CHAPTER 1

INTRODUCTION

1.1 Statement and significance of the problem: Malaria and world health

Malaria is a severely infections disease spreading in tropical and subtropical areas of the world.[1] Approximately 300-500 million people were infected and over 1 million patients die each year worldwide.[2] Malaria is an infectious disease caused by protozoa parasite of the genus *Plasmodium* and carried from person to person by anopheles mosquitoes. There are several types of parasites, but only five of them cause malaria in humans.[3] The parasites that cause malaria humans are:

1. *Plasmodium falciparum* (malaria tropica)
2. *Plasmodium vivax* (malaria tertiana)
3. *Plasmodium ovale* (malaria quartana)
4. *Plasmodium malariae* (malaria tertiana)
5. *Plasmodium knowlesi*

The first of two aforementioned species cause the most infections worldwide. *P. falciparum*, particularly, is the agent of severe, potentially fatal malaria because of anti-malaria drugs resistant.[4, 5]

The infectious stages of the malaria parasite reside in the salivary glands of female anopheles mosquitoes that bite humans for a blood meal. During blood extraction, the mosquito injects its saliva into the wound, thereby transferring approximately 15–20 so-called sporozoites into the blood stream. In a matter of minutes, these sporozoites are able to conceal themselves from the host's immune system by entering into the liver cells. Each sporozoite develops into a tissue schizont, containing 10,000–30,000 merozoites.[6] After one to two weeks, the schizont ruptures and releases the merozoites into the blood stream, starting the erythrocytic phase of the parasite's life cycle. In the cases of *P. vivax* and *P. ovale*, some sporozoites turn into hypnozoites, a form that can remain dormant in the liver cells, causing relapses months or even years after the initial infection. *P. falciparum*

and *P. malariae* lack this liver persistent phase, but *P. malariae* can persist in the blood for many years if inadequately treated.[6] Merozoites released into the bloodstream hide again from the host's immune system by invading erythrocytes. In the erythrocyte, the parasite develops from a ring stage *via* a trophozoite stage into a blood schizont. The erythrocyte ruptures and releases 16–32 new merozoites into the blood stream which in turn again invade erythrocytes, thereby starting a new erythrocytic cycle. This asexual life cycle, from invasion of the erythrocytes until the schizont ruptures, spans 48 h for *P. falciparum*, *P. vivax*, and *P. ovale*, and 72 h for *P. malariae*. After a number of asexual life cycles, some merozoites develop into sexual forms, the gametocytes, which are transferred to a mosquito during another blood meal. These gametocytes undergo sexual reproduction within the mosquito mid-gut producing thousands of infective sporozoites, which migrate to the salivary gland where they are ready for a new infection (Figure 1.1).[4, 5] With the rupture of the erythrocyte, the parasite's waste and cell debris are released into the blood stream causing some of the clinical symptoms of malaria. The main symptom is fever, but rarely in the classical tertian (every 48 h) or quartan (every 72 h) patterns. Further symptoms include chill, headache, abdominal and back pain, nausea, and sometimes vomiting. Thus, the early stages of malaria often resemble the onset of an influenza infection. *P. vivax*, *P. ovale*, and *P. malariae* show distinct selectivity toward the age of the infected erythrocytes. For that reason, the degree of total parasitemia is limited. In contrast, *P. falciparum* infects erythrocytes of all ages, leading to high parasitemia. Although the symptoms of *P. vivax, P. ovale*, and *P. malariae* infections can be severe in nonimmune persons, these parasites seldom cause fatal disease. Nevertheless, chronic infection with *P. malariae* can result in an (eventually fatal) nephrotic syndrome.[7] Malaria caused by these three parasites is often called benign malaria. In contrast, *P. falciparum malaria* (also known as malaria tropica) can progress within a few days from uncomplicated to severe malaria with a fatal outcome in 10–40% of all cases of severe malaria, depending on the time lag between the onset of the symptoms and effective treatment, as well as on the hospital facilities for the management of complications.[8] Observed complications can include coma (cerebral malaria), respiratory distress, renal failure, hypoglycemia, circulatory collapse, acidosis, and coagulation failure.[9]

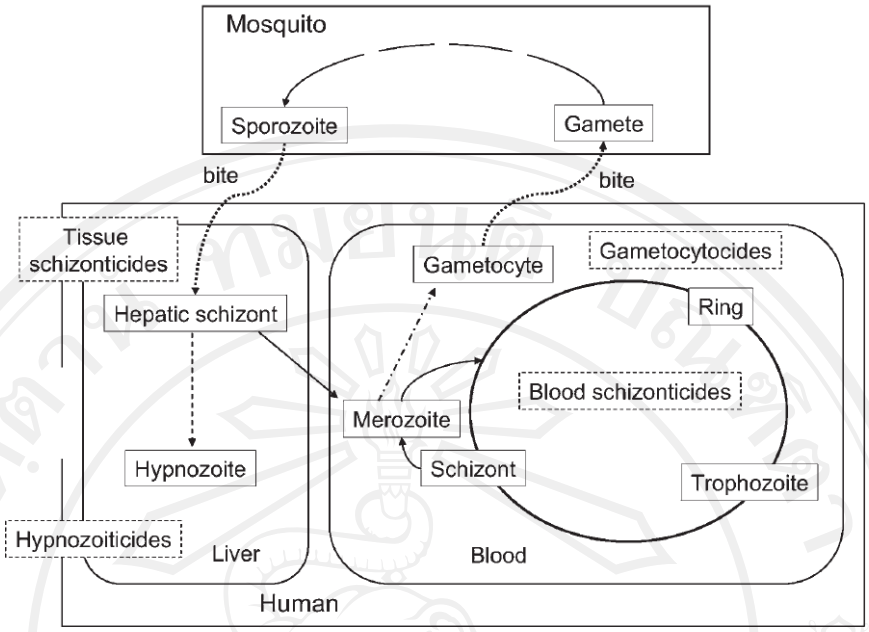


Figure 1.1 The life cycles of malaria disease[4, 5]

Traditionally, antimalarial agents are classified by the stages of the malaria life cycle that are targeted by the drug (Figure 1.1):[10, 11]

1. Blood schizonticides act on the asexual intra-erythrocytic stages of the parasites.
2. Tissue schizonticides kill hepatic schizonts, and thus prevent the invasion of erythrocytes, acting in a causally prophylactic manner.
3. Hypnozoiticides kill persistent intrahepatic stages of *P. vivax* and *P. ovale*, thus preventing relapses from these dormant stages.
4. Gametocytocidesdestroy intraerythrocytic sexual forms of the parasites and prevent transmission from human to mosquito.

As there are no dormant liver stages in *P. falciparum* malaria, blood schizonticidal drugs are sufficient to cure the infection. In cases of *P. vivax* and *P. ovale*, a combination of blood schizonticides and tissue schizonticides is required.[4, 5]

For antimalarial drugs, there are only a limited number of drugs which can be used to treat or prevent malaria as shown in Table 1.1. The most widely used are quinine and its derivatives and antifolate combination drugs.[12]

Table 1.1 Antimalarial drugs

Drug name	Use
SINGLE-AGENT THERAPY	
Chloroquine (CQ)	Treatment of non-*P. falciparum* infections
Amodiaquine (AQ)	Treatment of non-severe *P. falciparum* infections thought to be chloroquine resistant
Sulfadoxine/ Pyrimethamine (SP)	Treatment of non-severe *P. falciparum* infections thought to be chloroquine resistant
Mefloquine (MQ)	Treatment of non-severe *P. falciparum* infections thought to be chloroquine and SP resistant
Halofantrine	Treatment of suspected multidrug-resistant *P. falciparum*
Quinine	Treatment of severe malaria. Treatment of suspected multidrug-resistant *P. falciparum*
Tetracycline (tetra)/ Doxycycline (doxy)	In combination with quinine, can increase efficacy of treatment in areas with quinine resistance and/or reduce likelihood of quinine-associated side-effects by reducing duration of quinine treatment
Atovaquone/Proguanil	Treatment of multidrug-resistant *P. falciparum*
Artesunate	Treatment of multidrug-resistant *P. falciparum*
Artemisinin	
Artemether	
Primaquine	Treatment of *P. vivax* infections (reduce likelihood of relapse) Gametocytocidal agent

Table 1.1 Antimalarial Drugs (continued)

Drug name	**Use**
COMBINATION THERAPY	
Mefloquine + Artesunate	Treatment of non-severe *P. falciparum* infections thought to be chloroquine and pyrimethamine (SP) resistant
Sulfadoxine/ Pyrimethamine + Artesunate	Treatment of non-severe *P. falciparum* infections thought to be chloroquine
Lumefantrine + Artemether	Treatment of non-severe *P. falciparum* infections thought to be chloroquine and SP resistant

The currently available antimalarial agents can be classified according to their chemical structure and biological activity.

