Chapter 2

Literature review – Malaria and the treatment thereof

2.1 Epidemiology of malaria

The prevalence of malaria coincides with the occurrence of its vector, the female anopheline mosquito. There are 465 formally recognised and 50 unnamed species of anopheline mosquitoes of which 70 species have the capacity to transmit malaria. Of this 70 species, 41 are considered to be dominant vector species (DVS) that are of major concern to public health (Fig. 2.1) (Sinka *et al.*, 2012:1, White 2008:1201).

Malaria transmission cannot occur at temperatures below 20°C or above 35°C and at altitudes above 1000–2500 m, depending on the type of climate. Relative humidity (RH) values of 75% usually ensure the longest survival of the adult vectors and a value below 35% shortens the life span to a level incompatible with malaria transmission. Water is needed for breeding sites, but the optimum amount of water differs greatly from one species to the next. Some anopheline species requires small bodies of water for breeding whilst other species require lager bodies of water such as ponds and lakes (Wernsdorfer 2012:160).

As humans are the secondary host of pathogenic *Plasmodia*, a major factor in the epidemiology of malaria is the behaviour of man. Human related factors such as socio-economic status, health care access, migration, gender, and land use all play a pivotal role in the fight against malaria (Protopopoff *et al.*, 2009:3).

In areas where malaria is holoendemic and the population is constantly infected, the majority of people gain naturally acquired immunity (NAI). The first type of NAI is anti-disease immunity and confers protection against the clinical disease, affecting the risk and extent of morbidity associated with a given parasite density. The second type of NAI is anti-parasite immunity and confers protection against parasitemia, affecting the density of parasites. The third type of NAI is premonition and provides protection against new infections by maintaining a low-grade and generally asymptomatic parasitemia. NAI compromised individuals are usually infants, young children, pregnant women and adults removed from their routine infection resulting in an increase in morbidity and mortality amongst these individuals (Doolan *et al.*, 2009:14, Wernsdorfer 2012:160).
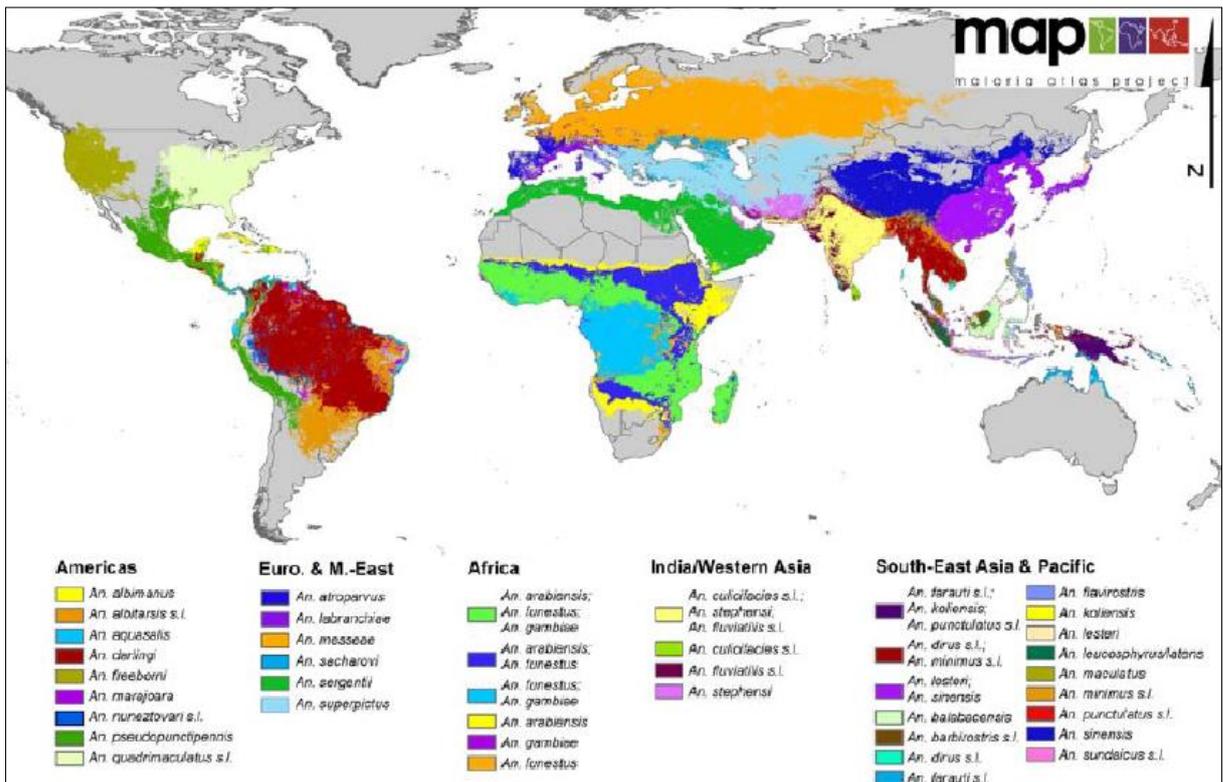
Figure 2.1: Global map of the 34 most dominant malaria vector species (Sinka *et al.* 2012:4).

2.2 Malaria parasite life cycle

The lifespan of the malaria parasite is spent in two hosts: the female *Anopheles* mosquito and the human (Fig. 2.2). The parasite's life cycle in the vertebrate host can be divided into the exoerythrocytic (pre-erythrocytic) and the erythrocytic stages, whilst the third phase takes place inside the mosquito (Bogitsh *et al.*, 2005:132, White 2008:1205). During a blood meal, the female mosquito secretes sporozoite-bearing saliva into the peripheral circulation of the host, inoculating the sporozoites into the bloodstream and regional lymph nodes. Soon after entering the peripheral circulatory system, the sporozoites infect the parenchymal cells of the liver and undergo asexual amplification called schizogony. After this period, the parasite is released from the hepatocytes as merozoites and invades the RBCs. Clinical symptoms of malaria are not prevalent during the liver stage, but manifest during the erythrocytic phase of the life cycle. The mosquito is then re-infected by the parasite when it takes a blood meal and ingests the micro- and macrogametocytes circulating in the bloodstream of an infected host (Doolan *et al.*, 2009:14, Stevenson *et al.*, 2011:1312).
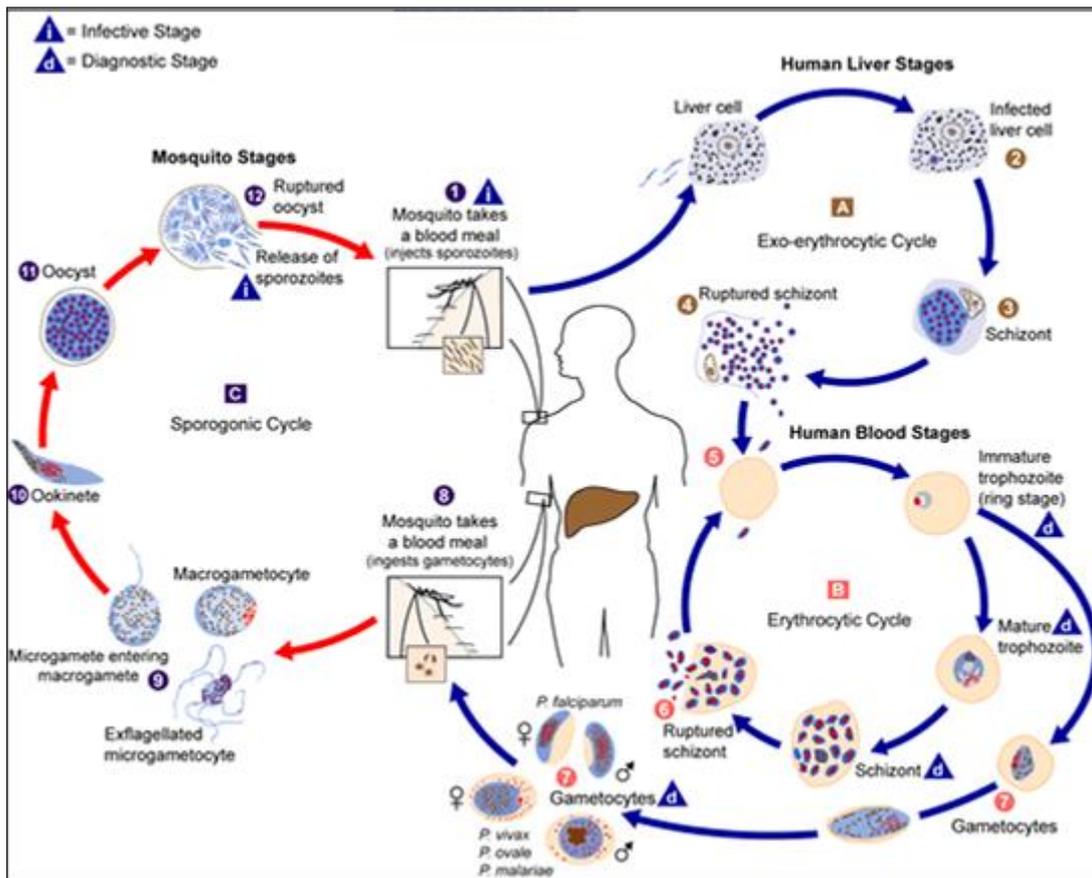
Figure 2.2: The life cycle of the malaria parasite (CDC 2009)

2.2.1 Exoerythrocytic phase

The exoerythrocytic phase of the malaria infection begins with the injection of sporozoites into the skin and ends when the first generation of merozoites are released into the surrounding bloodstream (Ménard *et al.*, 2008:564).

After injection, the sporozoites only have 1-3 hours of motility in which they have the capability to reach the target parenchyma cells on their own. Only a certain portion of the skin sporozoites invade the blood capillaries and make their way to the target liver cells. The other portions either enter the lymphatic vessels or stay in the skin itself (Amino *et al.*, 2006:220, Ménard *et al.*, 2008:565).

Once inside the hepatocytes, the sporozoites commence asexual amplification called schizogony that lasts for 2 to 10 days, and develops into liver-stage trophozoites (Doolan *et al.*, 2009:14). After 1 to 2 weeks (depending on the species) the nucleus of the trophozoites divides several times followed by division of the cytoplasm producing thousands of

merozoites, rupturing the hepatic schizont and releasing them into the bloodstream infecting erythrocytes and commencing the erythrocytic stage of the parasite's life cycle (Bogitsh *et al.*, 2005:132, White 2008:1206, Doolan *et al.*, 2009:14).

In both *P. vivax* and *P ovale* a certain portions of the sporozoites that have invaded the liver cells do not develop beyond the trophozoite stage but instead forms hypnozoites. These hypnozoites have the ability to generate infective merozoites many years after the parasite has been cleared from the blood, resulting in a malaria relapse (Dembele *et al.*, 2011:2).

2.2.2   Erythrocytic stage

The merozoites released from the hepatocytes are motile ovoid forms which swiftly invade passing erythrocytes. The process by which a merozoite invades the erythrocyte can be divided into four distinct steps: attachment of the merozoite to the erythrocytes surface, orientation so that the apical complex abuts to the red blood cell, tight-junction formation at the contact region and then the internalisation of the merozoite into the erythrocyte (Farrow *et al.*, 2011:954, White 2008:1206).

Once inside the erythrocyte the parasite flattens out and takes on an apparent ring form (Fig. 2.3). The parasite then ingests the cytosol of the infected RBC by endocytosis into its food vacuole (Bannister *et al.*, 2000:429). The ingested cytosol consists of more than 95% hemoglobin which is an essential source of amino acids for the parasite. This, however, leads not only to the release of amino acids but also the toxic by-product ferriprotoporphyrin IX (Dassonville-Klimpt *et al.,* 2011:26, Kumar *et al.*, 2005:178). To surmount the free ferriprotoporphyrin IX toxicity, the parasite has several detoxification systems that can be divided into two main groups. The first and primary means of detoxifying takes place in the food vacuole of the parasite where the acid conditions (pH = 5.2) promote the conversion of free haem into insoluble and non-toxic crystals termed hemozoin or malaria pigment. The second mechanism is by other systems situated mainly in the cytosol, such as detoxification by haem binding proteins and degradation of free haem by H_2O_2 (Dassonville-Klimpt *et al.,* 2011:26, Kumar *et al.*, 2007:815).

As the parasite grows, it starts to change into trophozoites, not differing from the ring forms in any fundamental internal structures, but rather in the size and shape of the parasitic cells. These trophozoites then undertake repetitive nuclear division forming schizonts, producing a new generation of merozoites in each infected RBC. Finally these newly formed merozoites

breach the parasitophorous vacuole and RBC membranes and are released into the bloodstream, ready to infect new RBCs (Bannister *et al.*, 2000:432).

