CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

Malaria remains a significant public health risk in endemic countries. Being a complex infectious disease, malaria is caused by a unicellular microorganism of the genus *Plasmodium.* The epidemiology, clinical manifestation and antimalarial susceptibility of malaria are species specific. Five species of the *Plasmodium* genus are known to cause human malaria, namely *P. falciparum, P. viviax, P. ovale, P. malariae and P. knowlesi*. Transmission occurs during a blood meal by an infected, female Anopheles mosquito. *Plasmodium falciparum* (*P. falciparum*) infection is the most prevalent and the cause of severe complications, resulting in 91% of all reported malaria cases. *Plasmodium vivax* is more benign, but has a larger geographical distribution (WHO, 2012).

Young children, pregnant women and non-immune visitors to malaria areas are most affected by this disease. Control and elimination strategies exist, but none are appropriate and infallible, as a successful combination is yet to be established as a single, global solution. Individually, designed strategies for specific environments should consider various contributing factors and the financial resources needed for successful deployment.

The fight against drug resistant malaria has become an essential strategy for malaria control and elimination today. The emergence of drug resistant malaria has been implicated in the surge of geographical distribution and the re-emergence of malaria in eradicated areas. The disparity between the humanitarian importance of malaria and the amount of resources invested, necessitates the development and implementation of novel, effective antimalarial drugs.

2.2 Geographical prevalence

Malaria is a devastating disease, causing high morbidity and mortality. The World Health Organization (WHO) estimated that 3.3 billion people live in malaria affected areas and are at risk, with 660 000 malaria related deaths reported in 2011 (WHO, 2012).

The prevalence and distribution of malaria is especially concentrated in the tropical and sub-tropical areas of sub-Saharan Africa, the Middle East, Latin America, the Indian sub-continent,

South-East Asia and Oceania (Figure 2.1). The disease is classified as endemic in 104 countries, with the sub-Saharan countries being most affected. The spread of drug resistant strains is the key factor in the resurgence and distribution of malaria in these geographical areas. 81% and 91% of the reported malaria cases and deaths, respectively, occurred in the African region, with children under five years and pregnant women still inexorably affected (WHO, 2012).

The number of people at risk of contracting malaria increases as the human population expands. During the 20th Century, the human population expanded from 1 - 6 billion people. In the 21st Century, therefore, 48% of the global population would be at risk of contracting malaria (Hay *et al*., 2004). There has been an evident reduction in malaria transmission in certain parts of sub-Saharan Africa, due to the Roll Back Malaria Partnership and the President's Malaria Initiative. However, progress has not been uniformly among and within countries (Mharakurwa *et al*., 2012).

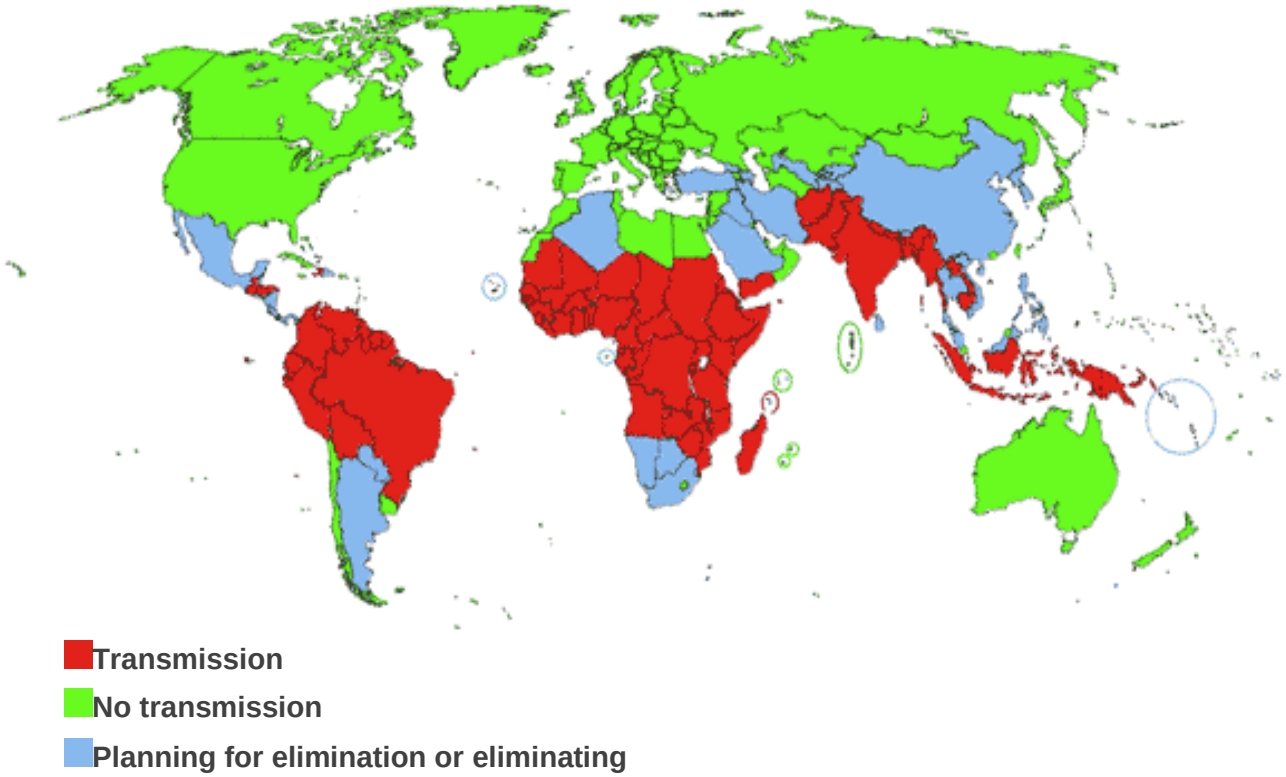

Figure 2.1: Areas of malaria transmission (Tambo *et al*., 2012).

2.3 South Africa

South Africa is generally malaria free (Figure 2.2), with 10% of the general population, i.e. approximately 5 million people, at risk (Moonasar *et al*., 2011). Out of the nine provinces, three are malaria endemic (Figure 2.3), i.e. KwaZulu-Natal, Limpopo and Mpumalanga. The North-West and Northern Cape provinces are affected with limited focal transmission (Moonasar *et al*., 2012; WHO, 2012). Malaria notifications in the Gauteng province are considered as traveller's malaria. Malaria transmission mainly occurs in the North-Eastern borders of the country during the rainy summer season, peaking from January to April (Moonasar *et al*., 2012; WHO, 2011).

Malaria in South Africa is well under control and well contained within endemic areas (Moonasar *et al.,* 2012; WHO 2011).

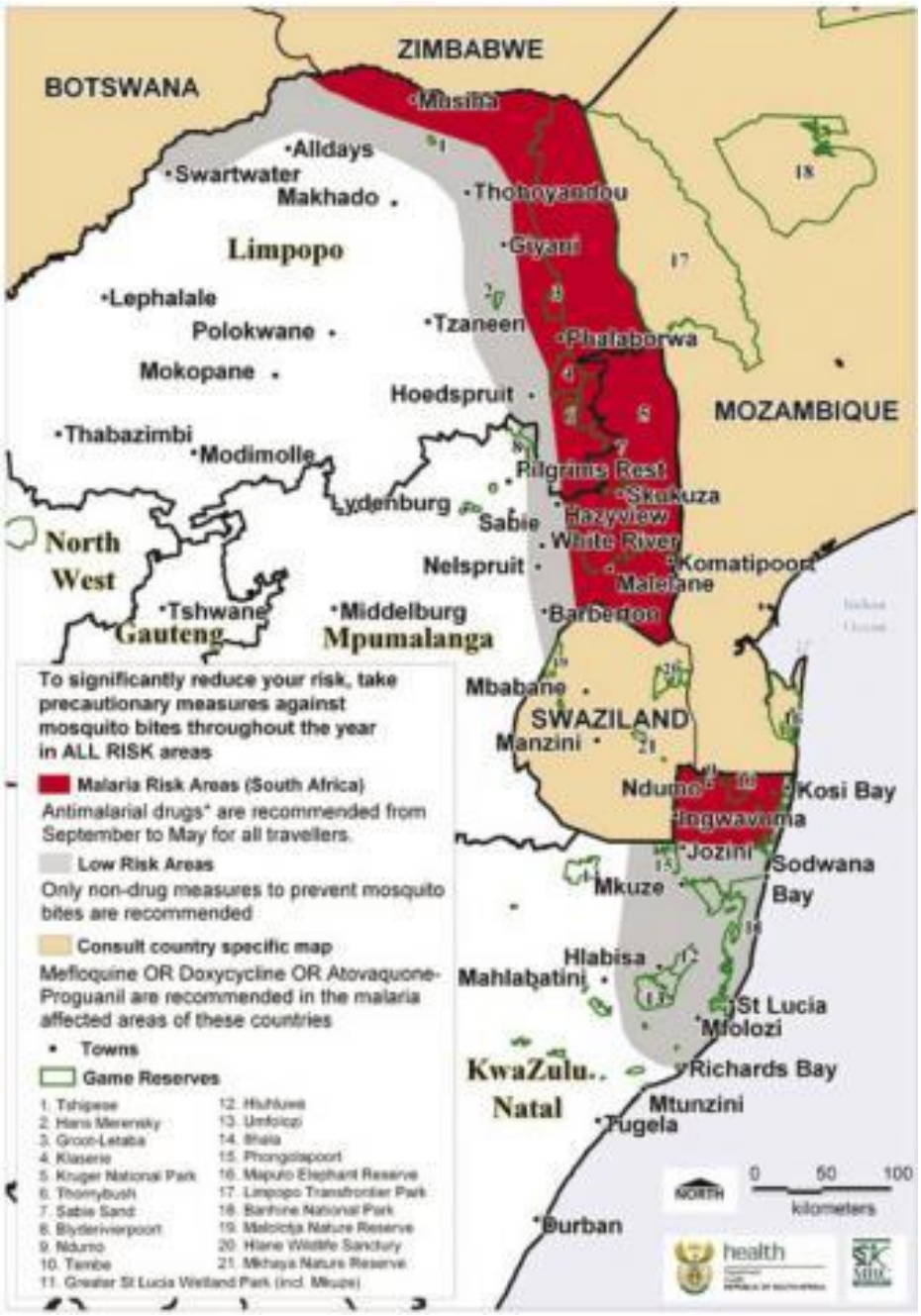

Figure 2.2: Malaria risk in South Africa (Moonasar *et al*., 2011).

