CHAPTER 1

Introduction

1.1 Statement and significant of the problems

Malaria is one of the most deadly infectious diseases and an enormous public health problem. Its endemicity is throughout most of the tropics and partial in subtropics. According to the World Health Organization's Report 2014, there were about 207 million cases of malaria and estimated 627,000 deaths in 2012. Malaria mortality rates have fallen by more than 25% globally and by 33% in the WHO African Region since 2000. Most deaths occur among children living in Africa where a child dies every minute from malaria (reported by WHO in 2011). 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 include *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 (Daneshvar *et al*, 2009). Most of malarial death is caused by *P. falciparum* because its high virulence.

Anti-malarial drug resistance is a major public health problem which obstructs the control of malaria spread. Research in recent years has established that the malaria parasites are resistant to chloroquine (CQ), pyrimethamine (PYR) or sulfadoxine (SDX) (Noranate *et al*, 2007). There are several genetic polymorphisms described in *Plasmodium* spp. that can provide reliable data about the prevalence of drug resistance (Jovel *et al*, 2011). Resistance to PYR is primarily conferred by a non-synonymous point mutation at codon 108 and is consecutively enhanced by mutations at codons 51, 59 and 164 of the *P. falciparum* dihydrofolate reductase gene (*pfdhfr*) located on chromosome 4. Dihydrofolate reductase (DHFR) enzyme is an enzyme in the folate pathway which is essential during DNA replication. Point mutations at the codons 437

and 540 of the dihydropteroate synthase gene (*pfdhps*) located on chromosome 8 of *P. falciparum* are considered responsible for sulfadoxin (SDX) resistance. Like DHFR, dihydropteroate synthase (DHPS) encoded by the *pfdhps* is a key enzyme in the folate pathway (Marks *et al*, 2005).

Iron metabolism is essential for many cellular functions in all living organisms. To cause infection, nearly all protozoa (such as plasmodial malaria parasites), fungi, and bacteria must obtain growth-essential iron from their hosts. To suppress infection, hosts have evolved iron-withholding defense systems. Iron deficiency and iron deprivation have been reported to inhibit their growth and development, and consequently protect against such microorganisms in humans. Enhancement of iron withholding is a potential target for the development of novel therapeutic agents. Appearance of widespread multiple drug resistance in human malaria has intensified the search for new anti-malarial compounds, particularly iron chelators (Weinberg & Moon, 2009). The chelators evidently exert this effect by sequestering iron which the pool(s) of iron being sequestered by the chelators have to be intracellular sources but may not include plasma transferrin (Heppner *et al*, 1988). The iron which is bioavailable for in the intracellular parasites originates from a labile erythrocyte cytosolic non-heme iron rather than from abundant heme iron. Indeed the parasite has to make its own heme within two separate organelles, the mitochondria and the apicomplast.

Paradoxically, despite the abundance of iron within the erythrocyte, iron chelators are cytocidal to the plasmodial parasite (Scholl *et al*, 2005). Numerous *in vitro* and *in vivo* studies have shown that iron chelators act as anti-malarial agents which eliminate internal iron pools and interfere with differentiation and growth of parasite components from iron supply, without interfering biological function of red blood cell (RBC). Green tea extract (GTE) contains many interesting phytochemicals, mainly catechin derivatives. Importantly, they show many biological activities such as anti-oxidative, anti-diabetic, anti-carcinogenic, anti-mutagenic, free radical-scavenging, hypolipidemic, hypocholesterolemic, anti-microbial and iron-chelating activities. The last two properties are relevant and attractive, so that the natural product may be used as therapeutic/preventive agent *per se* or in combination with standard

anti-malarial drug. Besides the anti-malarial drug target, it is expected that iron chelator and GTE would act directly on allosteric site(s) containing iron or starve the iron essential for the parasite growth. Beneficially, this concept can lower incidence of anti-malarial drug resistance and probably enhance anti-malarial drug efficiency. Emergence of drug resistant malaria has prompted an intensified search for new anti-malarial drugs or combinations of such drugs. Iron chelating agent may represent a new approach to anti-malarial treatment.

Therefore, the goal of this study was to examine if the new synthetic iron chelator 1-(*N*-acetyl-6-aminohexyl)-3-hydroxy-2-methylpyridin-4-one (CM1) and green tea extract (GTE) could inhibit growth and development of *P. falciparum* in cell culture and *P. berghei* in mouse and determine what possible mechanism the compounds exhibit. Additionally, combined treatments of iron-chelating agents with classical anti-malarial drugs were also investigate.