After a few asexual cycles, the sexual phase of the parasite commences where a certain portion of the parasite develops into gametocytes. These gametocytes freely circulate in the peripheral circulation and are ingested by the anopheline mosquito when it takes a blood meal (Kuehn *et al.*, 2010:210).
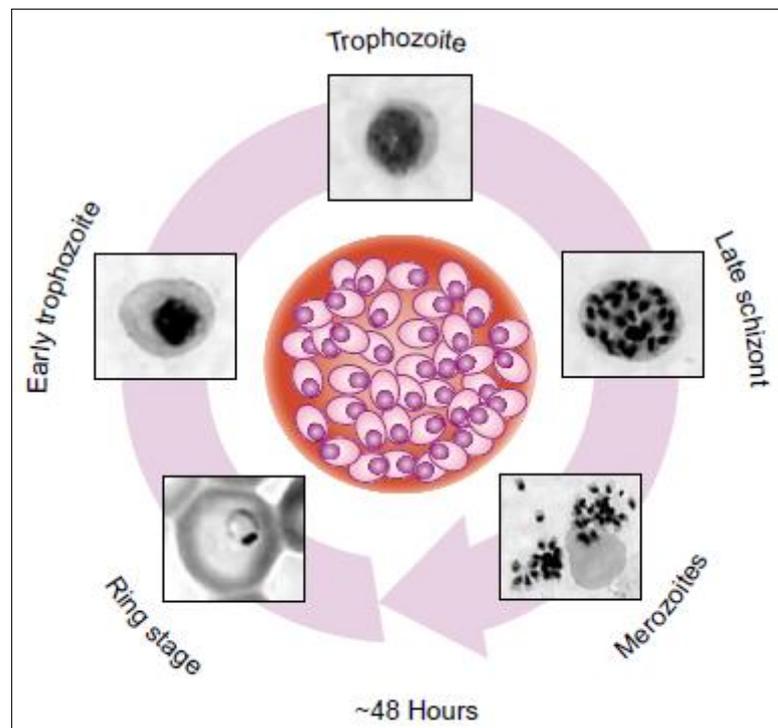

Figure 2.3: The *Plasmodium* intraerythrocytic development (Llinás *et al.*, 2004:383).

2.2.3 Sexual stage and development in the mosquito

The gametocytes circulating in the peripheral circulation are ingested with a blood meal and fertilise in the midgut lumen of the mosquito. The male gametocytes undergo rapid nuclear division and become motile by acquiring flagellums, seeking female macrogametes. Fusion and meiosis then takes place to form zygotes which develops into motile ookinetes that are able to cross the midgut epithelium of the mosquito. These ookinetes undergo meiosis, transforming them into oocysts capable of releasing thousands sporozoites that journey through the haemolymph into the salivary glands, where they await inoculation into the next human host (White 2008:1208, Whitten *et al.,* 2006:121).

2.3 Symptoms

The symptomatic manifestation of malaria is dependent on the previous immune status of the host. In areas of intense *P. falciparum* malaria transmission adults develop NAI, providing them with a low-grade asymptomatic malaria infection (Doolan *et al.*, 2009:14, Wernsdorfer 2012:160). Infants, children, pregnant women and adults removed from their routine infections have compromised NAI, and are in danger of acquiring severe malaria (Doolan *et al.*, 2009:14, Bunn 2004:648). With a lower or more seasonal transmission the age distribution of malaria shifts more upwards and severe malaria can be seen in older children as well. In these areas cerebral malaria is the most prominent manifestation. With an even more seasonal pattern of transmission, e.g. when travellers visits endemic areas, symptomatic malaria is seen at all ages (Luxemburger *et al.*, 1997:256).

With the end of the asymptomatic exoerythrocytic stage, merozoites are released from infected RBCs signalling the start of the erythrocyte stage. The erythrocyte stage is where most of the common symptoms of malaria are noticeable including fever, malaise, splenomegaly, anaemia, nausea, lassitude, perspiration, anorexia, vomiting, muscle and joint aches, abdominal discomfort and headache, most of which coincides with the cyclic release of new merozoites (Stevenson *et al.*, 2004:169, Stevenson *et al.*, 2011:1312, WHO 2010a:4). The most dangerous of all the malaria strains, *P. falciparum*, is responsible for the highest incidence of severe malaria, which presents as metabolic acidosis, cerebral malaria, severe anaemia, hypoxia, hypoglycaemia, lactic acidosis, renal failure, pulmonary oedema, black water fever, thrombocytopenia, gastrointestinal dysfunction and placental dysfunction (in woman) (Stevenson *et al.*, 2011:1312, Mishra *et al.*, 2006:282, Hisaeda *et al.*, 2005:702).

2.4 Pathophysiology

2.4.1 Sequestration and rosetting

In order to avoid clearance by the immune system in the spleen, *P. falciparum* has the ability to adhere to vascular endothelium through a process called sequestration (Fig. 2.4). The parasite has the capability to express its own proteins on the surface of the infected erythrocyte causing the RBC to adhere to endothelial cells (cytoadherence), uninfected erythrocytes (rosetting) and other infected cells (autoagglutination/clumping) (Heddini 2002:1587). The process of sequestration consists of three steps: firstly tethering (initial contact), secondly rolling and then thirdly firm adherence to the endothelium. Once adhered, the infected RBC remains stuck to the microvascular endothelium until schizogony and even afterwards (White 2008:1212).

Sequestration takes place in an assortment of organs including deep vascular beds of skeletal muscles, placenta, the heart and in the pulmonary circulation. Sequestration is not uniformly distributed and causes specific clinical features and organ dysfunction by a variety of possible mechanisms, e.g. poor local perfusion, local release of cytokines and nitric oxide (NO) and local metabolic derangements (Heddini 2002:1587).

As mentioned above, in addition to cytoadherence the parasite also has the ability to adhere to uninfected erythrocytes which is called rosetting. Rosetting is mainly associated with sequestering parasites that bind in the internal organs during schizogony. Although rosetting are also prevalent in malaria caused by *P. vivax*, *P. malariae* and *P. ovale* it is mainly caused by *P. falciparum* (Heddini 2002:1588).
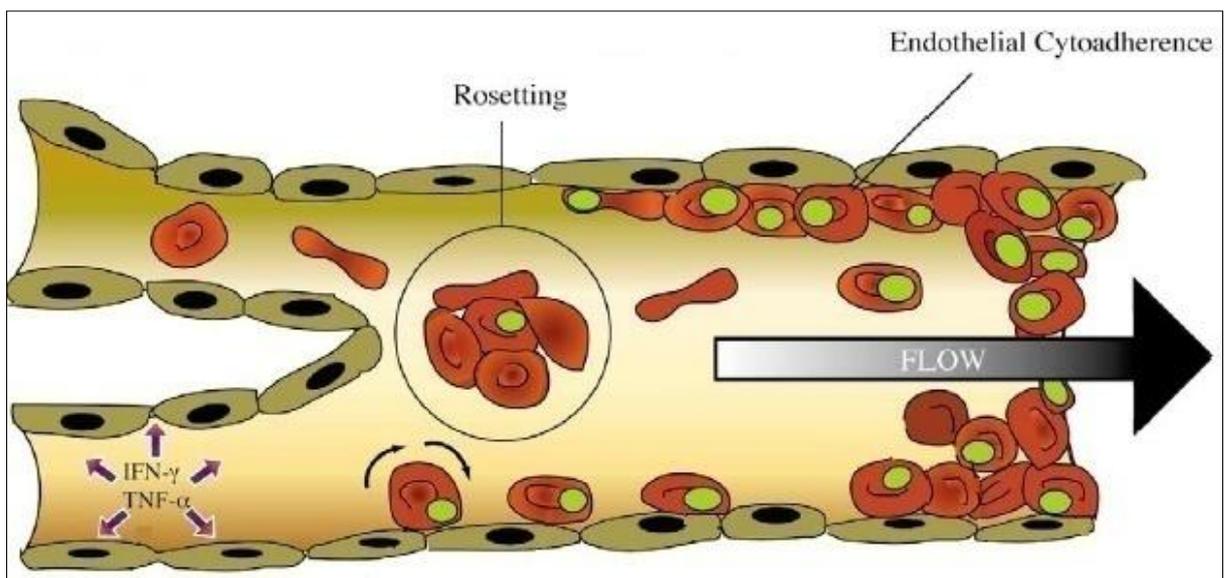

Figure 2.4: Sequestration and rosetting in the microcirculation (Heddini 2002:1588).

2.4.2 Cerebral malaria

Compared to mild and asymptomatic malaria, severe malaria is uncommon. However, due to the high incidence of malaria in Africa, Asia and South America, severe malaria still leads to a substantial amount of mortalities making it an enormous health problem (Turner 1997:570). Severe malaria affects several organs and presents with a number of symptoms including severe anaemia, respiratory complications, jaundice, renal failure, disseminated intravascular coagulation, hypoglycaemia, acidosis, multiple organ failure, shock and cerebral malaria (Turner 1997:570, Medana *et al.*, 2006:555, WHO 2010a:5). The best known and most studied of these symptoms are cerebral malaria which is potentially the

most fatal with a mortality of 15–20% even with active treatment and support (WHO 2010a:36).

Cerebral malaria is defined as a potentially reversible, scattered encephalopathy resulting in a coma that persists for more than 30 min after a seizure, in the absence of other factors that could cause unconsciousness (WHO, 2010:v, Medana *et al.*, 2006:555). The symptoms of cerebral malaria range from a state of confusion to a deep coma, and are frequently associated with convulsions (Crawley *et al.*, 2001:251). Although the coma resulting from cerebral malaria are characterised by rapid reversal with treatment, research has shown that permanent neurological complications following cerebral malaria are not uncommon and includes; weakness, hearing impairment, quadriplegia, epilepsy and cortical blindness (Turner 1997:570, Carter 2005:497).

The exact cause of cerebral coma is not known. There is undoubtedly an increase in cerebral anaerobic glycolysis caused by sequestration of infected RBCs and an increase in cerebral metabolic rates. The change in the metabolic milieu, increased production of nitric oxide and cytokines might all contribute to the pathogenesis of cerebral coma, but on their own do not provide a sufficient explanation for the coma. Although there has been considerable interest in the mechanism of cerebral coma and attempts to reverse it, it should be remembered that the brain in cerebral malaria has a severely compromised blood supply, and waking a patient from the coma may result in an increased cerebral metabolic demand and further damage (White 2008:1219).

2.4.3 Renal failure

Renal pathology is common in severe malaria and usually presents as electrolyte abnormality, abnormal urinary sediments, increased urinary protein excretion, oliguria, anuria, azotemia and acute renal failure (ARF) (Das 2008:89). ARF is almost exclusively caused by *P. falciparum* and is the result of cytoadherence of parasitized erythrocytes, dehydration, intravascular haemolysis, intravascular coagulation, sepsis, hyperbilirubinemia and hyperparasitaemia (Manan *et al.*, 2006:47, Das 2008:84).

ARF can be found in 1–4.8% of the patients in malaria endemic areas and in 25–30% of non-immune patients, highlighting the importance of NAI in preventing ARF (Barsoum 2000:2150). When prevalent, ARF is a serious complication of severe malaria with a mortality rate of 15-45% in certain areas (Barsoum 2000:2152).

2.4.4   Pulmonary oedema

Acute lung injury is most often found in non-immune individuals and can occur after a few days of infection or even after clearance of the parasite from the system. The initial signs of imminent pulmonary oedema are tachypnea, dyspnea, hypoxemia and respiratory failure. If left untreated these symptoms may progress to acute respiratory distress syndrome (ARDS) with an increased pulmonary capillary permeability (Trampuz *et al.,* 2003:318, Taylor *et al*., 2006:421).

Although the exact cause of ARDS is not known, contributing factors include respiratory compensation for metabolic acidosis, noncardiogenic pulmonary oedema, concomitant pneumonia and severe anaemia, all leading to an inflammatory mediated increase in the capillary permeability or to endothelial damage causing diffuse alveolar damage (Taylor *et al*., 2002:464).

2.4.5   Anaemia

Amongst all the symptoms, malaria induced anaemia is responsible for the greatest number of morbidity and mortality in holoendemic areas (Ong'echa *et al*., 2006:376). The greatest burden of malaria induced anaemia can be found in children under the age of three and in pregnant women, with a prevalence of 90 and 80%, respectively (Haldar *et al.,* 2009:87, Menendez *et al*., 2000:472).

During the erythrocyte stage of the parasite's life cycle, merozoites invade passing erythrocytes feeding on their hemoglobin, ultimately destroying the RBCs (Haldar *et al.,* 2009:87, Bannister *et al*., 2000:428). With high parasitemia, as is found in *P. falciparum*, there is substantial haemolysis of RBCs ultimately leading to anaemia (Menendez *et al*., 2000:469). Although haemolysis of RBCs are the leading cause of anaemia, other factors also play a variable role in this pathology, including phagocytosis of infected and uninfected RBCs by macrophages, clearance of infected RBCs from the circulation by the spleen, autoimmune haemolysis of RBCs and severe vascular haemolysis (Menendez *et al*., 2000:470, Ong'echa *et al*., 2006:376).

In addition to the increased destruction of RBCs there is also a decrease in production. During acute malaria, the suppression of the normal response to erythropoietin (EPO), a glycoprotein hormone that controls RBC production, leads to a decrease in RBC synthesis. Besides a decreased response, there are also a decrease in EPO synthesis, an observed

disturbance in nuclear division of bone marrow and an imbalance of cytokines leading to bone marrow suppression (Menendez *et al.*, 2000:470).

2.4.6   Blackwater fever

Blackwater fever presents as severe intravascular haemolysis and anaemia, producing dark or black urine often accompanied by abdominal pain, jaundice, hepatosplenomegaly, vomiting and renal failure (Khan *et al.*, 2009:380, Van den Ende *et al.*, 1998:632).

Blackwater fever can occur in several instances: Firstly, when patients with G6PD deficiency take oxidant drugs (e.g. primaquine, sulfones or sulfonamide) irrespective of whether they have malaria or not. Blackwater fever can also transpire in patients with G6PD deficiency that have malaria and receive quinine treatment. Thirdly, in some patients with severe *P. falciparum* malaria who have normal erythrocyte G6PD levels irrespective of the treatment given and lastly, when people who are exposed to malaria self-medicate with quinine (or related drugs) (White 2008:1221).

