanthren-2-ol (44).**




Yield (0.10 g, 80%), $R_f$ 0.78 (Hexane), mp 92-95 $^o$C; $\nu_{max}$ (Chloroform)/cm$^{-1}$ 3619 (free OH), 3029 (C-H), 1516, 1427 Ar (C = C), 1220 (C-O or C-N), 1042 (C-O or C-N), $\delta_H$ (400 MHz, $CDCl_3$) 6.73 (1H, s, H-11), 3.74 (1H, d, *J*=13.72 Hz, H-21a), 3.62 (1H, d, *J*=13.81 Hz, H-21b), 3.24 (1H, m, H-15), 2.94 (1H, dd, *J*=18.0, 5.80 Hz, H-7β), 2.74 (1H, ddd, *J*=18.0, 10.8 & 7.2, H-7α), 2.59 (4H, m, H-22 & 23), 2.23 (1H, d, *J*=12.79, H-1β), 1.92 (1H, dd, *J*=13.20, 7.78, H-6α), 1.55 - 1.78 (3H, m, H- 2, 6β), 1.47 (1H, d, *J*= 13.17, H-3β), 1.36 (3H, d, *J*=7.06, H-17), 1.35 (3H, d, *J*=7.06, H-16), 1.31-1.20 (3H, m, H-1α, 3α and H-5), 1.17 (3H, s, H-20), 1.08 (6H, t, *J*=7.2, H - 24 & 25), 0.94 (3H, s, H-18), 0.91 (3H, s, H-19); $\delta_C$ (100 MHz, $CDCl_3$) 155.0 (C-OH), 141.0 (C-9), 131.1 (C-8), 130.0 (C-14), 122.5 (C-11),

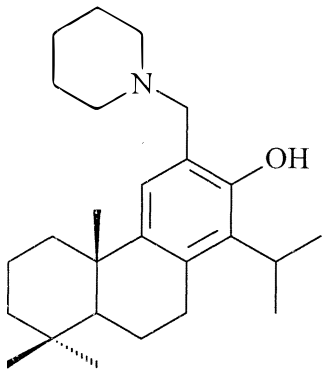
120.1 (C-12), 57.8 (C-21), 49.9 (C-5), 46.3 (C-22&23), 41.8 (C-3), 39.9 (C-1), 37.8 (C-10), 33.5 (C-4), 33.4 (C-18), 28.9 (C-7), 26.3 (C-15), 25.5 (C-20), 21.8 (C-19), 20.4 (C-16), 20.3 (C-17), 19.8 (C-6), 19.7 (C-2), 11.4 (C-24&25); [HRMS (FAB) Found: $M^+$ 371.31852. $C_{25}H_{41}ON$ requires 371.31852, Anal. (Calc. C, 80.8, H, 11.1, N, 3.7 Found: C, 80.5, H, 10.4, N, 3.7].

**1-Isopropyl-4b,8,8-trimethyl-3-pyrrolidin-1-ylmethyl-4b,5,6,7,8,8a,9,10-octahydro-phenanthren-2-ol (45).**



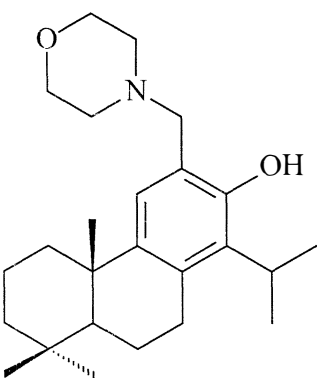

Yield (0.09 g, 74 %) as white powder., Rf 0.63 (Hexane), mp decompose on heating at 188-190 °C, $\nu_{max}$ (Chloroform)/$cm^{-1}$ 3619 (free OH), 3029 (C-H), 1516, 1427 Ar(C = C), 1220 (C-O or C-N), 1042(C-O or C-N), $\delta_H$ (300 MHz, $CDCl_3$) 6.73 (1H, s, H-11), 4.15 (1H, d, *J*=12.24 Hz, H-21α), 4.08 (1H, d, *J*=12.25 Hz, H-21β), 3.24 (1H, m, H-15), 2.94 (1H, dd, *J*=17.9, 5.80 Hz, H-7β), 2.74 (1H, ddd, J=18.0, 10.8 & 7.2, H-7α), 2.59 (4H, m, H-22 & 23), 2.18-2.05 (4H, m, H-24 / 25), 2.23 (1H, d, *J*=12.79, H-1), 1.92 (1H, dd, *J*=13.20,7.78, H-6α), 1.55 - 1.78 (3H, m, H-2 & H-6β), 1.47 (1H, d, *J*= 13.17, H-3β), 1.38 (3H, d, *J*=7.06, H-16), 1.36 (3H, d, *J*=7.06, H-17), 1.31-1.18 (3H, m, H-1α, H-3α & H-5), 1.15 (3H, s, H-20), 0.94 (3H, s, H-18), 0.90 (3H, s, H-19); $\delta_C$ (75 MHz, $CDCl_3$) 155.0 (C-OH), 144.7 (C-9), 131.1 (C-8), 130.0 (C-14), 122.5 (C-11), 120.1 (C-12), 55.6 (C-21), 53.1 (2C,22 & 23), 49.4 (C-5), 41.4 (C-3), 39.7 (C-1), 37.6 (C-10), 33.2 (C-4), 33.1 (C-18), 28.7 (C-7), 27.8 (C-15), 25.2 (C-20), 23.1 (C - 24 & 25), 21.5 (C-19), 20.7 (2C-16 & 17), 19.4 (C-6), 19.1 (C-2); [HRMS (FAB) Found: 370.31073. $C_{25}H_{39}ON$ requires (M+H): 370.31097, Anal. (Calc. C, 81.2, H, 10.6, N, 3.7 Found: C, 81.4, H, 10.9, N, 3.7].

**1-Isopropyl-4b,8,8-trimethyl-3-piperidin-1-ylmethyl-4b,5,6,7,8,8a,9,10-octahydro-phenanthren-2-ol (46).**



Yield (0.096 g, 71%) as white crystals, mp 118-121 °C; $R_f$ 0.73 (Hexane); $\nu_{max}$ (Chloroform)/$cm^{-1}$ 3619 (free OH), 3029 (C-H), 1516, 1427 Ar(C = C), 1220 (C-O or C-N), 1042 (C-O or C-N); $\delta_H$ (300 MHz, $CDCl_3$) 6.72 (1H, s, H-11), 3.61 (1H, d, *J*=13.72 Hz, H-21a), 3.52 (1H, d, *J*=13.81 Hz, H-21b) 3.24 (1H, m, H-15), 2.92 (1H, dd, *J*=17.9, 5.80 Hz, H-7β), 2.74 (1H, ddd, J=18, 10.8 & 7.2, H-7α), 2.46 (4H, br m, H-22&23), 2.21 (1H, d, *J*=12.07, H-1), 1.92 (1H, dd, *J*=13.20,7.78, H-6α), 1.73-1.55 (9H, m, H-2, 24, 25, 26 & H-6β), 1.47 (1H, d, *J*= 13.17, H-3β), 1.35 (3H, d, *J*=7.06, H-16), 1.37 (3H, d, *J*=7.06, H-17), 1.31-1.20 (3H, m, H-1α, H-3α & H-5), 1.16 (3H, s, H-20), 0.94 (3H, s, H-18), 0.91 (3H, s, H-19); $\delta_C$ (75 MHz, $CDCl_3$) 155.0 (C-OH), 140.8 (C-9), 133.0 (C-8), 131.1 (C-14), 122.3 (C-11), 119.6 (C-12), 62.7 (C-21), 53.7 (C - 22& 23), 49.7 (C-5), 41.6 (C-3), 39.7 (C-1), 37.5 (C-10), 33.27 (C-4), 33.23 (C-18), 28.7 (C-7), 26.7 (C-15), 25.8 (C-24&25), 25.2 (C-20), 24.1 (C-26), 21.6 (C-19), 20.2 (C-16), 20.1 (C-17), 19.5 (C-6), 19.4 (C-2); [HRMS (FAB) Found: 383.31789. $C_{26}H_{41}ON$ requires ($M^+$) 383.31879, Anal. (Calc. C, 81.4, H, 10.7, N, 3.6 Found: C, 81.5, H, 10.4, N, 3.6].

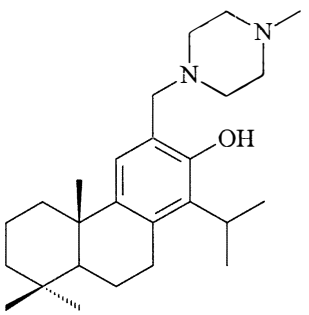
**1-Isopropyl-4b,8,8-trimethyl-3-morpholin-4-ylmethyl-4b,5,6,7,8,8a,9,10-octahydro-phenanthren-2-ol (47)**



Yield (0.07g, 51%) as white crystals, mp 116-120 °C, $R_f$ 0.76 (1:23 EtOAc: Hexane); $\nu_{max}$ (Chloroform)/$cm^{-1}$ 3619 (free OH), 3029 (C-H), 1516, 1427 Ar(C = C), 1220 (C-O or C-N), 1042(C-O or C-N), $\delta_H$ (400 MHz, $CDCl_3$) 10.29 (1H, broad s, OH), 6.74 (1H, s, H-11), 3.73 (4H, t, *J*= 4.5, H-24 & 25), 3.64 (1H, d, *J*=13.72 Hz, H-21a), 3.60 (1H, d, *J*=13.81 Hz, H-21b), 3.25 (1H, m, H-15), 2.92 (1H, dd, *J*=17.9, 5.80 Hz, H-7β), 2.73 (1H, ddd, *J*=18, 10.8 & 7.2, H-7α), 2.54 (4H, br m, H-22 & 23), 2.20 (1H, d, *J*=12.79, H-1), 1.90 (1H, dd, *J*=13.20,7.78 Hz, H-6α), 1.79-1.53 (3H, m, H-6β & H-2), 1.46 (1H, d, *J*= 13.17, H-3β), 1.36 (3H, d, *J*=7.06, H-16), 1.35 (3H, d, *J*= 7.06, H-17), 1.19 - 1.29 (3H, m, H-1α, H-3α and H-5), 1.16 (3H, s, H-

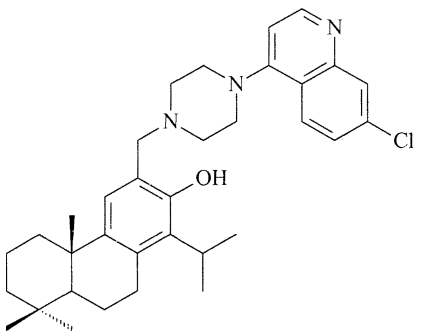
20), 0.94 (3H, s, H-18), 0.91 (3H, s, H-19); $\delta_C$ (100 MHz, $CDCl_3$) 153.3 (C-OH), 141.3 (C-9), 133.1 (C-8), 131.4 (C-14), 122.7 (C-11), 118.6 (C-12), 66.8 (C-24 & 25), 62.5 (C-21), 52.8 (C-22 & 23), 49.6 (C-5), 41.6 (C-3), 39.7 (C-1), 37.5 (C-10), 33.2 (C-4 & C-18), 28.7 (C-7), 27.4 (C-15), 25.2 (C-20), 21.5 (C-19), 20.2 (C-16), 20.1 (C-17), 19.5 (C-6), 19.4 (C-2). [HRMS (FAB) Found: 385.29876, $C_{25}H_{39}O_2N$ requires ($M^+$) 385.29806; Anal. (Calc. C, 77.8, H, 10.1, N, 3.6 Found: C, 78.2, H, 9.8, N, 3.6].