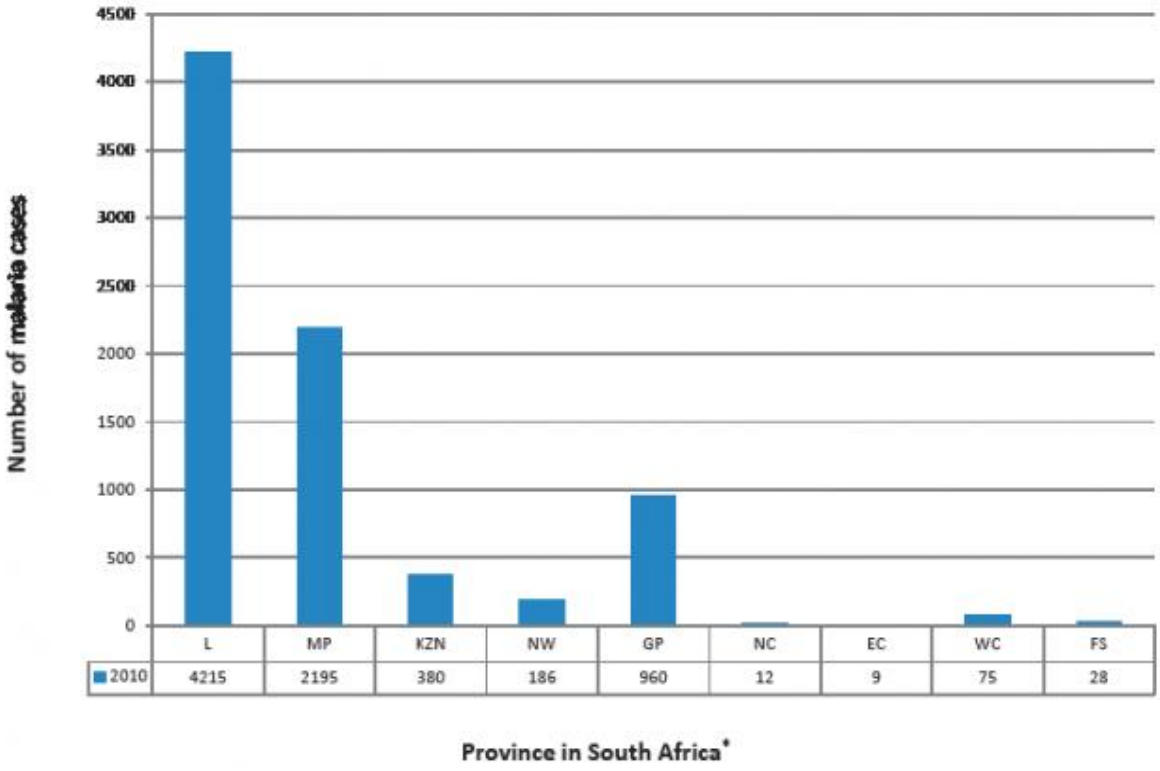

Figure 2.3: Malaria notifications for South Africa in 2010. Provinces: Limpopo (L); Mpumalanga (MP); KwaZulu-Natal (KZN); North-West (NW); Gauteng (GP); Northern Cape (NC); Eastern Cape (EC); Western Cape (WC); Free State (FS) (Moonasar *et al*., 2011).

2.4 Clinical manifestations

The clinical manifestations of uncomplicated malaria are non-specific and may present the following symptoms: tachycardia, tachypnea, chills, malaise, fatigue, diaphoresis, headaches, cough, anorexia, nausea, vomiting, abdominal pain, diarrhoea, arthralgias and myalgias (White & Breman, 2008). Complicated malaria, however, may present the following more severe manifestations: altered consciousness with or without seizures, respiratory distress or acute respiratory distress syndrome, circulatory collapse, metabolic acidosis, renal failure, haemoglobinuria (blackwater fever), hepatic failure, coagulopathy with or without disseminated intravascular coagulation, severe anaemia, or massive intravascular haemolysis and hypoglycaemia (Reyburn *et al*., 2005; White & Breman, 2008; WHO, 2012).

2.5 Diagnosis

The rapid and accurate diagnosis of malaria is an integral part of the efficient treatment of the disease. Due to the non-specific nature of malaria symptoms, the implementation of diagnostic methods is cardinal. A positive physical examination must therefore always be confirmed by laboratory results to ensure a sound diagnosis. Diagnosis is made *via* employing various methods, such as clinical diagnosis, microscopy, antigen based rapid diagnostic tests and serology.

2.5.1 Clinical diagnosis

A positive differential diagnosis can be made through identification of the clinical manifestations of malaria, but laboratory tests should be performed as confirmation. The early clinical manifestations are ambiguous and include symptoms, such as fever, chills, sweating, headaches, muscle pain, nausea and vomiting. Clinical symptoms of advanced stage *P. falciparum* malaria include confusion, coma, neurological focal signs, severe anaemia and respiratory difficulties (White, 2002; Wongsrichanalai *et al*., 2007).

2.5.2 Microscopy

Microscopy can be regarded as the “golden standard” for malaria diagnosis. It is still the most cost effective diagnostic method, with the added ability of differentiating between species and quantifying parasite density. Simple light microscopic examinations of thin and thick blood films (Romanovsky, Giemsa and Wright’s stain) are examined to determine the presence of a malaria infection. Thin films allow for species specific identification. Small quantities of blood are used to determine distinct parasite characteristics during stage development. Thick films are used for parasitemia quantification and comprise a more sensitive diagnostic method for ascertaining low levels of infection, since larger volumes of blood are inspected. (White, 2002; Wongsrichanalai *et al*., 2007; WHO, 2010). The effectiveness of blood smears is limited, as *P. malariae* and *P. knowlesi* display similar characteristics under the microscope. Low skill levels of personnel, and inadequate equipment and reagents can further decrease the efficacy of microscopy (Harchut *et al*., 2013).

2.5.3 Antigen based rapid diagnostic tests (RDT's)

Antigen based rapid diagnostic tests (RDT's) provide an effective diagnostic method to determine malaria in rural environments, with low skill levels of laboratory staff and inadequate equipment. Finger or venous blood is used and a positive result is visually determined by colour change on the dipstick (Warhurst & Williams 1996; Moody, 2002; Murray & Bennett, 2009). RTD's are immune-chromatographic tests that detect specific malaria antigens (i.e. histidine-rich protein 2 and *P. falciparum* specific parasite lactate dehydrogenase) in blood samples and yield rapid and highly sensitive diagnosis of *Plasmodium* infection (Maltha *et al*., 2010). In 2010, more than 50 million RTD's were reported in the African and South-Eastern Asian regions (WHO, 2010).

2.5.4 Serology

Serology is employed to measure past exposures to malaria. By employing indirect immune-fluorescence (IFA), or enzyme linked immune-sorbent assay (ELISA), antibodies against the malaria parasite are detected and then used to measure past exposure. Serology is not effective in cases of acute malaria, as detectable levels of malaria antibodies do not appear until weeks after infection. Antibodies also persist long after parasitemia has resolved. Moreover, the test is relatively expensive and not widely available (White, 2002; Wongsrichanalai *et al*., 2007).

2.6 Life cycle

The life cycle of *Plasmodium* parasites is complex and has adapted and specialised to endure various conditions in the two hosts, i.e. the female Anopheles mosquito and the human. The life cycle is divided into two asexual (schizogony) and one sexual (sporogony) stages. The sexual stage transpires in the vertebrate host, whilst the asexual stages transpire in the mosquito (Borstnik *et al.*, 2002; Frédérich *et al.*, 2002), as illustrated in Figure 2.4.

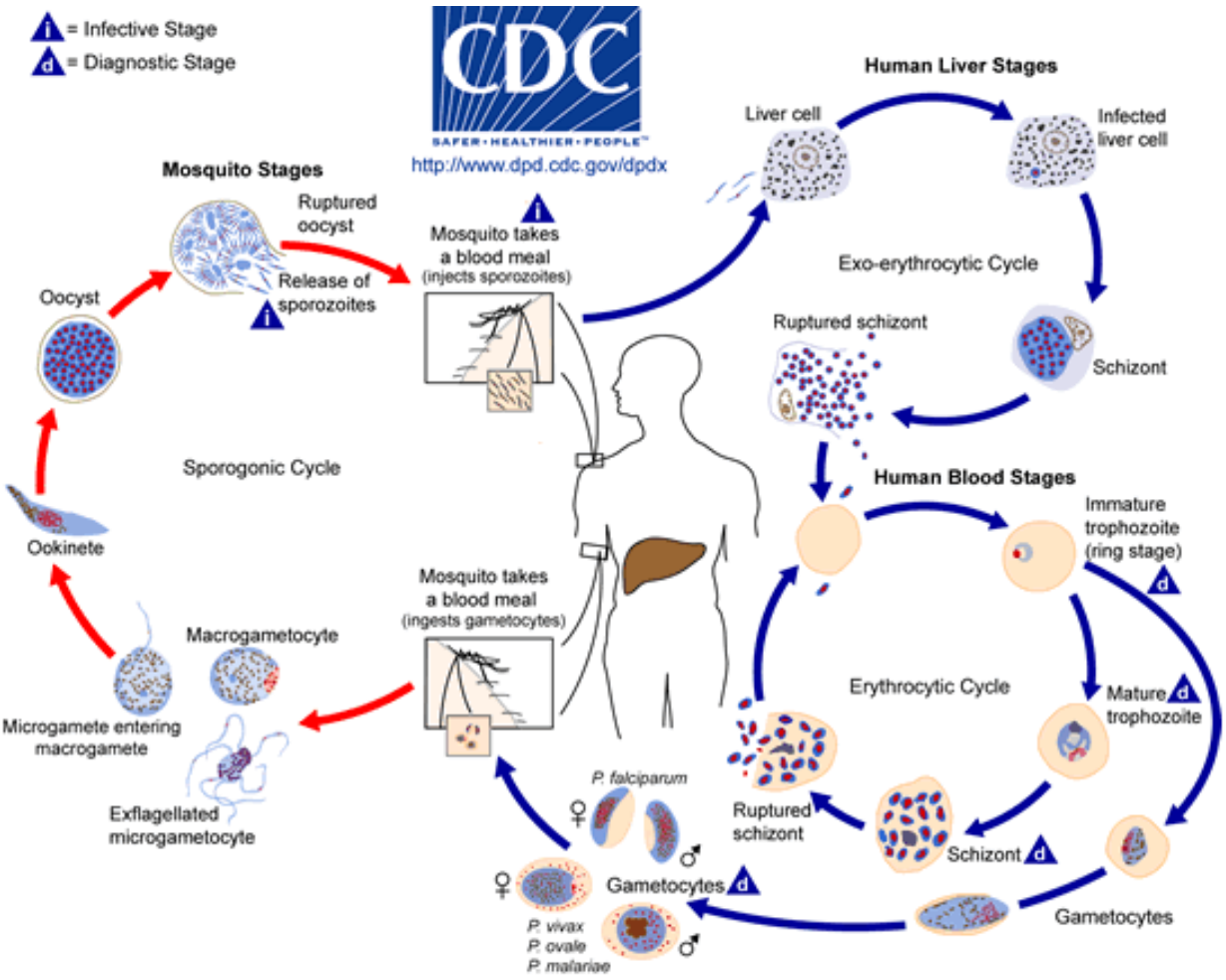

Figure 2.4: The life cycle of the malaria parasite (CDC, 2009).