The exact aetiology of blackwater is not yet known though there is an increase in haemolysis when *P. falciparum* are treated with quinine-like drugs (Tillyard 2004:187). An auto-immune mechanism was also investigated, but was found to be highly unlikely (Van den Ende *et al.*, 1998:637).

2.4.7   Enlarged spleen

The spleen plays an important role in limiting the spread of acute malaria infection by removing parasitized RBCs. As there is a massive increase in parasitemia with *P. falciparum* malaria, there is an associated upregulation of the reticuloendothelial system's phagocytosis function resulting in splenomegaly (Newton *et al.*, 1998:31, Tillyard 2004:188).

Although splenomegaly can be indicative of severe malaria, it is not fatal on its own. There have only been a few cases ever reported of death as a result of splenic rupture (Tillyard 2004:188).

2.4.8 Gastrointestinal dysfunction

Stress ulceration of the stomach and duodenum, and abdominal pain is common in severe malaria and is a result of both gut sequestration and visceral vasoconstriction (White 2008:1221). Massive gastrointestinal haemorrhage as been implicated in algid malaria, which is the circulatory collapse as a result of malaria (Mishra *et al.*, 2006:285, Tillyard 2004:193). Gastrointestinal haemorrhage and fluid loss have been implicated in a number of other pathologies, including ARF and hyponatraemia (Das 2008:89, Newton *et al.*, 1998:33). Although the absorption of most antimalarial drugs is unaffected by moderate gastrointestinal dysfunction, caution should be taken when prescribing medication as to not worsen the pathology (White 2008:1221, Newton *et al.*, 1998:40).

2.4.9 Acidosis

In severe malaria, it is often acidosis that is the major cause of death in both adults and children, and it has been considered to be mainly lactic acidosis, although ketoacidosis also sometimes plays a role (White 2008:1221). The rise in lactate is a result of both an increase in lactate synthesis and a decrease in lactate elimination from the body (Newton *et al.*, 1998:30).

Clinically, lactic acidosis is defined as blood or plasma lactate levels above 5 mmol/L and usually presents as tachypnea, deep gasping respiration, the use of secondary muscles for respiration, alar flaring, chest recession (intercostal or subcostal) and abnormally deep (acidotic) breathing (Newton *et al.*, 1998:23).

Normally, lactate plasma levels increase as a result of strenuous anaerobic exercise and are a short-term solution to the increased demand for ATP in skeletal muscle by allowing glycolysis to continue under these anaerobic conditions (Newton *et al.*, 1998:30). As soon as adequate oxygen supplies become available the excess lactate is metabolised into carbon dioxide and water in the liver, kidney, skeletal muscle and central nervous system (CNS) by metabolism *via* pyruvate. Without clearance of the excess lactate the buffering capacity of the blood and tissues is exceeded and lactic acidaemia ensues (Newton *et al.*, 1998:30).

In severe malaria, increased production of lactate can result from many causes. Sequestration of the microvascular circulation limits the availability of oxygen in the tissue, increasing the anaerobic glycolysis and leading to an increase in lactate plasma levels. Infected RBCs and the parasites themselves produce substantial amounts of lactate. Fever, anaemia and generalised seizures also cause an increase in metabolic rates resulting in a

substantial amount of lactate being synthesised. In addition to the increase in lactate production, the pathophysiological nature of the parasite decreases the normal excretion of lactate *via* the liver and kidneys, further increasing the lactate plasma concentration (Newton *et al.*, 1998:30, White 2008:1221). In severe malaria, the arterial, capillary, venous and cerebrospinal spinal fluid (CSF) concentrations of lactate rise in direct proportion to the severity of the disease and can be used to give an accurate prognosis (Newton *et al.*, 1998:30, White 2008:1221).

2.4.10 Hypoglycaemia

Hypoglycaemia, defined as plasma glucose levels of less than 2.2 mmol/L, presents as decreased consciousness, sweating, pupillary dilatation, tachycardia and seizures (Newton *et al.*, 1998:23). Hypoglycaemia is a common complication in malaria and is mainly as a result of; an increase in the demand for glucose in malaria resulting from stress, from the effects of fever and increased anaerobic glycolysis by both the host tissues and parasitized erythrocytes, from the effect of quinine or quinidine both of which stimulate the insulin secretion, and lastly from impaired gluconeogenesis within the liver secondary to acidosis (Newton *et al.*, 1998:29, Tillyard 2004:188, Ogetii *et al.*, 2010:1).

Hypoglycaemia has a prevalence of 8–20% and has been found to contribute to nervous system dysfunction and residual neurological deficit in survivors of cerebral malaria (White 2008:1222, Ogetii *et al.*, 2010:1)

2.5   Diagnosis

The preferred method of malaria diagnoses is the preparation of both thick and a thin blood smears examined under a light microscope. The thick smears is 20–40 times more sensitive than thin smears and is used for diagnoses, whilst the thin smear is used for identification of the species (Moody *et al.,* 2000:189, Trampuz *et al.,* 2003:316). In addition to identification, thin smears are also utilised in quantifying parasitemia and assessing the presence of schizonts, gametocytes and malarial pigment (Moody *et al.,* 2000:189). Using blood smears for diagnoses is however time consuming and requires the necessary personnel and equipment (Moody *et al.,* 2000:189).

In recent years, there has been an introduction of dipstick or card tests, which is simple, rapid, sensitive, highly specific and increasingly affordable. These tests are based on the

detection of specific malaria antigens in blood samples. Currently, histidine rich protein 2 (*Pf*HRP2), parasite lactate dehydrogenase and aldolase are some of the antigens used for detection (White 2008:1238, Trampuz *et al.,* 2003:317).

2.6   Chemotherapy

Despite enormous efforts, there is at present no effective malaria vaccine, leaving malaria therapy totally reliant on the use of drugs (Sardá *et al.*, 2009:3137, Todryk *et al.*, 2007:489, Matuschewski 2006:455, Moorthy *et al.*, 2004:155, Hyde 2002:165). The malaria parasite is rapidly acquiring resistance, fuelling the resurgence of the disease and making the need for new antimalarial drugs much greater (Le Bras *et al.*, 2003:148, Bloland 2001:1). The chromosomal mutations that confer antimalarial drug resistance are spontaneous and independent of the type of drug used, and not necessarily as a result of treatment failure (White 2004:1085, Le Bras *et al.*, 2003:148). This aggressive means of acquiring resistance has resulted in the parasite acquiring resistance against most drugs used against it, even the artemisinin class of compounds (Dondorp *et al.,* 2009:466).

Currently used antimalarial drugs fall into one of seven different pharmacological classes: the 4-aminoquinolines (chloroquine and amodiaquine), the aryl-amino alcohols (quinine, mefloquine, halofantrine and lumefantrine), the 8-aminoquinolines (primaquine), the antifolates (sulfadoxine, pyrimethamine, dapsone and proguanil), the hydroxynaphthoquinones (e.g. atovaquone), certain antibiotics (e.g. doxycyclin and clindamycin) and the artemisinin class of compounds (artemisinin, dihydroartemisinin, artemether and artesunate) (Schlitzer 2008:150).

2.6.1   4-Aminoquinolines

The precise mechanism of action of the 4-aminoquinoline compounds is not fully understood. The most widely accepted mechanism is thought to rely on their ability to accumulate to a great extent in the malaria infected erythrocyte, interfering with the normal process by which the parasite converts toxic free radical haem (ferriprotoporphyrin IX) into the insoluble nontoxic malaria pigment called hemozoin (Walczak *et al.*, 2011:1145, Sullivan 2002:1650). The 4-aminioquinolines behave as weak bases that concentrate in the food vacuoles of susceptible parasites. In the food vacuole they increase the vacuolar pH and inhibit the peroxidative activity of haem, disrupting its nonenzymatic polymerization into hemozoin. Failure to inactivate haem then kills the parasite *via* oxidative damage to its membranes,

digestive proteases and possibly other critical biomolecules. Although the inhibition of haem polymerization appears crucial to the 4-aminoquinoline's mechanism of action, the importance of other mechanisms e.g. the resulting accumulation of haem in the parasite, the formation of haem-quinoline complexes or other undefined actions, is still a topic of debate (Olliaro 2001:212, Walczak *et al.*, 2011:1145, Sullivan 2002:1650, Schlitzer 2008:150).
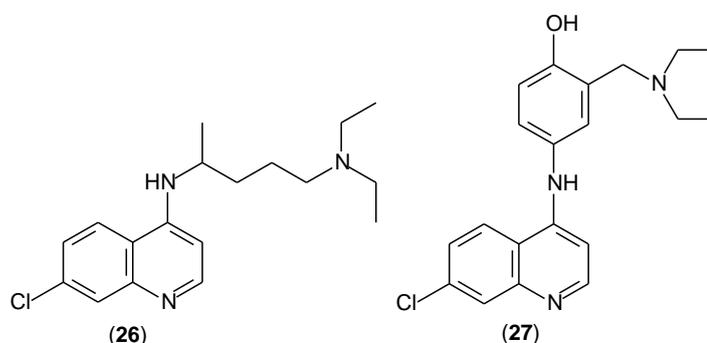

Figure 2.5: Structures of the 4-aminoquinoline compounds chloroquine (**26**) and amodiaquine (**27**).

2.6.1.1 Chloroquine

Chloroquine (CQ) (Fig. 2.5) is a 4-aminoquinoline and is formulated as a sulphate, phosphate or hydrochloric salt (WHO 2010a:73, White 2008:1251).

CQ and other synthetic quinoline antimalarials have been the foundation of malarial chemotherapy for much of the past 50 years (Mutai *et al.*, 2008:46). CQ has excellent clinical efficacy, limited toxicity, is easy to use and is very simple to synthesise (Bray *et al.*, 2005:9, Wellems *et al.*, 2001:770). The value of CQ as an antimalarial agent has unfortunately been eroded in recent years, mainly as a result of the development and spread of resistance (Wellems *et al.*, 2001:770).

CQ is more potent than quinine (see section 2.6.2.1), less toxic and is highly effective for prophylaxis and treatment of malaria caused by sensitive strains of *P. falciparum*. The drug rapidly controls the clinical symptoms and parasitemia of acute malaria attacks. Most patients become afebrile within 24–48 hours after their first dose, and thick blood smears of peripheral blood are generally negative by 48–72 hours. If the patient does not respond to CQ treatment within 48 hours, a resistant strain of *P. falciparum* should be suspected and quinine and another schizonticide should be used (Tracy *et al.*, 2001:1079).

CQ is administered over a period of 3 days. Against chloroquine sensitive non-*falciparum* malaria infections, 600 mg of chloroquine base is firstly given after which 300 mg is administered 6 hours later. After the initial two doses, 300 mg chloroquine base is given daily for a duration of two days (SAMF 2012:513).

When used for prophylaxis (only where non-*falciparum* malaria occurs), 300 mg of CQ base should be taken each weak divided into three doses (WHO 2001:45, SAMF 2012:513). Therapy should be initiated one week before entering the malarial area and continued for four weeks after returning (SAMF 2012:513).

CQ is generally well tolerated. Doses of CQ used for oral therapy might cause gastrointestinal upset, nausea, vomiting, headache, pruritus (mostly in dark-skinned persons), visual disturbances and urticaria. After an overdose, serious toxic manifestations relate primarily to the cardiovascular and central nervous systems. Cardiovascular effects include hypotension, vasodilatation, suppressed myocardial function, cardiac arrhythmias and eventual cardiac arrest (WHO 2010a:74, WHO 2001:47).

Resistance to CQ has been slow to develop but is now common worldwide (Wellems *et al.*, 2001:771). It is evident that resistant *P. falciparum* species reduce accumulation of CQ in the parasitic food vacuole by mechanisms that are still not clear. Proposed mechanisms include possible differences in CQ plasmodial uptake or efflux at the cytoplasmic membrane, altered H^+ flux at the parasite digestive vacuole, reduced CQ access to haematin and increased glutathione-mediated detoxification of CQ haematin complexes (Sullivan 2002:1649, Fidock *et al.*, 2000:862). One study found that the 13-exon gene, *Pf*CRT, have point mutations that is associated with CQ resistance. The *Pf*CRT protein is localised at the digestive vacuole and is thought to be responsible for alterations in the ion transport of the digestive vacuole membrane, leading to alterations in the acidity (Fidock *et al.*, 2000:869). A 0.3–0.5 unit decrease in vacuolar pH leads to a considerable decrease in the drug–haem interactions responsible for activity against the parasite. Drug flux across the digestive vacuole membrane is also affected by the structural changes in the *Pf*CRT protein, and by the effect of *Pf*CRT on other molecules (Fidock *et al.*, 2000:869).

2.6.1.2 Amodiaquine

Amodiaquine (AQ) (Fig. 2.5) is a 'Mannich base' 4-aminoquinoline with a similar mode of action to chloroquine (WHO 2010a:75).

With widespread resistance against CQ, AQ was seen as the cheapest alternative. Despite a certain amount of cross resistance, AQ has been found to retain activity in other areas where CQ has been rendered obsolete. AQ has a more palatable taste compared to CQ, has a decreased incidence of pruritus and was found to be significantly more effective than CQ in clearing parasites from the blood (Olliaro *et al.*, 1996:1197, WHO 2001:47). Unfortunately, prophylactic use of AQ is associated with unacceptable high incidents of hepatotoxicity and agranulocytosis, resulting in the drug being banned from certain countries (Olliaro *et al.*, 1996:1197, Schlitzer 2008:151). Research has, however shown that when only used in the treatment of malaria, AQ is arguably equally as safe as CQ (Olliaro *et al.*, 1996:1200).