1.2 The classification of antimalarial agent[10, 11]

1.2.1 4-Aminoquinolines and arylamino alcohols

1.2.1.1 4-Aminoquinolines

Chloroquine (CQ, **1**) has been the most successful single drug for the treatment and prophylaxis of malaria.[13] It is a safe and affordable drug. The mechanism of action of chloroquine is explained.[14-16] There is common that chloroquine interacts with the parasite's ability to digest hemoglobin. During its erythrocytic stages (Blood schizonticides), the parasite consumes large quantities of hemoglobin from its host cell, either for the purpose of amino acid (peptides) supply, or simply to create space inside the erythrocyte. Hemoglobin is shuttling by vesicles to a specialized organelle called digestive vacuole (DV). The protein component of hemoglobin is digested by the successive action of various proteolytic enzymes. First in sequence are the aspartate proteases plasmepsins I–IV, followed by falcipains (cysteine proteases) and the zinc protease falcilysine. The resulting small peptides and possibly free amino acids are transported across the vacuole membrane into the cytoplasm, leaving the heme part behind. Oxidation of the central iron yields

ferriprotoporphyrin IX (FPPIX) as shown in Figure 1.2.[17, 18] Higher concentrations of this molecule are toxic to the parasite, yet the precise mechanism by which FPPIX exerts its toxicity is not entirely clear. Membrane disruption and the generation of oxidative stress may be a factor in this context. The parasite disposes this hazardous waste through the formation of an insoluble polymer called hemozoin, which is microscopically visible in the digestive vacuole (DV) as the so-called malaria pigment. In addition to the formation of hemozoin, further mechanisms for the detoxification of FPPIX have been discussed. FPPIX could be destroyed by hydrogen peroxide formed during the conversion of oxyhemoglobin to methemoglobin.[19] Heme leaking out of the DV is degraded by the action of the glutathione system. Chloroquine, a dibasic compound (pKa values: 8.1 and 10.2), is trapped in the acidic digestive vacuole (pH 5.0–5.4) as a dication where it accumulates by some orders of magnitude. Similar to the other 4-aminoquinolines, chloroquine forms a complex with ferriprotoporphyrin IX and thereby prevents its polymerization into hemozoin.[20] FPPIX–CQ is toxic to the parasites. In chloroquine-resistant strains, the drug is expelled from the DV by the action of a membrane-bound transporter called the chloroquine resistance transporter (CRT).[21]

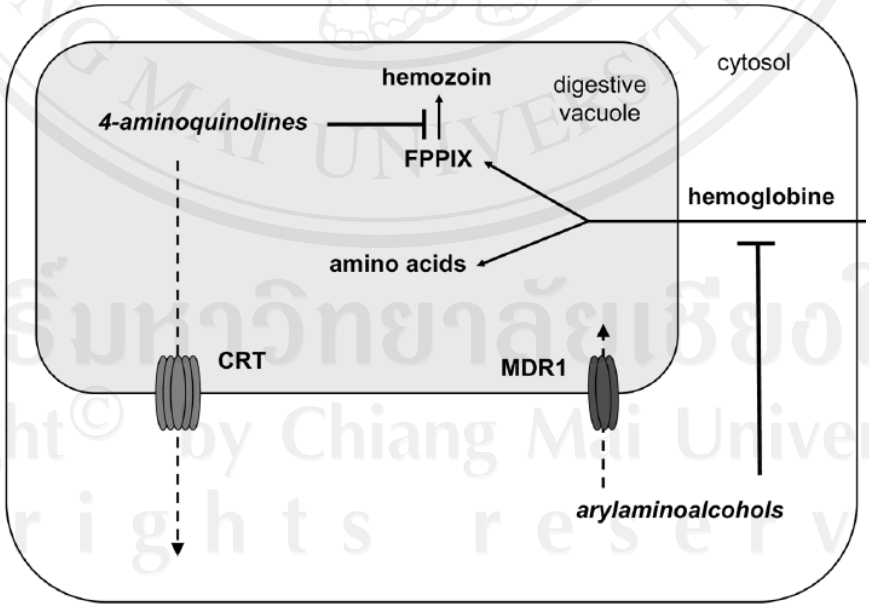


Figure 1.2 Mechanisms of action of 4-aminoquinolines. FPPIX: ferriprotoporphyrin IX; CRT: chloroquine resistance transporter; MDR1: multi drug resistance transporter 1[10]

The other compound structures such as mepacrine (**2**), sontoquine (**3**), amodiaquine (**4**), ferroquine (**5**), isoquine (**6**), tebuquine (**7**), naphthaquine (**8**) and pyronaridine (**9**) were shown in Figure 1.3.

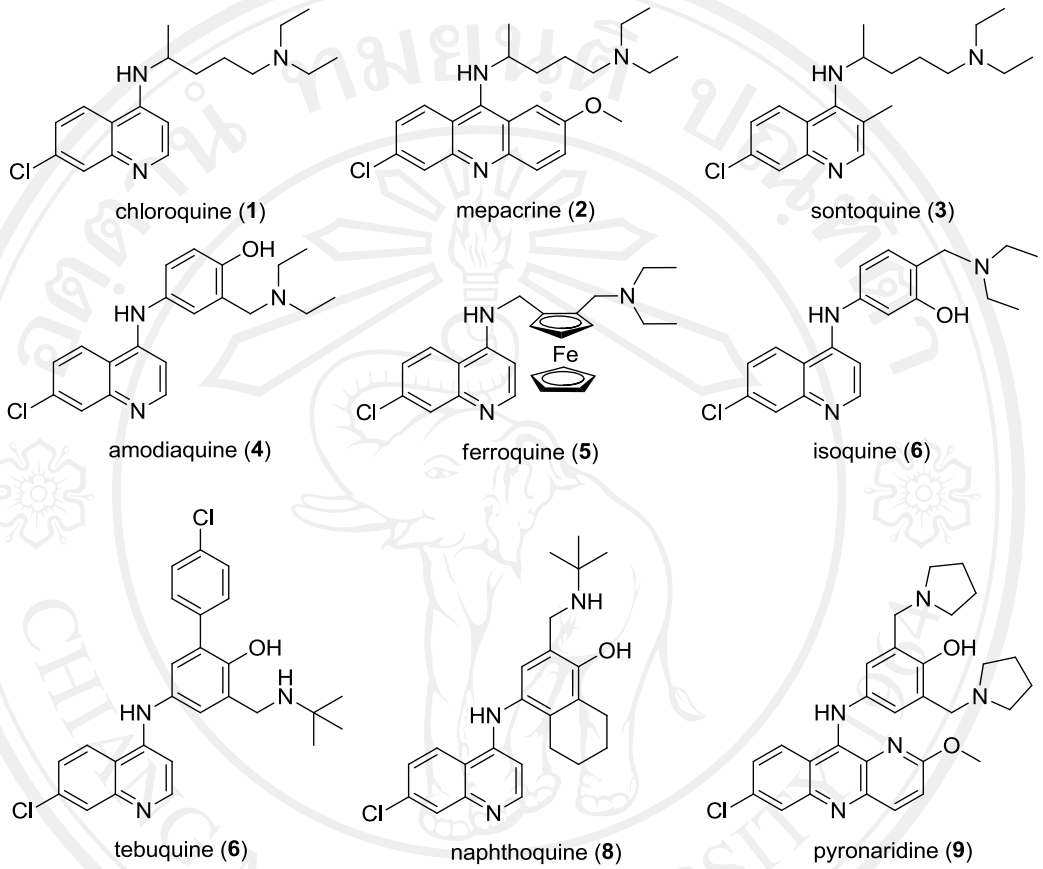


Figure 1.3 4-Aminoquinoline derivatives

1.2.1.2 Arylamino alcohols

Quinine (**10**) and quinidine (**11**) were isolated form the plant *Cinchona* tree. It was the first medicine to be used against malaria.[22] It acts on intra-erythrocytic stage (Blood schizonticides) of parasite.[23] The mechanism of action of arylaminoalcohols seems to be different from that of 4-aminoquinolines but is not known exactly. Arylamino alcohols seem to interfere with the heme digestion.[24, 25] Amplification of the *pfmdr1* gene appears to be the main factor in arylamino alcohol resistance[26–31] as shown in Figure 1.2. The other compounds such as mefloquine (**12**), halofantrine (**13**), lumefantrine (**14**) and desbutyllumefantrine (**15**) were shown in Figure 1.4.