**1-Isopropyl-4b,8,8-trimethyl-3-(4-methyl-piperazin-1-ylmethyl)-4b,5,6,7,8,8a,9, 10-octahydro-phenanthren-2-ol (48)**



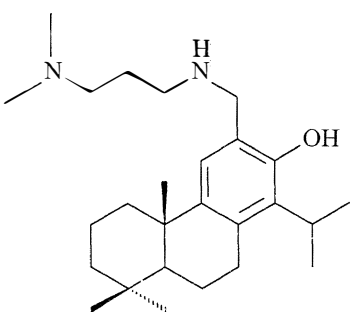

Yield (0.095 g, 68%), mp 159-162 °C, Rf 0.52 (Hexane); $\nu_{max}$ (Chloroform)/$cm^{-1}$ 3619 (free OH), 3029 (C-H), 1516, 1427 Ar (C = C), 1220 (C-O or C-N), 1042 (C-O or C-N), $\delta_H$ (300 MHz, $CDCl_3$) 10.42 (1H, br s, OH), 6.74 (1H, s, H-11), 3.64 (1H, d, *J*=13.72 Hz, H-21a), 3.60 (1H, d, *J*=13.81 Hz, H-21b), 3.25 (1H, m, H-15), 2.94 (1H, dd, *J*=17.9, 5.80 Hz, H-7β), 2.72 (1H, ddd, J=18.0, 10.8 & 7.2, H-7α), 2.52 (8H, br m, H-22 -25), 2.30 (3H, s, H-26), 2.21 (1H, d, *J*=12.79, H-1), 1.92 (1H, dd, *J*=13.20,7.78, H-6α), 1.80-1.52 (3H, m, H-6β & H-2), 1.47 (1H, d, *J*= 13.17, H-3β), 1.36 (3H, d, *J*=7.06, H-16), 1.34 (3H, 2d, *J*=7.06, H-17), 1.20-1.31 (3H, m, H-1α, H-3α and H-5), 1.16 (3H, s, H-20), 0.94 (3H, s, H-18), 0.91 (3H, s, H-19); $\delta_C$ (75 MHz, $CDCl_3$) 156.0 (C-OH), 141.1 (C-9), 133.0 (C-8), 131.3 (C-14), 122.5 (C-11), 119.1 (C-12), 61.9 (C-21),54.9 (C-22 & 23), 52.4 (C-24 & 25), 49.7 (C-5), 45.9 (C-26), 41.6 (C-3), 39.7 (C-1), 37.5 (C-10), 33.4 (C-4), 33.2 (C-18), 28.7 (C-7), 26.5 (C-15), 25.2 (C-20), 21.6 (C-19), 20.2 (C-16), 20.1 (C-17), 19.5 (C-6), 19.4 (C-2). [HRMS (FAB) Found: 398.32995, $C_{26}H_{42}N_2O$ requires (M+H) 398.32969; Anal. (Calc. C, 78.3, H, 10.6, N, 7.0 Found: C, 78.2, H, 10.6, N, 7.0].

**3-[4-(7-Chloro-quinolin-4-yl)-piperazin-1-ylmethyl]-1-isopropyl-4b,8,8-Trimethyl-4b,5,6,7,8,8a,9,10-octahydro-phenanthren-2-ol (49).**



Yield (0.12 g, 61%) as white powder, mp 92-95 °C, Rf 0.40 (1:4 EtOAc: Hexane), $\nu_{max}$ (Chloroform)/cm$^{-1}$ 3619 (free OH), 3029 (C-H), 1516, 1427 Ar (C = C), 1220 (C-O or C-N), 1042 (C-O or C-N), $\delta_H$ (300 MHz, $CDCl_3$) 8.72 (1H, d, *J*=5.12, H-2'), 8.05 (1H, d, J=2.01, H-8'), 7.93 (1H, d, *J* = 9.15 Hz, H-5'), 7.42 (1H, dd, *J* = 9.3, 2.19 Hz, H-6'), 6.85 (1H, d, *J* = 5.1 Hz, H-3'), 6.80 (1H, s, H-11), 3.80 (1H, d, *J*=13.72 Hz, H-21a), 3.73 (1H, d, *J*=13.81 Hz, H-21b), 3.28 (5H, br m, H-15, 22 & 23), 2.68 - 2.97 (6H, m, H-7β, H-7α, H-22 & 23), 2.23 (1H, d, *J*=12.82, H-1), 1.91 (1H, dd, *J*=13.20,7.78, H-6α), 1.80-1.56 (3H, m, H-6β & H-2), 1.47 (1H, d, *J*= 13.17, H-3β), 1.36 (3H, d, J=7.06, H-16), 1.34 (3H, d, *J*=7.06, H-17), 1.20-1.31 (3H, m, H-1α, H-3α and H-5), 1.18 (3H, s, H-20), 0.94 (3H, s, H-18), 0.91 (3H, s, H-19); $\delta_C$ (75 MHz, $CDCl_3$) 156.6 (C-OH), 153.7 (C-2'), 151.8 (C-4'), 150.0 (C-8a), 141.1 (C-9), 135.0 (C-7'), 134.9 (C-8), 131.5 (C-14), 128.9 (C-8'), 126.0 (C-6'), 124.8 (C-5'), 122.6 (C-11), 121.8 (C–4a), 118.6 (C-12), 109.2 (C-3'), 62.09 (C-21), 52.3 (C-24, 25), 52.0 (C-22 & 23), 49.6 (C-5), 41.6 (C-3), 39.7 (C-1), 37.5 (C-10), 33.27 (C-4), 33.22 (C-18), 28.6 (C-7), 27.4 (C-15), 25.2 (C-20), 21.6 (C-19), 20.2 (C-16), 20.1 (C-17), 19.5 (C-6), 19.4 (C-2); [HRMS (FAB) Found: 546.32538, $C_{34}H_{44}ClN_3O$ requires (M+H) 546.32509]; Anal. (Calc. C, 74.7, H, 8.1, N, 7.7 Found: C, 74.3, H, 7.9, N, 7.6].

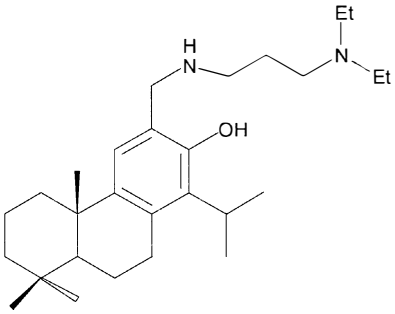
**3-[(3-Dimethylamino-propylamino)-methyl]-1-isopropyl-4b,8,8-trimethyl-4b,5,6,7,8,8a,9,10-octahydro-phenanthren-2-ol (50)**



Yield (0.98 g, 72%) as yellow oil, $R_f$ 0.24 (3:7 EtOAc: Hexane), $\nu_{max}$ (Chloroform)/cm$^{-1}$ 3619 (free OH), 3029 (C-H), 1516, 1427 Ar (C = C), 1220 (C-O or C-N), 1042 (C-O or C-N), $\delta_H$ (400 MHz, $CDCl_3$) 6.76 (1H, s, H-11), 3.88 (1H, d, *J*=13.5, H-21a), 3.82 (1H, d, J=13.4, H-21ß), 3.25 (1H, m, H-15), 2.92 (1H, dd, *J*=17.9, 5.80 Hz, H-7β), 2.72 (3H, m, H-7α & 22), 2.32 (2H, t, *J*=7.0 Hz, H-24), 2.20 (3H, s, H-25), 2.16 (4H, m, H-1β, H-26), 1.89 (1H, dd, *J*=13.20,

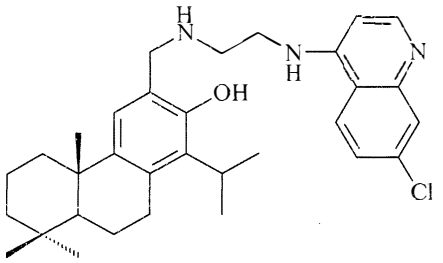
7.78, H-6α), 1.77-1.55 (5H, m, H-2, H-6β & H-23), 1.45 (1H, d, *J*= 13.17, H-3β), 1.36 (3H, d, *J*=7.06, H-16), 1.35 (3H, d, *J*=7.06, H-17), 1.33-1.19 (3H, m, H-1α, H-3α & H-5), 1.17 (3H, s, H-20), 0.94 (3H, s, H-18), 0.91 (3H, s, H-19); $\delta_C$ (100 MHz, $CDCl_3$) 156.5 (C-OH), 141.0 (C-9), 133.5 (C-8), 131.7 (C-14), 122.3 (C-11), 120.9 (C-12), 58.1 (C-22), 53.6 (C-21), 49.9 (C-5), 47.6 (C-24), 45.7 (C-25&26), 41.8 (C-3), 39.8 (C-1), 37.7 (C-10), 33.4 (C-4), 33.4 (C-18), 31.0 (C-23), 28.8 (C-7), 27.5 (C-15), 25.4 (C-20), 21.7 (C-19), 20.4 (C-16), 20.4 (C-17), 19.7 (C-6), 19.6 (C-2); [LRMS Found: (M+H) 401.4, $C_{26}H_{44}N_2O$, requires ($M^+$) 400.34536; Anal. (Calc. C, 77.9, H, 11.1, N, 7.0 Found: C, 77.7, H, 10.7, N, 6.56)].

**3-[(3-Diethylamino-propylamino)-methyl]-1-isopropyl-4b,8,8-trimethyl-4b,5,6,7,8,8a,9,10-octahydro-phenanthren-2-ol ( 51 )**



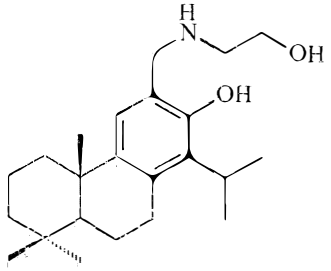

Yield (0.12 g, 80%) as yellow oil, *Rf* 2.35 (2:3 EtOAc: Hexane), $\nu_{max}$ (Chloroform)/$cm^{-1}$ 3619 (free OH), 3029 (C-H), 1516, 1427 Ar (C = C), 1220 (C-O or C-N), 1042 (C-O or C-N), $\delta_H$ (300 MHz, $CDCl_3$) 6.76 (1H, s, H-11), 3.88 (2H, br s, H-21a & 21b), 3.23 (1H, m, H-15), 2.93 (1H, dd, *J*=17.9, 5.80 Hz, H-7β), 2.69-2.79 (3H, m, H-7α & H-22), 2.45-2.53 (6H, m, H-24, H-25 & H-26), 2.21 (1H, d, *J*=12.79, H-1β), 1.90 (1H, dd, *J*=13.20, 7.78, H-6α), 1.53-1.78 (5H, m, H-2, 6β & 23), 1.47 (1H, d, *J*= 13.17, H-3β), 1.37 (3H, d, *J*=7.06, H-16), 1.35 (3H, d, *J*=7.06, H-17), 1.24-1.34 (3H, m, H-1α, H-3α & H-5), 1.17 (3H, s, H-20) 1.02 (6H, t, *J*=7.2, H-27&28), 0.94 (3H, s, H-18), 0.91 (3H, s, H-19); $\delta_C$ (75 MHz, $CDCl_3$) 154.7 (C-OH), 140.7 (C-9), 132.7 (C-8), 131.5 (C-14), 122.1 (C-11), 120.7 (C-12), 53.4 (C-21), 51.3 (C-22), 49.7 (C-5), 47.8 (C-24), 46.9 (C-25&26), 41.6 (C-3), 39.7 (C-1), 37.5 (C-10), 33.24 (C-4), 33.22 (C-18), 28.6 (C-7), 27.5 (C-23), 26.8 (C-15), 25.2 (C-20), 21.5 (C-19), 20.2 (C-16), 20.1 (C-17), 19.5 (C-6), 19.4 (C-2), 11.7 (C-27 & 28); [LRMS Found: (M+H) 429.6, $C_{28}H_{48}N_2O$, requires ($M^+$) 428.37666, Anal. (Calc. C, 78.45, H, 11.29, N, 6.5 Found: C, 78.9, H, 10.9, N, 6.39)].