Like CQ, AQ is also administered over a period of 3 days. Patients weighing more than 50 kg and are above 14 years of age, should take 600 mg daily for three days (WHO 2001:48).

The prophylactic use of AQ is associated with an unacceptable high incident of toxicity. Approximately 1 in 2 100 cases develops agranulocytosis, 1 in 15 500 hepatotoxicity and 1 in 30 000 aplastic anaemia with a total case fatality rate of 1 out of 15 650 cases (Olliaro *et al.*, 1996:1201).

Although there is a certain degree of cross-resistance between AQ and CQ, AQ still retains a much higher degree of activity in areas where CQ has long since been deemed ineffective (Fig. 2.13) (Holmgren *et al.*, 2006:309, Olliaro *et al.*, 1996:1201).

2.6.2 Aryl-amino alcohols

As with the 4-aminoquinoline compounds, the exact mechanism by which the aryl-amino alcohols exert their effect is not fully understood. Like the 4-aminoquinolines, it is believed that the aryl-amino alcohols act primarily on the erythrocyte stage of the malaria parasite by inhibiting the formation of hemozoin, resulting in the build-up of toxic ferriprotoporphyrin IX and ultimately killing the parasite (Foley *et al.*, 1998:67, Hoppe *et al.*, 2004:2370). It has also been observed that in addition to the hemozoin metabolic pathway, the aryl-amino alcohols have other targets by which they exercise their antimalarial activity, including their effects on volume-regulated anion channels, their inhibition of haemoglobin endocytosis and their ability to bind to phospholipids inside the malaria parasite (Foley *et al.*, 1998:67, Dassonville-Klimpt *et al.*, 2011:26). Although both the 4-aminoquinolines and aryl-amino alcohols prevent the

formation of hemozoin, only the 4-aminoquinolines inhibit the glutathione dependant destruction of ferriprotoporphyrin IX (Famin *et al.*, 2002:393).
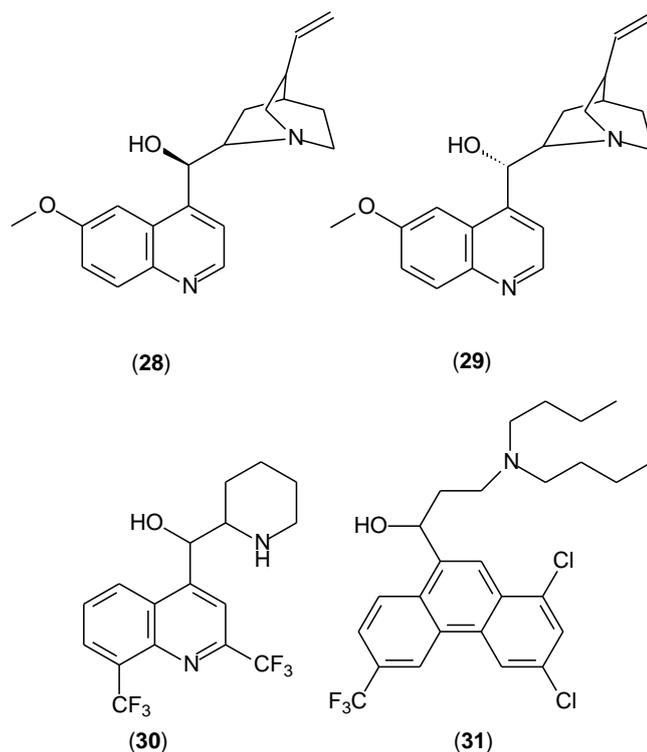

Figure 2.6: The aryl-amino alcohols, quinine (**28**), quinidine (**29**), mefloquine (**30**) and halofantrine (**31**).

2.6.2.1 Quinine

Quinine (QN) (Fig. 2.6) is one of four antimalarial alkaloids obtained from the bark of the chichona tree with the others being quinidine (the D-stereoisomer of QN), cinchonine and cinchonidine (WHO 2010a:90, White 2008:1246).

QN acts mainly on the mature trophozoite stage of the parasite's life cycle. Unlike the artemisinin class of compounds (see section 2.6.5), QN does not kill the exoerythrocytic or sexual stages of the parasite nor does it prevent sequestration or further development of formed meronts (WHO 2010a:90). Despite potential toxicity, QN is still the drug of choice in some countries. In severe cases, prompt use of QN can be life saving for non-immune patients. In the treatment of multidrug-resistant strains of *P. falciparum*, slower acting schizonticides such as sulphonamides and tetracyclines are given concurrently to enhance the action of quinine (Tracy *et al.*, 2001:1088, WHO 2001:63).

In areas with QN sensitive *P. falciparum*, 8 mg/kg of QN base is given three times a day for seven days. In areas where there are QN resistant *P. falciparum*, 8 mg/kg of QN base is given three times a day for seven days together with doxycyclin 100 mg daily for 7 days (WHO 2001:64). In the case of severe malaria a loading dose of 20 mg/kg QN should be diluted in 5% dextrose, 5–10 ml/kg, and given as a slow rate dependant IV infusion over 4 hours. Eight hours after the loading dose, a maintenance dose of 10 mg/kg QN should be diluted in 5% dextrose, 5–10 ml/kg, and given as a slow rate dependant IV infusion over 4 hours repeated 8 hourly until the patient is in a state where he/she can tolerate oral therapy (SAMF 2012:510).

A single dose > 3 g of QN is capable of causing serious adverse reactions in an adult (WHO 2001:66). Very often QN cause cinchonism that is characterised by tinnitus, impaired high tone hearing, headache, nausea, dizziness, dysphoria and disturbed vision (WHO 2010a:91, Foley *et al.*, 1998:72). Prolonged exposure to high doses may also produce gastrointestinal, cardiovascular and dermal manifestations (Tracy *et al.*, 2001:1088, WHO 2001:65). QN also has the ability to stimulate insulin secretion causing hypoglycaemia, especially during pregnancy (WHO 2001:65; WHO 2010a:91).

Despite being used in the treatment of malaria for more than 400 years, no widespread resistance against QN has been reported. A clear distinction should be made between treatment failure as a result of reduced *in vivo* efficiency, and treatment failure as a result of poor patient compliance or pharmacokinetic properties. With QN, most side effects manifest in the second half of the seven day treatment regime, when most malaria symptoms have passed, resulting in a large number of patients feeling no need to finish the course leading to sub-therapeutic QN plasma levels.   This results in treatment failure, not because of resistance, but because of poor patient compliance.   Another report found that early treatment failures with QN was in reality the result of unusual pharmacokinetic properties, again not as a result of reduced *in vivo* efficiency (Okombo *et al.*, 2011:78, Newton *et al.*, 2006:185). Actual resistance against QN appears to only be localised in South East Asia, with reports of resistance in Africa still needing further confirmation (Okombo *et al.*, 2011:78, Newton *et al.*, 2006:184).

2.6.2.2 Quinidine

Quinidine (Fig. 2.6) is the D-stereoisomer of QN and is more potent against malaria, but unfortunately has stronger cardiotoxic side effects making the drug less desirable (Foley *et al.*, 1998:66, WHO 2010a:90, WHO 2001:66). Quinidine's dosing regime is exactly the same

as QN's, where a loading dose of 20 mg/kg quinidine is diluted in 5% dextrose, 5–10 ml/kg, and given as a slow rate dependant IV infusion over 4 hours. Eight hours after the loading dose a maintenance dose of 10 mg/kg quinidine should be diluted in 5% dextrose, 5–10 ml/kg, and given as a slow rate dependant IV infusion over 4 hours repeated 8 hourly, until the patient is in a state where he/she can tolerate oral therapy (SAMF 2012:510, WHO 2001:66). Whilst administering quinidine *via* IV infusion, constant electrocardiographic monitoring is necessary (White 2008:1251).

Quinidine has a much greater effect on the cardiovascular system then QN and also stimulates insulin secretion resulting in hypoglycaemia (Foley *et al.*, 1998:66, White 2008:1250). Quinidine is, however less likely to cause deafness. Myocardial conduction, systemic hypotension and repolarisation abnormalities are much more common in patients receiving parental quinidine compared with those that receive QN (White 2008:1250).

Resistance against quinidine is the same as described for QN (see section 2.6.2.1).

2.6.2.3 Mefloquine

Mefloquine (MQ) (Fig. 2.6) is a 4-methanolquinoline derivative of QN and is effective against all forms of malaria (WHO 2010a:79). MQ was the first drug used to treat chloroquine–resistant *falciparum* infections in Thailand, where it was formulated with the pyrimethamine–sulphadoxine combination to delay development of resistance (Tracy *et al.*, 2001:1082).

MQ exists as both an *erythro* and *threo* racemic form, each consisting of a pair of enantiomers with only the *erythro* form being clinically used (Dassonville-Klimpt *et al.,* 2011:24). MQ is used against the *P. falciparum* strains that are resistant to 4-aminoquinolines and sulfa-pyrimethamine combinations, and is also active against *P. vivax* and *P. malariae*. It is a long acting blood schizonticide, not active against gametocyte or hepatocyte forms of malaria (WHO 2001:56). MQ is used both for therapy and as a chemoprophylactic agent and is especially useful as a prophylactic agent for non-immune travellers who stay for a brief period of time in endemic areas. The drug should, however, not be used for long term prophylaxis in order to prevent the development of MQ resistant parasites and to avoid the development of toxic adverse reactions (Tracy *et al.*, 2001:1083, WHO 2001:56).

For the treatment of uncomplicated malaria, 15-25 mg/kg is administered, divided into two doses given 24 hours apart, with meals and ample water (WHO 2001:57). For prophylaxis,

250 mg MQ is given once a week, initiated 1–2 weeks before arriving in the malaria endemic area and continued for 4 weeks after the last possible exposure (SAMF 2012:508).

MQ given in an oral dose of up to 1500 mg are generally tolerated well. Side effects like mild to moderate nausea, vomiting, abdominal pain, diarrhoea and dysphoria are frequently observed. Signs of central nervous system toxicities like dizziness, ataxia, headache, hallucinations, sleep disturbances, toxic encephalopathy and convulsions can occur (Tracy *et al.*, 2001:1083, WHO 2001:59).

Resistance to MQ started to appear in Thailand in 1982 and spread to other areas in Asia (Dassonville-Klimpt *et al.,* 2011:26). Resistance has increased gradually over the years to a point where the use of monotherapy has been compromised (Duraisingh *et al.*, 2005:181). In an effort to circumvent further development of resistance, the drug is combined with other potent antimalarials e.g. MQ-artesunate and MQ-artemether, successfully slowing down the spread of resistance. Areas previously affected by MQ resistance, however, remain less sensitive to these combinations indicating the presence of multidrug-resistant strains (Duraisingh *et al.*, 2005:181, Dassonville-Klimpt *et al.,* 2011:26).

2.6.2.4   Halofantrine

Halofantrine (HF) (Fig. 2.6) is a 9-phenanthrene methanol with one chiral carbon, but is used therapeutically as a racemate (White 2008:1253). As a member of the aryl-amino alcohols, HF possesses more structural similarities to QN and MQ than it does to the 4-aminoalcohols (Nateghpour *et al.,* 1993:2340).

HF is a blood schizonticide that is active against all *Plasmodium* species and is mainly used for the treatment of malaria due to CQ resistant *P. falciparum* infection. HF is, however not active against the gametocytes or hepatic stages of malaria (WHO 2001:67). HF is much more potent than QN and MQ but is unfortunately associated with a rare but potentially lethal ventricular tachycardia, which has restricted the use of this drug (White 2008:1253). Other disadvantages include a relatively high cost, variable bioavailability and cross-resistance with MQ, limiting the usefulness of the drug. HF might still be used in individual patients with a normal resting electrocardiogram in areas with fully sensitive malaria parasites (WHO 2001:67).

An oral dose of 8 mg/kg HF is given in three doses with a 6–8 hourly interval, and is repeated 1 week later in nonimmune patients (White 2008:1254, WHO 2001:67).

HF carries a significant risk of death, presumably resulting from ventricular tachyarrhythmia. HF slows atrioventricular conduction and produces the 'quinine effect' on myocardial repolarisation, reflected in a significant dose-related prolongation of the electrocardiograph QT interval. High HF doses may also cause diarrhoea, abdominal pain, vomiting, cough, rash, headache, pruritus and alleviated liver enzymes (Berman 2004:178, White 2008:1254, Rosenthal 2004:875, WHO 2001:68).

HF displays extensive cross-resistance with certain drugs that contain the same methanolic function e.g. MQ and QN. Interestingly, it was observed that an increase in CQ susceptibility leads to an increased HF resistance and *vice versa* (Wongsrichanalai *et al.*, 1997:156, Nateghpour *et al.,* 1993:2342).

2.6.3   8-Aminoquinolines

Over the years several 8-aminoquinolines have been tested against malaria, including pamaquine, bulaquine and isopentaquine, however only primaquine (PQ) (Fig. 2.7) had the desired antimalarial and toxicity profile (Vale *et al.*, 2009:937, WHO 2001:80).