1.2 Literature reviews

1.2.1 Incidence of malaria infection

Malaria remains the world most common parasitic disease caused by parasites, *Plasmodium* spp., causing approximately 207 million infections annually and 627,000 deaths, mostly in African children. International travelers are at risk of developing malaria when visiting endemic regions, and account for an estimated 30,000 cases of malaria annually (WHO Report in 2011). WHO and UNICEF's Child Health Epidemiology Reference Group (CHERG) have reported estimates of childhood death of malaria in the first 5 years of their life in 2010; 7.4% of global death, 15% seen in Africa, 1% seen in Eastern Mediterranean, and 1% seen in Southeast Asia (Liu *et al*, 2012). Malaria is a tropical disease caused by the plasmodial parasite whose life cycle is in both *Anopheles* mosquitoes and humans. It is transmitted to humans by female anopheles mosquitoes that bite mainly at night. The female mosquito needs blood in order for her egg development, while the male feeds only on plants. Five main species of plasmodium causing malaria in humans are *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi*. In pathogenesis, *P. falciparum* causes the most severe

illnesses and deaths whereas *P. vivax*, *P. ovale*, *P. malariae* cause mild disease. *P. knowlesi* was formerly reported found in monkeys and has recently documented to cause human infection in many countries of Southeast Asia (Jeslyn *et al*, 2010).

1.2.2 Life cycle of malaria parasite

Malaria infection in the human host begins when the sporozoites are injected into the blood circulation during the bite of the infectious mosquito. The sporozoites remain in the circulation for a short period. Kupffer cells in the liver may be invaded (or the parasite may be phagocytosed) but the sporozoites are not able to develop in those cells and die shortly after invasion. Most parasites however invade the hepatocytes and start the asexual exo-erythrocytic schizogonic cycle. The liver trophozoite initially appears as a mononucleated round body in the cytoplasm of the host cell, it subsequently begins to develop and multiply asexually. A mature schizont (the multinucleated stage of the parasite) is formed, and a large number of merozoites are released lastly. The mature schizont is 30-70 µm large, has no pigment (there is no hemoglobin in the hepatocyte), and occupies the entire cell cytoplasm. Number of merozoites produced at the end of the cycle is also species dependent; estimated as 2,000 for *P. malariae*, 10,000 for *P. vivax* and *P. ovale*, and up to 30,000 for *P. falciparum* (Garnham, 1966). *P. vivax* has a dormant stage in the human liver. After the sporozoites enter the hepatocytes not all will develop into schizonts, but some remains as hypnozoites (Mueller *et al*, 2009). The hypnozoites can remain dormant for months, or even years. Relapses indicating a dormant stage occur also in *P. ovale* (Richter *et al*, 2010).

The liver cycle ends when the mature schizont ruptures and releases the merozoites into the sinusoids of the liver. Released merozoites can only invade red blood cells. The early trophozoite is often referred to as 'ring form' because of its morphology. Trophozoite enlargement is accompanied by an active metabolism including the ingestion of host cytoplasm and the proteolysis of hemoglobin into amino acids. The end of the trophic period is manifested by

multiple rounds of nuclear division without cytokinesis resulting is a schizont. Merozoites bud from the mature schizont, also called a segmenter, and the merozoites are released following rupture of the infected erythrocyte. Invasion of erythrocytes reinitiates another round of the blood-stage replicative cycle (Miller *et al*, 2002). The repetitive intraerythrocytic cycle of invasion–multiplication–release–invasion continues, taking about 48 h in *P. falciparum*, *P. ovale* and *P. vivax* infections and 72 h in *P. malariae* infection (Tuteja, 2007).

As an alternative to the asexual replicative cycle, the parasite can differentiate into sexual forms known as macro- or microgametocytes. The gametocytes are large parasites which fill up the erythrocyte, but only contain one nucleus. Ingestion of gametocytes by the mosquito vector induces gametogenesis (i.e., the production of gametes) and escape from the host erythrocyte. Factors which participate in the induction of gametogenesis include: a drop in temperature, an increase in carbon dioxide, and mosquito metabolites. Microgametes, formed by a process known as exflagellation, are flagellated forms which will fertilize the macrogamete leading to a zygote. The zygote develops into a motile ookinete which penetrates the gut epithelial cells and then develops into an oocyst. The oocyst undergoes multiple rounds of asexual replication resulting in the production of sporozoites. Rupture of the mature oocyst releases the sporozoites into the hemocoel (i.e., body cavity) of the mosquito, and then migrate to and invade the salivary glands. Thus the life cycle is complete (Tuteja, 2007) **(Figure 1-1)**.

