CHAPTER I

Introduction

1.1 Statement and significance of the problems

Malaria is a mosquito-borne disease that is transmitted by *Plasmodium spp.* protozoa. The World Health Organization (WHO) reported that malaria kills an African children every 30 seconds (1). In 2013, the WHO estimated that 198 million cases of malaria occurred globally and this disease led to 584,000 deaths. Approximately 40% of the world's population live in malaria-endemic areas (2). 90% of all malaria deaths occur in tropical Africa and 78% of all deaths is children aged under 5 years (3). In **Figure 1-1** shows the endemic areas of malaria transmission including Southeast Asia, India, South and Central America, and particularly Sub-Saharan Africa.

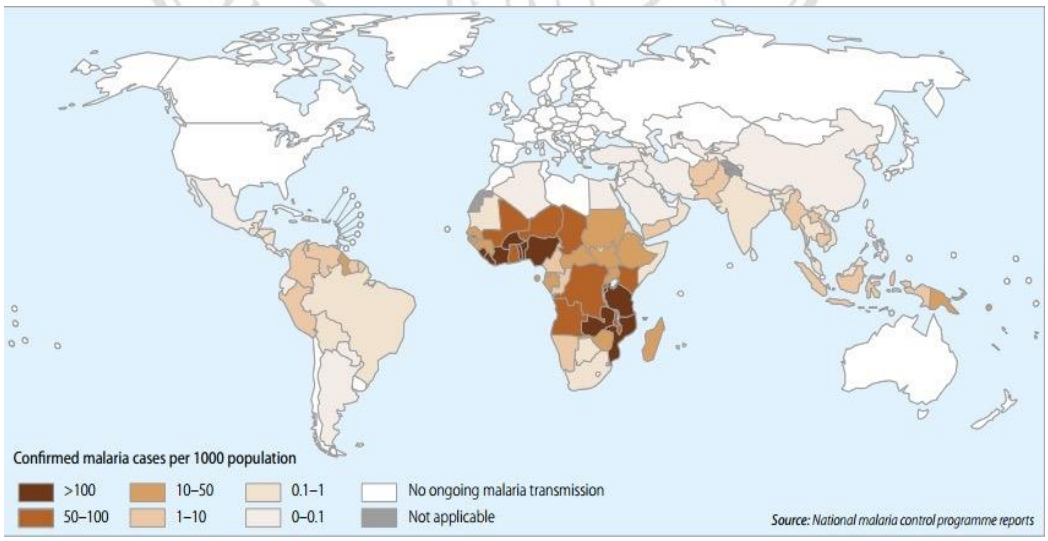


Figure 1-1 Countries with ongoing transmission of malaria, 2013 (World Health Organization; WHO 2014)

Malaria is a life-threatening disease caused by parasites that are transmitted to people through the bites of infected *Anopheles* spp. mosquitoes. Four common human malaria parasites are *Plasmodium falciparum* (*P. falciparum*), *Plasmodium vivax* (*P. vivax*), *Plasmodium ovale* (*P. ovale*), and *Plasmodium malariae* (*P. malariae*). The fifth one, *Plasmodium knowlesi* (*P. knowlesi*), has been recently documented to cause human infections in many countries of Southeast Asia, and also in Thailand (4). Almost every malarial death; including Thailand, is caused by *P. falciparum* because it is highly virulent.

Thailand is one of the countries in Southeast Asia that was severely affected with malaria. In the past two decades, there are about 150,000 malaria cases still occur in Thailand each year. Malaria endemic areas in Thailand include most of the forested and mountainous parts in the country, along the borders with Myanmar such as Tak, Chiang Rai, Mae Hong Son, Kanchanaburi, and along the borders with Combodia such as Trad, Chantaburi, Rayong, and Prachinburi. Furthermore, Laos and Malaysia are also highly endemic regions of malaria (5). Malaria also continues to be a major problem in Thailand, especially in the areas bordering Cambodia and Myanmar. *P. falciparum* infections do not respond to treatment with chloroquine or sulfadoxine-pyrimethamine, and sensitivity to quinine is reduced, while treatment with mefloquine shows a 50% failure rate. Reports of a similar situation have been coming in from western Cambodia. Such evidence confirms the increasing problem of drug resistance. There are many studies reporting about resistant parasites to antimalarial drugs in the region. In this study, we focused on the effect of antimalarial drug to parasites from Mae-Sariang district area, Mae Hong Son province.

Malaria disease can be categorized as uncomplicated or severe (complicated). Uncomplicated malaria have three mains symptoms of malaria such as a cold stage (sensation of cold, shivering), then a hot stage (fever, headaches, vomiting; seizures in young children), and finally a sweating stage (sweats, return to normal temperature, tiredness). While complicated malaria, infections are complicated by serious organ failures or abnormalities in the patient's blood or metabolism. The patients sometimes present symptoms like

common fever but these can lead to death. In general, malaria is a curable disease if diagnosed and treated promptly and correctly.

There are many antimalarial drugs that were used for treating malaria patients. In the past, Quinine (QN) was the most popular antimalarial drug, then Chloroquine (CQ), Pyrimethamine (PYR), Doxycycline (DOX), Sulfadoxine (SDX), and etc. were used for curing malaria; however, they are now resisted by the parasites. In decades, research has established that the malaria parasites are resistant to CQ, PYR or SDX. The parasite has an ability to select for resistance against antimalarial drugs quickly. There is no single antimalarial drugs in clinical use against which the parasite has not yet developed resistance. There are many cases resistance occur quickly, only a few years after wide-spread introduction of the antimalarial drugs (6). CQ resistance has been attributed to a single mutation at codon 76 in the *P. falciparum* Chloroquine resistance transporter gene (*Pfcrt* on chromosome 7; Lys→Thr [$Pfcrt^{K76T}$]) (7). Some research reported variants of parasite multidrug resistance genes (*Pfmdr*) have been found to contribute to CQ resistance, albeit inconsistently (8). Resistance to PYR is primarily conferred by a nonsynonymous point mutation at codon 108 and is consecutively enhanced by mutations at codons 51 and 59 of the *P. falciparum* dihydrofolate reductase gene (*Pfdhfr* on chromosome 4) (9). The enzyme is part of the folate pathway and, thus, of DNA replication. Selection for the Ser→Asn substitution at codon 108 [$Pfdhfr^{S108N}$] has been shown to be linked to parasite survival after treatment with PYR-containing regimens (10). Accordingly, the high frequencies of resistant parasite populations have been attributed to increased PYR consumption (11). An Asn→Ile substitution at codon 51 [$Pfdhfr^{N51I}$] and/or a Cys→Arg exchange at codon 59 [$Pfdhfr^{C59R}$] appears to enhance PYR resistance if one or both of these occur concurrently with $Pfdhfr^{S108N}$. Triple *Pfdhfr* mutation [$Pfdhfr^{S108N/N51I/C59R}$] is the combination of mutations most strongly associated with PYR resistance (12). An Ile→Leu exchange at codon 164 [$Pfdhfr^{I164L}$] mutation, which is shown to cause rapid spread of anti-folate resistance, has been observed in the western parts of Kenya (13). Nowadays, artemisinin; also known as *Qinghaosu*, and its derivatives are a first-line antimalarial drug group that possess the most rapid

