CHARPTER II

LITERATURE REVIEWS

2.1 General knowledge about malaria

Malaria is caused by a parasite called *Plasmodium*, which is transmitted *via* the bites of infected *Anopheleis ssp*. in red blood cells. It is widespread in tropical and subtropical regions, including much of Sub-Saharan Africa, Asia and America.[15,16] An estimated 200 millions clinical use of people are found across worldwide and about 2 millions deaths per year are due to *Plasmodium* infections. The majority occurs in children under 5 years of age in Sub-Saharan African countries.[17,18] The four common parasites that cause malaria are [19] :

1. *Plasmodium falciparum*
2. *Plasmodium vivax*
3. *Plasmodium ovale*
4. *Plasmodium malariae*

P. falciparum and *P. vivax* are the most severe form of malaria that are responsible for the vast majority of deaths associated with the disease.[20] Malaria caused by *P. ovale* and *P. malariae* causes milder disease in humans that are not generally fatal.

Malaria is passed on by person whom has malaria parasites in their blood to the other person by the female *Anopheles* mosquito biting (Figure 2.1).[21,22] When a mosquito bites an infected persons, a small amount of blood is taken, which contains microscopic malaria parasites. About one week later, when the mosquito takes its next blood meal, these parasites develop in the intestine and salivary glands of the

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mosquito and can be passed on to other people the next time when the mosquito bites. In the human body, the parasites multiply in the liver, and then infect red blood cells, causing symptoms such as fever, chills, nausea, flu-like illness, and, in severe cases, coma and death.[23,24,25] In addition, malaria can be transmitted by others means such as blood transfusion, infected needles and transmission from infected pregnant women to their babies in the placenta.[26,27,28,29]

Figure 2.1 Female Anopheles mosquito (*Anopheleis ssp.*).

2.2 Life Cycle of the Malaria Parasites [30]

When a female *Anopheles* mosquito penetrates human skin to obtain a blood meal it injects saliva mixed with an anticoagulant. If the mosquito is infected with *Plasmodium*, it will also inject elongated sporozoites into the bloodstream of its victim. The sporozoites travel to the liver and rapidly divide asexually to generate the next life cycle form, called merozoites. The released merozoites invade other liver cells and enter the host's blood stream, when they invade erythrocytes. Once insides the erythrocyte, the merozoite begin to enlarge as a uninucleate cell termed a ring trophozoite. The schizont then divides and produces mononucleated merozoites. The erythrocyte ruptures and releases toxins throughout the body of the host, bring about the well-known cycle of fever and chills that is characteristic of malaria. *Plasmodium*

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enters a sexual phase when some merozoites in the erythrocytes develop into gametocytes, cells capable of producing both male and female gametes. Gametocytes are incapable of producing gemetes within their human hosts and do so only when they are extracted from an infected human host by a mosquito. Within the gut of the mosquito, the gametocytes form male and female gametes. The resultant diploid zygotes develop within the mosquito's in intestinal walls and ultimately differentiate into oocytes. Within the oocytes, repeated mitotic division take place to produce large numbers of sporozoites which then migrate to the salivary glands of the mosquito. Then, they are injected into the blood stream of a human from this gland, thus starting the life cycle of the parasite again.

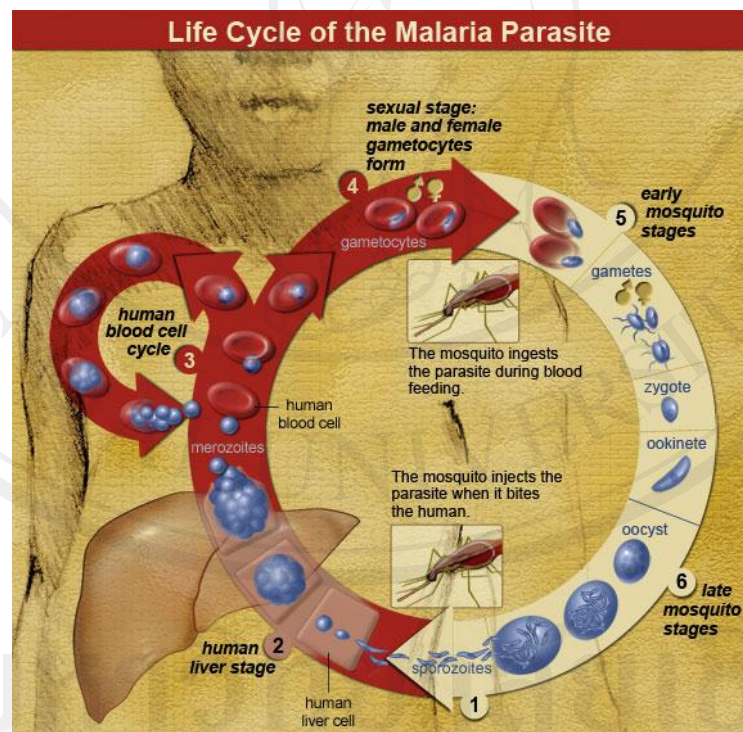

Figure 2.2 Life cycle of the Malaria parasites.[31]

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2.3 Treatment of malaria

Antimalarial drugs are prescribed as a preventive measure against infection with malaria and also as a treatment if the disease develops. There are various types of antimalarial drug. Some are suitable only for treatment of the infection, while others are used for both prevention and treatment. Chemotherapy has traditionally played an important role in the treatment and control of malaria. However, most of the drug resistance towards antimalarials was first documented and reported along the south-eastern border of Thailand and Cambodia.[32] Drug resistance in *P. falciparum* is not confined to chloroquine alone, but also to the other currently used antimalarials such as quinine and its derivatives and antifolate combination drugs.

2.3.1 Quinine and related compound [19]

In 1930, quinine was isolated from Cinchona bark. It is an alkaloid that acts as a blood schizonticidal and weak gametocide against *P.vivax* and *P.malariae*. Quinine (**1**) was the first drug used for malaria treatment in South America. Unfortunately, quinine was in short supply during World War II, therefore, synthetic quinoline and 4-aminoquinoline compounds such as chloroquine (**2**), mefloquine (**4**) and primaquine (**10**) were produced. These compounds have evolved from the structural modification of quinine and these compounds are more effective, cheap, safe and commonly available drug.

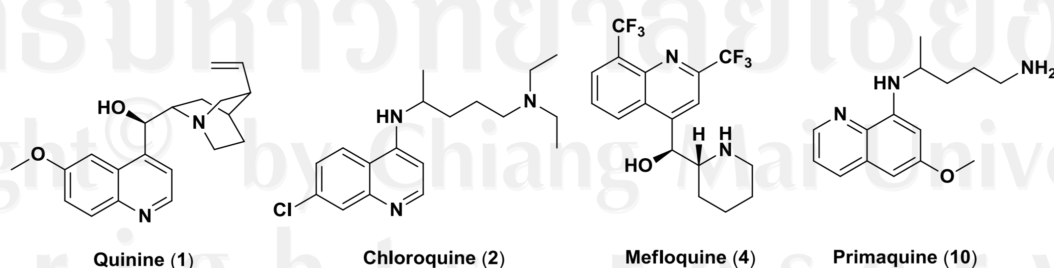

Figure 2.3 Structure of Quinine, Chloroquine, Mefloquine and Primaquine.

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Chloroquine (**2**) is a 4-aminoquinoline drug in the treatment of malaria.[33,34] Chloroquine is a compound with a complicated and still unclear mechanism of action. Chloroquine enters the red blood cell, inhabiting parasite cell, and digestive vacuole by simple diffusion. Chloroquine becomes protonated (to CQ2+), as the digestive vacuole is known to be acidic (pH 4.7); chloroquine then cannot leave by diffusion. Chloroquine caps hemozoin molecules to prevent further biocrystallization of heme, thus leading to heme buildup. Chloroquine binds to heme (or FP) to form what is known as the FP-Chloroquine complex; this complex is highly toxic to the cell and disrupts membrane function. Action of the toxic FP-Chloroquine and FP results in cell lysis and ultimately parasite cell autodigestion. In essence, the parasite cell drowns in its own metabolic products.[35,36,37] (Figure 2.4) Chloroquine is effective on all five species of parasites, including some strains of *P. falciparum*. But in many areas *P. falciparum* is resistant to chloroquine, and other medicines must be used.

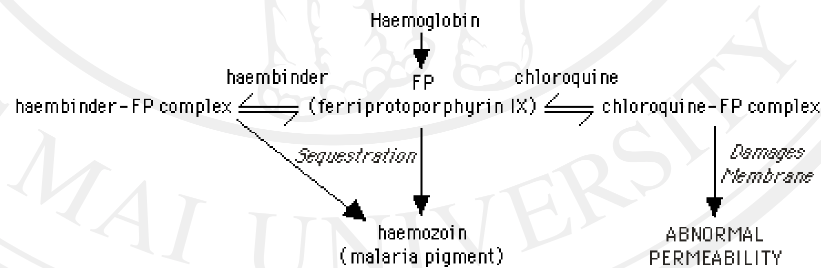

Figure 2.4 Mode of action of chloroquine.

Mefloqine (**4**) is an antimalarial agent which acts as a blood schizonticide and it is active against the erythrocytic stages of *Plasmodium* species.[38] This drug is recommended as a prophylactic drug for travelers to areas with significant risk of chloroquine-resistant *falciparum* malaria.

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Primaquine (**10**) is in a class of medications called antimalarials. It is not used in the prevention of malaria, but only used in the treatment to eliminate the liver stages of the life cycle of *P. vivax* or *P. ovale*.[39,40] It is commonly used following treatment with chloroquine to treat malaria parasite infection.

2.3.2 Antifolate combination drugs

Antifolate agents used in the treatment of malarial infection are subdivided into two classes[41]: inhibitors of dihydropteroate synthase (DHPS), known as class I antifolates and inhibitors of dihydrofolate reductase (DHFR), the class II antifolates. The combination of DHFR : pyrimethamine (**3**), proguanil (**11**), chlorproguanil (**12**) and trimethoprim (**13**) and DHPS : sulfa drugs; dapsone (**14**), sulfalene (**15**), sulfamethoxazole (**16**), sulfadoxine (**17**) inhibitors is synergistic, hence their use in combination in the treatment of malaria.[41] The classical such combination is sulphadoxine and pyrimethamine (SP) used as first line drug in Thailand and other parts of the world. Tetracycline (**18**) and its derivatives such as doxycycline (**19**) are very potent antimalarials and are used for both treatment and prophylaxis.[42] All of antimalarial drugs are shown in Figure 2.5.