2.6.1 Plasmodium falciparum life cycle in the human host

Transmission occurs during feeding, when the female mosquito inoculates the plasmodial sporozoites into the uninfected vertebrate host. To aspirate blood, the mosquito must probe through the skin in search of vascular space. The mosquito's saliva acts as a carrier for the small motile sporozoites (White, 2002; Opsenica & Šolaja, 2012). Following inoculation, the sporozoites migrate from the vascular space to the hepatic parenchymal cells *via* vascular or lymphatic pathways. Infection of the hepatic parenchymal cells occurs within 45 minutes of the mosquito's blood meal.

Sporozoites infiltrate the hepatocyte, epithelial parenchymatous cells of the liver, where asexual reproduction transpires. The duration of asexual reproduction is dependent on the type of *Plasmodium* genus, but averages six (*P. falciparum*) to fifteen (*P. malariae*) days. The hepatic schizonts rupture, due to the build-up of newly formed merozoites, liberating hundreds to thousands of merozoites into the circulatory system. Even though numerous merozoites are

released, hepatocyte infections are limited, leading to an asymptomatic hepatic stage. *Plasmodium vivax* and *P. ovale* have the ability to undergo no further hepatic development and form hypnozoites, causing the relapse in malaria.

When merozoites are released from the hepatocyte, they swiftly invade and attach themselves onto erythrocytes, orientating their apical complexes adjacent to the erythrocytes' membranes, which leads to the formation of a vacuole. Thereafter, the merozoites impregnate erythrocytes through their wriggling and boring motion. Erythrocyte binding proteins mediate the internal binding of the merozoite to the erythrocyte.

In the early stage of intra-erythrocytic development, the ring forms of all four parasite species are similar. Consummation of the erythrocyte occurs as the parasite increases in size and differentiation between the five genera of *Plasmodia* transpires. During development, the *P. falciparum* infected erythrocyte displays a *P. falciparum* erythrocyte membrane protein 1 *(Pf*EMP1) on its exterior surface. This antigen facilitates the adherence of the erythrocyte to vascular endothelium (sequestration), which is characteristic of *P. falciparum* infections. *Plasmodium falciparum* erythrocyte membrane protein 1 expression increases towards the middle of the 24-hour cycle.

As the parasites mature, *P. vivax* and *P. ovale* infected erythrocytes become distorted. Characteristic, readily identifiable red granules, known as Schuffner's dots, appear throughout the infected erythrocyte. *Plasmodium malariae* and *P. knowlesi* produce characteristic band forms during parasite maturation. The increase in parasitic growth and metabolic processes contribute towards the destruction of the erythrocyte. Finally, the erythrocyte ruptures and releases between 6 - 36 merozoites. The merozoites invade new erythrocytes, signalling the start of a new asexual stage. The asexual life cycles are approximately 24 hours for *P. knowlesi*, 45 hours for *P. falciparum*, *P. vivax* and *P. ovale* and 72 hours for *P. malariae* (White, 2002).

After various asexual stages of development cycles, 1% of merozoites invades erythrocytes and differentiates into gametocytes, signalling the start of the sexual stage. Transmission of the malaria parasite occurs in the gametocyte form.

2.6.2 Plasmodium falciparum life cycle in the mosquito

For infection to occur, one male and one female gametocyte must be inoculated with the female mosquito's blood meal. Gametocyte activation occurs in the mosquito's gut (mid-gut lumen). Male and female gametocytes undergo fusion and meiosis to procreate a zygote. This zygote then penetrates the mosquito's mid-gut epithelial cells, encysting (as an oocysts) within 24 hours. A mature oocyst contains thousands of fusiform mobile sporozoites. The dissolution of the oocysts liberates the sporozoites into the body cavity of the mosquito from where they transmigrate to the salivary glands of the mosquito, awaiting inoculation into the next host (White, 2002; Opsenica & Šolaja, 2012).

2.7 Malaria elimination, control and treatment

Worldwide eradication campaigns from 1940 to 1970 attempted to control and manage malaria, but the emergence of drug resistant malaria halted the efficacy of these programs. Recently, there has been an evident reduction in malaria transmission in certain parts of sub-Saharan Africa, due to the Roll Back Malaria Partnership, the President's Malaria Initiative and other similar campaigns (Mharakurwa *et al*., 2012).

One aspect of malaria control measures is vector control interventions. Vector control can either comprise insecticide treated nets (ITN's), or indoor residual spraying (IRS). Although the importance and efficacy of neither ITN's, nor IRS are debatable, the viability of replacing ITN's (maximum life span five years) to millions of households, as well as the potentially negative health and environmental impacts of IRS, pose risks to their future feasibility (WHO, 2012; Pinto & Machado, 2013).

The feasibility of a malaria vaccine had first been demonstrated in the 1970's. New initiatives of vaccine research and drug discovery that had been launched in the 1990's were spearheaded by Roll Back Malaria, the Medicines for Malaria Venture, Multi-lateral Initiative on Malaria and the Malaria Vaccine Initiative. Since their discovery, malaria vaccines have made good progress, with a number of candidates currently undergoing clinical trials (Nussenzweig & Long, 1994). These include pre-erythrocytic stage, asexual stage and transmission blocking vaccines (Bergmann-Leitner *et al*., 2006; Smith *et al*., 2006). Despite the enormous financial and research efforts being put into the development of malaria vaccines, malarial therapy currently is still totally reliant on the use of chemotherapy.

Antimalarial drugs possess selective actions at different stages of the parasite life cycle, namely:

- *Blood schizonticides*: Antimalarial drugs that act on erythrocytic parasites, by eliminating blood schizonts in the erythrocytes during the erythrocytic stage.

- *Tissue schizonticides*: Drugs that prevent invasion of malaria parasites into erythrocytes, by eliminating developing tissue schizonts, or hypnozoites in the liver.

- *Gametocides*: Drugs that destroy the sexual forms of the parasite in the blood and prevent transmission to mosquitoes.

- *Sporontocides*: Antimalarial drugs that prevent the development of oocysts in the mosquito and render gametocytes non-infective (Katzung, 2001).

2.8 Chemotherapy

Currently used chemotherapies are from seven drug classes, namely antifolates and sulphas, 4-aminoquinolines (chloroquine), arylaminoalcohols (quinine, mefloquine), 8-aminoquinolines (primaquine), antibiotics (doxycycline), artemisinins (artemisinin, artemether, artesunate, dihydroartemisinin) and hydroxynaphthoquinones (Schlitzer, 2008; Rudrapal, 2011). Segregation of antimalarial drugs into groups is according to differences in the pharmacophores, mechanisms of action and selective actions on the parasites' development stages.

2.8.1 Antifolates and sulphas

The *Plasmodium* parasite's *de novo* folate synthesis provides an exceptional species specific target for antimalarials (Müller & Hyde, 2013). Organisms that cannot synthesise *de novo* folate (**1**) need to liberate themselves from exogenous sources (Salcedo-Sora & Ward, 2013). Antifolates selectively inhibit steps during folate metabolism (Figure 2.6) (Müller & Hyde, 2013), where the inhibition of enzymes eradicates essential folate co-enzymes in the parasite (Yuthavong, 2002). This results in a perfect drug target for antimalarial treatment. Folic acid (Figure 2.5), an essential nutrient supplied by dietary intake in humans, needs to be synthesised in *Plasmodium* parasites. This is mediated by the production of dihydrofolic acid from dihydroperteroic acid. Folic acid is composed of three primary structures, namely a hetero-bicyclic pteridine ring, para-aminobenzoic acid (PABA) and glutamic acid (Müller & Hyde, 2013; Salcedo-Sora & Ward, 2013).

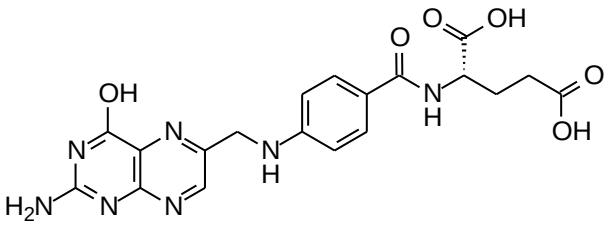

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Figure 2.5: Structure of folic acid (**1**).

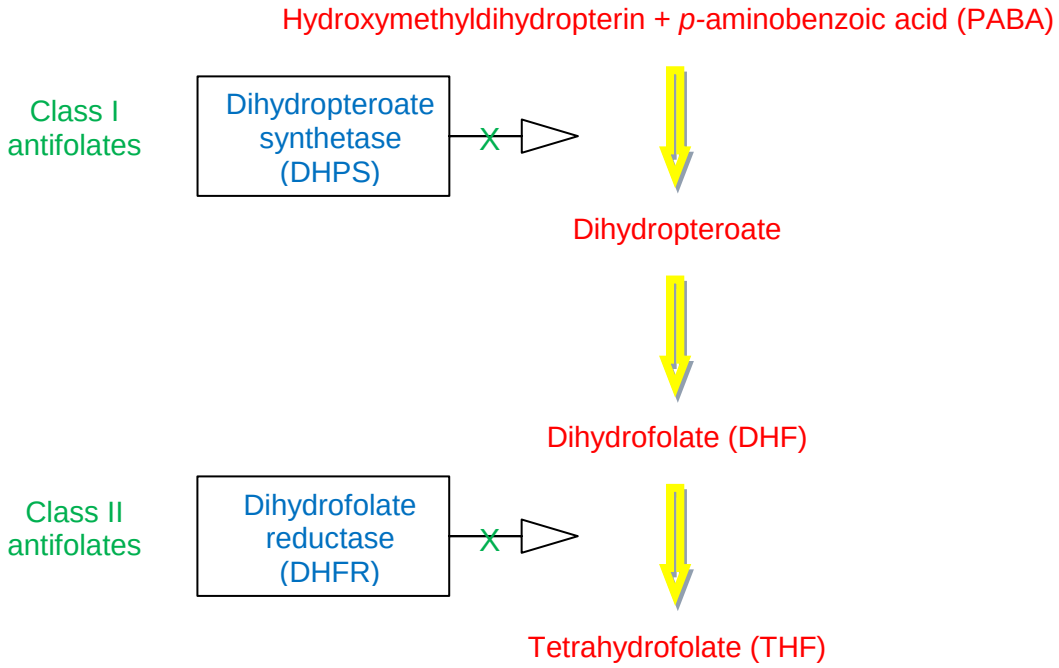

Figure 2.6: Biosynthetic pathway of tetrahydrofolic acid.