action of all current drugs against *P. falciparum* malaria as good efficiency as quinine in the past (14). Treatments containing an artemisinin derivative (artemisinin-combination therapies, ACTs) are now standard treatment worldwide for *P. falciparum*. Therapies that combine artemisinin with some other antimalarial drugs are the preferred treatment for adequate cure rate and delay development of parasite resistance in patients. The drug is also increasingly being used in *P. vivax* malaria (15). Unfortunately, clinical evidence for artemisinin resistance in Southeast Asia was first reported in 2008, they found that parasite clearance rate in the patient was a prolonged time; and *in vitro* susceptibility of parasite on Dihydroartemisinin (DHA) were reduced (16). Artemisinin resistance was subsequently confirmed by finding that *P. falciparum* has reduced *in vivo* susceptibility to artesunate in western Cambodia (17). In 2012, study of Phyo AP and team showed artemisinin resistance in *P. falciparum* emerged along the Thailand–Myanmar border at least 8 years ago and has since increased substantially (18). As drug resistance develops to existing drugs, new antimalarial drugs need to be introduced. Very few new antimalarial drugs were developed in the last quarter of the 20th century. An alarming increase in drug-resistant strains of the malaria parasite poses a significant problem for effective control. Recent advances in our knowledge of parasite biology as well as the availability of the genome sequence provide a wide range of novel targets for drug design. Gene products involved in controlling vital aspects of parasite metabolism and organelle function could be attractive targets. It is expected that the application of functional genomic tools in combination with modern approaches such as structure-based drug design and combinatorial chemistry will lead to the development of effective new drugs against drug-resistant malaria strains (2).

1.2 Literature reviews

1.2.1 Epidemiology

Malaria is a life-threatening disease of humans, rodents and avians caused by a protozoa *Plasmodium* species that is transmitted to different hosts by the female *Anopheles* mosquito leading to serious symptoms and death. Malaria is caused by five species of *Plasmodium*, a unicellular protozoan. Of the five species of *Plasmodium* that naturally infect humans, *P. vivax*, *P. malariae*, and *P. ovale* cause severe morbidity whereas *P. falciparum* is response for the most severe. While reported cases are still rare, *P. knowlesi* is recently added as human parasite. *P. falciparum* is the most dangerous not only because it digests the red blood cell's hemoglobin, but also because it changes the adhesion properties of the cell it inhabits. The parasite's asexual blood forms (merozoites and schizonts) are those life cycle stages responsible for plasmodial infection morbidity and mortality in the vertebrate host (19). This change in turn causes the cell to stick to the walls of blood vessels. It becomes especially dangerous when the infected blood cells stick to the capillaries in the brain, obstructing blood flow, a condition called cerebral malaria. Almost the cause of malarial death were occurred with *P. falciparum* which is mainly high virulent. These can be found in lower part of Sahara desert in Africa and some parts of Southeast Asia such as Myanmar, Cambodia and Thailand.

Younger children are at higher risk of dying; their bodies have not developed enough immunity to fight the disease, which can infect their brains and kill them. Each day malaria claims the lives of about 3,000 children in Africa—one every 30 seconds. Pregnant women are also highly susceptible since the natural defense mechanisms are reduced during pregnancy. Malaria also affects to spontaneous abortion, still-birth, premature delivery and low birth weight. In 2010, WHO reported that there were 107 countries suffered from malaria since last 50 years and 300-500 million patients per year were found. And 60 percent of cases were in Africa that had up to 1 million death cases (20-23).

1.2.2 Life cycle of the malaria parasites

All malaria parasites have a similar life cycle. The entire life span of *Plasmodium* that infect human is spent in two hosts: the insect vector, a female mosquito belonging to the genus *Anopheles*; and a human host. Only female mosquitoes are the vectors. The life cycle is the alternation of sexual and asexual phases in two hosts. The asexual phase, termed schizogony, occurs mainly in the human. The sexual phase, called sporogony, occurs mainly in the mosquito. The asexual phase in human is classified into two phases; the exoerythrocytic or hepatocytic schizogonic phase and the erythrocytic schizogonic phase. Malaria infection in the human or animal host begins when the sporozoites, a form of malaria parasites, are injected into the blood circulation during a blood meal by an infectious female mosquito. The sporozoites spend less than 30 minutes in blood circulation before move to liver cell. In the liver cell, a single sporozoite differentiate over next 5-8 days into 30,000-40,000 merozoites before rupturing out of the liver into the bloodstream. The merozoites can invade erythrocytes or red blood cells (RBCs) and start erythrocytic phase development called ring stage which they begin to metabolize hemoglobin. The next phase is trophozoite stage, during which the parasite metabolizes most of the hemoglobin, get larger, and prepares to reproduce more parasites. Finally, the parasites differentiate to form a multinucleated schizont. At the end of the cycle, the RBCs burst open and parasites are dispersed to infect more RBCs. When the erythrocytic schizont ruptures, the merozoites spill into the blood once again; it is during this phase that malaria–associated morbidity and mortality happen. The merozoites continue in a repeated cycle of infecting RBCs, multiplying, and bursting the RBCs. During this repeated cycle, some merozoites differentiate into male and female gametocytes. It is in this form where they can be taken up by the mosquito vector during a blood meal. Inside the mid-gut of the mosquito, fertilization occurs, producing zygotes, which develop into ookinetes. The ookinetes form oocysts, which then grow, divide and rupture to give rise to sporozoites which migrate to the salivary glands. Then the infectious cycle of malaria can repeat itself as shown in **Figure 1-2** (24).

