Synthesis and Anti-malarial Activity of Ethylene Glycol Oligomeric Ethers of Artemisinin

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"If we take as our standard of importance,
the greatest harm to the greatest number,
then there is no question that malaria is the
most
important
of all infectious diseases."

- Sir Macfarlane Burnet

ABSTRACT

Malaria continues to be a major serious health problem and public health threat, with over two billion people at risk of contracting this deadly disease. Malaria is endemic in 92 countries and more than one million deaths per year are attributed to malaria, the mortality in African children being the highest.

Drug-resistance to classical and existing anti-malarial drugs is a challenging problem in malaria control in most parts of the world, contributing to the need of developing new compounds for malaria treatment. Artemisinin is a sesquiterpene lactone endoperoxide and the first natural 1,2,4-trioxane isolated from *Artemisia annua.* Artemisinin and its derivates are of special biological interest because of their outstanding anti-malarial activity against chloroquine-resistant *P. falciparum* and cerebral malaria.

The reason for this is their unusual chemical structures and the difference in their mechanism of action compared to other anti-malarials. The endoperoxide bridge of artemisinin and a heme iron play critical roles in the mechanism of action of artemisinin. The reaction mechanism consists of two distinct steps, the first step an activation step and the second step an alkylation step. During the activation step, the heme iron breaks the endoperoxide linkage of artemisinin and an oxygen free radical is produced, which is subsequently rearranged to form a carbon-centered (C4) free radical. In the alkylation step, the carbon free radical alkylates specific malarial proteins, which causes a lethal damage to malarial parasites.

However, the use of such endoperoxides is restricted by their poor oral bioavailability, poor solubility in oil and water, a short plasma half-life (30 minutes in plasma) and the high rate of recrudescent infections when used as monotherapy in short-course treatments, even though these drugs have a rapid onset of action and low reported toxicity. In order to overcome these pharmacokinetic deficiencies, a number of new analogues with improved efficacy and increased solubility were introduced, including oil-soluble artemether and arteether, but these compounds still have a short plasma half-life. Artemisinin, dihydroartemisinin, artemether and arteether are all poorly water-soluble compounds, which results in slower and incomplete absorption of these drugs into the systemic circulation.

Therefore, it may be worthwhile to produce new artemisinin derivitives to hopefully develop a compound with enhanced pharmacokinetic properties resulting in better bioavailability and increased effectiveness.

The aim of this study was to synthesise ethylene glycol oligomeric ethers of artemisinin, determine certain physicochemical properties and evaluate their anti-malarial activity compared to artemether and chloroquine.

In this study eight ethylene glycol derivatives of artemisinin were synthesised by linkage of a polyethylene glycol chain of various chain lengths to C-10 of dihydroartemisinin. The structures of the prepared derivatives were confirmed by nuclear magnetic resonance spectroscopy (NMR) and mass spectroscopy (MS).

The experimental aqueous solubility of the synthesised compounds increased with the decrease in the experimental partition coefficients, as the polyethylene glycol (PEG) chain length increased, validating both structure-aqueous solubility and structure-lipophilicity relationships within the series.

The new ethylene glycol oligomeric ethers of artemisinin were tested *in vitro* against the chloroquine sensitive strain of *Plasmodium falciparum* (D-10). The results indicate that the anti-malarial activity increases with the elongating of the PEG chain length. The ethoxypoly(ethylene glycol) series (**6a-8**) showed higher anti-malarial activity than the methoxypoly(ethylene glycol) series (**3-5b**), thus showing that both hydrophilic and lipophilic properties are necessary for the enhancement of the anti-malarial activity. None of the synthesised compounds showed better anti-malarial efficacy than artemether. Compound (**8**), 2-[2-(2-ethoxyethoxy)ethoxy] ethoxy derivative, showed better anti-plasmodial activity than chloroquine and compounds (**5a**) and (**6a**) showed activity comparable to that of chloroquine. Compounds (**3**), (**4**), (**5b**), (**6b**) and (**7**) are less active than artemether and chloroquine. In all cases the anti-malarial activity of the β-isomers was higher than that of the α-isomers.

OPSOMMING

Malaria is steeds die grootste bestaande gesondheidsprobleem en bedreiging vir openbare gesonheid, met meer as twee miljard mense in gevaar om hierdie dodelike siekte op te doen. Malaria is endemies in 92 lande en meer as een miljoen sterftes word per jaar aan malaria toegeskryf, met die sterftesyfer van kinders in Afrika die hoogste.

Weerstandigheid teen klassieke en bestaande antimalariamiddels is 'n uitdagende probleem in die beheer van malaria in meeste dele van die wêreld, wat bydra tot die behoefte om nuwe verbindings vir behandeling van malaria te ontwikkel. Artemisinien is 'n seskwiterpeenlaktoon endoperoksied en die eerste natuurlike 1,2,4-trioksaan uit *Artemisia annua* geïsoleer. Artemisinien en sy derivate is van spesiale biologiese belang, omdat hulle uitstekende antimalaria-aktiwiteit teen chlorokienweerstandige *P. falciparum* en serebrale malaria toon.

Die rede hiervoor is dat hierdie verbindings ongewone chemiese strukture het en hul werkingsmeganisme verskil van die ander antimalariamiddels. Die endoperoksiedbrug van artemisinien en 'n heem-yster speel belangrike rolle in die werkingsmeganisme van artemisinien. Die meganisme van werking bestaan uit twee onderskeie stappe, die eerste stap, 'n aktiverende stap en die tweede stap, 'n alkilerende stap. Gedurende die aktiverende stap, breek die heem-yster die endoperoksiedskakel van artemisinien en 'n suurstofvrye radikaal word geproduseer, wat daarna herrangskik om 'n koolstofgesentreerde (C4) vrye radikaal te vorm. Gedurende die alkilerende stap, alkileer die koolstofvrye radikaal spesifieke malariaproteïene, wat tot dodelike beskadiging van die malariaparasiete lei.

Nogtans word die gebruik van hierdie endoperoksiede beperk deur hul swak orale biobeskikbaarheid, swak oplosbaarheid in beide olie en water, kort plasmahalfleeftyd (30 minute in plasma) en die hoë voorkoms van terugkerende infeksies wanneer dit in kort kursusse behandeling as monoterapie gebruik word, selfs al het hierdie geneesmiddels 'n vinnige intrede van werking en lae toksisiteit. Ten einde hierdie farmakokinetiese tekortkominge te oorkom, is 'n aantal nuwe analoë met beter effektiwiteit en hoër oplosbaarheid ontwikkel, waaronder die olie-oplosbare artemeter and arteeter, maar hierdie verbindings besit steeds 'n kort plasma half-leeftyd.

Artemisinien, dihidroartemisinien, artemeter and arteeter is almal swak wateroplosbare verbindings, wat gevolglik tot stadiger en onvoltooide absorpsie van hierdie geneesmiddels in die sistemiese sirkulasie lei.

Daarom kan dit nuttig wees om nuwe derivate van artemisinien te produseer om hopelik 'n verbinding te ontwikkel met betde farmakokinetiese eienskappe en gevolglik beter biobeskikbaarheid en hoër effektiwiteit.

Die doel van hierdie studie was om etileenglikool oligomeriese eters van artemisinien te sintetiseer, sekere fisiese-chemiese eienskappe te bepaal en om hulle antimalaria-aktiwiteit in vergelyking met die van artemeter en chlorokien te evalueer.

Agt etileenglikoolderivate van artemisinien is in hierdie studie gesintetiseer, deur die binding van 'n poli-etileenglikool (PEG)-ketting van verskeie kettinglengtes aan C-10 van dihidroartemisinien. Die strukture van die bereide derivate is met kernmagnetieseresonansiespektroskopie (KMR) en massaspektrometrie (MS) bevestig.

Die eksperimentele wateroplosbaarheid van die gesintetiseerde verbindings neem toe met die afname in die eksperimentele verdelingskoëffisiënt, soos wat die poli-etileenglikool-kettinglengte verleng, wat bevestig dat verwantskappe van sowel struktuur-wateroplosbaarheid en struktuur-lipofilisiteit binne die reeks bestaan.

Die nuwe etileenglikool oligomeriese eters van artemisinien is *in vitro* teen die chlorokinesensitiewe stam van *Plasmodium falciparum* (D-10) getoets. Die resultate toon dat die antimalaria-aktiwiteit onverwags met die verlenging van die PEG kettinglengte toeneem. Die etoksipoli(etileenglikool)reeks (**6a-8**) het 'n hoër anti-malaria-aktiwiteit getoon as die metoksipoli(etilee glikool reeks (**3-5b**), wat dus toon dat sowel hidrofiliese as lipofiliese eienskappe nodig is vir die verhoging in antimalaria-aktiwiteit. Geeneen van die gesintetiseerde derivate het beter antimalaria-effektiwiteit as artemeter getoon nie. Verbinding (**8**), die 2-[2-(2-etoksi-etoksi)etoksi]etoksiderivaat, het beter antimalaria-aktiwiteit getoon as chlorokien en verbindings (**5a**) en (**6a**) het aktiwiteit vergelykbaar met dié van chlorokien getoon . Verbindings (**3**), (**4**), (**5b**), (**6b**) en (**7**) is minder aktief as artemeter en chlorokien . In alle gevalle was die antimalaria-aktiwiteit van die β-isomere hoër as die van die α-isomere.

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INTRODUCTION AND PROBLEM STATEMENT

1.1 Introduction

Malaria continues to be a major serious health problem and public health threat, with treatment policies that have to be continuously revised and assessed by the World Health Organisation because of the failing therapeutic efficacy of existing anti-malarial drugs (Bosman & Olumese, 2004). The emergence of mono- and multi- drug resistant parasites which render treatment options as ineffective and limited are the direct cause of this problem (Bloland, 2001). This further leads to increased treatment dosages and the escalating prevalence of dose related adverse effects which is very disadvantageous to patient compliance (White, 2004).

Various factors are involved when the increase in malaria manifestations in recent years is discussed. Drug and insecticide resistance, climate stability, global warming, civil disturbances, escalating travel within endemic areas all contribute to increasing transmission rates (Greenwood *et al.,* 2005). The development of other chemotherapeutic anti-malarial drugs with different molecular mechanisms of action from those against which malaria parasites have developed resistance has therefore become a dominant focus area.

The most important reason for treatment failures of malaria is the emergence and spread of chloroquine and multi-drug resistant parasites. The predominance of this phenomenon drastically reduces our options of drugs to implement in treatment regimens. The implementation of artemisinin based combination therapy is recommended as a preventative measure for the emergence of drug resistance by the World Health Organisation (WHO, 2006).

A great need for alternative treatment options of the disease has therefore become prominent and this has encouraged researchers to search for other chemotherapeutic anti-malarial drugs with different molecular mechanisms of action from those against which malaria parasites have developed resistance. Artemisinin has been proven to comply with the previously mentioned qualities.

Artemisinin is a sesquiterpene lactone endoperoxide and the first natural 1,2,4-trioxane isolated from *Artemisia annua.*

This compound is of special biological interest because of its outstanding anti-malarial activity against chloroquine-resistant *P. falciparum* and cerebral malaria, as well as its *in vitro* activity against *Pneumocystis carinii* and *T. gondii* and good *in vitro* anti-neoplastic activity (Jung, 1997). Artemisinin and its derivatives are the only group of compounds that are still effective against multi-drug resistant *Plasmodium falciparum* (Tonmunphean *et al.*, 2006).

However, the use of such endoperoxides is restricted by their poor oral bioavailability, a short plasma half-life (30 minutes in plasma) and the high rate of recrudescent infections when used as monotherapy in short-course treatments, even though these drugs have a rapid onset of action and low reported toxicity (Gutpa *et al.*, 2002).

Therefore it is necessary to produce new derivatives of artemisinin to ultimately develop a compound with a longer plasma half-life, better bioavailability and increased effectiveness.

1.2 Aim and objectives of the study

The primary aim of this study was to synthesise ethylene glycol oligomeric ether derivatives of artemisinin, determine certain physicochemical properties and to evaluate their anti-malarial activity compared to the existing anti-malarial drugs artemether and chloroquine.

In order to achieve this goal, the following objectives were set:

- Synthesise polyethylene glycol (PEG) derivatives of artemisinin and confirm their structures.
- Experimentally determine and evaluate the relevant physicochemical properties such as the aqueous solubility and the partition coefficient for the synthesised artemisinin derivatives and to compare the experimental aqueous solubilities and the partition coefficients of the synthesised derivatives to that of the known anti-malarial artemether.
- Determine whether a relationship exists between the physicochemical properties like the aqueous solubility and partition coefficient of the artemisinin derivatives.
- Evaluate the *in vitro* anti-malarial efficacy of the artemisinin derivatives against the chloroquine sensitive D10 strain of *Plasmodium falciparum* in comparison to that of the reference drugs, artemether and chloroquine.

CHAPTER 2

2 MALARIA AND ANTI-MALARIAL COMPOUNDS

2.1 Malaria

2.1.1 Introduction

Malaria continues to be a major serious health problem and public health threat, with over two billion people at risk of contracting this deadly disease. Malaria is endemic in 92 countries and more than one million deaths per year are attributed to malaria, the mortality in African children being the highest (Breman, 2001). Malaria is by far Africa's most important tropical parasitic disease that kills more people than any other communicable disease, except perhaps tuberculosis and HIV-AIDS (Magardie, 2000). Serious concerns have been raised regarding the remarkably few drugs available for the treatment of malaria, particularly in rural Africa (White, 1992) where drug resistance is a major problem. Due to the emergence and spread of drug resistant parasites, mortality figures have risen in recent years. This poses eminent health and economic problems for populations situated in malaria endemic areas and undisputedly contribute to the worldwide burden of the disease (WHO, 2006). The pharmaceutical industry seeing little profit in a market confined to poor countries, has also abandoned further research concerning anti-malarial drugs (Brown, 1992). Therefore it is necessary to develop a new safe and effective chemotherapeutic anti-malarial drug with a different mechanism of action from those which against malaria parasites have developed resistance. Malaria vaccines have become an area of intensive research, however, there is no effective vaccine that has been introduced into clinical practice. There is one candidate vaccine, RTS,S/AS01, which started Pivotal Phase III evaluation in May 2009 and is designed not for travellers but for children resident in malaria-endemic areas who suffer the burden of disease and death related to malaria (Plassmeyer *et al.*, 2009).

The National Malaria Research Programme of South Africa, under the Medical Research Council (MRC) claims that the increase in malaria manifestations in recent years are caused by factors such as drug and insecticide resistance, drastic climate variations leading to heavy rainfalls in Southern Africa and elsewhere, and population migration (Smith *et al.*, 1977). The high risk groups include pregnant women, non-immune travellers, displaced people and labourers entering the endemic areas (Magardie, 2000).

2.1.2 Malaria in South Africa

South Africa has an estimated population of 49 million people and approximately 10% or roughly 5 million South Africans live in malaria risk areas. Malaria occurs in limited areas in South Africa, mainly in the low altitude (below a 1000 m) areas of the Limpopo province, Mpumalanga province and North-Eastern KwaZulu-Natal as shown in Figure 1.1. Limited focal transmissions may occasionally occur in the North West and Northern Cape provinces along the Molopo and Orange rivers. Malaria is distinctly seasonal in South Africa, with the highest risk being during the wet summer months (October to May) (www.doh.gov).

South Africa's malaria status is currently well within containable boundaries. Control measures, including chemoprophylaxis and treatment regimes, are firmly in place and with the scientific resources and adequate funding available, the South African Department of Health can ensure that this deadly disease will be managed according to international standards (Tren & Bate, 2004).

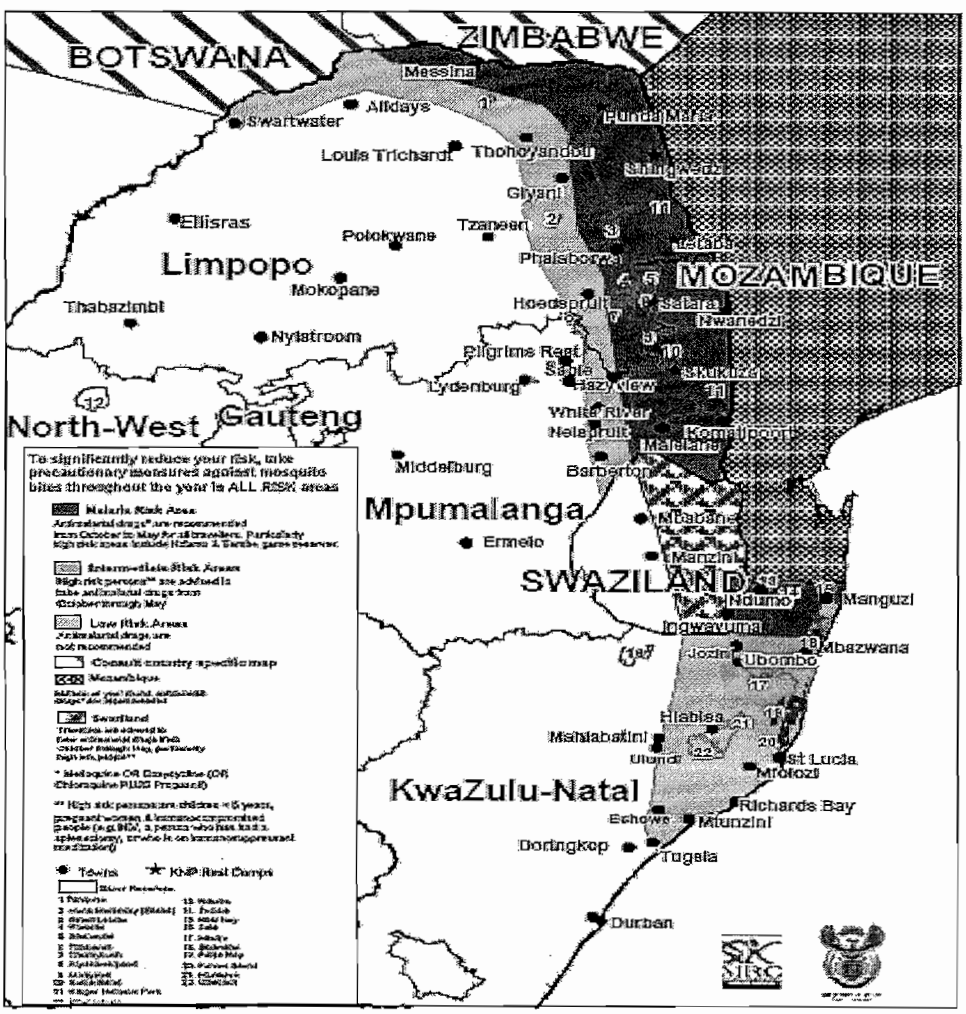


Figure 1.1 Map of malaria areas in South Africa (Malaria in Southern Africa, Updated 2005)

2.1.3 Epidemiology

2.1.3.1 Cause of malaria

Malaria has a probable origin in Africa and malarial parasites from fossils of mosquitoes have been dated back to 30 million years ago (Viswanathan, 1998). These unique protozoan parasites and causative agents of malaria belong to the *Plasmodium* genus consisting of four species of intracellular sporozoans: *P. falciparum, P. malariae, P. ovale and P. vivax. P. falciparum* is the deadliest of all the species because of its widespread resistance to chloroquine and is thus the biggest threat to mankind. Its etiology involves the invasion of the host red blood cells by the parasite (Behere & Goff, 1984).

The female *Anopheles* mosquito hosts the *Plasmodium* parasites and act as the vector, transmitting the protozoan organisms to humans while feeding.

2.1.3.2 Malaria incidence and distribution

The frequency of malaria is subjective to numerous variables. The most important key elements include: climate changes, the presence of humans, female *Anopheles* mosquitoes and malaria parasites. These elements also influence the global disease distribution (CDC, 2004b). Malaria is a worldwide burden and is especially concentrated in the tropical areas of sub-Saharan Africa as shown by Figure 1.2. It is clearly visible that Africa is the continent worst effected by this disease. Areas that are also affected to a slighter degree include: South East Asia, South America, Central America, India and the Pacific Islands (WHO, 2006).

According to the World Malaria Report 2008, 109 countries and territories can be classified as malaria endemic, or previously endemic with the risk of reintroduction. About half the world's population (3.3 billion) live in areas that have some risk of malaria transmission and one fifth (1.2 billion) live in areas with a high risk of malaria. Another 2.1 billion live in areas with a low risk of malaria transmission (WHO, 2006).