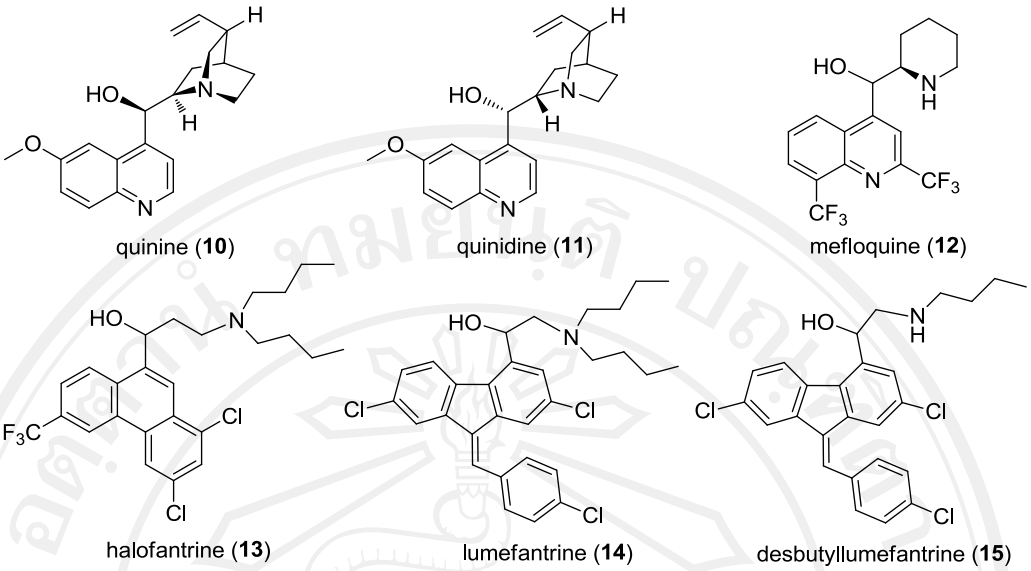


Figure 1.4 Arylamino alcohols derivatives

1.2.2 8-Aminoquinolines

Primaquine (**16**) distinguishes itself from other antimalarials, as it shows activity against the liver and the sexual blood stages of different *Plasmodia*.[32, 33] The mechanism of action of the 8-aminoquinolines is unclear, and there is no firm understanding of the mechanism of primaquine. The other compound structures such as quinocide (**17**), *tert*-butylprimaquine (**18**), tafenoquine (**19**), NCP1161B (**20**) and bulaquine (**21**) were shown in Figure 1.5.

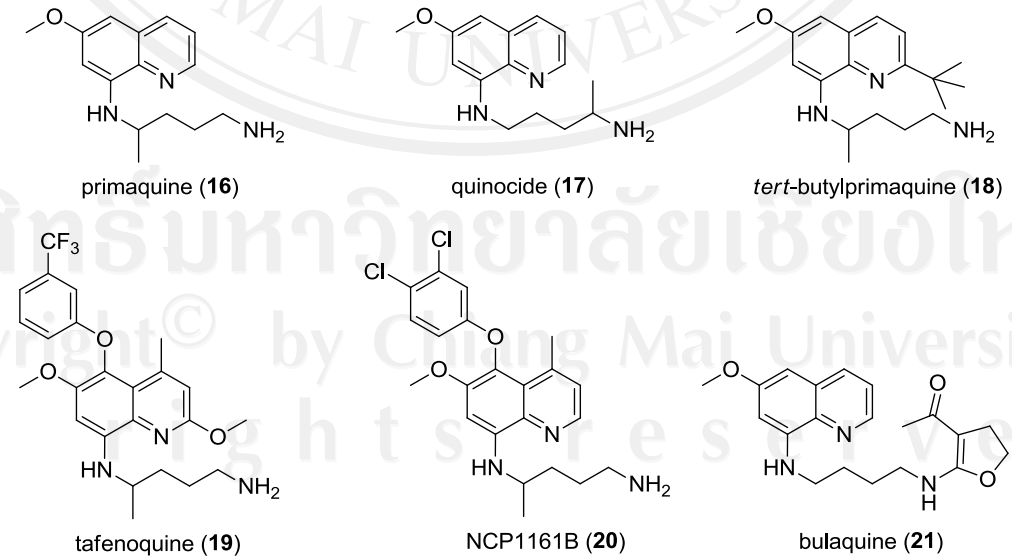


Figure 1.5 8-Aminoquinoline derivatives

1.2.3 Artemisinin and synthetic peroxide

Artemisinin (**22**) was isolated from the herb sweet wormwood (*Artemisia annua*).[34, 35] Artemisinin is highly active antimalarial agent. A key structural feature of artemisinin is the 1,2,4-trioxane substructure or the endoperoxide, which is mandatory for antimalarial activity. Artemisinin is active against sexual blood stages, thereby reducing transmission of the parasites.[36-38] Despite the growing importance of artemisinins, its exact mechanism of action is still unresolved and remains a matter of intense debate. It has been proposed that iron(II)-mediated cleavage of the endoperoxide leads to the formation of different carbon-centered radicals which may be primary or secondary in nature (Figure 1.6). The reactive carbon-centered radicals are thought to subsequently react more or less indiscriminately with different protein targets as well as with ferriprotoporphyrin IX itself, thus preventing heme detoxification and inhibiting a multitude of enzymes.[18, 39–42]

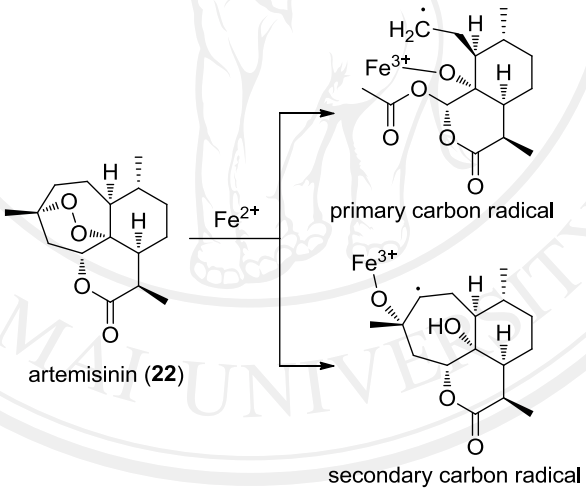


Figure 1.6 Iron (II)-mediated formation of primary or secondary carbon radicals from artemisinin

Whether these radicals unspecifically modify multiple targets like proteins and heme in the digestive vacuole[43] or whether they specifically inhibit an ER-located calcium pump (*PfATP6*)[44] is a matter of debate.[45]

O'Neill and Posner formulated the mechanism of artemisinins as "iron-triggered cluster bombs" (Figure 1.7).[43] Although very attractive-the development of resistance against a drug that acts nonspecifically against multiple targets is unlikely-this concept has been questioned owing to some contradictory findings: artemisinins

act against all developmental parasite stages, including those which do not produce hemozoin. Several experiments detected labeled artemisinin derivatives localized not within but only outside the digestive vacuole, and there are some highly active artemisinin derivatives that are more or less insensitive to Fe^{II}-mediated cleavage.[46, 47]

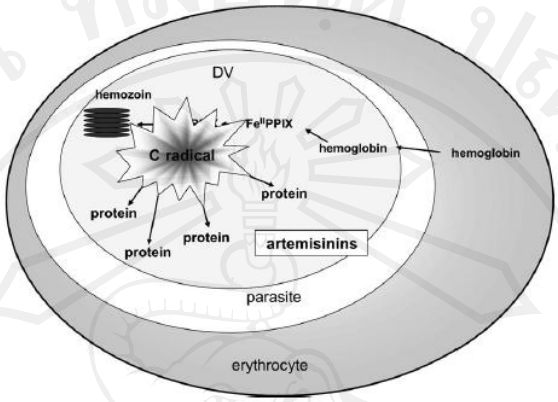


Figure 1.7 The "iron-triggered cluster bomb": the mechanism of action of artemisinins carbon-based radicals, deactivating proteins more or less indiscriminately

Recently, Krishna and co-workers put forward another theory: endoperoxide cleavage should take place in the cytoplasm catalyzed by a cytoplasmic iron(II) source.[44] The resulting reactive species then very specifically inhibits an ATP-dependent Ca^{2+} pump located on the endoplasmic reticulum (Figure 1.8). The pump, called *PfATP6*, is a homologue of a mammalian sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase.