P. falciparum is an intracellular parasite of human red blood cells, as shown in **Figure 1-3.** Pre-latent period of *P. falciparum* is shorter than other malaria parasites and its asexual cycle (48 hr) is also shorter than that of *P. malariae* (72 hr) and *P. ovale* (50 hr). Importantly, multiplication factor of *P. falciparum* is more than other malaria parasites. After invading into RBCs, *P. falciparum* can bind and develop within a parasitophorous vacuole (24). Moreover, they can harm human by remodelling the RBC membrane and its protein constituents to permit a higher flux of nutrients and waste product into or away from the intracellular parasite. (25).

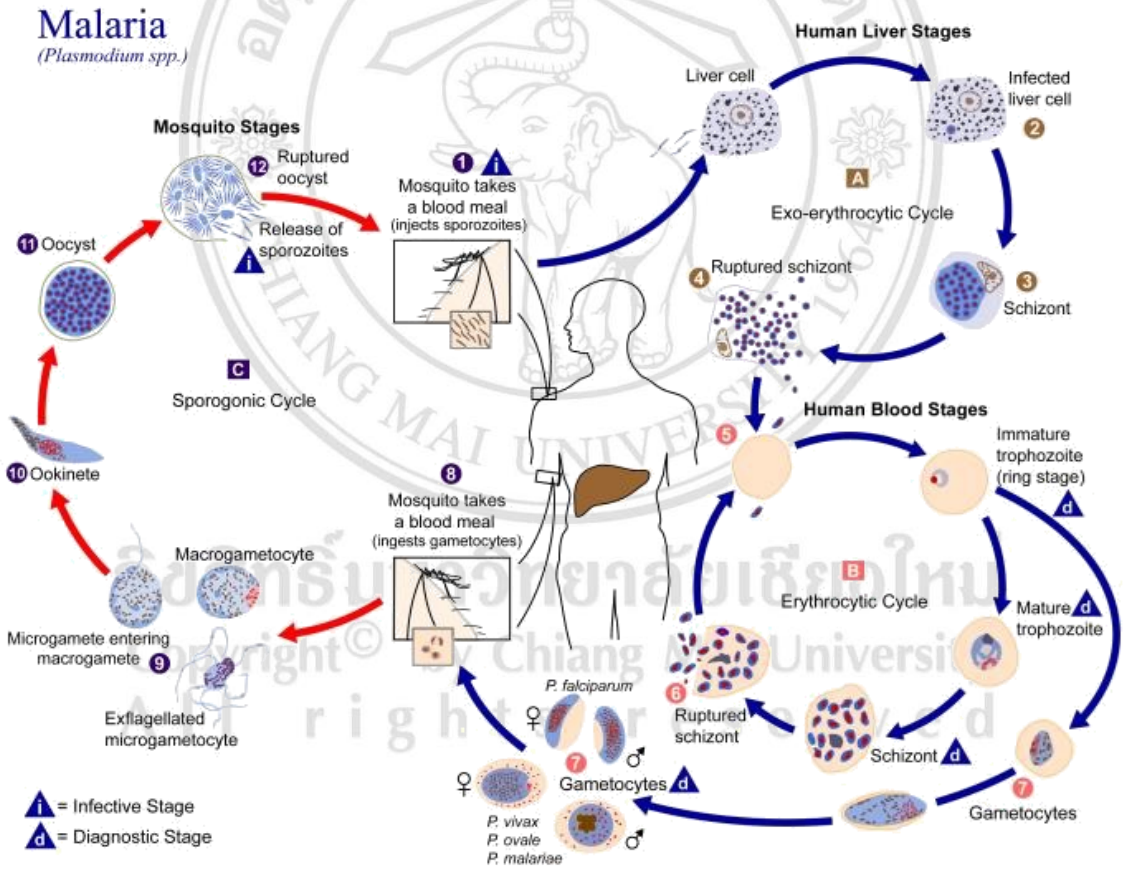


Figure 1-2 Life cycle of the *Plasmodium* species.
(from Centers for Disease Control and Prevention;
http://www.cdc.gov/malaria/about/biology/)

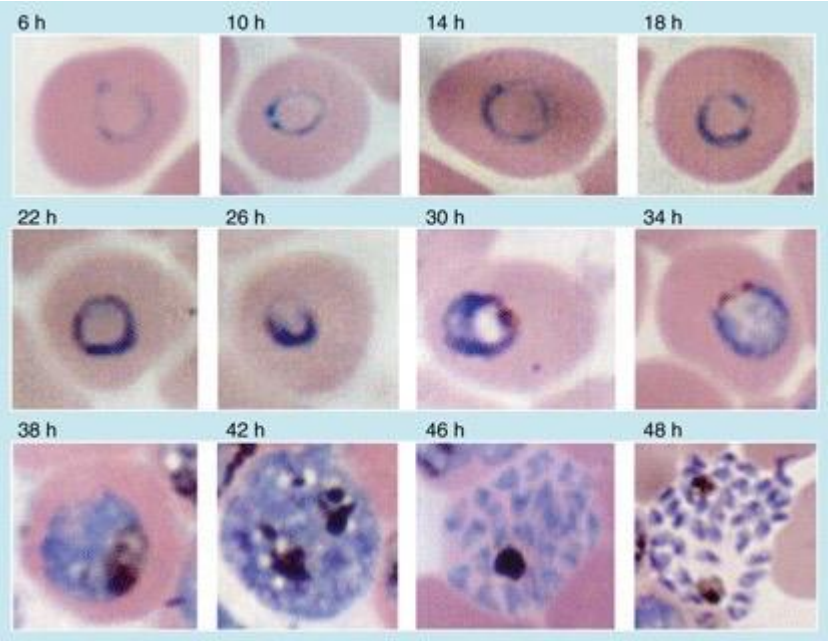


Figure 1-3 The stages in erythrocytic schizogonic phases of *P. falciparum* in 6-48 hour culture (26).