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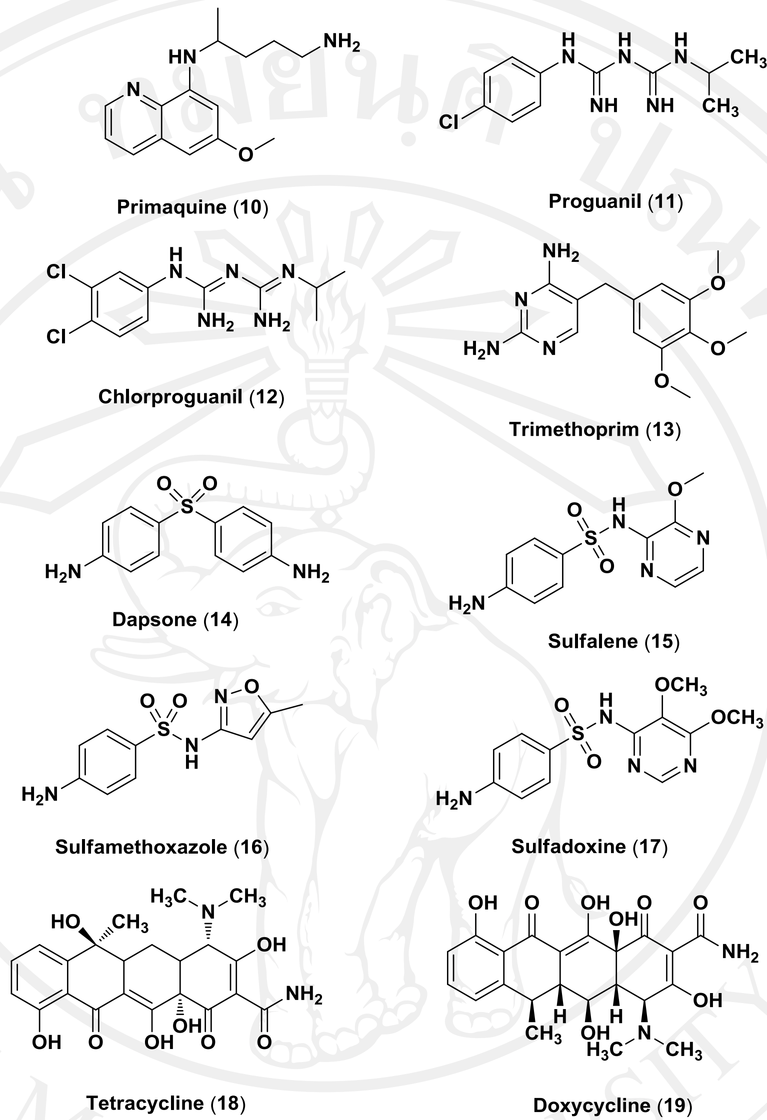

Figure 2.5 The structure of antimalarial drugs.

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2.3.3 Artemisinin compounds and its derivatives

Artemisinin was synthesized from the plant of *Artemisia annua Linn*. Artemisinin and its derivatives such as artesunate (**20**), arteether (**21**) and artemether (**22**) are a group of drugs that have been used for treatment of severe malaria. These drugs have shown very rapid parasites clearance in comparison to quinine compound.[43,2] Artemisinin compounds are usually used in combination with other antimalarial drug and these combination are called artemisinin-based combination therapies, or ACTs. For example, artemisinin and mefloquine combination is being used in some Southeast Asian countries where the multidrug-resistant strains of *P. falciparum* are highly prevalent for the treatment of uncomplicated malaria.[2]

2.4 Artemisinin (9)

Artemisinin or Qinghaosu is the active principal of the Chinese medicinal herb *Artemisia annua*. It is a sesquiterpene lactone that has an essential endoperoxide in the structure.[8] Artemisinin has a remarkable properties of the chemical structure and it can treat malaria patient for anti-malaria drug resistance that the patient can get better rapidly and the drug dosage is used at a low level. Moreover, there are currently no reported that malaria parasites resistance to artemisinin. Nevertheless, this drug has limited the option for clinical treatment due to its low solubility in both oil and water that make its difficult for the preparation of medicine, its poor oral bioavailability and recrudescence can occur after a complete treatment because the drug has a short half-life in plasma. In addition, there are neurotoxicity and cardiotoxicity effect in animal testing.[44] Therefore, semisynthetic artemisinins were performed to improve solubility in both oil and water for improving the efficiency of antimalarial drugs.[45] The lactone of artemisinin can easily be reduced using sodium borohydride to produce

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dihydroartemisinin (**23**) in high yield which has even more antimalarial activity *in vitro* than artemisinin and it has in turn led to the preparation of a series of semi-synthetic first generation analogues including sodium artesunate (**20**), arteether (**21**) and artemether (**22**) which are more oil soluble than artemisinin (**9**) and sodium artelinate (**24**) which is water soluble (Figure 2.6).[46,47] However, their use in monotherapy is associated with high incidences of recrudescent infection, suggesting that combination with other antimalarials might be necessary for maximum efficacy such as artemisinin based combinations (ACTs) that are known to improve cure rates, reduce the development of resistance and might decrease transmission of drug-resistant parasites.[48] The total effect of artemisinin combinations (which can be simultaneous or sequential) reduce the chance of parasite recrudescence, the within-patient selection pressure and prevent transmission. However, the drug group of artemisinin derivatives also has some disadvantages, for instance, artemether and arteether are neurotoxicity and cardiotoxicity effect in animal testing.[49]

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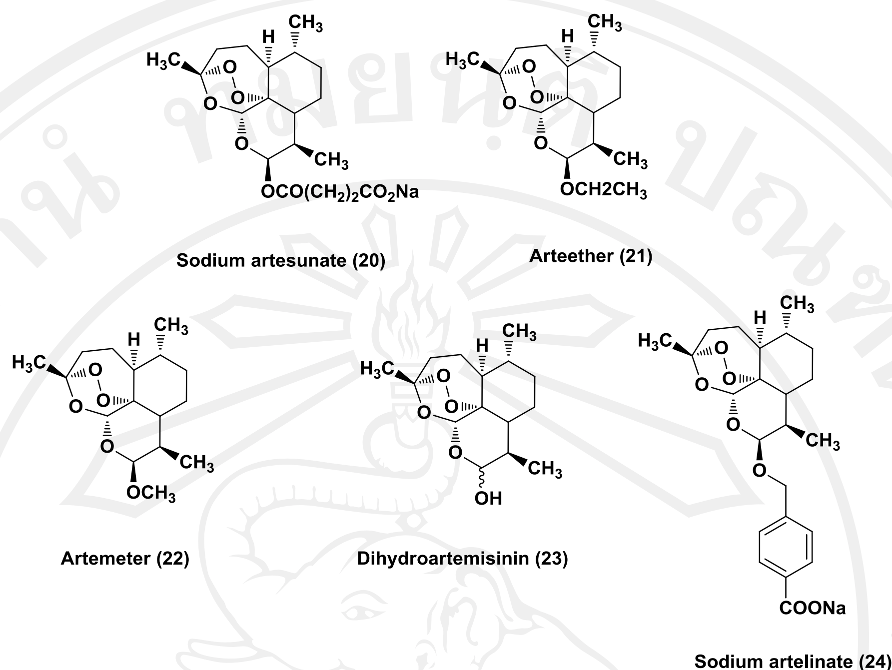

Figure 2.6 The structure of artemisinin derivatives.

2.5 Mechanism of action of artemisinin

Artemisinin is a peroxide-bridge sesquiterpene lactone compound which is essential for antimalarial activity. Its structure is unlike those of any other known antimalarial and is thus likely to have different mechanism of action. Mechanism action of artemisinin is specific that artemisinin does not interact with other molecule except it interacted with intraparasitic heme or iron. Intraparasitic heme or iron might function to activate artemisinin inside the parasite into toxic free radicals that alkylated protein and damage the microorganelles and membrane of the parasites.[50]

Mechanism of actions are as following[46] : First, the heme iron attacks and breaks the endoperoxide linkage of artemisinin to produce an oxy free radical, followed by rearrangement to give a carbon free radical. The resulting carbon then alkylate the specific malarial proteins causing lethal damage to malarial parasites. Two possible pathways were proposed for the formation of the oxy free radical

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(Figure 2.7). In pathway 1, the heme iron attacks at O_2 position of endoperoxide bridge giving the free radical at the O_1 position (**25**) and followed by an intramolecular 1,5-H shift and the C_4 free radical (**26**) is obtained. In pathway 2, the heme iron attacks the endoperoxide moiety at the O_1 position, giving the free radical at the O_2 position (**27**). This process is followed by a hemolytic cleavage of the C_3–C_4 bond, also resulting in the C_4 free radical (**28**).

Another interesting mechanism[51] is the hemoglobin digestion of red blood cells which provides the main source amino acid for the growth of parasites. Ferriprotophorphyrin IX (heme) is a product from the digestion of hemoglobin which is toxic to parasites. Therefore, the parasites convert it into an insoluble crystalline form called hemozoin that is essential to the survival of the parasites as shown in the Figure 2.7. In malaria parasites, hemozoin is often called malaria pigment. From some studies, artemisinin antimalarial drug can interact with Ferriprotophorphyrin IX (heme) so heme-artemisinin adduct can inhibit hemozoin formation and the new product is still toxic to parasites. Thus, artemisinin's drugs can kill malaria parasites.