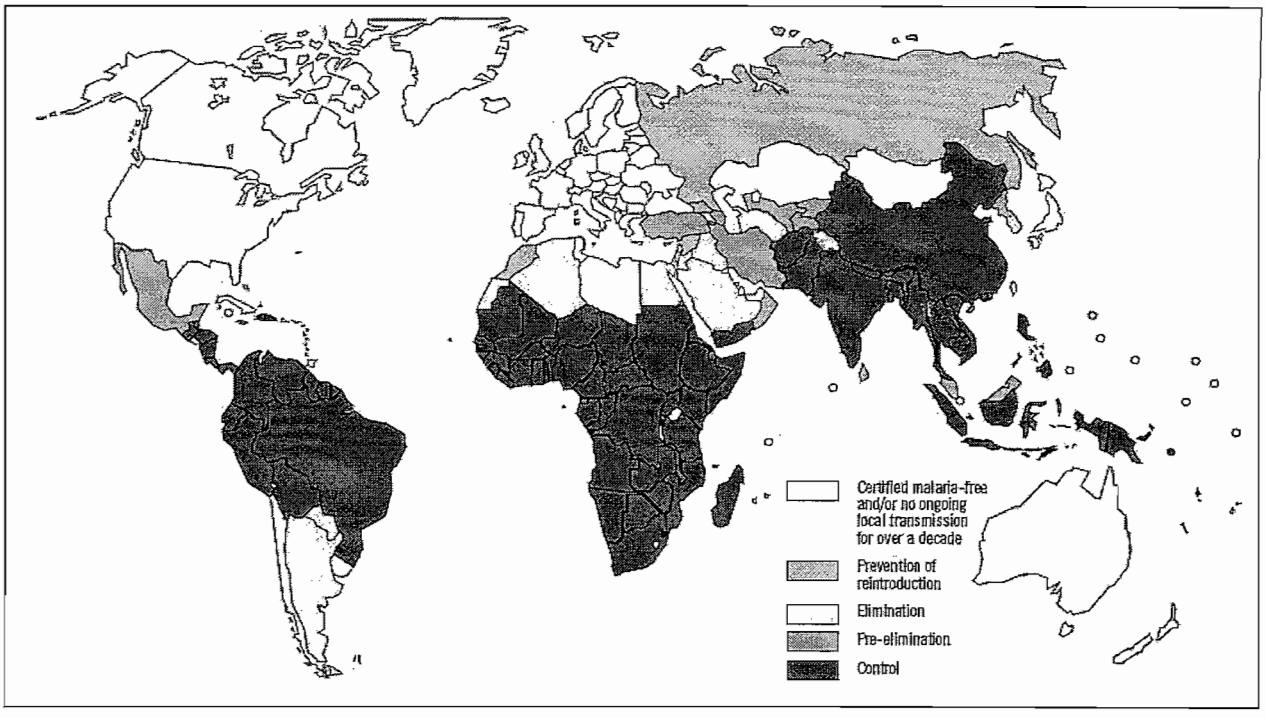


Figure 1.2 Malaria-free countries and malaria-endemic countries in phases of control, pre-elimination, elimination and prevention of reintroduction, end 2007 (World Malaria Report, 2008)

2.1.4 The parasitic lifecycle of *Plasmodium sp.*

The life cycle of *Plasmodium falciparum* is very complex, consisting of two asexual reproduction cycles in man and a sexual reproduction phase in the mosquito. The lifecycle can be categorised into three dominant stages:

1. Exo-erythrocytic schizogony;
2. Erythrocytic schizogony; and
3. Sporogony (Wiser, 2008)

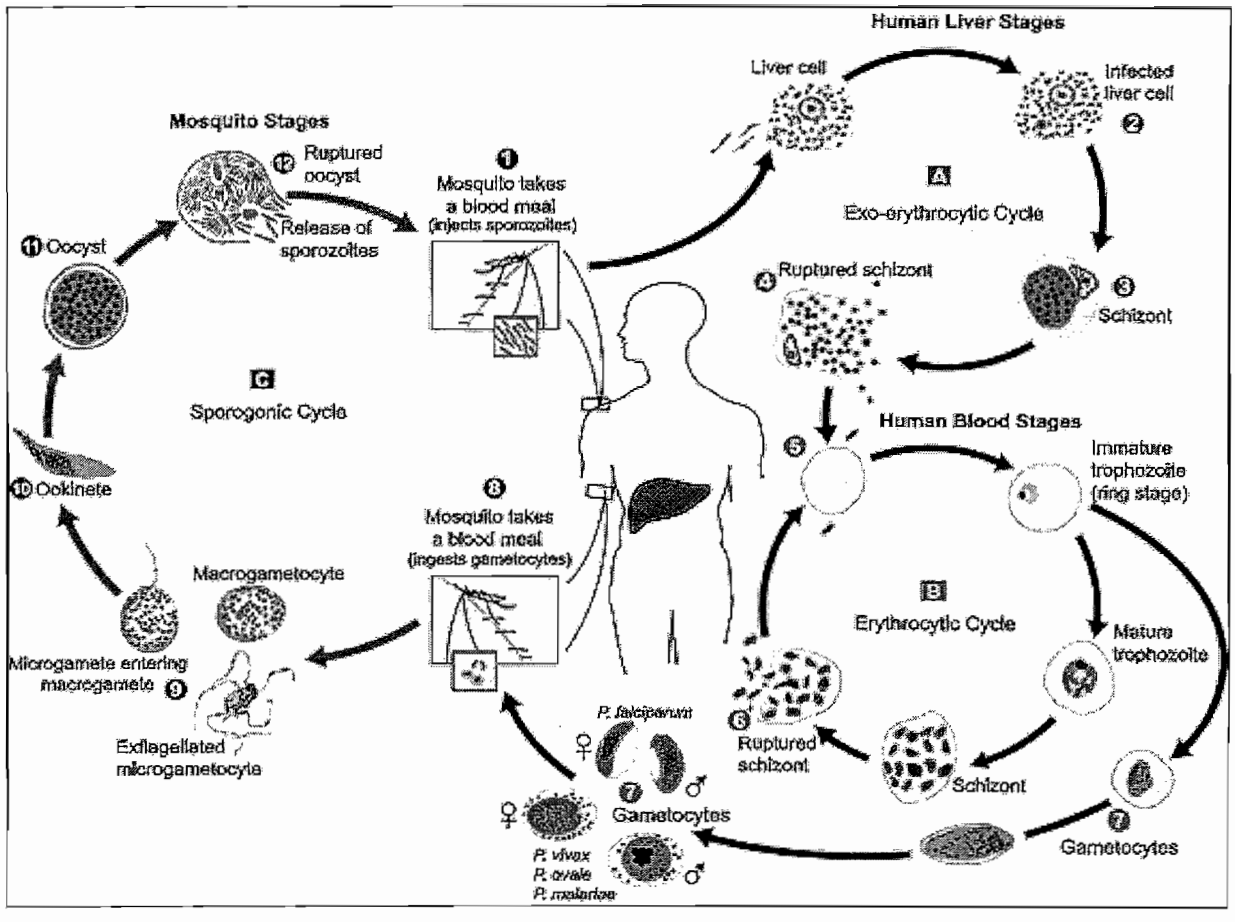


Figure 1.3 A schematic representation of the lifecycle of *Plasmodium sp.* (CDC, 2004a).

2.1.4.1 Pre-erythrocytic schizogony

Sporozoites enter the human bloodstream when the female *Anopheles* mosquito takes its blood meal (1). These sporozoites are immediately transported to the liver through hepatic circulation, where they penetrate hepatocytes (2) and then undergo asexual replication. Inside the liver cells they usually develop into exo-erythrocytic schizonts (3) that can contain thousands of merozoites. The hepatocyte host cells rupture and release these merozoites into the blood circulation were they invade red blood cells (4). Disease only occurs after the parasite leaves the liver and starts to invade and grow inside red blood cells (Wiser, 2008).

2.1.4.2 Erythrocytic schizogony

These merozoites infect the erythrocytes (5) and undergo schizogony which leads to the production of either asexual trophozoites or sexual gametocytes (7) in the blood cells. The asexual trophozoites multiply, eventually causing red blood cells to burst releasing more merozoites (6) into the bloodstream to invade uninfected erythrocytes (Quast, 1999).

When lysis of the erythrocytes occur not only are the merozoites released but antigens and toxins as well, resulting in the intermittent fever paroxysms associated with the clinical symptoms of the disease (Miller *et al.*, 2002). This cycle continuous repetitively and in synchronisation every 48 hours for most *Plasmodium* species (Tuteja, 2007). In contrast to the asexual pathway, the parasites may develop into immature sexual gametocytes (7).

2.1.4.3 Sporogony

The male and female gametocytes are taken up in the blood meal of a mosquito, when feeding on an infected human, (8) and then initiate the stages within the intermediate host. The gametocytes mature to micro- and macro-gametes (9) and the fertilized female macrogamete forms a zygote. The zygote is stimulated to form an ookinete (10) that penetrates the midgut wall of the mosquito, forming an oocyst (11). Within this oocyst reproduction takes place and numerous sporozoites form. When the oocyst reaches maturity it bursts, releasing the sporozoites (12), which migrate to the mosquito's salivary glands. From there they can enter the bloodstream of a new host, completing the lifecycle of the parasite (Wiser, 2008).

2.1.5 The Pathology of *Plasmodium falciparum*

During the lifecycle of the parasite, the molecular and cellular events influence the severity of the disease. All human *Plasmodium sp.* invade the bloodstream following the same mechanism, but *P. falciparum* reaches high parasitaemia when invading red blood cells because of greater flexibility in the receptor pathways it uses. *P. falciparum* infected blood cells must bind to placenta or endothelium for the parasite to avoid spleen-dependent killing mechanisms, but this binding also causes much of the pathology (Miller *et al.*, 2002).

The surface membrane of the infected erythrocyte becomes "sticky" in *P. falciparum* malaria, and can adhere to the surface epithelium of blood vessels of the internal organs like the brain, heart, lung, liver, kidney, placenta and subcutaneous tissues. The syncytiotrophoblasts in the placenta and the various endothelial cells in these organs express different and variable amounts of host receptors.

The variant antigen family of *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1) is central to host-parasite pathogenesis and interaction. Mature red blood cells infected with *P.falciparum* express PfEMP1 on the surface of the cells and can bind to many host receptors through its multiple adhesion domains.

The properties of PfEMP1 such as, antigenic variation for evading antibody-dependent killing and sequestration for evading spleen-dependent killing, contribute to the pathogenesis and virulence of *Plasmodium falciparum* and are essential for the survival of the parasite. Simultaneous binding to multiple receptors, binding of uninfected red blood cells and clumping of infected red blood cells are associated with the pathogenesis of malaria. The binding of parasite-infected erythrocytes to dendritic cells down regulates the host's immune response.

2.1.6 Symptoms and manifestations of malaria

In the early stages of malaria the symptoms are characteristically similar to flu and can be similar to symptoms of many other illnesses caused by parasitic, bacterial, or viral infections. The symptoms and manifestations of malaria can present as periodic fever paroxysms that occur in 48 or 72 hour intervals.

The severity of these paroxysms depends on various factors including the type of *Plasmodium* species causing the disease and the immunity level and general health of the infected individual. Malaria can be classified as uncomplicated or severe. The paroxysms can be categorised into three different stages as shown in Table 1.1. These symptoms are generally associated with uncomplicated malaria (Wiser, 2008).

Table 1.1 The three stages of malaria paroxysm symptoms (Wiser, 2008).

Stage	Symptoms
Cold stage	• Experiencing an intense cold sensation • Extreme shivering • Elevated body temperature • Lasts between 15 to 60 minutes
Hot stage	• Experiencing an intense hot sensation • Elevated body temperature • Severe headache, nausea, fatigue, dizziness, anorexia, myalgia • Lasts between 2 to 6 hours
Sweating stage	• Profuse sweating • Abating body temperature • Exhaustion and fatigue • Lasts between 4 to 6 hours

The symptoms may be visible in cycles and appear for different lengths of time and at different intensities. However, the symptoms may not follow this characteristic cyclic pattern, especially in the early stages of the illness. Severe malaria generates more complicated manifestations and it occurs in 90% of all *P. falciparum* infections. In most cases it is life threatening (Goldsmith, 1998b). Two distinctive features of severe malaria are cerebral malaria and severe anaemia.

Other important presentations include the following:

- Respiratory distress;
- Renal failure;
- Hypoglycaemia;
- Circulatory collapse;
- Coagulation failure; and
- Impaired consciousness (Pasvol, 2005)

2.1.7 Malaria diagnosis

Diagnosing malaria as quickly as possible is an integral part of efficiently treating the disease. Symptoms associated with uncomplicated malaria are not specific and can easily be confused with other illnesses caused by parasitic, bacterial, or viral infections. Therefore a sound diagnostic opinion should also be based on laboratory testing and not only on a physical analysis. Various methods have been developed to aid the process (CDC, 2004b).

2.1.7.1 Microscopy

This method of laboratory testing is still considered to be the "gold standard" for laboratory confirmation of the disease. Various thick and thin Giemsa stained blood smears are made and examined under a light microscope. Thick smears allow for the confirmation of parasites present and thin smears for specie identification and parasitaemia quantification (Gkrania-Klotsas & Lever, 2007; Basco, 2007).

2.1.7.2 Antigen detection methods

These methods were first and foremost designed to be used in the field and to produce fast results where microscopic methods are not available. Antigen detection tests detect antigens such as histidine rich protein-2 (HRP-2) present only in *P. falciparum* infections or parasite lactate-dehydrogenase (pLDH) found in infections caused by all four *Plasmodium* species (Gkrania-Klotsas & Lever, 2007; WHO, 2004).

2.1.7.3 Molecular diagnosis

A molecular diagnosis is based on polymerase chain reaction (PCR) techniques. These techniques identify *Plasmodium* DNA, mRNA and small subunit rRNA and can be used for diagnostic purposes or treatment follow-up evaluations (Gkrania-Klotsas & Lever, 2007).

2.1.8 Malaria control strategies

Malaria control strategies are a complex chain of measures and consist of various approaches to contain the disease. Optimum results for containing malaria will be achieved if all approaches are implemented concurrently.

2.1.8.1 Vector control

Vector control can be achieved by either (i) reducing vector density by implementing biological system modifications to control problematic populations, (ii) interrupting the lifecycle of the mosquito to completely eradicate mosquito populations by organisms feeding on mosquito larvae, destroying breeding sites or (iii) creating a barricade between the human host and the mosquito thus preventing the mosquito from feeding by the usage of insecticide treated bed-nets, indoor residual spraying of insecticides, repellents and wearing protective clothing (Tripathi *et al.*, 2005).

2.1.8.2 Chemoprophylaxis

Chemoprophylactic agents can be categorised according to two mechanisms of action, inhibiting the asexual blood stage development and inhibiting the development of parasites in the exo-erythrocytic stage in the liver (Ashley *et al.*, 2006). Before prescribing malaria chemoprophylactic agents a number of factors need to be taken into consideration including the patient's medical history, drug safety and tolerability, drug efficacy due to patterns of parasite drug resistance and the level of malaria endemicity of the travel destination. The prescriber should also inform the patient that even if the medication is administered correctly, chemoprophylaxis only provides 75% to 95% protection (Checkley & Hill, 2007).

2.2 Malaria treatment

2.2.1 Classification of anti-malarial compounds

Anti-malarial drugs can be classified according to their selective actions on different stages of the malaria parasite's life cycle (Figure 1.1).

Four basic categories exist:

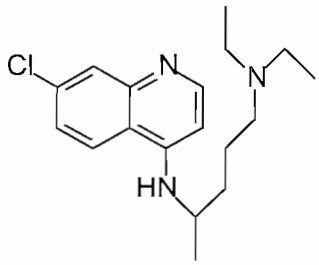
- *Tissue schizontocides:* Anti-malarial agents that prevent invasion of malaria parasites into red blood cells in the exo-erythrocytic stage by eliminating developing tissue schizonts or latent hypnozoites in the liver.
- *Blood schizontocides:* Anti-malarial agents that act on blood schizonts by eliminating parasites in the human red blood cells during the erythrocytic stage.
- *Gametocytocides:* Anti-malarial agents that prevent infection in mosquitoes by eliminating sexual forms of the parasite in hepatic circulation.
- *Sporontocides:* Anti-malarial agents that render gametocytes non-infective in the mosquito. (Sweetman, 2002; Goldsmith, 1998a)

2.2.2 Anti-malarial compounds

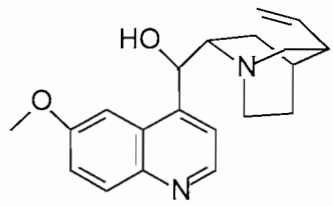
2.2.2.1 4-Aminoquinolines

The 4-aminoquinolines such as chloroquine (1) and amodiaquine are rapidly acting blood schizontozide with gametocytocidal activity and is proven highly effective, but controversy exists about their action mechanism. One hypothesis is that resistant strains of *P. falciparum* are able to efflux chloroquine by an active pump mechanism that releases the drug 40 times faster than sensitive strains, thereby causing the drug to be ineffective. Chloroquine resistance is maintained throughout the whole lifecycle and is then transferred to the progeny. Cross-resistance exists with other 4-aminoquinolines and mepacrine, but not with quinine (2), mefloquine (3), proguanil or pyrimethamine (6).

Chloroquine resistance has brought the focus on quinine back. This particular drug still remains very effective even after extensive use and reports of drug resistance are rare, but cases have been reported from East Africa and Thailand. Quinine is a naturally occurring compound with a narrow therapeutic range and relatively low potency, but the efficacy of this anti-malarial can be improved by combining it with tetracycline. However, poor compliance is a great drawback of this drug.



(1)

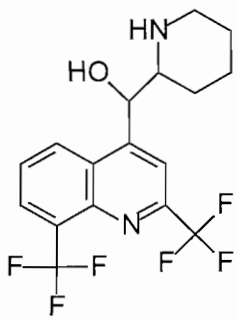


(2)

Figure 1.4 Chloroquine (1) Quinine (2).

2.2.2.2 4-Methanolquinolines

The 4-methanolquinoline derivatives such as mefloquine (3) and cinchona alkaloids are rapidly acting blood schizontocides. This anti-malaria drug is structurally related to quinine (2) and cross-resistance with quinine is common. The emergence of drug resistance is reduced when mefloquine is combined with sulphadoxine/pyrimethamine. It has been suggested that it should always be used in combination with other anti-malarials to prevent development of resistance to this drug. This compound was introduced for routine use in 1985.

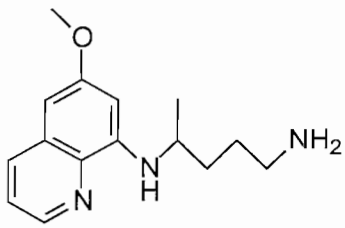


(3)

Figure 1.5 Mefloquine (3).

2.2.2.3 8-Aminoquinolines

The 8-aminoquinolines such as primaquine (4) is the only effective drug against the pre-erythrocytic stages (hypnozoites) of malaria, which is not eradicated by any of the other drugs mentioned above and is highly gametocidal (Baird, 1995). The 8-aminoquinolines are primarily used as tissue schizontocides to prevent relapses of the *ovale* and *vivax* malarias.

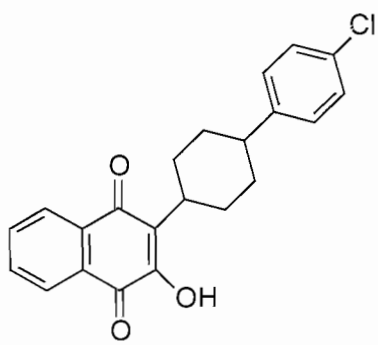


(4)

Figure 1.6 Primaquine (4)

2.2.2.4 Naphthalenes

The naphthalenes such as atovaquone (5) have weak anti-malarial activity and parasitaemia reoccurs in one-third of patients with *P. falciparum* when this drug is used as monotherapy. Atovaquone is thus combined with proguanil. Atovaquone-proguanil might be unaffordable for most African nations because it is expensive to produce (Goldsmith, 1998b).



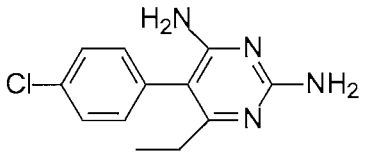
(5)

Figure 1.7 Atovaquone (5)

2.2.2.5 Diaminopyrimidines and biguanides

The diaminopyrimidines such as pyrimethamine (antifolate) (6) and the biguanides such as proguanil (PABA blocker) (7) and chlorproguanil (8), have dihydrofolate reductase inhibitory activity (Gutteridge & Trigg, 1971). The biguanides act as tissue schizontocides mainly for the prophylaxis of *falciparum* malaria. Pyrimethamine and proguanil are also slow acting blood schizontocides. They act on both the phase of development in the mosquito, as well as the pre-erythrocytic and erythrocytic stages of the parasite in the host (White, 1988). Over the past 30 years resistance to these drugs has developed and is now widespread. Resistance develops very quickly and remains stable due to a single point mutation.

The mechanism of resistance involves increased synthesis of blocked enzymes, increase in drug inactivating enzymes, modification of drug transport systems and the use of alternative pathways.



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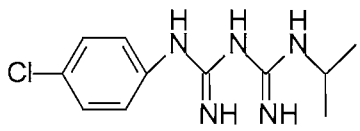

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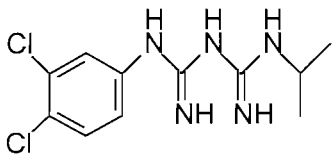


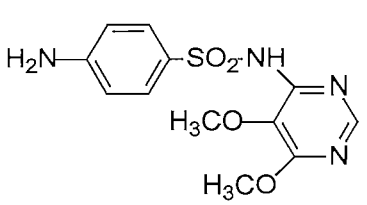
(8)

Figure 1.8 Pyrimethamine (6) Proguanil (7) Chlorproguanil (8)

2.2.2.6 Sulphonamides and sulphones

The sulphonamides such as sulphadoxine (9) and sulphones such as dapsone (10) are dihydropteroate and folate synthesis inhibitors and have blood schizontocidal action. Sulphadoxine and dapsone have been commonly used in combination with pyrimethamine (6). The combination shows synergy through sequential blockage of folic acid synthesis (White, 1988).

