จุฬาลงกรณ์มหาวิทยาลัย
ทุนวิจัย
กองทุนรัชดาภิเษกสมโภช

รายงานวิจัย

ความสัมพันธ์ระหว่างฤทธิ์ทางชีวภาพกับสมบัติโมเลกุลของสารกลุ่มอาร์ทีมิซินิน

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

โดย

ศิริรัตน์ ก๊กผล

CU
วท 15
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พฤศจิกายน ๒๕๔๔

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ศิริรัตน์ ก๊กผล

พฤศจิกายน 2544

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โครงการ...

ชื่อโครงการวิจัย	ความสัมพันธ์ระหว่างฤทธิ์ทางชีวภาพกับสมบัติโมเลกุลของสารกลุ่มอาร์ทีมิซินิน
ชื่อผู้ทำวิจัย	รศ. ดร. ศิริรัตน์ ก๊กผล
เดือนและปีที่ทำวิจัยเสร็จ	พฤศจิกายน 2544

ทำการศึกษาหาความสัมพันธ์ในเชิงปริมาณระหว่างฤทธิ์ทางชีวภาพกับสมบัติโมเลกุลของสารต้านมาลาเรียกลุ่มอาร์ทีมิซินินจำนวน 68 สาร โดยปรับโครงสร้างของสารทั้งหมดด้วยวิธี HF/3-21G และทำการคำนวณสมบัติโมเลกุลจากโครงสร้างที่ทำการปรับแล้วจำนวนทั้งสิ้น 102 สมบัติ ซึ่งครอบคลุมสมบัติของสภาพไฮโดรโฟบิก สภาพโพลาไรซ์ อิเล็กทรอนิคส์ และสเตอริก นำข้อมูลฤทธิ์ทางชีวภาพของสารมาจากเอกสารอ้างอิง ซึ่งเป็นการทดสอบฤทธิ์ทางชีวภาพกับเชื้อมาลาเรีย 2 สายพันธุ์คือ D-6 และ W-2 และข้อมูลสมบัติโมเกลุกลที่ได้จากการคำนวณนี้มาทำการวิเคราะห์ทางสถิติเพื่อหาความสัมพันธ์ ได้แบบจำลองที่มีประสิทธิภาพในการทำนายที่ดีถึงดีมากสำหรับฤทธิ์ทางชีวภาพทั้งสองสายพันธุ์ แบบจำลองเหล่านี้สามารถใช้ทำนายฤทธิ์ทางชีวภาพของสารในกลุ่มทดสอบได้ผลดี โดยให้ค่าที่ใกล้เคียงกับค่าจากการทดลอง นอกจากนี้ แบบจำลองยังให้ข้อมูลเกี่ยวกับการปรับเปลี่ยนโครงสร้างเพื่อทำให้ฤทธิ์ทางชีวภาพสูงขึ้น

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เลขหมู่ CU วท 15
เลขทะเบียน 012368
วัน, เดือน, ปี 3 ต.ค. 48

Project Title	Relationships between biological activity and molecular properties of artemisinin compounds
Name of the Investigator	Assoc. Prof. Dr. Sirirat Kokpol
Year	November 2001

Relationships between biological activity and molecular properties of 68 antimalarial artemisinin compounds were investigated in the quantitative manner. All compounds were geometrically optimized at the HF/3-21G level. Totally 102 molecular properties covering hydrophobicity, polarizability, electronic, and steric parameters were calculated from the optimized structures. The activities against 2 different strains of malarial parasites, D-6 and W-2, were taken from the literatures. Statistical analyses were performed to find the relationships between the activities and the calculated molecular properties. Models with good to excellent predictive ability were obtained for both strains. The models were shown to predict activities of compounds in the testing set very close to the experimental values. The models also supply information on how to modify the structure to enhance the antimalarial activities.

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CHAPTER 1
Introduction

1.1 Malaria

Malaria is one of the most widespread and prevalent endemic diseases, which threatens approximately 40 percent of the world's population in more than 90 countries. This disease is estimated to cause approximately 300 to 500 million illnesses and 1.5 to 2.7 million deaths each year [1-3]. In Thailand, malaria is found mostly in the west region and approximately 100,000 people are infected with this disease and around 800 people die from the disease annually [4].

Malaria is caused by parasitic protozoa in the genus *Plasmodium*. There are some 100 species of these protozoa but only four are responsible for the disease in humans; *P. falciparum*, *P. malariae*, *P. vivax*, and *P. ovale*. Among these four species, *P. falciparum* is the most dangerous and life-threatening. It produces cerebral and severe disease, which is often fatal if left untreated in non-immune individuals [5]. The last three species produce the mild forms of malaria by destroying red blood cells in peripheral capillaries and thus causing anemia. Humans are affected by inoculation with infected blood or by a bite of infected female *Anopheles* mosquito. Malaria symptoms usually develop 10 to 35 days after a person was infected. Frequently, the first symptoms are a mild fever, headache, muscle aches, and chills, together with a general feeling of illness. Sometimes symptoms begin with shaking chills followed by fever. These symptoms last 2 or 3 days and are very similar to those of the flu. Subsequent symptoms and patterns of disease vary among the four types of malaria.

Malaria can normally be cured by antimalarial drugs. The symptoms quickly disappear once the parasites are killed. If the person is untreated, the symptoms of vivax, ovale, or malariae malaria subside spontaneously in 10 to 30 days but may recur at variable intervals. Untreated falciparum malaria is fatal in up to 20 percent of patients.

Malaria control strategy changes with time. In the past, the main method was the eradication of mosquito, a vector of the disease. However, the resistance of vectors to pesticide is the major obstacle for this method. At the present, the strategy changes to

malaria prophylaxis, such as the use of insecticide-treated nets, together with the prompt treatment for malaria patients with antimalarial drugs. Researchers worldwide are currently investigating and developing new ways for malaria control. Promising projects are malaria vaccine, malaria genome project, and transgenic mosquito.

1.2 Antimalarial Drugs

In the ancient time, the bark of the cinchona tree had been used in the form of a powder to treat the chills and other symptoms associated with malaria. In 1820 Pierre Pelletier and Joseph Caventou isolated quinine from the bark. The quinine (Figure 1.1a) was found to be more palatable than the nauseating powder of cinchona bark [6]. Since then it became the main treatment for malaria. However, during the World War I (1914-1918) the normal supplies of quinine become unavailable. As a result, an extensive research program on synthetic antimalarials was settled in Germany.

From this extensive research program, pamaquine, a synthetic 8-aminoquinoline derivative, was synthesized in 1920s. A few years later, quinacrine or mepacrine was also developed and introduced for malarial therapy as a synthetic alternative to quinine. However, the quinine was still the chief antimalarial drug. During the World War II (1939-1945) quinine was again no longer available to the Allies since the Japanese cut off the supply of cinchona bark from Java. Therefore, quinacrine became the official drug for the treatment of malaria. But its toxicity and inability to cure benign tertian malaria, *P. vivax* malaria, or to act as a true causal prophylactic stopped its use and made it as an obsolete antimalarial drug. In order to search for more active compounds, some extensive antimalarial research programs were established in many countries such as the United States and the United Kingdom.

From the investigation on a large number of 4-aminoquinoline derivatives since 1941 by the cooperative program of antimalarial research in the United States, chloroquine (Figure 1.1b) was found to be a very effective drug, which has fewer side effects and does not turn the patient yellow [7]. However, it was just recognized that the compound had been synthesized and studied as early as 1934 under the name Resochin by the German scientists. Another successful drug, primaquine (Figure 1.1c), was found during the exhaustive search for more potent and less toxic compound than the pamaquine. The primaquine is particularly effective against *P. vivax*, the cause of benign tertian fever.

With the knowledge that pyrimidine compounds are of importance in the cell metabolism, the investigations on a large series of its derivatives were conducted under the antimalarial research program in the United Kingdom. This led to the discovery of proguanil or chloroguanide, a biguanidine compound, in 1945 [8]. The compound was proved to be an outstanding causal prophylactic agent for falciparum malaria and a satisfactory suppressive for vivax malaria. The investigations were further studied in the early 1950s by the joint research team of British and American. Pyrimethamine (Figure 1.1d) was then developed in 1951 [9]. Its antimalarial effects are identical to those of proguanil but, however, its potency is considerably greater. Undoubtedly, this is owing to the fact that it acts directly and its half-life is much longer than that of the active metabolite of proguanil. Therefore, pyrimethamine has been used widely for prophylaxis and suppression.

In the early 1960s the chloroquine-resistant strains of *P. falciparum* were reported in South America and South East Asia [10]. This prompted the U.S. Army to develop new effective antimalarial drugs. Resulting from the screening of about 300 of 4-quinolinemethanol derivatives since 1963, mefloquine (Figure 1.1e) displayed high activity against the chloroquine- and the pyrimethamine-resistant strains [11]. Due to its highly effectiveness, mefloquine was widespreadingly used since the late 1970s. However, the resistance to this drug since the early 1980s and its severe side effects [12] reduced its use.

The developments of parasite resistant strains to most common chemotherapeutic agents, e.g., chloroquine, quinine, sulfa/pyrimethanine combination, and mefloquine, have been reported in many parts of the world [13-15]. These made the malaria situation became serious once again. However, the Chinese discovery of a new and potentially valuable artemisinin could remit the situation since this drug and its derivatives are very effective against the drug-resistant strains of malaria.

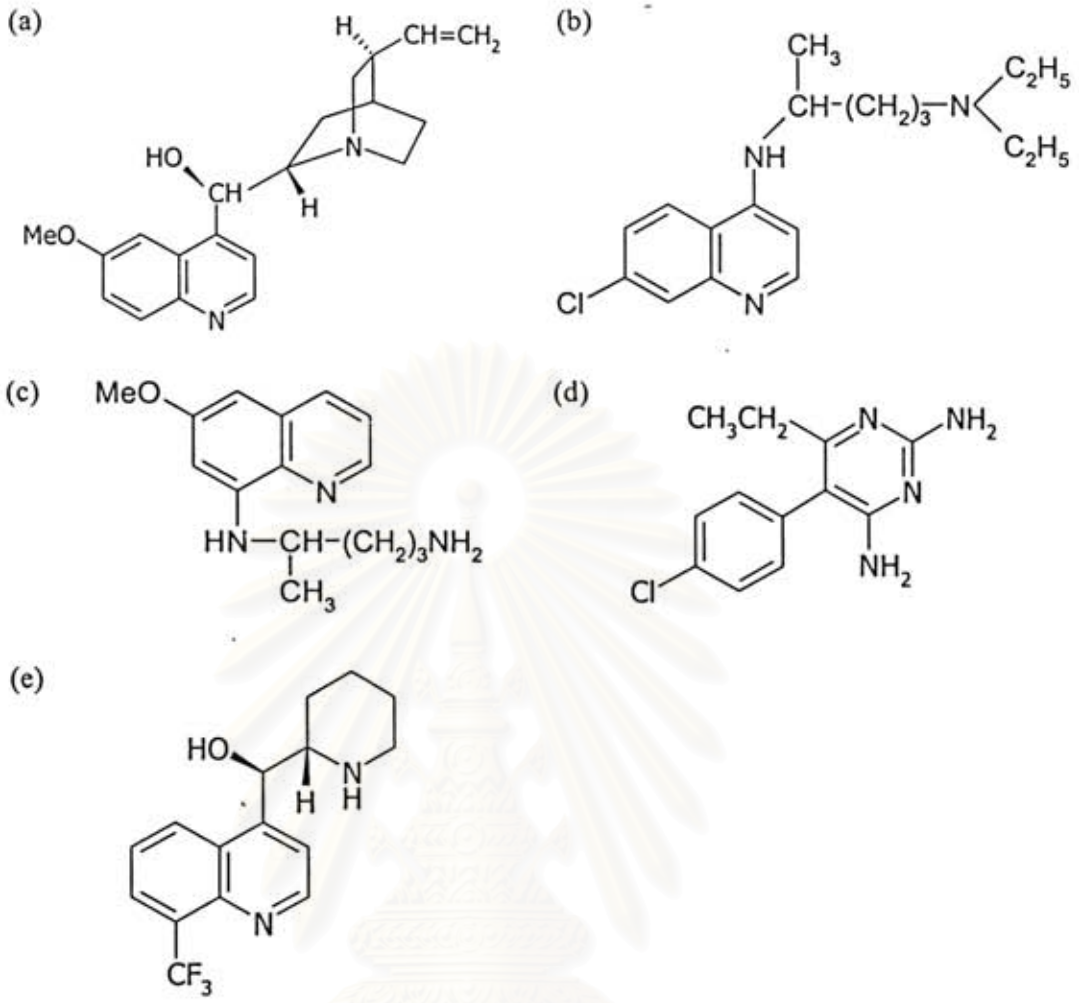

Figure 1.1 Structures of antimalarial drugs (a) quinine, (b) chloroquine, (c) primaquine, (d) pyrimethamine, (e) mefloquine

1.3 Artemisinin and its derivatives

1.3.1 Historical Outlines

The medicinal herb qinghao (*Artemisia annua* L., sweet wormwood, annual wormwood), a native plant in China, has been used as a remedy for fever in China since the ancient time. However, the first record was found in the treatise "Fifty-two Prescriptions" discovered in the Mawangdui Han Dynasty Tomb of 168 B.C. It was also recorded in the "Shennong Bencao Jing" published in the 1-2 century A.D. Nevertheless, its use as antimalarial agent was first mentioned in 341 A.D. by Ge Hong in the handbook of prescriptions for emergency treatments, "Zhouhou Bei Ji Fang". The next evidence was discovered in the "Bencao Gangmu" (compendium of materia

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medica) written by Li Shizhen, the famous herbalist, in 1596 A.D. Also, qinghao decoction was noted in the "Wenbing Tiaobian" for malaria treatment in 1798 A.D. Qinghao was prescribed in many ways, such as socked water, decoction, pill, and powder.

In 1967 the government of the People's Republic of China launched a systematic examination of indigenous plants used in traditional remedies as sources of drugs. By 1971, the crude extraction of qinghao with ethyl ether was shown to be highly effective in mice and simian malaria. Further investigation led to the isolation of an effective antimalarial compound from the aerial portions of the plant in 1972 [16]. The compound was named "qinghaosu", which means "active principle of qinghao", and the more Western sounding name, "artemisinine". However, the "-ine" suffix normally suggests the alkaloid or amine structures, may led to misunderstanding of its terpene structure. Therefore, the name "artemisinin" is preferred and used by the Chemical Abstracts.

1.3.2 Chemical Structure

Many experimental works have been done to elucidate the chemical structure of artemisinin. The compound, a colorless needle crystal, has a melting point of 156-157 ^{0}C. High resolution mass spectroscopy data (m/e = 282.1742, M^{+}) together with elemental analysis data (C = 63.72% and H = 7.86%) revealed the empirical formula of $C_{15}H_{22}O_5$, which suggested a sesquiterpene structure [17]. The absorption peaks in the IR region at 1745 cm^{-1} (strong, delta-lactone) and at 722, 831, 881, 1115 cm^{-1} (peroxide) were observed [17]. The ^{1}H-NMR and ^{13}C-NMR spectra indicated the presence of three methyl groups (one tertiary and two secondary), an acetal function, and several kinds of aliphatic carbon atoms. Qualitative and quantitative reaction experiments verified the presence of the lactone and peroxy-group. Later, its absolute structure was investigated by the X-ray diffraction [16] and was reconfirmed again [18-19]. The exact chemical name was assigned as octahydro-3,6,9-trimethyl-3,12-epoxy-12H-pyrano-[4,3-j]-1,2-benzodioxepin-10(3H)-one. Its stereochemistry and atomic numbering scheme according to the IUPAC is shown in Figure 1.2.