As with both the 4-aminoquinolines and the aryl-amino alcohols, the exact mechanism of action of this class is not known. Studies have shown that PQ interferes with the parasite's DNA structure and disrupts the mitochondrial membranes, leading to its death (Basso *et al.*, 2011:55). Another mechanism that is under investigation is the generation of reactive oxygen species by PQ that suppresses cellular defence and attack cell macromolecules, this in turn leads to the infected RBCs being damaged and consequently recognised by the spleen and then selectively removed (Bowman *et al.*, 2005:838). Some of the proposed mechanisms are as a result of PQ's metabolites, whilst others are as a result of the parent drugs itself, although the extent of every entity's contribution to the proposed mechanisms is not known (Basso *et al.*, 2011:55).
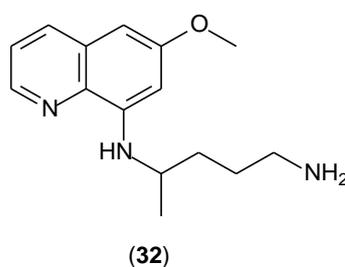

(**32**)

Figure 2.7:   The 8-aminoquinoline primaquine (**32**).

2.6.3.1 Primaquine

PQ is active against the intrahepatic forms of all species of malaria and also kills the hypnozoites resulting from *P. vivax* and *P. ovale* infections, making it the only drug currently on the market that is used for relapsing malaria. Primaquine is also gametocytocidal against *P. falciparum* and has significant blood stages activity against *P. Vivax* (WHO 2010a:87, WHO 2001:80). PQ is not only used in the treatment of malaria but is also utilised as a prophylactic agent (WHO 2001:81, Fryauff *et al.*, 1995:1190).

The required PQ dose differs for each species of parasite and whether it's used for treatment or prophylaxis.

The prophylactic treatment regime is 30 mg daily. In adults with normal glucose-6-phosphate dehydrogenase (G6PD), prophylactic treatment is generally well tolerated for periods of up to a year (WHO 2001:81, Fryauff *et al.*, 1995:1190). PQ prophylaxis is proficient against multiple *Plasmodium* species including *P. falciparum* and *P. vivax* (Fryauff *et al.*, 1995:1190).

When used for treatment, PQ dosage depends on the different species and location where treatment is taking place. *P. ovale* requires 15 mg PQ daily for 14 days, whilst *P. vivax* requires 30 mg daily for 14 days. Studies have shown that a dose of 15 mg for 14 days are sufficient for *P. vivax* prevalent north of the equator (WHO 2001:82, Baird *et al.*, 2003:116).

PQ may cause anorexia, nausea, vomiting and abdominal pain or cramps, weakness, uneasiness in the chest, anaemia, methemoglobinaemia, leukopenia and suppression of myeloid activity (WHO 2001:83). The most important adverse reaction, however, is haemolytic anaemia in patients with G6PD deficiency. In Mediterranean and Asian patients, the observed anaemia is potentially life threatening requiring blood transfusion in severe cases, whilst in African patients the anaemia is usually self-limiting (WHO 2010a:87, WHO 2001:83).

Although PQ has been used since 1946, resistance against the drug is extremely low, a fact that is not completely understood. Several hypotheses including PQ's short plasma half-life and its ability to sterilise the gametocyte form has been used to explain this phenomenon (Vale *et al.*, 2009:942, Baird *et al.*, 2004:1339). Those incidents of resistance that are however reported are very few and not necessarily conclusively linked to PQ resistance (Rajgor *et al.*, 2003:440).

2.6.4 The antifolates

The folate biosynthetic pathway in the parasite consists of a process in which six enzymes are involved in the conversion of guanosine triphosphate (GTP) to tetrahydrofolic acid (THF), a molecule that is of vital importance in the synthesis of purines, thymidine and some amino acids (Hawser *et al.,* 2006:941). The antifolates can be divided into two types. Type 1 antifolates consist of sulfonamides and sulfones that compete with *p*-aminobenzoic acid (PABA) for the active site on dihydropteroate synthase (DHPS), consequently inhibiting the formation of dihydropteroate. Type 2 antifolates consist of quinazolines, biguanides and triazine metabolites that inhibit dihydrofolate reductase (DHFR), preventing the nicotinamide adenine dinucleotide phosphate (NADPH) dependent reduction of dihydrofolate (DHF) to THF (Fig. 2.10) (Olliaro 2001:208). Combination of both type 1 and type 2 antifolates, e.g. sulfadoxine and pyrimethamine (SP), proved to have a synergistic effect against malaria, though resistance developed quickly compromising their use in many malaria endemic areas (Yuthavong 2002:176).

The 1,3,5-triazine sub-structure is a common moiety found in type-2 antifolate drugs (Fig. 2.8 & Fig. 2.9). The biguanide antifolate drugs do have activity on their own however, are in many cases also converted *in vivo* into the active triazine metabolite (Yuthavong 2002:175, Kinyanjui *et al.*, 1999:946). With various different substituents, the 1,3,5-triazine moiety has been found to have relative good activity against malaria and other bacteria both on its own and whilst attached to other pharmacophores (Agarwal *et al.*, 2005:532, Zhou *et al.*, 2006:5453, Manohar *et al.*, 2010:323, Kumar *et al.*, 2009:6997).
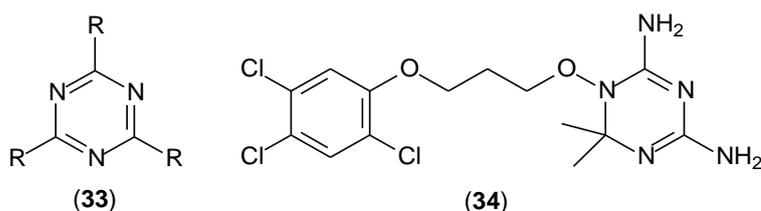

Figure 2.8:   The 2,4,6-trisubstituted-1,3,5-triazine (**33**) and the antimalarial triazine compound WR 99210 (**34**) (Kinyanjui *et al.*, 1999:943, Yuthavong 2002:175)
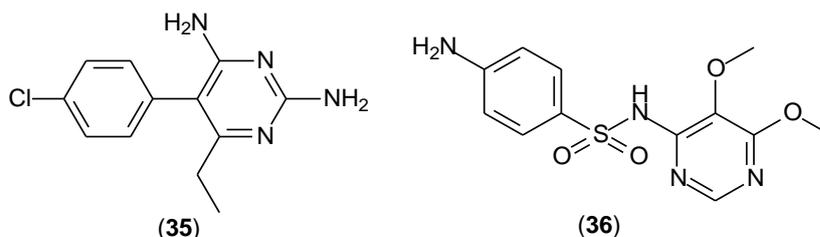

Figure 2.9:   Pyrimethamine (**35**) and sulfadoxine (**36**).

2.6.4.1 Pyrimethamine and sulfadoxine

Pyrimethamine (PYR) is a type-2 antifolate drug with a diaminopyrimidine structure. As a result of resistance, PYR is no longer used alone but always in combination with either sulfalene or sulfadoxine (SDX), with the PYR-SDX (SP) combination being more widely used (Fig. 2.9) (WHO 2010a:77, WHO 2001:51). SDX is a highly water soluble, slowly eliminated sulfonamide that is a structural analogue and competitive antagonists of PABA (WHO 2010a:76, Olliaro 2001:208).

PYR is a slow acting blood schizonticide with activity against the exoerythrocytic forms of the malaria parasite and is also known to inhibit sporozoite development in the mosquito vector (WHO 2010a:77). SP was initially used to treat chloroquine resistant *P. falciparum* infections but is now also plagued with very high levels of resistance (White 2008 1254). Initially this combination was used as both a treatment and prophylactic agent however, as a result of severe skin reactions they are now only utilised in the treatment of malaria (WHO 2001:50).

SP is given as a single oral dose consisting of three tablets, each containing 500 mg SDX and 25 mg PYR, resulting in a combined dose of 1500 mg SDX and 75 mg PYR (WHO 2001:51, SAMF 2012:511).

The most serious adverse reaction to the SP combination is the life threatening Steve-Johnson syndrome that is present in 1 out of 8 000 patients when the combination is used for prophylaxis. As a result the SP combination is today only used for the treatment of malaria. On its own, PYR are generally well tolerated occasionally causing hepatitis, thrombocytopenia, megaloblastic anaemia and leukopenia and rarely agranulocytosis and purpura (WHO 2001:53). Administration of PYR for prolonged periods may cause depression of haematopoiesis due to interference with folic acid metabolism (WHO 2010a:78).

There are no cross-resistance between the antifolate antimalarials and the 4-aminoquinolines, MQ, QN, HF or the artemisinin derivatives (WHO 2001:50). Not long after their introduction into the marked, worldwide resistance against this class spread rapidly making them obsolete in most areas afflicted by malaria (Uhlemann *et al.*, 2005:42). The extremely rapid emergence of resistance against the antifolates when compared to CQ indicates that there are alterations in the antifolates's target rather than in the mechanism by which the drugs reach their target, as is the case for CQ (Uhlemann *et al.*, 2005:43). The extent of resistance against PYR resulted in this drug only being used in combination therapy (Hyde 2002:166). Unfortunately, even the SP combination has fallen victim to resistance

resulting in it losing its effectiveness in a large number of the areas afflicted by malaria (Schlitzer 2008:154).
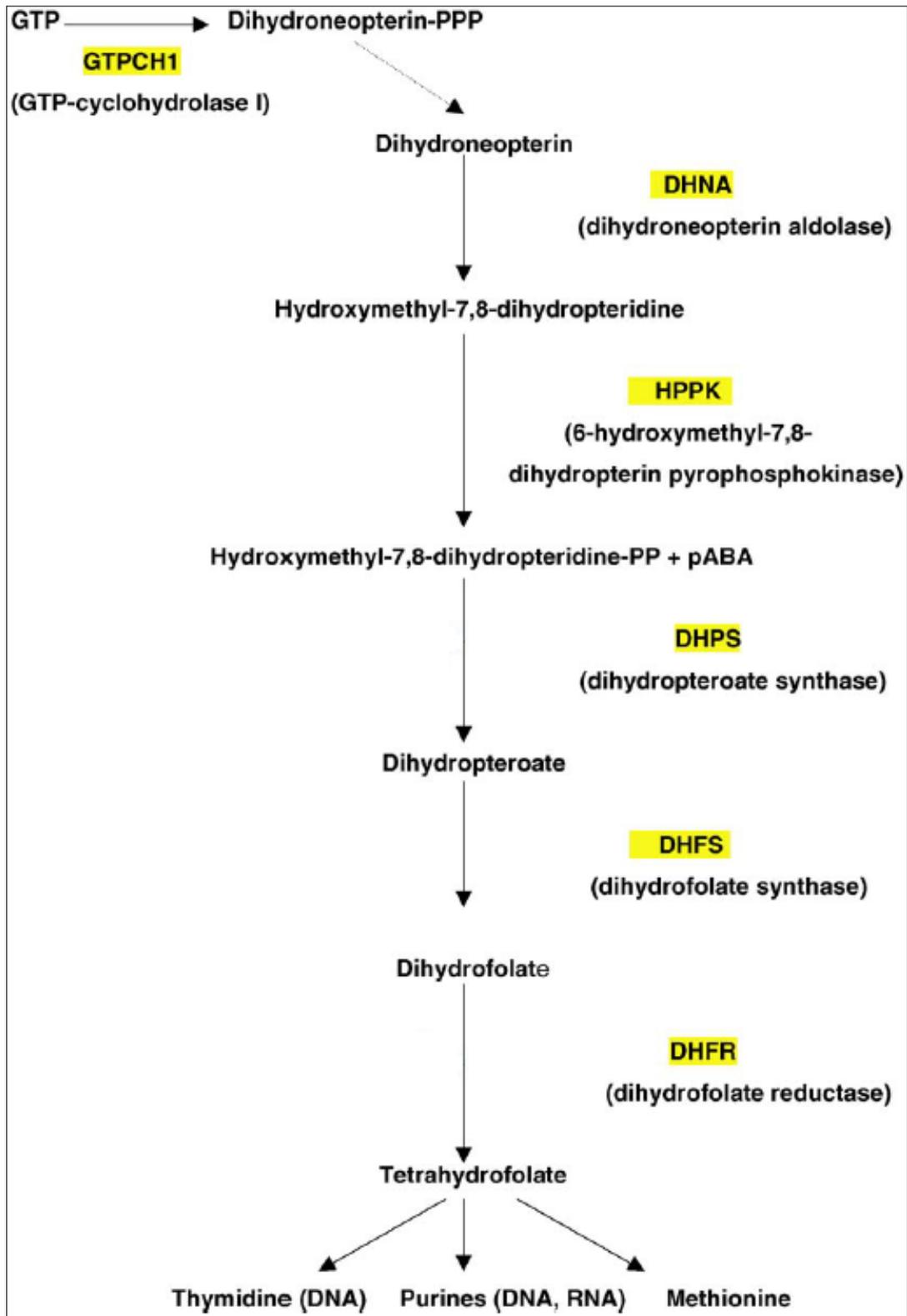

Figure 2.10:   Biosynthetic pathway of tetrahydrofolic acid (Hawser *et al.,* 2006:942).

2.6.4.1.1 Proguanil and atovaquone

Proguanil (PGN) is a biguanide dihydrofolate reductase inhibitor that is metabolised *in vivo* by the cytochrome P450 enzyme CYP2C19 into its active metabolite cycloguanil (CGN) (WHO 2001:54, WHO 2010a:89). Although the active metabolite is responsible for the antimalarial activity, PGN has been shown to exhibit some activity on its own through unknown mechanisms (Kinyanjui *et al.*, 1999:946, WHO 2001:54). PGN has a significant antiplasmodial effect on the tissue stages of *P. falciparum*, *P. vivax* and *P. ovale* and also exhibits a weak schizonticidal effect (WHO 2001:54). As a result of resistance against PGN, the drug is never used alone but always in combination with a variety of drugs, including CQ and atovaquone (WHO 2010a:89, WHO 2001:55, Schlitzer 2008:154). Atovaquone is a hydroxynaphthoquinone antimalarial and is active against all *Plasmodium* species, inhibiting the exoerythrocytic stage in humans and the oocyst development in the mosquito (Fig. 2.11) (WHO 2001:54, Schlitzer 2008:154, WHO 2010a:88).