Sulphones are chemical analogues of *p*-aminobenzoic acid (PABA), an essential precursor for the synthesis of folic acid (Milhous *et al.*, 1985). The most serious problem with these drugs is with hypersensitivity to the sulpha component, involving skin and mucous membranes (Winstanley, 2000).



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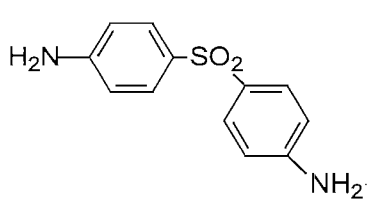
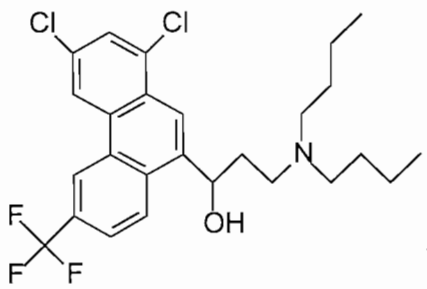


(10)

Figure 1.9 Sulphadoxine (9) Dapsone (10)

2.2.2.7 9-Phenanthrenemethanols

The 9-phenanthrenemethanols such as halofantrine (11) are blood schizontocides and expensive drugs without a parental formulation. Prolongation of the QTc interval and rare cases of fatal ventricular dysrhythmias have been reported (Malvy *et al.*, 2000)



(11)

Figure 1.10 Halofantrine (11)

2.2.2.8 Sesquiterpene lactones

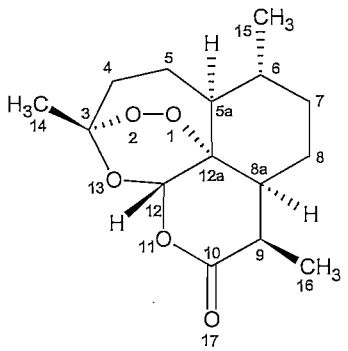
2.2.2.8.1 History of clinical use

Artemisinin was developed from an ancient Chinese herbal remedy, *Artemisia annua* (sweet wormwood or qinghao) and was used by Chinese herbal medicine practitioners for 2000 years. In 1967, a series of traditional remedies was screened by Chinese scientists for drug activities, and it was found that extracts of qinghao had potent anti-malarial activity. In 1972, the active ingredient of qinghao was extracted and purified and later named artemisinin (Meshnick, 2002). Artemisinin derivates were widely used in China by the 1980s and Western interest in these agents began to grow as multi-drug resistant strains of *Plasmodium falciparum* began to spread. Several artemisinin derivates are now being developed by Western pharmaceutical companies.

2.2.2.8.2 Chemical structures of artemisinin and its derivates

Artemisinin is a sesquiterpene lactone endoperoxide and the first natural 1,2,4-trioxane isolated from *Artemisia annua.* This compound is of special biological interest because of its outstanding anti-malarial activity against chloroquine-resistant *P. falciparum* and cerebral malaria, as well as its *in vitro* activity against *Pneumocystis carinii* and *T. gondii* and good *in vitro* anti-neoplastic activity (Jung, 1997).

Artemisinin and its derivates are the only group of compounds that are still effective against multi-drug resistant *Plasmodium falciparum* (Tonmunphean *et al.*, 2006). The reason for this is because of their unusual chemical structures and the difference in their mechanism of action compared to other anti-malarials.



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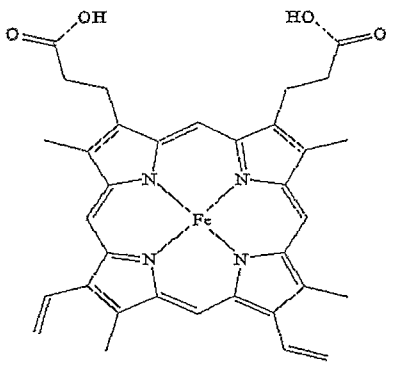


(13)

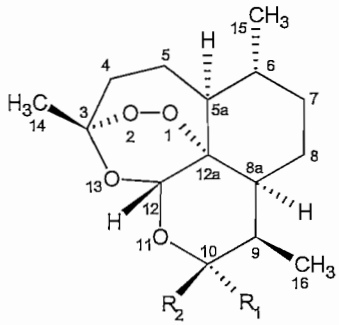
Figure 1.11 Artemisinin (12) Heme molecule (13)

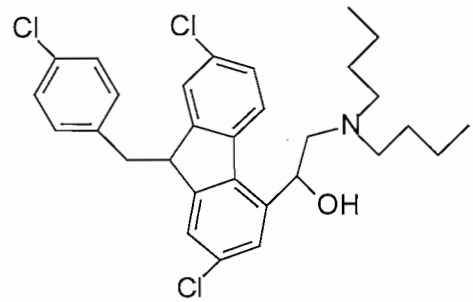
2.2.2.8.3 Mode of action

The mechanism of action of artemisinin is still not conclusive, but strong evidence suggests that the endoperoxide bridge of artemisinin and a heme iron play critical roles in the action mechanism (Tonmunphean *et al.*, 2006). The reaction mechanism consists of two distinct steps, the first step an activation step and the second step an alkylation step. During the activation step, the heme iron breaks the endoperoxide linkage of artemisinin and an oxygen free radical is produced, which is subsequently rearranged to form a carbon-centred (C4) free radical (Meshnick, 2002).

In the alkylation step, the carbon free radical alkylates specific malarial proteins, which causes a lethal damage to malarial parasites (Tonmunphean *et al.*, 2006).However, the use of such endoperoxides is restricted by their poor oral bioavailability, a short plasma half-life (30 minutes in plasma) and the high rate of recrudescent infections when used as monotherapy in short-course treatments, even if these drugs have a rapid onset of action and low reported toxicity (Gupta *et al.*, 2002). Therefore it is necessary to produce new compounds containing artemisinin to ultimately develop a compound with a longer plasma half-life, better bioavailability and increased effectiveness.

Artemisinin (12) is a pharmacologically active molecule and multiple derivates have been synthesized from dihydroartemisinin (17), such as arteether (14), artemether (15) and sodium artesunate (16) currently in use. Artemether and lumefantrine (18) (Coartem®) is currently in use against *P. falciparum* due to the development of resistance against pyrimethamine / sulphadoxine.





Arteether (14): R_1 = H, R_2 = OEt (18)

Artemether (15): R_1 = H, R_2 = OMe

Sodium artesunate (16): R_1 = H, R_2 = $OCO(CH_2)_2CO_2Na$

Dihydroartemisinin (17): R_1 = H, R_2 = OH

Figure 1.12 Artemisinin derivates (14-17) and Lumefantrine (18)

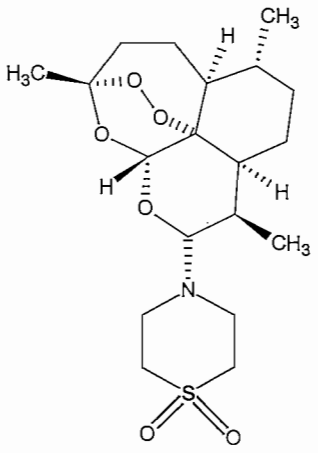
2.2.2.8.4 Artemisinin derivatives

A program aiming at modifying the chemical structure of artemisinin was launched in 1976, which resulted in a number of new analogues with improved efficacy and increased solubility: oil-soluble artemether (**2**) and arteether (Tongyin & Ruchang, 1985) and water-soluble sodium artesunate (Yang *et al.*, 1982). Artemether is the methyl ether derivative of artemisinin and arteether, the ethyl ether derivative, these compounds are lipophilic, more potent than artemisinin but still have a short plasma half-life. Artemisinin, dihydroartemisinin (**1**), artemether and arteether are all poorly water-soluble compounds, which results in slower and incomplete absorption of these drugs into the systemic circulation, sodium artesunate is much more hydrophilic which leads to better absorption (Ilett & Batty, 2004). However, the usefulness of sodium artesunate in the treatment of cerebral malaria and multi-drug resistant P. falciparum is offset by problems associated with its instability in aqueous solution (Lin *et al.*, 1989), the high rate of recrudescence and the drug's extremely short plasma half-life (Lin *et al.*, 1997).

2.2.2.8.5 Recent malaria research - Artemisone

The pharmaceutical company Bayer Healthcare in collaboration with the Hong Kong University of Science and Technology initiated a program to develop artemisinin derivatives with improved efficacy, stability, pharmacokinetic behaviour and reduced neurotoxicity. Artemisone (19) was selected as the lead candidate of various second-generation semi-synthetic artemisinin derivatives because of its increased anti-malarial efficacy, lack of neurotoxicity and comparably low costs of production (Ramharter *et al.*, 2006).

Artemisone is a new semi-synthetic 10-alkylaminoartemisinin that can be synthesised from dihydroartemisinin in a one-step process (Haynes *et al.*, 2004) Artemisone shows increased anti-plasmodial activity, improved metabolic stability and oral bioavailability, improved *in vivo* half-life and no neurotoxicity (Haynes *et al.*, 2006). This artemisinin derivative is also well tolerated in humans (Vivas *et al.*, 2007) with a curative effect at dose levels at least half those of artesunate in Phase IIa clinical trials in comparison with artesunate (Krudsood *et al.*, 2005). Biological studies confirm the increased efficacy of artemisone over artesunate against multi-drug resistant *P. falciparum* and provide the basis for the selection of potential partner drugs for future deployment as new artemisinin combination therapies (Vivas *et al.*, 2007).



(19)

Figure 1.13 Artemisone (19)

2.2.2.9 Malaria vaccine candidate RTS,S

The malaria vaccine candidate RTS,S (GlaxoSmithKline, Rixensart, Belgium), formulated with the adjuvant system AS02 or AS01, specifically targets the pre-erythrocytic stage of *Plasmodium falciparum*, and has been shown to confer complete or partial protection against experimental infection (Kester *et al.*, 2001; Stoute *et al.*, 1997). The goal is to ultimately register RTS,S for use in infants and children living in *P. falciparum* endemic areas. The plan consists of two main drivers: the need to protect the youngest age groups and to include RTS,S in the Expanded Program of Immunisation (EPI) scheme (Aponte *et al.*, 2007). In most areas with stable malaria transmission, children younger than 2 years have a large and disproportionate incidence of severe disease and death. There is growing recognition that malaria control strategies need to give top priority to protecting infants as soon as possible after birth (Macete *et al.*, 2006). The endemic countries of Sub-Saharan Africa have weak health systems, in this context, the EPI is the best functioning system of regular health contacts with infants, capable of delivering millions of doses across the continent including rural areas (Hutton & Tediosi, 2006).

The RTS,S antigen incorporates part of the pre-erythrocytic circumsporozoite of *P.falciparum*, which includes part of the central tetrapeptide repeat region (R), major T-cell - (T), which is fused to the entire surface antigen (S) of the hepatitis B virus and co-expressed in yeast as a particle with non-fused S antigen. (Heppner *et al.*, 2005).

RTS,S is formulated in AS02 or AS01, proprietary adjuvant systems containing an oil-in-water emulsion and the immune stimulants MPL and QS21 (Kester *et al.*, 2001).This malaria vaccine candidate consistently demonstrates significant protection against infection with *Plasmodium falciparum* malaria and also against clinical malaria and severe disease in children in areas of endemicity (Stewart *et al.*, 2007).

2.2.3 Drug resistance

Resistance to classical and existing anti-malarial drugs is a challenging problem in malaria control all over the world. Drug resistance is the ability of a parasite species to multiply and/or survive despite the administration and absorption of a drug given against the particular parasite. Resistance materialises with evolutionary single or multiple point mutations in the *Plasmodium* genome, rendering parasites that are drug insensitive (Shanks, 2006).

In 1945 chloroquine, a 4-aminoquinoline was introduced and proved very effective in combating malaria. Chloroquine could also be used as a prophylactic drug because of the drugs long half-life; it was also well tolerated and effective against all strains of *Plasmodia*.

However 12 years after its introduction the first cases of chloroquine resistant *Plasmodium falciparum* malaria were reported (Wongsrichanalai *et al.*, 2002). Since then resistance to classical anti-malarial agents such as mefloquine, chloroquine and sulphadoxine/pyrimethamine has spread all over the world, contributing to the emergence of developing new compounds for malaria treatment (WHO, 1999; Winstanley, 2000).

Reasons for the development of drug resistance include drug-use patterns, compound characteristics, human host, parasite, vector and environmental factors (Wongsrichanalai *et al.*, 2002). This has prompted researchers to search for other chemotherapeutic anti-malarial drugs with different molecular mechanisms of action from those against which malaria parasites have developed resistance. Artemisinin based combination therapy is currently the treatment of choice for drug resistant malaria and it is of great importance that the efficacy of this therapeutic regimen is maintained because no other effective alternate exist to surmount this hurdle (Wongsrichanalai *et al.*, 2002).

CHAPTER 3

ARTICLE FOR SUBMISSION

Chapter 3 contains the manuscript of an article to be submitted to Bioorganic and Medicinal Chemistry Letters. The article contains the background, aims, all the experimental details and results of this study, including the physicochemical properties and *in vitro* biological results of the artemisinin derivatives. The article is prepared according to the Guide for Authors that can be found on the website of this journal (http://www.elsevier.com/wps/find/journaldescription.authors/972/authorinstructions.), except that for easy reading figures, schemes and tables are inserted at their logical places as they would appear in the printed version.

Synthesis and anti-malarial activity of ethylene glycol oligomeric ethers of artemisinin

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Abstract

The objective of this study was to synthesise derivatives of the anti-malarial drug artemisinin with more favourable physicochemical properties for systemic delivery in an effort to increase the anti-malarial efficacy. Artemisinin derivatives (**3-8**) more water-soluble than artemether were synthesised as new potential anti-malarial agents. The synthesis, aqueous solubility, log P values and anti-malarial activity of a series of polyethylene glycol (PEG) ethers of artemisinin are reported. The ethers were synthesised in a one-step process by coupling the PEG moiety of various chain lengths to C-10 of dihydroartemisinin. The aqueous solubility of all compounds increased as the ethylene oxide chain lengthened while the log P values decreased. The new derivatives were tested *in vitro* against the D10 strain of *Plasmodium falciparum*. Compound (**8**) was the most active of the series, IC_{50} = 14.90 ng/ml, making it more potent than chloroquine. Thus, the physicochemical properties of all the synthesised compounds were improved, and the anti-malarial efficacy of compound (8) is higher than that of chloroquine.

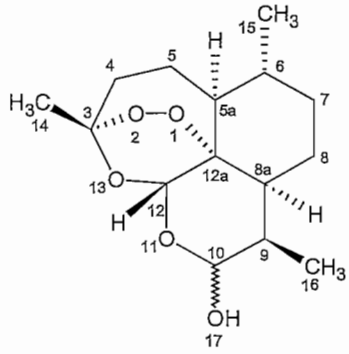
Keywords: Artemisinin, malaria, *Plasmodium falciparum*, polyethylene glycol (PEG)

1. Introduction

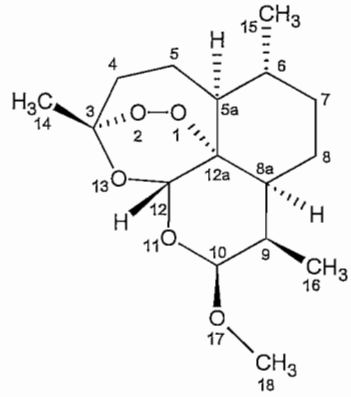
Malaria remains a major cause of morbidity and death in tropical countries all over the world and a substantial number of people are exposed to the risk of contracting this deadly disease each year. The relentless increase in resistance of malaria parasites to existing and classical anti-malarial drugs, such as chloroquine, sulphadoxine/pyrimethamine and mefloquine, caused researchers to search for other chemotherapeutic anti-malarial drugs with different molecular mechanisms of action from those which malaria parasites have developed resistance (Davis *et al.*, 2005).

The artemisinin group of drugs was first discovered and developed in China. A crude extract of the wormwood plant *Artemisia annua* (qinghao) was used as an anti-pyretic approximately 2000 years ago, and its specific effect on the fever of malaria was reported in the 16th century (Klayman, 1985). The active constituent of the extract was identified and purified in the 1970s, and was known as qinghaosu or artemisinin. Artemisinin proved effective in clinical trials in the 1980s, but a number of semi-synthetic derivatives were developed to improve the drug's pharmacological properties and anti-malarial potency (Hien & White, 1993). Several million patients have been treated with these compounds during the past three decades, due to the increasing prevalence of multi-drug resistant *Plasmodium falciparum* (De Vries & Dien, 1996).

Artemisinin and its derivatives showed a rapid onset of action, low toxicity and high anti-malarial activity against both drug-resistant and drug-sensitive malaria, in early clinical studies (China Cooperative Research Group, 1982b). The practical values of these anti-malarial agents, nevertheless, are impaired by their poor solubility in oil and water, poor oral bioavailability, high rate of parasite recrudescence after treatment (China Cooperative Research Group, 1982a) and short plasma half-life (Lee & Hufford, 1990).



(**1**)



(**2**)

Figure 1. Dihydroartemisinin (**1**) and artemether (**2**)

In order to overcome these pharmacokinetic deficiencies, a program aiming at modifying the chemical structure of artemisinin was launched in 1976, which resulted in a number of new analogues with improved efficacy and increased solubility: oil-soluble artemether (**2**) and arteether (Tongyin & Ruchang, 1985) and water-soluble sodium artesunate (Yang *et al.*, 1982). Artemether is the methyl ether derivative of artemisinin and arteether the ethyl ether derivative; these compounds are lipophilic and more potent than artemisinin, but still have a short plasma half-life. Artemisinin, dihydroartemisinin (**1**), artemether and arteether are all poorly water-soluble compounds, which results in slower and incomplete absorption of these drugs into the systemic circulation; sodium artesunate is much more hydrophilic which leads to better absorption (Ilett & Batty, 2004). However, the usefulness of sodium artesunate in the treatment of cerebral malaria and multi-drug resistant *P. falciparum* is offset by problems associated with its instability in aqueous solution (Lin *et al.*, 1989), the high rate of recrudescence and the drug's extremely short plasma half-life (Lin *et al.*, 1997).

In the search for stable, more water-soluble, high potency, long acting and orally active anti-malarial agents, we modified the artemisinin molecule by introducing polyethylene glycol groups (PEGs) at the C10-position of artemisinin. PEGs are amphiphilic and relatively inert polymers consisting of repetitive units of ethylene oxide (Hamidi *et al.*, 2006). Pegylation, generally described as the molecular attachment of polyethylene glycols with different molecular weights to active drug molecules, is one of the most promising and extensively studied strategies with the goal of improving the pharmacokinetic behaviour of therapeutic drugs (Hamidi *et al.*, 2006). The main pharmacokinetic outcomes of pegylation are summarised as changes occurring in overall circulation life-span, tissue distribution pattern, and elimination pathway of the parent drug (Nucci, 1991). The attachment of a PEG moiety to drug molecules increases the overall size of the parent drug and the circulation half-life of PEGs increase with the increase in molecular weight (Bailon *et al.*, 1999). Many studies indicated a dramatic enhancement in the biological half-life of particular drug molecules as a result of pegylation (Koumenis *et al.*, 2000). Pegylated drugs are also more stable over a range of pH and temperature changes (Monfardini *et al.*, 1995) compared with their unpegylated counterparts. Consequently, pegylation confers on drugs a number of properties that are likely to result in a number of clinical benefits, including sustained blood levels that enhance effectiveness, fewer adverse reactions, longer shelf-life and improved patient convenience (Kozlowski *et al.,* 2001).

The aim of this study was to synthesise ethylene glycol oligomeric ethers of artemisinin, determine certain relevant physicochemical properties and evaluate their anti-malarial activity compared to that of artemether and chloroquine.

2. Materials and methods

2.1. Materials

2-(2-methoxyethoxy) ethan-1-ol, 2-[2-(2-methoxyethoxy)ethoxy] ethan-1-ol, 2-[2-(2-ethoxyethoxy)ethoxy] ethan-1-ol and boron trifluoride diethyl etherate were all purchased from Fluka South Africa, Ltd. 2-methoxyethan-1-ol and 2-(2-ethoxyethoxy) ethan-1-ol were purchased from Acros Organics, Ltd. 2-ethoxyethan-1-ol was purchased from BDH Chemicals, Ltd. Dihydroartemisinin was purchased from HuBei Enshi TianRanYuan Science and Technology Herbal Co., Ltd. HPLC grade acetonitrile was obtained from Labchem South Africa, Ltd. All the reagents and chemicals were of analytical grade.

2.2. General procedures

Thin-layer chromatography (TLC) was performed using silica gel plates ($60F_{254}$ Merck). Preparative flash column chromatography was carried out on silica gel (230-240 mesh, G60 Merck) and silica gel 60 (70-230 mesh ASTM, Fluka). Analytical quantities of samples were weighed on a Sartorius/BP211D balance (capacity, resolution: 210 g, 0.0001; and 80 g, 0.00001).