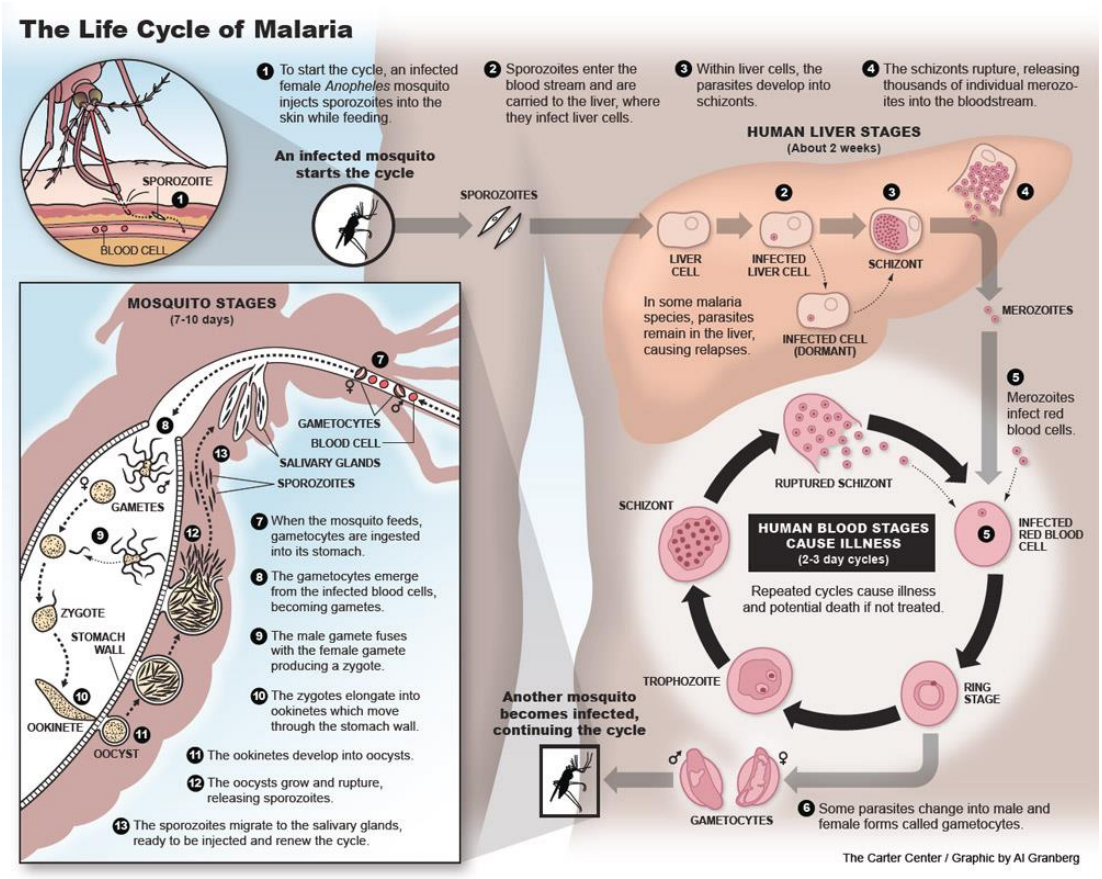


Figure 1-1 Life cycle of malaria parasite (www.cartercenter.org)

P. berghei was discovered by Vinckey and Lips in 1948, belonging to *Plasmodium* spp. that infect murine rodents found in Central Africa. Other rodent malaria include *P. vinckei, P. chabaudi* and *P. yoelii.* There are small differences between the four rodent malaria parasite species, for example differences in morphology, developmental time and size of different stages and isoenzymes. These variable characteristics influence host-parasite interaction and have been found to be responsible for differences in the course of infection, virulence and pathology. Rodent malaria models are used routinely in preclinical studies. These models have been used extensively for the screening of anti-malarial compounds to test the activity and also to study the mechanism of drug action, resistance and host-parasite interaction. The rodent malaria models are particularly valuable in studies of the erythrocytic stage of malaria infection, because the morphology and parasite developmental stages are similar to those in

human malaria infections. An important advantage of the rodent malaria models is that all parasite stages can be observed in the peripheral blood circulation.

Rodent malaria parasites represent the practical models for the experimental study of mammalian malaria (Janse & Waters, 1995). These parasites have proved to be analogous to the malarias of man and other primates in most essential aspects of structure, physiology and life cycle (Meisner & Carter, 1977). *P. berghei* is useful as *in vivo* models to study the interactions between the host and the erythrocytic stage of the parasite. In addition, *P. berghei* is a good model for research on developmental biology of malaria parasites, because of availability of technologies for *in vitro* cultivation and large scale production and purification of the different life cycle stages.

1.2.3 Anti-folate drugs used in malaria

Anti-folate drugs are molecules directed to interfere with the folate metabolic pathway at some levels and can be used in treatment of malarial infection. The drugs are divided into two classes, of which class I is the inhibitors of dihydropteroate synthase (DHPS) and class II is the inhibitors of dihydrofolate reductase (DHFR) **(Figure 1-2)**. Combination of DHFR and DHPS inhibitors is synergistic in the treatment of malaria (Nzila, 2006). Disruption of folate synthesis by DHFR and DHPS inhibitors leads to decrease in levels of fully reduced tetrahydrofolate (THF), a necessary cofactor in important one-carbon transfer reactions in the purine, pyrimidine, and amino acid biosynthetic pathways (Ferone, 1977). 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 (Gritzmacher & Reese, 1984; Gutteridge & Trigg, 1971; Newbold *et al*, 1982; Schellenberg & Coatney, 1961; Triglia & Cowman, 1999). These are examples of commonly used anti-malarial drugs.