**3-{[2-(7-Chloro-quinolin-4-ylamino)-ethylamino]-methyl}-1-isopropyl-4b,8,8-trimethyl-4b,5,6,7,8,8a,9,10-octahydro-phenanthren-2-ol ( 52 )**



Yield (0.13 g, 86%) as white powder, *Rf* 0.32 (3:7 EtOAc: Hexane), $\nu_{max}$ (Chloroform)/cm$^{-1}$ 3619 (free OH), 3029 (C-H), 1516 & 1427 Ar (C = C), 1220 (O-H), 1042 (C-O & C-N); $\delta_H$ (300 MHz, $CDCl_3$) 8.54 (1H, d, *J*=5.3 Hz, H-2') 7.97 (1H, d, *J*=2.1 Hz, H-8'), 7.68 (1H, d, *J*=8.9 Hz, H-5'), 7.37 (1H, dd, *J*= 8.9 & 2.1 Hz, H-6'), 6.73 (1H, s, H-11), 6.42 (1H, d, *J*=5.3 Hz, H-3'), 5.73 (1H, broad, NH), 4.03 (1H, d, *J*=16.8 Hz, H-21a), 3.97 (1H, d, *J*=16.8 Hz, H-21b), 3.42 (2H, t, *J*=6.4 Hz, H-23), 3.25 (1H, m, H-15), 3.17 (2H, t, *J* = 6.2 Hz, H-22), 2.93 (1H, dd, *J*=17.9, 6.40 Hz, H-7β), 2.72 (1H, ddd, J=18.0, 10.8 & 7.2 Hz, H-7α), 2.60 (1H, d, *J*=12.7, H-1β), 1.92 (2H, m, H-6α & N-H or OH), 1.80-1.52 (3H, m, H-6β & H-2), 1.47 (1H, d, *J*= 13.17, H-3β), 1.32 (3H, d, *J*=7.06, H-16), & 1.30 (3H, d, *J*=7.06, H-17), 1.20-1.31 (3H, m, H-1α, H-3α and H-5), 1.17 (3H, s, H-20), 0.94 (3H, s, H-18), 0.91 (3H, s, H-19); $\delta_C$ (75 MHz, $CDCl_3$) 151.7 (C-2'), 150.4 (C-OH), 149.8 (C-4'), 148.7 (C-8a), 143.2 (C-9), 135.1 (C –7'), 132.5 (C-14), 132.2 (C-8), 128.5 (C-8'), 125.4 (C-6'), 121.2 (C-5'), 121.0 (C-11), 117.2 (C-4a), 116.7 (C-12), 99.3 (C-3'), 50.2 (C-21), 49.5 (C-22), 49.3 (C-5), 41.5 (C-3), 40.1 (C-23), 39.6 (C-1), 37.6 (C-10), 33.3 (C-4), 33.2 (C-18), 28.5 (C-7), 27.3 (C-15), 25.2 (C-20), 21.5 (C-19), 20.3 (C-16), 20.2 (C-17), 19.4 (C-6), 19.3 (C-2), [LRMS Found: (M+H) 520.4, $C_{28}H_{48}N_2O$, requires ($M^+$) 519.30164, Anal. (Calc. C, 73.8, H, 8.14, N, 8.08 Found: C, 73.8, H, 8.0, N, 7.96).

**3-[(2-Hydroxy-ethylamino)-methyl]-1-isopropyl-4b,8,8-trimethyl-4b,5,6,7,8,8a,9,10-octahydro-phenanthren-2-ol (53)**



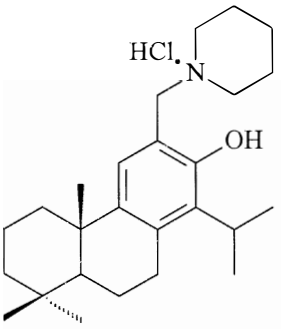
Yield (0.0.81 g, 65%), *Rf* 0.15 (2:3 EtOAc: Hexane), $\nu_{max}$ (Chloroform)/cm$^{-1}$ 3619 (free OH), 3029 (C-H), 1516 & 1427 Ar (C=C), 1220 (O-H), 1042 (C-O or C-N), $\delta_H$ (300 MHz, $CDCl_3$) 6.77 (1H, s, H-11), 3.93 (1H, d, *J*=13.70 Hz, H-21a), 3.90 (1H, d, *J*=13.81 Hz, H-21ß), 3.77 (2H, t, J = 5.12, H-23), 3.25 (1H, m, H-15), 2.94 (1H, dd, *J*=17.90, 5.80 Hz, H-7β), 2.84 (2H, t, *J* = 5.10, H-22), 2.71 (1H, ddd, *J*=18.0, 10.8 & 7.2, H-7α), 2.21 (1H, d, *J*=12.79, H-1β), 1.95 (1H, dd, *J*=13.20, 7.78, H-6α),

1.55-1.80 (3H, m, H-2 & 6β), 1.47 (1H, d, *J*= 13.17, H-3β), 1.37 (3H, 2d, *J*=7.06, H-17), 1.35 (3H, 2d, *J*=7.06, H-16), 1.24 -1.29 (3H, m, H-1α, H-3α and H-5), 1.17 (3H, s, H-20), 0.94 (3H, s, H-18), 0.91(3H, s, H-19); $\delta_C$ (75 MHz, $CDCl_3$) 154.30 (C-OH), 141.1 (C-9), 133.0 (C-8), 131.7 (C-14), 122.2 (C-11), 120.4 (C-12), 61.4 (C - 23), 53.0 (C-21), 50.3 (C-22), 49.7 (C-5), 41.6 (C-3), 39.6 (C-1), 37.5 (C-10), 33.3 (C-4), 33.2 (C-18), 28.6 (C-7), 27.4 (C-15), 25.2 (C-20), 21.5 (C-19), 20.24 (C-16), 20.20 (C-17), 19.5 (C-6), 19.4 (C-2). [LRMS Found: ($M^+$) 359.3, $C_{28}H_{48}N_2O$, requires ($M^+$) 359.28243, Anal. (Calc. C, 76.8, H, 10.37, N, 3.9 Found: C, 76.3, H, 10.2, N, 4.0].

### 5.3 General procedure for the preparation of salts of Mannich bases

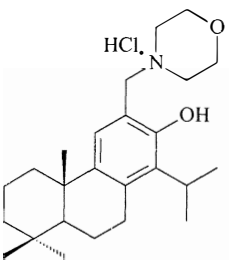
A 30 mmol of sample compound (Mannich base) was dissolved in 2 - 3 ml of diethyl ether, cooled to 0 $^0$C and treated drop wise with stoichiometric amount of cold methanolic hydrochloric acid (2 M). The resulting precipitate was then filtered and dried .

### Hydrochloride salt of compound number 3 (46b)



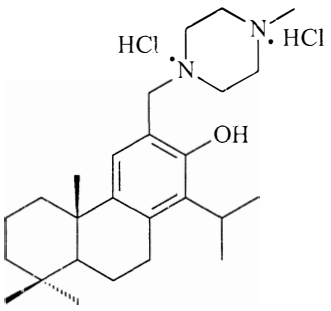

Yield (84%) as white powder, $\delta_H$ (300 MHz, $CDCl_3$) 7.13 (1H, s, H-11), 4.22 (1H, d, *J*=13.72 Hz, H-21a), 4.18 (1H, d, *J*=13.81 Hz, H-21b), 3.31-3.01 (5H, m, C-15, 22 & 23), 2.97 (1H, dd, *J*=17.9, 5.80 Hz, H-7β), 2.74 (1H, ddd, *J*=18, 10.8 & 7.2, H-7α), 2.46 (4H, br, H-22&23), 2.34 (1H, d, *J*=12.07, H-1), 1.96 (1H, dd, *J*=13.20,8.10, H-6α), 1.9-1.55 (9H, m, H-2, 6β, 24, 25 & 26), 1.50 (1H, d, *J*= 13.17, H-3β), 1.35 (3H, d, *J*=7.06, H-16), 1.37 (3H, d, *J*=7.06, H-17), 1.31-1.21 (3H, m, H-1α, H-3α & H-5), 1.20 (3H, s, H-20), 0.97 (3H, s, H-18), 0.94 (3H, s, H-19); $\delta_C$ (75 MHz, $CDCl_3$) 153.2 (C-OH), 145.8 (C-9), 137.5 (C-8), 136.3 (C-14), 127.6 (C-11), 118.2 (C-12), 58.1 (C-21), 53.9 (C-22 & 23), 51.4 (C-5), 42.7 (C-3), 40.8 (C-1), 38.9 (C-10), 34.22 (C-4), 33.7 (C-18), 29.9 (C-7), 28.5 (C-15), 25.5 (C-24&25), 24.1 (C-20), 22.9 (C-26), 21.9 (C-19), 20.8 (C-16), 20.7 (C-17), 20.4 (C-6), 20.3 (C-2). [HRMS (FAB) Found: 384.32615, $C_{26}H_{41}NO$-HCl, requires (M+H) 384.32662; Anal. (Calc. C, 74.3, H, 10.0, N, 3.33 Found: C, 74.6, H, 9.7, N, 3.3)].

**Hydrochloride salt of compound number 4 (47b)**



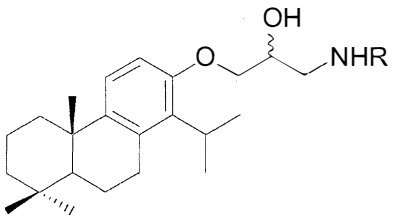

Yield (76 %) as white powder, $\delta_H$ (400 MHz, $CDCl_3$) 10.29 (1H, br s, OH), 7.19 (1H, s, H-11), 4.33 (1H, d, *J*=13.72 Hz, H-21a), 4.29 (1H, d, *J*=13.20 Hz, H-21b), 4.00 (2H, t, *J*= 4.5 Hz, H-22), 3.79 (2H, t, *J*= 4.5 Hz, H-23), 3.34-3.42 (3H, m, H-15 & H-24), 3.18 (2H, br, H-25), 2.97 (1H, dd, *J*=17.9, 5.80 Hz, H-7β), 2.75 (1H, ddd, *J* = 18.4, 10.4 & 7.6, H-7α), 2.34 (1H, d, *J*=13.2 Hz, H-1), 1.96 (1H, dd, *J*=13.20,7.78 Hz, H-6α), 1.85 -1.60 (3H, m, H-6β & H-2), 1.49 (1H, d, *J*= 13.17, H-3β), 1.35 (3H, d, *J*=7.06, H-16), 1.34 (3H, d, *J*= 7.06, H-17), 1.19 - 1.29 (3H, m, H-1α, H-3α and H-5), 1.20(3H, s, H-20), 0.96 (3H, s, H-18), 0.94 (3H, s, H-19); $\delta_C$ (100 MHz, $CDCl_3$) 151.9 (C-OH), 144.5 (C-9), 136.9 (C-8), 135.5 (C-14), 126.8 (C-11), 116.2 (C-12), 63.6 (C-24 & 25), 57.0 (C-21), 51.6 (C-22 & 23), 49.9 (C-5), 41.5 (C-3), 39.6 (C-1), 37.7 (C-10), 33.0 (C-4), 32.4 (C-18), 28.7 (C-7), 27.3 (C-15), 24.2 (C-20), 20.8 (C-19), 19.6 ( C-16 & C-17), 19.2 (C-6), 19.1 (C-2). [HRMS (FAB) Found: 386.30553, $C_{25}H_{39}NO_2$.HCl, requires (M+H) 386.30588; Anal. (Calc. C, 71.1, H, 9.5, N, 3.3 Found: C, 70.7, H, 9.3, N, 3.3)].

**Hydrochloride salt of compound number 5 (48b)**



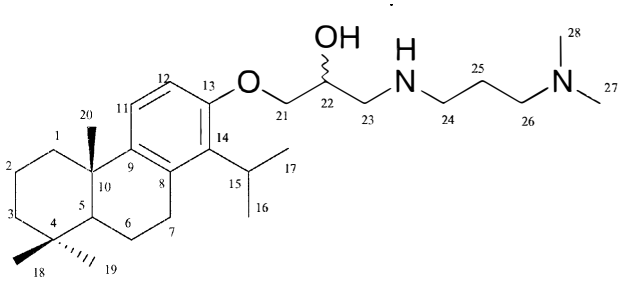

Yield (87%), $\delta_H$ (400 MHz, $CD_3OD$) 7.25 (1H, s, H-11), 4.41 (1H, d, *J*=13.71 Hz, H-21a), 4.39 (1H, d, *J*=13.81 Hz, H-21b), 3.45-3.90 (8H, br m, H-22, 23, 24&25), 3.34 (1H, m, H-15), 2.94 – 3.01 (4H, m, H - 7β & 26), 2.74 (1H, ddd, *J* = 18.4, 10.4 & 7.6, H-7α), 2.36 (1H, d, *J*=12.79, H-1β), 1.95 (1H, dd, *J*=13.20,7.78, H-6α), 1.52 - 1.85 (3H, m, H-6β & H-2), 1.49(1H, d, *J*= 13.17, H-3β), 1.36 (3H, d, *J*=7.06, H-16), 1.34 (3H, 2d, *J*=7.06, H-17), 1.23-1.30 (3H, m, H-1α, H-3α and H-5), 1.20 (3H, s, H-20), 0.96 (3H, s, H-18), 0.94 (3H, s, H-19); $\delta_C$ (100 MHz, $CDCl_3$) 156.0 (C-OH), 141.1 (C-9), 133.0 (C-8), 131.3 (C-14), 122.5 (C-11), 117.1 (C-12), 61.9 (C-21),54.9 (C-22 & 23), 52.4 (C-24 & 25), 49.9 (C-5), 45.9 (C-26), 41.5 (C-3), 39.7 (C-1), 37.8 (C-10), 33.0 (C-4), 32.4 (C-18), 28.7 (C-7), 27.3 (C-15), 24.2 (C-20), 20.7 (C-19), 19.63 (C-16), 19.61 (C-17), 19.2 (C-6), 19.1 (C-2). [HRMS (FAB) Found: 399.33765, $C_{26}H_{42}N_2O$.HCl, requires (M+H) 399.33752; Anal. (Calc. C, 66.2, H, 9.4, N, 5.9 Found: C, 66.13, H, 9.6, N, 5.6)].