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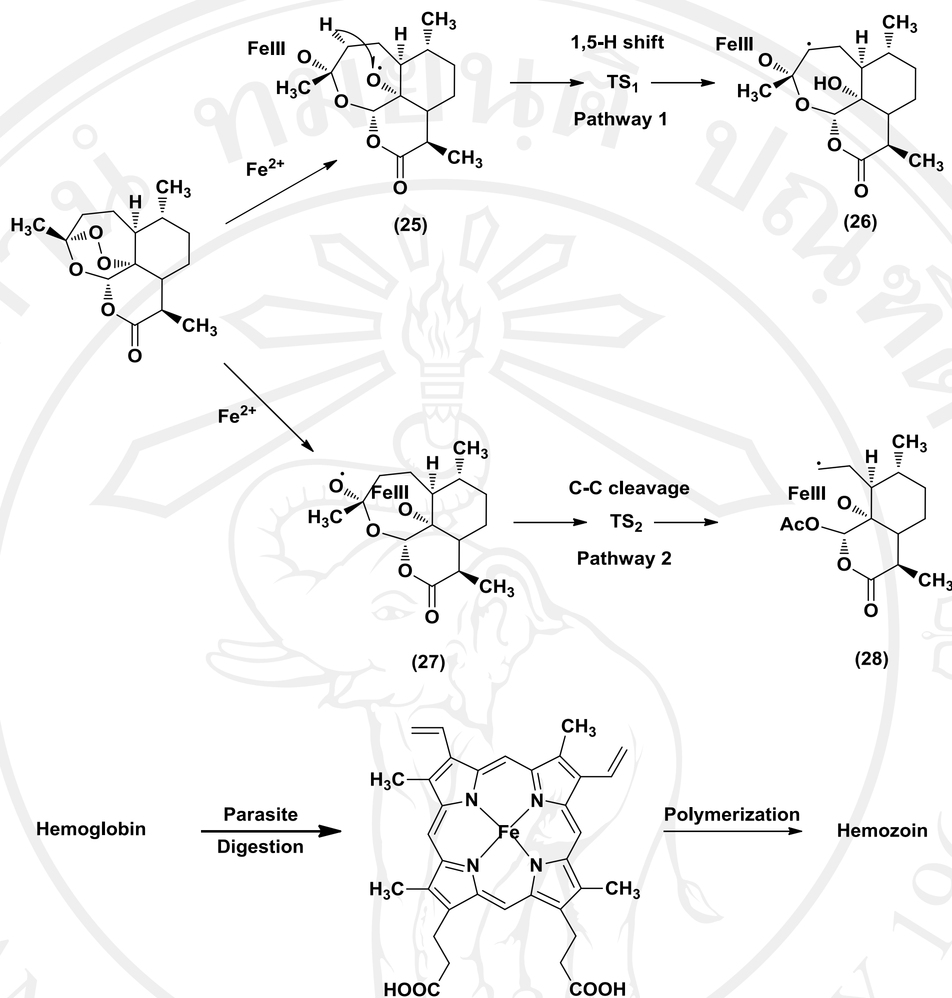

Figure 2.7 Propose mechanism of action of artemisinin.

2.6 Artemisinin and its derivatives as antimalarial agents.

Artemisinin has a long history of use as an antimalarial remedy. Because of its poor bioavailability (low solubility in oil or water) for oral administration, C-10 of artemisinin was modified to be the C-10 acetal type for improving solubility and antimalarial efficacy such as arteether (**21**) and artemether (**23**). Unfortunately, arteether and artemether have few side effects including potential neurotoxicity developing if high dose are given.[52] In addition, the C-10 acetal type of artemisinins showed lower stability than the C-10 non acetal type in simulated stomach acid.[53]

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Jung *et al.*[53] (1998) synthesized non-acetal type analogs of artemisinin containing C-C bond at position-10 (compound **29-30**) (Figure 2.8). It was found that the half-life of non-acetal type analogs **29-30** was 15-22 times longer than acetal (C-O) type analog **23, 31** and **32** under simulated stomach acid (pH = 2.0, 37 ^{o}C). In addition, the *in vitro* activity of non-acetal type prodrugs **29** and **30** against *P. falciparum* were comparable to eight to nine times more active than the acetal type prodrugs **23, 31** and **32**.

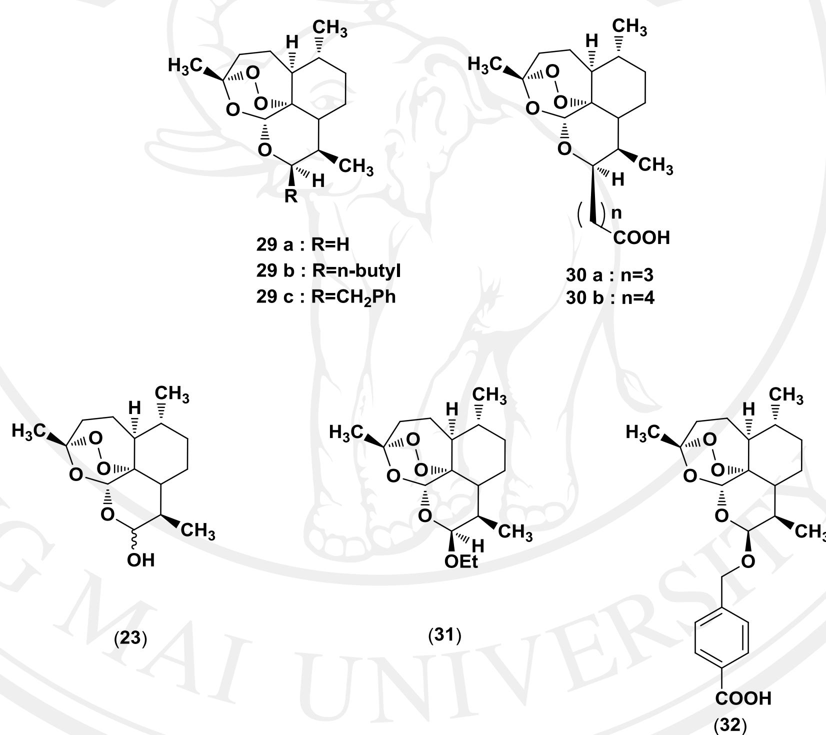

Figure 2.8 Structure of the acetal and non acetal (C-C) type prodrugs of artemisinin.

Zhu *et al.*[54] (2000) synthesized and evaluated antimalarial activity of a new type of water soluble derivatives (compound **33a-c**) in which the amino group is bonded to the C-10 acetal artemisinin using an ethereal linkage. (Figure 2.9)

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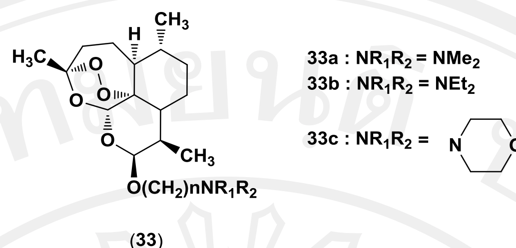

Figure 2.9 Structure of artemisinin water soluble derivatives.

The results showed that these synthesized compounds (**33a**, **33b** and **33c**) were more active against *P. berghei* than artesunic acid (**20**) by oral administration. However, their oral efficacies were poorer than artesunic acid (**20**) against *P. knowlesi* in monkey.

Further study of (+)-deoxoartemisitene (**34**) and its novel C-11 derivatives **36**, **37**, **38** and **39** to overcome the expected acid instability and neurotoxicity were showed that the solubility of compounds **36**, **37** and **38** in water was four times greater than that of artemisinin (**9**) (0.97 mg/mL). The half-life of compounds **36**, **37**, **38**, and **39** in simulated stomach acidic conditions (pH = 2.0, 37 °C) was 15 times longer than that of artemisinin (**9**) ($t_{1/2}$ = 23.5 h).[55]

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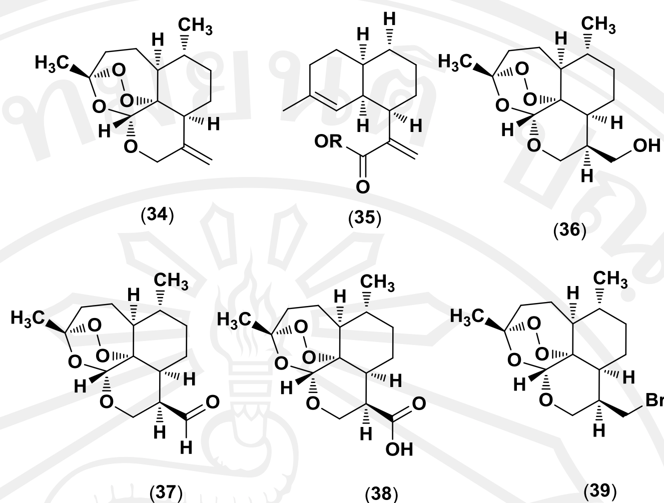

Figure 2.10 Structure of (+)-deoxoartemisitene (**34**) and its novel C-11 derivatives (**36**-**39**).

Modification of artemisinin at C-10 was done by the same research group.[56] (+)-Deoxoartelinic acid (**40**) was synthesized from artemisinic acid (**35**). It was found that compound **40** showed superior antimalarial activity *in vitro* against chloroquine-resistant malaria when compare with artemisinin and artelinic acid and showed higher suppression *in vivo* than either arteether or artelinic acid. Futhermore, (+)-Deoxoartelinic acid (**40**) was 23 times more stable than clinically useful arteether and artelinic acid and was 10 times more stable than artemisinin in simulated stomach acid. In addition, it was four times more stable than artemisinin in water.

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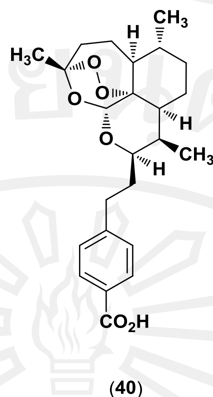

Figure 2.11 Chemical structure of (+)-Deoxoartelinic acid (**40**).

In addition to its antimalarial activity, artemisinin and its derivatives were also found to posses cytotoxicity which inhibited various cancer cell lines such as leukemia, brain cancer, lung cancer, colon cancer, breast cancer, cervical cancer, ovarian cancer and prostate cancer in the nanomolar to micromolar range.[35, 12, 57, 58]

2.7 General knowledge about cancer

Cancer, medically known as a malignant neoplasm, is a large group of different diseases, all involving unregulated cell growth. In cancer, cells divide and grow uncontrollably, forming malignant tumors, then they can affect to function of cells and nearby organs and those organs loses their ability to carry out their normal functions. In addition, cancer cell has spread to other tissues by bloodstream and lymph in the body and invade to other part in the body until lead to death.[59] The name of cancer cell will be called based on where the cancer cell occurs in the body such as lung cancer, cervical cancer and breast cancer.