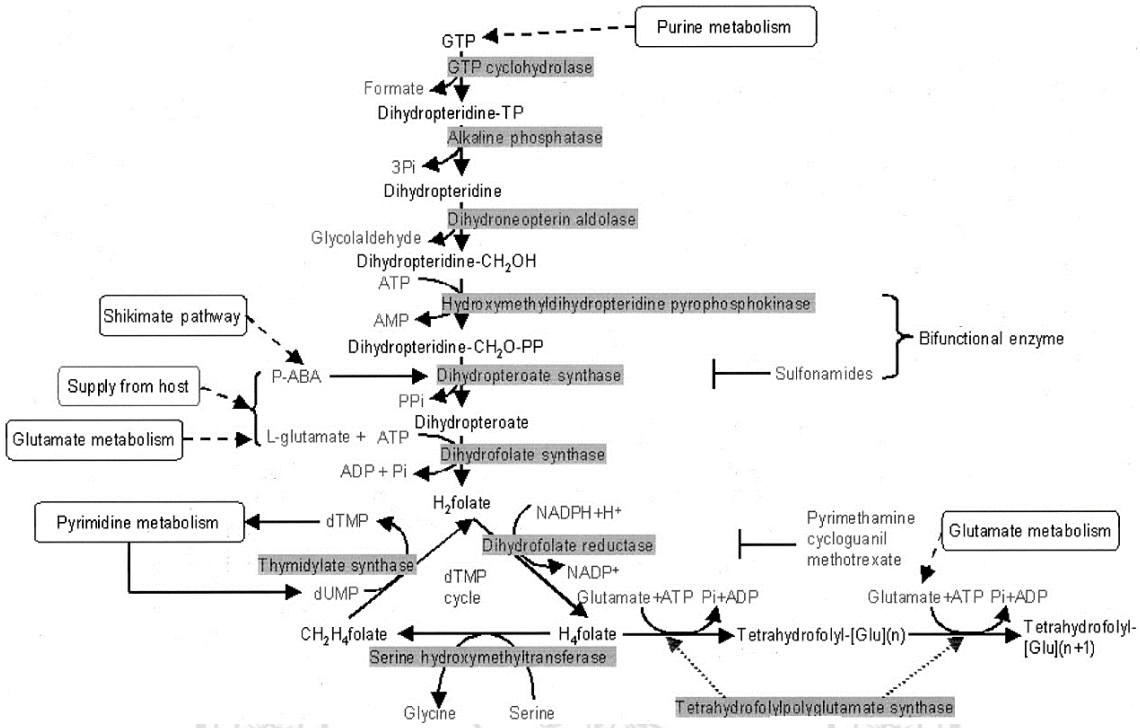


Figure 1-2 Pathway of folate biosynthesis in *Plasmodium* spp.(Gregson & Plowe, 2005)

Proguanil is a prodrug which is metabolized to its triazine form cycloguanil **(Figure 1-3)**, and also an inhibitor of the parasite DHFR. This drug has been deployed largely as a prophylactic agent against malaria or in combination with chloroquine (Shanks *et al*, 2001; Wernsdorfer, 1990). It has also been used in combination with atovaquone, an inhibitor of electron-transport to the cytochrome bc1 complex (coenzyme Q); this combination, known as Malarone, is synergistic and is used as a prophylactic agent against malaria (Kain *et al*, 2001). The mechanism of drug synergism between this combination is still poorly understood (Nzila, 2006).

Chlorproguanil **(Figure 1-3)** is metabolized to active metabolite chlorcycloguanil that inhibits parasite's DHFR enzyme. Chlorproguanil was recommended for prophylaxis but has not been used as much as proguanil (Esposito, 1991; Wernsdorfer, 1990). This is due to its higher efficiency and toxicity when compared with proguanil. Therefore, chlorproguanil is recommended for prophylaxis at a lower dose. One study has demonstrated the inadequacy of

the recommended dose to provide prophylactic protection (Watkins *et al*, 1987). This anti-folate has now been combined with dapsone as an anti-malarial anti-folate combination.

Pyrimethamine (PYR) **(Figure 1-3)** is in the 2,4-diaminopyrimidine derivative family of anti-DHFR inhibitors and most widely used anti-folate for malaria treatment (Hitchings & Burchall, 1965; Hitchings *et al*, 1952a). The interest in the anti-malarial activity of this family of compounds was sparked in the late 1940s when they were synthesized and tested as analogues of folic acid in the treatment of tumors (Hitchings *et al*, 1952b). Structures of these compounds and proguanil are similar and hypothesized that 2,4-diaminopyrimidine could have anti-malarial activity. It is generally used in combination with sulfadoxine (SFX) or sulfalene than used in monotherapy (Hurly, 1959; McGregor *et al*, 1963).