## 5.4 General procedure for the preparation of the Aminoalcohols (55-58)



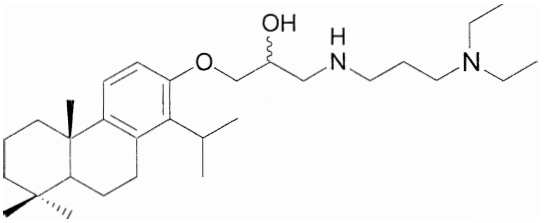

A mixture of 0.2 mmol of preformed epoxy totarol (**66**) and 4 equivalents of the primary amine was dissolved in methanol. The reaction mixture was then allowed to reflux for ca. 20 hours at 65 $^{0}$C. After refluxing for 20 hours the solvent was removed under reduced pressure and the crud product was chromatographed on a silica gel to give NMR pure compounds.

**1-(3-Dimethylamino-propylamino)-3-(1-isopropyl-4b,8,8-trimethyl-4b,5,6,7,8,8a,9,10-octahydro-phenanthren-2-yloxy)-propan-2-ol (55)**



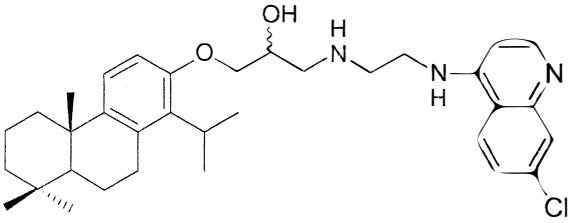

Yield (60.4mg, 60%), $R_f$ 0.24 (1:4 EtOAc:Hex); $\nu_{max}$ (Chloroform)/cm$^{-1}$ 3597 (OH), 3394 (N-H), 3021 Ar (C-H), 2965-2810 (C-H), 1665 (N-H), 1601 -1591(C = C), 1468 (C = C), 1262 & 1222 (C-O or C-N), 1051 (C-O or C-N), $\delta_H$ (300 MHz; $CDCl_3$) 7.69 (1H, d, J=8.87 Hz, H-11), 6.71 (1H, d, J=8.82 Hz, H-12), 4.09 (1H, m, H-22), 3.97 (1H, ddd, J=9.74, 5.4 & 2.4, Hz, H-21α), 3.89 (1H, dd, J=9.24, 5.3 Hz, H-21β), 3.29 (1H, m, H-15), 2.92 (2H, m, H-23α, H-7α), 2.72 (4H, m, H-7β, H-23β & H-24), 2.49 (8H, m, H-26, 27,28, NH & OH), 2.24 (1H, d, J=12.89, H-1β), 1.90 (1H, dd, J=13.32, 8.09 Hz, H-6α), 1.56 - 1.78 (5H, m, H-2, H-6β & H-25), 1.45 (1H, d, *J*= 13.58, H-3β), 1.31 (3H, d, *J*=7.06, H-16), 1.29 (3H, d, *J*=7.04, H-17), 1.27-1.20 (3H, m, H-1α, H-3α & H-5), 1.17 (3H, s, H-20), 0.94 (3H, s, H-18), 0.91 (3H, s, H-19); $\delta_C$ (100 MHz, $CDCl_3$) 155.2 (C-13), 143.4 (C-9), 133.7 (C-8), 133.1 (C-14), 122.7 (C-11), 109.9 (C-12), 70.3 (C-21), 68.5 (C-22), 52.3 (C-23), 51.1 (C-24), 49.5 (C-5), 47.5 (C-26), 45.7 & 45.8 (C-27&28), 41.6 (C-3), 39.6 (C-1), 37.6 (C-10), 33.24 (C-4 & C-18), 30.8 (C-25), 28.7 (C-7), 27.4 (C-15), 25.1 (C-20), 21.5 (C-19), 20.6 (C-2), 20.6 (C-17), 19.4 (C-6), 19.3 (C-16); [HRMS (EI) Found: $(M+H)^+$, 445.37940. $C_{28}H_{48}N_2O_2$ requires ($M^+$), 444.37158].

**1-(3-Diethylamino-propylamino)-3-(1-isopropyl-4b,8,8-trimethyl-4b,5,6,7,8,8a,9,10-octahydro-phenanthren-2-yloxy)-propan-2-ol ( 56)**



Yield (70.9mg, 75%); $R_f$ 0.88 (1:4 EtOAc:Hex); $\nu_{max}$ (Chloroform)/$cm^{-1}$ 3597 (OH), 3394 (N-H), 3022 Ar (C-H), 2967-2810 (C-H), 1664 (N-H), 1601 -1591(C = C), 1468 (C = C), 1262 & 1222 (C-O or C-N), 1051 (C-O or C-N), $\delta_H$ (300 MHz; $CDCl_3$) 7.69 (1H, d, J=8.87 Hz, H-11), 6.71 (1H, d, J=8.82 Hz, H-12), 4.09 (1H, m, H-22), 3.97 (1H, ddd, J=9.74, 5.4 & 2.4, Hz, H-21α), 3.89 (1H, dd, J=9.24, 5.3 Hz, H-21β), 3.29 (1H, m, H-15), 2.92 (2H, m, H-23α, H-7α), 2.72 (4H, m, H-7β, H-23β & H-24), 2.49 (8H, m, H-26, 27,28, NH & OH), 2.24 (1H, d, J=12.89, H-1β), 1.90 (1H, dd, J=13.32, 8.09 Hz, H-6α), 1.56 - 1.78 (5H, m, H-2, H-6β & H-25), 1.45 (1H, d, *J*= 13.58, H-3β), 1.31 (3H, d, *J*=7.06, H-16), 1.29 (3H, d, *J*=7.04, H-17), 1.27-1.20 (3H, m, H-1α, H-3α & H-5), 1.17 (3H, s, H-20), 0.94 (3H, s, H-18), 0.91 (3H, s, H-19); $\delta_C$ (100 MHz, $CDCl_3$) 155.2 (C-13), 143.4 (C-9), 133.7 (C-8), 133.1 (C-14), 122.7 (C-11), 109.9 (C-12), 70.3 (C-21), 68.5 (C-22), 52.3 (C-23), 51.1 (C-24), 49.5 (C-5), 48.5 (C-26), 46.86 & 46.83 (C-27&28), 41.6 (C-3), 39.6 (C-1), 37.6 (C-10), 33.24 (C-4 & C-18), 30.8 (C-25), 28.7 (C-7), 27.4 (C-15), 25.1 (C-20), 21.5 (C-19), 20.6 (C-2), 20.6 (C-17), 19.4 (C-6), 19.3 (C-16), 11.7 (C-29 & 30); (Found: $(M)^+$, 472.39678. $C_{30}H_{52}N_2O_2$ requires ($M^+$), 472.40288].

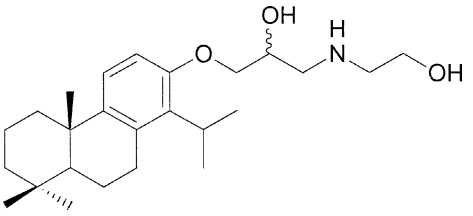
**1-[2-(7-Chloro-quinolin-4-ylamino)-ethylamino]-3-(1-isopropyl-4b,8,8-trimethyl-4b,5,6,7,8,8a,9,10-octahydro-phenanthren-2-yloxy)-propan-2-ol (57)**



Yield (73mg, 65%), $R_f$ 0.54 (1:4 MeOH:DCM); $\nu_{max}$ (Chloroform)/$cm^{-1}$ 3529 (OH), 3395 (N-H), 3063 Ar (C-H), 3008-2973 (C-H), 1612 -1591(N-H & C = C), 1232 & 1216 (C-O or C-N), 1052 (C-O or C-N), $\delta_H$ (300 MHz; $CDCl_3$) 8.48 (1H, d, *J*=5.41 Hz , H-2'), 7.93 (1H, d, *J*=2.11 Hz, H-8'), 7.69 (1H, dd, *J*=8.92, 2.07 Hz, H-6'), 7.26 (1H, m, H-5'), 7.07 (1H, d, *J*=8.79 Hz, H-11), 6.69 (1H, d, *J*=8.76 Hz, H-12), 6.36 (1H, d, *J*=5.48 Hz, H-3'), 4.21 (1H, m, H-22), 4.01 (2H, m, H-21), 3.39 (2H, t, *J*=5.47 Hz, H-25), 3.29 (1H, m, H-15), 3.12 (2H, m, H-24), 3.02-2.89 (3H, m, H-23, H-7β), 2.74 (1H, ddd, *J*=18.0, 10.8 & 7.2 Hz, H-

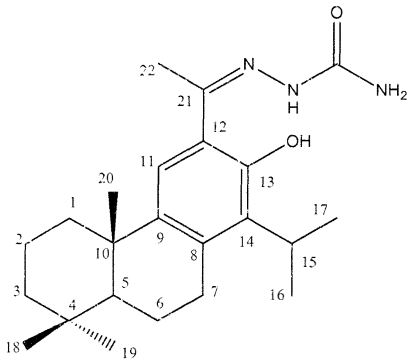
7α), 2.25 (1H, d, *J*=12.40 Hz, H-1β), 2.19 (3H, br, NH & OH), 1.92 (1H, dd, *J*=13.32, 8.09 Hz, H-6α), 1.79-1.55 (3H, m, H-2 & H- 6β), 1.47 (1H, d, *J*= 12.33 Hz, H-3β), 1.30 (3H, d, *J*=7.50 Hz, H-16), 1.29 (3H, d, *J*=7.50 Hz, H-17), 1.27-1.24 (3H, m, H-1α, H-3α & H-5), 1.18 (3H, s, H-20), 0.94 (3H, s, H-18), 0.91 (3H, s, H-19); $\delta_C$ (75 MHz, $CDCl_3$) 151.6 (C-2'), 150.3 (C-13), 149.5 (C-4'), 148.7 (C-8a), 144.0 (C-9), 134.3 (C–7'), 133.4 (C-8 &14), 128.4 (C-8'), 125.7 (C-6'), 123.1 (C-11), 121.6 (C-5'), 117.2 (C-4a), 110.2 (C-12), 99.3 (C-3'), 70.36 (C-21), 69.5 (C-22), 51.9 (C-23), 49.7 (C-5), 47.5 (C-25), 42.3 (C-24), 41.7 (C-3), 39.8 (C-1), 37.9 (C-10), 33.5 (C-4), 33.4 (C-18), 28.9 (C-7), 27.5 (C-15), 25.4 (C-20), 21.8 (C-19), 21.1 (C-16), 21.0 (C-17), 19.4 (C-6), 19.3 (C-2); [HRMS (EI) Found: ($M^+$), 563.32733. $C_{34}H_{46}ClN_3O_2$ requires ($M^+$), 563.32786].