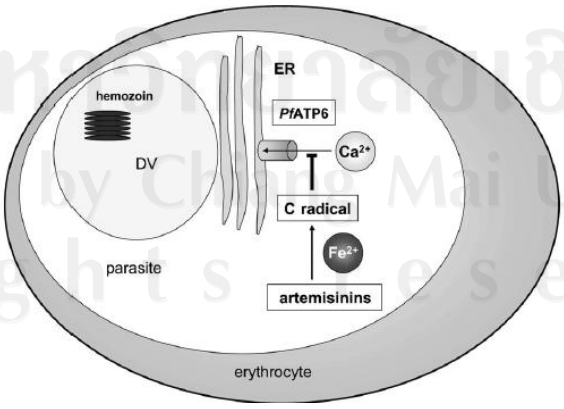


Figure 1.8 Recent results suggest that: these radicals specifically inhibit a sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase called *PfATP6*

The other compound structures such as dihydroartemisinin (**23**), artemether (**24**), arteether (**25**), artesunate (**26**), artelinate (**27**), artemisone (**28**), 1,2,4-trioxolan (**29**), artemisinin dimer (**30**), endoperoxide (**31**) and 1,2,6,7-tetraoxalan (**32**) were shown in Figure 1.9.

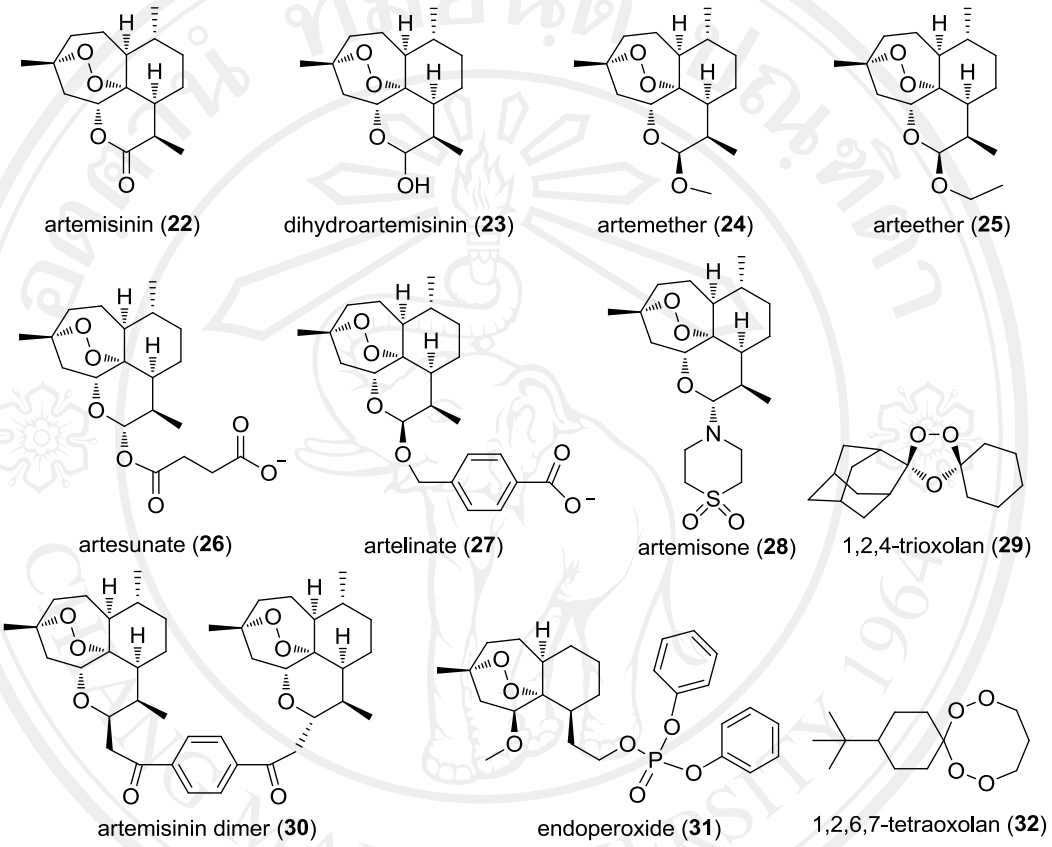


Figure 1.9 Artemisinin and synthetic peroxide derivatives

1.2.4 Chimeric molecules

Based on the assumption that both 4-aminoquinolines and trioxanes act on heme, chimeric molecules have been designed to contain the 4-aminoquinoline moiety of chloroquine (**1**) and a trioxane moiety such as 4-aminoquinoline-trioxane (**33**) show antimalarial activity more than each part.[48] Figure 1.10 showed the other compound structures such as artemisinin-mefloquine ester linker (**34**) and artemisinin-mefloquine amine linker (**35**).[49]

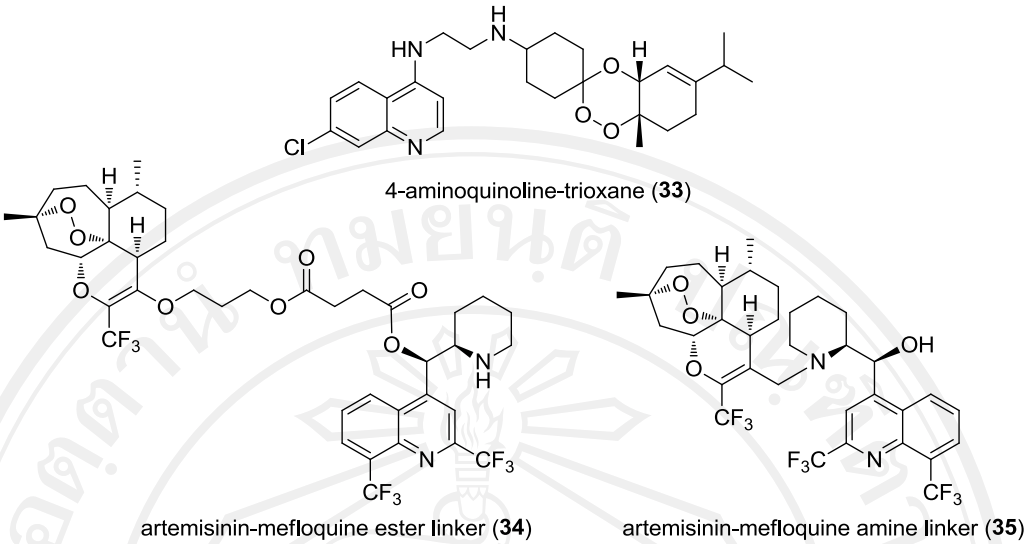


Figure 1.10 Chimeric molecules

1.2.5 Antifolates

In most species, tetrahydrofolic acid plays a key role in the biosynthesis of thymine, purine nucleotides, and several amino acids (Met, Gly, Ser, Glu, and His). Which are then reduced to tetrahydrofolic acid, pathogenic microorganisms including *Plasmodia* can synthesize dihydrofolic acid from simple precursors. Inhibitors of two key enzymes of the folate biosynthetic pathway, dihydropteroate synthase (DHPS) and dihydrofolate reductase (DHFR), have long been used in the treatment of bacterial and protozoal infections.[50] Antifolates act in all growing stages of the asexual erythrocytic cycle and on young gametocytes.[51, 52]

The first antifolate to be used against malaria was the well-known DHPS inhibitor (competitive *p*-aminobenzoic acid (PABA)) such as sulfachrysoidine (**36**), dapsone (**37**) and sulfanilamide (**38**). In contrast to many other antimalarials, the interaction of DHFR inhibitors with their target is pyrimethamine (**39**), cycloguanil (**40**), chlorcyclo-guanil (**41**), proguanil (**42**), chlorproquanil (**43**), sulfanilamide (**44**), trimethoprim (**45**) and methotrexate (**46**), which structures were shown in Figure 1.11.