The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance III 600 spectrometer (at a frequency of 600.17 and 150.913 MHz, respectively) in deuterated chloroform ($CDCl_3$). Chemical shifts are reported in parts per million (δ ppm) using tetramethylsilane (TMS) as internal standard. The splitting pattern abbreviations are as follows: s (singlet), d (doublet), dd (doublet of doublets), ddd (doublet of doublet of doublets), dt (doublet of triplets), t (triplet), td (triplet of doublets), tt (triplet of triplet) and m (multiplet).

The low resolution FAB (Fast Atom Bombardment) mass spectra (MS) were recorded on a VG70SE mass spectrometer using a xenon atom beam at 8kV and a 3-nba matrix in all cases. Positive ions ($M+H^+$) and ($M+Na^+$) were recorded.

2.3. High performance liquid chromatography (HPLC)

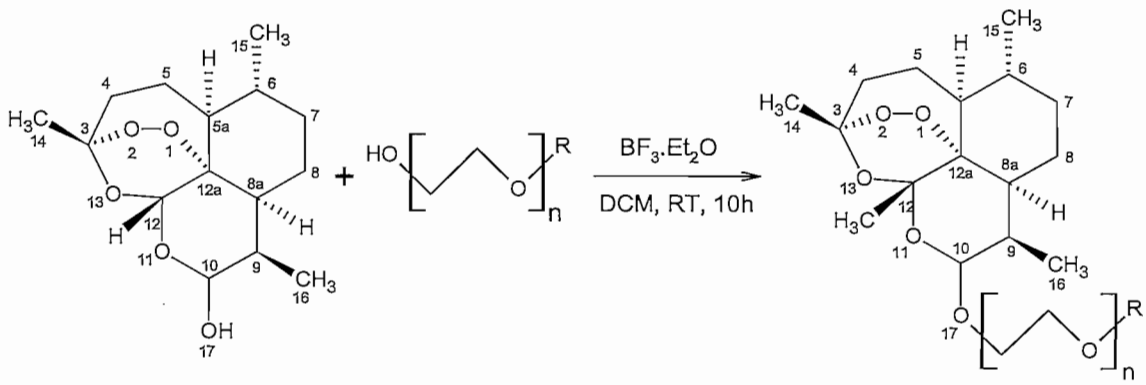
The HPLC system consisted of an Agilent 1100 series auto sampler, Agilent 1100 series variable wave detector (VWD) and Agilent 1100 series isocratic pump. A Zorbax Eclipse XDB C18, 5 µm (150 x 4.60 mm) column was used and the Agilent Chemstation rev A08.03 for LC systems software package for data analysis.

The compounds were quantified using a gradient method (A = 0.2 % triethylamine in H_2O, pH 7.0, B = acetonitrile) at a flow rate of 1 ml/min with 20 µl standard sample injections. The gradient consisted of 25 % of solvent B (ACN) until 1 min, then increased linearly to 95 % of B after 8 min, and held for 15 min, where after the instrument was re-equilibrated to the starting conditions for 5 min. A calibration plot of peak area versus drug concentration for each compound showed excellent linearity ($0.993 < r^2 \leq 1$) over the concentration range (0–2000 µg/ml) employed for the assays. The absorption maximum for dihydroartemisinin and all its derivatives was at 205 nm; this wavelength was consequently used for the HPLC detection. New mobile phase was prepared for each sample batch that was analysed by HPLC. The peak retention times (t_R) were 10.17 min for (**2**), 9.86 min for (**3**), 9.78 min for (**4**), 9.57 min for (**5a**), 9.57 min for (**5b**), 10.42 min for (**6a**), 9.84 min for (**6b**), 10.34 min for (**7**) and 10.12 min for (**8**).

3. Experimental procedures

*3.1. Synthesis of ethylene glycol oligomeric ethers of artemisinin (**3-8**) (Scheme 1)*

The synthesis of ethylene glycol oligomeric ethers of artemisinin (Scheme 1) was achieved by using with slight modifications the general method reported by Li *et* al (2000) and is described as follows: to a solution of dihydroartemisinin, (**1**) (2.0 g, 7 mmol) and an methoxypoly(ethylene glycol) (MPEG) (**3-5b**) or ethoxypoly(ethylene glycol) (EPEG) (**6a-8**) (14 mmol, 2.0 equiv relative to dihydroartemisinin) dissolved in 50 ml of anhydrous dichloromethane (DCM, CH_2Cl_2) was added boron trifluoride diethyl etherate ($BF_3.Et_2O$) (1.0 ml) portion wise at 0 °C. The mixture was stirred at 0 °C for 0.5 h, then at room temperature for 10 h. The progress of the reaction was monitored by thin layer chromatography (TLC). After completion, the reaction mixture was washed sùccessively with a saturated $NaHCO_3$ solution, water and brine. The organic layer was dried over $MgSO_4$ and evaporated to dryness under reduced pressure. The resultant oil was purified by flash chromatography eluting with DCM:EtOAc (ratios as described below) as mobile phase. All the synthesised compounds were oils, and failed to crystallise. ^1H and ^{13}C NMR chemical shifts as well as FAB-MS data of compounds (**3-8**) (Scheme 1) are reported.



(**1**) (**3-8**)

Compound	Isomer	R	n
(**3**)	10-β	βCH_3	1
(**4**)	10-β	CH_3	2
(**5a**)	10-β	CH_3	3
(**5b**)	10-α	CH_3	3

(**6a**)	10-β	CH_2-CH_3	1
(**6b**)	10-α	CH_2-CH_3	1
(**7**)	10-β	CH_2-CH_3	2
(**8**)	10-β	CH_2-CH_3	3

Scheme 1. Synthesis of ethylene glycol oligomeric ethers of artemisinin

3.1.1. 2-methoxyethoxy-10β-dihydroartemisinin (**3**) *(R = CH_3, n = 1)*

Ether (**3**) was purified by flash silica gel column chromatography eluting with DCM:EtOAc (20:1) to give a clear oil: 0.91 g (38%) yield. $C_{18}H_{30}O_6$. ^{1}H NMR δ (ppm): (600 MHz, $CDCl_3$) ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.41 (s, 1H, H-12), 4.80 (d, *J* = 4.8 Hz, 1H, H-10), 3.95 – 3.87 (m, 1H, H-18a), 3.58 – 3.46 (m, 3H, H-18b-19), 3.37 – 3.31 (m, 3H, H-21), 2.64 – 2.54 (m, 1H), 2.34 (td, *J* = 14.2, 3.7 Hz, 1H), 2.00 (d, *J* = 14.4 Hz, 1H), 1.88 – 1.57 (m, 5H), 1.51 – 1.38 (m, 4H), 1.35 – 1.15 (m, 3H), 0.95 – 0.82 (m, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 103.04 (C-10), 102.89 (C-3), 88.98 (C-12), 81.60 (C-12a), 71.64 (C-19), 67.53 (C-18), 58.94 (C-21), 51.83 (C5a), 46.52 (C-8a), 39.45 (C-9), 37.16 (C-4), 36.59 (C-7), 34.47 (C-6), 31.48 (C-14), 25.99 (C-5), 24.64 (C-8), 20.11 (C-15), 19.46 (C-16). MS FAB 343.3 (($M+H^+$) 8%), 365.4 (($M+Na^+$) 4%).

3.1.2. 2-(2-methoxyethoxy) ethoxy-10β-dihydroartemisinin (***4***) *(R = CH_3, n = 2)*

Derivative (**4**) was purified by flash silica column chromatography eluting with DCM:EtOAc (15:1) to produce a light yellow oil: 1.28 g (47%) yield. $C_{20}H_{34}O_7$. ^{1}H NMR δ (ppm): (600 MHz, $CDCl_3$) 5.41 (s, 1H, H-12), 4.80 (d, *J* = 2.4 Hz, 1H, H-10), 3.95 – 3.87 (m, 1H, H-18a), 3.58 – 3.46 (m, 7H, H-18b-19-21-22), 3.37 – 3.31 (m, 3H, H-24), 2.64 – 2.54 (m, 1H), 2.34 (td, *J* = 14.2, 3.7 Hz, 1H), 2.00 (d, *J* = 14.4 Hz, 1H), 1.88 – 1.57 (m, 5H), 1.51 – 1.38 (m, 4H), 1.35 – 1.15 (m, 3H), 0.95 – 0.82 (m, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 104.10 (C-10), 101.99 (C-3), 87.91 (C-12), 81.13 (C-12a), 71.95 (C-22), 70.52 (C-19), 70.40 (C-21), 67.39 (C-18), 59.03 (C-24), 52.60 (C-5a), 44.41 (C-8a), 37.36 (C-9), 36.40 (C-4), 34.60 (C-7), 30.82 (C-6), 26.16 (C-14), 24.66 (C-5), 24.48 (C-8), 20.25 (C-15), 12.87 (C-16). MS FAB 387.3 (($M+H^+$) 12%), 409.3 (($M+Na^+$) 11%).

3.1.3. 2-[2-(2-methoxyethoxy)ethoxy] ethoxy-10β-dihydroartemisinin (**5a**) (R = CH_3, n = 3)

A yield of 1.45 g (48%) of yellowish oil was obtained after purification with flash chromatography eluting with DCM:EtOAc (10:1). $C_{22}H_{38}O_8$. ^{1}H NMR δ (ppm): (600 MHz, $CDCl_3$) 5.36 (s, 1H, H-12), 4.76 (d, *J* = 4.3 Hz, 1H, H-10), 3.91 – 3.82 (m, 1H, H-18a), 3.62 – 3.48 (m, 11H, H-18b-19-21-22-24-25), 3.31 (s, 3H, H-27), 2.55 (d, *J* = 3.1 Hz, 1H), 2.29 (td, *J* = 14.1, 3.5 Hz, 1H), 1.96 (d, *J* = 14.4 Hz, 1H), 1.83 – 1.65 (m, 4H), 1.55 (d, *J* = 12.9 Hz, 1H), 1.43 – 1.34 (m, 4H), 1.29 – 1.11 (m, 3H), 0.86 (dd, *J* = 23.3, 6.8 Hz, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 103.96 (C-10), 102.05 (C-3), 87.90 (C-12), 81.12 (C-12a), 71.92 (C-25), 70.68 (C-19), 70.54 (C-21), 70.50 (C-22), 70.25 (C-24), 67.39 (C-18), 58.94 (C-27), 52.60 (C-5a), 44.41 (C-8a), 37.32 (C-9), 36.40 (C-4), 34.61 (C-7), 30.84 (C-6), 26.16 (C-14), 24.66 (C-5), 24.47 (C-8), 20.41 (C-15), 12.87 (C-16). MS FAB 431.4 ((M+H$^+$) 5%), 453.4 ((M+Na$^+$) 25%).

*3.1.4. 2-[2-(2-methoxyethoxy)ethoxy] ethoxy-10α-dihydroartemisinin (**5b**) (R = CH_3, n = 3)*

Ether (**5b**) was purified by flash silica gel column chromatography eluting with DCM:EtOAc (10:1) to give a yellow oil: 0.36 g (12%) yield. $C_{22}H_{38}O_8$. ^{1}H NMR δ (ppm): (600 MHz, $CDCl_3$) 5.26 (s, 1H, H-12), 4.44 (d, *J* = 9.2 Hz, 1H, H-10), 3.97 (t, *J* = 8.7 Hz, 1H, H-18a), 3.59 (dd, *J* = 16.2, 6.2 Hz, 9H, H-18b-19-22-24-25), 3.49 – 3.46 (m, 2H, H-21), 3.31 (s, 3H, H-27), 2.32 (ddd, *J* = 16.7, 13.5, 5.0 Hz, 2H), 1.95 (d, *J* = 14.4 Hz, 1H), 1.80 (d, *J* = 13.6 Hz, 2H), 1.68 (d, *J* = 13.4 Hz, 1H), 1.61 (d, *J* = 13.1 Hz, 1H), 1.50 – 1.35 (m, 5H), 1.27 – 1.17 (m, 3H), 0.95 – 0.81 (m, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 104.22 (C-10), 100.31 (C-3), 91.10 (C-12), 80.40 (C-12a), 71.90 (C-25), 70.86 (C-19), 70.71 (C-21), 70.57 (C-22), 70.42 (C-24), 68.13 (C-18), 58.93 (C-27), 51.53 (C-5a), 45.34 (C-8a), 37.32 (C-9), 36.28 (C-4), 34.28 (C-7), 32.49 (C-6), 26.01 (C-14), 24.67 (C-5), 22.21 (C-8), 20.25 (C-15), 12.55 (C-16). MS FAB 431.1 ((M+H$^+$) 5%), 453.1 ((M+Na$^+$) 11%).

3.1.5. 2-ethoxyethoxy-10β-dihydroartemisinin (**6a**) *(R = CH_2-CH_3, n = 1)*

Compound (**6a**) afforded 0.86 g (35%) yield as a clear oil after purified by flash silica gel column chromatography eluting with DCM:EtOAc (15:1). $C_{19}H_{32}O_6$. ^{1}H NMR δ (ppm): (600 MHz, $CDCl_3$) 5.41 (s, 1H, H-12), 4.93 (d, *J* = 4.5 Hz, 1H, H-10), 3.95 – 3.90 (m, 1H, H-18a), 3.62 – 3.45 (m, 5H, 18b-19-21), 2.29 – 2.20 (m, 1H), 1.99 (dd, *J* = 42.6, 25.3 Hz, 1H), 1.85 – 1.80 (m, 1H), 1.74 – 1.67 (m, 1H), 1.58 – 1.48 (m, 3H), 1.39 – 1.33 (m, 5H), 1.24 – 1.13 (m, 8H), 0.89 (dd, *J* = 21.7, 9.5 Hz, 4H).

^{13}C NMR (151 MHz, $CDCl_3$) δ 102.95 (C-10),102.85 (C-3), 88.80 (C-12), 81.46 (C-12a), 69.45 (C-19), 67.51 (C-21), 66.36 (C-18), 51.80 (C-5a), 46.32 (C-8a), 39.45 (C-9), 37.15 (C-4), 36.39 (C-7), 34.45 (C-6), 31.38 (C-14), 25.82 (C-5), 24.58 (C-8), 19.97 (C-15), 19.40 (C-16), 15.08 (C-22). MS FAB 357.4 (($M+H^+$) 10%), 379.3 (($M+Na^+$) 7%).

*3.1.6. 2-ethoxyethoxy-10α-dihydroartemisinin (**6b**) (R = CH_2-CH_3, n = 1)*

Derivative (**6b**) was purified by flash silica column chromatography eluting with DCM:EtOAc (15:1) to produce a clear oil: 0.17 g (7%) yield. $C_{19}H_{32}O_6$. ^1H NMR δ (ppm): (600 MHz, $CDCl_3$) 5.27 (s, 1H, H-12), 4.45 (d, *J* = 9.3 Hz, 1H, H-10), 3.97 (dd, *J* = 10.7, 4.3 Hz, 1H, H-18a), 3.60 – 3.46 (m, 5H, H-18b-19-21), 2.55 (d, *J* = 3.3 Hz, 1H), 2.33 (ddd, *J* = 28.3, 13.7, 5.7 Hz, 2H), 2.00 – 1.90 (m, 1H), 1.85 – 1.51 (m, 3H), 1.51 – 1.32 (m, 4H), 1.32 – 1.07 (m, 5H), 1.00 – 0.75 (m, 8H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 104.23 (C-10), 100.35 (C-3), 91.16 (C-12), 80.40 (C-12a), 69.95 (C-19), 68.13 (C-21), 66.48 (C-18), 51.52 (C-5a), 45.34 (C-8a), 37.33 (C-9), 36.28 (C-4), 34.28 (C-7), 32.46 (C-6), 26.00 (C-14), 24.67 (C-5), 22.22 (C-8), 20.25 (C-15), 15.11 (C-16), 12.40 (C-22). MS FAB 357.4 (($M+H^+$) 10%), 379.3 (($M+Na^+$) 8%).

*3.1.7. 2-(2-ethoxyethoxy) ethoxy-10β-*dihydroartemisinin (**7**) *(R = CH_2-CH_3, n = 2)*

A yield of 1.34 g (48%) of light yellow oil was obtained after purification with flash chromatography eluting with DCM:EtOAc (10:1). $C_{21}H_{36}O_7$. ^1H NMR δ (ppm): (600 MHz, $CDCl_3$) 5.37 (s, 1H, H-12), 4.76 (d, *J* = 4.6 Hz, 1H, H-10), 3.92 – 3.86 (m, 1H, H-18a), 3.62 – 3.45 (m, 9H, H-18b-19-21-22-24), 2.55 (s, 1H), 2.29 (dd, *J* = 19.4, 8.6 Hz, 1H), 1.96 (d, *J* = 14.5 Hz, 1H), 1.77 (dd, *J* = 28.0, 11.6 Hz, 1H), 1.70 – 1.54 (m, 3H), 1.44 – 1.35 (m, 5H), 1.26 (d, *J* = 5.7 Hz, 1H), 1.16 (dt, *J* = 13.9, 6.0 Hz, 4H), 0.90 – 0.82 (m, 7H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 103.90 (C-10), 101.99(C-3), 87.91 (C-12), 81.14 (C-12a), 70.52 (C-19), 70.27 (C-22), 69.94 (C-21), 67.39 (C-24), 66.65 (C-18), 52.56 (C-5a), 44.57 (C-8a), 37.34 (C-9), 36.41 (C-4), 34.77 (C-7), 30.82 (C-6), 26.17 (C-14), 24.67 (C-5), 24.33 (C-8), 20.24 (C-15), 15.11 (C-16), 12.97 (C-25). MS FAB 401.5 (($M+H^+$) 8%), 423.4 (($M+Na^+$) 7%).

3.1.8. 2-[2-(2-ethoxyethoxy)ethoxy] ethoxy-10β-dihydroartemisinin (**8**) *(R = CH_2-CH_3, n = 3)*

Ether (**8**) was purified by flash silica gel column chromatography eluting with DCM:EtOAc (5:1) to give a yellow oil: 1.60 g (51%) yield. $C_{23}H_{40}O_8$. ^{1}H NMR δ (ppm): (600 MHz, $CDCl_3$) 5.35 (s, 1H, H-12), 4.75 (d, *J* = 4.4 Hz, 1H, H-10), 3.90 – 3.83 (m, 1H, H-18a), 3.60 – 3.52 (m, 12H, H-18b-19-21-22-24-25-27), 3.45 (m, 1H, H-27), 2.54 (d, *J* = 3.4 Hz, 1H), 2.32 – 2.26 (m, 1H), 1.93 (dd, *J* = 13.4, 10.8 Hz, 1H), 1.73 (ddd, *J* = 41.2, 22.1, 8.8 Hz, 3H), 1.54 (d, *J* = 13.1 Hz, 1H), 1.48 – 1.35 (m, 5H), 1.25 – 1.13 (m, 6H), 0.82 (dd, *J* = 9.9, 7.6 Hz, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 104.00 (C-10), 102.06 (C-3), 87.81 (C-12), 81.09 (C-12a), 70.65 (C-19), 70.59 (C-25), 70.55 (C-24), 70. 47 (C-21), 69.78 (C-22), 67.37 (C-27), 66.67 (C-18), 52.62 (C-5a), 44.42 (C-8a), 37.38 (C-9), 36.38 (C-4), 34.66 (C-7), 30.88 (C-6), 26.13 (C-14), 24.64 (C-5), 24.30 (C-8), 20.33 (C-15), 15.15 (C-16), 12.94 (C-28). MS FAB 467.0 ($(M+Na^+)$ 38%).

3.2. Physicochemical properties

3.2.1. Solubility

The aqueous solubility values (S_w) of compounds (**3**) - (**5a**) and (**6a**) – (**8**) were obtained by preparing solutions in phosphate buffer at pH 7.4 (Table 1). The S_w value of artemether was also obtained by preparing a saturated solution in the buffer. The slurries were stirred with magnetic bars in a water bath at 37 °C for 24 h. An excess of solute was present at all times to provide saturated solutions. After 24 h, the solutions were filtered and analyzed directly by HPLC to determine the concentration of solute dissolved in the solvent. The experiment was performed in triplicate. It was not possible to determine the aqueous solubility of compound (**5b**) due to insufficient supply of this derivative.

3.2.2. Experimental log P

Equal volumes of *n*-octanol and phosphate buffer solution of pH 7.4 were saturated with each other under vigorous stirring for at least 24 h. Accurately weighed 2 mg of each derivative was dissolved in 0.75 ml of pre-saturated *n*-octanol, the solution was then stoppered and agitated for 10 min in 2 ml graduated tubes (0.5 ml division). Subsequently 0.75 ml of pre-saturated buffer was transferred to the tubes containing the before mentioned solutions.

The tubes were stoppered and agitated for 45 min then centrifuged at 4 000 rpm (1503 G) for 30 min. The *n*-octanol and aqueous phases were allowed to separate at room temperature for 5 min, where after their volume ratio (v/v; *n*-octanol:buffer) was determined. The volume ratio was found in all cases to be 1.

The *n*-octanol and aqueous phases were diluted with acetonitrile (ACN) and analysed by HPLC. From this data the concentrations of the derivative in both phases were determined. The log P values (log (octanol: pH 7.4 buffer partition coefficient)) were calculated as logarithmic ratios of the concentrations in the *n*-octanol phase to the concentrations in the buffer. The experiment was performed in triplicate and the results expressed as means are listed in Table 1.