**(1-(2-Hydroxy-ethylamino)-3-(1-isopropyl-4b,8,8-trimethyl-4b,5,6,7,8,8a,9,10-octahydro-phenanthren-2-yloxy)-propan-2-ol (58)**



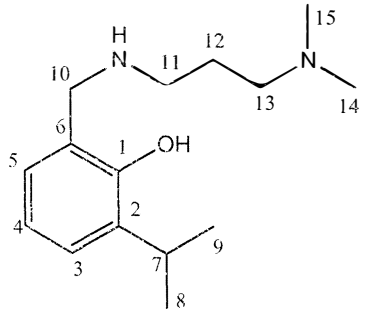

Yield (44.0mg, 54%), $R_f$ 0.92 (EtOAc); $\nu_{max}$ (Chloroform)/$cm^{-1}$ 3509 (OH), 3398 (N-H), 3008 Ar (C-H), 2929, 2782 (C-H), 1644 (N-H), 1591(C = C), 1468 (C = C), 1261 & 1239 (C-O or C-N), 1050 (C-O or C-N),$\delta_H$ (300 MHz; $CDCl_3$) 7.08 (1H, d, J=8.83 Hz, H-11), 6.70 (1H, d, J=8.77 Hz, H-12), 4.16 (1H, m, H-22), 3.97 (2H, m, H-21), 3.71 (2H, m, H-25), 3.29 (1H, m, H-15), 2.94 (2H, m, H-23α, H-7β), 2.87(3H, m, H-23β & H-24), 2.75 (1H, m, H-7β), 2.52 (2H, br, NH & OH), 2.24 (1H, d, J=13.73 Hz, H-1β), 1.91 (1H, dd, J=13.36, 8.05 Hz, H-6α), 1.56 - 1.78 (5H, m, H-2, H-6β & H-25), 1.46 (1H, d, *J*= 13.18, H-3β), 1.31 (3H, d, *J*=7.07, H-16), 1.29 (3H, d, *J*=7.05, H-17), 1.27-1.20 (3H, m, H-1α, H-3α & H-5), 1.17 (3H, s, H-20), 0.94 (3H, s, H-18), 0.91 (3H, s, H-19); $\delta_C$ (100 MHz, $CDCl_3$) 151.8 (C-13), 143.6 (C-9), 133.9 (C-8), 133.2 (C-14), 122.8 (C-11), 110.0 (C-12), 70.2 (C-21), 68.7 (C-22), 60.7 (C - 25), 51.9 (C-23), 51.2 (C-24), 49.5 (C-5), 41.6 (C-3), 39.6 (C-1), 37.7 (C-10), 33.2 (C-4), 33.2 (C-18), 28.7 (C-7), 27.4 (C-15), 25.1 (C-20), 21.5 (C-19), 20.8 (C-16), 20.7 (C-17), 19.5 (C-6), 19.4 (C-2). [HRMS (EI), Found: ($M^+$) 403.30864. $C_{23}H_{35}N_3O_2$ requires 403.30668. Anal. (Calc. C, 74.50; H, 10.24; N, 3.67; Found: C, 74.76, H, 10.19, N, 3.60].

**[1-(2-Hydroxy-1-isopropyl-4b,8,8-trimethyl-4b,5,6,7,8,8a,9,10-octahydro-phenanthren-3-yl), 1-Methyl ]-Semicarbazone (59)**



A 32mg (0.286 mmol) of semicarbazide hydrochloride and 24mg (0.286 mmol) of Sodium acetate were added in to a solution containing 40gm (0.143 mmol) of compound 3b in ethanol (4.0 ml). The reaction mixture was then allowed to reflux for 50 hr. After refluxing for 50 hours the solvent was removed under reduced pressure and the crude product was chromatographed on a silica gel with 100% ethyl acetate to give 12.5 mg of NMR pure Semicarbazone derivative (**59**). Yield (12.5mg, 38%), $R_f$ 0.69 (EtOAc); $\nu_{max}$ (Chloroform)/$cm^{-1}$; 3619 (OH), 3473 (N-H, $NH_2$), 3161(N-H), 1720 (C=O), 1583 (C=N), 1516, 1427 Ar (C = C), 1220 (C-N), 1042 (C-N), $\delta_H$ (400 MHz, $CDCl_3$) 11.10 (OH), 8.53 (1H, s, NNH), 7.22 (1H, s, H-11), 5.35 (2H, br s, NH2), 3.24 (1H, m, H-15), 2.94 (1H, dd, *J*=18.0, 5.80 Hz, H-7β), 2.74 (1H, ddd, *J*=18.0, 10.8 & 7.2, H-7α), 2.32 (3H, s, H-26), 2.23 (1H, d, *J*=12.79, H-1β), 1.92 (1H, dd, *J*=13.20, 7.78, H-6α), 1.57 - 1.78 (3H, m, H- 2, 6β), 1.47 (1H, d, *J*= 13.17, H-3β), 1.36 (3H, d, *J*=7.06, H-17), 1.35 (3H, d, *J*=7.06, H-16), 1.31-1.20 (3H, m, H-1α, 3α and H-5), 1.18 (3H, s, H-20), 0.95 (3H, s, H-18), 0.92 (3H, s, H-19); $\delta_C$ (100 MHz, $CDCl_3$) 156.9 (C=O), 154.6 (C-OH), 153.5 (C=N), 141.7 (C-9), 132.1 (C-8&14), 121.9 (C-11), 117.5 (C-12), 49.9 (C-5), 41.5 (C-3), 39.7 (C-1), 37.8 (C-10), 33.4 (C-4), 33.2 (C-18), 28.9 (C-7), 27.3 (C-15), 25.5 (C-20), 21.6 (C-19), 19.9 (C-16), 19.8 (C-17), 19.4 (C-6), 19.2 (C-2), 13.4 (C-22); [HRMS (EI) Found: $M^+$ 385.27293. $C_{23}H_{35}N_3O_2$ requires 385.27294.

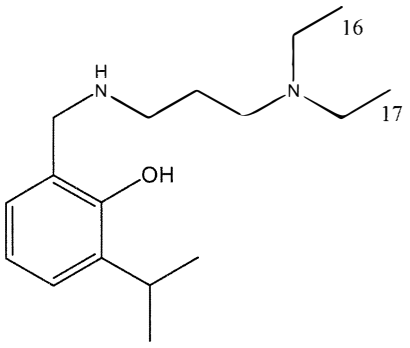
**2-[(3-Dimethylamino-propylamino)-methyl]-6-isopropyl-phenol (74)**



Yield (92mg, 50%); $R_f$ 0.17 (1:4 EtOAc:Hex); $\nu_{max}$ (Chloroform)/$cm^{-1}$ 3605 (free OH), 3303 (N-H), 3049 Ar (C-H), 2959-1769 (C-H), 1644 & 1594 (N-H or C = C), 1227 (C-O or C-N), $\delta_H$ (300 MHz; $CDCl_3$) 7.11 (1H, dd, J=7.50, 1.68 Hz, H-5), 6.83 (1H, dd, J=7.36, 1.76 Hz, H-3), 6.74 (1H, t, J=7.48 Hz, H-4), 5.28 (1H, s, OH), 3.97 (2H, br s, H-10), 3.33 (1H, sept, J=6.92 Hz, H-7), 2.73 (2H, t, J=6.66 Hz, H-11), 2.36 (2H, t, J=6.97 Hz, H-13), 2.22 (6H, s, H-

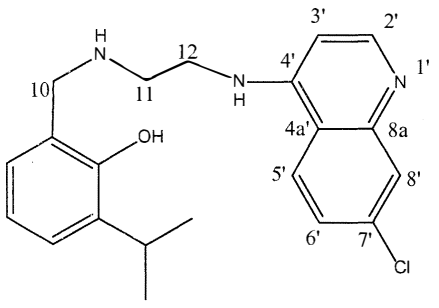
14 & 15), 1.71 (2H, pent, J=6.94 Hz, H-12), 1.23 (6H, d, J=6.94 Hz, H-9 & 8); $\delta_C$ (CDCl3; 75 MHz), 155.5 (C-1), 135.6 (C-2), 125.7 (C-5), 125.1 (C-3), 122.1 (C-4), 118.6 (C-6), 57.9 (C-10), 52.9 (C-13), 47.5 (C-11), 45.4 (C-14 & 15), 27.1 (C-12), 26.5 (C-7), 22.6 (C-8 & 9), [HRMS (EI) Found: 250.20122. $C_{15}H_{26}ON_2$ requires $M^+$ 250.20451.]

**2-[(3-Diethylamino-propylamino)-methyl]-6-isopropyl-phenol (75)**




Yield (174.9mg, 95.8%); $R_f$ 0.11 (1:4 EtOAc:Hex); $\nu_{max}$ (Chloroform)/$cm^{-1}$ 3605 (free OH), 3303 (N-H), 3049 Ar (C-H), 2959-1769 (C-H), 1644 &1594 (N-H or C = C ), 1227 (C-O or C-N), $\delta_H$ (300 MHz; $CDCl_3$) 7.10 (1H, dd, J=7.55, 1.71 Hz, H-5), 6.83 (1H, dd, J=7.40, 1.76 Hz, H-3), 6.74 (1H, t, J=7.45 Hz, H-4), 5.28 (1H, s, OH), 3.96 (2H, br s, H-10), 3.33 (1H, sept, J=6.92 Hz, H-7), 2.73 (2H, t, J=6.57 Hz, H-11), 2.59 (4H, q, J=7.20 Hz, H-14 &15) 2.57 (2H, t, J=7.15 Hz, H-13), 1.75 (2H, quint, J=6.60 Hz, H-12), 1.23 (6H, d, J=6.93 Hz, H-9 & 8), 1.05 (6H, t, J=7.15 Hz, H-16&17); $\delta_C$ (CDCl3; 75 MHz), 155.5 (C-1), 135.6 (C-2), 125.7 (C-5), 125.2 (C-3), 122.0 (C-4), 118.6 (C-6), 52.7 (C-10), 51.2 (C-13), 47.6 (C-11), 46.6 (C-14 & 15), 26.5 (C-12), 26.0 (C-7), 22.6 (C-8 & 9), 11.0 (C-16 & 17); [HRMS (EI) Found: 278.23897. $C_{17}H_{30}N_2O$ requires $M^+$ 278.23581].

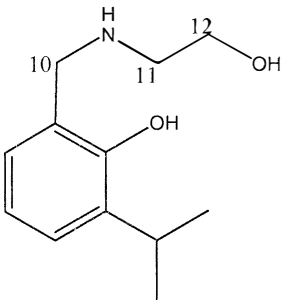
**2-{[2-(7-Chloro-quinolin-4-ylamino)-ethylamino]-methyl}-6-isopropyl-phenol (76)**




Yield (108mg, 80%); $R_f$ 0.24 (30% EtOAc:Hex); $\nu_{max}$ (Chloroform)/$cm^{-1}$ 3535 (OH), 3396-3419 (N-H), 3064 Ar (C-H), 2961-2872 (C-H), 1611 & 1582 (N-H or C=C) , 1208-1235 (C-O or C-N), $\delta_H$ (400 MHz; $CDCl_3$), 8.53 (1H, d, J=5.39 Hz, H-2'), 7.98 (1H, d, J=2.20, H-8'), 7.72 (1H, d, J= 8.94 Hz, H-5'), 7.37 (1H, dd, J=8.93, 2.14 Hz, H-6'), 7.10 (1H, dd, J=7.40, 1.45, H-3), 6.87 (1H, t, J=7.51 Hz, H-4), 6.80 (1H, dd, H-5), 6.41 (1H, d, J=5.43 Hz, H-3'), 6.82 (1H, br s, OH), 4.95 (2H, br s, 2xNH), 4.06 (2H, br s, H-10), 3.43 (2H, t, J=5.79 Hz, H-12), 3.27 (2H, quintet, J=6.9 Hz , H-7), 3.19 (2H, t, J=5.81 Hz, H-11), 1.22 (6H, d, J=6.94 Hz, H-8 & 9); $\delta_C$ (CDCl3; 100 MHz), 151.6 (C-1), 150.9 (C-2'),

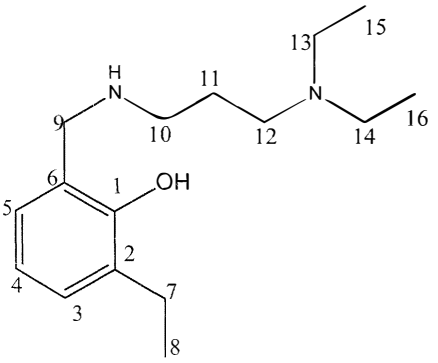
149.8 (C-4'), 148.6 (C-8a), 136.3 (C-2), 135.2 (C-7'), 128.4 (C-8'), 125.5 (C-6'), 124.9 (C-5), 124.5 (C-3), 121.2 (C-5'), 120.6 (C-4), 118.8 (C-4a), 117.2 (C-6), 99.3 (C-3'), 50.1 (C-10), 49.3 (C-11), 40.1 (C-12), 26.3 (C-7), 22.6 (C-8 & 9), [HRMS (EI) Found: 369.15503. $C_{21}H_{24}ClN_3O$ requires $M^+$ 369.16079].