1.2.3 Symptoms and diagnostic of malaria disease

The initial symptoms of malaria are flu-like and include a high temperature (fever), headache, sweats, chills and vomiting. These symptoms can sometimes be difficult to identify as malaria. Other symptoms of malaria can include muscle pains, diarrhea, and generally feeling unwell. As complications of severe malaria can occur within hours or days of the first symptoms, it is important to seek urgent medical help as soon as possible. There are many symptoms of complicated malaria such as anemia, cerebral malaria, liver failure and jaundice, shock, pulmonary edema, hypoglycemia, kidney failure, swelling and rupturing of the spleen, or dehydration lead to death.

Malaria is diagnosed by the clinical symptoms and microscopic examination of the blood film on glass slide. They may have no symptoms but still malaria parasite can be found in their bloodstream called “asymptomatic parasitemia”. When health status is weaker, malaria parasite will grow faster and

cause malaria. Normally, gender and age have no relation to severity of disease. Characteristics of malaria that affect to epidemiology are time and multiplication factors such as virulence, drug susceptibility and areas of endemicity. Antimalarial drugs can normally cure it. The symptoms, fever, shivering, pain in the joints or muscles, and headache will quickly disappear once the parasites were killed. In certain regions; however, the parasites have developed resistance to certain antimalarial drugs, particularly pyrimethamine and chloroquine. So patients in these area require treatment with other more expensive and effective drugs.

1.2.4 Antimalarial drugs (Medications) and resistance

Malaria can be treated effectively early in the course of the disease, but delay of therapy can have serious or even fatal consequences. There are many malaria chemotherapeutics which were used for treatment malaria disease. According to the review about antimalarial drugs by Martin Schlitzer (27), currently used antimalarial drugs are classified into seven classes:

1. 4-Aminoquinolines

4-Aminoquinolines form complexes with ferriprotoporphyrine IX (FPPIX), thereby preventing its polymerization into non-toxic hemozoin. FPPIX is formed in large quantities during the parasite's digestion of hemoglobin and represents a toxic waste product to the parasite (28-30). Resistance against 4-aminoquinolines results from a single mutation at condon 76 by changing amino acid from Lysine to Threonine in the gene of a transport protein (Chloroquine resistance transporter; CRT, on chromosome 7) located in the membrane of the digestive vacuole which facilitates the removal of 4-aminoquinolines from the digestive vacuole (in **Figure 1-4**) (31-34).

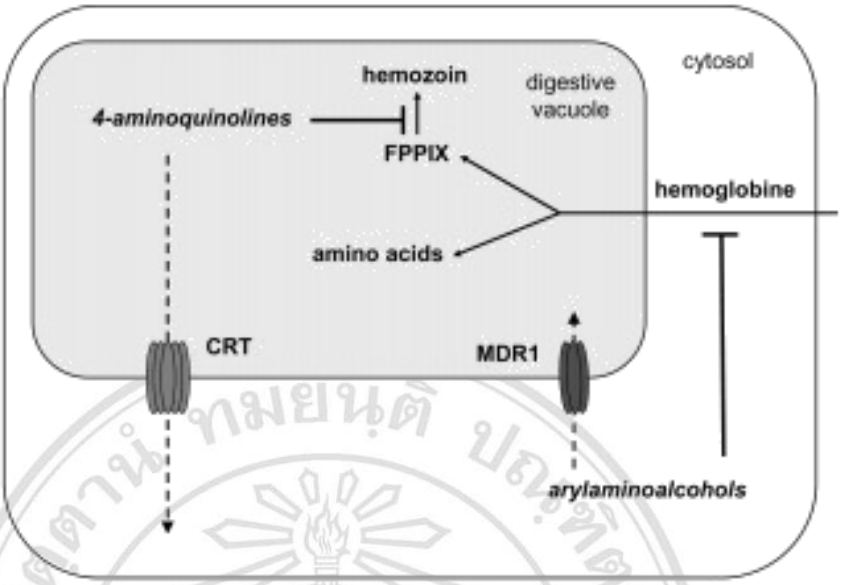


Figure 1-4 Mechanisms of action of 4-aminoquinolines. FPP: ferriprotoporphyrine IX; CRT: chloroquine resistance transporter; MDR1: multi drug resistance transporter 1 (27).

- *Chloroquine (CQ)*

As shown in **Figure 1-5**, Chloroquine has been the most successful single drug for the treatment and prophylaxis of malaria (28). Before resistant strains began to emerge in the 1960s, CQ is a safe and affordable drug, and it was effective. These resistant malaria strains have developed independently at four different regions in the world and successively spread over almost the entire malaria-endangered area (34). Nowadays, more than 80% of field isolates are resistant to CQ (35), in several regions this number can reach 100% (36). In contrast, most strains of *P. vivax, P. ovale, and P. malariae* are still sensitive. More than 50% of *P. vivax* acquired in Papua New Guinea and eastern parts of Indonesia are chloroquine-resistant. Chloroquine-resistant *P. vivax* has also been reported from South-eastern and Southern Asia, and Southern America (37).

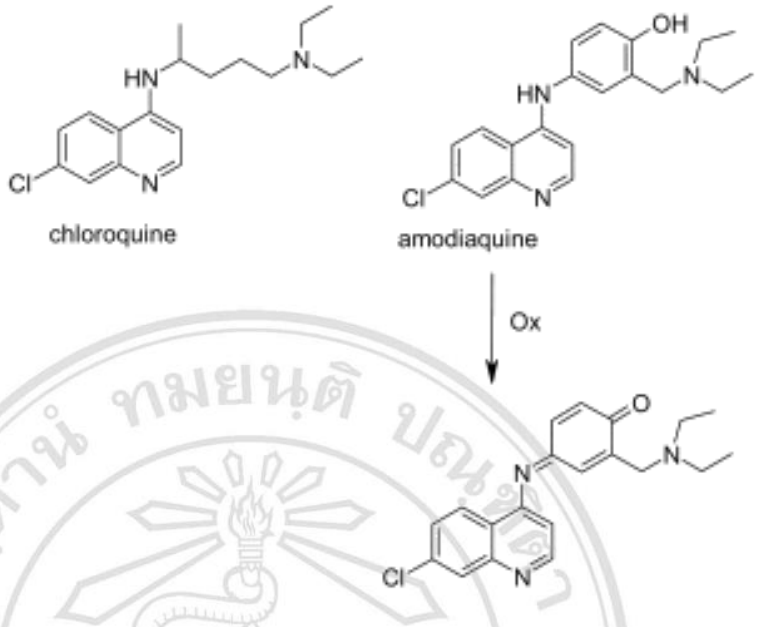


Figure 1-5 Chemical structure of 4-Aminoquinolines; such as Chloroquine and Amodiaquine (27).