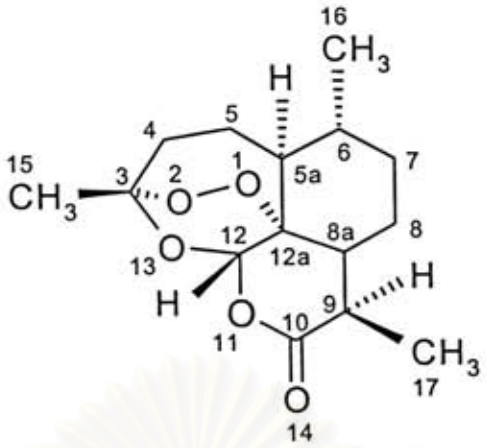


Figure 1.2 Stereochemistry and atomic numbering scheme of artemisinin.

1.3.3 Antimalarial Activity

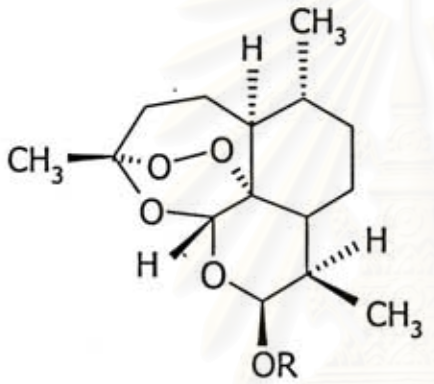
From early *in vitro* experiments, artemisinin has potency comparable to chloroquine and mefloquine [20-21]. Moreover, it was shown to be effective against the chloroquine-resistant strains of *P. falciparum*. *In vivo* experiments in mice, chickens, and monkeys also shown that clearance of parasitemia could be accomplished by the administration of artemisinin. However, the high recrudescence rate of parasitemia (up to 70%) was found [16].

In human, the clinical cures of 1,511 patients with *P. vivax* and of 588 patients with *P. falciparum* during 1973-1978 were reported by the qinghaosu antimalaria coordinating research group, China in 1979 [16]. In addition, 143 cases of chloroquine-resistant falciparum malaria and 141 cases of cerebral malaria were treated with good results. Artemisinin were administrated in four different dosage forms: tablets, oil, oil suspension, and water suspension. In *P. vivax* patients, the order of rapidity in parasite clearance is tablets > oil > oil suspension > water suspension. In *P. falciparum* patients, the oil form was the most rapid acting.

Artemisinin is a rapid acting drug as indicated by the parasite clearance time of about 40 hours (in tablet form) compared with 56 hours of the chloroquine in *P. vivax* malaria [16]. The drug was considered safe in normal patients and also in patients complicated by heart, liver, and renal diseases of pregnancy. Neither obvious adverse reactions nor noticeable side effects were seen during the treatment. Unfortunately, a high rate of recrudescence is its main problem [22]. In addition, the drawbacks are also contributed from its insolubility in both water and oil [23], its poor efficacy by oral

administration [24], and short plasma half-life [22]. However, there exist solutions for all these problems.

In order to solve the problem of its poor solubility, some more soluble derivatives were synthesized [23], e.g., dihydroartemisinin (Figure 1.3a), artemether (Figure 1.3b), arteether (Figure 1.3c), and artesunate (Figure 1.3d). These derivatives were found to be more active than artemisinin and hence, they are now increasingly being used for malaria treatment against drug resistant strains of *P. falciparum* [25-27]. Combining these drugs with other antimalarials having a longer half-life, such as mefloquine, could solve the recrudescence and the short plasma half-life problem [25, 28-31].



(a) R = H

(b) R = CH_3

(c) R = CH_2CH_3

(d) R = $COCH_2CH_2COOH$

Figure 1.3 Structures of artemisinin derivatives (a) dihydroartemisinin, (b) artemether, (c) arteether, (d) artesunate

1.3.4 Mechanism of Action

The mechanism of action for artemisinin compounds still remains uncertain. However, it is generally accepted that the endoperoxide group in artemisinin compounds plays an important role in its biological activity. The evidence is that deoxyartemisinin analogs, which lack the endoperoxide moiety, are devoid of antimalarial activity [17, 32]. The endoperoxide moiety is believed to produce free radicals which are necessary for mediating the effects. One evidence that supports the importance of free radicals is the enhancement of antimalarial activity by oxidant agents (free-radical-generating compounds) and the retard of antimalarial activity by antioxidants (free-radical scavengers) [33]. In additional to the endoperoxide group, some amount of iron is required as suggested from *in vitro* experiments [34-36]. The

mechanism of action was proposed to involve two sequential steps, i.e., activation and alkylation steps [33].

A. Activation Step

In the activation step, the endoperoxide linkage is attacked by ferrous ion, Fe (II), to produce reactive species, such as oxygen free radicals and hydroperoxide compound [37-39]. The specific source of iron for this activation step is still uncertain. Malarial parasites, which live in the red blood cell, an extremely Fe rich source, digest up to 80% of hemoglobin contained in the host cell to give heme (Figure 1.4) and globin as products. Globin is then hydrolyzed to provide amino acids as their sources for protein synthesis. Heme portion is normally discarded but owing to its toxicity, it is mostly detoxified by polymerization process to hemozoin. However, some free heme may be transiently present. Therefore, it is very probable that the free iron may be released from hemoglobin heme [40]. It was proposed that the reaction between artemisinin and free heme could intercede the hemoglobin degradation and the heme detoxification processes which may cause the parasites to die [41]. This idea is supported by the observation that the chloroquine-resistant strains of *P. berghei* which lack hemozoin are extremely resistant to artemisinin [42].

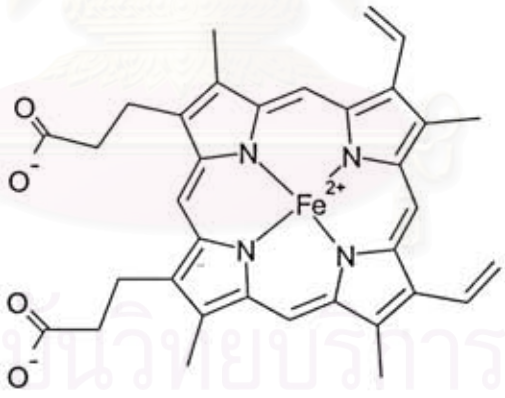


Figure 1.4 The structure of heme.

There are three proposed mechanisms for the approaching of heme iron to the endoperoxide linkage of artemisinin compounds. Posner and co-workers [38] proposed that the iron attacks the compound at the O_2 position and produces a free radical at the O_1 position (1A) (see Figure 1.5). It is then rearranged to form a C_4 free radical (1B) via the intermolecular 1,5-hydrogen shift process. This radical (1B) was suggested to be an important substance for antimalarial activity [43]. The supporting evidence is that

trioxanes compound having substituent group at the α-C_4 position, which makes it difficult or even impossible to produce such a radical, is virtually devoid of activity [44].

The radical 1B is then changed to the vinyl ether 1C by a beta-scission reaction, which also generates the Fe(IV)=O as another product. Subsequently, the intermolecular reaction between compound 1C and Fe(IV)=O leads to an epoxide compound 1D. Alternatively, the epoxide 1D could be derived directly from the radical 1B by a direct expulsion of iron. Finally, a C_4-hydroxylated product 1E is formed. The compound 1D is able to alkylate the specific proteins of the malarial parasites and possibly causes damage to the parasites [45].

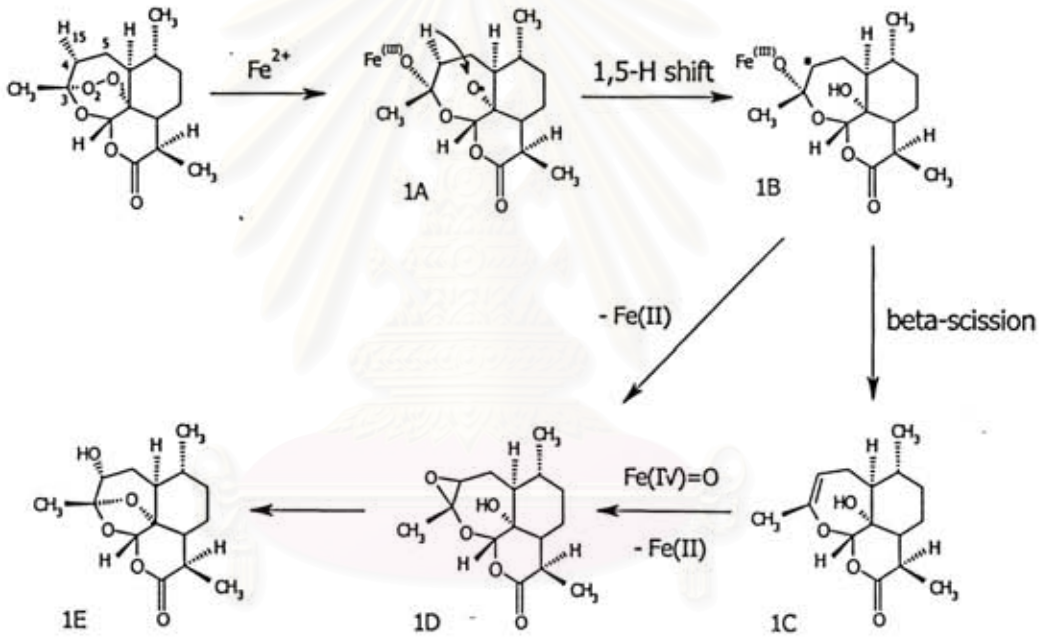


Figure 1.5 The mechanism of action of artemisinin compound as proposed by Posner et al. (pathway 1) [38].

On the other hand, Jefford and co-workers [39] believed that the iron attacks the compound at the O_1 position and produces a free radical at the O_2 position (2A) (Figure 1.6). After that the homolytic C_3-C_4 bond cleavage is occurred giving a carbon radical at the C_4 (2B). This radical (2B) could also be very harmful to the parasites [39] in the similar way to the compound 1D. In the last step, expulsion of iron leads to tetrahydrofuran 2C.

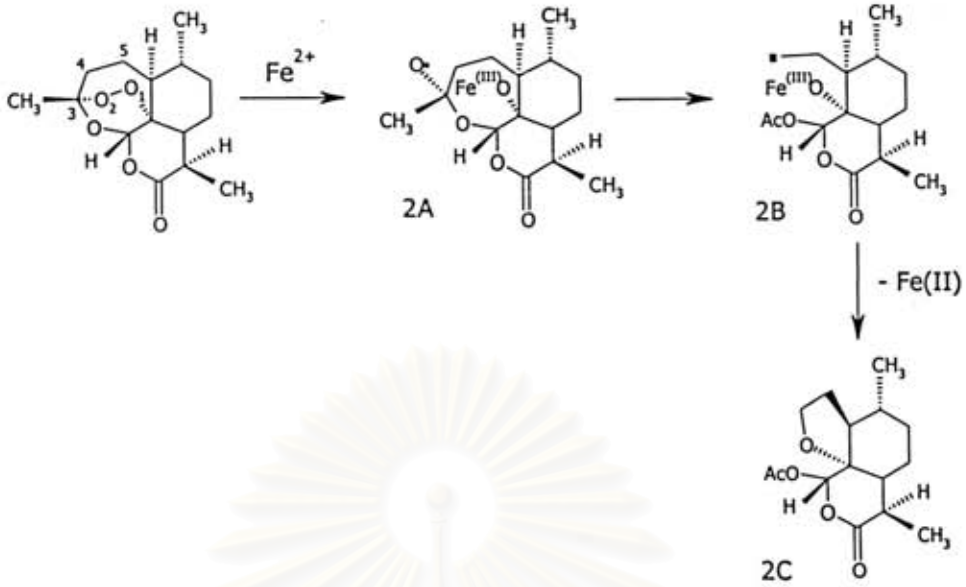


Figure 1.6 The mechanism of action of artemisinin compound as proposed by Jefford et al. (pathway 2) [39].

Haynes and Vonwiller presented a different hypothesis [46]. Instead of the O-O bond breaking, a heterolytic bond cleavage between O_2-C_3 by iron yielding the iron-oxo olefin (3A) was proposed as the first step in the mechanism of action (Figure 1.7). The loss of iron via protolysis reaction takes place producing the hydroperoxide 3B. The lactone 3C and diketo acid 3D are then resulted as the final products. Since hydrogen peroxide is known to have antimalarial activity, the compound 3B was postulated to be the active species. However, this mechanism is still in doubt. Because the formation of free radical compound was strongly evident [47-48] but no free radical was proposed in this mechanism.

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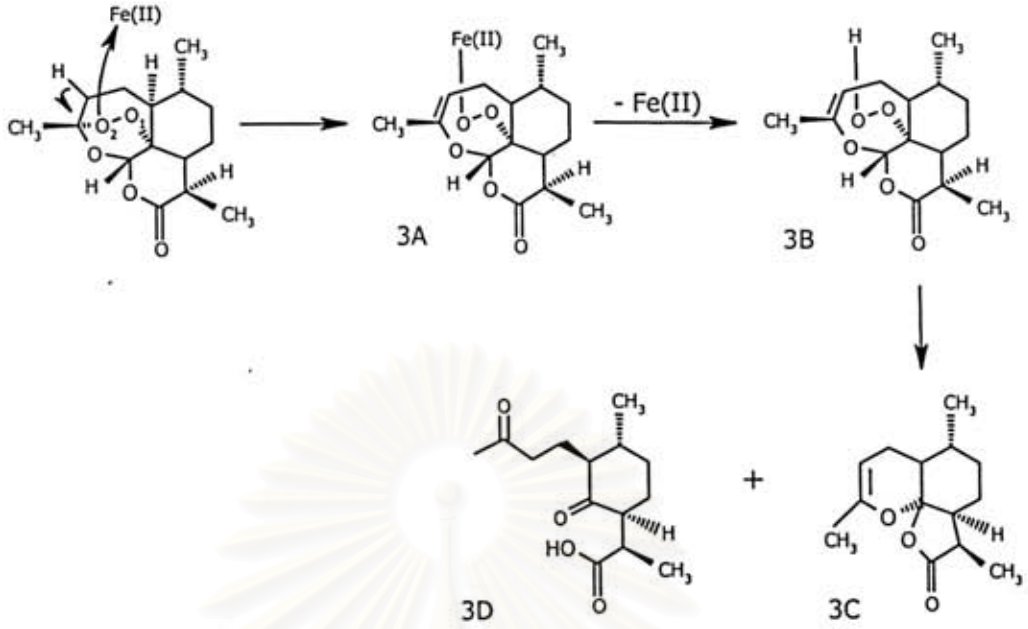

Figure 1.7 The mechanism of action of artemisinin compound as proposed by Haynes et al. (pathway 3) [46].