**2-[(2-Hydroxy-ethylamino)-methyl]-6-isopropyl-phenol (77)**




Yield (91.6mg, 60 %); $R_f$ 0.24 (30% EtOAc:Hex); $\nu_{max}$ (Chloroform)/$cm^{-1}$ 3605 (free OH), 3394 (N-H), 3049 Ar (C-H), 2966 & 2874 (C-H), 1644 &1594 (N-H or C = C ), 1200 & 1223 (C-O or C-N), 1053 (C-O), $\delta_H$ (300 MHz; $CDCl_3$) 7.15 (1H, dd, J=7.55, 1.51 Hz, H-5), 7.03 (1H, dd, J=7.50, 1.52 Hz, H-3), 6.85 (1H, t, J=7.22 Hz, H-4), 4.91 (2H, br s, 2xOH), 4.35 (1H, s, NH), 4.10 (2H, s, H-10), 3.74 (2H, t, J=5.12 Hz, H-12), 3.33 (1H, sept, J=6.91 Hz, H-7), 2.87 (2H, t, J=5.10 Hz, H-11), 1.24 (3H, d, J=6.93 Hz, H-9), 1.20 (3H, d, J=6.93 Hz, H-8); $\delta_C$ (CDCl3; 75 MHz), 155.1 (C-1), 135.9 (C-2), 124.8 (C-5), 124.4 (C-3), 120.3 (C-4), 118.6 (C-6), 59.2 (C-12), 53.4 (C-11), 50.2 (C-10), 26.3 (C-7), 22.6 (C-8 & 9); [HRMS (EI) Found: 209.13771. $C_{12}H_{19}NO_2$ requires $M^+$, 209.14158].

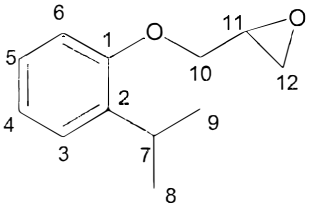
**2-[(3-Diethylamino-propylamino)-methyl]-6-ethyl-phenol (78)**




Yield (105.4mg, 65%); $R_f$ 0.13 (1:4 EtOAc:Hex); $\nu_{max}$ (Chloroform)/$cm^{-1}$ 3605 (free OH), 3303 (N-H), 3049 Ar (C-H), 2959-1769 (C-H), 1644 & 1594 (N-H or C=C), 1227 (C-O or C-N), $\delta_H$ (300 MHz; $CDCl_3$) 7.05 (1H, dd, J=7.44, 1.71 Hz, H-5), 6.83 (1H, dd, J=7.44, 1.62 Hz, H-3), 6.71 (1H, t, J=7.45 Hz, H-4), 5.53 (2H, br s, OH & NH), 3.96 (2H, br s, H-9), 2.74 (2H, t, J=6.55 Hz, H-10), 2.64 (2H, q, J=7.53 Hz, H-7) 2.61 (4H, q, J=7.19 Hz, H-13 & 14), 2.59 (2H, t, J=6.85 Hz, H-12), 1.76 (2H, quintet, J=6.56 Hz, H-11), 1.21(3H, t, J=7.52 Hz, H-8), 1.06 (6H, t, J=7.20 Hz, H-15&16); $\delta_C$ (CDCl3; 75 MHz), 156.0 (C-1), 131.3 (C-2), 128.2 (C-3), 126.0 (C-5), 121.9 (C-4), 118.6 (C-6), 52.7 (C-9), 51.1 (C-10), 47.6 (C-12), 46.5 (C-13 & 14), 25.9 (C-11), 22.9

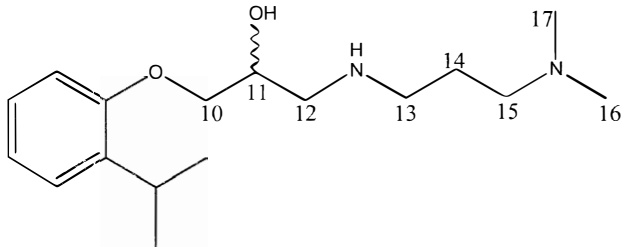
(C-7), 14.1 (C-8), 10.9 (C-15 & 16); [HRMS (EI) Found: 264.22045. $C_{16}H_{28}N_2O$ requires $M^+$ 264.22016].

**2-(2-Isopropyl-phenoxymethyl)-oxirane (79)**



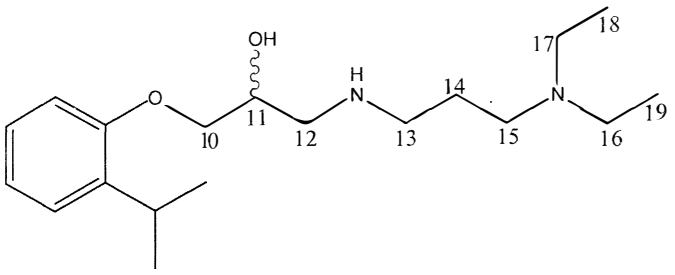

Yield (570.6 mg, 80.4%); $R_f$ 0.57 (1:20 EtOAc:Hex); $\nu_{max}$ (Chloroform)/$cm^{-1}$) 3064 Ar (C-H), 2956-2872 (C-H), 1600, 1585 & 1492 (C=C), 1245 & 1044 (C-O), $\delta_H$ (300 MHz; $CDCl_3$) 7.23 (1H, dd, J=7.53, 1.67 Hz, H-3), 7.15 (1H, td, J=7.63, 1.74 Hz, H-5), 6.96 (1H, td, J=7.47, 1.09 Hz, H-4), 6.83 (1H, dd, J=8.14, 1.00 Hz, H-6), 4.24 (1H, dd, J=11.05, 3.11 Hz, H-$10^{\alpha}$), 4.00 (1H, dd, J=11.05, 5.35 Hz, H-$10^{\beta}$), 3.37 (2H, m, H-7 & H-11), 2.91 (1H, dd, J=5.00, 4.16 Hz, H-$12^{\alpha}$), 2.79 (1H, dd, J=5.00, 2.64 Hz, H-$12^{\beta}$), 1.24 (6H, d, J=6.92 Hz, H-9 & 8); $\delta_C$ (CDCl3; 75 MHz), 155.7 (C-1), 137.4 (C-2), 126.5 (C-5), 126.2 (C-3), 121.2 (C-4), 111.6 (C-6), 68.8 (C-10), 50.3 (C-11), 44.6 (C-12), 26.9 (C-7), 22.65 (C-8), 22.61(C-9); [HRMS (EI) Found: $M^+$, 192.115237. $C_{12}H_{19}NO_2$ requires 192.1503].

**1-(3-Dimethylamino-propylamino)-3-(2-isopropyl-phenoxy)-propan-2-ol (80)**



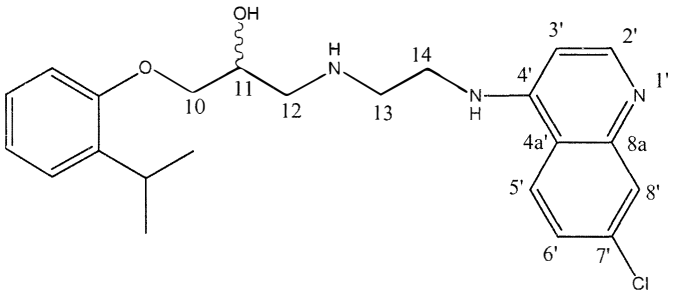

Yield (96mg, 63%); $R_f$ 0.37 (80:20:1 DCM:MeOH:$NEt_3$); $\nu_{max}$ (Chloroform)/$cm^{-1}$) 3604 (OH), 3395 (N-H), 3064 Ar (C-H), 2961-2873 (C-H), 1664 (N-H), 1591, 1600 & 1462 (C=C), 1236 & 1221(C-O or C-N), 1046 & 1033 (C-O or C-N), $\delta_H$ (300 MHz; $CDCl_3$) 7.19 (1H, dd, J=7.52, 1.62 Hz, H-3), 7.12 (1H, td, J=7.67, 1.73 Hz, H-5), 6.92 (1H, td, J=7.48, 0.98 Hz, H-4), 6.83 (1H, dd, J=8.15, 0.93 Hz, H-6), 4.10 (1H, m, H-11), 4.01 (1H, dd, J=9.34, 5.23, H-10α), 3.94 (1H, dd, J=9.34, 5.42, H-10β), 3.30 (1H, sept, J=6.91 Hz, H-7), 2.97 (2H, br s, NH & OH), 2.91 (1H, dd, J=12.31, 3.80 Hz, H-12α), 2.75 (3H, m, H-12β & H-13), 2.35 (2H, t, J=7.04 Hz, H-15), 2.21 (6H, s, H-16 & 17), 1.68 (2H, quint, J=6.98 Hz, H-14), 1.20 (6H, d, J=6.92 Hz, H-8 & 9); $\delta_C$ (CDCl3; 75 MHz), 155.7 (C-1), 136.9 (C-2), 126.6 (C-5), 126.0 (C-3), 120.9 (C-4), 118.4 (C-6), 70.5 (C-10), 68.2 (C-11), 52.9 (C-12), 52.0 (C-13), 48.1 (C-15), 45.3 (C-16 & 17), 27.4 (C-14), 26.8 (C-7), 22.6 (C-8 & 9); [HRMS (FAB) Found: 295.14298. $C_{17}H_{31}N_2O_2$ requires $(M+H)^+$ 295.23855].

**1-(3-Diethylamino-propylamino)-3-(2-isopropyl-phenoxy)-propan-2-ol (81)**




Yield (120.5mg, 72.0%); $R_f$ 0.44 (80:20:1 DCM:MeOH:$NEt_3$); $\nu_{max}$ (Chloroform)/$cm^{-1}$) 3604 (OH), 3395 (N-H), 3064 Ar(C-H), 2961-2873 (C-H), 1664 (N-H), 1591, 1600 & 1462 (C=C), 1236 & 1221(C-O or C-N), 1046 & 1033 (C-O or C-N), $\delta_H$ (300 MHz; $CDCl_3$) 7.21 (1H, dd, J=7.52, 1.65 Hz, H-3), 7.14 (1H, td, J=7.64, 1.74 Hz, H-5), 6.93 (1H, td, J=7.45, 1.00 Hz, H-4), 6.84 (1H, dd, J=8.15, 0.94 Hz, H-6), 4.09 (1H, m, H-11), 4.01 (1H, dd, J=9.26, 5.22 Hz, H-10α), 3.95 (1H, dd, J=9.24, 5.22 Hz, H-10β), 3.31 (1H, sept, J=6.91 Hz, H-7), 2.90 (1H, dd, J=12.19, 3.78 Hz, H-12α), 2.79 (1H, dd, J=12.15, 7.53 Hz, H-12β), 2.71 (2H, m, H-13), 2.51 (4H, q, J=7.15 Hz, H-16 & 17), 2.49 (2H, t, J=7.00 Hz, H-15), 1.60 (2H, quint, J=6.91 Hz, H-14), 1.22 (6H, d, J=6.92 Hz, H-9 & 8), 1.02 (6H, J=7.13 Hz, H-18 & 19); $\delta_C$ (CDCl3; 75 MHz), 155.7 (C-1), 136.9 (C-2), 126.6 (C-5), 126.0 (C-3), 120.9 (C-4), 111.4 (C-6), 70.5 (C-10), 68.3 (C-11), 52.0 (C-12), 51.18 (C-13), 48.6 (C-15), 46.9 (C-16), 46.8 (C-17), 27.1 (C-14), 26.8 (C-7), 22.6 (C-8 & 9), 11.5 (C-18 & 19); [HRMS (FAB) Found: $(M+H)^+$ 323.27541. $C_{19}H_{34}N_2O_2$ requires $M^+$ 322.26985, Anal. (Calc. C, 70.76, H, 10.63, N, 8.69 Found: C, 69.52, H, 10.43, N, 8.47].