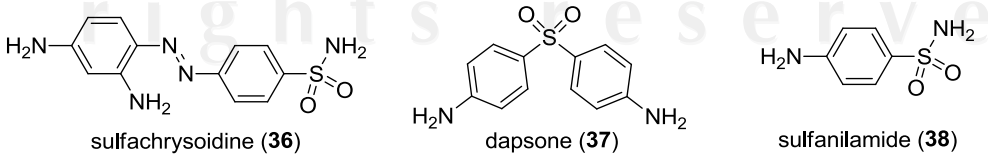


Figure 1.11 Antifolates

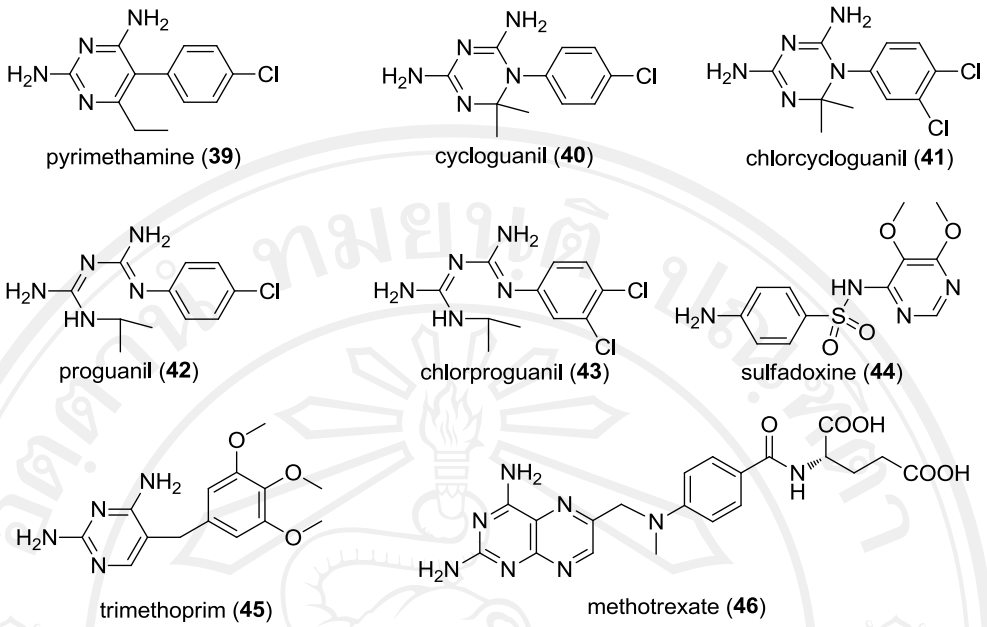


Figure 1.11 Antifolates (continued)

1.2.6 Compounds acting on the respiratory chain

In contrast to higher eukaryotic organisms, the mitochondrial electron transport of *P. falciparum* seems not to be coupled with the synthesis of ATP.[53] The mean source of this high-energy compound for *P. falciparum* is anaerobic glycolysis. Atovaquone (**51**) displays of electron transport leads to a rapid collapse of the mitochondrial membrane potential, which causes a complete shutdown of mitochondrial metabolism (electron-transport inhibitor).[53] Figure 1.12 showed the other naphthoquinone strucures such as hydrolapachol (**47**), parvaquonel (**48**), menoctone (**49**), buparvaquone (**50**) and 2-aziridylnaphthoquinone (**52**).

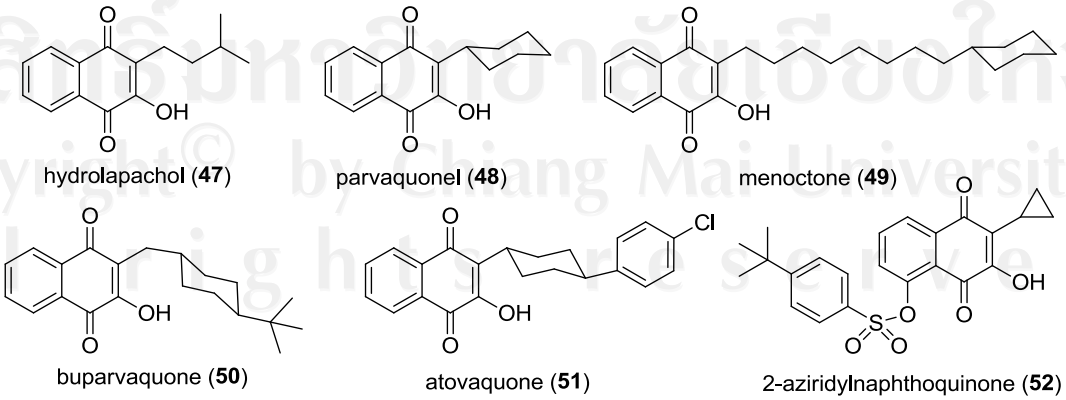


Figure 1.12 Hydroxynaphthoquinone derivatives

1.2.7 Antibiotics

Several antibacterial agents display considerable activity against the eukaryotic malaria parasites, as antibiotics are known to specifically target prokaryotic structures. This apparent contradiction can be explained by the presence of two organelles, the mitochondrion and the apicoplast. A variety of antibiotics are all translation inhibitors in prokaryotic systems, are also considered to inhibit protein synthesis inside the apicoplast.[54-56] Rifampicin (**53**) is inhibitor of bacterial RNA polymerase. A similar RNA polymerase was shown to be encoded on the plastid genome.[57] Other compounds such as ciprofloxacin (**54**), lincomycin (**55**), tetracycline (**56**), doxycycline (**57**) and chloramphenicol (**58**) were shown in Figure 1.13.

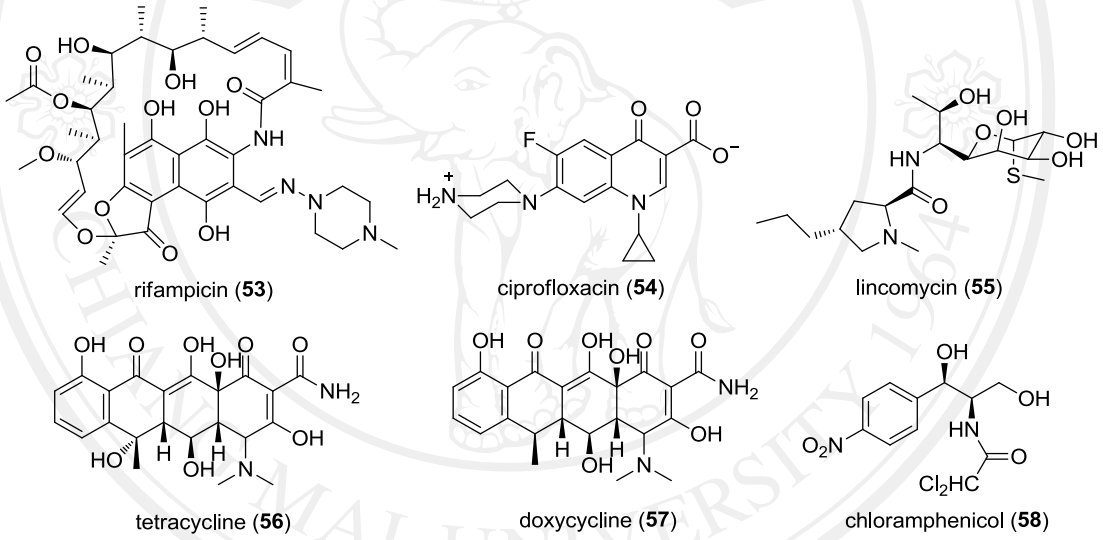


Figure 1.13 Antibiotics

1.2.8 Diamidines

The explanation for the mechanism of action of diamidines, as the activity of pentamidine (**59**) is antagonized by the inhibition of hemoglobin degradation.[58] Pentamidine (**59**) have been demonstrated to cause a collapse of the mitochondrial membrane potential at least with yeast as a model organism.[59] Other compound structures such as propamidine (**60**), bisamidine (**61**), stilbamidine (**62**), furamidine (**63**) and two linear diamidine (**64**) were shown in Figure 1.14.