The antifolates and sulphas can be classified into two sub-classes, based on their modes of action, namely:

- *Class I*: Sulphonamides, sulphadoxine, sulphones, dapsone and sulphalene.
- *Class II*: Proguanil, chlorproguanil, cycloguanil and pyrimethamine.

2.8.1.1 Class I

Sulphonamides and sulphones mimic PABA and disrupt folic acid synthesis (Figure 2.6). The class I inhibitors compete with mammalian enzyme DHFS on the active binding site. The inability of the parasite to synthesise folic acid leads to cell death. The resultant synergisms from combining pyrimethamine and sulphonamides, or sulphones are postulated to the

probability of inhibiting two critical steps in a metabolic pathway (Olliaro, 2001). Although these drugs are blood schizonticides, resistance in monotherapy can develop rapidly. Fixed combinations with pyrimethamine include dapsone (**2**) (Maloprim®), sulphadoxine (Fansidar®) and sulphalene (**3**) (Metakelfin®) (Nzila, 2006; Shapiro & Goldberg, 2006).

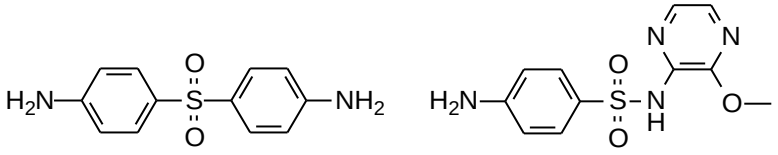

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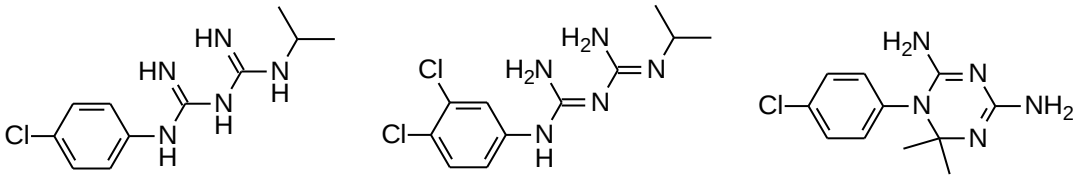
Figure 2.7: Structures of dapsone (**2**) and sulphalene (**3**).

2.8.1.2 Class II

In 1945, British antimalarial research had led to the discovery of chloroguanidine, more readily known as proguanil (**4**). This is a biguanide derivative, consisting of a cyclic triazine metabolite that provides the antifolate its action of antimalarial activity. Class II inhibits DHFR that facilitates the enzymatic reduction of 7,8-dihydrofolate (DHF) to 5,6,7,8-tetrahydrofolate (THF) (Nzila, 2006; Shapiro & Goldberg, 2006; Müller & Hyde, 2013).

The prodrug, chlorproguanil (**5**) (LAPUDRINE®), is more potent than proguanil (Nzila, 2006; Shapiro & Goldberg, 2006). A genetic deficiency of an oxidation phenotype occurs in 20% of Asians and Kenyans, and in 3% of Caucasians. CYP2C19 ability to facilitate the biotransformation of chlorproguanil into its active metabolite is inhibited, rendering the drug therapeutically inactive (Shapiro & Goldberg, 2006). Proguanil had demonstrated activity in both the liver and asexual red cell stages of *P. falciparum*. It has proven effective for acute *P. vivax* attacks, but unfortunately fails to diminish gametocytes and exhibits the rare ability to disrupt the development of fertilised gametes, encysted in the mosquito's gut (Nzila, 2006; Shapiro & Goldberg, 2006).



4 **5** **6**

Figure 2.8: Structures of proguanil (**4**), proguanil prodrug, chlorprogaunil (**5**) and proguanil's active metabolite, cycloguanil (**6**).

Responsible treatment regimens with proguanil can only occur when used in combination therapy. In sub-Saharan Africa, proguanil is used for the treatment of chloroquine and pyrimethamine-sulphadoxine resistant strains of *P. falciparum*. The proguanil and atovaquone combination (MALARONE®) is used for chloroquine and multi-drug resistant *P. falciparum* and *P. vivax* (Nzila, 2006; Shapiro & Goldberg, 2006).

A series of 2,4-diaminopyrimidines, structurally similar to progaunil, had been evaluated for their antimalarial activity against malaria parasites in the 1940's. Pyrimethamine (**7**) had then been selected for further testing, due to its promising therapeutic properties that included potent parasitic inhibition, an ability to overcome drug resistance and documented synergistic antimalarial activity (White, 2002; Nzila, 2006; Shapiro & Goldberg, 2006). Pyrimethamine is a slow acting blood schizontocide that inhibits the mature trophozoite stage with pre-erythrocytic sporontocidal activity (White, 2002; Shapiro & Goldberg, 2006). In comparison to cycloguanil, pyrimethamine has similar antimalarial activity, with both a higher potency and longer half-life, but a decreased activity against *P. falciparum* hepatocytes. It completely fails at eradicating hypnozoites or gametocytes (White, 2002; Shapiro & Goldberg, 2006; Hawkins *et al*., 2007).

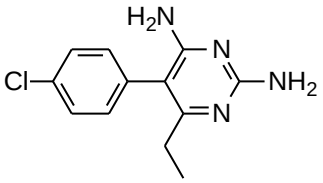


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Figure 2.9: Structures of pyrimethamine (**7**).

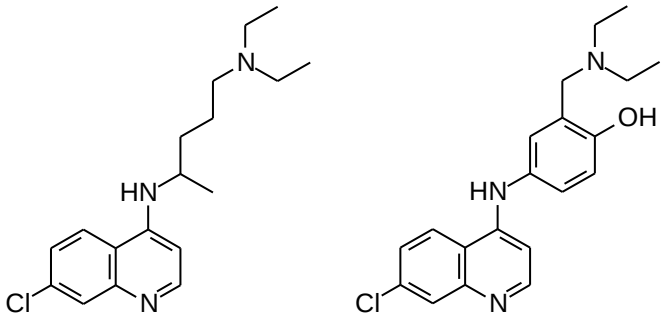
2.8.1.3 Resistance to antifolate drugs

Specific point mutations in the *dhfr* or *dhps* domains are linked to drug resistance. Point mutations in *dhfr* domain amino acids N511, C59R, S108N and I164L are associated with pyrimethamine resistance. For sulpha resistance, amino acid mutations on S436/F, A437G, K540E, A581G and A613S/T are reported. Similarly, mutations on A16V and S108T for proguanil and A437G, K540E and A581G for sulphadoxine are noted. These mutations cancel folate antagonists' intrinsic activities, as the mutated enzymes cannot be recognised (Mberu *et al.,* 2002; Hawkins *et al*., 2007; Müller & Hyde, 2013).

2.8.2 4-Aminoquinolines

During World War II, the American soldiers had suffered a vast number of deaths, attributed to malaria. In 1943, chloroquine (**8**) had distinguished itself as the most promising compound out of thousands that had been synthesised and screened (Ponts & Le Roch, 2013).

The 4-aminoquinolines are weak bases, diprotonated and hydrophilic at a neutral pH. Protonation of the weak base drug in the parasite's acid food vacuole results in the protonated compound being unable to permeate through the lipophillic biological membranes. Consequently, the drug localises in the parasite's digestive vacuole (White, 2002; van Heerden *et al*., 2012). Chloroquine inhibits both haem dimerisation and glutathione mediated haem degradation, interfering with the parasite's haem detoxification pathway (White, 2002). Chloroquine binds to free ferriprotoporphyrin IX, a waste product of haem metabolism, inhibiting the sequestration of toxic ferriprotoporphyrin IX into non-toxic haemozoin (Pretorius *et al*., 2013). Chloroquine mediated DNA intercalation occurs only in concentrations exceeding the required concentration for the antiprotozoal effect (White, 2002).



8 **9**

Figure 2.10: Structures of the 4-aminoquinoline compounds, chloroquine (**8**) and amodiaquine (**9**).

Chloroquine is a cost effective and easily available drug that can be used as prophylactic treatment, or as treatment in the absence of resistance. It is indicated in the treatment against the erythrocytic forms and gametocytes of *P. vivax*, *P. malariae*, *P. ovale* and *P. falciparum.* Chloroquine is ineffective against *P. falciparum* gametocytes (Shapiro & Goldberg, 2006; Ponts & Le Roch, 2013). It is also ineffective against the latent tissue forms of *P. vivax* and *P. ovale* (Shapiro & Goldberg, 2006). Amodiaquine (**9**), a 4-aminoquinoline, is an inexpensive, readily available drug and is effective against chloroquine resistant strains of *P. falciparum* (Adjuik *et al.,* 2002; White, 2002; Shapiro & Goldberg, 2006). Amodiaquine had been discovered in the 1950's, but was withdrawn in the 1970's, due to its toxicity (Johansson *et al.,* 2009).

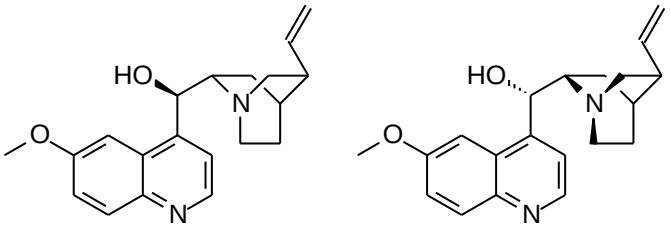
2.8.2.1 Resistance to 4-aminoquinolines

The development of resistance towards 4-aminoquinolines is connected to multiple parasite gene mutations, especially on codons A220S and K76T (Olliaro, 2001). Various resistance mechanisms have been elucidated that include the following: (1) altered drug accumulation mechanisms leading to sub-therapeutic drug concentrations in the digestive vacuole, (2) altered transport at the parasite cytoplasmic and/or digestive vacuole membrane and (3) altered proton flux or transporter at the digestive vacuole membrane (Olliaro, 2001).