3.3. In vitro *biological studies*

The derivatives were tested in triplicate on two separate occasions against the chloroquine sensitive (CQS) strain of *Plasmodium falciparum* (D10). Continuous *in vitro* cultures of asexual erythrocyte stages of *P. falciparum* were maintained using a modified method reported by Trager and Jensen (1976). Quantitative assessment of anti-plasmodial activity *in vitro* was determined *via* the parasite lactate dehydrogenase assay using a modified method described by Makler (1993).

The test samples were prepared as a 2 mg/ml stock solution in 10% dimethyl sulfoxide (DMSO) and sonicated to enhance solubility. The samples were tested as a suspension if not completely dissolved. Stock solutions were stored at -20 °C. Further dilutions were prepared on the day of the experiment. Chloroquine (CQ) and artemether were used as the reference drugs in all experiments. A full-dose response was performed for all compounds to determine the concentration inhibiting 50% of parasite growth (IC_{50} value) (Table 2). The samples were tested at a starting concentration of 1000 µg/ml, which was then serially diluted 2-fold in complete medium to give 10 concentrations, with the lowest concentration being 2 µg/ml. The same dilution technique was used for all samples. Test samples were re-tested at a starting concentration of 100 µg/ml. The solvents to which the parasites were exposed to had no measurable effect on the parasite viability. The IC_{50}-values were obtained using a non-linear dose-response curve fitting analysis via Graph Pad Prism v.4.0 software. The *in vitro* anti-plasmodial activity is reported as IC_{50} values in Table 2.

4. Results and Discussion

4.1. Chemistry (Scheme 1)

The new ether derivatives of artemisinin were prepared by treatment of dihydroartemisinin (**1**) with an appropriate methoxypoly(ethylene glycol) (**3-5b**) or ethoxypoly(ethylene glycol) (**6a-8**) in the presence of boron trifluoride diethyl etherate ($BF_3.Et_2O$) at room temperature (Scheme 1). The yield of the purified products ranged from 7-51% (Table 1). The purification was achieved by flash column chromatography in silica gel. An oxonium ion at position 11 was proposed to be involved in the ether formation (Lin *et al.,* 1989). In some cases a mixture of two isomers, as seen from their proton NMR spectra (**5b** and **6b**) was formed and they were separated by flash silica column chromatography. The configuration at the C-10 position of the ethers was assigned based on the vicinal couplings $J_{H9:H10}$ (Venugopalan *et al.,*1995). The large coupling constant between the protons at positions 10 and 9 in the case of the α-series, $J_{H9:H10}$ = 9-10 Hz (compounds **5b** and **6b**) (Lin *et al.,*1997), indicates that the relative configuration at the position 10 and 9 is *trans.* The β-series have a small coupling constant, $J_{H9:H10}$ = 3.6-5 Hz (compounds **3-5a**, **6a**, **7** and **8**) (Lin *et al.,* 1989) between these protons. The relative configuration at positions 10 and 9 of the β-series is *cis.* In the α-series the signals, due to OCH_2 and H-10 appears upfield compared with those in the β-series (Venugopalan *et al.,* 1995).

Polyethylene glycols (PEGs) were chosen because of their useful properties like a wide range of solubility in both organic and aqueous solvents, good biocompatibility meaning less toxicity, no antigenicity and immunogenicity, no interference with enzymatic activities and conformations of polypeptides, and easy excretion (glomular filtration in the kidneys) from living organisms, making PEGs an ideal choice for preparing derivatives (Zalipsky & Lee, 1992). Bonora *et al.* (1997) found that PEGs may cause an increase in the stability of the conjugated molecules. PEGs may also extend circulation life of drugs in the body, reduce toxicity and enhance protection from proteolytic degradation (Veronese & Pasut, 2005). It is one of only a few synthetic polymers that are approved by the US Food and Drug Administration (FDA) to be used internally in food, cosmetics and pharmaceutical products (Moore & Roberts, 1982). Two series of PEGs were used in this study, methoxypoly(ethylene glycol) (MPEG) and ethoxypoly(ethylene glycol) (EPEG).

In the ^{1}H NMR spectrum of dihydroartemisinin the signals for H-12 and H-10 for the 10β-isomer, having a higher R_f value, appeared at δ_H 5.41 (s) and 4.8 (s) respectively whereas for the 10α-isomer they appeared at δ_H 5.33 (s) and 4.43 (s) respectively. In the α-series the signals due to OCH_2 and H_{10} appear up field compared with those in the β-series (Venugopalan *et al.*, 1995). The ^{13}C NMR spectrum of dihydroartemisinin showed signals at 104.99, 99.40, 88.62, 81,42, 52.87, 45.51, 37.65, 36.84, 34.61, 32.28, 25.60, 22.99, 22.72, 20.00 and 14.87 ppm corresponding to C-10, C-3, C-12, C-12a, C-5a, C-8a, C-9, C-4, C-7, C-6, C-14, C-5, C-8, C-15 and C-16 respectively. Thus, the presence of the dihydroartemisinin moiety is confirmed in the spectra of all the compounds.

The chemical structures of the title compounds (**3-8**) (Scheme 1) were confirmed by NMR and FAB-MS data. The presence of the MPEG chain (**3-5b**) was confirmed by the resonance of carbons $-OCH_3$ between 58.77-59.03 ppm and $-OCH_2-CH_2O-$ between 71.64-67.53 ppm and the EPEG chain (**6a-8**) was confirmed by the resonance of carbons $-CH_3$ between 15.08-12.40 and $-OCH_2-CH_2O-$ between 70.86–66.36 ppm in ^{13}C NMR. ^{1}H NMR spectra of compounds (**3-8**) exhibited resonances in the 3.99-3.31 ppm region characteristic of methylene protons $-O\mathit{CH_2}\text{-}\mathit{CH_2}O-$ belonging to MPEG chain and resonance between 3.97-3.45 ppm region characteristic of methylene protons $-O\mathit{CH_2}\text{-}\mathit{CH_2}O-$ belonging to EPEG chain with the confirmation of the $-CH_3$ protons as signals between 1.25-1.00 ppm.

The FAB-MS data for the compounds confirmed the presence of molecular ions (*m/z*) at 343.3 ($M+H^+$) and 365.4 ($M+Na^+$) for (**3**) at 387.3 ($M+H^+$) and 409.3 ($M+Na^+$) for (**4**), at 431.4 ($M+H^+$) and 453.4 ($M+Na^+$) for (**5a**), at 431.1 ($M+H^+$) and 453.1 ($M+Na^+$) for (**5b**), 357.4 ($M+H^+$) and 379.3 ($M+Na^+$) for (**6a**), at 357.4 ($M+H^+$) and 379.3 ($M+Na^+$) for (**6b**), at 401.5 ($M+H^+$) and 423.4 ($M+Na^+$) for (**7**), at 467.0 ($M+Na^+$) for (**8**) corresponding to the molecular formulae $C_{18}H_{30}O_6$, M_w = 342.43 (**3**), $C_{20}H_{34}O_7$, M_w = 386.48 (**4**), $C_{22}H_{38}O_8$, M_w = 430.53 (**5a** and **5b**), $C_{19}H_{32}O_6$, M_w = 356.45 (**6a** and **6b**), $C_{21}H_{36}O_7$, M_w = 400.51 (**7**) and $C_{23}H_{40}O_8$, M_w = 444.56 (**8**). These formulae in turn indicate the number of oxyethylene units, **n**, in the series (R = CH_3) to be 1 for (**3**), 2 for (**4**) and 3 for (**5a** and **5b**) respectively, as well as the number of oxyethylene units, **n**, in the series (R = CH_2-CH_3) to be 1 for (**6a** and **6b**), 2 for (**7**) and 3 for (**8**) respectively.

4.2. Aqueous solubility and experimental log P (Table 1)

Aqueous solubility and lipophilicity influence the way a drug molecule passes through biological membranes and barriers to ultimately enter the systemic circulation. The drug molecules must posses some lipophilic properties to permeate biological membranes and hydrophilic properties to be taken up in the systemic circulation, but a more hydrophilic nature is needed in order for the efficient drug delivery. The two characteristics expressed as logarithm of the partition coefficient (log P) between *n*-octanol and phosphate buffer (pH 7.4) are listed in Table 1 for compounds (**3-8**). Polyethylene glycols (PEGs) exhibit the ability to increase molecules' hydrophilicity by the increasing number of oxyethylenic units in the polyethylene glycol moiety. Solomons & Fryhle (2007) states that the aqueous solubility of polyethylene glycols increases as result of increased association of water molecules with the intrachain oxygen atoms of PEG. Thus, increasing the chain length automatically increases the number of intra-chain oxygen atoms that can interact with the water molecules, leading to an increase in aqueous solubility. However, as the chain length increased so did the number of ethylene units which ultimately increases lipid solubility. The EPEG derivatives (**6a-8**) showed higher log P values than the MPEG derivatives (**3-5b**) (Table 1). All the derivatives showed lower log P values than artemether (log P = 1.92).

The S_w values (aqueous solubility) for (**3**) - (**5a**) and (**6a**) – (**8**), as expected, increased with the decrease of log P values, as the chain length increased thus firmly validating both structure-aqueous solubility and structure-lipophilicity relationships within the series. The S_w values for all the compounds were lower than that of artemether (S_w = 30 µg/ml).

Table 1. Physicochemical properties of ethylene glycol oligomeric ethers of artemisinin (**2-10**)

Compound	R	n	Emp. Formula	M_w (g/mol)	Yield (%)	*J* (Hz)	Isomer	Sw (µg/ml) [a]	STD	Log P [a]	STD
artemether	NA	NA	$C_{16}H_{26}O_6$	298.38	NA	NA	NA	30	0.04	1.92	0.18
(**3**)	CH_3	1	$C_{18}H_{30}O_6$	342.43	38	4.8	β	471	0.03	1.27	0.24
(**4**)	CH_3	2	$C_{20}H_{34}O_7$	386.48	47	4.5	β	640	0.02	0.96	0.20
(**5a**)	CH_3	3	$C_{22}H_{38}O_8$	430.53	48	4.3	β	1386	0.05	0.53	0.03
(**5b**)	CH_3	3	$C_{22}H_{38}O_8$	430.53	12	9.2	α	-[b]	0.06	0.78	0.24
(**6a**)	CH_2-CH_3	1	$C_{19}H_{32}O_6$	356.45	35	4.5	β	50	0.01	1.42	0.17
(**6b**)	CH_2-CH_3	1	$C_{19}H_{32}O_6$	356.45	7	9.3	α	65	0.01	1.69	0.06
(**7**)	CH_2-CH_3	2	$C_{21}H_{36}O_7$	400.51	48	4.6	β	208	0.03	1.17	0.23
(**8**)	CH_2-CH_3	3	$C_{23}H_{40}O_8$	444.56	51	4.4	β	889	0.04	0.80	0.02

Substituent (R), index (n), empirical formula (Emp. Formula), molecular weight (M_w), coupling constant between H-10 and H-9 (J), aqueous solubility (S_w), standard deviation (STD), partition coefficient, log P (*n*-octanol-PBS, pH 7.4).

[a] Determined experimentally, each value represents the mean and STD of 3 measurements. [b] Compound not submitted.

4.3. In vitro *anti-malarial activity (Table 2)*

The new ethylene glycol oligomeric ethers of artemisinin were tested *in vitro* against one clone of human malaria, *Plasmodium falciparum* D-10 (chloroquine sensitive strain). The anti-malarial activity of the test compounds was compared with that of chloroquine and artemether (Table 2). The results indicate that the anti-malarial activity increases as the number of oxyethylenic units in the polyethylene glycol moiety increase, elongating the chain length. The ethoxypoly(ethylene glycol) series (**6a-8**) which is more lipophilic, showed higher anti-malarial activity than the methoxypoly(ethylene glycol) series (**3-5b**) which is more hydrophilic, even if the overall anti-malarial activity increased with the increase in hydrophilicity, thus showing that both hydrophilic and lipophilic properties are necessary for the enhancement of the anti-malarial activity. None of the compounds showed better anti-malarial efficacy than artemether (IC_{50} = 7.4 ng/ml). Compound (**8**), 2-[2-(2-ethoxyethoxy)ethoxy] ethoxy derivative, was the most active compound of the two series, with the IC_{50} value of 14.90 ng/ml, making it more potent than chloroquine (16.46 ng/ml). Compounds (**5a**) and (**6a**) showed activity comparable to that of chloroquine. Compounds (**3**), (**4**), (**5b**), (**6b**) and (**7**) are less active than artemether or chloroquine. In all cases the anti-malarial activity of the β-isomers was higher than that of the α-isomers.

Table 2. Anti-malarial activity of ethylene glycol oligomeric ethers of artemisinin (**3-8**), artemether and chloroquine

Compound	IC_{50} (ng/ml) [a]	STD
(**3**)	88.5	0.05
(**4**)	40.8	0.57
(**5a**)	17.2	1.78
(**5b**)	22.1	1.99
(**6a**)	18.2	0.97
(**6b**)	32.8	2.01
(**7**)	20.8	1.73
(**8**)	14.9	0.94
artemether	7.4	1.81
chloroquine	16.46	2.48

Concentration inhibiting 50% of parasite growth (IC_{50}), standard deviation (STD)

[a] determined experimentally, data represents the mean and STD of 3 measurements

5. Conclusion

In conclusion, we have successfully synthesised a series of ethylene glycol oligomeric ethers of artemisinin (**3-8**) through derivatisation at its biological sensitive 10-OH position. The structures of the synthesised compounds were confirmed by physical means, i.e. ^{1}H and ^{13}C NMR and FAB-MS spectroscopy and their log P values and aqueous solubility were measured experimentally. The EPEG derivatives (**6a-8**) showed higher log P values than the MPEG derivatives (**3-5b**) (Table 1). All the derivatives showed lower log P values than artemether (log P = 1.92). The S_w values (aqueous solubility) for the MPEG derivatives (**3-5a**) were higher than that of the EPEG derivatives (**6a–8**), as expected, the overall aqueous solubility increased with the decrease of log P values, as the chain length increased, thus firmly validating both structure-aqueous solubility and structure-lipophilicity relationships within the series. The S_w values of all the compounds were lower than the aqueous solubility of artemether (S_w = 30 µg/ml). These compounds were tested for anti-malarial activity against artemether and chloroquine as standards. The results indicate that the overall anti-malarial activity unexpectedly, increases as the number of oxyethylenic units in the polyethylene glycol moiety, elongating the chain, increases. The ethoxypoly(ethylene glycol) series (**6a-8**) which is more lipophilic, showed higher anti-malarial activity than the methoxypoly(ethylene glycol) series (**3-5b**) which is more hydrophilic, but the overall anti-malarial activity increased with the increase in hydrophilicity, thus showing that both hydrophilic and lipophilic properties are necessary for the enhancement of the anti-malarial activity. None of the compounds showed a better anti-malarial effect than artemether (7.4 ng/ml). Derivative (**8**) showed promising anti-malarial activity, better than the known anti-malarial chloroquine. Derivatives (**5a**) and (**6a**) showed anti-malarial efficacy comparable to that of chloroquine.

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SUMMARY AND FINAL CONCLUSIONS

This section strives to unify the outcomes of this study and draws overall conclusions from all data, to point out relevance and specific advances made by this study, to point out remaining scientific questions and to propose appropriate prospective studies.

Malaria continues to be a major serious health problem and public health threat, with over two billion people at risk of contracting this deadly disease. Malaria is endemic in 92 countries and more than one million deaths per year are attributed to malaria, the mortality in African children being the highest (Breman, 2001). Malaria treatment policies have to be continuously revised and assessed by the World Health Organisation because of the failing therapeutic efficacy of existing anti-malarial drugs (Bosman & Olumese, 2004). The emergence of mono- and multi- drug resistant parasites which render treatment options as ineffective and limited are the direct cause of this problem (Bloland, 2001).

A great need for alternative treatment options of the disease has therefore become prominent and this has encouraged researchers to search for other chemotherapeutic anti-malarial drugs with different molecular mechanisms of action from those against which malaria parasites have developed resistance. Artemisinin has been proven to comply with the previously mentioned qualities.

Artemisinin is a sesquiterpene lactone endoperoxide and the first natural 1,2,4-trioxane isolated from *Artemisia annua.* This compound is of special biological interest because of its outstanding anti-malarial activity against chloroquine-resistant *P. falciparum* and cerebral malaria, as well as its *in vitro* activity against *Pneumocystis carinii* and *T. gondii* and good *in vitro* anti-neoplastic activity (Jung, 1997). Artemisinin and its derivatives are the only group of compounds that are still effective against multi-drug resistant *Plasmodium falciparum* (Tonmunphean *et al.*, 2006).

However, the use of such endoperoxides is restricted by their poor oral bioavailability, a short plasma half-life (30 minutes in plasma) and the high rate of recrudescent infections when used as monotherapy in short-course treatments, even though these drugs have a rapid onset of action and low reported toxicity (Gupta *et al.*, 2002).

Artemether is the methyl ether derivative of artemisinin and this compound is lipophilic, more potent than artemisinin but still have a short plasma half-life. Artemisinin, dihydroartemisinin, artemether are all poorly water-soluble compounds, which results in slower and incomplete absorption of these drugs into the systemic circulation (Yu *et al.*, 2004). One of the ways to address these problems is to synthesise artemisinin derivatives that have more suitable physicochemical properties for drug delivery in the systemic circulation and a higher anti-malarial efficacy than existing drugs.

The primary aim of this study was to synthesise ethylene glycol oligomeric ether derivatives of artemisinin, attempting to overcome the pharmacokinetic deficiencies of the artemisinin endoperoxides and the existing anti-malarial artemether.

In order to achieve this goal, the following objectives were set:

- Synthesise polyethylene glycol (PEG) derivatives of artemisinin and confirm their structures.
- Experimentally determine and evaluate certain physicochemical properties such as the aqueous solubility and the partition coefficient for the synthesised artemisinin derivatives and to compare the experimental aqueous solubilities and the partition coefficients of the synthesised derivatives to the known anti-malarial artemether.
- Determine whether a relationship exists between the physicochemical properties like the aqueous solubility and partition coefficient of the artemisinin derivatives.
- Evaluate the in vitro anti-malarial efficacy of the artemisinin derivatives against the chloroquine sensitive D10 strain of Plasmodium falciparum in comparison to the reference drugs, artemether and chloroquine.

The artemisinin derivatives were successfully synthesised via synthetic organic methods and their structures were verified by NMR and MS spectroscopy.

It was not possible to determine the aqueous solubility (S_w) of compounds (**5b**) due to insufficient supply of this derivative. The derivatives showed an increase in aqueous solubility (ranging from 50 to 1386 µg/ml) in both series of compounds. This could be due to the fact that as the intra-chain oxygen atoms increased, more bonds were formed with surrounding water molecules and thus increased the aqueous solubility. The S_w values for all the compounds calculated were lower than that of artemether (30 µg/ml).

The experimental partition coefficient (log P) of artemether (1.92) was higher than that of all the synthesised derivatives (ranging from 0.53 to 1.69). As the chain length of the polyethylene glycol increased the partition coefficient value decreased in both series of compounds. This correlates with the aqueous solubility values which showed that the S_w values increased as the chain length increased due to an increase in the intra-chain oxygen atoms.

The *in vitro* biological data showed that the overall anti-malarial activity unexpectedly, increases as the number of oxyethylenic units in the polyethylene glycol moiety increase, elongating the chain length. The ethoxypoly(ethylene glycol) series (**6a-8**) which is more lipophilic, showed higher anti-malarial activity than the methoxypoly(ethylene glycol) series (**3-5b**) which is more hydrophilic, even if the overall anti-malarial activity increased with the increase in hydrophilicity, thus showing that both hydrophilic and lipophilic properties are necessary for the enhancement of the anti-malarial activity. None of the compounds showed better anti-malarial efficacy than artemether (7.4 ng/ml). Compound (**8**), 2-[2-(2-ethoxyethoxy)ethoxy] ethoxy derivative, was the most active compound of the two series, with the IC_{50} value (50% inhibitory concentration) 14.90 ng/ml, making it more potent than chloroquine (16.46 ng/ml). Compounds (**5a**) and (**6a**) showed activity comparable to chloroquine. Compounds (**3**), (**4**), (**5b**), (**6b**) and (**7**) are less active than artemether and chloroquine. In all cases the anti-malarial activity of the β-isomers was higher than that of the α-isomers.

In this study it has been shown that the synthesised derivatives possess better pharmacokinetic properties (log P and S_w) than the lipophilic anti-malarial artemether. The biological results indicated that only one compound (**8**) showed better anti-malarial efficacy than one of the reference drugs chloroquine and compounds (**5a**) and (**6a**) showed anti-malarial activity comparable to that of chloroquine.