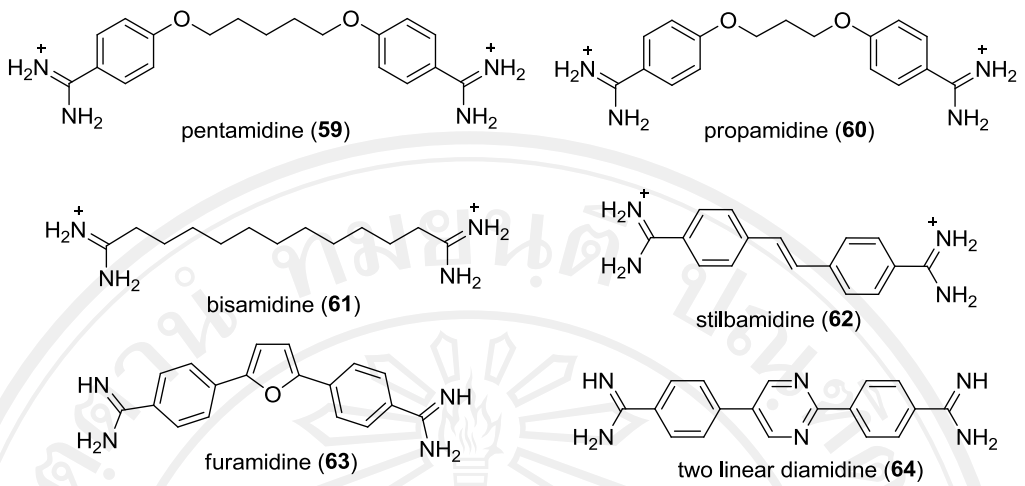


Figure 1.14 Diamidines

1.2.9 Inhibitors of phospholipid metabolism

During the intraerythrocytic stages of parasites produce large quantities of membrane constituents through phospholipid metabolism.[60] Important points learned from these early investigations are that two quaternary ammonium groups connected by a flexible chain lead to the most active compounds. The bis(triethyl-ammonium) (**65**) turned out to be the most active compound. It inhibits the growth of cultured parasites.[61] Other compounds such as bis(triethylammonium) derivative (**65**), aromatic amidinium salt (**66**, MS1) and bis-thiazolium salt (**67**) were shown in Figure 1.15.

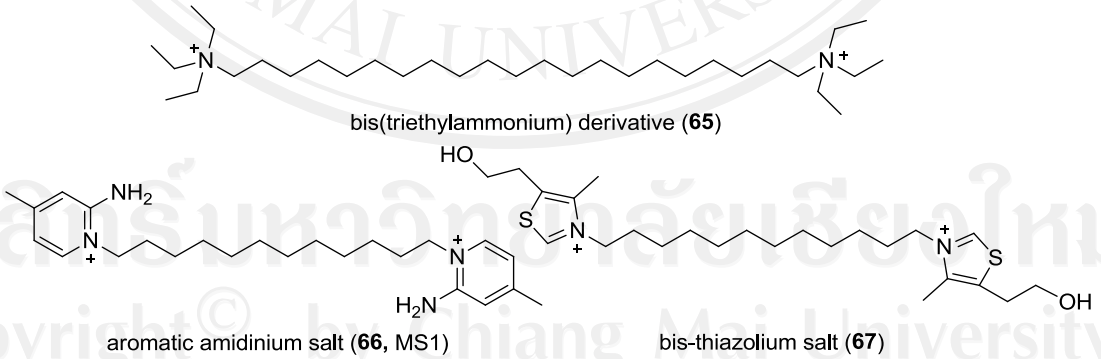


Figure 1.15 Inhibitors of phospholipid metabolism

1.2.10 Inhibitors of isoprenoid biosynthesis

Fosmidomycin (**68**) was rediscovered as an inhibitor of 1-desoxy-D-xylulose-5-phosphate reductoisomerase (DXR) which is enzyme to produce isoprenoids.[62] Other compounds such as cyclopropane derivative (**69**), FR900098

(**70**), Schl-7168 (**71**), dichlorophenyl derivative (**72**) and diphosphate derivative (**73**) were shown in Figure 1.16.

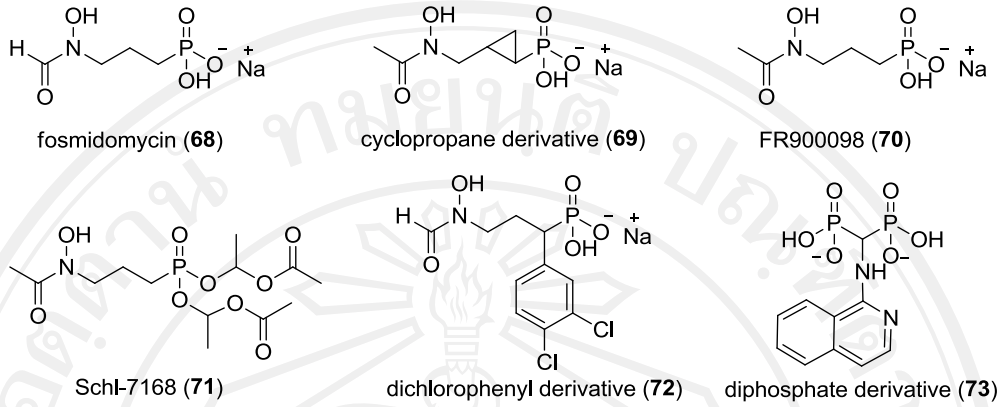
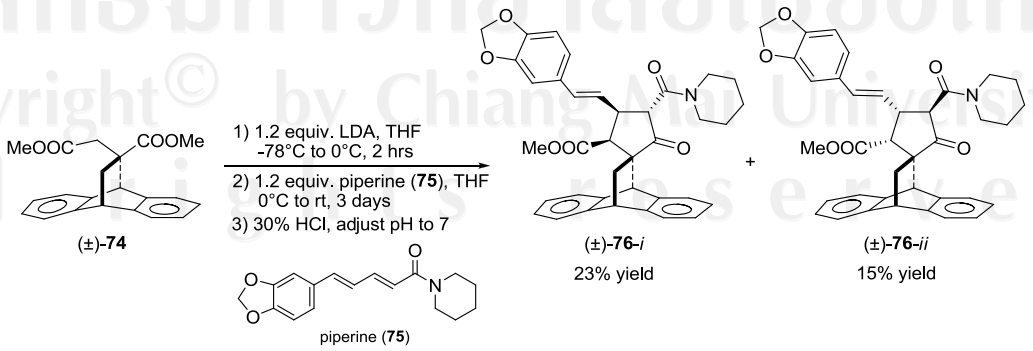


Figure 1.16 Inhibitors of isoprenoid biosynthesis

Out of the thousands of compounds investigated only a few have achieved a significant place in the chemotherapy of malaria. However, the malaria diseases have increase damages and drug resistances have severely limited the available anti-malarial drugs.

1.3 Literature reviews

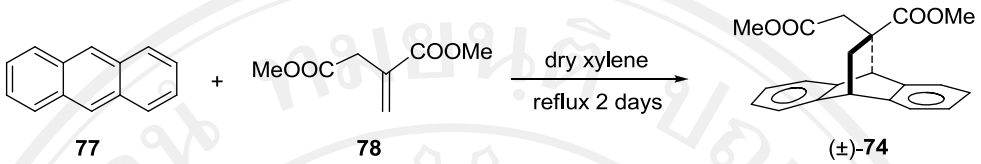
In 2007, Choommongkol Ruangrat[63] reported the spirocyclo pentanone–anthracene adducts ((±)-**76**-*i* and (±)-**76**-*ii*) were generated from dimethyl itaconate–anthracene adduct (**74**) and piperine (**75**) *via* tandem Michael addition-Dieckmann condensation reactions as shown in Scheme 1.1. It shows antimalarial activity against *P. falciparum* (K1, chloroquine–resistance strain) IC_{50} 4.7 and 3.4 μg/ml, respectively. Importantly, it is non-cytotoxicity against Vero cells.