2.8.3 Aryl-amino alcohols

Quinine (**10**), also known as peruvian, jesuit's, or cardinal's bark, is the main alkaloid in the bark of the cinchona tree. Since 1633, the powdered bark had been recognised for its antipyretic properties (Shapiro & Goldberg, 2006; Dinio *et al.,* 2012; Ponts & Le Roch, 2013).

Quinidine (**11**) is a stereoisomer of quinine. This dextrorotatory diastereomer has a higher intrinsic antimalarial activity than quinine. Extensive and dangerous side effects have rendered quinidine as a last choice antimalarial drug, when artemisinin or quinine is unavailable (White, 2002). Quinine's principal mode of action targets the asexual erythrocytic forms of all *Plasmodium* species. It has antigametocidal activity against *P. vivax* and *P. malariae*, with little to zero effect on hepatic forms of the *Plasmodium* parasite. Where *Plasmodium* parasites are susceptible to both drugs, quinine shows a higher toxicity and lower potency than chloroquine (Shapiro & Goldberg, 2006).

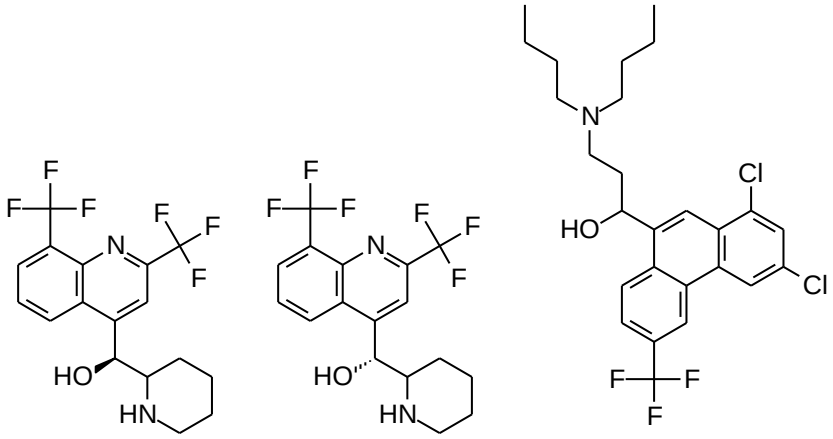


10 **11**

Figure 2.11: Structures of the diastereomers, quinine (**10**) and quinidine (**11**).

Quinine and quinidine are exceptionally valuable as parenteral treatments against drug resistant strains of *P. falciparum* (Shapiro & Goldberg, 2006) and have similar antimalarial mechanisms of action to that of chloroquine.

In 1963, the Walter Reed Army Institute for Medical Research (WRAIR) had tested quinoline methanols structurally related to quinine (White, 2002; Kitchener, 2003; Shapiro & Goldberg, 2006; Croft, 2007). Of the 250 000 compounds being screened, only mefloquine (**12**) and halofantrine (**13**) had been selected for further use (Croft, 2007). With high antimalarial activity and safety, mefloquine was earmarked for the treatment of drug resistant *P. falciparum*. Mefloquine is a blood schizonticide, (Schlagenhauf *et al.,* 2010), with no activity against hepatic forms or gametocytes of *P. falciparum*. It has no activity against latent tissue forms of *P. vivax* (Shapiro & Goldberg, 2006). Mefloquine's exact mechanism of action is still unknown, but is it hypothesised to be similar to that of chloroquine (Shapiro & Goldberg, 2006). FANSIMEF®, a combination of mefloquine and pyrimethamine-sulphadoxine, had failed to delay the development of drug resistant parasites in Thailand (Shapiro & Goldberg, 2006). The combination had shown no pronounced beneficial therapeutic activity, similar to mefloquine monotherapy (White, 2002). Loss of activity could be attributed to mefloquine's slow elimination time, thus presenting parasites with sub-therapeutic drug levels (Shapiro & Goldberg, 2006).



12 **13**

Figure 2.12: Structures of mefloquine (**12**) as a racemate and halofantrine (**13**).

Halofantrine is a 9-phenanthrene methanol chiral molecule with similar activity as the quinoline antimalarials (White, 2002; Shapiro & Goldberg, 2006). Side effects, erratic pharmacokinetics and extensive cross resistance with mefloquine prevent its usage (White, 2002; Shapiro & Goldberg, 2006; Bouchaud *et al.,* 2009). Lumefantrine is used in combination with artemether (COARTEM^{TM}) as first line treatment for acute, uncomplicated *P. falciparum* malaria (Shapiro & Goldberg, 2006; Manyando *et al*., 2010).

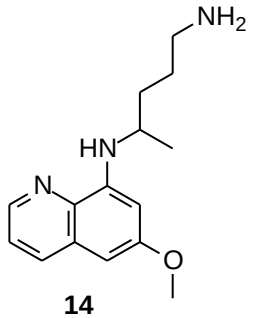
2.8.3.1 Resistance to aryl-amino alcohols

The increase of PfMDR1 and *P. falciparum* chloroquine resistance transporter (Pfcrt) gene expression, as well as multiple parasite gene mutations are indicative of the development of aryl-amino alcohol resistance (Witkowski *et al*., 2009).

2.8.4 8-Aminoquinolines

In 1891, Ehrlich had discovered that methylene blue exhibited weak plasmodicidal activity. Consequently, the 8-aminoquinolines were synthesised, based on his finding (Ehrlich, as quoted by Shapiro & Goldberg, 2006). World War II had prompted the United States to search for a more potent, but less toxic 8-aminoquinoline (Vale *et al.,* 2009). Primaquine (**14**) had then been selected on the basis of its exo-erythrocytic activity. It had prevented the relapse of malaria, but had a devastating effect on patients' erythrocytes. The erythrocyte toxicity of primaquine had then led to the discovery of G6PD deficiency (Shapiro & Goldberg, 2006; Vale *et al.,* 2009; Fernando *et al.,* 2011).

The destruction of the primary and latent hepatic stages of the parasite by primaquine prevents *P. vivax* and *P. ovale* relapse. Primaquine has activity against all four species of gametocytes. Combining a blood shizontocide with primaquine would beneficially cause total erythrocytic eradication and prevent the emergence of resistance (Shapiro & Goldberg, 2006; Vale *et al.,* 2009; Fernando *et al.,* 2011).



14

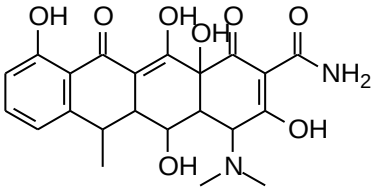
Figure 2.13: Structures of primaquine (**14**).

2.8.4.1 Resistance to primaquine

Primaquine has been used as antimalarial drug over the past fifty years, with no confirmed resistance (Baird & Hoffman, 2004). Confounding factors, such as the use of combination therapy, treatment duration and daily dosing regiments should be taken in account before resistance can be confirmed (Fernando *et al.,* 2011).

2.8.5 Antibiotics

The tetracyclines, discovered in the 1940's, are very potent antimalarials and are used for both treatment and prophylaxis. Antibiotics inhibit protein synthesis *via* the prevention of aminoacyl-tRNA attachment to the ribosomal acceptor site. Doxycycline (**15**) and tetracycline are slow acting blood schizontocides. Monotherapy of tetracyclines for malaria treatment is ineffective, but combining tetracyclines with quinine or quinidine increases the therapeutic uses of both drugs (White, 2002; Briolant *et al.,* 2008). To date, no resistance to the tetracyclines has been reported.

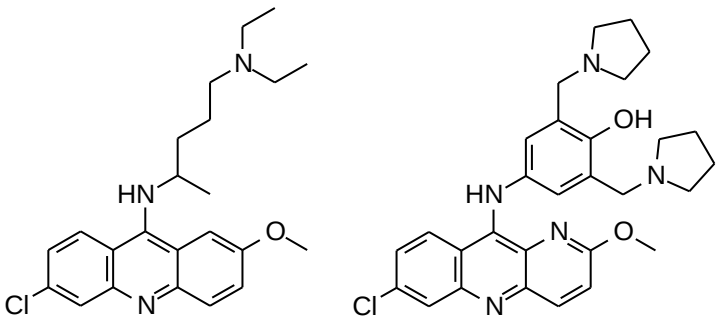


15

Figure 2.14: Structures of doxycycline (**15**).

2.8.6 Synthetic acridines

Acridine based compounds, with advanced antimalarial activity (Valdés, 2011), had been the foundation of antimalarial drug research (Pérez *et al*., 2013). During 1933, an intensive search for a synthetic surrogate of quinine had resulted in to the discovery of quinacrine (**16**) (Coggeshall, 1952; Pérez *et al*., 2013). Although quinacrine had consequently become the primary antimalarial drug used by the American soldiers during World War II, the discovery of chloroquine ever since had made quinacrine usage obsolete (Elueze, 1996). During the 1970's, the Institute of Parasitic Diseases Shanghai, had synthesised pyronaridine (**17**), which is still used as monotherapy in China today (Croft, 2012; Elueze, 1996). Pyronaridine is an azacrine type mannich base. It has antimalarial activity against *P*. *falciparum, P. vivax* and cerebral malaria, and against chloroquine, mefloquine and multi-drug resistant cases (Elueze, 1996). PYRAMAX®, ratio 3:1, is a fixed dose of pyronaridine and artesunate. It is indicated in the treatment of uncomplicated *P. falciparum* and blood stage infections of *P. vivax* (Kurth *et al.,* 2009; Croft, 2012). Pradines *et al*. (2010) assessed the *in vitro* cross resistance of pyronaridine with other antimalarials. Attempts were made to elucidate whether genetic polymorphisms in the genes, involved in the reduction of quinoline susceptibility, could apply to pyronaridine. Screened results reported the absence of cross resistance between pyronaridine and quinolines. As an end result, pyronaridine's IC_{50} values were unrelated to genetic polymorphisms in genes (Pradines *et al*., 2010), validating the absence of cross resistance occurrences.

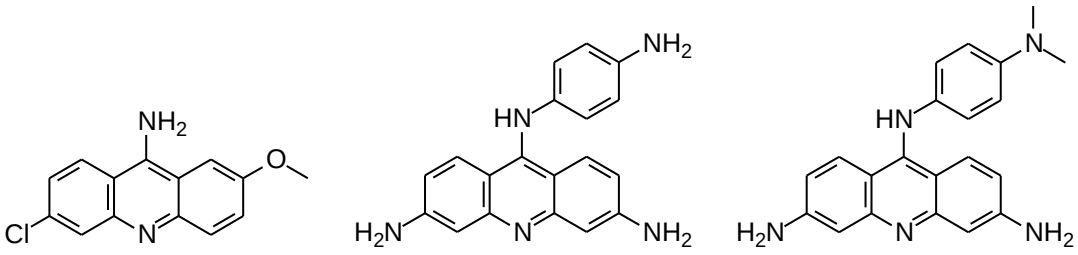


16 **17**

Figure 2.15: Structures of quinacrine (**16**) and pyronaridine (**17**).