- *Amodiaquine (AQ)*

As shown in **Figure 1-5**, Amodiaquine has an aromatic structure into chloroquine's side chain resulted in AQ is effective against low level chloroquine-resistant *P. falciparum* but not against highly chloroquine-resistant parasites (38). An elevated rate of treatment failures (40 – 80%) is seen in some Asian countries. But its mechanism can cause severe hepatotoxicity and life-threatening agranulocytosis (39). As a result, in western countries AQ is no longer on the market. It is assumed that these serious side effects occur only when AQ is used prophylactically over a prolonged period of time, and that shorter therapeutic regimes are sufficiently safe (40-42). Hence, because of its activity against CQ-resistant strains and its affordability, AQ is still used for malaria therapy in the developing world (43, 44).

2. Arylaminoalcohols

Arylaminoalcohols mechanism seems to be different from 4-aminoquinolines but is not known exactly. Chemical structure of Arylaminoalcohols, as shown in **Figure 1-6**, seem to interfere with the heme digestion (45). Amplification of the *Pfmdr1* gene appears to be the main factor in arylaminoalcohol resistance (3, 46, 47). This gene codes for another transport protein (*P. falciparum* multi drug resistance 1 – *Pf*MDR1) located in the membrane of the digestive vacuole which has been shown to transport arylaminoalcohols into the digestive vacuole, as shown in **Figure 1-4** (48).

- *Quinine (QN)*

  Quinine; as shown in **Figure 1-6**, was the first effective treatment for malaria caused by *P. falciparum*. QN is still one of the most important drugs for the treatment of uncomplicated malaria, and often the only therapeutic option for the treatment of severe malaria because preparations for intravenous applications are available and the cost is low (49). Clinical resistance to QN occurs in Southeast Asia and Western Oceania. Resistance is less frequent in South America and Africa (50). Generally, a combination of QN with tetracycline or doxycyclin or clindamycin is recommended (51, 52). QN presents multiple side effects, most of them are reversible, but some are severe in nature, like its arrhythmogenic potential and the release of insulin leading to severe hypoglycemia.

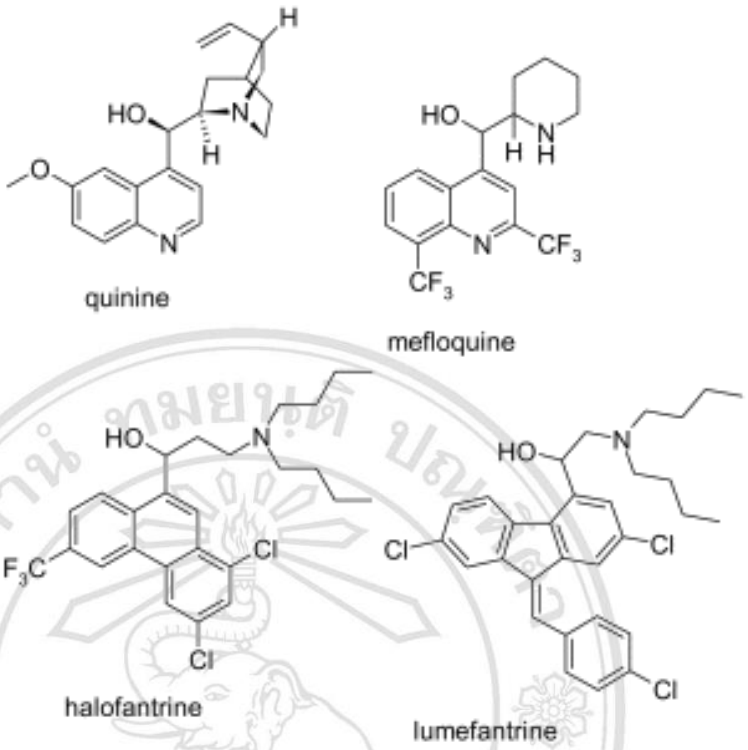


Figure 1-6 Chemical structure of Arylaminoalcohols such as Quinine, Mefloquine, Halofantrine, and Lumefantrine (27).

- *Mefloquine (MQ)*

As shown in **Figure 1-6**, Mefloquine, which is used as the erythro racemate, was developed in the 1970s as a synthetic analogue of quinine. It displays high activity against most chloroquine-resistant *Plasmodium* strains (53). MQ has been widely used, especially in Asia. Here, in some areas, efficiency of the monotherapy has dropped to 40% while in Africa MQ is still effective in 90% of the cases (WHO). For Asia, a combination with artesunate is recommended (54), but for this combination a recrudescence rate of 17% has already been reported (55). MQ does not eliminate parasites in the liver phase of the disease, and people with *P. vivax* malaria should be treated with a second drug

that is effective for the liver phase, such as primaquine. Prophylactic use of MQ is associated with neuropsychiatric side effects, such as insomnia, depression, and panic attacks.

- *Halofantrine*

Halofantrine, as shown in **Figure 1-6**, is active against chloroquine-resistant *Plasmodium* strains (56), but is associated with a high risk of cardiac arrhythmias. Therefore, halofantrine has been withdrawn from the market in several countries.

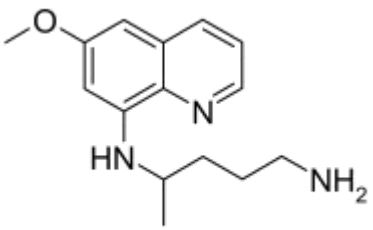
- *Lumefantrine*

As shown in **Figure 1-6**, Lumefantrine (also known as benflumetol) is an antimalarial drug. It is only used in combination with artemether (57), but it has lower antimalarial drug activity than halofantrine.