The PGN-atovaquone combination is utilised in both prophylaxis and the treatment of malaria, whilst the PGN-CQ combination is only used for prophylaxis (Van Der Berg *et al.*, 1999:746, Matsika-Claquina *et al.*, 2006:382, Høgh *et al.*, 2000:1888).
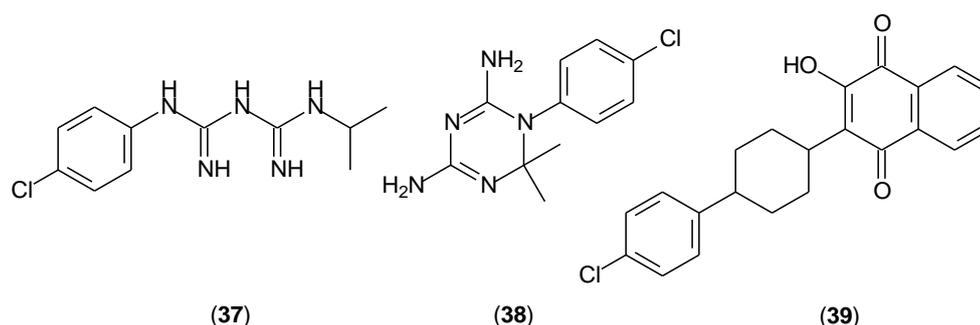

Figure 2.11: Proguanil (**37**), its active metabolite cycloguanil (**38**) and atovaquone (**39**).

The prophylactic PGN-atovaquone regime consists of 250 mg atovaquone and 100 mg PGN taken daily at the same time with food or milk. Treatment should commence 1-2 days before entering the malaria area and should continue for 7 days after leaving the risk area (Van Der Berg *et al.*, 1999:746, WHO 2010b:146). The prophylactic PGN-CQ regime consists of 250 mg CQ taken twice a week, and 100 mg PGN twice daily (Høgh *et al.*, 2000:1889). CQ treatment should commence 1 week before entering the malaria endemic area whilst PGN treatment should commence 1-2 days before entering the area. The PGN-CQ combination should be stopped 4 weeks after returning from the endemic area (WHO 2010b:146).

For treatment of uncomplicated malaria, 4 tablets of the PGN-atovaquone combination (consisting of 250 mg atovaquone and 100 mg PGN) are given for 3 days with food (Wichmann *et al.*, 2004:2).

PGN has almost no adverse reactions, with only mild gastric intolerance, diarrhoea and occasional aphthous ulceration and hair loss (WHO 2010a:90).

Resistance against both PGN and atovaquone developed extremely fast when used on their own, barring their use as monotherapy (Schlitzer 2008:154, WHO 2010a:89). The use of the PGN-atovaquone combination however, has proved to be highly effective with minor resistance reported thus far (Wichmann *et al.*, 2004:3). It was, however observed that when a certain strain develops resistance against atovaquone, that same strain will exhibit resistance against the PGN-atovaquone combination (Schlitzer 2008:154).

2.6.5   The artemisinin compounds

Artemisinin (qinghaosu) (Fig. 2.12) is a sesquiterpene lactone with a peroxide bridge linkage, and is extracted from the leaves of the sweet wormwood plant (*Artemisia annua*), and has been used in China for more than 1500 years to treat malaria (Woodrow *et al.*, 2005:71). Artemisinin's low solubility in both oil and water led Chinese researchers to synthesise the oil-soluble artemether and arteether derivatives, the water-soluble artesunate derivative and dihydroartemisinin (DHA) (Bray *et al.*, 2005:17, White 2008:1239, WHO 2001:69).

Artemether, arteether, artemisinin and sodium artesunate (Fig. 2.12) are all metabolised *in vivo* into the active form DHA, although each parent compound also contributes to the overall antimalarial effect (Krishna *et al.*, 2004:234). The artemisinin class of compounds are active against all species of malaria that invade humans, not only killing the large ring stage parasites, but also the tiny ring stage that are present only a few hours after RBC invasion. These drugs have the capability to induce a 10 000 fold reduction in parasitemia during the asexual stage, killing the parasite and resulting in its removal from the RBC allowing the "pitted" RBC to go back into circulation (Woodrow *et al.*, 2005:72). In addition to the antimalarial action on the erythrocyte stage, the artemisinins also inhibit parasite metabolism, cytoadherence of infected RBCs and kill the early gametocytic stage of the parasite, preventing transmission to the mosquito (Woodrow *et al.*, 2005:74).

Although the endoperoxide bond is now known to be necessary for antimalarial activity, the exact mechanism of action is still a topic of debate with each proposed mechanism raising more questions than answers. For a long time the ability of the artemisinin class to generate

free radicals after forming adducts with free haem was thought to be the reason for their antimalarial activity (Woodrow *et al.*, 2005:74). This theory originates from the knowledge that peroxides are a good source of free reactive oxygen species (ROS) and that the Fe^{2+} dependant Fenton process enhance the production of ROS.
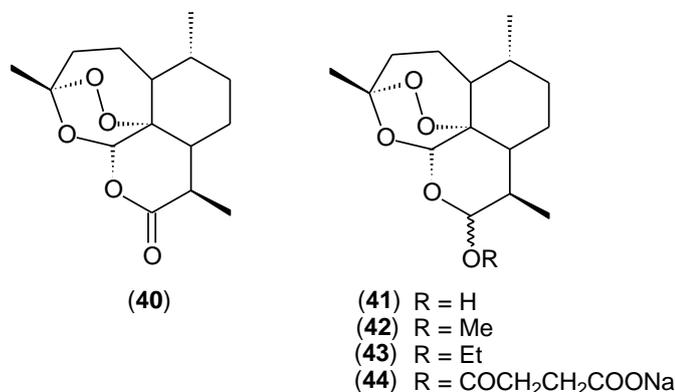

(**40**)	(**41**) R = H
	(**42**) R = Me
	(**43**) R = Et
	(**44**) R = COCH$_2$CH$_2$COONa

Figure 2.12:   Artemisinin (**40**), dihydroartemisinin (**41**), artemether (**42**), arteether (**43**) and artesunate (**44**).

A source inside the infected RBC that have abundant Fe^2 ions is the food vacuole of the parasite, where digestion of haemoglobin takes place and free iron is detoxified by conversion into hemozoin.   This mechanism hypothesises that parasite death and haemolysis are caused by ROS, whose increase in numbers eventually overwhelms the parasites' anti-oxidant defence mechanisms killing it (Krishna *et al.*, 2004:236, Krishna *et al.*, 2010:517).   Although this proposed mechanism explains some of the observations made whilst investigating the antimalarial action of the artemisinins, some critical questions still remain, e.g. why does haem-artemisinin adducts not have antimalarial activity *in vitro*, why are there artemisinin derivatives that are unable to chemically react with haem but still possess potent antimalarial activity, how can artemisinins kill non-haem generating parasites and why are there correlations between polymorphisms in *Pf*ATP6 and artemisinin susceptibilities (Krishna *et al.*, 2004:236, Krishna *et al.*, 2010:517)?   A new proposed mechanism of action that is a bit more compatible with the antimalarial mechanism observations for the artemisinin compounds, is that there are an inhibition of the parasites' sarcoplasmic reticulum Ca^{2+}-transporting ATPases (SERCAs). This theory originates from the observation that the artemisinins and thapsigargin, a known inhibitor of SERCAs, have structural similarities between their sesquiterpene moieties (Krishna *et al.*, 2010:517, Krishna *et al.*, 2004:241).   Artemisinin might induce its activity in an analogous manner, more specifically targeting the SERCA of malarial (*Pf*ATP6) and not mammalian pumps. Proof corroborating this hypothesis includes a demonstrated specificity for inhibition of the SERCA of *P. falciparum*, an exceptional correlation between assays for inhibiting *Pf*ATP6 and killing

of parasites, an antagonistic effect between thapsigargin and artemisinins and also an appropriate Fe^{2+}-dependency for inhibition of PfATP6 (Krishna $et\ al.$, 2004:241).

The use of artemisinin has now largely been replaced by DHA, artemether and artesunate, with the latter two derivatives used most frequently (Schlitzer 2008:152, WHO 2010a:80). Arteether is used far less than artemether because of a lack of clear advantages and much less clinical data available (Woodrow $et\ al.$, 2005:71). DHA is not soluble in water but can, however, with suitable excipients be administered via the oral and rectal routes (WHO 2010a:83). The lipid soluble artemether can be administered as an oil-based intramuscular (IM) injection or orally, whilst the water soluble artesunate can be administered orally, rectally or via IM injection (WHO 2010a:83). Artemisinin derivatives are used against uncomplicated and severe malaria in adults, children and in pregnant women (Woodrow $et\ al.$, 2005:74). These compounds act against chloroquine sensitive, chloroquine-resistant and multidrug-resistant strains of $P.\ falciparum$ with potencies 100-fold greater than any other antimalarial drug on the market. They have the broadest antimalarial effect and produce more rapid clearance than any other drug (Tracy $et\ al.$, 2001:1072, White 2008:1258). Artesunate, artemether and artemisinin have elimination half-lives of <10min, <2.8 h and <5 h respectively (Krishna $et\ al.$, 2004:234, Karbwang $et\ al.$, 1997:309, de Vries $et\ al.$, 1996:818). Because of their short elimination half-lives, the active metabolite DHA exerts most of the antimalarial effect. The advantage of their short half-lives is that selection for drug-resistant parasites is less likely to happen. The disadvantage, however, is that there is also a higher associated risk of recrudescence when these drugs are used in monotherapeutic regimens (Krishna $et\ al.$, 2004:234). To reduce the occurrence of recrudescences and to prevent the development of resistance, the WHO now recommends combining the artemisinin class of compounds with other antimalarials of a distinct different class to form artemisinin-based combination therapies (ACTs) (Na-Bangchang $et\ al.$, 2009:391, WHO 2010a:13). Combining a fast-acting highly effective artemisinin derivative, e.g. artesunate, with a longer acting partner drug, e.g. MQ, forms a combination that is highly effective, reducing the parasite biomass with 4-logs in each asexual life cycle (Na-Bangchang $et\ al.$, 2009:391).

As a result of this class's short half-life, the artemisinins are only used in the treatment of malaria and not as prophylaxis. All artemisinin compounds should preferably not be administered as monotherapy but as ACTs. When monotherapy is unavoidable, as a result of known adverse reaction to combination therapy, treatment should preferably not be shorter than 7 days and adherence to the correct treatment regime should be insured (WHO 2001:72).

On occasion when artemisinin is still used, combined therapy constitutes a loading dose of 20 mg/kg on the first day, then 10 mg/kg for the next two days plus MQ 25 mg/kg as a single dose on the second day of treatment (WHO 2001:73). Monotherapy consists of a loading dose of 20 mg/kg on the first day, then 10 mg/kg for the next 6 days (WHO 2001:72).

For the treatment of uncomplicated malaria, combined therapy with artemether entails 4 mg/kg once a day for 3 days plus mefloquine 25 mg/kg as a single dose on the second day of treatment. Artemether monotherapy consists of a loading dose of 4 mg/kg on the first day, followed by 2 mg/kg once a day for 6 days. For the treatment of severe malaria a loading dose of 3.2 mg/kg artemether is administered *via* IM injection on the first day, followed by 1.6 mg/kg daily for a minimum of 3 days or until the patient can take oral therapy to complete a 7 day course. The daily dose can be given as a single injection (WHO 2001:75).

The treatment regime of artesunate for uncomplicated malaria, for both mono- and combined therapy are exactly the same as for artemether. When treating severe malaria with artesunate a loading dose of 2.4 mg/kg is administered *via* IM injection followed by 1.2 mg/kg at 12 and 24 hours, after which 1.2 mg/kg is administered daily for 6 days. Intravenously, 2.4 mg/kg is injected on the first day followed by 1.2 mg/kg daily for 6 days (WHO 2001:77).

For the treatment of uncomplicated malaria with DHA, both mono- and combined therapy regimes are exactly the same as for artesunate/artemether (WHO 2001:79).

Remarkably this potent class of compounds are extremely well tolerated with very few adverse reactions. There have been reports of mild gastrointestinal disturbances, dizziness, tinnitus, reticulocytopenia, neutropenia and a rare severe allergic reaction prevalent in only 1 in 3 000 people taking the drug (WHO 2010a:81, Woodrow *et al*., 2005:77). Potentially the most deadly adverse reaction is neurotoxicity that has been observed in several animal models, but has yet to be substantiated in humans (Woodrow *et al*., 2005:76).