Winter *et al*. (2006) had synthesised novel acridones and had evaluated their antimalarial activities against CQS and CQR strains of *P. falciparum* (Winter *et al*., 2006*).* The outcomes from screen tests of 2-methoxy-6-chloro-9-aminoacridine (**18**) were IC_{50} values of 18 nM and 42 nM against D6 and Dd2 strains of *P. falciparum*. Structure-activity relationship studies reported substitution on position 3 of the acridine pharmacophore, with an electron donating group and the increase in linker length, enhanced antimalarial activity. The high resistance index of most of the acridine derivatives illustrates acridine's tendency to lose activity from a sensitive to a resistant strain (Winter *et al.,* 2006).

Various synthetic acridines, with increased hydrophilic properties had been synthesised. Screen tests against the K1 CQR strain of *P. falciparum*, 3,6-diamino-1'-amino-9-anilinoacridine (**19**) and 1'-dimethylamino-3,6-diamino-9-anilinoacridine (**20**), exhibited IC_{50} values in the nanomolar range (Chavalitshewinkoon *et al.,* 1993; Auparakkitanon *et al*., 2003). The physicochemical properties of the compounds had promoted cell penetration.

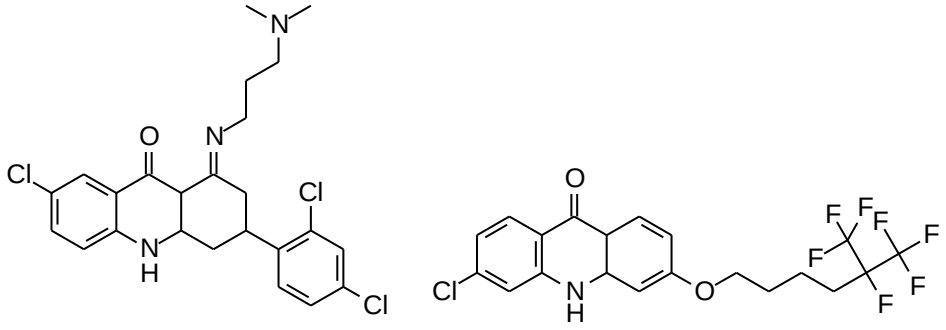


18 **19** **20**

Figure 2.16: Structures of 2-methoxy-6-chloro-9 aminoacridine (**18**) 3,6-diamino-1'-amino-9-anilinoacridine (**19**) and 1'-dimethylamino-3,6-diamino-9-anilinoacridine (**20**).

The quinacrine-floxacrine hybrid, WR 243251 (**21**) and 3-(5,6,6,6-Tetrafluoro-5-trifluoromethyl-hexyloxy)-6-chloroacridinone (**22**), showed nanomolar IC_{50} values with limited side effects

(Kesten *et al.,* 1992; Werbel *et al*., 1985; Biagini *et al.,* 2008). The reported IC_{50} values were 11 nM and 25 nM against CQS and CQR strains, respectively, whilst demonstrating WR 243251 blood schizonticidal potential by the inability to induce resistance (Berman *et al*., 1994). Coleman *et al*. (2001) had determined the ability of WR 243251 to inhibit both oocyst and sporozoite development and reported sporogonic development inhibition of *P. vivax* (Coleman *et al*., 2001). Atovaquone's cross resistance, however, halted further progress (Winter *et al*., 2006). 3-(5,6,6,6-Tetrafluoro-5-trifluoromethyl-hexyloxy)-6-chloroacridinone exhibited an IC_{50} value of 1 pM against the D6 CQS strain of *P. falciparum* and low cytotoxicity to murine splenic lymphocytes (Winter *et al*., 2006).



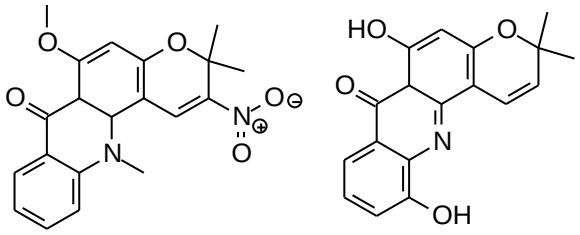
21 **22**

Figure 2.17: Structures of WR 243251 (**21**) and 3-(5,6,6,6-Tetrafluoro-5-trifluoromethyl-hexyloxy)-6-chloroacridinone (**22**).

2.8.6.1 Acridine alkaloids

Acridinone alkaloids are isolated from plants of the *Rutaceae* family. Acronycine, 5-hydroxyacronycine and 2-nitroacronycine (**23**) have comparable antineoplastic and antiparasitic activities (Svoboda *et al.,* 1966; Schneider *et al.,* 1972; Basco *et al.,* 1994). Only 2-nitroacronycine has reportable antimalarial activity, with IC_{50} values of 2 µg/ml against susceptible and resistant strains (Basco *et al.,* 1994).

Glycocitrine-1, des-N-methylnoracronycine, atalaphillidine (**24**) and glycobisamine-A have antimalarial activities comparable to that of chloroquine. Atalaphillidine stands out, with total parasite inhibition *in vivo* and no toxic effects. In total, forty-seven acridinone alkaloid derivatives by Fujioka *et al*. (1989) had been tested for possible antimalarial activity, of which four exhibited potent antimalarial activity in the 23 - 150 ng/ml range (Fujioka *et al.,* 1989; Fujioka *et al.,* 1990).



23 **24**

Figure 2.18: Structures of 2-nitroacronycine (**23**) and atalaphillidine (**24**).

2.8.6.2 Mechanism of action

Various mechanisms of the 9-aminoacridines had been postulated. The 9-aminoacridines are known DNA intercalators (Jaycox *et al*., 1987), inhibitors of mammalian topoisomerase I and II, acetylcholinesterase inhibitors and are responsible for the inhibition of β-haemation production (Ciesielska *et al*., 1997; Valdés, 2011). The most widely reported mechanism is thought to be that of DNA intercalators.

2.8.6.3 Toxicity

The use of mepacrine had been discontinued, due to hepatotoxicity. The synthetic acridine, pyronaridine, is currently the only acridine based drug being used in antimalarial chemotherapy (Croft *et al*., 2012; Elueze, 1996). Pyronaridine is well tolerated, showing lower toxicity than chloroquine. Most common side effects present as abdominal pain, diarrhoea, pruritus, headaches, dizziness and nausea (Croft *et al*., 2012).

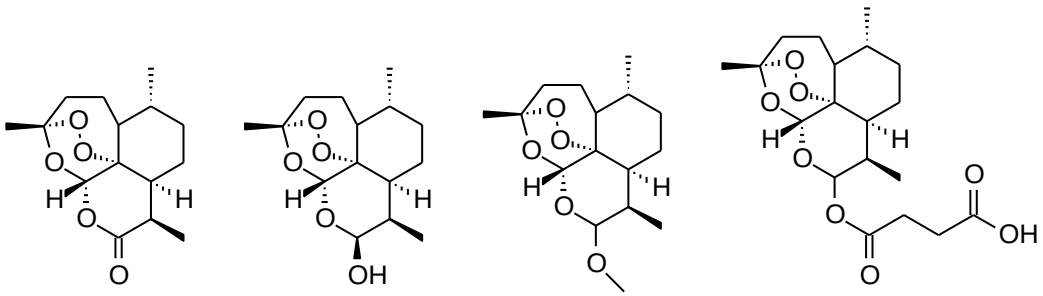
2.8.7 Artemisinins

The re-discovery of artemisinin's (**25**) antimalarial activity has high significance. The potentially crippling malaria death toll of Vietnamese soldiers during the Vietnam War had necessitated North-Vietnamese leaders to find a solution. Project 523 had originated from the strategic meeting held on May 23, 1967, where the possibility of developing novel antimalarial therapies had been discussed.

Project 523 had yielded various antimalarial compounds, of which the most valuable were the artemisinin derivatives, artemether (**27**) and artesunate (**28**), discovered in 1987, and DHA (**26**) in 1992 (Cui & Su, 2009). Artemisinin is a sesquiterpene lactone and consists of three isoprene units bound to cyclic organic peroxide esters. The endoperoxide ring is essential for antimalarial activity (O'Neill *et al*., 2010). The poor pharmacokinetics of artemisinin had resulted in the

synthesis of semi-synthetic and synthetic structural variations of artemisinin. The reduction of artemisinin's carbonyl group yields DHA and its various derivatives. Artesunate is more water soluble, while artemether and arteether are oil soluble (White, 2002; Cui & Su, 2009).

The artemisinins are the most potent antimalarial drugs currently used and they suitably exhibit antimalarial activity against both the asexual and sexual stages of parasitic development (Kumar & Zheng, 1990; Chen *et al*., 1994; Skinner *et al*., 1996). Artemisinin is a fast acting, potent blood schizontocide, with selective activity during the early ring stages up to the schizonts of the erythrocytic cycle. Artemisinin also inhibits the early gametocytic stage of the parasite, preventing the transmission of the parasite from human to mosquito. It has a rapid parasite clearance time, reducing the parasitemia 10 000-fold. Unfortunately, a short biological half-life (~1 hour) precludes long lasting activity and excludes the artemisinins use as a possible drug in chemoprophylaxis (White, 2002; Woodrow *et al*., 2005). Favourable physicochemical properties of semi-synthetic and synthetic artemisinins promote formulation into suppositories. This is beneficial, as the administration route bypasses the clinical manifestation of malaria, namely vomiting, prostration and impaired consciousness (Karunajeewa *et al*., 2007; Gomes *et al*., 2008).



25 **26** **27** **28**

Figure 2.19: Structures of artemisinin derivitives, artemisinin (**25**), dihydroartemisinin (**26**), artemether (**27**) and artesunate (**28**).

2.8.7.1 Metabolism

Artemisinins undergo rapid enzymatic biotransformation into their active metabolite, DHA (White, 2002). Artemisinin automatically induces the hepatic enzymes, cytochrome P450 and CYP2B6 that facilitate its biotransformation (Svensson *et al*., 2003). The resulting metabolite, DHA, is then eliminated by glucuronidation (White, 2002). The short half-life and quick elimination time contribute to poor cure rates and recrudescence in monotherapy (Cui & Su, 2009).