3. 8-Aminoquinolines

- *Primaquine*

Primaquine, as shown in **Figure 1-7**, is different from other antimalarial drugs as it is active against the liver and the sexual blood stages of different plasmodia. Primaquine is still the only antimalarial drug licensed for the radical cure (or antirelapse therapy) of *P. vivax* infections (58, 59). Most serious side effect of primaquine is a potentially life-threatening hemolysis in humans with glucose-6-phosphate-dehydrogenase (G6PD) deficiency, a genetic polymorphism particularly abundant in Africa and Asia (38).



primaquine

Figure 1-7 Chemical structure of primaquine (27).

4. Anti-folates

Anti-folate drugs, their chemical structures shown in **Figure 1-8**, are molecules directed to interfere with the folate metabolic pathway. There are two enzyme targets involved in biosynthesis of tetrahydrofolate; the dihydropteroate synthase (DHPS) and dihydrofolate reductase (DHFR). Combination of DHPS and DHFR inhibitors is synergistic in the treatment of malaria (24, 60, 61). As shown in **Figure 1-9**, disruption of folate synthesis by two inhibitors leads to decrease in levels of tetrahydrofolate (THF), a necessary cofactor in important one-carbon transfer reactions in the purine, pyrimidine, and amino acid biosynthetic pathways (62). Lower levels of THF result in decreased conversion of glycine to serine, reduced methionine synthesis and lower thymidylate levels with a subsequent arrest of DNA replication (63). These are examples of commonly used antimalarial drugs**.**

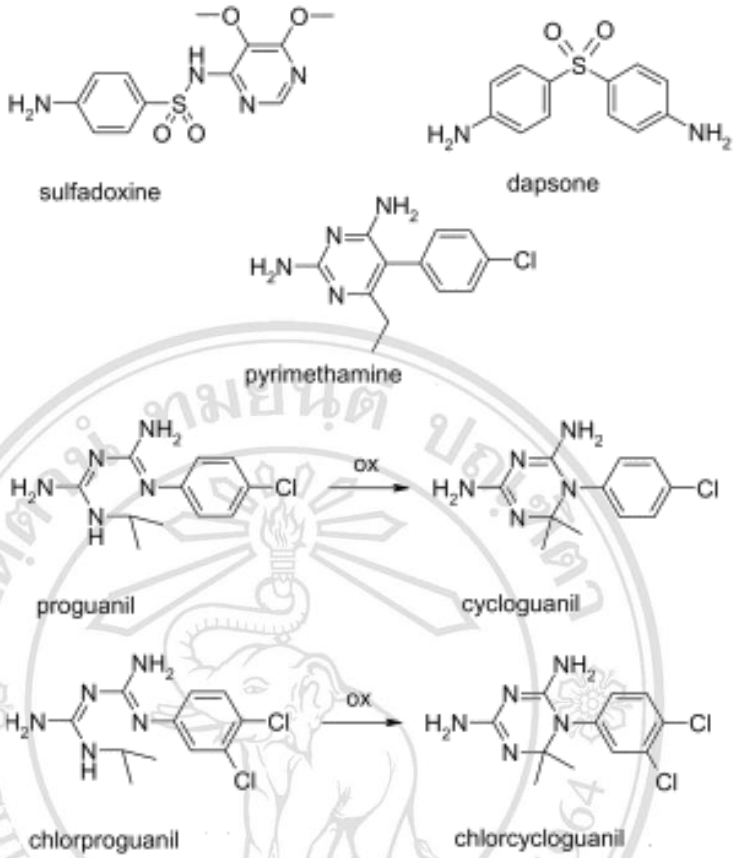


Figure 1-8 Chemical structure of Anti-folate drugs (27).

- *Sulfadoxine*

Sulfonamide like sulfadoxine acts as competitive inhibitors of 4-aminobenzoic acid or false substrates against dihydropteroate synthase (DHPS). DHPS inhibitors have only weak antimalarial drugs activity but are synergistic with dihydrofolate reductase inhibitors. Stepwise accumulation of mutations has led to considerable resistance against dihydropteroate synthase inhibitors.

- *Pyrimethamine (PYR) and cycloguanil*

Pyrimethamine and cycloguanil inhibit dihydrofolate reductase (DHFR). In therapy, open-chain of biguanid prodrug proguanil is transformed into cycloguanil via oxidative ring closure. Main determinant of the therapeutic success of anti-folate combinations is the mutational status of DHFR (64). Stepwise accumulation of mutations has led to considerable resistance (65, 66). PYR and cycloguanil have low toxicity, with little or no side effects when used at the recommended doses. Unfortunately, resistance to PYR and cycloguanil are widespread. Mutations in the malarial gene for dihydrofolate reductase reduce its effectiveness (67). These mutations decrease the binding affinity between pyrimethamine and dihydrofolate reductase via loss of hydrogen bonds and steric interactions (68).

In the past, PYR was used in combination with SFX in the name trade as 'Fansidar'. It acts by interfering with folate metabolism lead to reduce synthesis of thymidylate and DNA. Resistance to Fansidar is now widespread and serious side effects have been reported. It is no longer recommended.

PYR/SFX resistance is conferred by mutations in *P. falciparum* dihydrofolate reductase (*Pfdhfr*) and *P. falciparum* dihydropteroate synthase (*Pfdhps*) genes, whose enzymes are target for PYR and SFX respectively (9, 69). A point mutation at codon 108 from amino acid Ser→Asn [$Pfdhfr^{S108N}$] has been linked to PYR resistance. Additional point mutations at codon 51 from amino acid Asn→Ile [$Pfdhfr^{N51I}$] and at codon 59 from amino acid Cys→Arg [$Pfdhfr^{C59R}$] lead to increased resistance (70, 71). High grade pyrimethamine resistance is linked to the occurrence of the point mutations at codon 164 from amino acid Ile→Leu [$Pfdhfr^{I164L}$] which has been observed together with

the *Pfdhfr* gene triple *Pfdhfr* $^{N51I/C59R/S108N}$ mutant in Southeast Asia and the Americas (72, 73). *Pfdhfr*^{I164L} mutation, which is shown to cause rapid spread of anti-folate resistance, has been observed in the western parts of Kenya (13).