Scheme 1.1 Syntheses of spirocyclopentanone–anthracene adducts ((±)-**76**-*i* and (±)-**76**-*ii*)

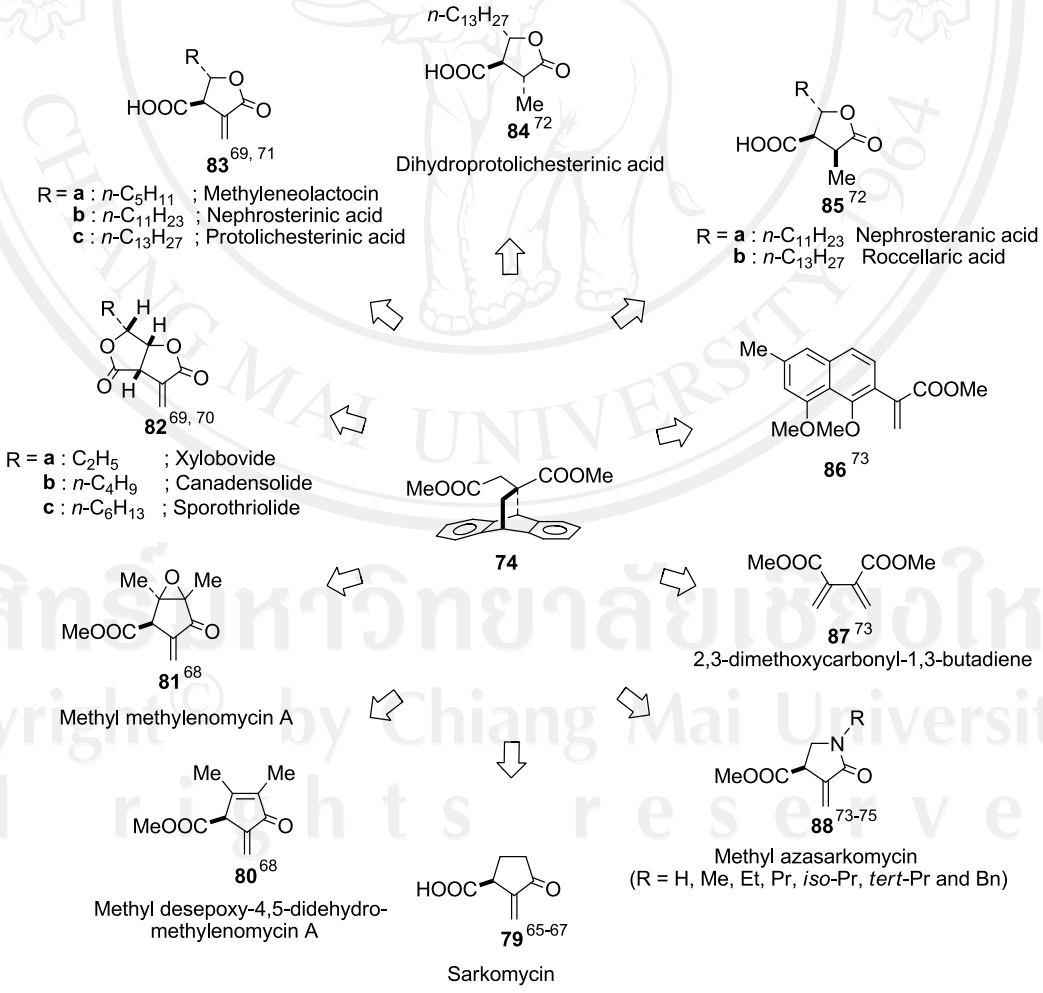
1.3.1 Dimethyl itaconate–anthracene adduct ((±)-74)

Dimethyl itaconate–anthracene adduct ((±)-**74**) was prepared from anthracene (**77**) and dimethyl itaconate (**78**), as shown in Scheme 1.2.[64]



Scheme 1.2 Synthesis of dimethyl itaconate–anthracene adduct ((±)-**74**)

Adduct (±)-**74** was used as starting material to synthesize biologically active natural product such as sarkomycin (**79**),[65-67] methyl deepoxy-4,5-didehydro methylemomycin A (**80**),[67] methyl metylenomycin A (**81**),[68] α-methyllene-γ-butyro lactone (**82**, **83**)[69-71] and α-methyl-γ-butyrolactone (**84**, **85**).[72]



Scheme 1.3 Applications of dimethyl itaconate–anthracene adduct ((±)-**74**)

Bruno Pradines *et al.*[76] synthesized dihydroethanoanthracenic derivatives to test *in vitro* against *P. falciparum* chloroquine resistant parasite W2, Palo Alto, FCR3, and Bres1. Some of DEA derivatives (**89**, **90**, and **91**) displayed IC_{50} 0.4, 0.2 and 0.3 μM respectively, as shown in Figure 1.17.

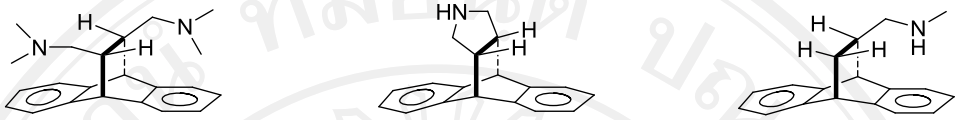


Figure 1.17 Dihydroethanoanthracenic (DEA) derivatives

1.3.2 Piperine and their derivatives

Piperine (**75**) is a major alkaloid compound in *Piper nigrum* (popularly known as black pepper) which was extracted from the dry fruits to give 3-7% yield.[77] Pepper (*Piper nigrum* L.) is used as a spice worldwide and distributed in the tropical and subtropical region of the world.[78] It had various structural categories, such as terpenes, steroids, lignans, flavones and alkaloids/amides. Some amides were shown in Figure 1.18. Piperine displays a variety of pharmacological activities, antifungal,[79] antidiarrhoeal,[80] antiinflammatory,[81] biological activities such as insecticidal,[82] nematocidal,[83] inhibition of life metabolism,[84] immunomodulatory and antitumor.[85]

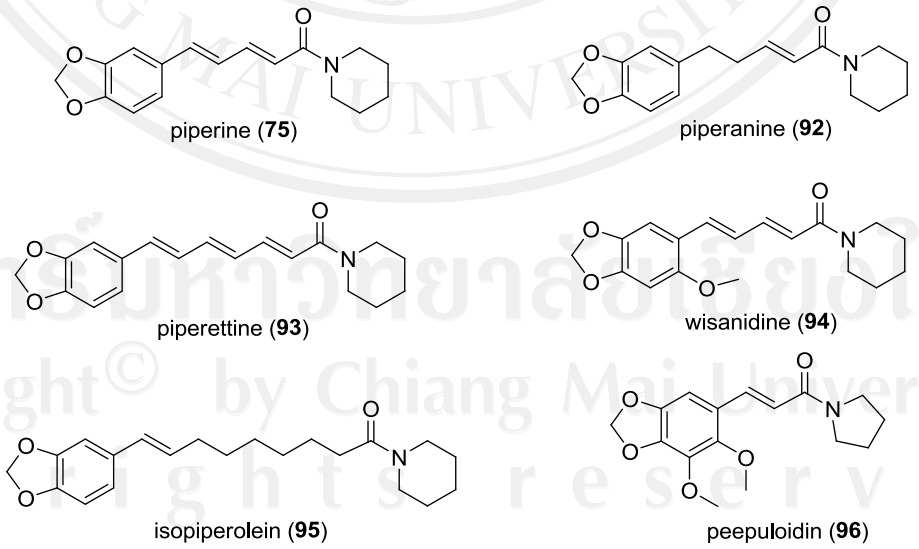


Figure 1.18 Alkaloid compounds from piper[86]

Sachiko Tsukamoto *et al.*[87] isolated dipiperamides A (**97**), B (**98**) and C (**99**) (Figure 1.19) from the white pepper which inhibited CYP3A4 activity. Cytochrome

P450 enzymes are heme-containing monooxygenases and recognized to be responsible for drug metabolism.[88]

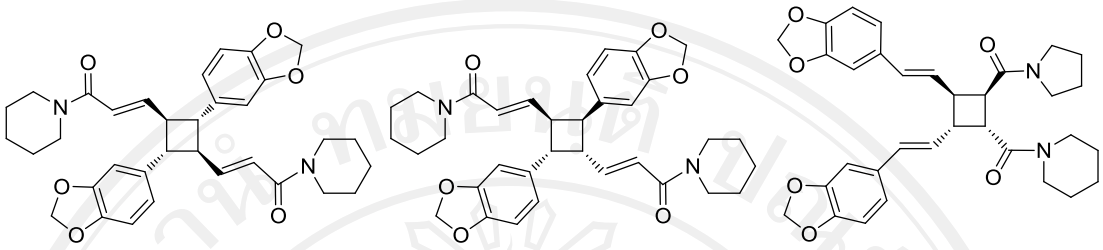


Figure 1.19 Structure of dipiperamides

Thitima Rukachaisirikul *et al.*[89] reported a novel piperine dimer, named chabamide (**100**) as shown in Figure 1.20. It was isolated from stems of *Piper chaba Hunter* and its structure was elucidated on the basis of spectroscopic evidence. Chabamide has efficient to antimalarial activity with an IC_{50} 2.7 μg/ml and antituberculosis activity with the minimum inhibitory concentration (MIC) 12.5 μg/ml.