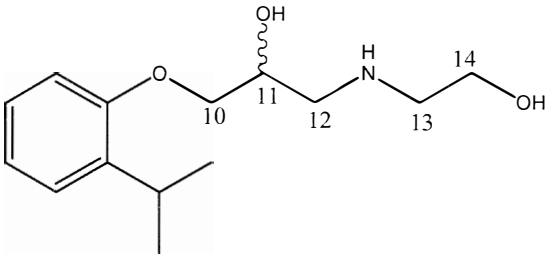
**1-[2-(7-Chloro-quinolin-4-ylamino)-ethylamino]-3-(2-isopropyl-phenoxy)-propan-2-ol (82)**




Yield (100mg, 62%); $R_f$ 0.16 (80:20:1 DCM:MeOH:$NEt_3$); $\nu_{max}$ (Chloroform)/$cm^{-1}$); 3529 (OH), 3395, 3421 (N-H), 3064 Ar(C-H), 2997-2973 (C-H), 1612 & 1585 (N-H and C=C), 1236 & 1221(C-O or C-N), 1046 & 1033 (C-O or C-N), $\delta_H$ (300 MHz; $CDCl_3$), 8.40 (1H, d, J=5.51 Hz, H-2'), 7.88 (1H, d, J=2.11 Hz, H-8'), 7.74 (1H, d, J= 8.95 Hz, H-5'), 7.22 (1H, dd, J=8.93, 2.10 Hz, H-6'), 7.19 (1H, dd, J=7.57, 1.67 Hz, H-3), 7.12 (1H, td, J=7.96, 1.71 Hz, H-5), 6.94 (1H, td, J=7.50, 1.10 Hz, H-4), 6.84 (1H, dd, J=8.15, 0.94 Hz, H-6), 6.30 (1H, J=5.56 Hz, H-3'), 4.24 (1H, m, H-11), 4.03

(2H, m, H-10), 3.39 (2H, t, J=5.62 Hz, H-14), 3.27 (1H, sept, J=6.92 Hz, H-7), 3.12 (2H, td, J=5.31, 2.28 Hz, H-13), 3.00 (1H, dd, J=12.29, 3.76 Hz, H-12α), 2.93 (1H, dd, J=12.15, 7.51 Hz, H-12β), 2.65 (3H, br s, 2NH & OH), 1.18 (3H, d, J=6.92 Hz, H-8), 1.17 (3H, d, J=6.92 Hz, H-8); $\delta_C$ (CDCl3; 100 MHz), 155.5 (C-1), 150.7 (C-2'), 150.4 (C-4'), 147.6 (C-8a), 136.9 (C-2), 135.3 (C-7'), 127.24(C-8'), 126.7 (C-5), 126.2 (C-3), 125.45 (C-6'), 121.8 (C-5'), 121.2 (C-4), 117.0 (C-4a), 111.3 (C-6), 98.8 (C-3'), 70.3 (C-10), 68.9 (C-11), 51.8 (C-12), 47.4 (C-13), 42.1 (C-14), 26.8 (C-7), 22.6 (C-8 & 9); [HRMS (EI) Found: 413.18776, $C_{12}H_{19}NO_2$ requires $M^+$, 413.18701, Anal. (Calc. C, 66.74, H, 6.82, N, 10.15 Found: C, 66.35, H, 6.92, N, 9.93].

**1-(2-Hydroxy-ethylamino)-3-(2-isopropyl-phenoxy)-propan-2-ol (83)**




Yield (80 mg, 60.8%); $R_f$ 0.18 (80:20:1 DCM:MeOH:$NEt_3$); $\nu_{max}$ (Chloroform)/$cm^{-1}$) 3604 (OH), 3395 (N-H), 3064 Ar (C-H), 2961-2873 (C-H), 1666 (N-H), 1585, 1600 & 1492 (C=C), 1236 & 1221(C-O or C-N), 1046 &1033 (C-O or C-N), $\delta_H$ (300 MHz; $CDCl_3$) 7.19 (1H, dd, J=7.52, 1.70 Hz, H-3), 7.12 (1H, td, J=7.53, 1.75 Hz, H-5), 6.92 (1H, td, J=7.47, 1.09 Hz, H-4), 6.82 (1H, dd, J=8.17, 1.05 Hz, H-6), 4.62 (1H, br s, OH), 4.09 (1H, m, H-11), 4.00 (1H, dd, J=9.44, 5.30 Hz, H-10α), 3.94 (1H, dd, J=9.44, 5.30 Hz, H-10β), 3.29 (1H, sept, J=6.92 Hz, H-7), 2.73-2.93 (8H, m, H-12, 13, 14, NH & OH), 1.20 (6H, d, J=6.92 Hz, H-9 & 8); $\delta_C$ (CDCl3; 75 MHz), 155.7 (C-1), 136.9 (C-2), 126.6 (C-5), 126.1 (C-3), 121.0 (C-4), 111.5 (C-6), 70.5 (C-10), 68.6 (C-11), 52.0 (C-12), 51.4 (C-14), 41.3 (C-13), 26.8 (C-7), 22.7 (C-8 & 9). [HRMS (EI) Found: $M^+$, 253.15273, $C_{12}H_{19}NO_2$ requires $M^+$ 253.16779.

## REFERENCES


1) Frederich, M.; Dogne J., Anjenot, L., De Mol, P., *Current Medicinal Chemistry*, **2002**, 9, 1435-1456.
2) The History of malaria : http://www.rph.wa.gov.au/malaria/history.html
3) Malaria Site: http://www.malariasite.com/malaria/WhatIsMalaria.htm
4) Chauhan, P. M. S.; Srivastava, S. K., *Current Medicinal Chemitry*, **2001**, 8, 1535-1542.
5) Egan T. J., *Mini Reviews in Medicinal Chemistry*, **2001**, 1, 113-123
6) Ziegler J., Linck R., Wright D. W., *Current Medicinal Chemistry*, **2001**, 8, 171-189.
7) Africa Malaria Report, **2003,** [On-line], Available: http:// www.rbm.who.int/amd2003/amr2003/amr-toc.html
8) Miller, L. H.; Baruch, D. I.; Marsh, K.; Doumbo, O. K., *Nature*, **2002**, 415, 673-679.
9) Kolacovich, K. A.; Gluzman, I. Y., Duffin, K. L.; Goldberg, D.E., *Mol. Biochem. Parasitol.* **1997**, 87, 123-135.
10) Francis, S. E.; Sullivan, D. J.; Jr. ; Goldberg, D . E ., *Annu. Rev. Microbiol.* **1997**, 51, 97-123
11) Olliaro,P.L., Goldberg, D. E., *Parasitol. Today* **1995**, 11, 294
12) Shenai, B.; Sijwali, P.; Singh, A.; Rosental, P., *J. Biol. Chem.*, **1999**, 275, 29000-29010.
13) Coombs, G. H., Goldberg, D. E., Klemba, M., Berry, C., Kay, J., Mottram, J. C., *Trends Paracitol.,* **2001**, 17, 532-536.
14) Sajid, M., McKerrow, J. H., *Mol. Biochem. Parasitol.* **2002**, 120, 1-21.
15) Goldberg, D. E., Slater, A. F. G., Beavis, R., Chait, B., Cerami, A., Henderson, G. B., *J. Exp. Med.*, **1991**, 173, 961-968.
16) Sijwali, P. S.; Shenai, B. R.; Gut, J.; Singh, A. and Rosenthal, P., *J. Biochem.,* **2001**, *360, 481*
17) Eggleson, K. K., Duffine, K. L., Goldberg, D. E., *J. Biol. Chem.*, **1999**, 274, 32411-32417.
18) Green M. D., Xiao L., Lal A. A., *Mol. Biochem. Parasitol.*, **1996**, 83, 183-188.
19) Gluzman, L. Y., Francis, S. E., Oksman, A., Smith, C. E., Duffin, K. L., Goldberg, D. E., *J. Clin. Invest.,* **1994**, 93, 1602-1608.
20) Atamna H., Ginsburg H., *Mol. Biochem. Parasitol.*, **1993**, 61, 231-241.
21) Olliaro P., *Pharmacol. Therap.*, **2001**, 89, 207-219.
22) Ridley R. G., *Nature*, **2002**, 415, 686-693.

23) Wellems T. E., Plowe C. V., *J. Inf. Dis.*, **2001**, 184, 770-776.

24) Hyde J. E., *Microbes and Infection*, **2002**, 4, 165-174.

25) Olliaro, P., Nevill, C., Lebras, J., Ringwald, P., Mussano, P., Garner, P., Brasseur, P., *The Lancet*, **1996**, 348, 1196-1201.

26) Raynes, K. J., Stocks P. A. O`Neill, P. M., Park, P. K., Ward, S. A., *J. Med. Chem.*, **1999**, 42, 2747-2751.

27) Srivastava, I. K., Rottenberg, H., Vaidya, A. B., *J. Biol. Chem.*, **1997**, 272, 3961-3966.

28) Looareesuwan S., Viravan C., Webster, H. K., kyle, D. E., Canfield, C. J., *Am. J. Trop. Med. Hyg.*, **1996**, 54, 62-63.

29) Rosenthal, P. J., *J. Exp. Biol.*, **2003**, 206, 3735-3744.

30) Stocks, P. A., Raynes, K. J., Ward, S. A., *Antimalarial Chemotherapy: Mechanism of Action, Resistance, and New Directions in Drug Discovery*; Rosenthal, P. J. Ed; Humana Press, Totowa, N J: **2001**; 235-253.

31) De. D., Krogstad, F. M., Cogswell, F. B., Krogstad, D. J., *Am. J. Trop. Med. Hyg.*, **1996**, 55, 579-583.

32) Biot, C., Glorian, G., Masiejewski, L. A., Brocard, J. S., *J. Med. Chem.*, **1997**, 40, 3715-3718.

33) Stocks, P. A., Raynes, K. J., Bray, P. G., Park, P. K., O`Neill, P. M., Ward, S. A., *J. Med. Chem.*, **2002**, 45, 4975-4983.

34) Clough, B., Wilson, R. J. M., *Antimalarial Chemotherapy: Mechanism of Action, Resistance, and New Directions in Drug Discovery*; Rosenthal, P. J. Ed; Humana Press, Totowa, N J: **2001**; 265-286.

35) Rasoanaivo, P., Ratsimamanga-Urverg, S., Frappier, F., *Curr. Med. Chem.* **1996**, 3, 1-10.

36) Batra, S., Srivastava, P., Roy, K., Pandey, P. C., Bhaduri, A. P., *J. Med. Chem.*, **2000**, 43, 3428-3433.

37) Chibale, K., *Life*, **2002**, 53, 249-252.

38) Bray, P. G., Ward, S., *Pharmacol. Ther.*, **1998**, 77, 1-28.

39) Foote, S. J., Kyle, D. E., Martin, R. K., Oduola, A. M. J., Forsth, K., Kemp, D. J., Cowman, A. F., *Nature*, **1990**, 345, 255-258.

40) Fidock, D. A., Nomura, T., Talley, A. K., Cooper, R.A., Dzekunov, S. M., Ferdig, M. T., Ursos, L. M., Sidhu, A. B., Naude, B., Deitsch, K. W., Su, X-Z, Wootton, J. C., Roepe, P. D., Wellems, T. E., *Mol. Cell*, **2000**, 6, 861-871.

41) Reed, M. B., Saliba, K. J., Caruana, S. R., Kirk, K., Cowman, A. F., *Nature*, **2000**, 403, 906-909.

42) Martin, S. K., Oduola, A. M., Milhous, W. K., *Science*, **1987**, 235, 899-901.

43) Bitoniti, A. J., Sjoerdsma, A., McCann, P. P., Kyle, D. E., Oduola, A. M., Rossan, R. N., Milhous, W. K., Davidson, D. E., *Science*, **1988**, 242, 1301-1303.

44) Guan, J., Kyle, D. E., Gerena, L., Zhang, Q., Milhous, W. K., Lin, A. J., *J. Med. Chem.*, **2002**, 45, 2741-2748.