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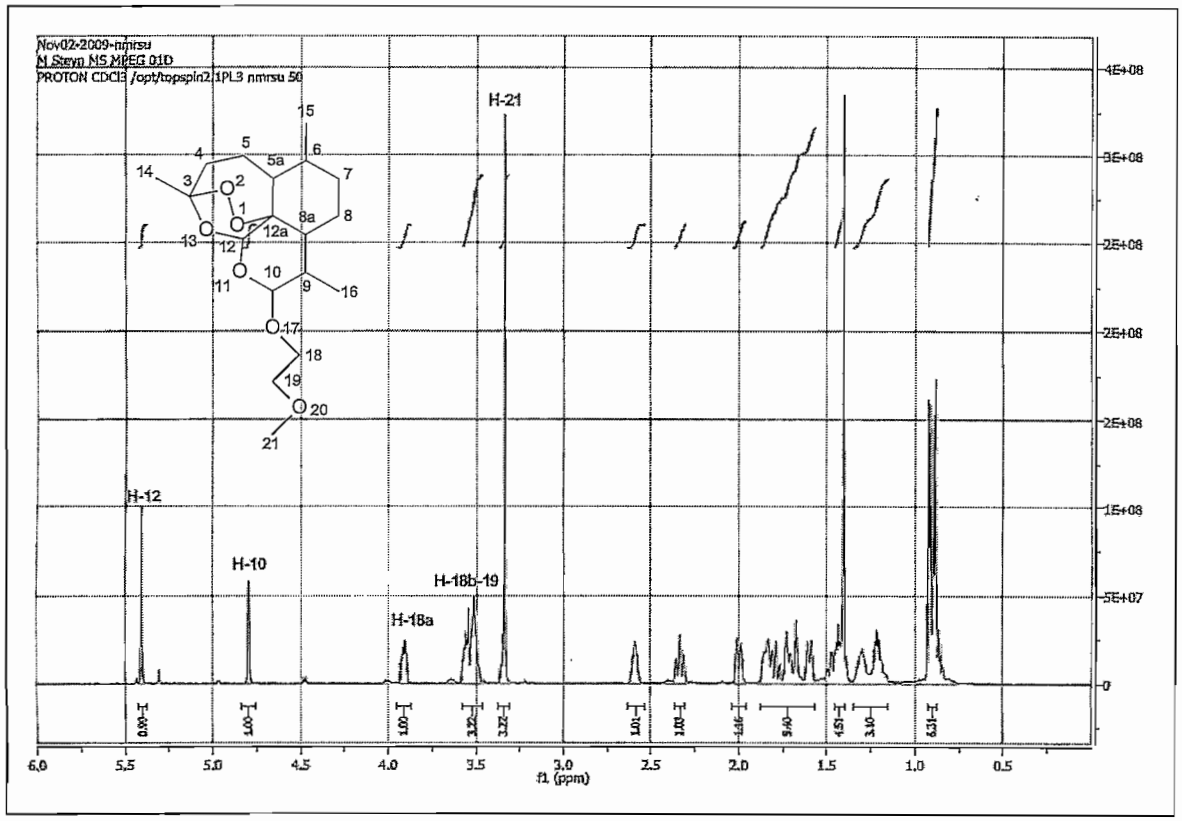
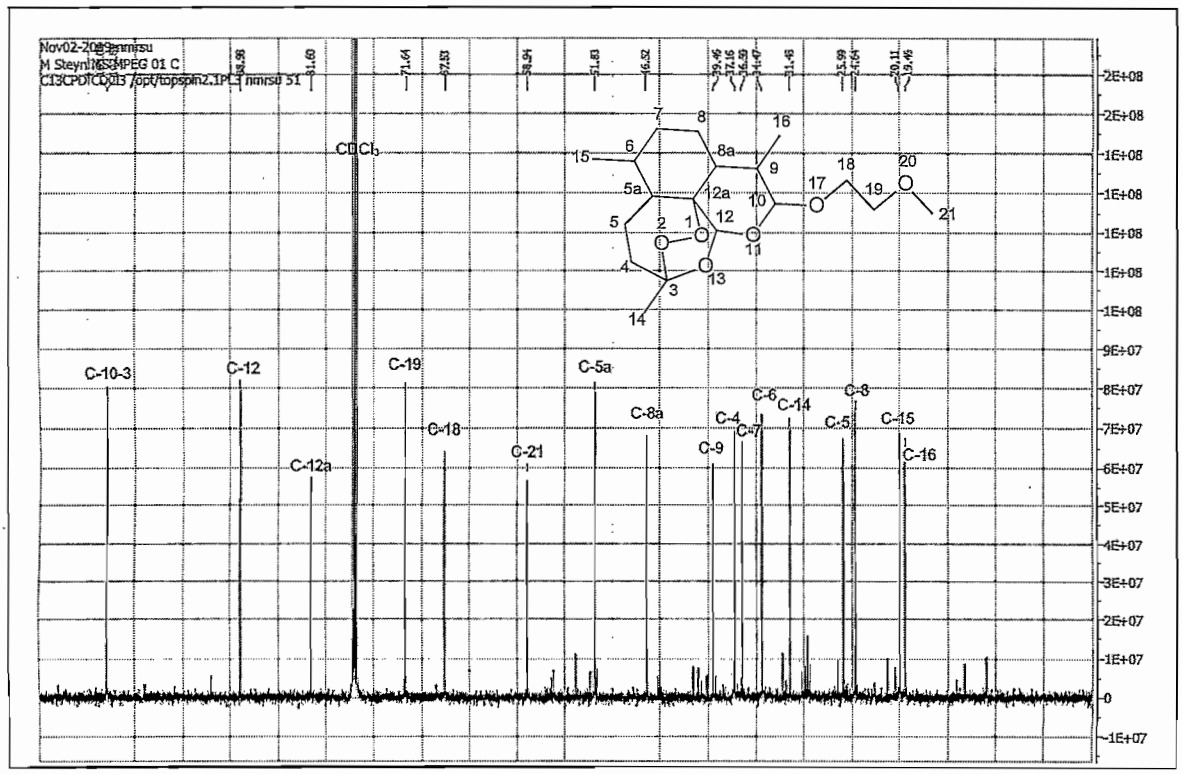
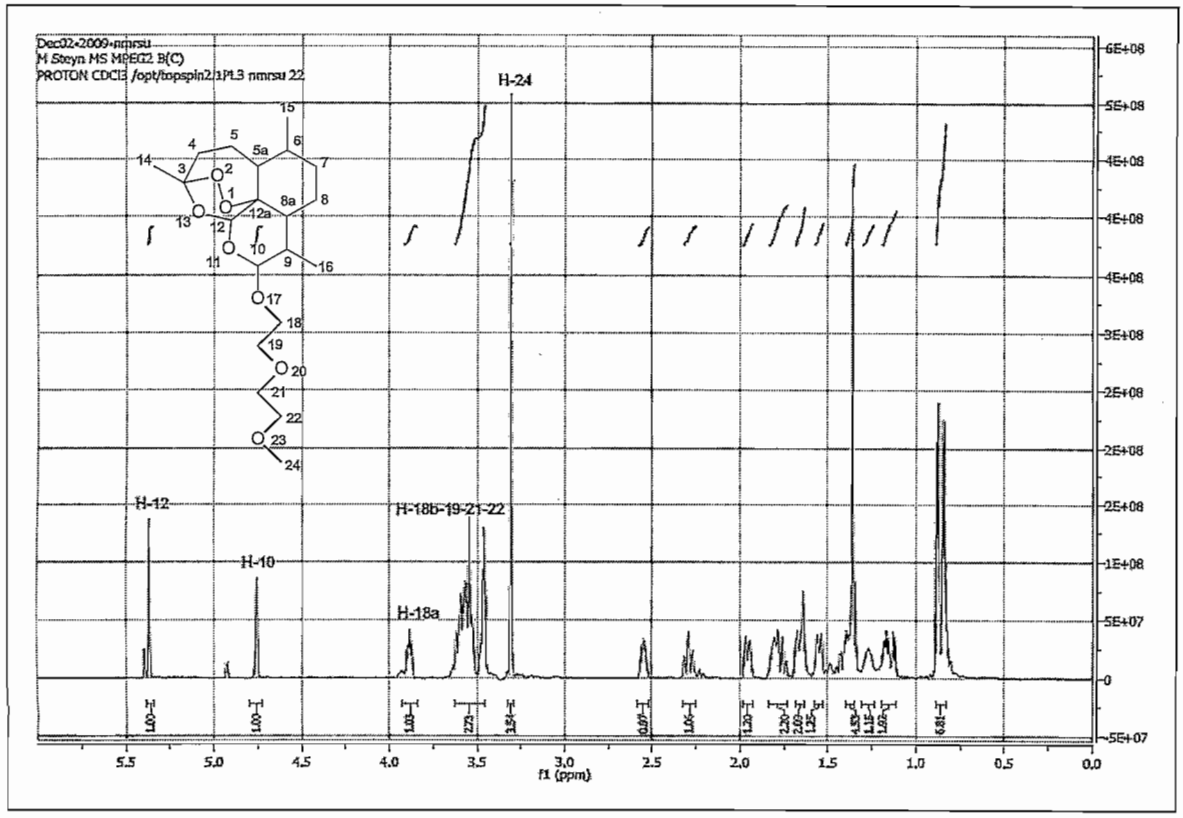
Spectrum 1: ^{1}H NMR of (3)



Spectrum 2: ^{13}C NMR of (3)



Spectrum 3: ^{1}H NMR of (4)



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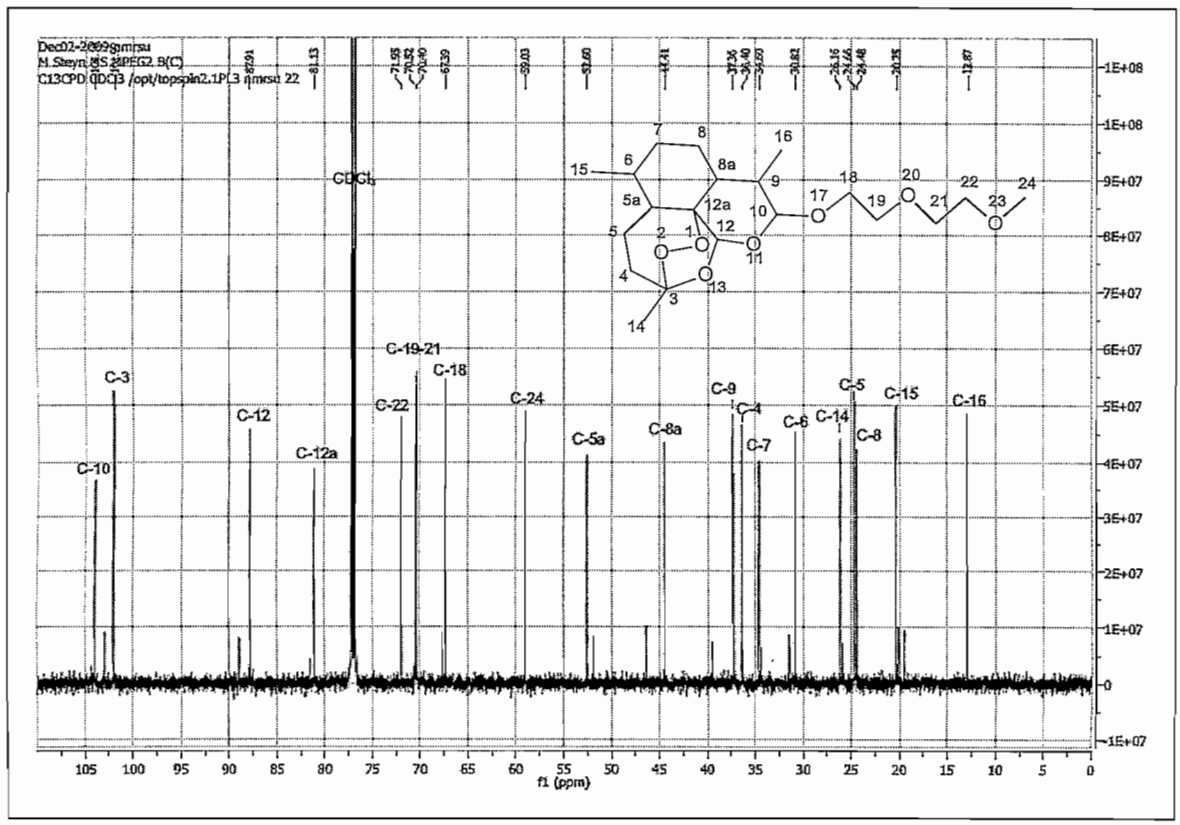
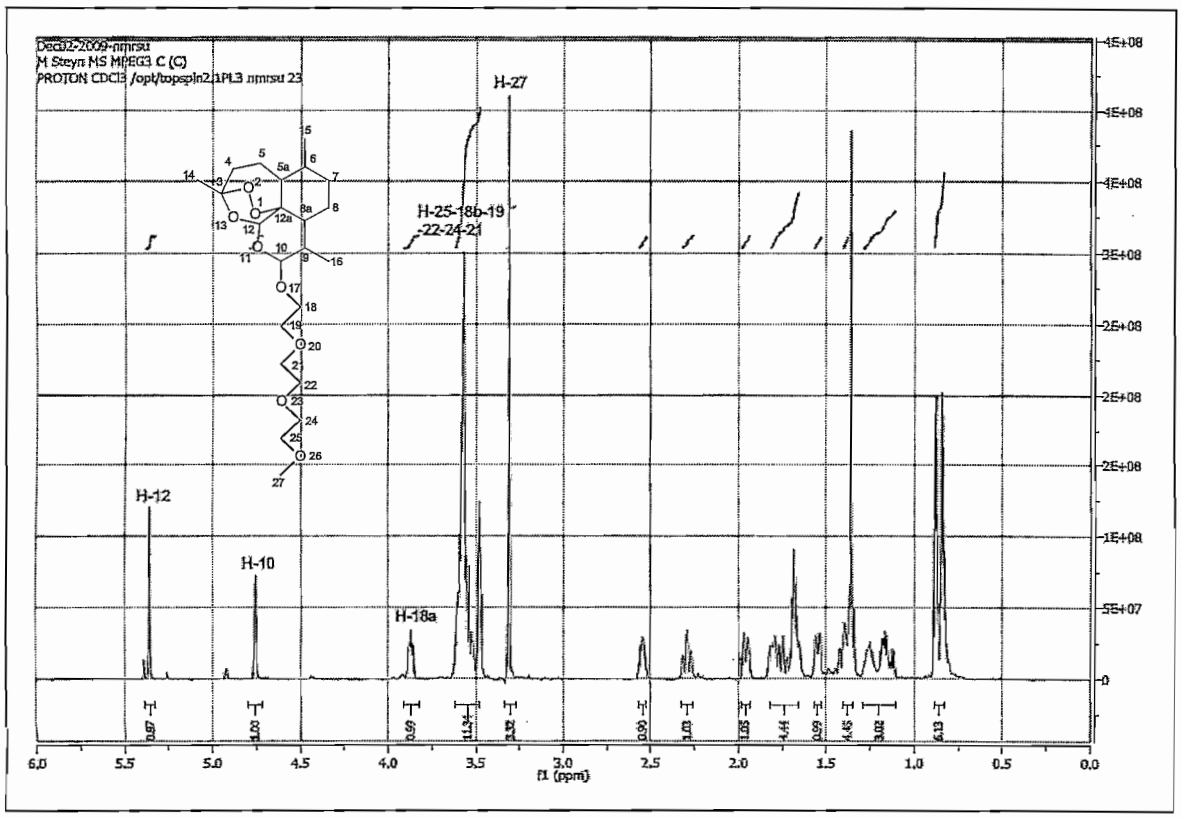
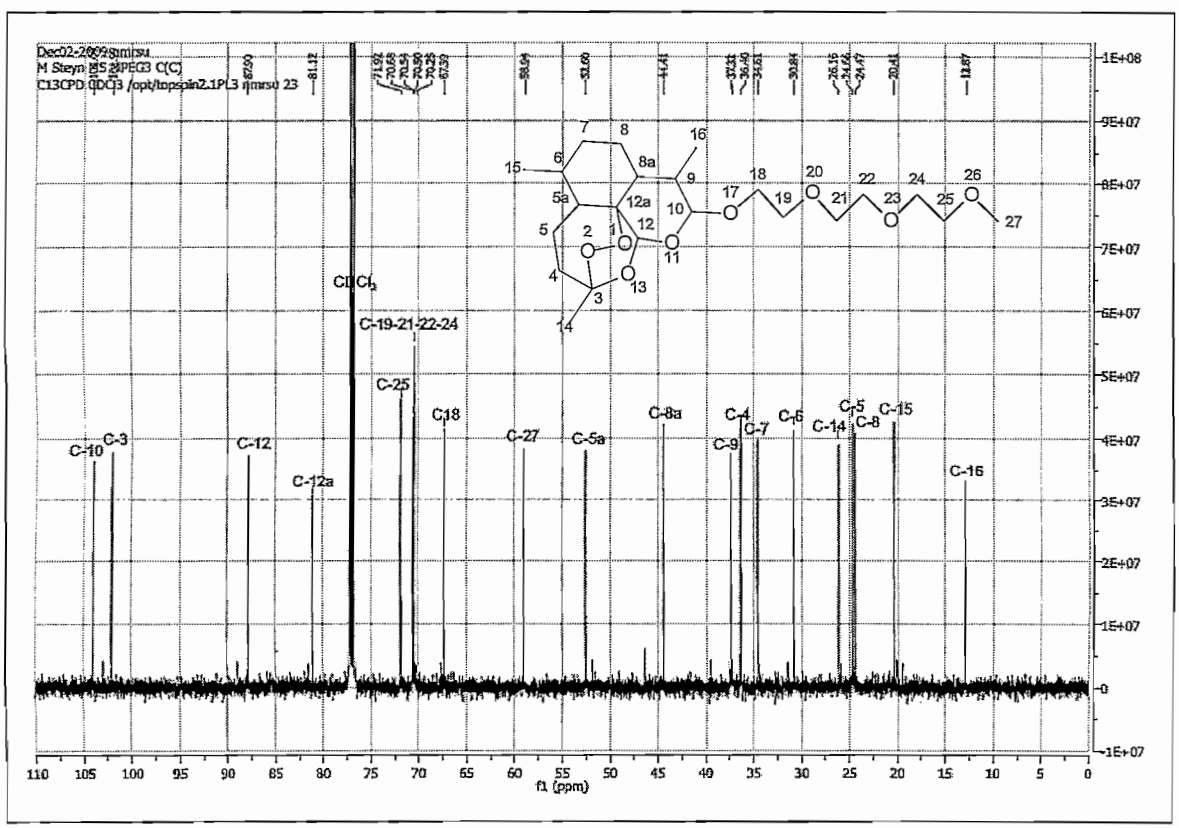


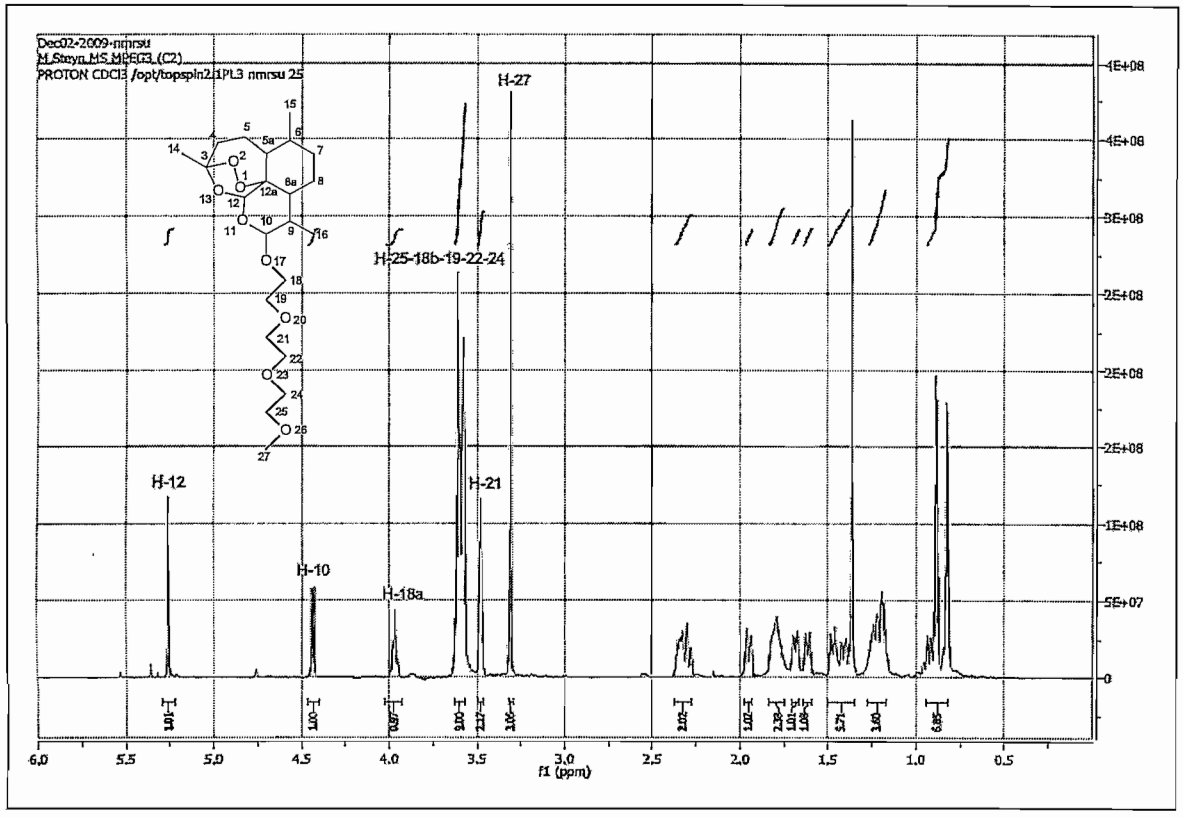
Spectrum 5: ^{1}H NMR of (5a)