B. Alkylation Step

In the alkylation step, radicals and reactive intermediates formed from the activation step would rapidly react with nearby molecules due to their high reactivity. Alkylations of proteins [45, 49-50] and heme [51-52] but not of DNA [50] have been reported. The alkylation reaction was observed with many proteins, including human serum albumin, glycoprotein, hemoproteins, catalase, and cytochrome *c*. But most importantly, artemisinin and its derivatives were found to alkylate specific malaria proteins which are 25, 32, 42, 50, 65, and > 200 kDa in size via covalent linkage [45]. It seems that the heme alkylation does not play any significant role in the mode of action since the performed adduct(s) of artemisinin and heme have almost no antimalarial activity [51].

Despite the fact that artemisinin and its derivatives are active and are now being used worldwide, their long term uses possibiy cause malarial parasite to give lower response to these compounds in a near future as happened to the other antimalarial drugs. Hence, new more effective derivatives are needed. Therefore, the QSAR techniques were applied on these compounds.

1.4 Computer-Aided Drug Design (CADD)

1.4.1 Drug Design and Development Process

The discovery and development of a new effective drug is a long and very expensive process as a new drug must not only produce desired responses with minimum side effects but also must be better than the existing therapies. The process is schematically represented in Figure 1.8.

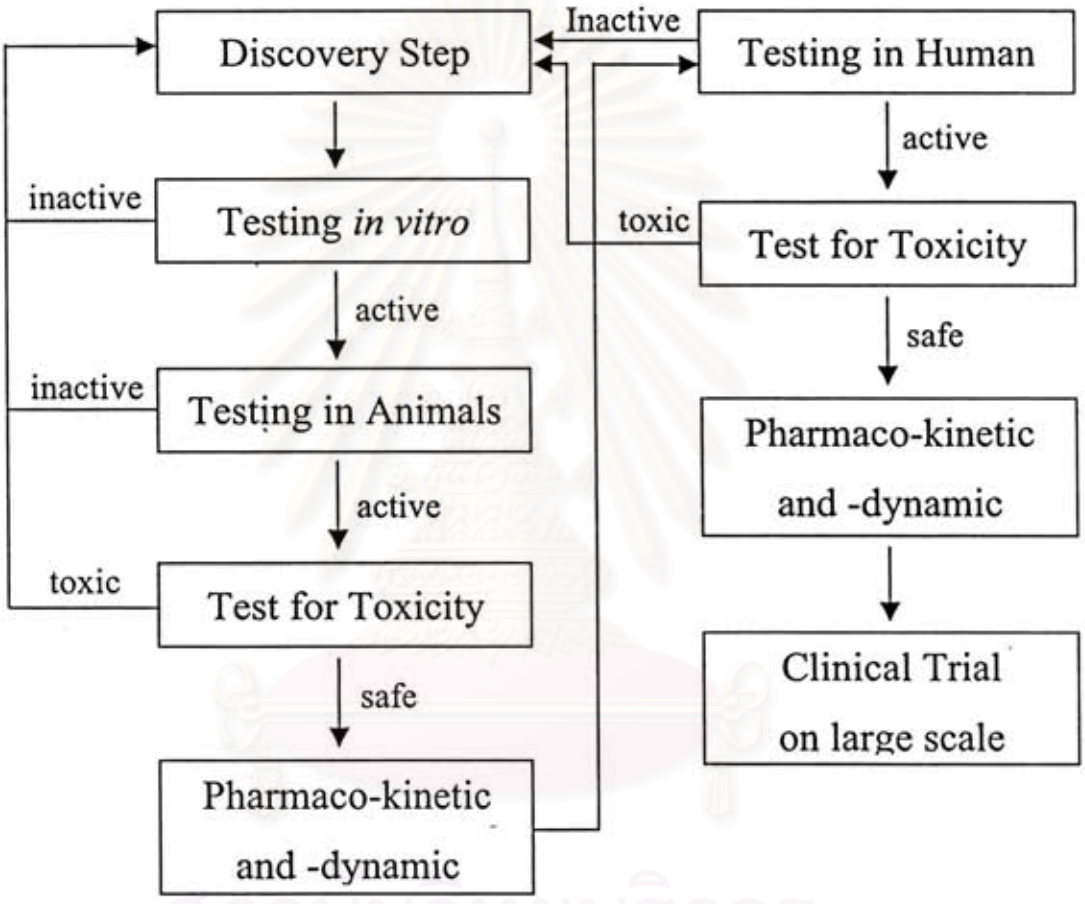

Figure 1.8 Steps in drug discovery and development process.

In the first step, the discovery step, a lead compound is discovered and identified, which can be done in many ways, for example, by extraction from herbs or animals, by chemical synthesis in laboratory, and by modification from known drugs. In the next step, the compound undergoes testing *in vitro* to access its biological activity. If the compound does not exhibit any acceptable potency, it is then disregarded and we have to return to the first step for a new compound. Until an active compound is found, we can then proceed to the next step, testing its biological activity in animal. This *in*

vivo test is much more complicated than the *in vitro* test, and the active compound found in the *in vitro* test does not necessary be active compound in the *in vivo* test. Again, if the compound does not show good activity, it is ignored and we have to go back to the discovery step again. But if the compound is considerably active, we can go further to the next step to test for toxicity and side effect. Since the compound may cause the side effect in long term, this step will require quite a long time, in average 3-5 years. During this time, the pharmaco-kinetic and pharmaco-dynamic of the compound are studied. After the compound was certainly proved to have low toxicity and no serious side effect, we can move to test the activity in human. As in animal, testing process for its toxicity and investigating the pharmaco-kinetic and pharmaco-dynamic should be carefully done. The time required for these steps are around 5-10 years. Moreover, in order to account for all races of human, the compound has to be tested on a large scale, the so-called clinical trial. Finally, it must undergo a review and approval process by the responsible government agency before it can be marketed. In summary, all the processes require in average 12 years and more than 200 million-dollar budget [53].

From the above paragraph, a lot of money and time must definitely be spent for a production of one new drug. Therefore, it is an ideal to have such a new drug with low budget and in a short time. As a result, many new technologies have been invented to assist the drug development. With the great advances in computer technology, Computer-Aided Drug Discovery (CADD) has been developed and it has been employed successfully in all leading pharmaceutical companies.

Quantitative Structure-Activity Relationship (QSAR) is the very first invented method of the CADD. It relates molecular properties expressed in numerical values to the activity via a mathematical model. Therefore, it enables the prediction of biological activity of new compounds in advance based on knowledge of the chemical structure alone. As a result, it will cut down the number of analogues which have to be made and hence reduces the syntheses and testing biological activity efforts. Definitely, this wiii save a lot of time and money. Moreover, from this method, it can also help in deciding which features of a molecule give rise to its activity and how to make modification to enhance the activity of a compound. The QSAR method has been used successfully for some decades.

1.4.2 Molecular Property

The numerical value of molecular properties can be obtained experimentally or theoretically. However, the latter approach seems to be more convenient. Quantum mechanical methods are increasingly being used to calculate molecular and electronic properties due to some advantages over the experimental works. There are two main advantages. Firstly, it is cheaper and more convenient while gives very reliable values as compared to those of experiments. It is, therefore, no need to synthesize compounds. Furthermore, the power in terms of hardware and software is increasing while the costs of computing are steadily decreasing. Secondly, it is able to calculate some properties that are very hard or impossible to measure experimentally, such as electronic properties. Moreover, from these methods, it is possible to derive properties that depend upon the electronic distribution and in particular to investigate chemical reactions in which bonds are being broken and formed.

Hundreds of different physical, structural, and chemical parameters have been used in the QSAR studies. In the early era (1970s), only a few parameters were recorded. The numbers of parameters used were gradually increased to more than 220 different parameters by 1980s. And they were exponentially increased to more than 17,000 different parameters by 1990s [54]. Although new parameters are steadily invented, there is still a lack of adequate parameters to describe some important interactions like the membrane partitioning of drugs, the strength of hydrogen bonds, the influence of desolvation energies on drug-receptor affinity, and steric interactions with a binding site.

With the improvement of hardware and software, numbers of parameters are being developed and tried. Among these, the most commonly used are hydrophobic, polar, electronic, and steric properties. Brief details for these four types of parameters are discussed below.

A. Hydrophobicity Parameters

No other physicochemical property has attracted as much attention in QSAR as hydrophobicity. The hydrophobicity determines how easy for a drug to cross cell membranes and is also important in assessing the drug-receptor interactions. A drug should have a suitable hydrophobicity. If it is too hydrophilic, it could not cross the hydrophobic cell membrane to the target site. But if it is too hydrophobic, it would

quickly be extracted from an aqueous bloodstream and be stored in the fatty tissues of the body. Consequently, it does not reach the intended target site. This is the reason why parabolic relationships between hydrophobicity and activity are frequently seen. Changing substituents on a drug may well have significant effects on its hydrophobic character and hence its biological activity.

The hydrophobic character of a drug is widely presented by the partition coefficient (P), which is defined as the ratio of drug concentrations in the organic and aqueous phases of a two-compartment system under equilibrium conditions (equation 1.1). Although many organic/aqueous systems have been used since the past time, an *n*-octanol/water system is now accepted as the standard system for the experimentally measurement of the P.

$$P = \frac{\text{Concentration of drug in organic phase}}{\text{Concentration of drug in aqueous phase}} \qquad \text{.....(1.1)}$$

From the definition of P, it is obvious that hydrophobic compounds will have a high P value, whereas hydrophilic compounds will have a low P value. However, the main drawback of measuring P experimentally is that the compound has to be synthesized. Moreover, the measurement is sometimes not easy. Therefore, it would be much better if we could calculate P theoretically.

Since the scale of numbers involved in measuring P usually cover several factors of ten, the logarithm unit is used instead to allow more manageable numbers. The log P value can be calculated by using its additive property and there are a lot of molecular modeling softwares available for this purpose. In the software database, the hydrophobicity contribution of various substituents and the experimental log P values of as many compounds as possible are stored. By adding and/or subtracting these contributions to an experimental log P value of a parent compound, the log P value of the desired compound could be obtained. Both experimental and theoretical log P values play an important role in the QSAR studies as indicated by its contributions to many QSAR equations.

B. Polarizability Parameters

Since polarizability is the ability of electrons to change position in response to the presence of an outside electrical field, it is thus related to the electronic properties,

size, and polarity of a compound. Therefore, it may have an influence on the drug-receptor interactions. Molar refractivity (MR), which is calculated by the equation (1.2), is the most utilized polarizability parameter.

$$MR = \frac{MW}{d} \cdot \frac{n^2 - 1}{n^2 + 2} \qquad .....(1.2)$$

where MW = molecular weight

d = density

n = refractive index

The refractive index-related correction term in MR accounts for the polarizability and thus for the size and the polarity of a certain group. The larger the polar part of a molecule is, the larger its MR value will be. Molar refractivity normally has significant contributions to the QSAR equations of ligand-enzyme interactions.

C. Electronic Parameters

The electronic properties of various substituents clearly have an effect on a drug's ionization or polarity. This in turn may have an effect on how easily a drug can pass through cell membranes or how strongly it can bind to a receptor. The Hammett electronic constant (σ) was the first parameter used to describe electronic effects. However, it could account for only substituents on an aromatic ring. This disadvantage limits its use. Therefore, many new electronic parameters have been applied in the QSAR study, such as dipole moments (μ), moment of inertia (I), hydrogen-bonding parameters, and parameters derived from quantum chemical calculation, e.g., orbital energies and partial atomic charges.

D. Steric Parameters

Generally, a drug has to approach and interact with its receptor to mediate its effects. The bulk, size, and shape of the drug must be appropriate for the binding. In some cases, a bulky substituent may act like a shield, which hinders the drug-receptor interaction. Alternatively, it may help a drug to orient properly for the maximum drug-receptor binding which then increases the activity. Therefore, it is worth to investigate a relationship between activity and steric character. Steric effects are sometimes difficult to describe since the 3D structures of the binding sites of drug are most often unknown, however, if the structure is known, there is still some problems to calculate the steric

effects quantitatively. Such problems are, for example, different conformations of different ligands, small (or even large) differences in the binding modes of different analogs, and variations in the positions of side chains and even backbone atoms of the protein in different ligand-protein complexes.

Bond length, bond angle, and dihedral angle parameters, which can be simply measured from the structure, are members of this group. The topological indices, which are calculated using the chemical graph theory [55] as the basis, are also widely used. Examples of these indices are the Wiener index [56], molecular connectivity indices (Chi) [57], valence-modified molecular connectivity indices (ChiV) [57], and molecular shape indices (Kappa) [57]. The Wiener index is the sum of distances between all pairs of heavy atoms in the molecule. The Chi and ChiV indices reflect the atom identities, bonding environments, and number of bonding hydrogens. Molecules that are drawn without hydrogen atoms can be decomposed into fragments of length *m*, which may be divided into four different categories: Path, Cluster, Path/cluster, and Ring. The spread and numbers of substructure fragment membership for each category is determined by molecular connectivity. The main difference between these two types of indices is that only the valence electrons involved in skeletal bonding (sigma orbitals) are counted for the Chi indices whereas all the valence electrons are counted in the ChiV indices to take into account electron configuration of the atom. The kappa indices are molecular shape indices based on the assumption that the shape of a molecule is a function of the number of atoms and their bonding relationship (without considering hydrogen atoms). The values are derived from counts of one-bond (Kappa 1), two-bond (Kappa 2), and three-bond (Kappa 3) fragments, each count being relative to fragment counts in reference structures which possess a maximum and minimum value for that number of atoms. Therefore, the Kappa 1 shows the degree of complexity of a binding pattern. The Kappa 2 indicates the degree of linearity or star-likeness of bonding patterns. The Kappa 3 indicates the degree of branching at the center of a molecule. More details about topological indices can be found elsewhere [58].

1.4.3 Statistical Analysis

After the desired physicochemical properties were calculated, the next step is to find relation with the biological activity in a quantitative manner. For this purpose, a statistical analysis is needed. The regression analysis is one of the most frequently used statistical analyses to find a correlation equation. The general form of multiple linear regression (MLR) models is depicted in equation (1.3).

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \ldots + \beta_m x_m \qquad \ldots..(1.3)$$

where y = dependent variable

$x_1, x_2, x_3, \ldots$ = independent variables

$\beta_0, \beta_1, \beta_2, \ldots$ = regression coefficients

Since one of the goals in QSAR studies is the ability to describe a biological activity of a compound from its physicochemical properties, it is important to achieve this ability by using a statistical analysis method that can minimize an error between actual and calculated biological values. Therefore, a least-squares method, which has a strategy to minimize the residual sum of squares (sum of squares of the errors), is usually employed. Details about this method can be found in a statistical textbook.

After the model is evaluated, it is necessary to have some indicators to justify the significance and quality of the correlation equations. The first indicator is the standard deviation, s, which is based on variance. It is defined as a sum of squared errors per degree of freedom in a calculation. The lower the s value, the better is the regression model.

The second and most popular indicator used to measure the quality of the QSAR model is the Pearson correlation coefficient, r, which is calculated by the formula shown in equation 1.4. The r statistics has a value between -1 and 1 ($-1 \leq r \leq 1$), where r = 1 implies a perfect positive correlation, r = -1 implies a perfect negative correlation, and r = 0 implies no correlation. Therefore, a value of r close to 1 or -1 indicates a strong degree of linear relationship.

$$r = \frac{\Sigma(xy) - (\Sigma x).(\Sigma y)/n}{\sqrt{\Sigma(x^2) - (\Sigma x)^2/n} \cdot \sqrt{\Sigma(y^2) - (\Sigma y)^2/n}} \qquad \ldots..(1.4)$$

Generally, r^2 is used instead of the r itself, thus $0 \leq r^2 \leq 1$. The r^2 statistics is a ratio of the variance explained by a regression model to the total variance (equation 1.5). Therefore, it gives an information on how many percentage of the variation in the biological activity (Y variable) can be explained by the physicochemical properties (X variables) presented in the equation.

$$r^2 = \Sigma(y_{calculate} - y_{mean})^2 / \Sigma(y_{observe} - y_{mean})^2 \quad .....(1.5)$$

The third indicator is the F value, which measures the level of statistical significance of the regression model. Only F values being larger than the 95 % significance limits prove the overall significance of a regression equation.