Cycloguanil (Cyc) is an active metabolite of proguanil. A chemical structure as **Figure 1-3**, cyclic dihydrotriazine is similar to PYR. The drugs owe their effectiveness to their structural comparable to the natural substrate dihydrofolate (DHF) and bind to the malarial DHFR more strongly than to the vertebrate host DHFR. Cyc is used alone or combined with sulfa drug for prophylaxis and treatment of malaria infection (Sirawaraporn, 1998). All these anti-folates have a higher affinity of binding with *P. falciparum* than human DHFR. It was accepted that differences of binding affinity account for their good therapeutic index.

PYR and Cyc have low toxicity, with little or no side effects when used at the recommended doses. Unfortunately, PYR and Cyc were challenged by the emergence and spread of malaria parasite strains that are resistant to their action, thereby limiting their use in the treatment of malaria (Hyde, 1990). All these anti-folates have a higher affinity of binding to *P. falciparum* than human DHFR. It was accepted that differences of binding affinity account for their good therapeutic index. Sulfa drugs are DHPS inhibitor that can block *de novo* folate synthesis. The drugs belong to sulfonamide and sulfone family can

be used successfully as anti-malarial agents since the parasite needs to synthesize folate (Nzila, 2006). PYR/SFX resistance is attributable to parasites that carry point mutations at codons 51, 59, 108 and 164 of *dhfr*, and resistance is augmented by point mutations at codons 437 and/or 540 or 437 and/or 581 of the *dhps* gene (Gregson & Plowe, 2005; Sibley *et al*, 2001). Development of the resistance to PYR/SFX by plasmodial parasites is a major problem for the effective treatment of malaria, especially *P. falciparum* malaria. Although the molecular basis for parasite resistance is known, the factors promoting the development and transmission of these resistant parasites are little known.

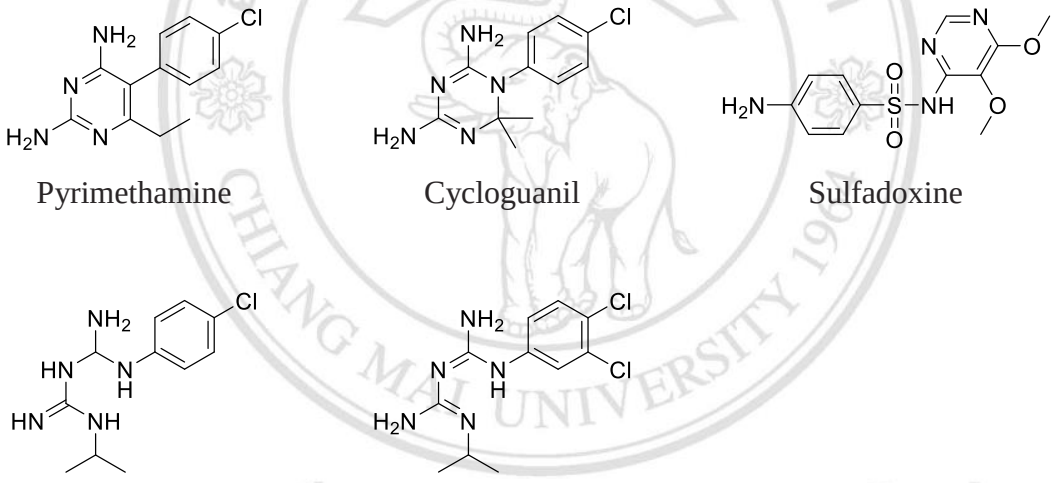
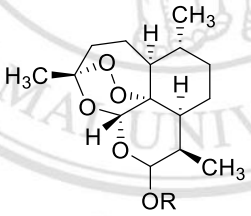


Figure 1-3 Chemical structures of pyrimethamine, cycloguanil, proguanil, chlorproguanil (Volpato & Pelletier, 2009) and sulfadoxine

Artemisinin is isolated from the plant *Artemisia annua*, sweet wormwood and it is an herb employed in Chinese traditional medicine known as Qinghaosu. Artemisinin and its derivatives are a group of drugs that have the most rapid action of all current drugs against *P. falciparum* (Cao *et al*, 1997). The compound has a sesquiterpene lactone ring containing an unusual peroxide bridge. This peroxide is believed to be responsible for the drug's mechanism of action. Combined treatments along with an artemisinin derivative are now standard treatment worldwide for *P. falciparum* infection. Therapies that combine artemisinin with some other anti-malarial drug are the preferred treatment for malaria and are both effective and well tolerated for the patients. The drug is also increasingly used in *P. vivax* infection as well as a topic of research in cancer treatment (Douglas *et al*, 2010). Since artemisinin itself has poor bioavailability, which limits its effectiveness. Semi-synthetic derivatives of artemisinin have been developed. These are artesunate, artemether, dihydroartemisinin (DHA), artelinic acid, artenimol and artemotil **(Figure 1-4)**.