45) Alibert, S., Santelli-Rouvier, C., Pradines, B., Houdouin, C., Parzy, D., Karolak-Wojciechowska, J., Barbe J., *J. Med. Chem.* **2002**, *45*, 3195-3209

46) Schwikkard, S., van Heeren, F. R., *Nat. Prod. Rep.*, **2002**, 19, 675-692.

47) Phillipson, J. D., *Trans R Soc Trop. Med. Hyg.*, **1994**, 88, 17-19.

48) Aaron, J. T., Ranking, E. A., A Nurse`s guid to herbal remedies, [On-line], Available: www. Nursingnetwork.com/altmed2.html

49) Farnsworth, N. R., *Natural Products and drug Development*; P. Krogsraard-Larsen, S. Brogger Christensen and H. Kofod, Eds; Ballier, Tindall and Cox, London, **1984**, 9-98.

50) Breinbauer R., Manger M., Scheck, M., Waldmann, H., *Curr. Med. Chem.*, **2002,** 9, 2129-2145.

51) Cragg, G. M., Newman, D. J., Snader, K. M., *J. Nat. Prod.* **1997**, 60, 52-60.

52) Newman, D. J., Cragg, G. M., Snader, K. M., *J. Nat. Prod.* **2003**, 66, 1022-1037.

53) Nicolaou, K. C., Winssinger, N., Vourloumis, D., Ohshima, T., Kim, S., Pfefferkorn, J., Xu, J. Y., Li, T. J., *J. Am. Chem. Soc.*, **1998**, 120, 10814-10826.

54) Oketch-Rabah, H. A., Mwangi, J.W., Malaria and infectious disease, 1998, 9, 15. [O-line]: http://www.chez.com/malaria/09ansu15.htm.

55) Meshnick, S. R., *Antimalarial Chemotherapy: Mechanism of Action, Resistance, and New Directions in Drug Discovery*; Rosenthal, P. J. Ed; Humana Press, Totowa, N J: **2001**; 191-201.

56) Tang, Y., Dong, Y., Vennerstrom, J. L., *Med. Res. Rev.*, **2004**, 24, 425-448.

57) Posner, G. H., Paik, I., Sur, S., McRiner, A. J., Borstnik, K., Xie, S., Shapiro, T. A., *J. Med. Chem.*, **2003**, 46, 1060-1065.

58) Meseguer, B., Alonso-Diaz, D., Griebenow, N., Herget, T., Waldmann, H., *Chem, Eur. J.* **2000**, 6, 3943-3957.

59) Akritopoulou-Zanze, I., Sowin, T. J., *J. Comb. Chem.* **2001**, 3, 301-311.

60) Nicolaou, K. C., Winssinger, N., Pastor, J., Nincovic, S., Sarabia, F., He, Y., Vourloumis, D., Yang, Z., Li, T., Giannakakou, P., Hamel, E., *Nature*, **1997**, 387, 268-272.

61) Evans, B. E., Rittle, K. E., Bock, M. G., DiPardo, R. M., Freidinger, R. M., Whitter, W. L., Lundell, G. F., Veber, D. F., Anderson, P. S., Chang, R. S. L., Lotti, V. J., Cerino, D. J., Chen, T. B., Kling, P. J., Kunkel, K. A., Springer, J. P., Hirshfield, J., *J. Med. Chem.* **1988**, 31, 2235-2246.

62) Nicolaou, K. C., Pfefferkorn, J. A., Roecker, A. J., Cao, G. -Q., Barluenga, S., Mitchell, H. J., *J. Am. Chem. Soc.* **2000**, 122, 9939-9953.

63) Nicolaou, K. C., Pfefferkorn, J. A., Mitchell, H. J., Roecker, A. J., Barluenga, S., Cao, G. –Q., Affleck, R. L., Lillig, J. E., *J. Am. Chem. Soc.* **2000**, 122, 9954-9967.

64) Bendall, J. G., Cambie, R. C., *Aust. J. Chem.*, **1995**, 48, 884-916.

65) Cambie R. C., Mander L. N., *Tetrahedron*, **1962**, 18, 465-475.

66) Short, W. F., Wang, H., *J. Chem. Soc.*, **1951**, 2979.

67) Barltrop, J. A., Rogers, N. A. J., *J. Chem. Soc.*, **1958**, 2566.

68) Chow, Y-L., Erdtman, H., *Acta Chem. Scand.*, **1960**, 14, 1852.

69) Geissman T. A., Crout D. H. G., *Organic Chemistry of Secondary Plant Metabolism*, Freeman Cooper and Company, San Francisco, **1969,** 298.

70) Wenkert E., Jackson B. G., *J. Am. Chem. Soc.*, **1958**, 80, 211.

71) Matsumoto, T., Suetsugu, A., Bull. *Chem. Soc. Jpn*, **1979**, 52, 1450.

72) (a) Marcos I. S., Cubillo M. A., Moro R. F., Diez d., Basabe F., Sanz F., Urones J. G., *Tetrahedron Letters*, **2003**, 44, 8831-8835. (b) Marcos I. S., Moro R. F., Carballares, S., Urones J. G., Synlett 2002, 458.

73) Kubo I., Muroi H., Himejima M., *J. Nat. Prod.*, **1992**, 55, 1436.

*74)* Evans G. B., Furneaux R. H., Gravestock M. B., Lynch G. P., Kenneth scott G., *Bioorg. Med. Chem.,* **1999**, 7, 1953-1964.

75) Evans G. B., Furneaux R. H., *Bioorg. Med. Chem.,* **2000**, 8, 1653-1662.

*76)* Evans G. B., Furneaux R. H., Gainsford G. J., Murphy M. P., *Bioorg. Med. Chem.,* **2000**, 8, 1663-1675.

77) Constantine G. H., Karchesy J. J. Franzblau S. G., Lafleur L. E., *Fitoterapia*, **2001**, 72, 572-574.

*78)* Clarkson C., Musonda C. C., Chibale K., Campbell W. E., Smith P., *Bioorg. Med. Chem.*, **2003**, 11, 4417-4422.

79) Clarkson C., "Isolation and Characterisation of Two Antiplasmodial Diterpenes from Harpagophytum procumbens (Devil's Claw) and Chemical Modification of a Related Analogue". Thesis presented for the degree of Doctor of Philosophy in Pharmacology, University of Cape Town, **2002**.

80) World health Organization. Practical chemotherapy of malaria: report of a WHO scientific group. Technical Report Series no. 805, **1990**.

81) O`Neill, P.M., Willock, D. J., Hawley, S. R.; Bray, P. G., Storr, R. C., Ward, S. A., Park, B. K. *J. Med. Chem*. **1997**, 40, 437 – 448.

82) O'Neill, P.M.; Bray, P. G.; Hawley, S. R.; Ward, S. A.; Kevin Park, B., *Pharmacol. Ther.,* **1998**, 77, 29 – 58.

83) O'Neill, P. M.; Mukhtar, A.; Stock, P. A.; Randle, L. E.; Hindley, S.; Ward, S. A.; Storr, R. C., Bickley, J. F., O`Neil, I. A.; Maggs, J. L.; Hughes, R. H.; Winstanley, P. A.; Bray, P. G.; Kevin Park, B., *J. Med. Chem*., **2003**, 46, 4933 – 4945.

84) Vippagunta, S. R.; Dorn, A.; Matila, H.; Bhattacharjee, A. K.; Karle, J. M., Ellis, W. Y.; Ridley, R. G.; Vennerstrom, J. L., *J. Med. Chem*., **1999**, 42, 4630 – 4639.

85) Koh, H. L.; Go, M. L.; Ngiam, T. L.; Mak, J. W.; *Eur. J. Med. Chem*., **1994**, 29, 107-l 13.

86) Bray, P. G., Mungthin, M., Ridley, R. G., Ward, S. R., *Mol. Pharmacol.*, **1998**, 54, 170-179.

87) Bray, P. G. Janet, O., Rynes, K. J., Mungthin, M.,Ginsburg, H., Ward, S. R., *J. Cell Biol.*, **1999**, 145, 363-376.

88) Egan, T. J., Hunter, R., Kaschula, c. H., Marques, H. M., Misplon, A., Walden, J., **2000**, 43, 283-291.

89) Ringwald, P.;* Eboumbou, E. C. M.; Bickll, J.; Basco, L. K., *Antimicro. Agents Chemother.*, 1999, 1525 – 1527.

90) http:// www.mmv.org-[MMV] << Pyramax (pyronaridine + artesunate) >>

91) Portela, C., Afonso, C. M.M., Pinto, M. M.M., Ramos, Maria, J., *FEBS Lett.*, **2003**, 27435, 217-222.

92) Lynn, A., Chandra, S., Malhotra, P., Chauhan, V. S., *FEBS Lett*., **1999**, 459, 267-271.

93) Bradbury, J., *The Lancet*, **2003**, 3, 677.

94) Harrison, T., Samuel, B. U., Kompong, T. A., Hamm, H., Mohandas, N., Lomasney, J. W., Haldar, K., *Science*, **2003**, 301, 1734-1736.

95) Fattori, D., **2004**, *Drug Discov. Today*, 9, 229-238.

96) Mueller, G., **2003**, *Drug Discov. Today* 8, 681-691.

97) Otto, H.; Schirmeister, T.; *Chem. Rev.*, **1997,** 97, 133-171

98) Rich, D. H.; Brouwn, M. A.; Barrettt, A. J., *Biochem. J.*, **1986**, 235, 731-734.

99) Cerecettoa, H.; Di Maioa, R.; Gonzálezb, M.; Rissoa, M.; Sagrerab, G.; Seoanea, G.; Denicolac, A.; Peluffod, G.; Quijanoc, d, C.; Stoppanie, A.O.M.; Paulinof; M.; Olea-Azarg, C.; Basombríoh, M. A., *Eur. J. Med. Chem.*, **2000**, 35, 343- 350.

100) Cerecettoa, H.; Di Maioa, R.; Gonzálezb, M.; Rissoa, M.; Saenza, P.; Seoanea, G.; Denicolac, A.; Peluffod, G.; Quijanoc, d, C.; Olea-Azarg, C., *J. Med.chem.*, **1999**, 42, 1941-1950.

101) Pandeyau, S. N.; Vibha Mishra, P. N. S.; Rupainwar, D. C., *Pharmacol. Res.*, **1998**, 37, 17-23.

102) Warren, J. D.; Woodward, D. L.; Hargreaves, R. T., *J. Med. Chem.*, **1977**, 20, 1520-1521.

103) Ramu, K.; Lam, G. N.; Hughes, H.; *Drug Metab. Dispos.*, **2000**, 28, 1153–1161.

104) Kleinman, E. F.; Comprehensive Organic Synthesis: "Selectivity, Strategy & Efficiency", Trost, B. M., Fleming, B. Ed; University of California, Berekeley, CA, **1991**, 2, 893 - 950.

105) Lorand, T.; Kocsis B.; Sohar, P.; Nagy, G.; Kispal, G.; Krane, HG.; Schmitt, H.; Weckert, E.; *Eur. J. Chem.* **2001**, 36, 705 – 717.

106) Ronda, J. C., Serra, A., Cadiz, V., *Macromol. Chem. Phys.*, **1999**, 200, 221-230.

107) Cruickshank, P. A.; Fishman, M., *J. Org. Chem.*, **1969**, 34, 4060.

108) Trager, W., Jensen, J. B., *Science*, **1976**, 193, 673-675.

109) Makler, M. T., Ries, J. M., Williams, J. A., Bancroft, J. E., Piper, R. C., Gibbins, B. L., Hinriches, D. J., 1993, *Amer. Soc. Trop. Med. Hyg.*, **1993**, 48, 739-741.

110) Noedl, H., Wongsrichanalai, C., Wernsdorfer, W. H., *Trends in Parasitology*, **2003**, 19, 175-181.

111) Tacon, C., BSc (honors) research project, **2003**, department of Pharmacology, University of Cape Town.

112) Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; and Feeney, P. J. *Adv. Drug Delivery Rev.* **1997**, 23, 4-25.

113) Lipinski, C. A. *J. Pharmacol. Toxicol. Methods* **2000**, 44, 235-249.

114) Navia, M. A., and Chaturvedi, P. R., *D D T* , **1996**, 1, 179-189.