In general, the regression equation can be accepted in QSAR studies if the following four criteria are met. Firstly, the correlation coefficient r is around or better than 0.8 ($r^2 \geq 0.64$) for *in vivo* data and 0.9 ($r^2 \geq 0.81$) for *in vitro* data [54]. Secondly, the standard deviation s is not much larger than the standard deviation of the biological data. Thirdly, the overall significance level is better than 95 % as indicated by the F value. Fourthly, the confidence intervals of all individual regression coefficients prove that they are justified at the 95 % significance level, i.e., their confidence intervals are smaller than the absolute values of the regression coefficients. In addition, there should be no fewer than five compounds for each chemical descriptor used in the final equation to prevent the chance correlations. Moreover, the descriptors should not be intercorrelated, i.e., interdescriptor correlation coefficients should be less than 0.5 [59].

Using the r^2 alone to justify the QSAR model is not recommended. The predictability of the model should also be considered. This is because the r^2 gives an information on the reproducibility, how well the model reproduces the biological activity of the compounds included in the model, not the predictability. The predictability, an ability to predict a biological activity of a new compound outside the model, could be measured by various approaches, e.g., cross-validation [60], bootstrapping, random change of the values of the dependent variable, and dividing the original set into the training set and testing set. However, the most widely used method is the cross-validation. In this method, the predictability of the model is estimated by repeatedly leaving out one (or more) compound(s) at a time until each compound is excluded exactly once. Using the reduced set of data, the model is derived and is used to predict the activity of the left out compound. During the cross-validation test, the

sum of the squared prediction errors called the predictive residual sum of squares (PRESS), the cross-validated correlation coefficient (r_{cv}^2 or q^2), and the cross-validated standard error of estimate (s_{cv}) are evaluated using the formula shown in equation 1.6 to 1.8. A smaller s_{cv} and a larger q^2 indicate the model's good predictability. Generally, a model with the q^2 value of greater than 0.50 is accepted as a good model [61].

$$PRESS = \Sigma(y_{observed} - y_{predicted})^2 \quad .....(1.6)$$

$$q^2 = 1 - PRESS/SST \quad .....(1.7)$$

$$s_{cv} = (PRESS/n)^{1/2} \quad .....(1.8)$$

The main goal for QSAR study is the ability to predict biological activity of other compounds outside the model rather than the ability to reproduce the biological activity of the compounds included in the model. Therefore, we should test the model by predicting the activity of the "new compound", which is not included in the process of deriving the model. Therefore, the real predictive ability of the model could not be determined by the q^2 value. In order to investigate the real predictive ability, the compounds are randomly divided into 2 sets, training set and testing set. Compounds in the training set are used to derive the model. Subsequently, the obtained model is used to predict the biological activity of compounds in the testing set. By comparing between predicted and actual values, its real predictive power is obtained.

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CHAPTER 2
Calculations

2.1 Structures and Biological Data

With the aim to explore effects of structural differences in artemisinin compounds concerning their biological activities, totally 68 artemisinin derivatives with significantly different structures and biological activities [32, 62-68] were used in this study. All compounds were categorized into 3 groups according to their structural similarity. The activities were measured as the IC_{50} values, the inhibitory concentration of a compound required for 50% inhibition of the parasitemia, against the Sierra Leone (D-6) and the Indochina (W-2) clones of *P. falciparum*. The D-6 clone is mefloquine-resistant but chloroquine-sensitive while the W-2 clone is chloroquine-resistant but mefloquine-sensitive. Since the biological data arise from different sources, there might occur an inconsistency from individual experimental testing procedure. Therefore, the relative activity, the ratio of activity of artemisinin and the drug compound, was used. Moreover, in order to compare drug activities of different compounds of various molecular weights, it is necessary to convert the biological activities of the compounds (IC_{50}) in ng/mL unit to nmol/mL unit according to the formula shown below. Furthermore, the relative activities were reported in the logarithm unit. As a result, compounds with relative activities of positive values are more active than artemisinin. On the other hand, compounds with relative activities of negative values are less active than artemisinin. The structures of 68 compounds are given in Figure 2.1 to 2.7 and their corresponding biological data are depicted in Table 2.1 to 2.6. Note that the activities for D-6 and W-2 strains were denoted as log(D-6) and log(W-2), respectively.

activity = (IC_{50} of artemisinin / IC_{50} of analog) x (MW of analog / MW of artemisinin)

2.1.1 Compounds Group 1

There are 22 compounds belonging to this group. All the structures in this category have C=O functional group at the C_{10} position. Compounds 1 and 3 to 22 are analogues of artemisinin (compound 2) with substituent groups at either C_3 or C_9 positions (Figure 2.1). Their biological activities are given in Table 2.1.

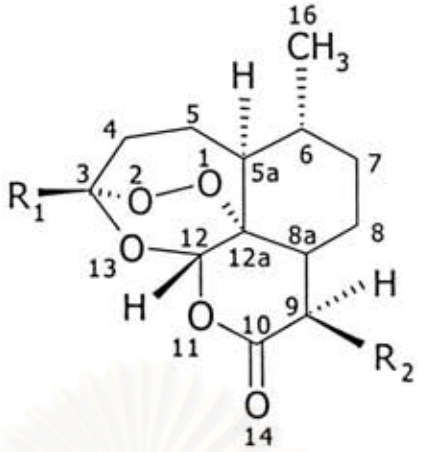


Figure 2.1 Structures of artemisinin derivatives number 1 to 22.

Table 2.1 Structures and biological data of compounds number 1-22 in group 1.

No	R_1	R_2	log(D-6)	log(W-2)
1	CH_3	H	0.23	0.79
2	CH_3	CH_3	0.00	0.00
3	CH_3	C_2H_5	0.83	1.11
4	CH_3	*n*-C_3H_7	0.78	1.13
5	CH_3	*i*-C_3H_7	0.35	-0.04
6	CH_3	*n*-C_4H_8	0.04	0.17
7	CH_3	*i*-C_4H_8	-0.64	-0.55
8	CH_3	*n*-C_5H_{11}	0.64	1.02
9	CH_3	*n*-C_6H_{13}	0.77	0.84
10	CH_3	*i*-C_6H_{13}	-0.41	-0.04
11	CH_3	$(CH_2)_2C_6H_5$	0.35	0.12
12	CH_3	$(CH_2)_3C_6H_5$	0.92	0.78
13	CH_3	$(CH_2)_4C_6H_5$	0.29	0.63
14	CH_3	CH_2COOH	-2.70	-2.52
15	CH_3	$CH_2CH{=}CH_2$	-0.47	-0.10
16	CH_2CH_3	H	-0.06	0.05
17	$(CH_2)_3CH_3$	H	-0.70	-0.74
18	$CH_2CH(CH_3)_2$	H	-0.28	-0.35
19	$(CH_2)_2COOC_2H_5$	H	0.37	0.37
20	$(CH_2)_2C_6H_5$	H	-1.52	-2.00

Table 2.1 (Continued)

No	R_1	R_2	log(D-6)	log(W-2)
21	*p*-$ClC_6H_4(CH_2)_3$	H	0.06	0.10
22	$C_6H_5(CH_2)_4$	H	0.34	0.45

2.1.2 Compounds Group 2

Compounds in this category have -OR substituent group at the C_{10} position. This makes their structures significantly different from those in the group 1, especially the lactone ring. The reason is clearly due to the difference in hybridization of the C_{10} atom, i.e., sp^2 in the group 1 and sp^3 in the group 2. This structural difference is demonstrated in Figure 2.2.

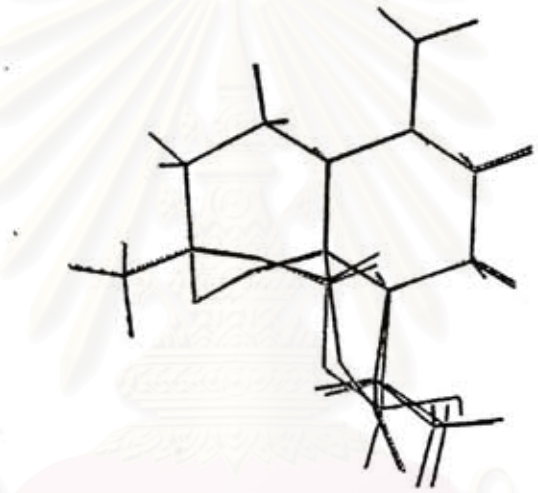


Figure 2.2 Structural difference between compound in group 1 (blue) and 2 (red).

Totally 30 compounds were chosen in this group. Substituent groups are varied at only the O_{14} position for compounds 23 to 34 (Figure 2.3) and at both the C_9 and the O_{14} positions for compound 35 to 38 (Figure 2.4). Compounds 39 to 49 have the substituent groups at only the C_{18} position (Figure 2.5). Their biological activities are shown in Table 2.2 to 2.5.

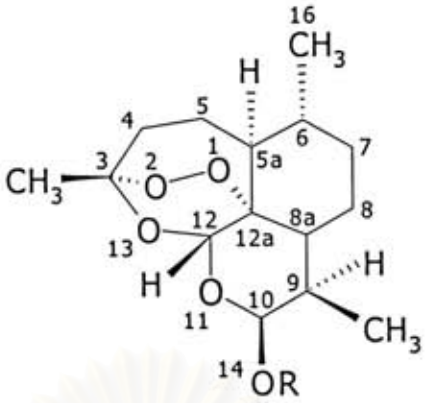


Figure 2.3 Structures of artemisinin derivatives number 23 to 34.

Table 2.2 Structures and biological data of compounds number 23-34 in group 2.

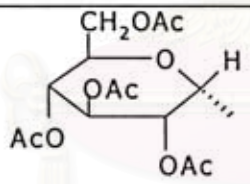
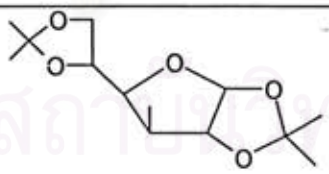
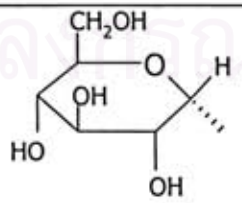
No	R	log(D-6)	log(W-2)
23	H	0.86	-0.02
24	CH_3	0.45	0.55
25	$CH_2COOCH_2CH_3$	0.81	0.52
25	$(CH_2)_2COOCH_3$	0.32	0.13
26	$(CH_2)_3COOCH_3$	0.11	-0.02
28	$CH_2C_6H_4COOCH_3$	0.77	0.44
29	$CH_2C_6H_4COOH$	0.03	-0.15
30		0.41	0.89
31		0.58	0.61
32		-1.40	-0.51

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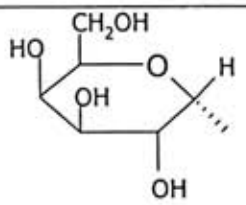
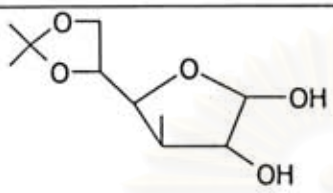
Table 2.2 (Continued)

No	R	log(D-6)	log(W-2)
33		-0.46	-1.52
34		0.29	0.16

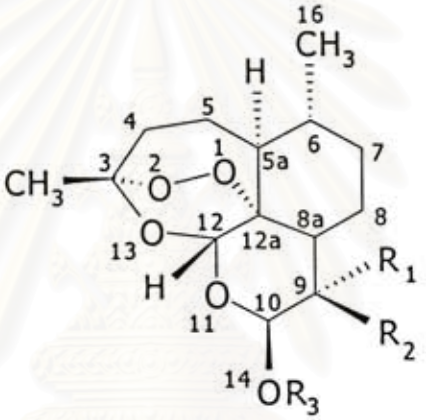


Figure 2.4 Structures of artemisinin derivatives number 35 to 38.

Table 2.3 Structures and biological data of compounds number 35-38 in group 2.

No	R_1	R_2	R_3	log(D-6)	log(W-2)
35	OH	CH_3	CH_2CF_3	0.39	0.31
36	CH_3	OH	CH_2CF_3	-0.70	-0.72
37	OH	CH_3	CH_2CH_3	-0.28	-0.46
38	CH_3	OH	CH_2CH_3	-1.05	-1.15

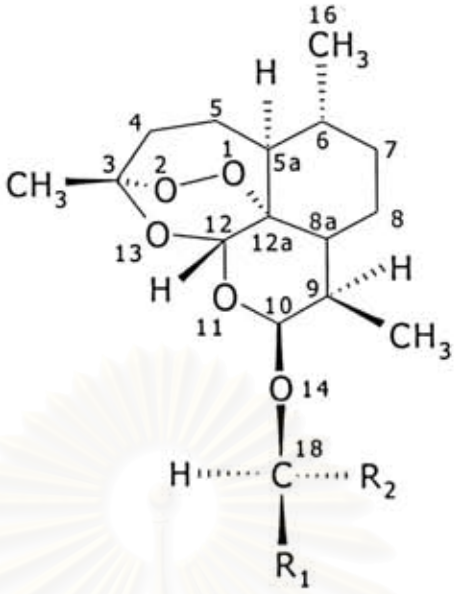


Figure 2.5 Structures of artemisinin derivatives number 39 to 49.

Table 2.4 Structures and biological data of compounds number 39-49 in group 2.

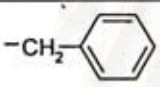
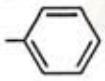
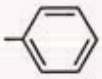
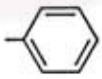
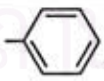
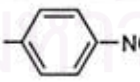
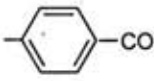
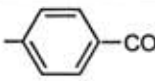
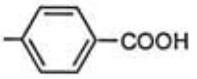
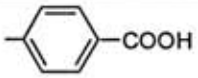
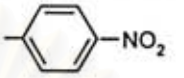
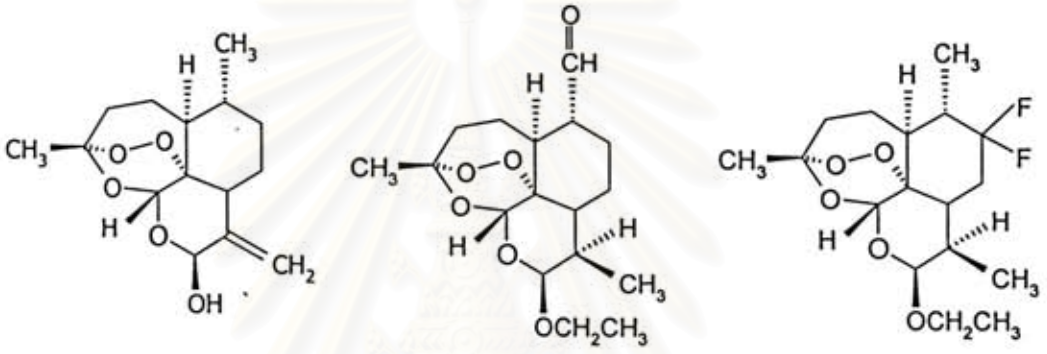
No	R_1	R_2	log(D-6)	log(W-2)
39		$-CH_2CH_2CH_3$	0.56	0.35
40		$-COOCH_2CH_3$	1.10	1.23
41	$-COOCH_2CH_3$		0.85	0.85
42	$-CH_2COOCH_2CH_3$		0.88	0.89
43		$-CH_2COOCH_2CH_3$	0.91	1.06
44	$-CH_2COOCH_2CH_3$		0.86	0.90
45	$-CH_3$		0.60	0.68
46		$-CH_3$	0.72	0.68

Table 2.4 (Continued)

No	R_1	R_2	log(D-6)	log(W-2)
47	$-CH_3$		-0.21	-0.21
48		$-CH_3$	0.08	0.10
49	$-CH_2COOH$		0.12	0.09



50 51 52

Figure 2.6 Structures of artemisinin derivatives number 50 to 52.