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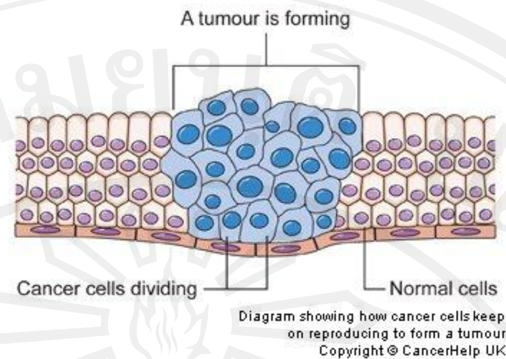

Figure 2.12 Cells divide and grow uncontrollably, forming malignant tumors.

2.8 Treatment of cancer

The treatment of cancer disease can be treated several ways such as operation, irradiation and chemotherapy. The operation and irradiation are the methods that tumor is removed from the body and chemotherapy is the use of anticancer drugs to kill cancers. The treatment usually needs combination of several methods for more effective treatment. The treatment methods are as following[60,61] :

- Surgery is the oldest form of treatment for cancer. Surgery is performed in order to remove the cancerous tumor as well as some of the surrounding tissue and lymph nodes near it.
- Radiation therapy (also called radiotherapy) uses high-energy particles or waves, such as x-rays or gamma rays, to destroy cancer cells.
- Hormone therapy is used against certain cancers that depend on hormones for their growth. This treatment may include the use of drugs that stop the production of certain hormones or that change the way they work. Hormone production or hormone action can also be stopped by the surgical removal of hormone-producing glands.

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- Immunotherapy (also called biological therapy) is the use of treatments that promote or support the body`s immune system response to a disease such as cancer.
- Chemotherapy[62] is the use of anticancer drugs to treat cancerous cells that has been used for many years and it is one of the most common treatment for cancer. The current anticancer regimens are frequently associated with significant levels of toxicity and the emergence of drug resistance. Therefore, one major challenge to relieve cancer burden is to develop highly effective drugs with specificity on cancers but little or no side effects on normal mammalian cells.

2.9 Artemisinin and its derivatives as anticancer agents

Artemisinin has a unique chemical structure that prevents cancer resistance. Cancer cells require extra iron for cell division, hence cancer cells typically absorb a significantly larger amount of iron than normal cells.[63,64,65,66] Then, the active molecule in artemisinin reacts with free iron, releasing highly-reactive free radicals that attack the cancer cell membranes, breaking them apart and killing them.[67] Therefore, mechanism action of artemisinin to cancer cells might be the same mechanism to kill malaria parasites. Moreover, artemisinin and its derivatives kill cancer cells mainly by inducing apoptosis.[12,68,69,70] Artemisinin was found to be most active against leukemia and colon cancer cell lines. Other cancer cell lines tested that indicated some responsiveness to artemisinin’s actions were melanoma, breast, ovarian, prostate, renal and central nervous system cancers.[12]

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A great deal of works was devoted to synthesize artemisinin derivatives to improve the anticancer activity. Mankil *et al*.[71] (1997) synthesized artemisinin and its related trioxanes analogs that have been prepared from artemisinic acid (**35**) to get ethyl deoxoartemisinin (**41a**), n-butyl deoxoartemisinin (**41b**), homo deoxo-artemisinin (**42**) and *N,N*-diethylaminomethylbenzoyl deoxoartemisinin (**43**). These compounds were assayed *in vitro* cytotoxicity and showed a good cytotoxicity against murine leukemia (P-388) and human epidermoid carcinoma (KB).

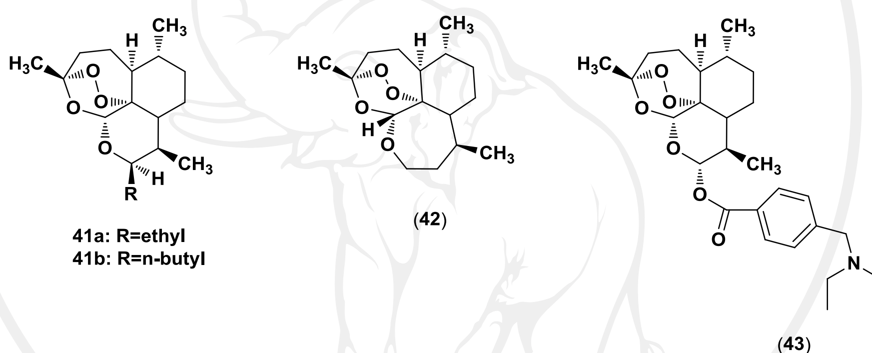

Figure 2.13 Chemical structure of artemisinin-related analogs **41a,b**, **42** and **43**.

Ying *et al*.[57] (2003) synthesized a new type of dihydroartemisinin ether containing cyano and aryl group (**44-45**) as shown in Figure 2.14. These compounds were tested the cytotoxicity against human lung adenocarcinoma epithelial cell line (A549 and P388), mouse lymphocytic leukemia cell line (L1210) and human colon adenocarcinoma cell line (HT-29) using MTT assay. Compound **44c** and **45c** were the most active when compared with compound **44a, 44b, 45a** and **45b** with Br substituent on the phenyl ring. Moreover, compound **46a** showed 1000-fold less activity than compound **46b**. The result indicated that the peroxy group is essential for anticancer activity. Furthermore, the inactive compound **47** was found to be inactive

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to cytotoxicity. The result indicated that the position of cyano groups was also important to cytotoxicity.

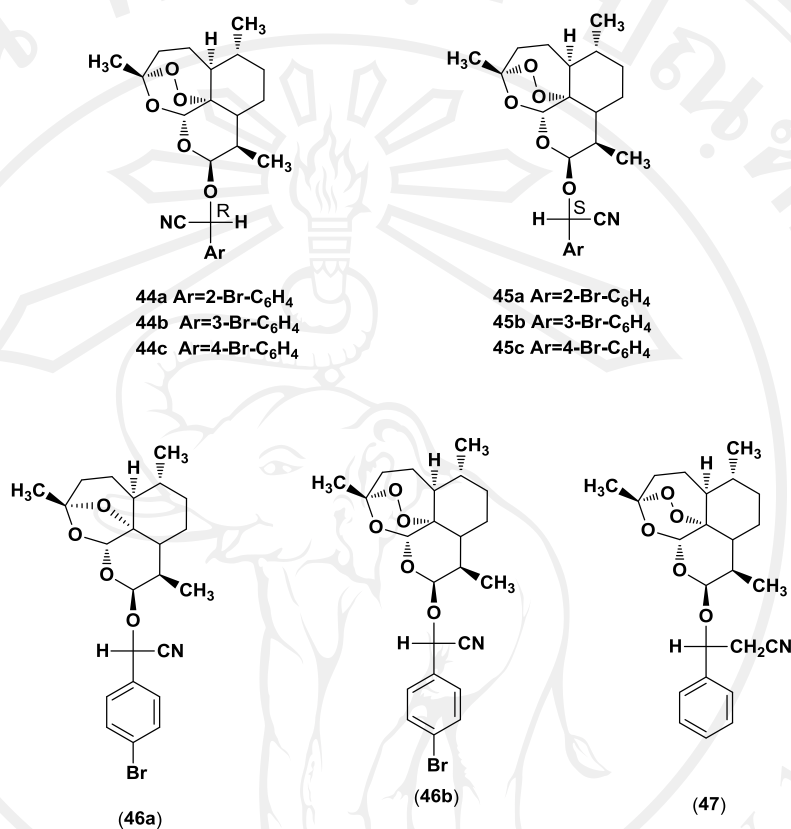

Figure 2.14 Chemical structure of dihydroartemisinin containing CN and Ar group.

Yungen *et al.*[72] (2005) demonstrated the syntheses of amide compounds **48a-i** bearing linear alkyl carbon chains of different length were synthesized (Figure 2.15) and studied for their *in vitro* cytotoxicity against a human hepatocellular carcinoma cell line (HepG2). It was found that the cytotoxicity of artemisinin increased with the length of alkyl substituted side chain of amides. The compound **48g** with $C_{14}H_{29}$ carbon chain exhibited the most potent cytotoxicity (IC_{50} = 0.46 μM) which was almost 200-fold more potent than artemisinin (**9**) (IC_{50} = 97 μM).

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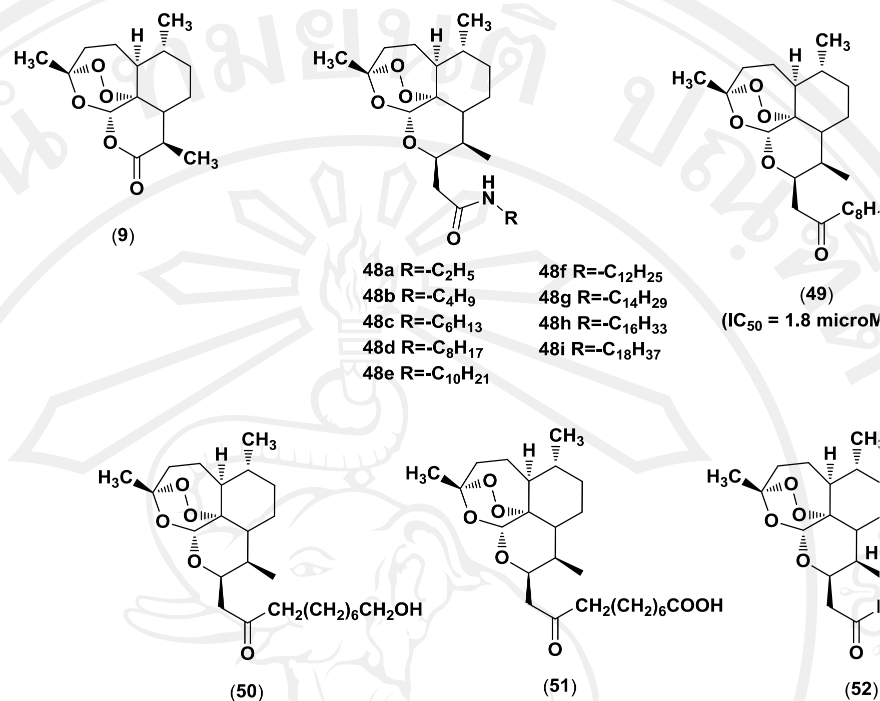

Figure 2.15 Artemisinin derivatives with amide bearing linear alkyl carbon chains.