For *Pfdhps* mutation, a mutation at codon 437 which amino acid was changed from Ala→Gly [*Pfdhps*^{A437G}] is mainly associated with SFX resistance with increased resistance conferred in the presence of additional point mutations at Ser→Ala/Phe/His [*Pfdhps*$^{S436A/F/H}$], Ala→Gly [*Pfdhps*^{A581G}], Lys→Glu [*Pfdhps*^{K540E}], and Ala→Ser/Thr [*Pfdhps*$^{A613S/T}$] (74). Differing extents of antimalarial drug resistance to PYR/SFX are subject to the varying numbers and combinations of mutations present in the *Pfdhfr* and *Pfdhps* genes. Primarily, the *Pfdhfr*/*Pfdhps*$^{N51I, C59R, S108N/A437G, K540E}$ quintuple mutation has strongly been associated with clinical PYR/SFX treatment failure (71, 75).

- *The new antimalarial drug candidate 'P218'*

Researcher teams at BIOTEC, NSTDA and their collaborators, have developed a new anti-folate antimalarial drug candidate 'P218' aiming against PYR-resistant parasite strains and isolates. **Figure 1-10** shows structures of PYR and P218. P218 binds to the active site of *Pf*DHFR in a substantially different fashion from the human enzyme, which is the basis for its high selectivity. Unlike pyrimethamine, P218 binds both wild-type and mutant *Pf*DHFR in a slow-on/slow-off tight-binding mode, which prolongs the target residence time. P218, when bound to *Pf*DHFR-TS, resides almost entirely within the envelope mapped out by the dihydrofolate substrate, which may make it less susceptible to resistance mutations. The high *in vivo* efficacy in a SCID mouse model of *P. falciparum* malaria, good oral bioavailability, favorable enzyme selectivity, and good safety characteristics of

P218 make it a potential candidate for further development (76). Currently, with the co-sponsored by NSTDA and Medicines for Malaria Venture (MMV), P218 is during the good laboratory practice (GLP)-certified preclinical studies with the aim to be processed for phase 1 clinical trial within year 2016.

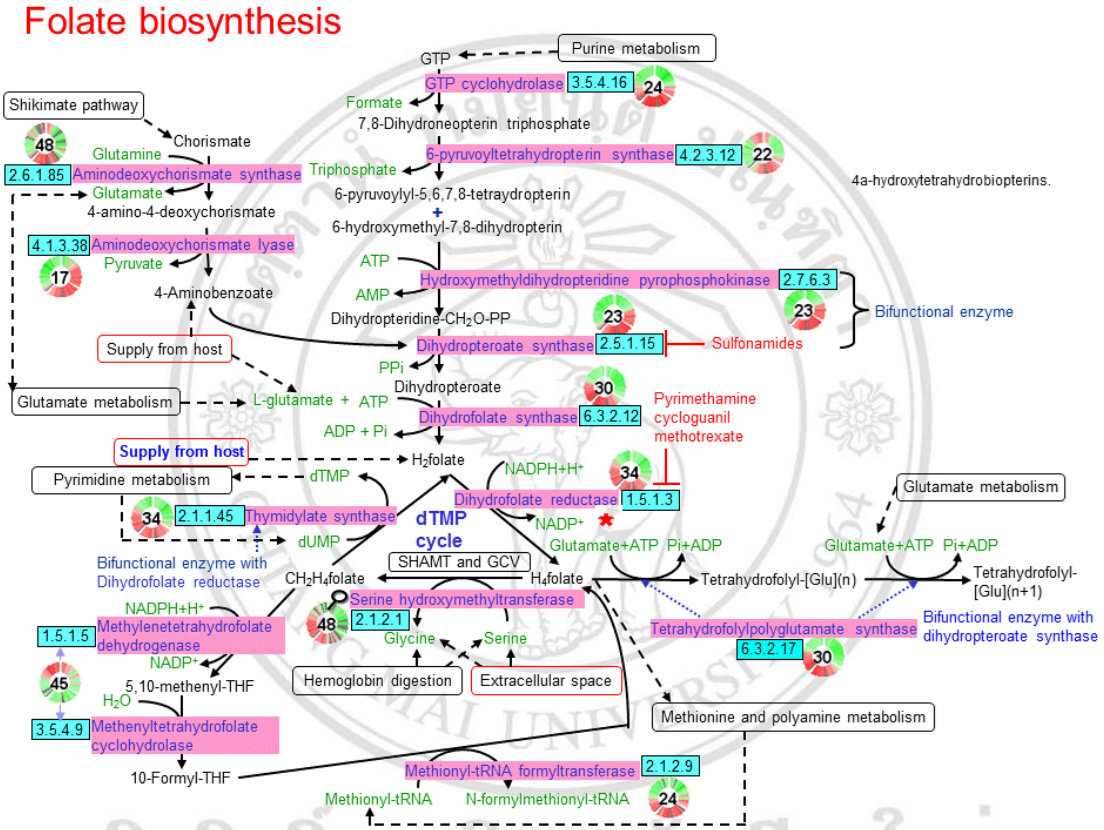


Figure 1-9 Pathway of folate biosynthesis in *Plasmodium* spp.

(http://mpmp.huji.ac.il/maps/folatebiopath.html)

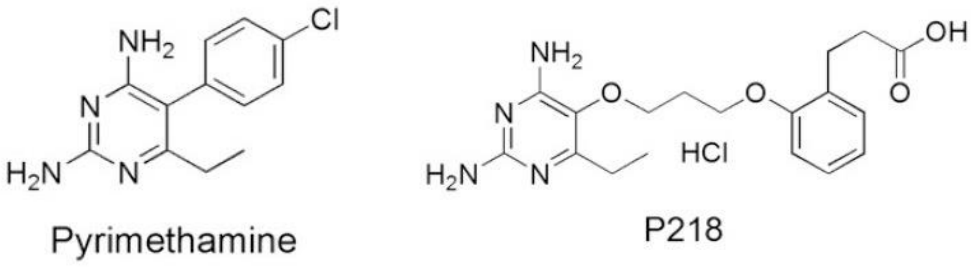


Figure 1-10 Anti-folate antimalarial drug structure; Pyrimethamine and the new antimalarial drug candidate 'P218' (76).