2.8.7.2 Cytotoxicity

The artemisinins are extremely well tolerated, with a favourable general toxicity profile (Nosten & White, 2007). There have been reports of mild gastrointestinal disturbances, acute nausea, vomiting, dizziness, tinnitus and neutropenia. These symptoms usually present with the commencement of therapy, but dissipate towards day seven. The most deadly adverse reaction is selective neurotoxicity. These compounds have a selective pattern of causing damage to certain brain stem nuclei, particularly those involved in auditory processing in mammal species. The degree of toxicity can be correlated with the drug administration route. The depot effect of intramuscular injections continuously exposes the central nervous system to toxic drug levels. The transient high plasma drug levels after oral administration are unlikely to cause neurotoxicity though. Although these findings are unsubstantiated in human studies, the importance of this observation cannot be disregarded (Price *et al*., 1999).

2.8.7.3 Clinical tolerance

No artemisinin resistant strains of *Plasmodium* parasites have yet been isolated from patients. Sporadic treatment failures occur, usually due to poor therapy responses to artesunate, or artemether. Reports from Western Thailand and China, India, Sierra Leone, Nigeria and Madagascar indicate a reduced susceptibility of the artemisinins by parasites (Oduola *et al*., 1992; Luxemburger *et al*., 1998; Gogtay *et al*., 2000; Randrianarivelojosia *et al*., 2001; Sahr *et al*., 2001; Yang *et al*., 2003). Elevated treatment failure and recrudescence rates on the Thai-Cambodian border and Southern Cambodia indicate the implosion of artesunate-mefloquine therapy, partly due to widespread mefloquine resistance development (Rogers *et al*., 2009). The increase of parasitemia and the occurrence of recrudescence are indicative of the development of clinical tolerance, but not yet of resistance.

2.8.7.4 Artemisinin based combination therapies (ACT's)

ACT's are used as first line treatments in seventy-nine of the eighty-eight *P. falciparum* endemic countries and have been adopted by general health care services in seventy countries (WHO, 2012). In 2010, eighty-four countries have adopted ACT's for first line treatment as a national policy. This had led to 278 million ACT treatment courses having been delivered to public and private sectors globally, compared to 11 million in 2005 (WHO, 2011).

The rationale behind artemisinin combination therapy is to decrease the risk of parasite mutation by treating it with two distinct chemical entities, consisting of different mechanisms of action, simultaneously. The artemisinin derivative would rapidly clear 95% of the parasites,

while the remaining 5% would be cleared by the slower eliminating partner drug (Araujo *et al*., 2009). The partner drug should be structurally dissimilar, therapeutically viable and possess a long half-life.

Today, a number of ACT's are being used and clinically tested. Artemisinin-lumefantrine, Coartem™, and artesunate-pyronaridine are found highly effective in treating uncomplicated *P. falciparum* malaria (Kokwaro *et al*., 2007). Widespread resistance towards mefloquine on the Thai-Cambodian border, however, has decreased ACT's therapeutical efficacy and has become the root course of a 28% treatment failure with artemisinin-lumefantrine treatment (Davis *et al*., 2005). This predicament is worrying, as the effectiveness of ACT's may be compromised by the use of unsuitable partner drugs.

2.8.7.5 Modes of action and potential cellular targets

The plasmodium parasite is dependent on host haemoglobin for its development and for creating space in its digestive vacuole. Haemoglobin biodegradation is facilitated intracellularly *via* protease enzymes to yield haematin, peptides and amino acids. The build-up of toxic haematin, formed by hydrogen bonding of the haem monomer, undergoes biomineralisation into non-toxic, insoluble haemozoin (O'Neill *et al.,* 2010). Remarkable efforts to elucidate artimisinin's mechanism of action have raised more questions than having delivered answers and have yielded nothing concrete so far. Various mechanisms have been postulated, namely:

- Activation of artemisinin and production of free radicals.
- Targeting haem polymerisation.
- Targeting PfATP6 and mitochondrial targets.

2.8.7.5.1 Activation of artemisinin (open ring model) and production of free radicals

The artemisinins are considered prodrugs, with an endoperoxide bridge that is essential for antimalarial activity. The substitution of peroxide oxygen with a carbon results in a total loss of antimalarial activity (Krishna *et al*., 2004; Haynes & Krishna, 2004). Activation of the endoperoxide bridge generates carbon centred free radicals, or reactive oxygen species (ROS). Artemisinins induce oxidative stress and decrease anti-oxidant and glutathione levels in the parasite (Krungkrai & Yuthavong, 1987; Meshnick, 2002; Ittarat *et al*., 2003). Ring opening of artimisinins can be facilitated by the protonation of the endoperoxide bridge, or through

complexation with Fe^{2+} (Olliaro *et al*., 2001). The parasite is rich in haem, an iron ring molecule, whereupon artemisinin can react as a chelator.

2.8.7.5.2 Targeting haem polymerisation

Artemisinin chelates freely onto haem, forming haem-artemisinin adducts. These adducts can also be seen with haem-trioxane interactions (Hong *et al*., 1994; Kannan *et al*., 2002; Kannan *et al*., 2005). These adducts interact with *P. falciparum* histadine-rich protein II (PfHRP), inhibiting haem polymerisation and haemozoin formation (Kannan *et al*., 2002; Loup *et al*., 2007). Furthermore, artemisinins can directly influence haemozoin degradation (Pandey *et al*., 1999). This hypothesis has, however, been contradicted by studies reporting no inhibition of haemozoin formation *in vivo* (Meshnick, 1996; Haynes *et al*., 2003).

2.8.7.5.3 Targeting *Pf*ATP6 and mitochondrial targets

The sarco/endoplasmic reticulum (SR) calcium transport ATPase (SERCA) lowers the cytosolic free calcium concentration, by actively transporting calcium into membrane bound stores *via* efflux. As this calcium transportation mechanism is essential to cellular survival, inhibition of SERCA will ultimately facilitate cell death (Eckstein-Ludwig *et al*., 2003).

Genetic analysis demonstrates gene manipulation of NADH dehydrogenase in the mitochondrial electron transport chain, which may result in either an increase or a decrease in artemisinin sensitivity. The mitochondria may have a dual purpose in artemisinins' activity. The mitochondrial electron transport chain may activate artemisinin, generating ROS, which in turn would cause mitochondrial damage (Li *et al*., 2005).

2.9 Hybrid theory

One of the challenges in antimalarial chemotherapy is the identification and development of compounds with innovative chemical scaffolds and enzyme targets.

A hybrid molecule is defined as a chemical entity with more than one structural domain, covalently linked together, each possessing its own biological function (Meunier, 2008). A hybrid is the chemical combination of two or more pharmacophores (Figure 20), by co-formulating two or more traditional antimalarial agents into a single tablet. The linking of two distinct chemical moieties has been reported to have improved antimalarial activity and reduced the susceptibility to resistance development (Jones *et al*., 2009).

Hybridisation can lead to a synergistic therapeutic effect, i.e. the ability to be more active than a 1:1 ratio of the individual parent components, the optimisation of physicochemical properties and the inhibition of drug resistance development (Walsh *et al*., 2007; Walsh & Bell, 2009). The choice of a linker is important, as it can determine the characteristics of the hybrid moiety. The desired ability of the linker to be either metabolically stable or undergo cleavage, must be considered in the design of hybrid molecules. To overcome resistance, the use of a metabolically stable linker is necessitated. Fragmentation into the individual moieties after biotransformation is beneficial, if the two individual moieties have independent sites of action (Walsh & Bell, 2009). Fused hybrids with short chain linkers have the ability to impart favourable properties from one entity to another, whereas flexible, longer chain linkers can promote dual receptor activation.

The disadvantages of hybrids include increased molecular weight and bulkiness, steric impediment on receptor bindings, increased toxicity and inferior activity (Walsh & Bell, 2009).

Hybrid molecules are classified as follows (Morphy & Rankovic, 2005):

- *Conjugates*: An entity containing the pharmacophores from both targets that are separated by a distinct stable linker group that is not found in any of the individuals.

- *Cleavage conjugates*: An entity with a linker that is designed to be metabolised, releasing the individual entities to interact independently.

- *Fused hybrid:* The linker's length between the two moieties is reduced in such a manner that the frameworks of the different pharmacophores are touching.

- *Merged hybrid*: The two pharmacophores are bound together at a commonality in the structures, rendering a smaller, simpler molecule.

Figure 2.20: Illustration of hybrid drug theory (Meunier, 2008.)

The length of the methylene linker on chloroquine has shown to be a principal determinant of activity against CQR *P. falciparum.* The increase of linker length had proven to cause potent

parasite inhibition (Ridley *et al*., 1996; De *et al*., 1998). Chibale *et al*. (2000) had elucidated the antimalarial effect, chemical diversity and the length of the linker on a series of ferrochloroquine analogues (Chibale *et al*., 2000). Their findings were that increments of linker length, the addition of electron withdrawing groups and their proximity to the individual pharmacophores, had rendered enhanced antimalarial and stability characteristics.

2.9.1 Acridine based hybrids

The previously neglected acridine pharmacophore is now used for its intrinsic antimalarial and fluorescent properties in hybrid moieties (Jones *et al*., 2009). Kumar *et al*. (2009, 2010) had synthesised series of 9-anilinoacridine triazines and quinoline-acridine hybrids, with reportable antimalarial activity (Kumar *et al*., 2009; Kumar *et al*., 2010). The 9-anilinoacridine triazine hybrids had exhibited suppression rates into the 90 percentile (Kumar *et al*., 2009). Interestingly, the substitution of the *p*-phenylenediamine linker with an *m*-phenylenediamine linker in quinoline-acridine hybrids resulted in a five-fold increase in activity (Kumar *et al*., 2010). The steric impediment of the linker had played an important role in the overall activity and stability of hybrids.

2.9.2 Artemisinin based hybrids

Artemisinin based hybrids have the advantage of targeting the parasite *via* two distinct mechanisms, thereby delaying or circumventing the development of resistance.

Artemisinin-acridine hybrids were developed by Jones *et al*. (2009) during attempts to amplify antitumor activity. Acridine, a known DNA-intercalator, had been chosen as a hybrid scaffold. The artemisinin-acridine hybrids had shown an increased antineoplastic effect, but had only displayed comparable antimalarial activity to DHA (Jones *et al*., 2009).