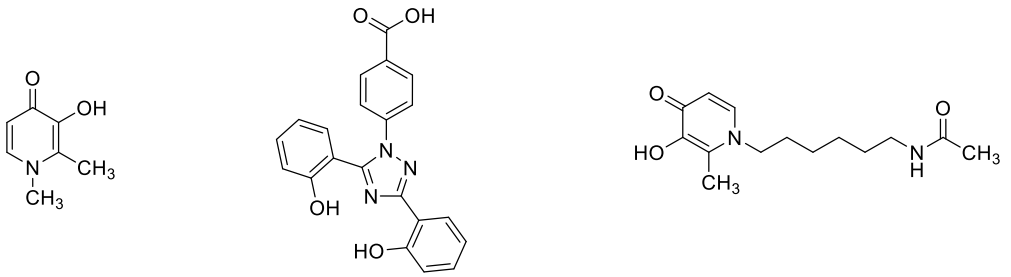
OR = O	Artemisinin
R = H	Dihydroartemisinin
R = Me	Artemether
R = Et	Arteether
R = $CO(CH_2)_2CO_2H$	Artesunate

Figure 1-4 Chemical structures of artemisinin and its derivatives

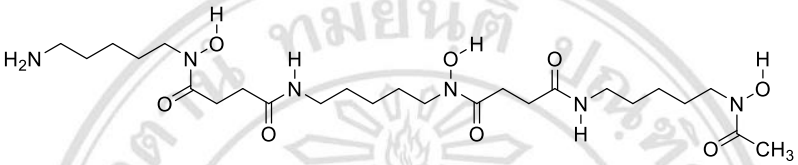
1.2.4 Iron chemistry and chelator

Iron is an essential trace element for all cellular forms of life and plays a crucial role in oxygen sensing and transport, electron transfer and catalysis (Aisen *et al*, 2001). Excessive active iron catalyzes the production of a variety of reactive oxygen species (ROS), such as superoxide anion radicals, hydrogen peroxide and hydroxyl radicals via Haber-Weiss and Fenton reactions. The ROS are attributed to the ability of the metal in the redox cycle and can damage a variety of cells and tissues including the heart, liver, pancreas, erythrocytes and endocrine glands resulting in dysfunctions of the organs (Emerit *et al*, 2001). Hydrogen peroxide is normally applied to kill microorganisms in neutrophils; nevertheless, an excess amount can be toxic.

Iron components such as non-heme iron, heme iron and iron-sulfur in metabolic pathways are possible targets of effective iron chelators in treatment of malaria infection. The quinolines like chloroquine and quinine interfere with iron protoporphyrin IX crystallization in the digestive vacuole (Sullivan *et al*, 1996). Artemisinins are activated by iron to generate carbon-centered radicals that rapidly kill parasites (O'Neill & Posner, 2004). Iron chelators have been explored as alternative anti-malarial drugs for decades. Importantly, the chelators have to permeate through erythrocytic and parasite membranes readily, so that they can kill the intracellular parasites effectively. It has been shown that iron(III) chelators have anti-malarial activity *in vitro*, apparently through the mechanism of withholding or depleting iron from vital metabolic pathways of the intra-erythrocytic parasite. Certain iron(II) chelators also have anti-malarial activity, probably the mechanistic action appears to be a chemical catalyst in formation of toxic reactive oxygen species (ROS) via Fenton/Haber-Weiss reactions rather than the withholding of iron. Nowadays, desferrioxamine (DFO), deferiprone (DFP) and deferasirox (DFX) are iron chelators **(Figure 1-5)** of choice used for the treatment of β-thalassemia patients with iron overload (Cappellini *et al*, 2009; Hershko *et al*, 2003).



DFP DFX CM1



DFO

Figure 1-5 Chemical structures of DFO, DFP, DFX and CM1

1) Desferrioxamine (DFO)

DFO is the first drug that was introduced in the 1970s to treat iron overload. The hexadentate chelator has an extremely high affinity for iron(III) (DFO : Fe = 1:1, K_a =1029) and a much lower affinity for other metal ions such as zinc, calcium and magnesium (Hershko & Rachmilewitz, 1978). DFO is poorly absorbed from the GI tract and rapidly excreted in the urine (plasma half-life of 5-10 min), it must be therefore administered intravenously, intramuscularly and subcutaneously (Porter, 1997). Surprisingly, one iron(III)-chelator like DFO exhibits anti-malarial activity in experimental animals infected with malaria parasites (Mabeza *et al*, 1999). It was found that DFO inhibits the growth of *P. falciparum* culture, probably interfering with the completion of schizogony (Raventos-Suarez *et al*, 1982). Previous studies demonstrated that DFO suppresses the parasitemia in *P. berghei*- and *P. vinckei*-infected mice (Fritsch *et al*, 1985; Singh *et al*, 1985), *P. berghei*-infected rats (Hershko & Peto, 1988), *P. falciparum*-infected Aotus monkey (Pollack *et al*, 1987) and *P. vivax*-infected human (Bunnag *et al*, 1992).