The only reported cases of resistance against these drugs are at the Thai-Cambodian and Thai-Myanmar borders were resistance against artesunate were characterised by a markedly prolonged time to clear the parasite from the blood (Dondorp *et al.,* 2009:466, Phyo *et al*., 2012:6). Artemisinin and its derivatives should not be given as monotherapy, but in combination with other drugs in order to avoid the development of resistance (White 2008:1259).
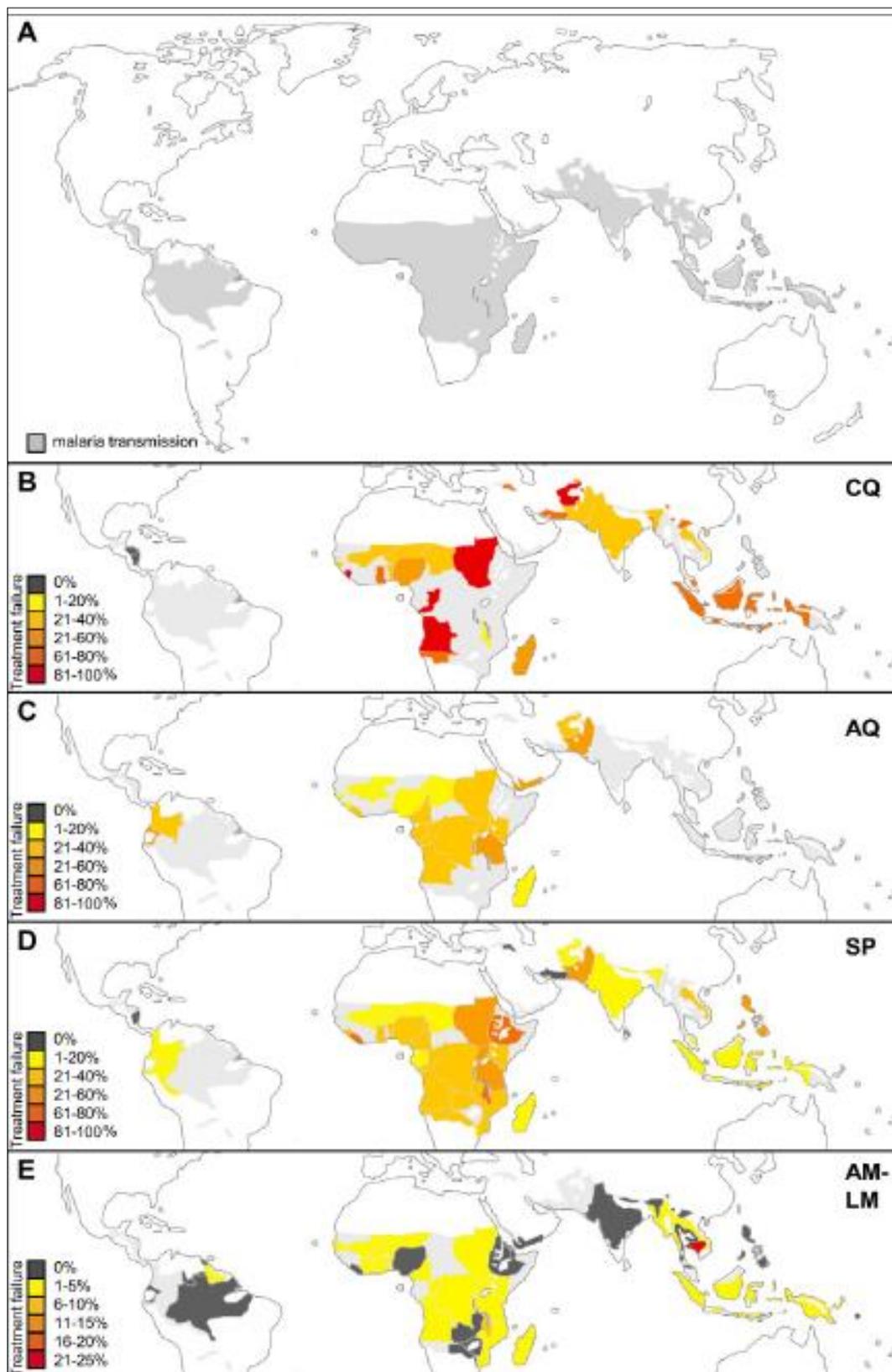
Figure 2.13: (A) Areas of malaria transmission, (B) CQ treatment failure, (C) AQ treatment failure, (D) sulfadoxine-pyrimethamine treatment failure, (E) artemether-lumefantrine treatment failure (Petersen *et al.*, 2011:1552).

2.7   Ion trapping

The success of the aryl-amino alcohols can be attributed, amongst other things, to their ability to hyperconcentrate inside the parasitic food vacuole to the concentration needed for inhibition of the detoxification of haem, thereby killing the parasite (Fig. 2.14) (Olliaro 2001:212, Sullivan 2002:1648). The major mechanism by which the necessary intra parasitic concentrations are achieved is by a process called ion trapping (Egan 2003:118).   Ion trapping (also known as pH trapping) relies of the notion that amino groups in certain drugs, e.g. CQ, act as weak bases. In the normal *in vivo* environment (pH 7.4), these amines are neutral and unprotonated increasing the amount of drug capable of crossing lipophillic membranes *via* passive diffusion. When these unprotonated drug molecules reach the acidic food vacuole (pH 4.5 - 5.5) of the malaria parasite, they become protonated and less membrane permeable resulting in their accumulation in the digestive vacuole to the necessary concentrations (Egan 2003:118).
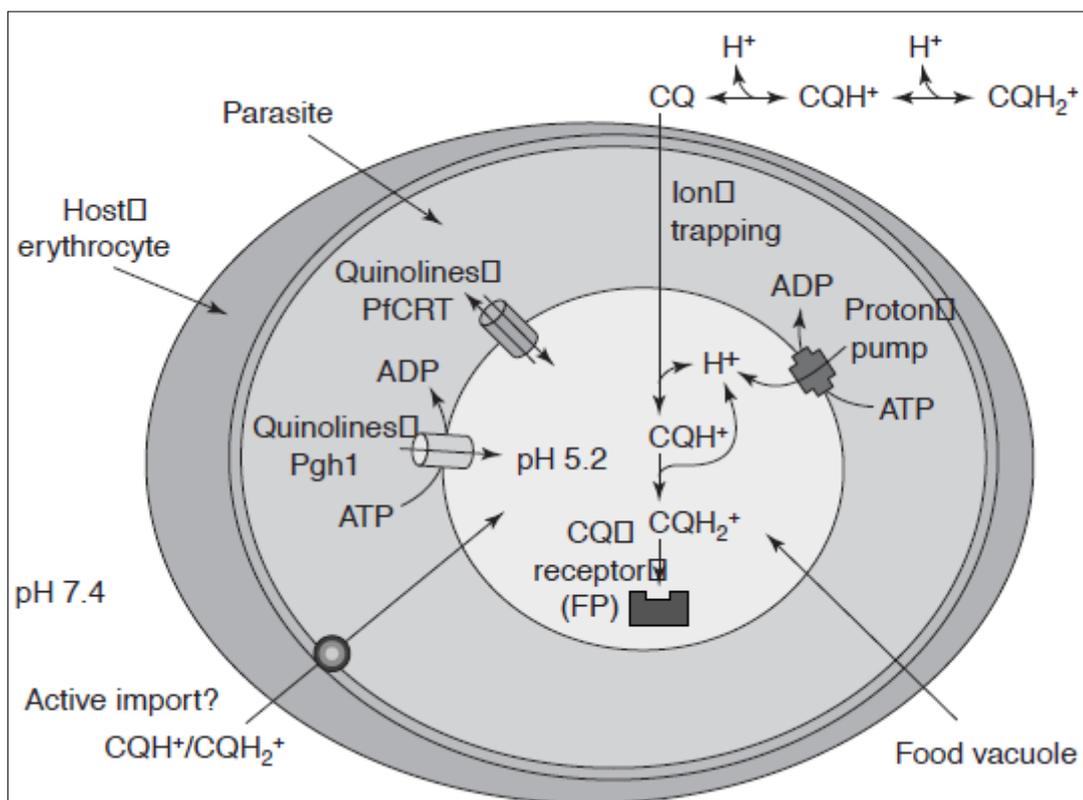

Figure 2.14:   pH trapping in the malaria parasite (Macreadie *et al*., 2000:439).

The pK_a value of each amino group in a molecule has the possibility to effect the drug's potency through its contribution to ion trapping. With a pH gradient of about 2.2, it is calculated that CQ will accumulate 20 000 fold in the parasitic food vacuole, assuming

passive diffusion of the free base, partitioning along pH gradients and trapping of both the mono- and di-protonated CQ in acidic parasitic compartments (Kuhn *et al.*, 2007:1010). At physiological pH, CQ is partially ionised with an aromatic and aliphatic amino group with pK_a values of 8.55 and 9.81, respectively. The non-ionised molecules can easily cross the parasite vacuole membrane, however once inside the acidic food vacuole the molecule becomes protonated, trapping the CQ inside (Wright 2007:903).

Ion trapping has been found to contribute to the activity of the aryl-amino alcohols, QN, CQ derivatives and novel antimalarial drugs (Ryckebusch *et al.*, 2003:3784, Egan *et al.*, 2000:284, Sanchez *et al.*, 2008:1089, Kaschula *et al.*, 2002:3533, Kumar *et al.*, 2008:713). Previous studies designed to increase the concentration of artemisinin *via* ion trapping resulted in a modest increase in antimalarial activity (Hindley *et al.*, 2002:1056, O'Niel *et al.*, 1996:4513).

2.8   Mono- and bis-quaternary ammonium salt drug theory

The asexual erythrocyte stage of the malaria parasite synthesises a huge quantity of membranes, leading to an increase in the erythrocytic phospholipid (PL) content of about 600%. Phosphatidylcholine (PC) and phosphatidylethanolamine (PE) makes up 85% of the parasite's PLs. The parasite draws 'polar heads' (choline, ethanolamine and serine) and fatty acids from the plasma and synthesises the bulk of the PLs needed using its own intracellular machinery (Fig. 2.15) (Calas *et al.*, 1997:3557). The parasite is totally reliant on this machinery, as the mature erythrocyte is devoid of any lipid biosynthetic activity (Calas *et al.*, 2007: 6307).

PL biosynthesis has been identified in previous studies as a vital metabolic pathway, with choline transport into the infected erythrocyte one of the rate limiting steps (Ancelin *et al.*, 2003:2598). It was found that certain antimalarial compounds can block the *de novo* synthesis of PC, the major malarial phospholipid, by competing with or substituting the polar head analogs (e.g. choline) (Fig. 2.16) (Calas *et al.*, 1997:3557, Calas *et al.*, 2007:6307). These choline antagonists possess mono- or bis-quaternary ammonium groups, which are potent inhibitors of the PL-related choline carrier in eukaryotic cells, such as erythrocytes. These structures selectively inhibit the *de novo* malaria PC synthesis by mimicking the choline structure (Fig. 2.17) (Calas *et al.*, 2007:6307).
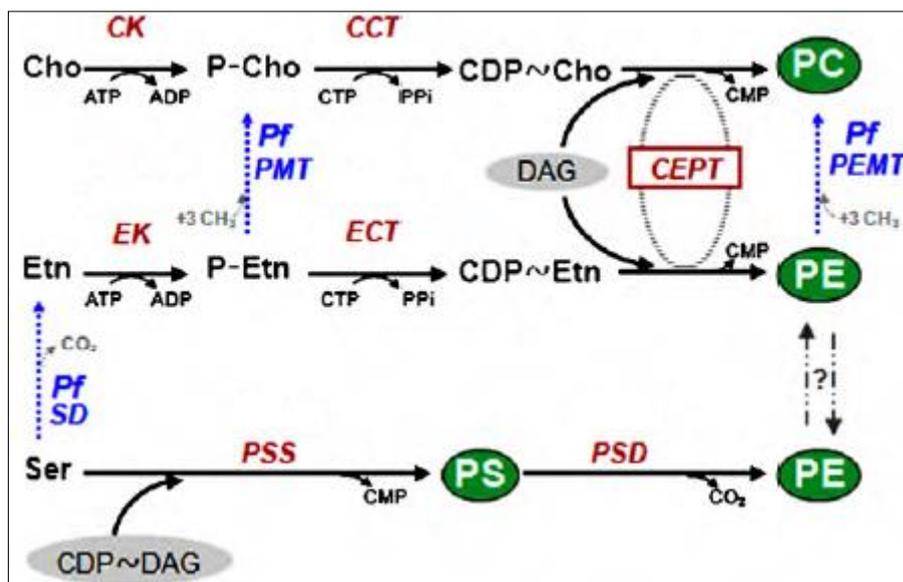
Figure 2.15: Biosynthetic pathways for PC, PE and PS in *Plasmodium*. Lipids are represented as grey ovals and enzyme names are in red italics. Specific pathways to *P. falciparum* are marked by blue dotted arrows. *Plasmodium* species (Pf: *P. falciparum*) in which enzyme pathways take place are indicated above the enzyme names. DAG, diacylglycerol; CDP-, cytidine-diphospho-; Cho, choline; P-Cho, phosphocholine; Etn, ethanolamine; P-Etn, phosphoethanolamine; Ser, serine; CK, choline kinase; CCT, CTP: phosphocholine cytidylyltransferase; EK, ethanolamine kinase; ECT, CTP: phosphoethanolamine cytidylyltransferase; CEPT, choline/ethanolamine-phosphotransferase; PSS, phosphatidylserine synthase; PSD, phosphatidylserine decarboxylase; PEMT, phosphatidylethanolamine N-methyltransferase; SD, serine decarboxylase; PMT, phosphoethanolamine N-methyltransferase (Déchamps *et al.*, 2010:70).

This pharmacological approach has been fully validated *in vivo* against rodent malaria and *P. berghei* and *P. cynomolgi* malaria in monkeys. In these studies, the authors claimed to have successfully cleared parasitemia in animals by specifically targeting the choline uptake with the synthesised ammonium salt compounds (Ancelin *et al.*, 2003:2600).