Walsh *et al*. (2007) had synthesised a novel artemisinin-quinine hybrid with increased activity against drug sensitive and drug resistant strains of *P. falciparum*. They concluded that the hybrid had retained the antimalarial activity of both parent compounds, emphasising the hybrid's ability to enhance antimalarial activity and overcome drug resistance (Walsh *et al*., 2007).

Two novel series of endoperoxides, incorporating an aminoquinoline or acridine moiety, had also been synthesised by Araújo *et al*. (2009). It was found that both series of hybrid molecules had expressed high levels of antimalarial activity of 3 - 24.2 nM and 3 – 26 nM *in vitro* against 3D7 and K1 strains, respectively (Araújo *et al*., 2009).

2.10 Antimalarials in the pipeline

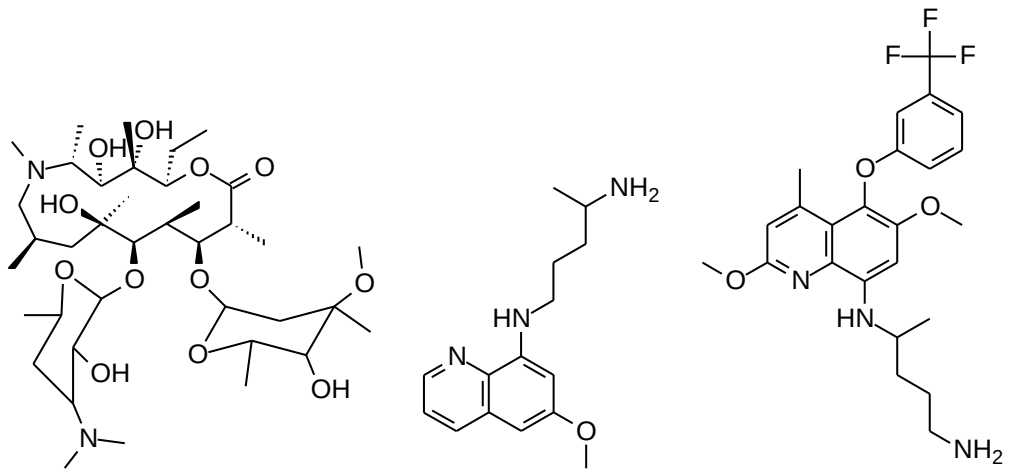
Considerable research and financial resources have been invested into the development of malaria vaccines. Unfortunately, to date chemotherapy still remains the main treatment regime in the antimalarial combat. Factors that undermine drug efficiency are toxicity, or side effects and the increasing development of resistance. The development of resistance to each anti-infective chemotherapy is inevitable, with the antimalarial chemotherapies being no exception (Wells & Poll, 2010). It is therefore of paramount importance that the search for novel and effective antimalarials continues. The following are antimalarials in the pipeline:

- Euartesim is the combination of DHA and piperaquine. This co-formulation has diverse pharmacokinetic properties, in which the recrudescent of malaria is addressed through the longer half-time drug, piperaquine (Eastman *et al*., 2011). Euartesim is indicated in the treatment of uncomplicated malaria, as recommended by the WHO (Schlagenhauf & Petersen, 2013).

- Pyramax is the physical combination of artesunate and pyronaridine. It is indicated in the treatment of uncomplicated *P. falciparum* malaria and for erythrocytic forms in *P. vivax* malaria (Pradines *et al*., 2010). This combination has shown equipotent activity against other ACT's and has completed phase III clinical trials in humans (Kurth *et al*., 2011).

- Azithromycin (**29**) is a macrolide antibiotic that inhibits parasite growth through the inhibition of the apicoplast's protein biosynthesis (Pradel & Schlitzer, 2010). Results of various clinical trials are contradictory, but a synergetic effect in combination with chloroquine *in vivo* has been reported (Dunne *et al*., 2005; van Eijk & Terlouw, 2011).

- Quinocide (**30**), elubaquine and tafenoquine (**31**) are 8-aminoquinolines. These drugs have slow elimination times and are currently undergoing further testing (White, 2002). Tafenoquine (**31**) has been evaluated as a radically potential cure against *P. vivax* (Nasveld *et al*., 2010).



29 **30** **31**

Figure 2.21: Structures of azithromycin (**29**), quinocide (**30**) and tafenoquine (**31**).

- Arteolane (OZ277 or RBx11160) (**32**) is a fully synthetic 1,2,4-trioxolane with a peroxidic pharmacophore and has shown significant and rapid antimalarial activity in preclinical studies. Arteolane has *in vitro* activity, superior to chloroquine, mefloquine, artemether and artesunate against CQS and CQR strains of *P. falciparum* (Valecha *et al*., 2010). The reductive activation of the endoperoxide bond and the subsequent carbon centred radicals, or carbocations are hypothesised to interfere with lipid components of the parasite's digestive vacuole (Fügi *et al*., 2010). This interference weakens the integrity of the food vacuole's membrane. In malaria infected patients, however, the bioavailability of arterolane was limited. This could have been attributed to an increased Fe^{2+} load, resulting in rapid iron mediated degradation (Charman *et al*., 2011). The toxicology of rat models demonstrated a remarkably, benign toxicological profile that lacked neurotoxicity, due to a diminished drug accumulation in the brain (Vennerstrom *et al*., 2004). Arteolane is now being evaluated as combination therapy with piperaquine (Valecha *et al*., 2012). OZ439 was developed to overcome arteolane's instability. The structural modification led to a potent compound with a single dose cure rate, as observed in *P. berghei* infected mice (Charman *et al*., 2011).

- Naphthoquine is a 4-aminoquinoline, used in a fixed dose combination with artemisinin (ARCO®). A single dose is significant in curing uncomplicated *P. falciparum* malaria in adults (Tun *et al*., 2009; Hombhanje *et al*., 2009; Wang *et al*., 2004; Hombhanje & Huang, 2010). Recent studies promoted a more suitable two-day regimen as preventive measure against drug resistance development (Benjamin *et al*., 2012; Batty *et al*., 2012).

- Artemisone (**33**) is a second generation artemisinin derivative. It has improved physicochemical properties, bioavailability and negligible neurontoxicity and cytotoxicity *in vitro* and *in vivo* (Haynes *et al*., 2006). In order to gain insight into this compound's mechanism of action, the time and course of artemisone's cellular uptake into uninfected and infected erythrocytes were determined (Pooley *et al*., 2011). The outcomes strengthened the hypothesis that the artemisinin's cellular uptake is through a common pathway.

  Artemisone has *in vitro* IC_{50} values of 1.49 nM (K1 strain) and 1.59 nM (TM90-C2A strain) (Nagelschmitz *et al*., 2008) and exhibits efficacy against cerebral malaria in *P. berghei* infected mice (Waknine-Grinberg *et al*., 2010). Enhanced antimalarial activity can be linked to superior inhibition of cellular target, similar to the parasite's Ca^{2+} pump.

- Coumarins are important natural flavonoid compounds with anti-HIV, anticoagulant, antibacterial, anti-oxidant, dyslipidemic and antimalarial pharmacological properties. The coumarin pharmacophore is readily synthesised *via* a Perkin reaction between salicylaldehyde and acetic anhydride. Daphnetin (**34**), 5,7-methoxy-8-(3-methyl-1-buten-3-ol)-coumarin (**35**) and pachyrrhizine (**36**) are naturally occurring coumarins with established antimalarial activity (Sashidhara *et al*., 2012).

  Sashidhara *et al*. (2012) had synthesised coumarin-trioxane hybrids as a new class of antimalarial compounds. The *in vitro* evaluation of these hybrids resulted in moderate antimalarial activity, with IC_{50} values of 39 - 360 ng/mL against the 3D7 *P. falciparum* strain (Sashidhara *et al*., 2012). The coumarins are well tolerated compounds. Liver function impediment and hepatic toxicity are rare, but when occurring, these symptoms disappear within a few days after treatment. Toxicity is also dose dependent (Lake, 1999).

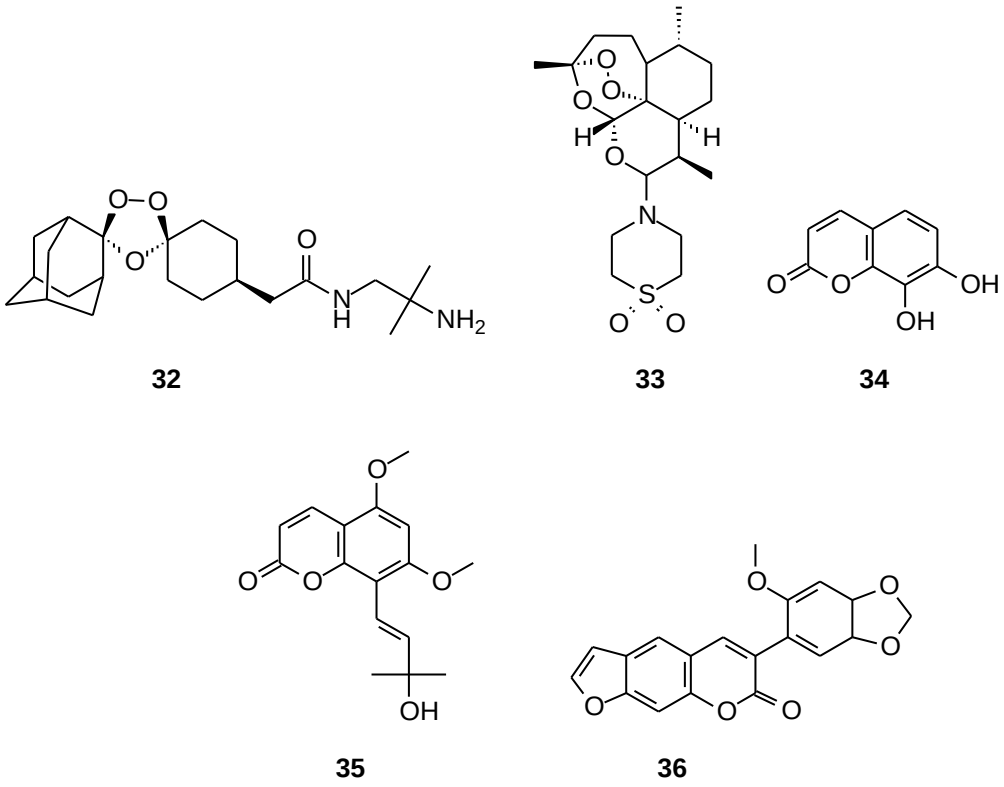


Figure 2.22: Structures of arteolane (**32**) artemisone (**33**) daphnetin (**34**), 5,7-methoxy-8-(3-methyl-1-buten-3-ol)-coumarin (**35**) and pachyrrhizine (**36**).