Two experimental iron chelators, alkylthiocarbamates and 8-hydroxyquinoline, selectively inhibit glycolysis in *P. falciparum*-infected erythrocytes by the mechanism attributed to toxicity of lethal chelator-iron complex (Scheibel & Stanton, 1986). In addition, DFO was found to inhibit the liver schizogony of both the *P. yoelii*-infected rat hepatocyte and *P. falciparum*-infected human hepatocyte cultures (Stahel *et al*, 1988). Other iron chelators were also investigated for their anti-malarial activity (Hershko *et al*, 1991; Iheanacho *et al*, 1990; Yinnon *et al*, 1989).

2) Deferiprone (DFP)

DFP (1,2-dimethyl-3-hydroxypyrid-4-one, L1), a synthetic bidentate chelator, has been the first orally active drug available for clinical use. A previous study has demonstrated DFP decreases serum ferritin and liver iron concentrations in transfusion-dependent patients (Hoffbrand *et al*, 2003). The drug is able to reduce iron in many cells and tissues, especially heart, liver and erythroid cells (Prus & Fibach, 2009; Westwood *et al*, 2003; Wong *et al*, 1997). However, its side-effects include nausea, vomiting, GI disturbance, leucopenia and thrombocytopenia and zinc deficiency which are typically observed (Cohen *et al*, 2000). Recently, the Government Pharmaceutical Organization (GPO) of Thailand has manufactured and launched the DFP (GPO-L-One®) for the treatment of Thai thalassemia patients with iron overload. Single and combined treatments with DFP are effective in inhibition of *P. falciparum* growth (Gordeuk & Loyevsky, 2002; Mohanty *et al*, 2002; Thuma *et al*, 1998).

3) Deferasirox (DFX)

DFX (ICL670), a tridentate synthetic oral chelator with a high affinity and specificity for iron, has been clinically used for the treatment of transfusion-dependent thalassemia patients since 2003 (Galanello *et al*, 2003; Nisbet-Brown *et al*, 2003). Efficacy and safety of DFX usage have previous been evaluated and reported (Cappellini *et al*, 2006; Porter, 2006). Common side effects of DFX are abdominal symptoms (usually diarrhea), skin exanthems, elevated serum creatinine level and renal tubular dysfunction (Cappellini & Pattoneri, 2009). DFO chelation along with DFP or DFX has been designed to improve the efficacy and to avoid the adverse effects in the treated patients (Cappellini *et al*, 2007). Ideally, the iron chelator should be orally active, cheap, high specific for iron but not for other metal ions, freely penetrate into target tissues, get the patients compliant and show minimal side effects. A previous study showed that standard IC_{50} values for *P. falciparum* culture are similar for DFX and DFO, probably the chelators inhibit parasite growth via deprivation of iron from critical targets within the malaria parasites (Goudeau *et al*, 2001).

4) CM1

CM1 or 1-(*N*-Acetyl-6-aminohexyl)-3-hydroxy-2-methylpyridin-4-one, a hydroxypyridin-4-one derivative, has been chemically synthesized at the Department of Biochemistry, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand and proposed to be a new oral iron chelator. Dr. Somdet Srichairatanakool and colleagues have identified its chemical structure to be $C_{14}H_{22}N_2O_3$ with a molecular mass of 266 g/mol **(Figure 1-5)**. Preliminary results have demonstrated that the CM1 is a bidentate chelator which is more lipophilc than DFP and can bind the iron efficiently. Interestingly, the chelator is able to chelate plasma non-transferrin-bound serum iron (NTBI), but is not toxic to peripheral blood mononuclear cells (PBMC) *in vitro*. CM1 reduces iron-

induced redox damage and decreases levels of the intracellular iron pool or labile iron pool (LIP) in iron-loaded hepatocyte cultures (Pangjit *et al*, 2012). In treatments of iron-loaded mice, CM1 could reduce levels of tissue iron content, the RBC membrane non-heme iron, NTBI, labile plasma iron (LPI) and a lipid-peroxidation product malondialdehyde (MDA) (Srichairatanakool *et al*, 2013).

1.2.5 Green tea

Tea (*Camellia sinensis*) is an excellent source of polyphenols, namely catechins, including (-)-epicatechin (EC), (-)-epicatechin 3-gallate (ECG), (-)-epigallocatechin (EGC), (-)-epigallocatechin 3-gallate (EGCG), (+)-catechin (C) and (-)-gallocatechin (GC) **(Figure 1-6)**. Among them, EGCG is the most abundant catechins found in green tea extract (GTE) and exerts the strongest antioxidant capacity. It has been reported that catechins possess free radical scavenging abilities and iron chelating properties (Srichairatanakool *et al*, 2006). Green tea also shows a protective effect under various oxidative-related pathologic conditions such as the oxidative stress-related renal disease (Yokozawa *et al*, 2012) and the cardiovascular diseases (CVD) (Crespy & Williamson, 2004; Lee *et al*, 2005).