Table 2.5 Biological data of compounds number 50-52 in group 2.

No	log(D-6)	log(W-2)
50	-1.80	-2.40
51	0.14	0.21
52	0.24	0.17

2.1.3 Compounds Group 3

Compounds in this group have no substituent at the C_{10} position (Figure 2.7). Totally 16 compounds with different substituent groups at either the C_3 position (compounds 53 to 60) or the β-C_9 position (compounds 61 to 68) were selected. Most compounds in this group have higher activities than artemisinin itself. Hence, the O_{14} atom seems to be not necessarily required for high antimalarial activity.

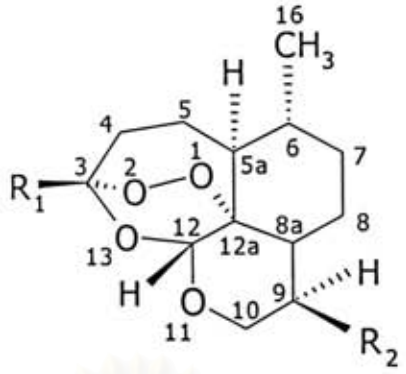


Figure 2.7 Structures of artemisinin derivatives number 53 to 68.

Table 2.6 Structures and biological data of compounds number 53-68 in group 3.

No	R_1	R_2	log(D-6)	log(W-2)
53	CH_3	H	0.37	0.28
54	CH_3	CH_3	0.82	0.75
55	CH_3	CH_2CH_3	0.96	0.67
56	CH_3	$(CH_2)_2CH_3$	0.67	0.74
57	CH_3	$(CH_2)_3CH_3$	1.77	1.32
58	CH_3	$(CH_2)_4CH_3$	0.23	0.16
59	CH_3	$(CH_2)_3C_6H_5$	1.71	1.40
60	CH_3	*p*-ClC_6H_4 $(CH_2)_3$	1.84	1.52
61	CH_2CH_3	H	-1.00	-1.00
62	$(CH_2)_2CH_3$	H	0.86	0.84
63	$(CH_2)_3CH_3$	H	0.81	0.75
64	$CH_2CH(CH_3)_2$	H	0.26	0.40
65	$(CH_2)_4C_6H_5$	H	0.53	0.58
66	$(CH_2)_2C_6H_5$	H	-1.22	-1.70
67	*p*-ClC_6H_4 $(CH_2)_3$	H	-0.89	-0.55
68	$(CH_2)_2COOH$	H	-3.05	-3.05

The main reason to distinguish this group from the two previous ones is the substituent at the C_{10} position. All compounds in the group 2 are proposed to change rapidly to dihydroartemisinin upon entering the body [33] but this process can not occur for compounds in the group 3.

Comparing between some compounds in group 1 and group 3, the substituent at either C_9 (Table 2.7) or C_3 positions (Table 2.8) usually lead to contrary changes in activities between the two groups. For example, when the hydrogen atom at the β-C_9 position is changed to a methyl group, the activities of compounds in group 1 increase (compound 1 vs. compound 2) whereas this change in compounds group 3 leads to a decrease in activities (compound 53 vs. compound 54). Note that the activities of each compound are reported as relative values (in logarithm unit) to those of the compound with R = -H for substituent at the C_9 position and with R = -CH_3 for substituent at the C_3 position.

Table 2.7 Effect of substituent at C_9 position in compounds of group 1 and 3.

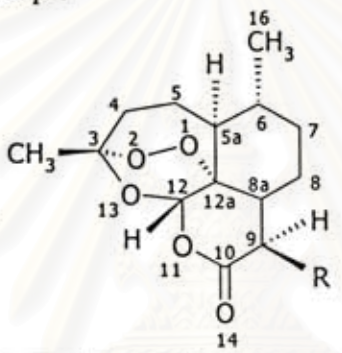
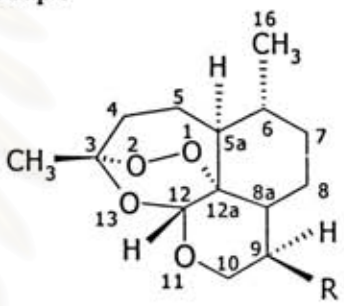
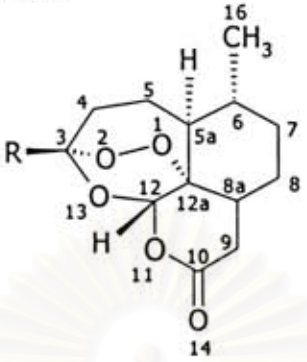
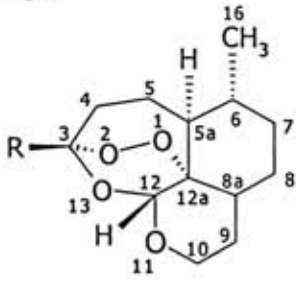
	Group 1			Group 3		
						
R	No.	log(D-6)	log(W-2)	No.	log(D-6)	log(W-2)
H	1	0.00	0.00	53	0.00	0.00
CH_3	2	-0.24	-0.80	54	0.44	0.47
CH_2CH_3	3	0.60	0.32	55	0.59	0.39
$(CH_2)_2CH_3$	4	0.55	0.34	56	0.30	0.46
$(CH_2)_3CH_3$	6	-0.19	-0.62	57	1.39	1.04
$(CH_2)_4CH_3$	8	0.41	0.23	58	-0.14	-0.12
$(CH_2)_3C_6H_5$	12	0.69	0.00	59	1.33	1.12

Table 2.8 Effect of substituent at C_3 position in compounds of group 1 and 3.

	Group 1 			Group 3 		
R	No.	D-6	W-2	No.	D-6	W-2
CH_3	1	0.00	0.00	53	0.00	0.00
CH_2CH_3	16	-0.29	-0.74	61	-1.40	-1.30
$(CH_2)_3CH_3$	17	-0.92	-1.52	63	0.44	0.47
$CH_2CH(CH_3)_2$	18	-0.51	-1.15	64	-0.11	0.12
p-$ClC_6H_4(CH_2)_3$	21	-0.17	-0.68	67	-1.30	-0.82
$(CH_2)_4C_6H_5$	22	0.11	-0.35	65	0.15	0.30
$(CH_2)_2C_6H_5$	20	-1.70	-2.70	66	-1.52	-2.00

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2.2 Structural Optimization

Since the experimental structures are not available for all the compounds used in this study, theoretical calculation is needed to determine the geometry of each compound. Therefore, the quantum chemical method was used. However, it is necessary to establish a suitable level of accuracy for the geometry optimization. For this purpose, the artemisinin structure was optimized using CNDO, AM1, Hartree Fock with 3-21G (HF/3-21G) and 6-31G** (HF/6-31G**) basis sets, and Density Functional Theory (DFT) level of theory with B3LYP functional and 6-31G** basis set (B3LYP/ 6-31G**). Subsequently, all optimized structures were compared with the X-ray structure [19] to determine the suitable structural optimization method. Finally, this method will be used for the optimizations of all compounds. Artemisinin derivative structures with (group 1) and without (group 2 and 3) C=O at the C_{10} position were built using the X-ray structure of artemisinin and dihydroartemisinin, respectively, as template. All the quantum chemical calculations were carried out using the Gaussian 98 program [69].

2.3 Calculations of Properties

There are a lot of parameters that can be used in the field of QSAR. Totally, 102 physicochemical parameters were calculated using the TSAR [70] and Gaussian [69] softwares.

A. Hydrophobicity properties

For the hydrophobicity parameter, the log P was calculated. Moreover, the desolvation free energies for water (F_{h2o}) and for octanol (F_{oct}), which indicated the ease for desolvating solute molecule (water or octanol) from a drug compound, were also included. This is important for the drug-receptor binding process since both drug and receptor must become at least partially desolvated before the binding.

B. Polarizability properties

For the polarizability parameter, the molar refractivity was calculated using the TSAR software.

C. Electronic properties

For the electronic parameters, atomic charges obtained from the Gaussian software were used. Since the Mulliken Population Analysis (MPA) method is known to be dependent on the basis set used, therefore, the Natural Population Analysis (NPA) method, which is much less sensitive to the basis set, was used instead for the atomic charge calculations. Atomic charges of 17 atoms namely O_1, O_2, C_3, C_4, C_5, C_{5a}, C_6, C_7, C_8, C_{8a}, C_9, C_{10}, O_{11}, C_{12}, C_{12a}, O_{13}, and O_{14} were computed at the HF/3-21G level.

Twenty bond orders [71] were calculated at the HF/3-21G level which are denoted as follows: B(1-2), B(1-12a), B(2-3), B(3-4), B(3-13), B(4-5), B(5-5a), B(5a-6), B(5a-12a), B(6-7), B(7-8), B(8-8a), B(8a-9), B(8a-12a), B(9-10), B(10-11), B(10-14), B(11-12), B(12-12a), and B(12-13). The bond order parameter indicates types of bonding. The bond order values of 1.0, 2.0, and 3.0 correspond to the single, double, and triple bonds, respectively.

Dipole moment, HOMO energy, and LUMO energy were also calculated at the HF/3-21G level. Moreover, six moments of inertia were computed using the TSAR software. They are calculated in all three perpendicular axes, which pass through the center of mass of a molecule. And these results are reported in the project as Inertia Moment 1 Size, Inertia Moment 2 Size, Inertia Moment 3 Size, Inertia Moment 1 Length, Inertia Moment 2 Length, and Inertia Moment 3 Length. The moment of inertia indicates the ability for rotation, which may involve the binding.

D. Steric properties

Structural parameters, 12 bond lengths (R), 14 bond angles (A), and 16 torsion angles (T), were taken from the HF/3-21G optimized structures. In order to represent these parameters, the atom number corresponding to the structure of artemisinin in Figure 1.2 was given in the parenthesis. For example, the R(1-2) parameter represents the bond length between atom 1 and 2, the A(1-2-3) means the bond angle between atom 1, 2, and 3, and the T(1-2-3-4) is the torsion angle between atom 1, 2, 3, and 4. All structural parameters are as follows: R(1-2), R(1-12a), R(2-3), R(3-4), R(3-13), R(4-5), R(5-5a), R(5a-12a), R(10-11), R(11-12), R(12-12a), R(12-13), A(1-2-3), A(1-12a-5a), A(2-1-12a), A(2-3-4), A(2-3-13), A(3-4-5), A(3-13-12), A(4-3-13), A(4-5-5a), A(5-5a-12a), A(5a-12a-12), A(10-11-12), A(11-12-13), A(12a-12-13), T(1-2-3-4), T(1-12a-5a-5), T(1-12a-12-13), T(2-1-12a-5a), T(2-1-12a-12), T(2-3-4-5), T(2-3-13-12), T(3-2-1-

12a), T(3-4-5-5a), T(3-13-12-12a), T(4-3-13-12), T(4-5-5a-12a), T(5-4-3-13), T(5-5a-12a-12), T(5a-12a-12-13), and T(10-11-12-13).

Topological index after the Balaban method [70] and the following 6 connectivity indices were calculated using the TSAR software: Chi0 (atoms), ChiV0 (atoms), Chi1 (bonds), ChiV1 (bonds), Chi2 (path), and ChiV2 (path). In addition, three shape indices, i.e., Kappa 1, Kappa 2, and Kappa 3, were also computed.

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CHAPTER 3

Results and Discussions

3.1 Structural Optimization

Important structural parameters of artemisinin obtained from the optimization using CNDO, AM1, HF/3-21G, HF/6-31G**, and B3LYP/6-31G** were compared with those of the X-ray structure. The difference between the optimized values and the X-ray values was calculated for each optimization method in term of percent error (% error). The above results as well as CPU time for optimizations are shown in Table 3.1

Table 3.1 Comparison of important structural parameters of artemisinin between the X-ray structure, semiempirical, and *ab initio* optimized structures.

Parameter	X-ray	CNDO	AM1	HF/ 3-21G	HF/ 6-31G**	B3LYP/ 6-31G**
Bond Distance (Å)						
O_1-O_2	1.475	1.229	1.289	1.462	1.390	1.460
O_1-C_{12a}	1.450	1.410	1.469	1.477	1.430	1.455
O_2-C_3	1.417	1.394	1.447	1.441	1.396	1.414
C_3-C_4	1.518	1.488	1.532	1.537	1.537	1.546
C_{5a}-C_{12a}	1.538	1.504	1.540	1.537	1.546	1.555
C_5-C_{5a}	1.537	1.486	1.526	1.551	1.543	1.547
C_4-C_5	1.533	1.477	1.513	1.544	1.535	1.539
C_3-O_{13}	1.448	1.390	1.427	1.436	1.409	1.441
C_{12}-O_{13}	1.388	1.387	1.416	1.408	1.376	1.396
C_{5a}-C_6	1.550	1.495	1.531	1.549	1.545	1.552
C_{8a}-C_{12a}	1.520	1.538	1.532	1.529	1.532	1.540
C_{12}-O_{11}	1.455	1.401	1.421	1.428	1.408	1.439
C_6-C_7	1.532	1.486	1.521	1.543	1.533	1.539
C_8-C_{8a}	1.533	1.490	1.517	1.535	1.533	1.538
C_3-C_{15}	1.517	1.478	1.519	1.512	1.513	1.519
C_9-C_{16}	1.530	1.474	1.516	1.542	1.532	1.536

Table 3.1 (Continued)