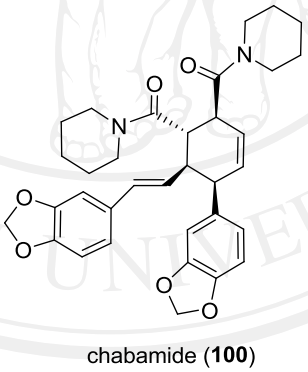


Figure 1.20 Structure of chabamide

1.3.3 Cinnamic acid derivatives

Brozic Petra *et al.*[90] studied 3-trifluoromethylcinnamic acid (**101**) and α-methylcinnamic acid (**102**) inhibited 17β-hydroxysteroid dehydrogenase type 5 (AKR1C3) which is involve in the pre-receptor regulation of androgen and estrogen action in the human shown IC_{50} 50 μM and 6.4 μM, respectively as shown in Figure 1.21.

Manoj Kakwani *et al.*[91] reported cinnamicpierazine diamine (**103**) and cinnamic-homopiperazine diamine (**104**) which have against mycobacterial (Mtb H37Rv). Diamine (**103** and **104**) shows MIC 9.0 μM and 17.3 μM, respectively.

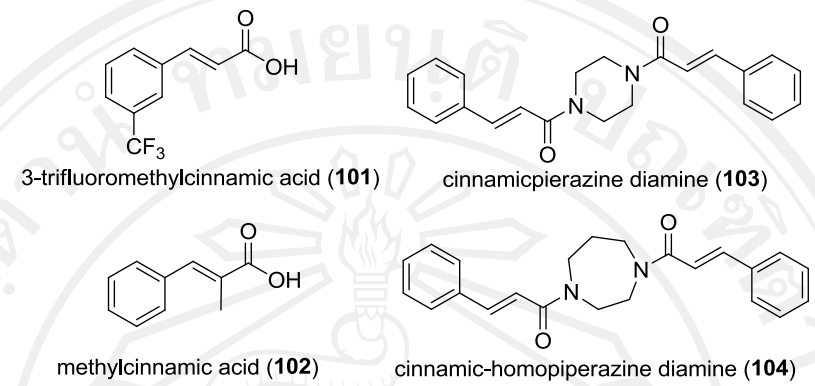
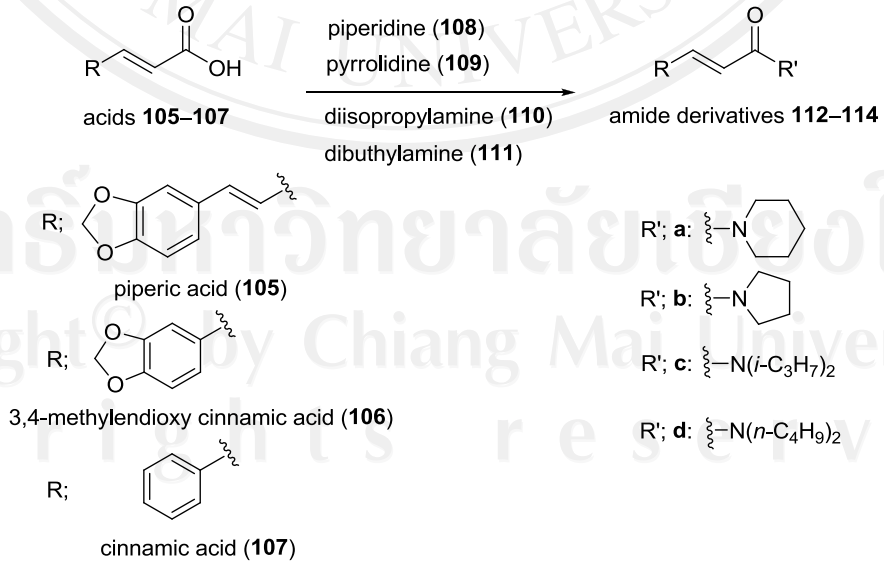


Figure 1.21 Structure of cinnamic acid derivatives

1.4 Objectives

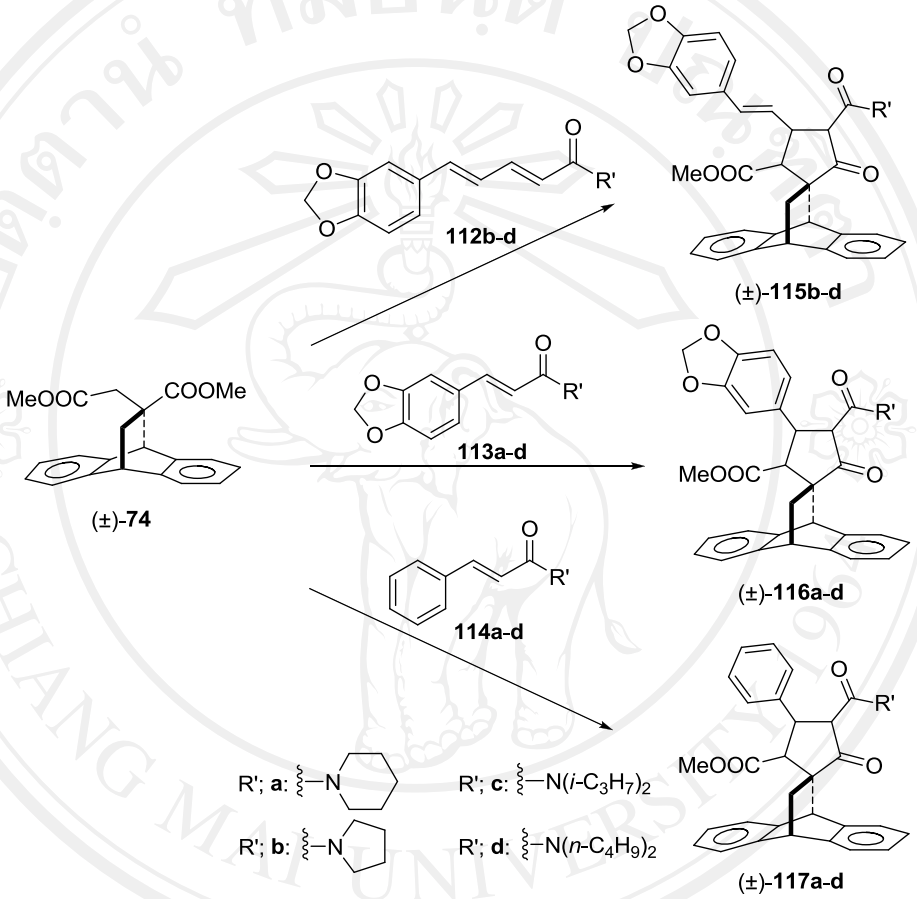
In this thesis, we focused on design and syntheses of racemic spirocyclopentanone–anthracene adduct derivatives ((±)-**115b-d** – (±)-**117a-d**) for antimalarial activity. The steps of experiment including:

1. To prepare amide derivatives **112b-d** – **114a-d** from acids (piperine acid (**105**), 3,4-methylenedioxy cinnamic acid (**106**) and cinnamic acid (**107**)) react with amines **108** – **111** as shown in Scheme 1.4.



Scheme 1.4 Preparation of amide derivatives (**112b-d** – **114a-d**)

2. To synthesize racemic spirocyclopentanone–anthracene adduct derivatives ((±)-**115b-d** – (±)-**117a-d**) from amide derivatives (**112b-d** – **114a-d**) and racemic dimethyl itaconate–anthracene adduct ((±)-**74**), as starting material *via* tandem Michael addition–Dieckmann condensation reactions as shown in Scheme 1.5.



Scheme 1.5 Summarization of the synthesis of spirocyclopentanone–anthracene adduct derivatives ((±)-**115b-d** – (±)-**117a-d**)

3. To examine the antimalarial activity against *P. falciparum* (K1 multidrug resistant strain)[92] by microculture radioisotope technique[93] and cytotoxicity against Vero cells (African green monkey kidney) by green fluorescent protein (GFP)-based assay[94] of racemic spirocyclopentanone–anthracene adduct derivatives ((±)-**115b-d** – (±)-**117a-d**).

4. To study the effects of spirocyclopentanone–anthracene adducts ((±)-**76**-*i* and (±)-**76**-*ii*) on activity of CYP450 from porcine liver microsomes.