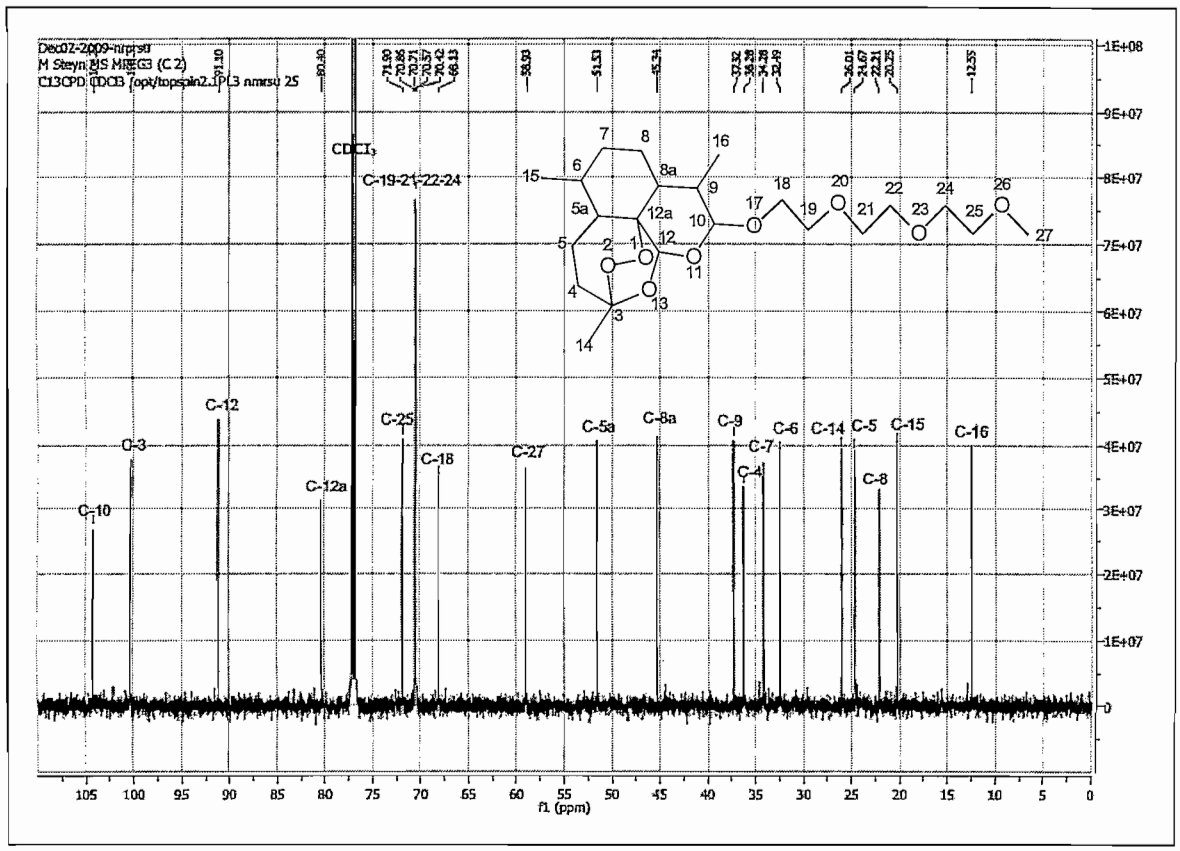
Spectrum 6: ^{13}C NMR of (5a)



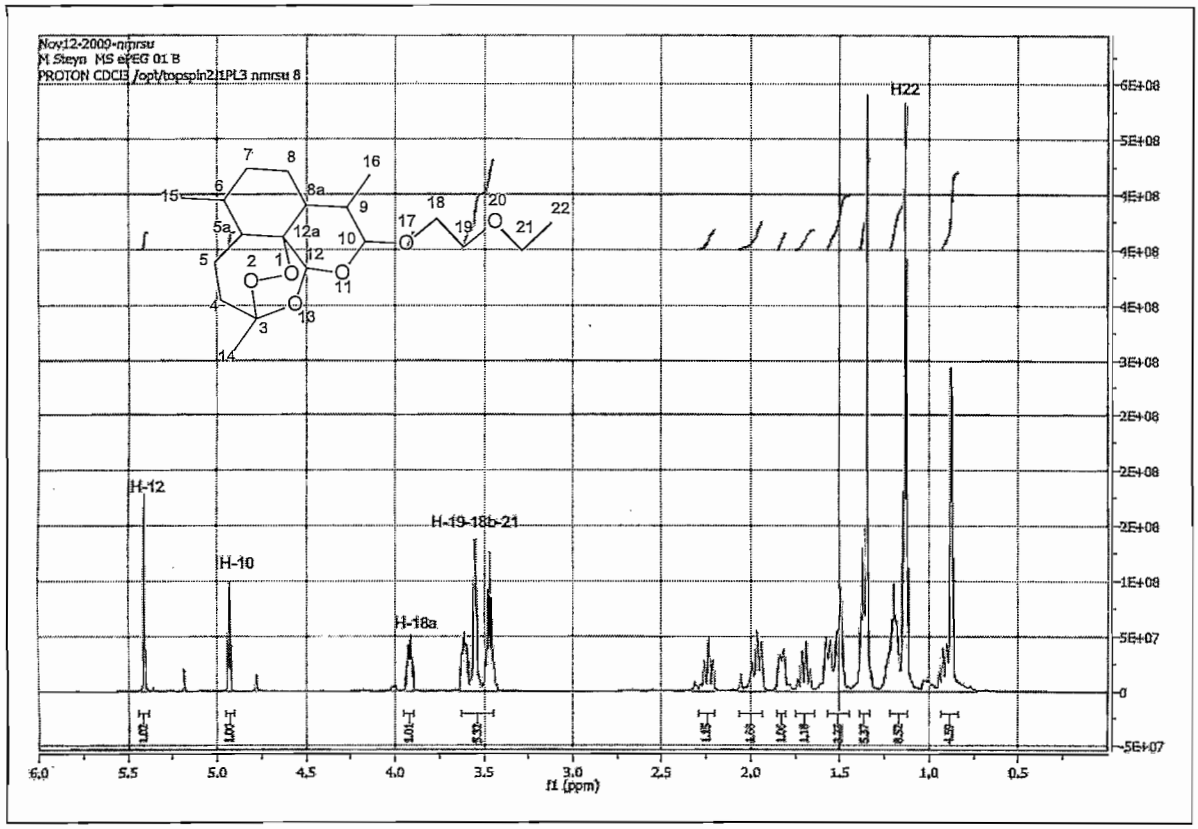
Spectrum 7: ^{1}H NMR of (5b)



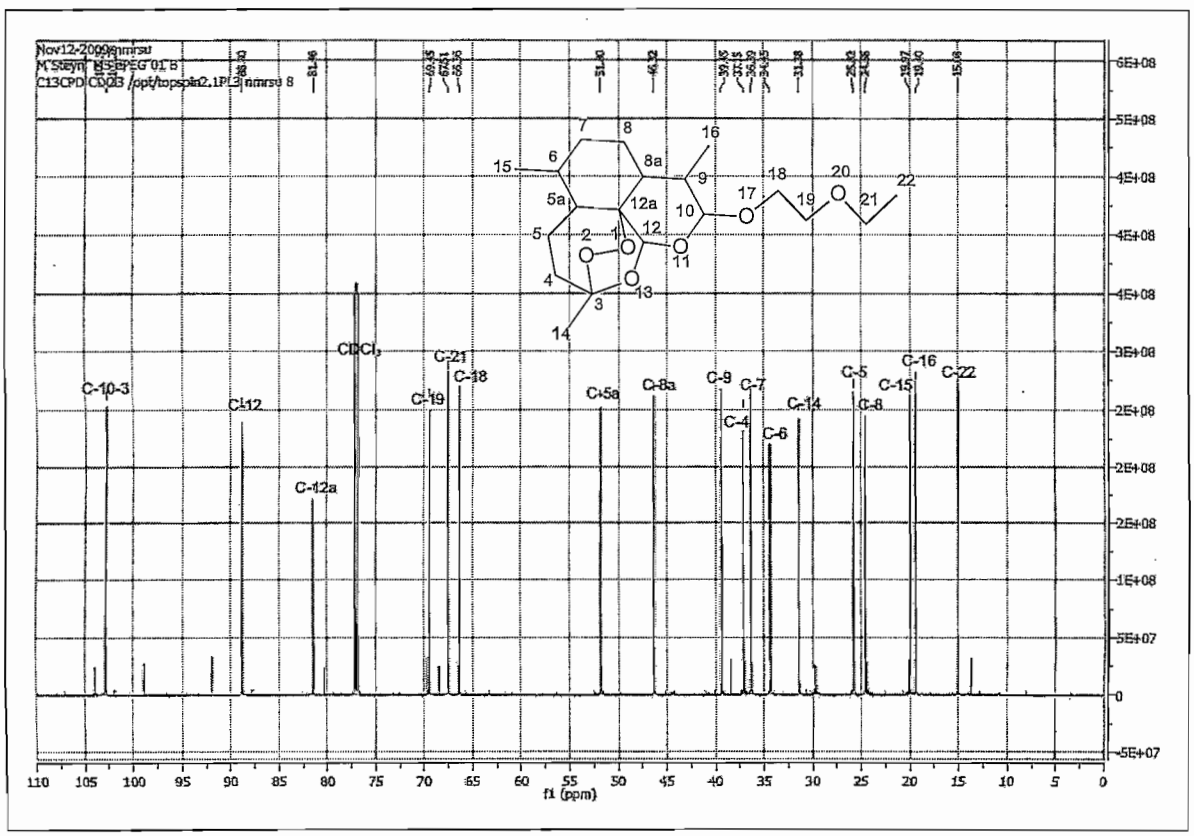
Spectrum 8: ^{13}C NMR of (5b)



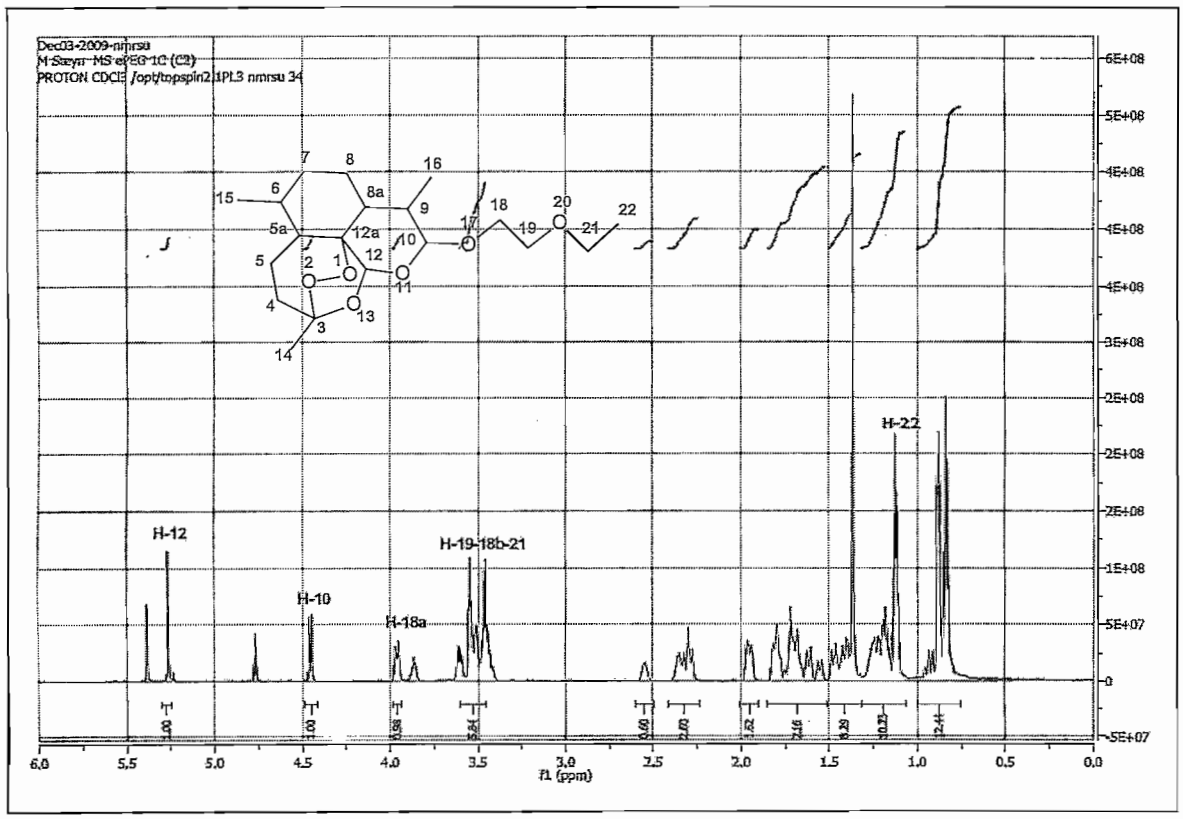
Spectrum 9: ^{1}H NMR of (6a)



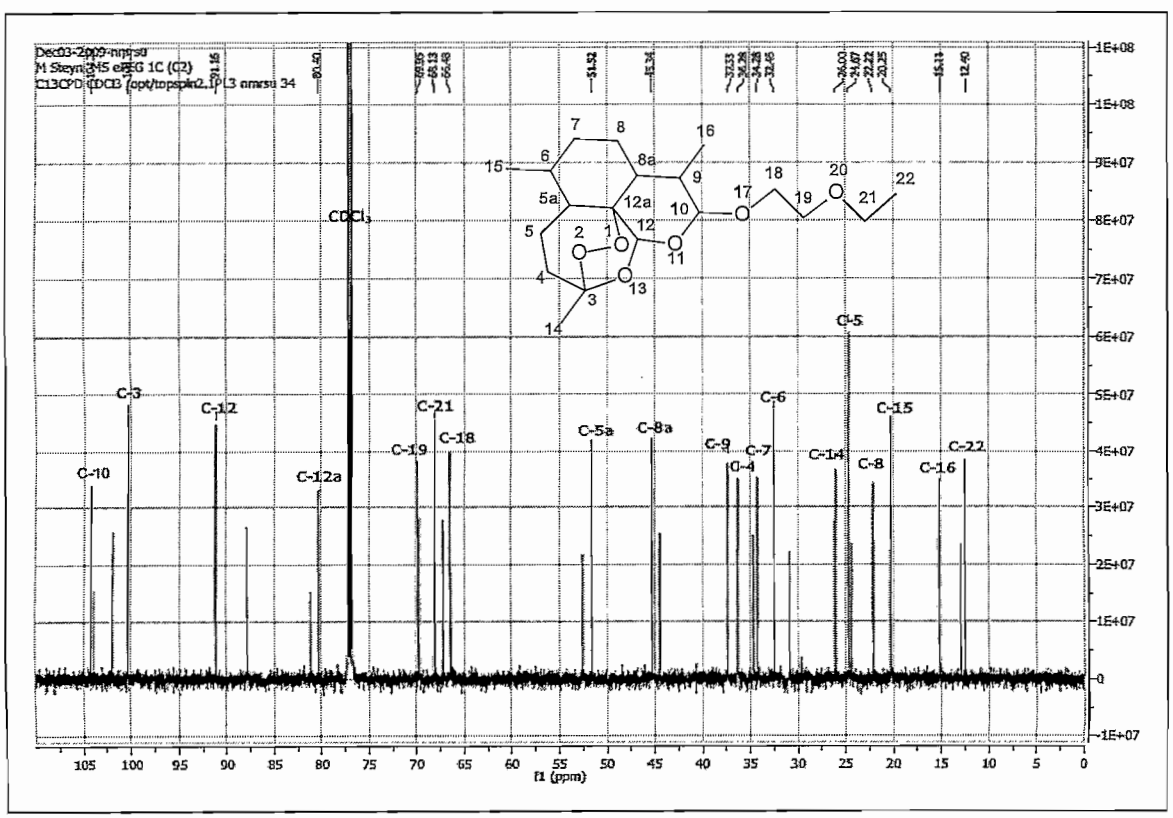
Spectrum 10: ^{13}C NMR of (6a)



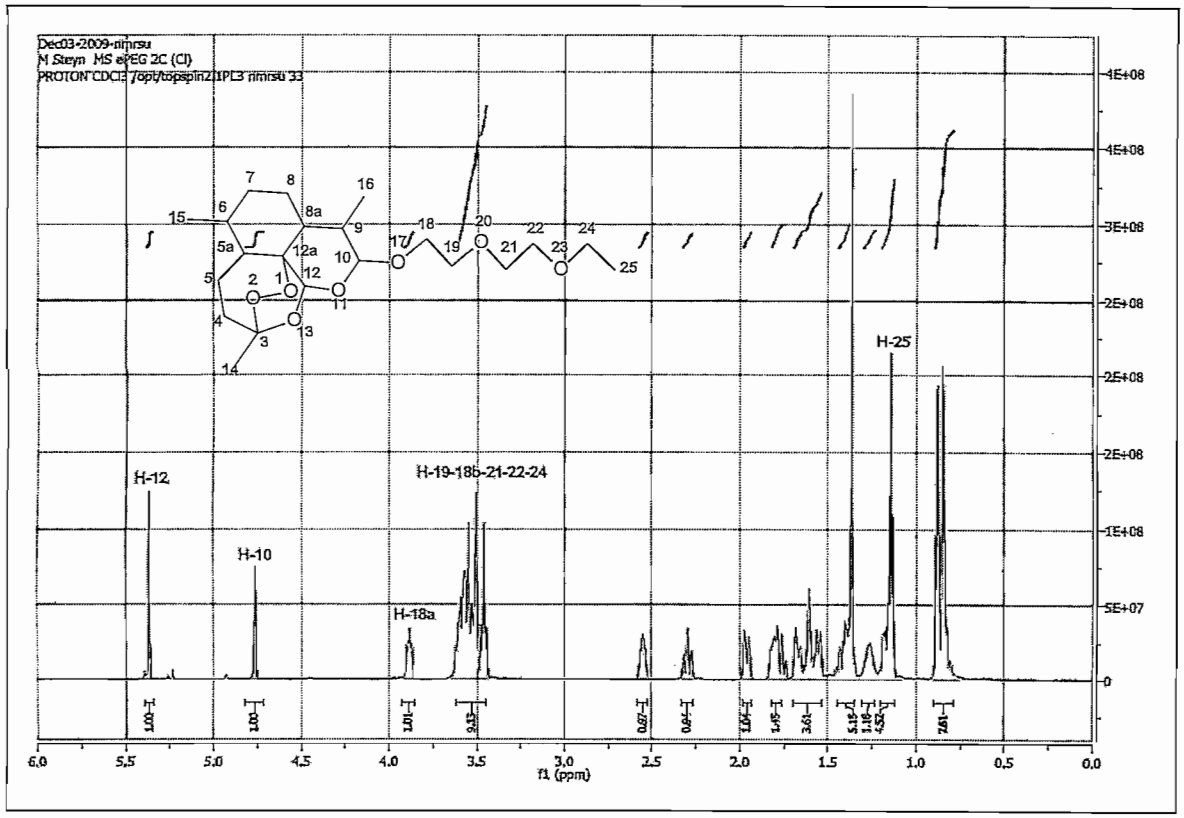
Spectrum 11: ^{1}H NMR of (6b)



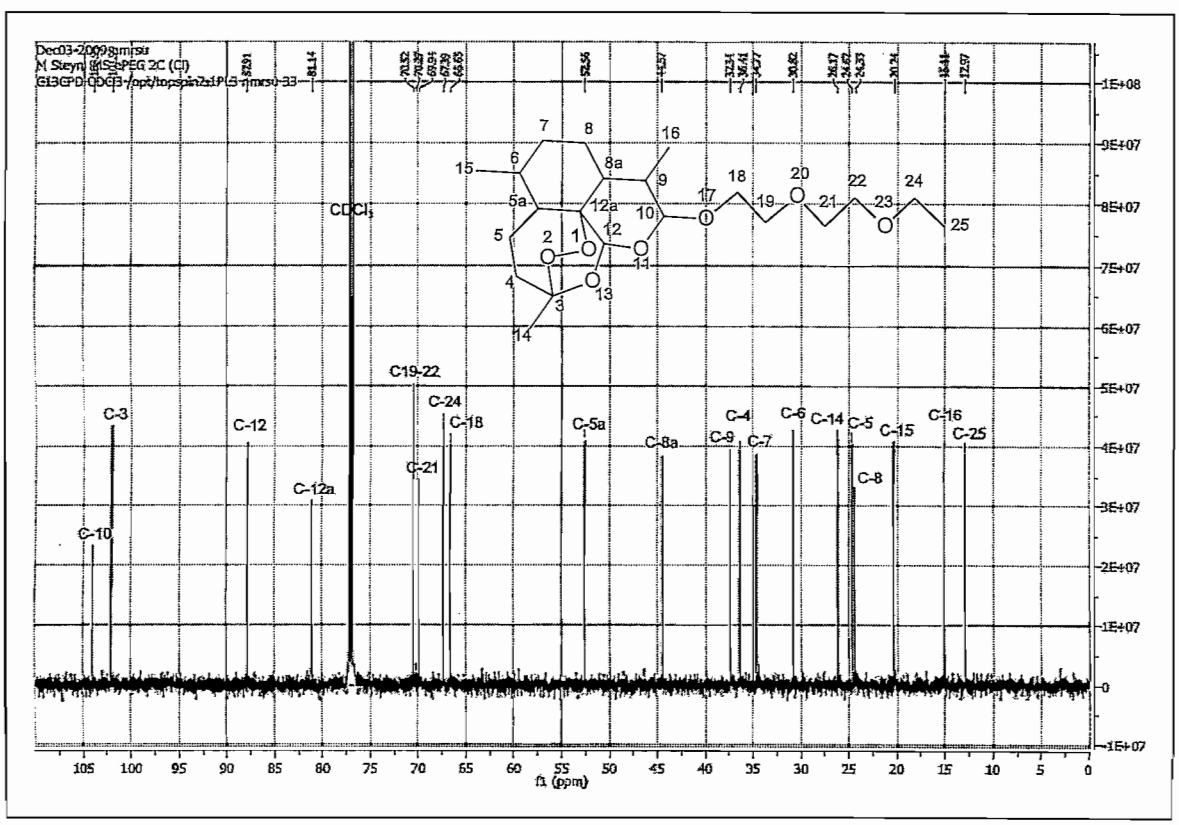
Spectrum 12: ^{13}C NMR of (6b)