Parameter	X-ray	CNDO	AM1	HF/ 3-21G	HF/ 6-31G**	B3LYP/ 6-31G**
C_{8a}-C_9	1.540	1.504	1.524	1.539	1.537	1.543
C_{10}-O_{11}	1.351	1.409	1.373	1.366	1.338	1.365
C_{10}=O_{14}	1.201	1.340	1.231	1.197	1.183	1.207
C_9-C_{17}	1.540	1.464	1.513	1.532	1.528	1.531
% error	-	4.00	1.83	0.81	1.14	0.64
Bond Angle (°)						
C_5-C_{5a}-C_{12a}	112.6	112.1	111.2	111.8	112.0	112.1
C_4-C_5-C_{5a}	116.3	115.2	114.6	115.7	116.5	116.6
C_3-C_4-C_5	114.5	115.2	113.9	113.0	114.1	114.1
O_{13}-C_3-C_4	110.4	111.7	112.4	109.6	109.6	109.4
C_5-C_{5a}-C_{12a}	112.6	112.1	111.2	111.8	112.0	112.1
C_4-C_5-C_{5a}	116.3	115.2	114.6	115.7	116.5	116.6
C_3-C_4-C_5	114.5	115.2	113.9	113.0	114.1	114.1
O_{13}-C_3-C_4	110.4	111.7	112.4	109.6	109.6	109.4
C_{12}-O_{13}-C_3	113.5	115.0	115.5	115.7	115.3	114.1
C_6-C_{5a}-C_{12a}	113.2	112.5	111.9	111.7	113.0	112.8
C_{8a}-C_{12a}-C_{5a}	112.8	111.1	112.9	113.3	112.8	112.9
O_2-C_3-C_4	113.2	113.1	111.6	111.0	111.3	111.9
C_{5a}-C_6-C_7	111.6	108.6	111.7	111.0	111.1	111.4
C_8-C_{8a}-C_{12a}	111.0	118.0	110.6	110.3	111.3	111.2
C_{15}-C_3-C_4	114.3	113.1	113.6	115.3	114.2	114.5
C_{16}-C_6-C_{5a}	110.8	113.8	110.8	111.3	112.1	112.1
C_9-C_{8a}-C_{12a}	109.2	107.3	108.8	108.5	109.0	109.1
C_{10}-O_{11}-C_{12}	124.6	74.2	121.0	125.9	125.6	124.6
C_{17}-C_9-C_{8a}	113.4	122.4	112.6	114.1	114.5	114.5
% error	-	3.47	0.93	0.81	0.52	0.42

Table 3.1 (Continued)

Parameter	X-ray	CNDO	AM1	HF/ 3-21G	HF/ 6-31G**	B3LYP/ 6-31G**
Torsion Angle (°)						
C_4-C_5-C_{5a}-C_{12a}	323.9	313.6	315.1	321.4	324.7	324.0
C_{12}-O_{13}-C_3-C_4	272.7	283.9	281.4	271.7	269.7	270.6
O_1-C_{12a}-C_{5a}-C_5	69.0	73.7	73.2	68.8	67.5	68.5
O_2-C_3-C_4-C_5	265.8	273.3	269.7	263.3	267.2	265.4
O_{11}-C_{12}-O_{13}-C_3	258.1	241.5	245.7	262.1	260.7	259.2
C_7-C_6-C_{5a}-C_{12a}	46.1	63.8	49.9	50.0	49.3	48.8
C_8-C_{8a}-C_{12a}-C_{5a}	55.5	33.0	53.8	56.4	53.1	53.1
% error	-	11.37	3.29	1.56	1.81	1.36
CPU time	-	1 min.	1 min.	10 hrs.	2 days	2.5 days

Comparing between the optimized and X-ray structures, the HF/3-21G method gives better results than the CNDO and AM1 methods as indicated by its much lower % errors. It also yield better structures than the HF/6-31G** method especially the bond length of the endoperoxide linkage, which is believed to be responsible for the antimalarial activity [17]. The B3LYP/6-31G** method produces the lowest % errors, indicating the best method for optimization. However, in order to determine the suitable structural optimization method, we must consider not only the structure but also the CPU time. The HF/3-21G method requires computational time only 10 hours compared to 2 and 2.5 days for the HF/6-31G** and the B3LYP/6-31G** methods, respectively. As the HF/3-21G method gives geometrical parameters within acceptable accuracy to the X-ray data and requires reasonable computational time, it is the most suitable level of theory for the structural optimization both in terms of accuracy and time. Hence, it was used for the optimizations of all 68 compounds.

3.2 Calculations of Properties

Since there are totally 102 calculated physicochemical parameters for each compound, it seems to be not possible to show all the values. Therefore, only 13 selected parameters are shown. These include 3 atomic charges, 2 bond orders, 2 bond lengths, 2 bond angles, 1 torsion angle, F_{H2O}, log P, and molecular shape index order 2

(Kappa 2). The biological activities for both D-6 and W-2 strains were also included in the tables, log(D-6) and log(W-2) columns. For convenience, all the properties are given according to each group of compounds, i.e., Table 3.2, 3.3, and 3.4 for compounds group 1, 2, and 3, respectively.

Table 3.2 Some selected properties of compounds group 1.

No	log(D-6)	log(W-2)	C_3	O_{13}	O_1	B(3-13)	B(9-10)	R(1-12a)
1	0.23	0.79	0.585	-0.620	-0.327	0.783	0.848	1.476
2	0.00	0.00	0.585	-0.620	-0.328	0.783	0.830	1.477
3	0.83	1.11	0.585	-0.619	-0.327	0.783	0.831	1.477
4	0.78	1.13	0.585	-0.619	-0.327	0.783	0.831	1.477
5	0.35	-0.04	0.585	-0.619	-0.327	0.782	0.839	1.477
6	0.04	0.17	0.585	-0.619	-0.327	0.783	0.831	1.477
7	-0.64	-0.55	0.585	-0.619	-0.327	0.783	0.832	1.478
8	0.64	1.02	0.585	-0.619	-0.327	0.783	0.831	1.477
9	0.77	0.84	0.585	-0.619	-0.327	0.783	0.831	1.477
10	-0.41	-0.04	0.585	-0.619	-0.327	0.783	0.831	1.477
11	0.35	0.12	0.585	-0.619	-0.327	0.782	0.832	1.477
12	0.92	0.78	0.585	-0.619	-0.327	0.782	0.832	1.477
13	0.29	0.63	0.585	-0.619	-0.327	0.783	0.831	1.477
14	-2.70	-2.52	0.585	-0.619	-0.327	0.780	0.835	1.476
15	-0.47	-0.10	0.585	-0.618	-0.327	0.782	0.831	1.477
16	-0.06	0.05	0.592	-0.619	-0.325	0.778	0.848	1.477
17	-0.70	-0.74	0.593	-0.621	-0.325	0.774	0.848	1.477
18	-0.28	-0.35	0.596	-0.624	-0.326	0.769	0.848	1.475
19	0.37	0.37	0.594	-0.612	-0.318	0.791	0.850	1.479
20	-1.52	-2.00	0.592	-0.624	-0.327	0.768	0.848	1.475
21	0.06	0.10	0.590	-0.623	-0.322	0.774	0.848	1.477
22	0.34	0.45	0.593	-0.622	-0.325	0.774	0.848	1.476

Table 3.2 (Continued)

No	R(3-4)	A(1-2-3)	A(10-11-12)	T(4-5-5a-12a)	F_{H2O}	log P	Kappa 2
1	1.537	107.2	126.5	-38.5	-24.79	2.523	4.014
2	1.537	107.1	125.9	-38.6	-24.38	3.085	4.263
3	1.537	107.2	125.2	-38.8	-24.11	3.482	4.747
4	1.537	107.1	125.3	-38.8	-23.91	3.878	5.250
5	1.537	107.1	124.4	-39.0	-23.76	3.812	4.997
6	1.537	107.1	125.3	-38.8	-23.70	4.274	5.772
7	1.537	107.2	125.4	-38.8	-23.60	4.208	16.467
8	1.537	107.1	125.3	-38.8	-23.50	4.670	17.416
9	1.537	107.2	125.3	-38.8	-23.30	5.067	18.367
10	1.537	107.1	125.4	-38.8	-23.20	5.001	18.367
11	1.537	107.1	125.5	-38.8	-23.93	5.095	18.993
12	1.537	107.2	125.3	-38.8	-23.73	5.491	19.934
13	1.537	107.1	125.4	-38.8	-23.53	5.888	20.878
14	1.537	107.1	125.8	-38.5	-42.85	2.249	16.467
15	1.537	107.2	123.6	-39.0	-23.91	3.663	15.523
16	1.538	107.3	126.5	-38.7	-24.59	3.091	4.497
17	1.538	107.4	126.5	-38.7	-24.19	3.884	5.523
18	1.539	107.7	126.2	-38.3	-24.25	3.594	4.747
19	1.540	107.3	127.7	-38.5	-37.45	2.577	6.311
20	1.541	107.0	127.1	-39.5	-24.62	4.308	6.270
21	1.537	107.6	125.9	-38.4	-24.07	5.223	7.053
22	1.538	107.5	125.9	-38.6	-24.21	5.101	7.356

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Table 3.3 Some selected properties of compounds group 2.

No	log(D-6)	log(W-2)	C_3	O_{13}	O_1	B(3-13)	B(9-10)	R(1-12a)
23	0.86	-0.02	0.585	-0.624	-0.325	0.789	0.870	1.483
24	0.45	0.55	0.585	-0.625	-0.325	0.789	0.874	1.483
25	0.81	0.52	0.585	-0.624	-0.325	0.788	0.879	1.482
26	0.32	0.13	0.585	-0.624	-0.326	0.788	0.875	1.482
27	0.11	-0.02	0.585	-0.625	-0.324	0.788	0.875	1.483
28	0.77	0.44	0.585	-0.624	-0.325	0.787	0.875	1.482
29	0.03	-0.15	0.585	-0.624	-0.326	0.787	0.875	1.482
30	0.41	0.89	0.585	-0.622	-0.327	0.788	0.877	1.483
31	0.58	0.61	0.585	-0.624	-0.325	0.788	0.877	1.482
32	-1.40	-0.51	0.585	-0.626	-0.323	0.790	0.876	1.484
33	-0.46	-1.52	0.585	-0.624	-0.325	0.788	0.878	1.482
34	0.29	0.16	0.585	-0.623	-0.326	0.790	0.881	1.482
35	0.39	0.31	0.587	-0.621	-0.342	0.790	0.840	1.493
36	-0.70	-0.72	0.587	-0.622	-0.324	0.786	0.855	1.485
37	-0.28	-0.46	0.587	-0.623	-0.341	0.794	0.839	1.493
38	-1.05	-1.15	0.588	-0.623	-0.324	0.791	0.853	1.486
39	0.56	0.35	0.585	-0.625	-0.325	0.789	0.874	1.483
40	1.10	1.23	0.585	-0.623	-0.326	0.787	0.880	1.482
41	0.85	0.85	0.586	-0.631	-0.325	0.781	0.874	1.482
42	0.88	0.89	0.585	-0.624	-0.325	0.788	0.877	1.482
43	0.91	1.06	0.585	-0.623	-0.326	0.789	0.875	1.483
44	0.86	0.90	0.584	-0.623	-0.325	0.784	0.874	1.481
45	0.60	0.68	0.585	-0.625	-0.325	0.788	0.877	1.482
46	0.72	0.68	0.585	-0.624	-0.325	0.788	0.875	1.483
47	-0.21	-0.21	0.585	-0.624	-0.325	0.787	0.876	1.482
48	0.08	0.10	0.585	-0.625	-0.325	0.789	0.876	1.482
49	0.12	0.09	0.584	-0.623	-0.325	0.785	0.877	1.481
50	-1.80	-2.40	0.584	-0.623	-0.318	0.785	0.901	1.480
51	0.14	0.21	0.584	-0.623	-0.326	0.789	0.874	1.480
52	0.24	0.17	0.584	-0.623	-0.323	0.787	0.873	1.478

Table 3.3 (Continued)

No	R(3-4)	A(1-2-3)	A(10-11-12)	T(4-5-5a-12a)	F_{H2O}	log P	Kappa 2
23	1.534	108.1	117.1	-39.6	-24.38	3.053	4.263
24	1.534	108.0	117.2	-39.5	-18.76	3.331	4.747
25	1.534	108.0	117.8	-39.4	-31.41	3.247	7.111
26	1.534	108.0	117.3	-39.5	-21.41	3.086	7.111
27	1.534	108.0	117.2	-39.5	-31.21	3.337	7.680
28	1.534	108.0	117.7	-39.5	-31.29	4.838	8.652
29	1.534	108.0	117.7	-39.5	-36.97	4.806	8.094
30	1.535	107.9	119.4	-39.9	-72.85	2.352	13.619
31	1.535	108.0	118.3	-39.4	-38.60	4.597	8.507
32	1.534	108.1	117.3	-39.5	-58.97	1.836	8.045
33	1.534	108.0	118.0	-39.5	-58.97	1.836	8.045
34	1.535	107.9	118.4	-39.4	-48.60	3.217	8.250
35	1.532	108.4	117.1	-39.9	-30.40	3.357	5.998
36	1.534	107.7	115.6	-40.7	-30.40	3.357	5.998
37	1.533	108.5	115.9	-40.0	-27.69	2.724	5.247
38	1.534	107.7	114.7	-40.9	-27.69	2.724	5.247
39	1.534	108.1	117.2	-39.5	-17.63	6.637	8.982
40	1.534	108.0	118.4	-39.4	-31.09	5.218	9.223
41	1.535	107.9	117.8	-38.8	-31.09	5.218	9.223
42	1.534	108.0	117.8	-39.4	-30.89	5.275	9.806
43	1.534	108.0	117.8	-39.6	-30.89	5.275	9.806
44	1.534	107.9	118.6	-39.2	-51.96	3.808	10.873
45	1.534	108.0	117.7	-39.4	-30.95	5.251	8.897
46	1.534	108.0	117.8	-39.5	-30.95	5.251	8.897
47	1.534	108.0	117.7	-39.4	-36.63	5.219	8.340
48	1.535	108.0	117.9	-39.4	-36.63	5.219	8.340
49	1.534	108.0	118.3	-39.3	-57.84	3.434	9.708
50	1.534	108.1	116.4	-39.6	-24.38	2.832	4.263
51	1.536	108.3	116.9	-40.7	-27.99	2.261	5.772
52	1.535	108.1	116.6	-40.0	-25.57	3.595	5.497

Table 3.4 Some selected properties of compounds group 3.