On the other hand, incorporation of polar hydroxyl and carboxylic group at the terminal ends of carbon chains (**50**, **51** and **52**) showed lower toxicity than methyl analogue **49** (Figure 2.15). These findings indicated that the lipophilic end of the carbon chain is essential for their cytotoxicity. Moreover, the position of polar group on the carbon chain **52** has a significant effect on the cytotoxicity.

Recent reports showed that formation of artemisinin oligomer by connecting artemisinin moiety to each other is particularly effective against malarial parasites and cancer cells. Pras *et al.*[73] (1998) reported that the endoperoxide group is essential to cytotoxicity to human granulocyte/macrophage progenitor cells (CFU-GM) (Figure 2.16). It was found that artemisinin (**9**) was more toxic to CFU-GM cells than deoxyartemisinin (**53**). Moreover, the stereochemistry of the ether linkage of dimers was of importance for the CFU-GM cell-killing activity. The results showed that the

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nonsymmetrical dimer **54** and the symmetrical dimer **55** were respectively 100 times and 7 times more cytotoxic than artemisinin (**9**) while, the nonsymmetrical dimer **54** was more active than the symmetrical dimer **55**

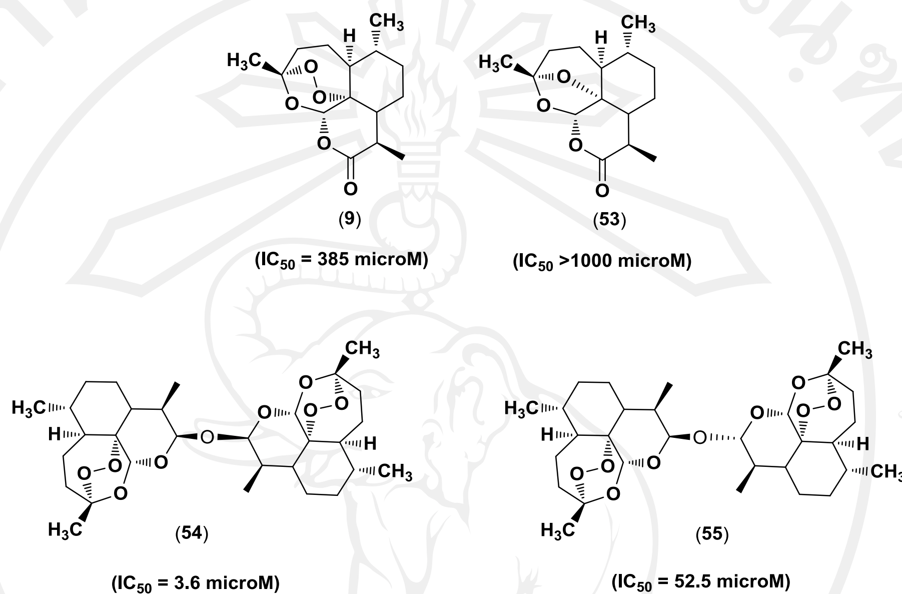

Figure 2.16 Structure of artemisinin and selected derivatives.

Thebtaranonth *et al.*[10] (2001) prepared C-16 derivatives of artemisinin from the reaction of **56** with various types of nucleophile by nucleophilic addition of lithium keto and ester enolates and mono- and bifunctional Grignard reagent to provide C-16 derived artemisinin derivatives, which include artemisinin monomers, dimers, trimers and tetramers as shown in Figure 2.17. The evaluation of these synthesized compounds showed that artemisinin monomers **59**, dimers **60-62**, trimers **63** and tetramers **64** exhibited antimalarial activity better than artemisinin with no or low cytotoxicity against KB and epidermoid carcinoma of the mouth cell line (BC).

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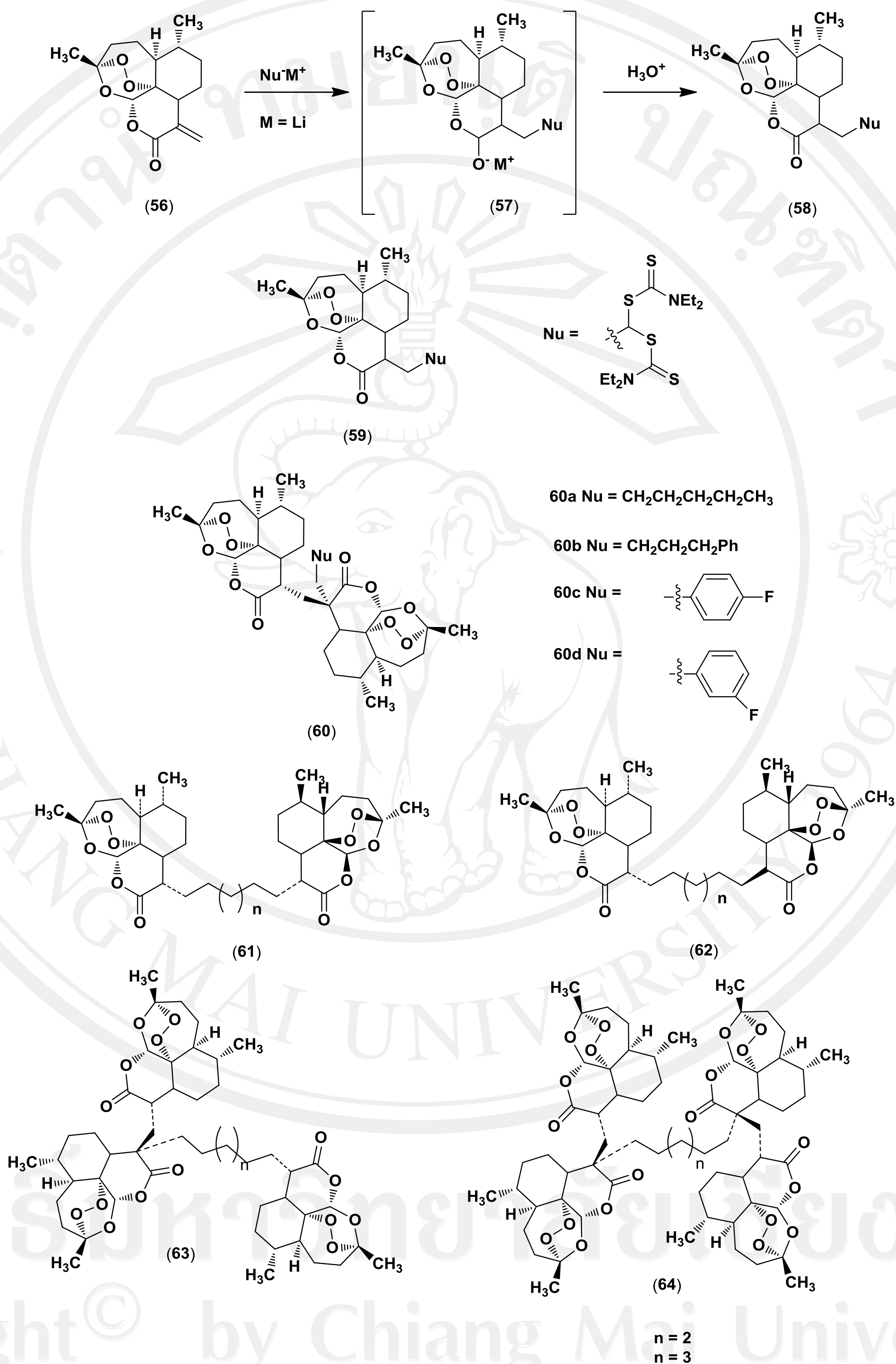

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Figure 2.17 C-16 derived artemisinin monomers, dimers, trimers and tetramers.

Jung *et al.*[74] (2003) prepared C-10 non-acetal type derivatives of deoxo-artemisinin monomers (**65** and **66**), dimers (**67** and **68**) and trimer (**69**) from the first primary amines intermediate **65** and bromoalkyl analogues intermediates **66** as shown in Figure 2.18. The results showed that aminobutyldeoxoartemisinin **65b**, amide-linked dimer **67**, sulfide-linked dimer **68a**, sulfone-linked dimer **68b** and trimer **69** were highly active against human breast adenocarcinoma cell line (MCF7).

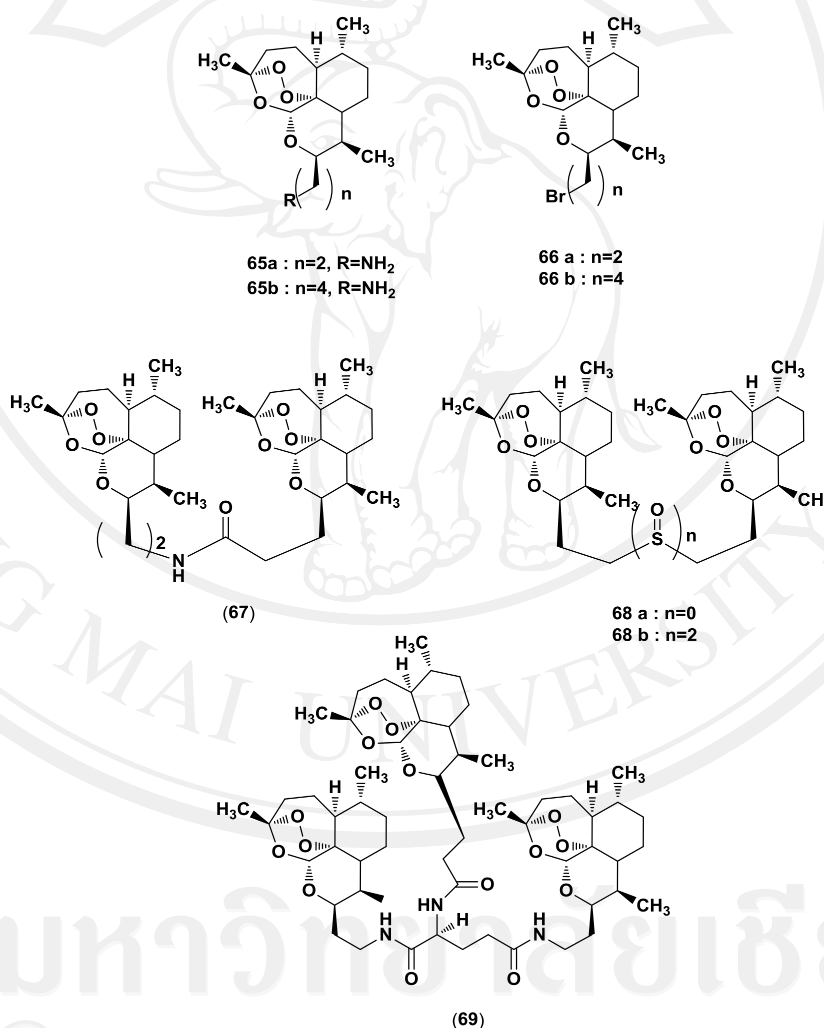

Figure 2.18 C-10 non-acetal type derivatives of deoxoartemisinin as monomers (**65**), (**66**), dimers (**67, 68a,b**) and one trimer (**69**).