5. Artemisinin and derivatives

Artemisinin (ART) is the active ingredient of the herb sweet wormwood (*Artemisia annua*) in Chinese traditional medicine known as *Qinghaosu*. Artemisinin and its derivatives (**Figure 1-11)** are a group of drugs that have the most rapid action of all current drugs against *P. falciparum* (77). The compound has an artemisin's lactone ring containing an unusual endoperoxide bridge. The endoperoxide which is believed to be cleaved by intraparasital iron-II sources to yield carbon-centered radicals. Whether these radicals unspecifically modify multiple targets like proteins and heme in the digestive vacuole (78, 79) or whether they specifically inhibit an ER-located calcium pump (*Pf*ATP6) is a matter of debate (80, 81). Artemisinin based combination therapy (ACT) is used as the first line treatment of uncomplicated *falciparum* malaria in over 100 countries. Therapies that combine artemisinin with some other antimalarial drugs are the preferred treatment for malaria and are both effective and well tolerated for the patients.

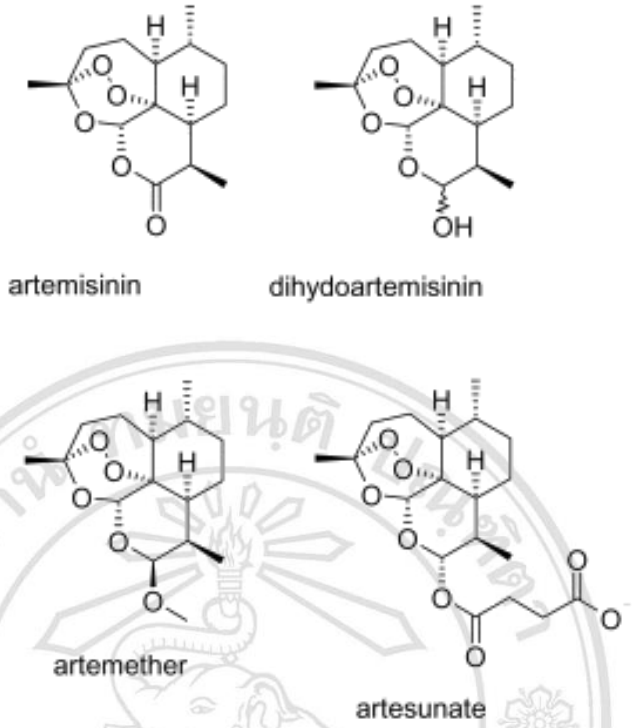


Figure 1-11 Chemical structure of artemisinin and derivatives (27).

Artemisinin is highly active in reducing the parasite biomass by 10,000 fold in a single asexual cycle (14, 82). This makes artemisinin the most active and rapid acting antimalarial drugs known today. Reduced sensitivity (2- to 3-fold) has been involved in multiple copies of the *Pfmdr1* gene, what is also responsible for resistance against arylaminoalcohols (3, 46, 47, 83, 84). In addition, West African isolates with significantly reduced sensitivity have been shown to carry a single mutation in the *Pfatp6* gene (85). Clinical evidence for artemisinin resistance in Southeast Asia was first reported in 2008. It was found that parasite clearance rate in the patient was prolonged and *in vitro* susceptibility of parasite on Dihydroartemisinin (DHA) was reduced (16). Artemisinin resistance in *P. falciparum* was reported along the Thailand–Myanmar border (18). Hence, resistance against artemisinin appears as a real threat.

6. Inhibitors of the respiratory chain

This drug group, as shown in **Figure 1-12**, inhibits the mitochondrial electron transport chain and leads to a rapid breakdown of the mitochondrial membrane potential. Then, it binds to the ubiquinone binding side of the cytochrome bc1 complex and blocks the movement of an iron-sulfur cluster containing a protein domain which is normally involved in electron transport (86-88).

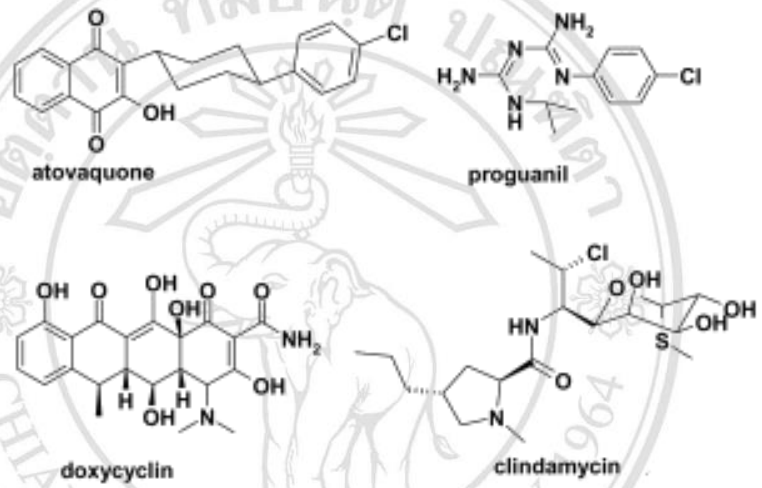


Figure 1-12 Chemical structure of antimalarial drugs. Atovaquone and Proguanil are inhibitors of the respiratory chain class. Doxycycline and Clindamycin are antibiotics class (27).

7. Antibiotics

- *Doxycyclin*

Doxycyclin, as shown in **Figure 1-12,** is the most widely used antibiotic of the tetracycline class against malaria. In therapy, it is combined with quinine or artesunate for the treatment of uncomplicated as well as severe malaria (89). Doxycyclin has also become the mainstay in malaria prophylaxis in cases where mefloquine is contra-indicated or in areas with multi-resistant *P.*

falciparum strains. It cannot be used in children under the age of eight or in pregnant women due to its incorporation in developing bones and teeth.

- *Clindamycin*

  Since clindamycin is considered save in pregnancy and in young children, it represents an alternative to doxycyclin (90). Clindamycin was used in combination with a faster acting antimalarial drug is currently recommended by several national authorities or committees for the treatment of uncomplicated as well as severe malaria. Due to its short elimination half life it is unsuited for prophylaxis.

1.3. Objectives of the study

1.3.1 To determine antimalarial drug sensitivity in *P. falciparum* isolates from malaria patients in Mae-Sariang District, Mae Hong Son province.

1.3.2 To identify mutations of drug resistance genes (*Pfcrt and Pfdhfr*) in *P. falciparum* isolates from malaria patients in Mae-Sariang District, Mae Hong Son province.