No	log(D-6)	log(W-2)	C_3	O_{13}	O_1	B(3-13)	B(9-10)	R(1-12a)
53	0.37	0.28	0.585	-0.624	-0.325	0.790	0.885	1.481
54	0.82	0.75	0.585	-0.624	-0.326	0.791	0.881	1.481
55	0.96	0.67	0.585	-0.624	-0.326	0.791	0.881	1.481
56	0.67	0.74	0.585	-0.624	-0.326	0.791	0.881	1.481
57	1.77	1.32	0.585	-0.624	-0.326	0.791	0.882	1.481
58	0.23	0.16	0.585	-0.624	-0.326	0.791	0.882	1.481
59	1.71	1.40	0.585	-0.624	-0.326	0.791	0.882	1.480
60	1.84	1.52	0.585	-0.624	-0.326	0.790	0.881	1.480
61	-1.00	-1.00	0.588	-0.626	-0.323	0.781	0.885	1.480
62	0.86	0.84	0.591	-0.625	-0.323	0.781	0.885	1.480
63	0.81	0.75	0.592	-0.625	-0.323	0.781	0.885	1.480
64	0.26	0.40	0.593	-0.622	-0.322	0.782	0.885	1.480
65	0.53	0.58	0.592	-0.626	-0.322	0.781	0.885	1.480
66	-1.22	-1.70	0.589	-0.626	-0.321	0.780	0.885	1.480
67	-0.89	-0.55	0.592	-0.626	-0.320	0.783	0.885	1.480
68	-3.05	-3.05	0.590	-0.627	-0.318	0.783	0.885	1.481

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Table 3.4 (Continued)

No	R(3-4)	A(1-2-3)	A(10-11-12)	T(4-5-5a-12a)	F_{H2O}	log P	Kappa 2
53	1.535	107.9	116.0	-39.6	-15.50	2.855	3.765
54	1.535	107.9	115.9	-39.4	-15.15	3.258	4.014
55	1.535	107.9	115.9	-39.5	-14.95	3.654	4.497
56	1.535	107.9	115.9	-39.5	-14.75	4.050	5.000
57	1.535	107.9	115.9	-39.5	-14.54	4.447	5.523
58	1.535	107.9	115.9	-39.5	-14.34	4.843	6.064
59	1.535	107.9	116.0	-39.4	-14.57	5.664	7.356
60	1.535	107.9	116.0	-39.5	-14.22	6.182	7.602
61	1.536	108.3	115.4	-39.6	-15.29	3.423	4.247
62	1.536	108.4	115.3	-39.6	-15.09	3.820	4.750
63	1.536	108.3	115.4	-39.6	-14.89	4.216	5.274
64	1.539	108.2	116.1	-40.2	-14.75	4.150	5.000
65	1.536	108.3	115.4	-39.6	-14.71	5.830	7.680
66	1.535	108.4	115.4	-39.6	-15.12	5.037	6.558
67	1.535	108.3	115.5	-39.5	-14.57	5.951	7.356
68	1.534	108.4	115.4	-39.6	-33.83	2.587	5.523

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3.3 QSAR Models

All the calculated physicochemical properties were related to the antimalarial activity by the multiple linear regression analysis using the stepwise procedure. The methodology of the stepwise method is to start with the best single variable to build the model and then add further significant variables, according to their contribution to the model. During the process, there is a proof whether already introduced variables are no longer significant at a later stage. If it is, this variable is excluded from the equation. The adding and proofing process continues until a static model is reached.

3.3.1 All 68 Compounds

In order to access the real predictive ability, compounds were divided into the training set (90%) and testing set (10%). Therefore, seven compounds were randomly chosen from each group of compounds for the testing set, i.e., compound number 5, 18, 30, 38, 41, 54, and 67, which include both high and low activities. The remaining 61 compounds, the training set, were used to derive the model. For the log(D-6) activity, three parameters were presented in the model as shown in equation (3.1) with the r^2 and q^2 of 0.444 and 0.386, respectively. For the log(W-2) activity, also three parameters were employed in the model, giving the r^2 and q^2 values of 0.413 and 0.318, equation (3.2). These two models have r^2 values of less than 0.81 and q^2 values of less than 0.50, hence poor models.

$$\log D\text{-}6 = 96.23*B(3\text{-}13) - 0.278*A(1\text{-}2\text{-}3) + 0.412*\log P - 47.09 \quad .....(3.1)$$

$r^2 = 0.444, \quad q^2 = 0.386, \quad s = 0.709, \quad n = 61, \quad F = 15.193$

$$\log W\text{-}2 = 117.66*B(3\text{-}13) - 109.52*R(5a\text{-}12a) + 0.427*\log P + 74.73 \quad .....(3.2)$$

$r^2 = 0.413, \quad q^2 = 0.318, \quad s = 0.751, \quad n = 61, \quad F = 13.358$

The variables in the two models are quite similar, i.e., they both have the B(3-13) and log P variables in the equations. However, the only difference is the A(1-2-3) variable in the log(D-6) model and the R(5a-12a) variable in the log(W-2) model. As the activity depends on all the variables presented in the model, using only one variable to predict the activity is not appropriate and it may not give a proper activity value. However, the analysis on each individual variable could give useful information

although in qualitative manner. The obtained information provides the understanding in the mode of action, which is very helpful for the design of new more effective drugs.

The B(3-13) variable is referred to a strength of the bond between atom 3 and 13, hence, it is related to how easily to break this bond which may involve in the mechanism of action. Therefore, the plus sign of its coefficient in both models indicates that compounds with higher B(3-13), i.e., difficult to break this bond, are more active. Considering the log P variable, a plus sign of its coefficient points out that compounds with higher log P are more active. The log P is defined as the ratio of drug concentrations in the *n*-octanol and water solvents, therefore, compound that can be dissolved more favorably in *n*-octanol than in water (high log P) should have high activity.

The comparisons between actual and predicted values of 7 compounds in the testing set for log(D-6) and log(W-2) activities are displayed in Figure 3.1 and 3.2, respectively.

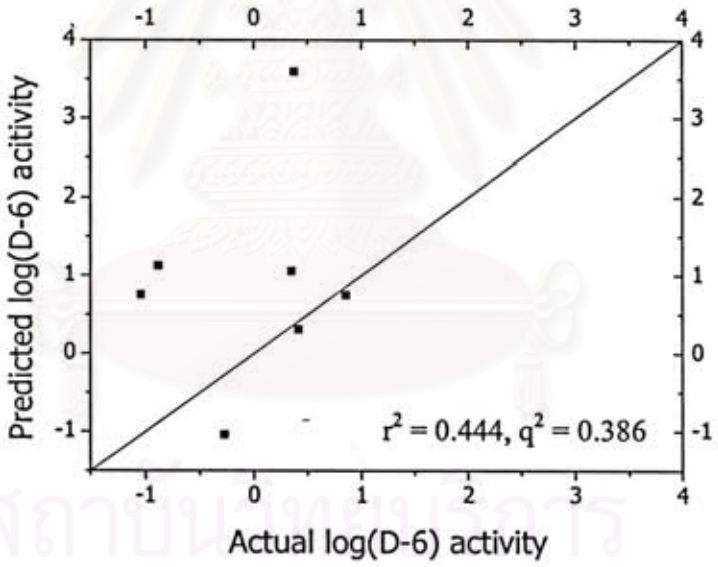

Figure 3.1 Comparison between actual and predicted log(D-6) activities for 7 compounds in the testing set.

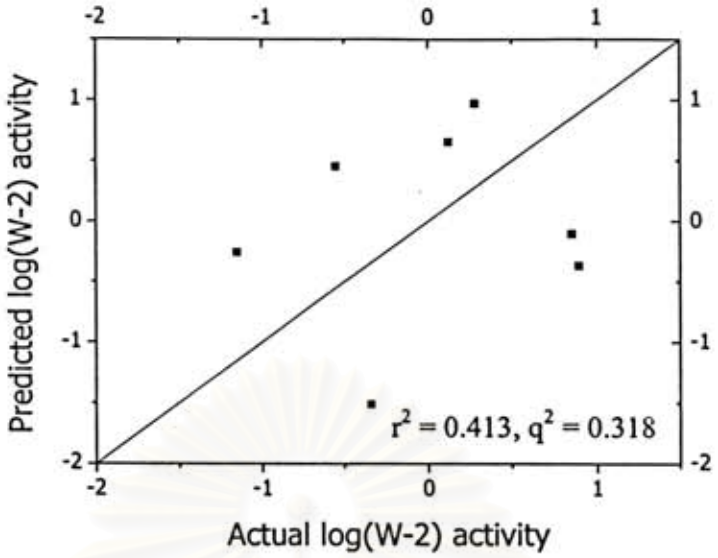

Figure 3.2 Comparison between actual and predicted log(W-2) activities for 7 compounds in the testing set.

From the models in equations (3.1) and (3.2) as well as the predicted activities in Figure 3.1 and 3.2, it can be seen that a good QSAR model for the whole set of compounds could not be achieved from our calculated physicochemical properties. This may be attributed by the structural differences among the compounds especially compounds with C=O group at the C_{10} position. This group brings about the change in conformation of the lactone ring, which makes a compound different from other compounds without this substituent. Moreover, such a substituent also causes the substituent groups at C_3 and C_9 position to manifest their effects differently from those in other compounds (see Chapter 2). This is also possibly one of the reasons for the poor predictive models when compounds of this group were considered together with other compounds. Therefore, the QSAR analysis was separately performed for each individual group of compounds with more closely related structures.

3.3.2 Compounds with C=O at C_{10} position (Group 1)

Considering only the compounds having C=O group at the C_{10} position (group 1 in Chapter 2), there are 22 compounds belonging to this group. Compound number 5 and 18 were randomly selected for the testing set. However, the log(W-2) activity of both compounds has a minus sign (low activity). Therefore, in order to check whether the model can be used for both high and low activities, the testing set for log(W-2) model was randomly selected from each high and low activity compounds, i.e.,

compound number 11 and 18. The remaining 20 compounds were used for the training set. The results for log(D-6) and log(W-2) activities are shown in equations (3.3) and (3.4), respectively. Moreover, the correlation coefficients between each pair of variables presented in the models are given in Table 3.5.

$$\log D\text{-}6 = 221.30*O_{13} + 1.063*T(4\text{-}5\text{-}5a\text{-}12a) + 0.193*F_{h2o} - 0.00453*\text{Kappa 2} + 182.378 \quad .....(3.3)$$

$r^2 = 0.818$, $q^2 = 0.504$, $s = 0.531$, $n = 20$, $F = 29.158$

$$\log W\text{-}2 = 246.79*O_{13} + 1.599*T(4\text{-}5\text{-}5a\text{-}12a) + 0.205*F_{h2o} - 0.00409*\text{Kappa 2} + 219.43 \quad .....(3.4)$$

$r^2 = 0.826$, $q^2 = 0.467$, $s = 0.543$, $n = 20$, $F = 20.971$

Table 3.5 Correlation coefficients between each pair of variables presented in the models of compound group 1.

	log D-6	log W-2	O_{13}	T(4-5-5a-12a)	F_{h2o}	Kappa 2
log D-6	1.000	0.969	0.130	0.185	0.546	-0.027
log W-2	0.969	1.000	0.163	0.262	0.511	-0.011
O_{13}	0.130	0.163	1.000	0.349	-0.497	0.096
T(4-5-5a-12a)	0.185	0.262	0.349	1.000	-0.323	-0.154
F_{h2o}	0.546	0.511	-0.497	-0.323	1.000	0.009
Kappa 2	-0.027	-0.011	0.096	-0.154	0.009	1.000

The r^2 and q^2 values for the log(D-6) model are quite good. For the log(W-2) model, the r^2 value is greater than 0.81, hence, acceptable model. However, its q^2 value is slightly lower than the acceptable value of 0.50. The correlation coefficients between each pair of variables shown in Table 3.5 shows that the F_{h2o} parameter has the highest relationship with both activities. Moreover, all the interdescriptor correlation coefficients are less than 0.5, which indicate no intercorrelation between each variable in the model, hence, acceptable model [59].

Both models use the same set of parameters. The F_{h2o} parameter (the desolvation free energy for water) refers to the ease for desolvating water molecule from a drug compound. Hence, a plus sign of its coefficient points out that compounds with higher F_{h2o} values (hard to desolvate water molecules) have higher activities. For example,

compound 14 having low F_{h2o} (-42.85 kJ/mol) has low activity. Therefore, this suggests that water molecules may play some important roles in the mechanism of action. The T (4-5-5a-12a) parameter, which is presented in both models, indicates that this torsion angle may involve in the structural change during reactions in the mechanism of action (see section 1.3.4 in Chapter 1). Compound with smaller T(4-5-5a-12a), i.e., less negative, has higher activity as indicated by a plus sign of its coefficient in the equations. For example, the angles in compound 15 and 20 are large (-39.0° and -39.5°, respectively), hence low activities. Note that a negative value of the torsion angle is assigned to the counter-clockwise measurement. The reason for this relationship may be that the compound must change its structure to have a positive T(4-5-5a-12a) angle during the reaction mechanism. Therefore, compound with less negative (smaller) T(4-5-5a-12a) angle is easier to achieve this change. A plus sign of O_{13} parameter refers that compounds with less negative O_{13} charge and therefore stabilize O free radicals have higher activities. A minus sign of Kappa 2 parameter indicates that compounds with low degree of linearity have higher activities.

The relationships between actual and predicted values for log(D-6) and log(W-2) activities of 20 compounds in the training set are shown in Figure 3.3 and 3.4, respectively.

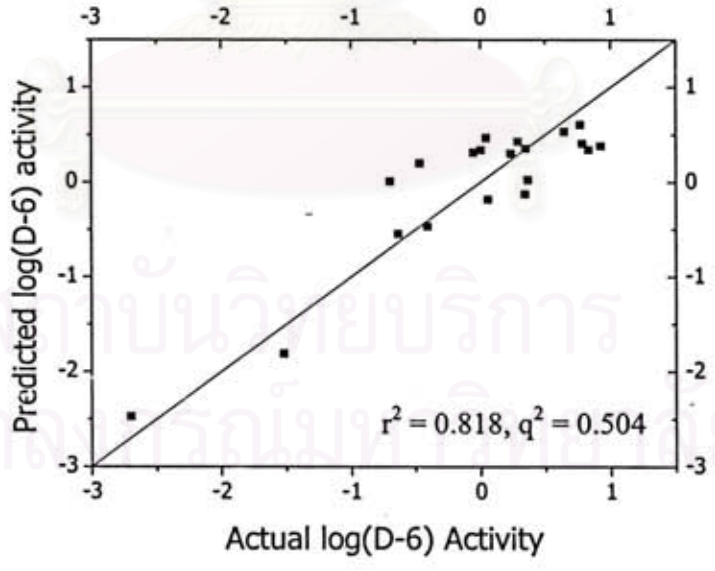

Figure 3.3 Relationship between actual and predicted log(D-6) activities for 20 compounds in training set of group 1.

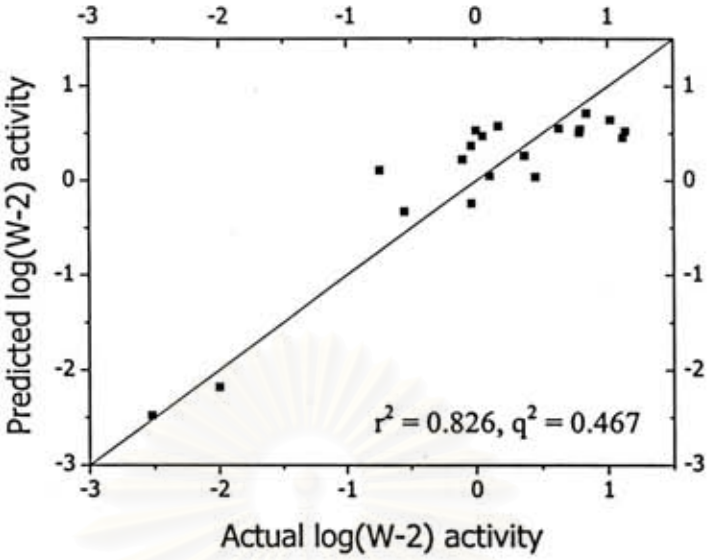

Figure 3.4 Relationship between actual and predicted log(W-2) activities for 20 compounds in training set of group 1.

The comparisons between actual and predicted values of 2 compounds in the testing set for log(D-6) and log(W-2) activities are given in Table 3.6. The predictions for log(D-6) activity are quite good and those for log(W-2) are fairly good.

Table 3.6 Activity predictions of 2 compounds in the testing set of group 1.