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Further, O'Neill *et al.*[75] (2004) observed that phosphate ester linker plays a crucial role in imparting potent anticancer activity. A new series of non-acetal C-10 carba dimers **70-73** were synthesized and examined for antimalarial and antitumor activities (Figure 2.19). The results showed that all of the dimers display potent low nanomolar antimalarial activity versus the K1 and HB3 strains of *Plasmodium falciparum.* In contrast to their potent activity versus malaria parasites, virtually all of the dimers expressed poor anticancer activity apart from the trioxane phosphate ester dimers **73a** and **73b**, which expressed nanomolar growth inhibitory (GI_{50}) values versus a range of cancer cell lines in the NCI 60 human cancer cell lines.

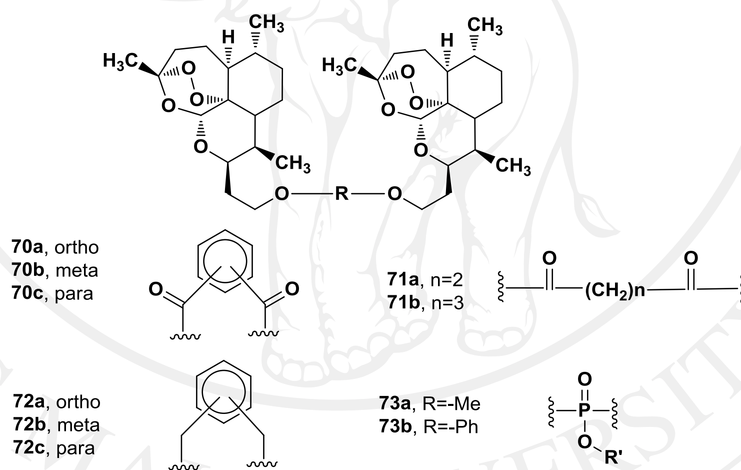

Figure 2.19 A new series of non-acetal C-10 carba trioxane analogues of dimers (**70a-70c, 71a, 71b, 72a-72c, 73a, 73b**).

Paik *et al.*[76] (2006) also reported the design synthesis of a new series of hydrolytically stable, C-10 non-acetal dimer **74-79** as shown in Figure 2.20. It was found that bis-benzyl alcohol dimer **77** is the most potent against malaria parasites which was 10 times more potent than artemisinin (**9**). The phthalate dimer **75** also showed anticancer activity against lung cancer cell line (HOP-92), human skin

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melanoma cell line (SK-MEL-5) and human breast carcinoma cell line (BT-549). Moreover, both of dimer **75** and dimer **77** are not cytotoxic toward several human cancer cell lines such as human cervival cancer cells.

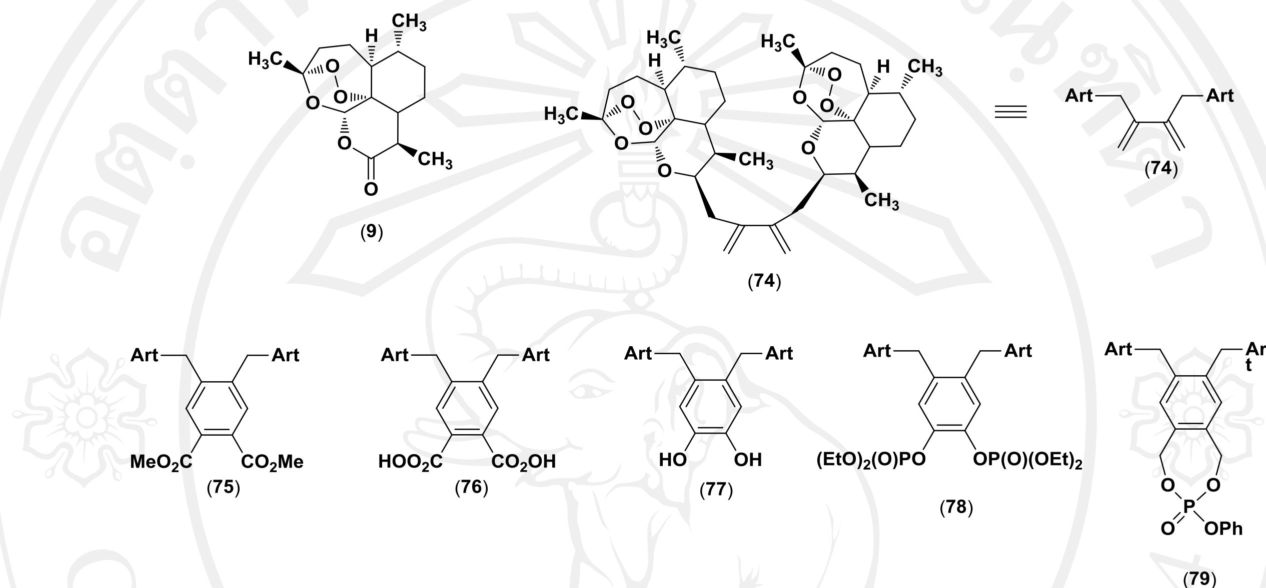

Figure 2.20 The new series of hydrolytically stable, C-10 non-acetal dimers **74-79**.

Chadwick *et al.*[77] (2009) prepared a series of C-10 carba-linkage dimers **80-83** to explore the effects of the nature and length of linker upon biological activity as shown in Figure 2.21. The C-10 carba artemisinin dimers with amide **80**, carbonate **81a**, urea **81b**, 4,4'-bipiperidine-linker **82** and phosphonate-linked dimer **83** were synthesized and evaluated the antimalarial and anticancer activities.

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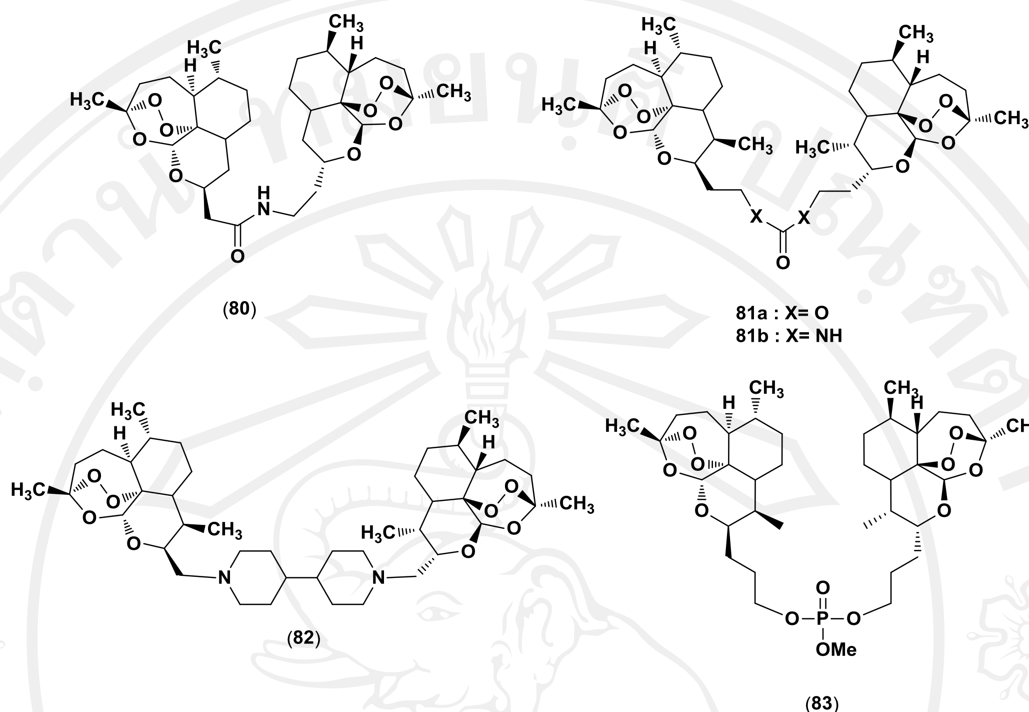

Figure 2.21 The C-10 carba artemisinin dimers with amide **80**, carbonate **81a**, urea **81b** and 4,4'-bipiperidine-linker **82** and phosphonate-linked dimer **83**.

It was revealed that amide-linked dimer **80** was the most potent compound assayed against 3D7 *P. falciparum*. In addition, methyl phosphate dimer **83** was the most potent of the compounds assayed against human promyelocytic leukemia cell line (HL-60). The further study (2010)[78], N^1-, N^4-, N^8-artemisinin-spermidine conjugated **84-86** and N^1-, N^4-, N^8- amide linked conjugates **87-89** were prepared and evaluated for anticancer activity against HL-60 cancer cells and antimalarial activity against chloroquine-sensitive 3D7 *P. falciparum* as shown in Figure 2.22. The most potent compounds were the terminally substituted amine-linked and amide-linked Boc precursors (**84, 86, 87** and **89**).