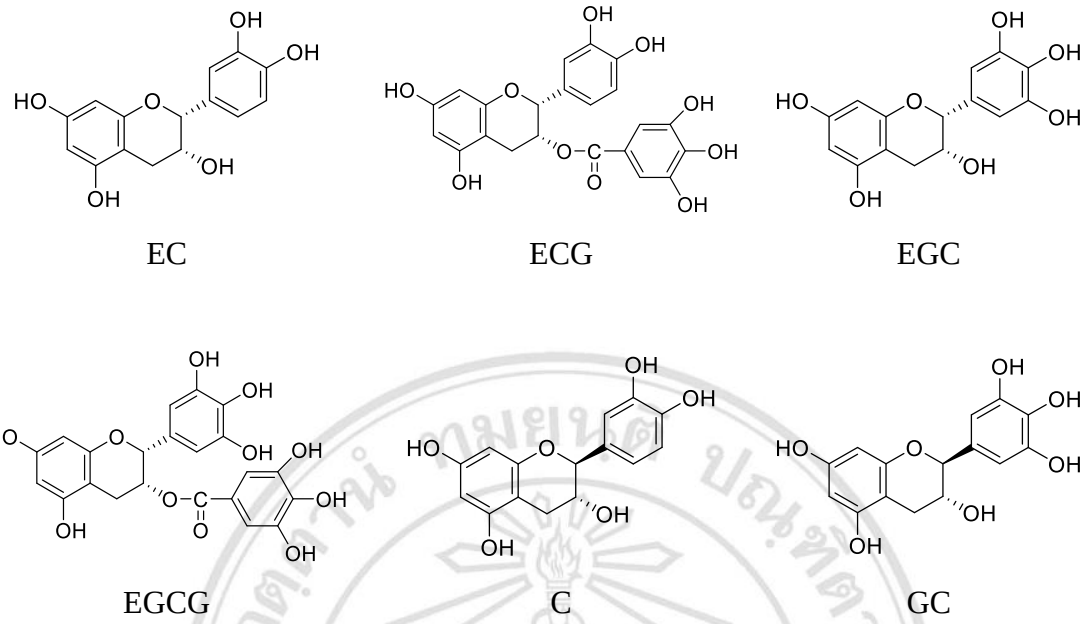


Figure 1-6 Chemical structures of catechins in green tea (*Camellia sinensis*) (Zuo *et al*, 2002)

De Alarcon and coworkers showed that drinking tea produces a 41 – 95% inhibition of dietary iron absorption in β-thalassemia intermediate patients, whose the iron absorption increases strikingly (de Alarcon *et al*, 1979; Pippard *et al*, 1979). It has been elucidated that GTE and EGCG fraction decrease iron (as NTBI) in plasma, eliminate plasma lipid-peroxidation product and destroyed erythrocyte ROS *in vitro* (Srichairatanakool *et al*, 2006; Thephinlap *et al*, 2007) and in iron-loaded rats (Ounjaijean *et al*, 2008). The GTE inhibits or delays the deposition of hepatic iron in regularly iron-loaded β-thalassemic knockout (BKO) mice effectively. This implies a prevention of iron-induced ROS generation and consequently liver damage and fibrosis by green tea consumption (Saewong *et al*, 2010). It has been found that elevated levels of plasma NTBI and lipid peroxidation tended to be normalized in the BKO mice in response to oral therapy with GTE. The mice exhibit a decrease of the lipid peroxidation product and an improvement in the oxidant–antioxidant balance in erythrocytes. It has been shown that the treatment of iron-loaded mouse hepatocytes and human hepatoma (HepG2) cells with GTE (0 – 100 mg/dl) and EGCG (0 – 200 μM)

remove intracellular LIP and ROS efficiently, and relieve the mitochondrial membrane collapse, implying a hepatoprotective effect of green tea catechins in the hepatocytes with iron overload (Srichairatanakool *et al*, 2012). Green tea crude extract and its catechin derivatives strongly inhibit growth of *P. falciparum* culture by the mechanism of inhibition of parasite enoyl-acyl carrier protein reductase and hexose transporter, but not interfering with the folate pathway (Sannella *et al*, 2007; Sharma *et al*, 2007; Slavic *et al*, 2009). Currently, It has been demonstrated green tea can protect renal failure during *P. berghei* malaria infection (Somsak *et al*, 2013). Extraordinary, EGCG inhibits *P. berghei* sporozoite motility and liver cell infection and shows additive effect with digitonin (Hellmann *et al*, 2010).

1.3 Objectives of the study

1) To study inhibitory effect of 1-(*N*-acetyl-6-aminohexyl)-3-hydroxy-2-methylpyridin-4-one (CM1) and green tea extract (GTE) on the growth of *P. falciparum*

2) To evaluate efficacy of CM1 and GTE in inhibition of *P. berghei* growth in mice

3) To compare the combined effect of CM1 and GTE together with PYR on *P. falciparum*-infected RBC and *P. berghei*-infected mice