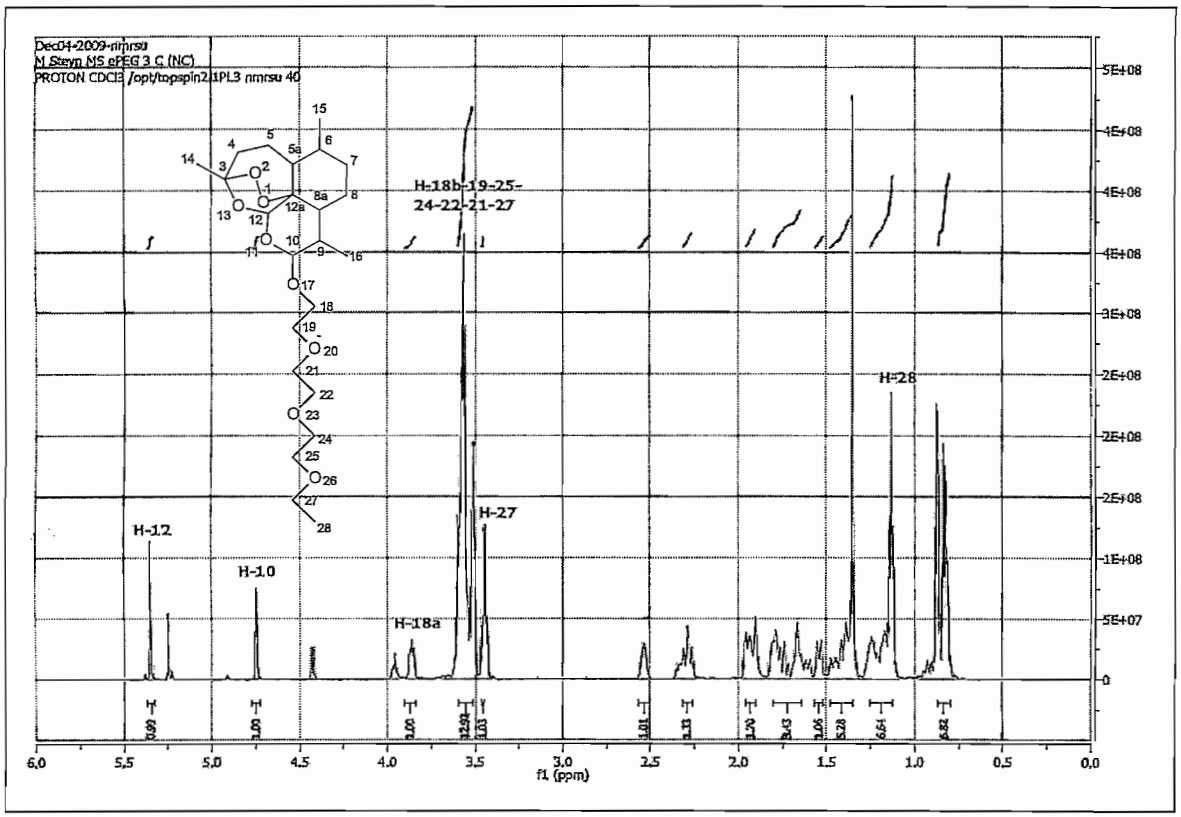
Spectrum 13: ^{1}H NMR of (7)



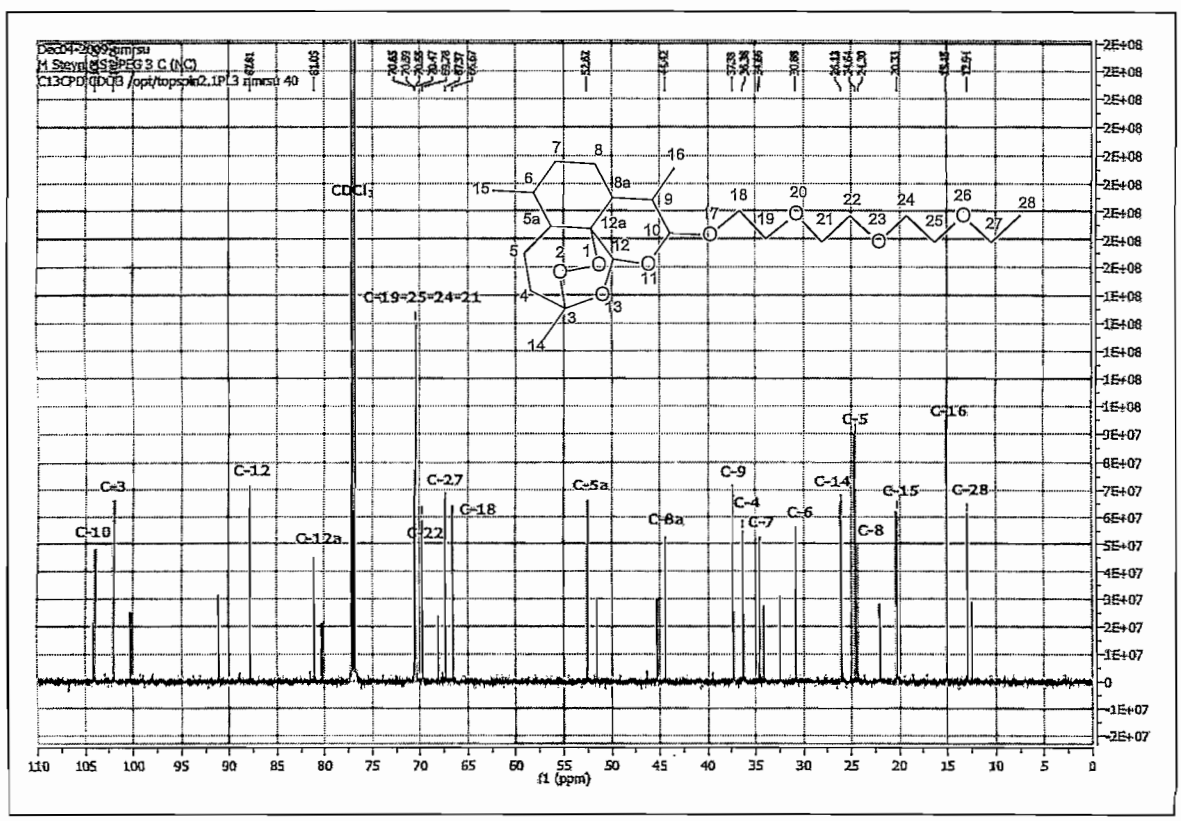
Spectrum 14: ^{13}C NMR of (7)



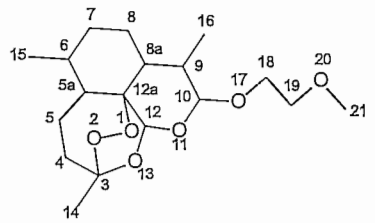
Spectrum 15: ^{1}H NMR of (8)



Spectrum 16: ^{13}C NMR of (8)



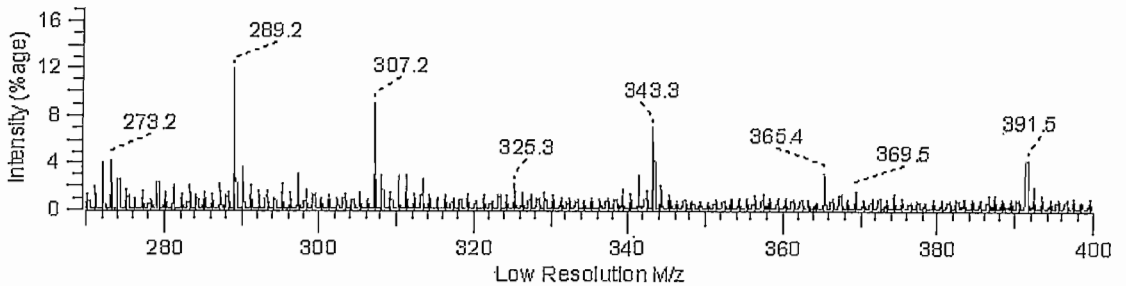
Spectrum 17: FAB-MS of (3)



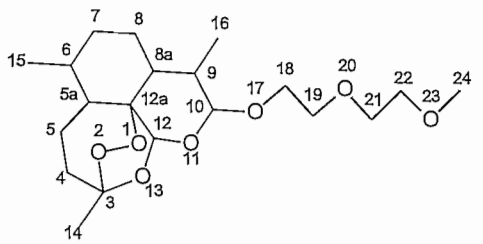
$C_{18}H_{30}O_6$

342.43

343.3 ($M+H^+$), 356.4 ($M+Na^+$)



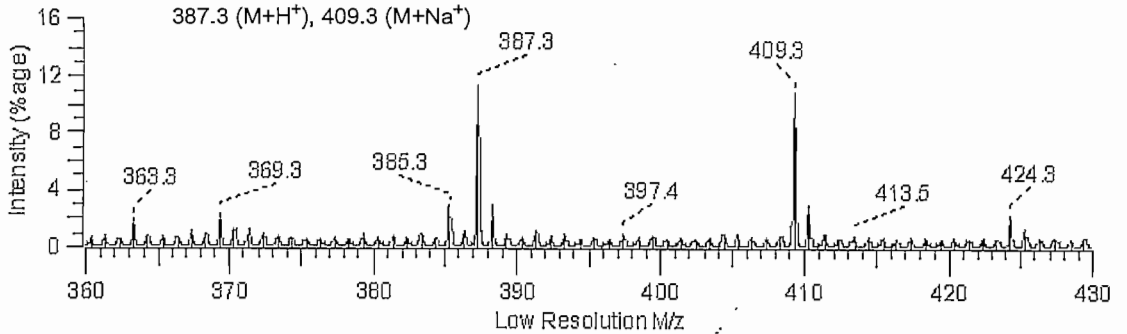
Spectrum 18: FAB-MS of (4)



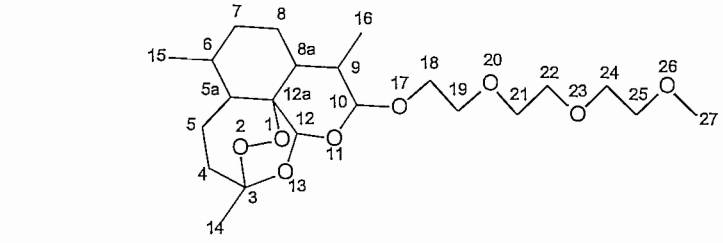
$C_{20}H_{34}O_7$

386.48

387.3 ($M+H^+$), 409.3 ($M+Na^+$)



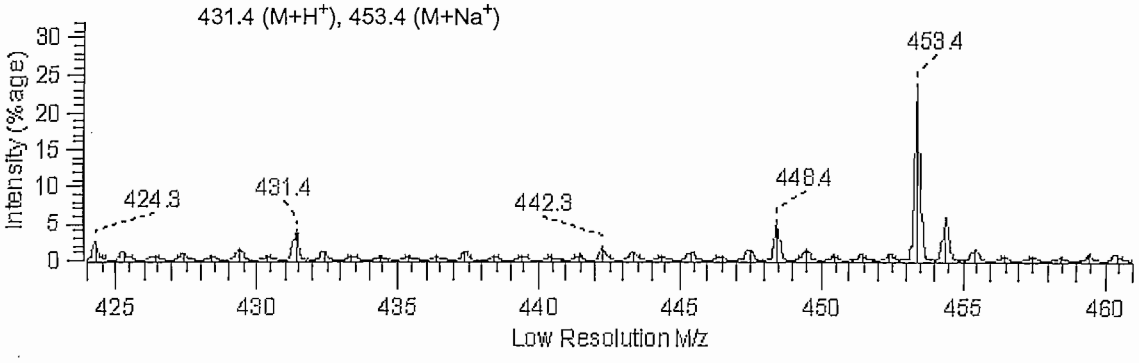
Spectrum 19: FAB-MS of (5a)



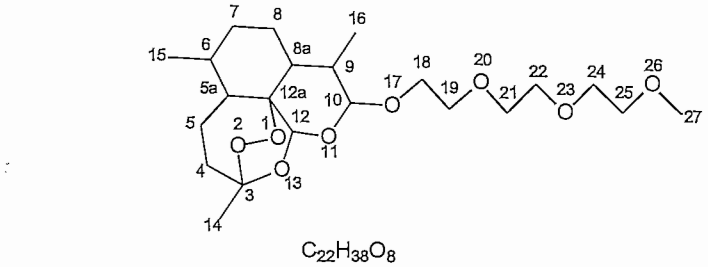
$C_{22}H_{38}O_8$

430.53

431.4 ($M+H^+$), 453.4 ($M+Na^+$)



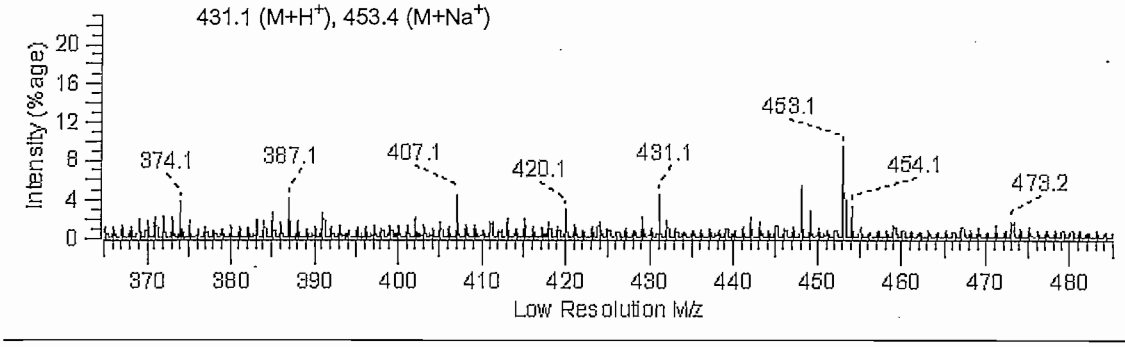
Spectrum 20: FAB-MS of (5b)



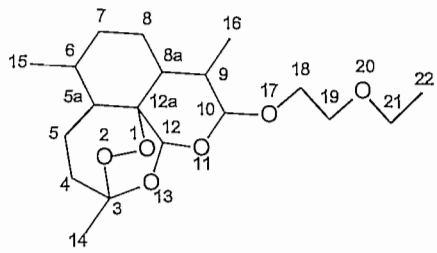
$C_{22}H_{38}O_8$

430.53

431.1 ($M+H^+$), 453.4 ($M+Na^+$)



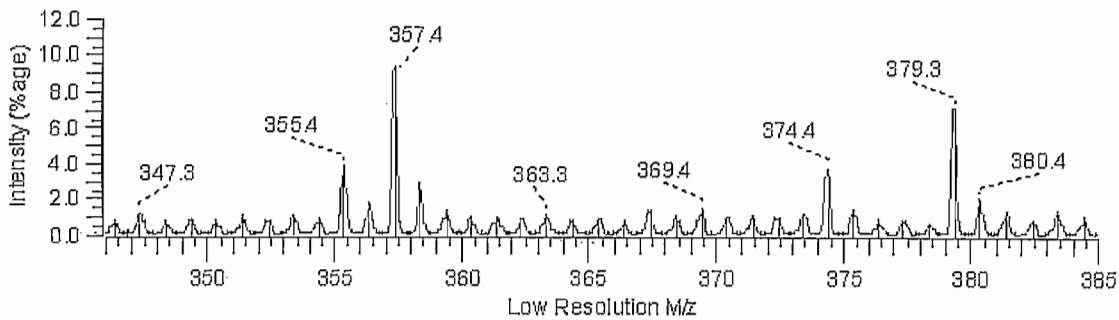
Spectrum 21: FAB-MS of (6a)



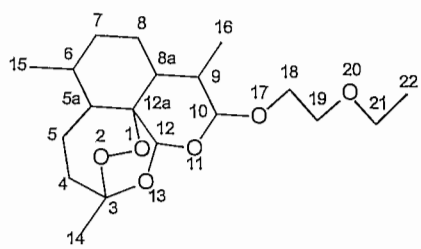
$C_{19}H_{32}O_6$

356.45

357.4 ($M+H^+$), 379.3 ($M+Na^+$)



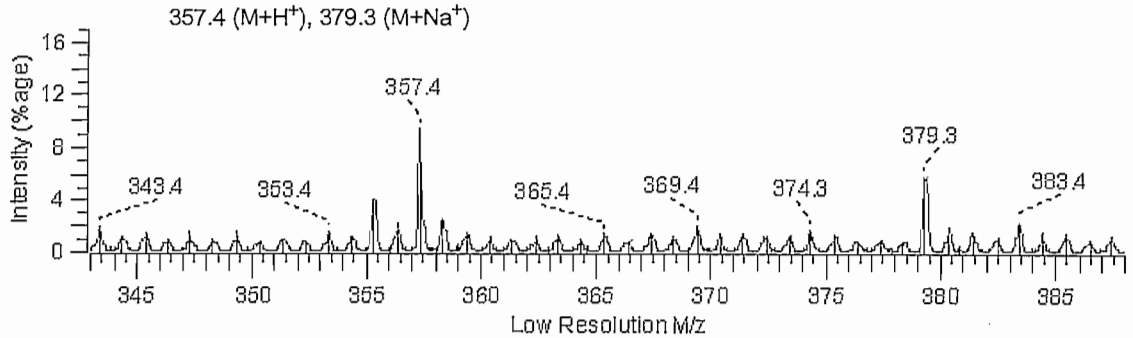
Spectrum 22: FAB-MS of (6b)



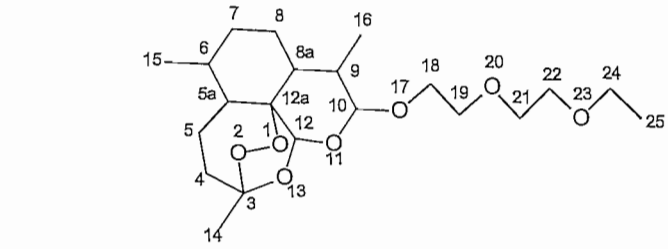
$C_{19}H_{32}O_6$

356.45

357.4 ($M+H^+$), 379.3 ($M+Na^+$)



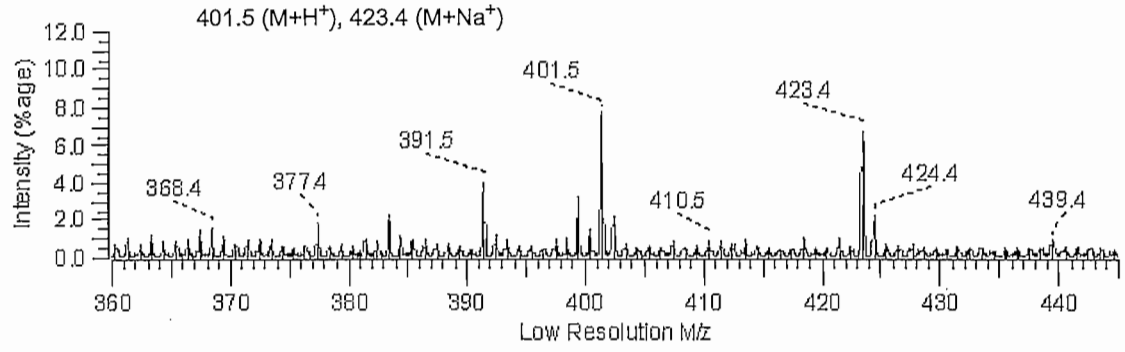
Spectrum 23: FAB-MS of (7)



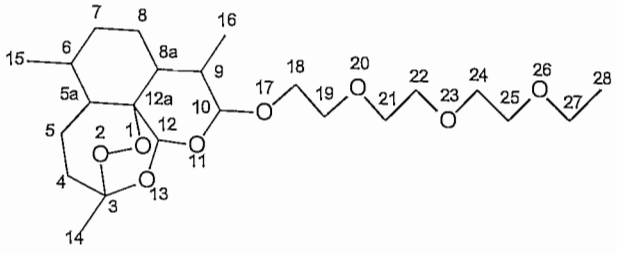
$C_{21}H_{36}O_7$

400.51

401.5 ($M+H^+$), 423.4 ($M+Na^+$)



Spectrum 24: FAB-MS of (8)



$C_{23}H_{40}O_8$

400.51

467.0 ($M+Na^+$)