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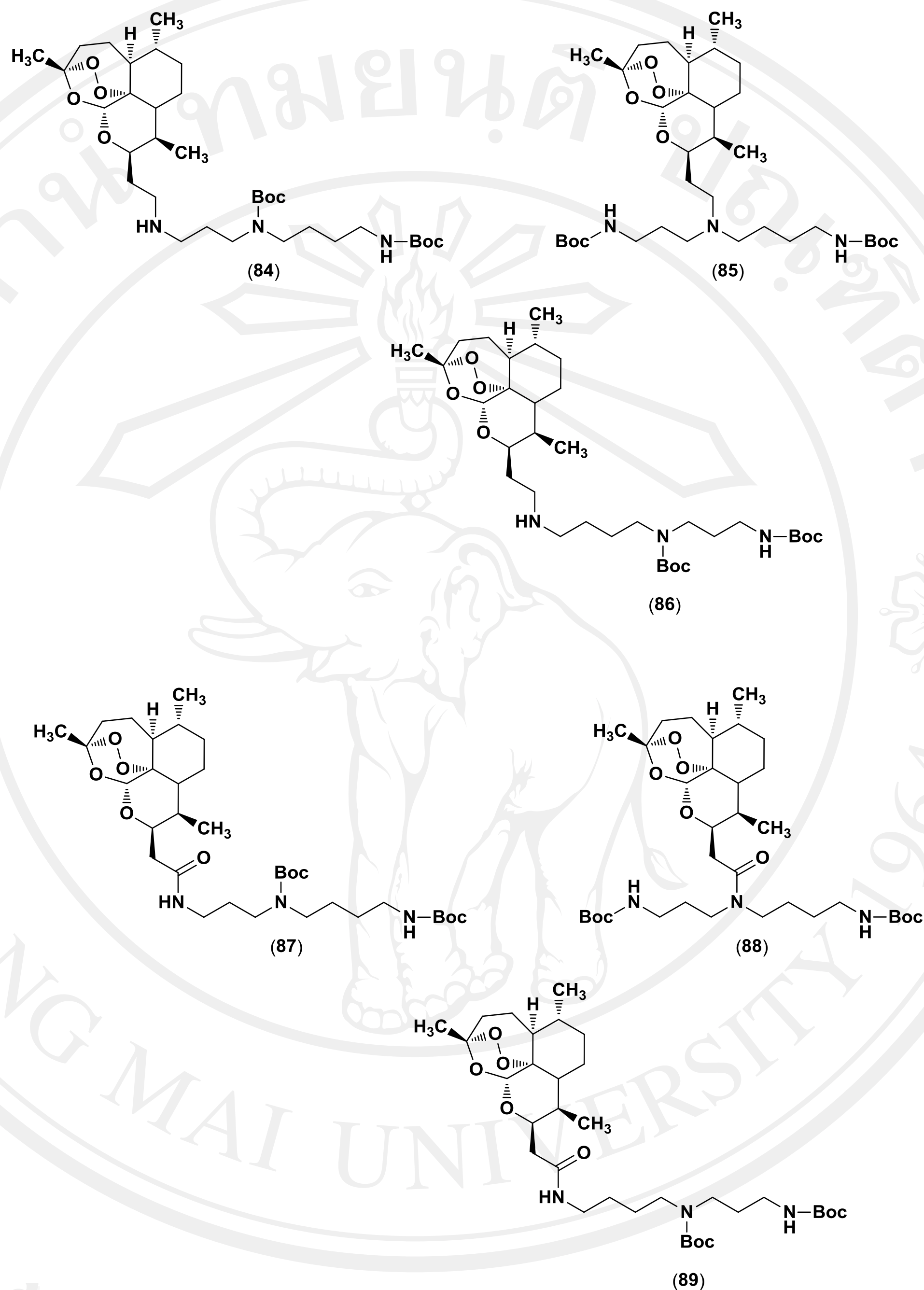

Figure 2.22 N^1-, N^4- and N^8- artemisinin-spermidine conjugated **84-89**.

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2.10 Peptide Nucleic Acid

Peptide Nucleic Acid (PNA) is an achiral and uncharged nucleic acid analogue constructed from a polyamide backbone composed of *N*-(2-aminoethyl) glycine to which the nucleobase are attached by a methylene carbonyl group (Figure 2.23).[79] It was first introduced by Peter E. Nielson (Univ. Copenhagen) in 1991.[80] Unlike DNA or DNA analogs, PNAs do not contain sugar moieties or phosphate groups. It was therefore a surprise that PNA in many respects mimiced the behavior of DNA, and in some applications demonstrated superior properties. By convention, PNAs are depicted like peptides, with the N-terminus on the first (left) position and the C-terminus on the right. The remarkable properties of PNA are[81,82] :

- The binding between PNA/DNA strands is stronger than between DNA/DNA strands due to the lack of electrostatic repulsion because PNA backbone is uncharged.
- PNA oligomers shows greater specificity in binding to complementary DNAs, with a PNA/DNA base mismatch being more destabilizing than a similar mismatch in a DNA/DNA duplex.
- PNA possess a remarkable chemical stability and is neither degraded by nucleases nor proteases.
- PNA are stable over a wide pH range.

Because of these properties, PNA oligomers have great potential as therapeutic agents, diagnostic tools and probes in molecular biology.

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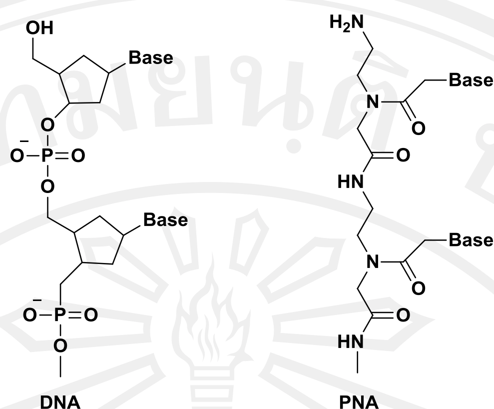

Figure 2.23 Comparison structure of PNA and DNA.

A large number of chemical modifications of original amino ethylglycine PNA backbone have been reported. For example, Woski *et al.*[83] (1999) prepared the *N*-(2-aminoethyl) glycine backbone unit of PNA with pyreneacetic acid (**93**) and acetic acid moieties to produce 9-fluorenylmethoxycarbonyl (Fmoc)-protected PNA monomers (**91, 92**) for the synthesis of potential polyintercalators (**94**) (Figure 2.24).

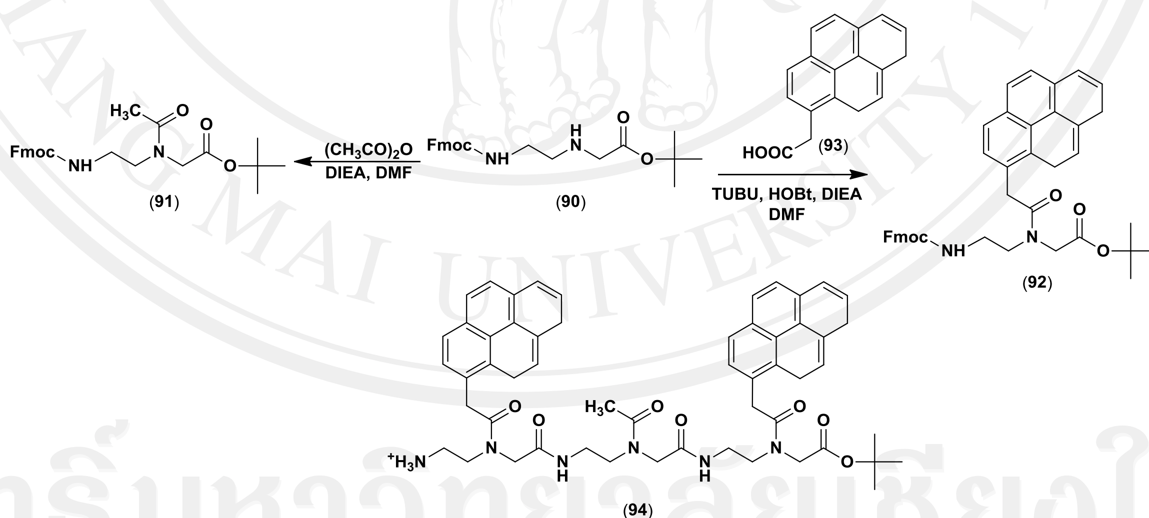

Figure 2.24 Synthesis of Fmoc-Py-*t*Bu (**92**), Fmoc-Ac-*t*Bu (**91**) and intercalator **94**.

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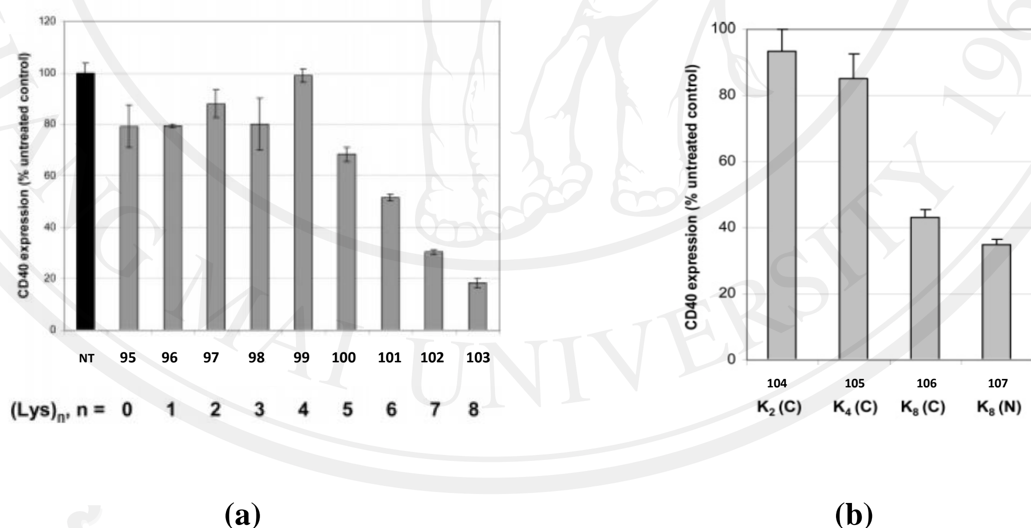
Introducing a few lysine-based monomer into a PNA oligomer has been demonstrated to gently increase the aqueous solubility. A number of basic amino acids of PNA-peptide conjugates and C- vs N- terminal position of the peptide were evaluated for inhibition of CD40 expression in mouse B cell lymphoma cell line (BCL_1).[84] The PNA conjugated carrying hexa-, hepta- and octa lysine residues at the N-terminus showed higher activity than the parent compound **95**, while activity of conjugates which carrying 1-5 lysines showed no significantly increase (Figure 2.25a). In addition, the oligo (*L*-lysine) peptide with two (**104**), four (**105**) and eight lysine (**106**) attached to the C-terminal end of PNA exhibited slightly lower activity than eight lysine attached to the N-terminal end of PNA analogue (**107**) (Figure 2.25b).



(a) **(b)**

Figure 2.25 (a) Effect of the number of N-terminal lysine residues on the reduction of CD40 expression in mouse BCL1 cells (b) Effect of both C and N terminally conjugated *L*-lysine peptides on the inhibition of CD40 expression.