The potency of these compounds seems to be related to their ability to accumulate inside the infected erythrocytes in high concentrations. It was found that primary, secondary and tertiary amine compounds all had lower activity than the quaternary ammonium salts against the malaria parasite, with the activity increasing as the substitution on the nitrogen atom

increases. It was also found that if the nitrogen atom had a positive charge, the antimalarial activity increased dramatically (Calas *et al.*, 1997:3560).
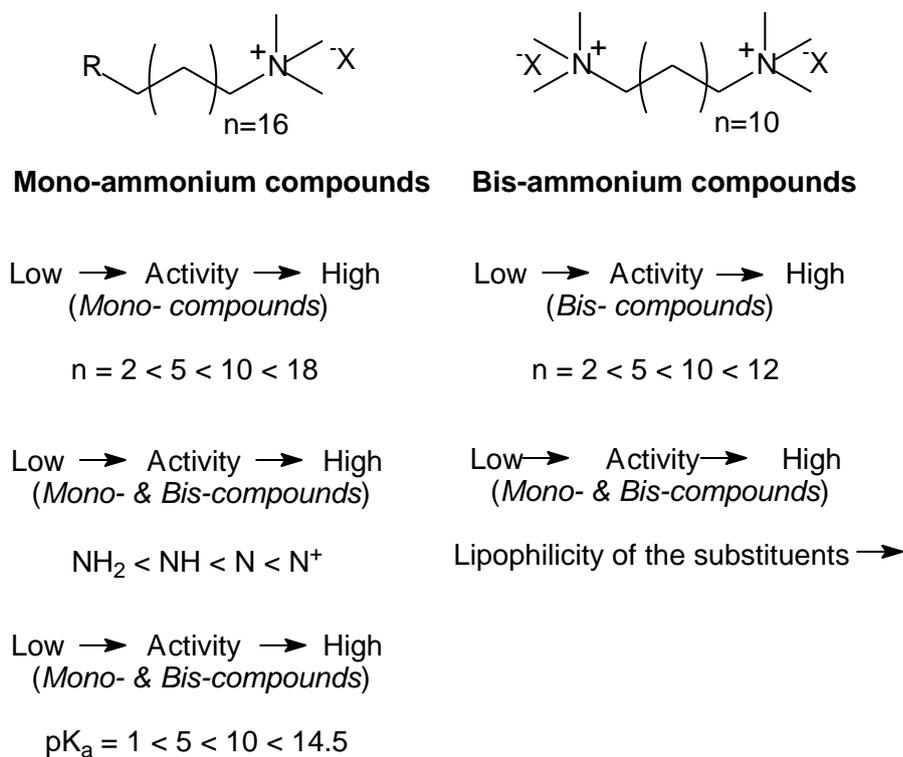

Mono-ammonium compounds **Bis-ammonium compounds**

Low ⟶ Activity ⟶ High   Low ⟶ Activity ⟶ High
(*Mono- compounds*)   (*Bis- compounds*)

n = 2 < 5 < 10 < 18   n = 2 < 5 < 10 < 12

Low ⟶ Activity ⟶ High   Low ⟶ Activity ⟶ High
(*Mono- & Bis-compounds*)   (*Mono- & Bis-compounds*)

$NH_2 < NH < N < N^+$ Lipophilicity of the substituents ⟶

Low ⟶ Activity ⟶ High
(*Mono- & Bis-compounds*)

$pK_a = 1 < 5 < 10 < 14.5$

Figure 2.16: Pharmacophore of the general mono- and bis-amine compounds as described by Calas and co-workers (Calas *et al.*, 2007:6309, Ancelin *et al.*, 2003:2599).

Concerning the mono-quaternary ammonium salts, it was found that by increasing the alkyl chain length from 2 – 18 carbon atoms, an increase in the activity was observed whether they contained an alcohol in the chain or not. Increasing the lipophilicity of the substituents on the nitrogen also had a beneficial effect on antimalarial activity (Calas *et al.*, 1997:3560). Another important parameter is the basicity of the polar head. It was observed that as the pK_a value increased, so did the antimalarial activity of the compounds, with the optimum pK_a value being between 12.5 and 14.5 (Calas *et al.*, 2007:6312).

The bis-quaternary ammonium salts showed the highest activity of all the compounds tested. It was found that a chain length of 12 carbon units separating the two polar heads was optimum for antimalarial activity. The presence of an oxygen atom in the linker had no negative effect on the activity of the compounds (Calas *et al.*, 2007:6310).

One major drawback of the quaternary ammonium salts is their very low oral bioavailability. These compounds carry a positive charge making it difficult to cross the intestinal wall. One way to circumvent this problem is to replace the quaternary ammonium salt with a bioisosteric group that is highly basic, ionise at physiological pH and is capable of creating bonds with the target(s) similar to those of the bis-quaternary ammonium salt. Tertiary amines, amidines or guanidines are perfect bioisosteric groups and should cross the intestinal wall more readily (Calas *et al.*, 2007:6308).
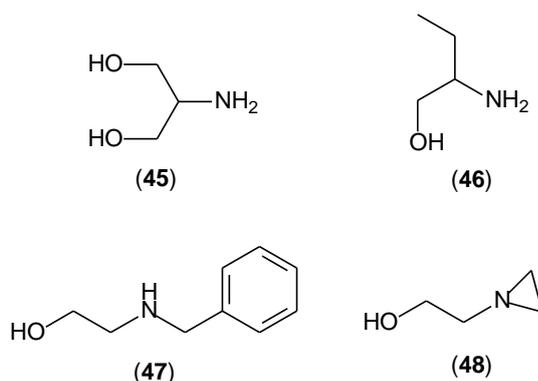

Figure 2.17: Mono-ammonium compounds with activity against malaria, (**45**) (IC_{50}: 50 µmol/L), (**46**) (IC_{50}: 80 µmol/L), (**47**) (IC_{50}: 40 µmol/L) and (**48**) (IC_{50}: 50 µmol/L) (Ancelin *et al.*, 1998:1427).

2.9   Hybrids

A hybrid drug is defined as a chemical entity with more than one structural domain each having its own biological function, indicating that the hybrid acts as two distinct pharmacophores either on the same or different biological targets (Fig. 2.18) (Meunier 2008:72).

Hybrid molecules should not be confused with either prodrugs (that regenerates an active pharmacophore *in vivo*), the fragment-based approach (the addition of multiple fragments that are able to bind to adjacent regions of the active site when the protein target is known), or physically mixing two drug powders and administering them together (e.g. ACTs) (Meunier 2008:72).
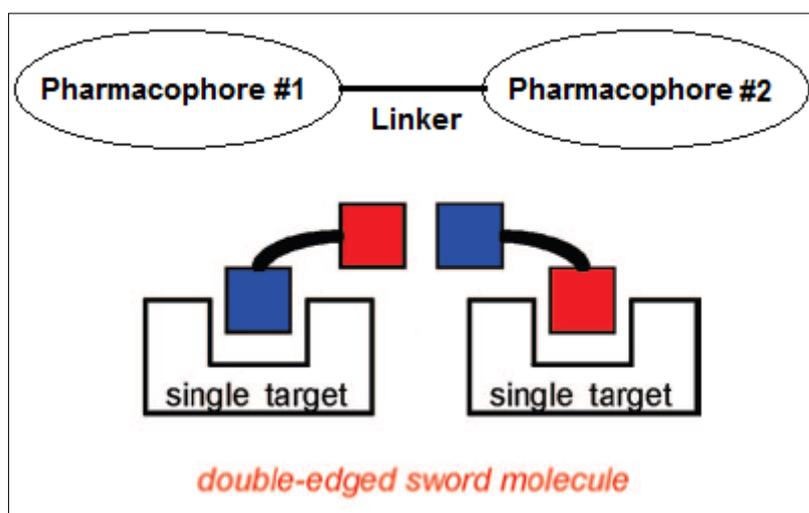
Figure 2.18: Hybrid drug theory (Meunier 2008:69).

Aside from the dual mechanism of action, other advantages of hybrid molecules include more predictable pharmacokinetic properties, the ability of one entity to impart favourable qualities onto the other entities, e.g. increased solubility, and a tendency to be more active than merely the physical mixing of two pharmacophores in a 1:1 ratio and administering them together (Meunier 2008:75, Walsh *et al.*, 2007:3600). Hybrid drug molecules have been utilised against malaria numerous times, showing a propensity for increased activity and the ability to overcome resistance (Walsh *et al.*, 2007:3600, Kumar *et al.*, 2009:6997, Manor *et al.*, 2010:324).

2.10 Dimers

In addition to coupling different pharmacophores to one another, the attachment of the same pharmacophore to itself, thus forming a dimer, trimer, etc., has been shown to increase activity and overcome resistance (Galal *et al.,* 2009:746, Chaturvedi *et al.*, 2010:20, Posner *et al.*, 2007:2516, Kaur *et al.*, 2010:3247).

Piperaquine is a bisquinoline dimer that was synthesised in the 1960s and used on a large scale in China (Fig. 2.19) (Davis *et al.*, 2005:76). Piperaquine shows good activity and a lower degree of resistance as compared to its monomer counterpart, CQ (Davis *et al.*, 2005:77, Keating 2012:940). In this case the lower resistance was attributed to the bulky nature of piperaquine, which inhibit the transporter mediated drug efflux, protecting the drug against CQ resistant strains of *P. falciparum* (Davis *et al.*, 2005:77).
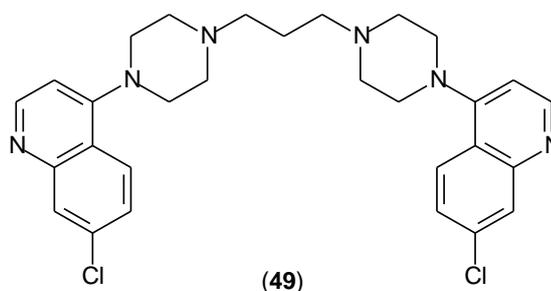
(**49**)

Figure 2.19: Piperaquine (**49**), a CQ dimer.

Artemisinin dimers were also found to have increased anticancer and antimalarial activity compared to artemisinin monomers and related derivatives (Posner *et al.*, 2008:1035, Posner *et al.*, 2007:2516, Grellepois *et al.*, 2005:5222, Chaturvedi *et al.*, 2010:20, Galal *et al.,* 2009:744).

2.11 Targeted drug delivery

Targeted drug delivery has the potential to increase the activity and decrease the toxicity of a given drug compound (Chadwick *et al.*, 2010:2586). In both cancer and malaria research the exploitation of the polyamine transportation in specific targeting of malicious cells have received much attention in the past decade (Müller *et al.*, 2001:246, Chadwick *et al.*, 2010:2586). The parasitic polyamine transportation system presents itself as a perfect means of specifically delivering a cytotoxic agent, as it is distinctively different from mammalian systems and is required for normal cell growth in the malaria parasite (Müller *et al.*, 2001:242, Chadwick *et al.*, 2010:2589).

Polyamine compounds are required in several important processes that are integral to macromolecular syntheses, cell proliferation and differentiation in the malaria parasite. The natural occurring polyamines spermidine, putrescine and spermine (Fig. 2.20) are found in most eukaryotes. These polyamine compounds exist as polycations *in vivo*, and are taken up by the malaria parasite through a polyamine transport system that recognises these specific positive charges on the compounds and then actively transports them into the parasitic cells (Chadwick *et al.*, 2010:2586). The pK$_a$ value of each amine is important, for if the amine is not protonated at physiological pH the molecule will not be recognised by the polyamine transport system. It is, however very important to note that even though the charge characteristics for recognition must be present, the polyamine transport system is not

stringent on the exact position of each amine in the molecule, resulting in a wide assortment of structural derivatives being able to utilise this uptake system (Chadwick *et al.*, 2010:2587).
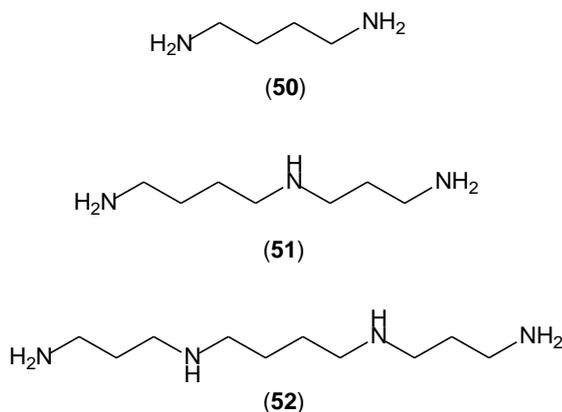

Figure 2.20: The natural occurring polyamines putrescine (**50**), spermidine (**51**) and spermine (**52**) (Chadwick *et al.*, 2010:2587).

The rapidly growing malaria parasite can synthesise a certain amount of polyamines on its own, but is however dependant on a significant amount of exogenous polyamines to be transported by the polyamine transport system to provide it with the necessary quantities (Chadwick *et al.*, 2010:2586, Müller *et al.*, 2001:246, Ramya *et al.*, 2006:580). If a moiety that has the capability to be recognised by the polyamine transport system is attached to a certain drug, the derivative might be actively transported into the parasitic cell thereby increasing the activity and lowering the toxicity (Chadwick *et al.*, 2010:2586).

Researchers have reacted spermidine and Boc-protected spermidine with artemisinin and have observed potent activity in the Boc-protected artemisinin-spermidine product. They postulated that the supposed poor activity of the spermidine derivatives was as a result of poor membrane permeability of the artemisinin-spermidine compounds, insufficient accumulation in the food vacuole of the parasite, or failure of the compounds to be recognised by the polyamine transport system (Chadwick *et al.*, 2010:2591).