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Rios *et al.*[85] (2009) designed and synthesized ultra-short peptides with various amino acid such as isoleucine, valine, proline, phenylalanine, lysine and ornithine amino acid. These peptides were analyzed the influence of the amphiphatic character of ultra-short peptides on antimalarial activity. These results indicated that tetrapeptides Phe-Orn-Phe-Orn (**108**) and Lys-Phe-Phe-Orn (**109**) showed antimalarial activity with IC_{50} values of 3.31 and 2.57 μM, respectively.

Ryszard *et al.*[86] (2001) demonstrated that the effect of the PNA oligomers were observed to be dependent on the number of *L*-lysine (Lys) residue at the C-terminus. It was suggested that the PNA containing Lys was taken up by a mechanism similar to that of cell-penetrating homeodomain proteins and that the Lys tail enhanced intracellular accumulation of PNA oligomer without affecting its ability to reach and hybridize to three target sequence (Table 2.1). The result showed that the PNA containing four Lys residue (PNA-4) (**112**) was the most effective in generating EGFP fluorescence in treat cells : its EC_{50} (2.1 μM) was almost 2.5 times lower than that of PNA-1 (**110**) (4.7 μM).

Table 2.1 Antisense activities of PNA containing Lys.

Oligomers	Sequence 5' → 3'	Target site	EC_{50} (μM)
PNA-1 (**110**)	H-GCT ATT ACC TTA ACC CAG-Lys-NH_2	654	4.7 ± 0.6
PNA-2 (**111**)	H-GCT ATT ACC TTA ACC CAG-$(Lys)_2$-NH_2	654	3.3 ± 0.4
PNA-4 (**112**)	H-GCT ATT ACC TTA ACC CAG-$(Lys)_4$-NH_2	654	2.1 ± 0.2

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2.11 Other oligopeptides and their biological activities.

It is known that peptides play central roles as regulators of nearly all vital processes. The advantages natural oligopeptides can offer as drugs including specificity, potency, and low toxicity. However, the major disadvantages of *in vivo* usage of whole natural oligopeptides and protein are low material availability, relatively high cost, high molecular weight, enzymatic degradation and sterilization problem. On the other hand, synthetic small peptides have several advantages over natural oligopeptides and small molecular drugs due to their ease of synthesis and their handling convenience. It is also regarded as the ideal lead compound for the development of antifungal reagents and insecticides.[87] Dipeptide derivatives also have been recognized for decades and showed diverse biological activities, including anti-inflammatory, antinoceptive, antimalarial.[88,89] For instance, peptides incorporating *d*-amino acids in nature were found to be antimicrobial compounds and signal molecules. For examples, Alitame, *L*-Asp-*D*-Ala fenchyl ester, is known to be sweetener.[87] Cyclo(*D*-Pro-*L*-Arg) can act as a specific inhibitor of family chitinase. Recent studies also showed that certain peptides containing phenylalanine, serine, or alanine residues have shown antitumor activity by enhancing nucleic acid or protein metabolism.[90]

Stewart *et al.*[91] (2009) also reported that Fmoc-OC2Y-Atmp with bulky and significantly hydrophobic O-(2,6-dichloro-benzyl)-tyrosine (**113**) showed a remarkable high growth inhibition of PC3 prostate cancer (55-78%) that was higher than cisplatin (39%). These results suggested that Fmoc-amino acid amide derivatives may be worth therapeutic agents for cancer treatment.

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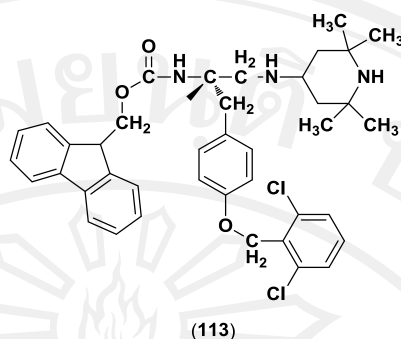

Figure 2.26 Structure of Fmoc-amino acid amides.

Quite recently, the studies of the cytotoxicity of Fmoc dipeptide analogs with a hydroxyl group have been reported by Yang-Chang *et al*.[92] The results revealed that Fmoc dipeptide **114**, **115**, **116, 117**, **118**, **119**, **120**, **121** and **122** showed potent activity against human liver hepatocellular carcinoma cell line (HepG2, Hep3B), human breast adenocarcinoma cell line (MCF-7 and MDA-MB-231), human gingival carcinoma cell line (Ca9-22) and human lung carcinoma cell line (A549) (Figure 2.27). Additionally, compound **123** was three fold more potent than doxorubicin against the Ca9-22 cell line. Furthermore, compound **123** showed a synergistic effect and increased the cytotoxicity of doxorubicin against the human breast cancer cell line (MDA-MB-231).

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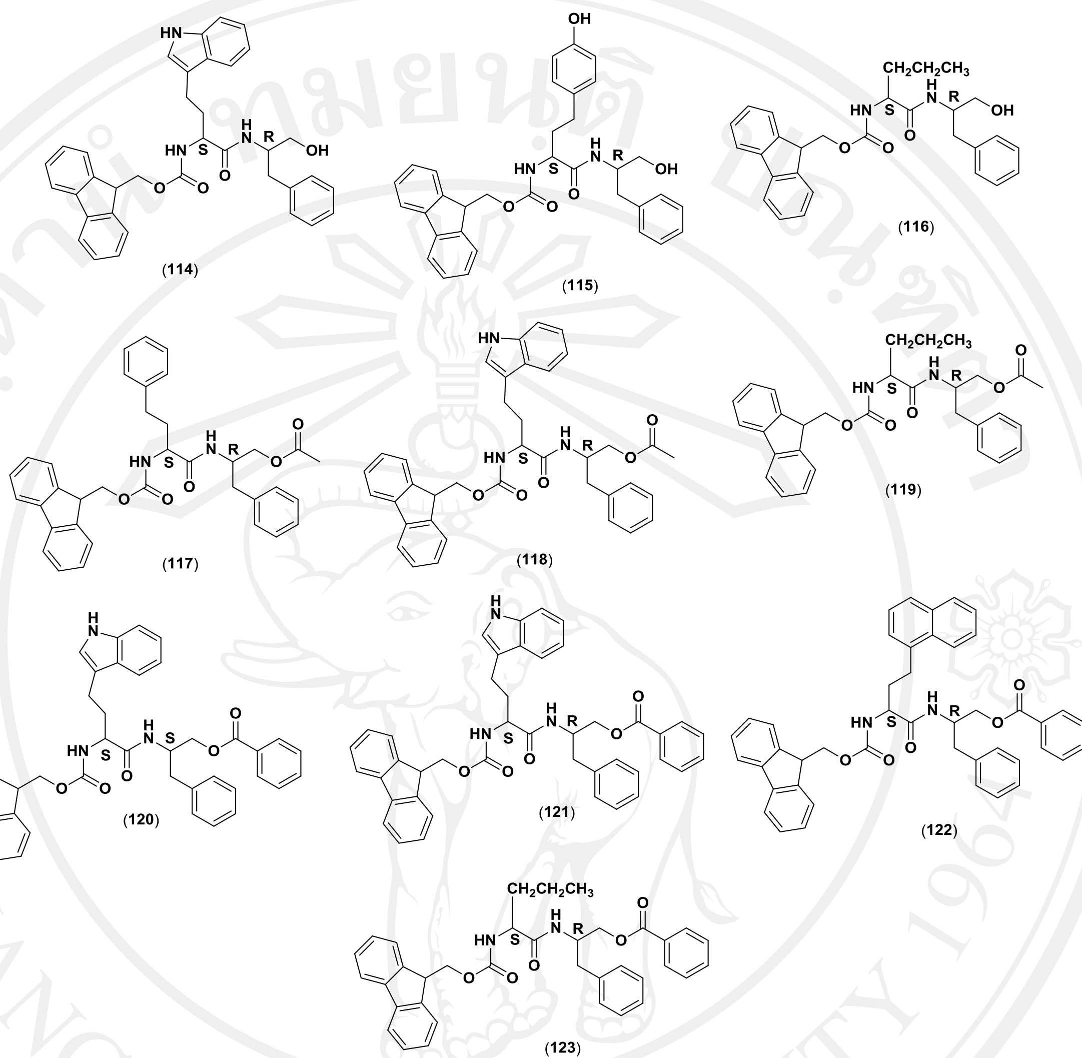

Figure 2.27 Structure of Fmoc-acetate dipeptides (**117**-**119**), Fmoc-alcohol dipeptides (**114**-**116**) and Fmoc-benzoate dipeptides (**120**-**123**).

In addition, cyclization of appropriate oligopeptides to give cyclic peptide can enhance the biological stability by stabilize the conformation suitable for better binding to receptor and improved biological activity. Maryna *et al*.[93] (2004) demonstrated that cyclo(Phe-Pro) (**124**) inhibited the growth of HT-29, MCF-7 and human epithelial carcinoma cell line (HeLa) and induced apoptosis in HT-29 colon cancer cells, suggesting a potential antitumor activity as shown in Figure 2.28.

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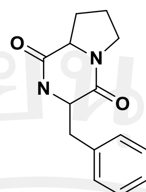

Figure 2.28 Chemical structures of cyclo(Phe-Pro) (**124**).

In recent years, the cytotoxicity of cyclodecapeptides such as cyclo(Lys-Lys-Leu-Leu-Lys-Phe-Lys-Lys-Leu-GIn) (**125**), cyclo(Leu-Lys-Leu-Lys-Lys-Phe-Lys-Lys-Leu-GIn) (**126**), cyclo(Leu-Leu-Lys-Lys-Lys-Phe-Lys-Lys-Leu-GIn) (**127**) and cyclo(Lys-Lys-Leu-Lys-Lys-Phe-Lys-Lys-Leu-GIn) (**128**) against human carcinoma cell lines have been reported.[94] The cyclodecapeptide **125**-**128** displayed specificity and high cytotoxicity against HeLa cells with IC_{50} 22.5-38.5 μM and showed low hemolytic activity, low cytotoxicity to non-malignant fibroblasts, high stability to protease degradation and inducing apoptosis in human cancer cells.

These findings lead us to design, synthesize and explore the biological activities of linear deoxoartemisinin pseudo peptide nucleic acid oligomers and cyclic deoxoartemisinin pseudo peptide nucleic acid oligomers.

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