Compound No.	log(D-6) activity		Compound No.	log(W-2) activity	
	Actual	Predicted		Actual	Predicted
5	0.35	0.34	11	0.12	0.51
18	-0.28	-0.36	18	-0.35	-0.12

3.3.3 Compounds with -OR at C_{10} position (Group 2)

Group 2 consists of 30 compounds with -OR group at the C_{10} position. Compounds number 30, 38, and 41 were randomly selected for the testing set. The QSAR results for 27 compounds in the training set are given in equations (3.5) and (3.6). The correlation coefficients between each pair of variables presented in the models are given in Table 3.7.

$$\log D\text{-}6 = -56.85*B(9\text{-}10) - 122.49*R(1\text{-}12a) + 0.99*A(10\text{-}11\text{-}12) + 0.0467*F_{h2o} + 0.0290*\text{Kappa 2} + 116.29 \quad \text{.....(3.5)}$$

$r^2 = 0.902$, $q^2 = 0.834$, $s = 0.241$, $n = 27$, $F = 38.456$

$$\log W\text{-}2 = -50.52*B(9\text{-}10) + 544.29*R(3\text{-}4) + 0.75*A(10\text{-}11\text{-}12) + 0.0444*F_{h2o} + 0.16*\text{Kappa 2} - 878.765 \quad .....(3.6)$$

$r^2 = 0.838$, $q^2 = 0.576$, $s = 0.345$, $n = 27$, $F = 21.694$

Table 3.7 Correlation coefficients between each pair of variables presented in the models of compound group 2.

	log D-6	log W-2	B(9-10)	R(3-4)	A(10-11-12)	F_{h2o}	Kappa 2
log D-6	1.000	0.891	-0.103	-0.048	0.517	0.243	0.411
log W-2	0.891	1.000	-0.134	-0.030	0.497	0.157	0.415
B(9-10)	-0.103	-0.134	1.000	-0.459	0.440	-0.137	0.236
R(3-4)	-0.048	-0.030	-0.459	1.000	-0.361	0.108	-0.228
A(10-11-12)	0.517	0.497	0.440	-0.361	1.000	-0.416	0.452
F_{h2o}	0.243	0.157	-0.137	0.108	-0.416	1.000	-0.492
Kappa 2	0.411	0.415	0.236	-0.228	0.452	-0.492	1.000

The r^2 and q^2 values for both models are very impressive. The correlation coefficients between each pair of variables shown in Table 3.5 confirmed that our models are acceptable, since all variables in the model have no intercorrelation.

In both models, the A(10-11-12) parameter has the highest correlation coefficient with both activities (see Table 3.7), which suggests that this parameter may have a contribution to the structural change in the reaction mechanism. A minus sign of the B(9-10) parameter, bond order between atom 9 and 10, in both models indicates that compounds with lower B(9-10) values (i.e., weak bond) have higher activities. This parameter may related to the reaction mechanism involving the change in lactone ring from compound 3B to compound 3C and 3D (see Figure 1.7 in Chapter 1). The weak bond between C_9 and C_{10} could facilitate this lactone ring rearrangement. The explanations for the importance of the F_{h2o} parameter and Kappa 2 in both models are the same as described for the model (3.3) and (3.4) in the section 3.3.2.

The relationships between actual and predicted values for log(D-6) and log(W-2) activities of 27 compounds in the training set are shown in Figure 3.5 and 3.6, respectively.

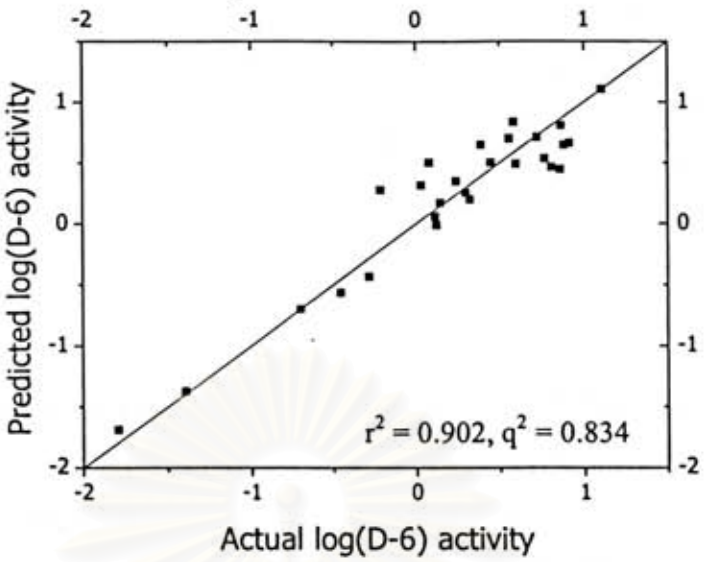

Figure 3.5 Relationship between actual and predicted log(D-6) activities for 27 compounds in training set of group 2.

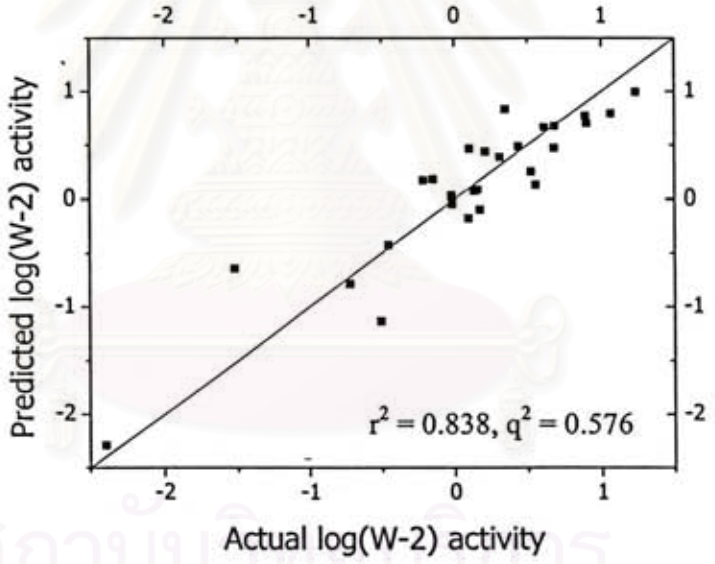

Figure 3.6 Relationship between actual and predicted log(W-2) activities for 27 compounds in training set of group 2.

The comparison between actual and predicted values of 3 compounds in the testing set for log(D-6) and log(W-2) activities are given in Table 3.8. The predictions are excellent.

Table 3.8 Activity predictions of 3 compounds in the testing set of group 2.

Compound No.	log(D-6) activity		log(W-2) activity	
	Actual	Predicted	Actual	Predicted
30	-1.05	-1.55	0.89	0.91
38	0.85	0.90	-1.15	-1.22
41	0.41	0.25	0.85	0.89

3.3.4 Compounds without a substituent at C_{10} position (Group 3)

Compounds in group 3 have no substituent group at the C_{10} position. Totally 16 compounds are belonging to this group. Compounds number 54 and 67 were randomly selected for the testing set. The QSAR models were derived from 14 compounds in the training set. Two parameters, C_3 and O_1 atomic charges, were statistically selected for the models of both activities. The r^2 and q^2 values of 0.876 and 0.846 for log(D-6) activity and 0.917 and 0.885 for log(W-2) activity indicate the excellent models. The QSAR models are given in equation (3.7) and (3.8). The correlation coefficients between each pair of variables presented in the models are given in Table 3.9.

$$\log \text{D-6} = 234.19*C_3 - 688.34*O_1 - 360.115 \quad \text{.....(3.7)}$$

$r^2 = 0.876$, $q^2 = 0.846$, $s = 0.510$, $n = 14$, $F = 38.921$

$$\log \text{W-2} = 273.23*C_3 - 705.18*O_1 - 388.642 \quad \text{.....(3.8)}$$

$r^2 = 0.917$, $q^2 = 0.885$, $s = 0.405$, $n = 14$, $F = 60.779$

Table 3.9 Correlation coefficients between each pair of variables presented in the models of compound group 3.

	log D-6	log W-2	C_3	O_1
log D-6	1.000	0.989	-0.387	-0.853
log W-2	0.989	1.000	-0.324	-0.838
C_3	-0.387	-0.324	1.000	0.451
O_1	-0.853	-0.838	0.451	1.000

As in the models of compound group 1 and 2, there is no intercorrelation between variables in the QSAR models, i.e., correlation coefficient between C_3 and O_1 is only 0.451, which is less than 0.5. The presence of C_3 and O_1 atomic charges in both models indicates that compounds with more negative O_1 charge and less C_3 charge would have higher activities. From Table 3.9, the correlation coefficients between O_1 charge and activities are higher than those between C_3 charge and activities (-0.853 and -0.838 compared to -0.387 and -0.324). Therefore, the O_1 charge is statistically more important.

The relationships between actual and predicted activity values of 14 compounds in the training set are shown in Figure 3.7 and 3.8, respectively. These relationships together with the activity predictions of 2 compounds in the testing set (Table 3.10) indicate the high predictive ability of the models.

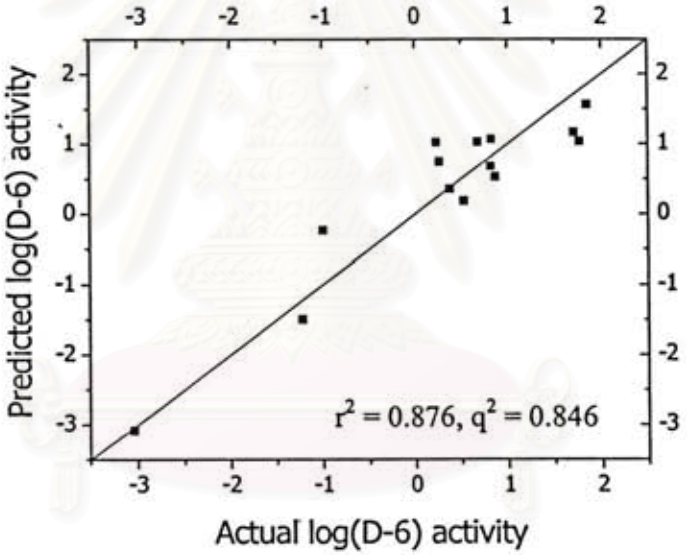

Figure 3.7 Relationship between actual and predicted log(D-6) activities for 14 compounds in training set of group 3.

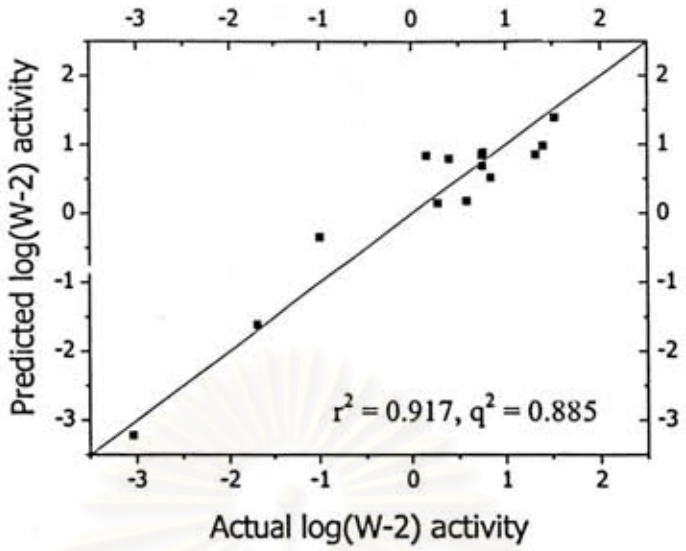

Figure 3.8 Relationship between actual and predicted log(W-2) activities for 14 compounds in training set of group 3.

Table 3.10 Activity predictions of 2 compounds in the testing set of group 3.

Compound No.	log(D-6) activity		log(W-2) activity	
	Actual	Predicted	Actual	Predicted
54	0.37	0.34	0.28	0.11
67	-0.89	-1.05	-0.55	-1.07

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CHAPTER 4
Conclusions

4.1 QSAR Models

The relationships between antimalarial activities and molecular properties of 68 artemisinin compounds were investigated in the quantitative manner (QSAR) using activities against two strains of malarial parasite, i.e., D-6 and W-2. As the X-ray data is not available for all the compounds used in this study, the structures were obtained theoretically using the quantum chemical calculations with the HF/3-21G method, which was justified to be a suitable method for geometry optimization of our compounds.

From the statistical analysis, good relationships could not be derived for the group of all compounds, i.e., both r^2 and q^2 values are below the acceptable value. However, if the compounds were divided into 3 groups according to the structural similarity, impressive relationships with high predictive power were obtained for each group of compounds. For compounds with C=O at the C_{10} position (group 1), models with quite good quality were obtained. For the compounds with -OR at the C_{10} position (group 2) excellent models were derived for both activities, i.e., r^2 and q^2 values of 0.902 and 0.838 for the log(D-6) and r^2 and q^2 values of 0.834 and 0.576 for the log(W-2). In case of compounds without substituent at the C_{10} position (group 3), excellent predictive models were found with r^2 values of 0.876 and 0.917, and q^2 values of 0.846 and 0.885.

The F_{h2o} parameter was presented in models of group 1 and 2. This implies the important effect of water molecules in reaction mechanism for antimalarial activities. However, this parameter appeared to have no significant role for the compounds in group 3 since it was not included in the models. Comparing the structures of the compounds in group 3 with the other two groups, water molecules possibly interact with the substituent group (C=O, and -OR) at the C_{10} position. Also, the shape index order 2 (Kappa 2) was included in models for group 1 and 2. The Kappa 2 indicated the degree of linearity. The A(10-11-12) parameter was presented only in the models for the compounds in group 2. The reason is that the compounds in group 1 and 3 have no

variation in the substituent group at the C_{10} position. Therefore, this angle was kept nearly unchanged and does not related to the activities. From all the results, the antimalarial activities of artemisinin compounds could be mainly described by their structures.

Interestingly, our QSAR results support the docking results between artemisinin compounds and heme [72-73], which involved the interaction between heme iron and endoperoxide moiety of artemisinin compound. From the docking calculations, the heme iron prefers to attack the O_1 of the endoperoxide. This is possibly due to the more negative charge of O_1 over O_2. Our QSAR models indicate that compound with more negative O_1 charge becomes more active, which is in agreement with the docking results.

Finally, the real predictive ability of each model was judged from the comparison between actual and predicted activities of compounds in the testing set. The obtained models can predict the activities very close to the experimental values, thus confirming their high predictive power. Furthermore, our models are statistically better than those of the previous works [74-78].

4.2 Suggestions for New Compounds

Using the information obtained from all investigations in this study, we could afford some suggestions on the structural modification of artemisinin compounds to enhance the activities as follows. Firstly, it is not necessary to include a substituent group at the C_{10} position. Secondly, the new compound must have moderate solubility in both water and oil. Finally, it is advised to include substituent at either C_3 or C_9 positions that causes the atomic charge of O_1 atom to be more negative and of C_3 atom to be more positive.

According to the above suggestions, we proposed the structures of 3 new potent compounds (compound number 69 to 71), which belong to compound group 3. As all $-R_1$ groups (substituent at the C_3 position) in compound group 3 are electron donating groups (EDG), varying the EDG at this position to make the O_1 charge more negative and C_3 charge more positive should yield compounds with higher activities. Therefore, $-C_6H_5$ group was selected in compound 69. Changing the substituent at the C_9 position ($-R_2$ group) can also make an enhancement in activities. As a result, we changed the

$-R_2$ group to be –F and $-C{=}OCH_3$ for compounds 70 and 71, respectively, while the $-R_1$ group is $-CH_3$. The biological activities of these 3 proposed compounds were predicted using our QSAR models. The structures and predicted activities of all 3 proposed artemisinin compounds are given in Figure 4.1 and Table 4.1.

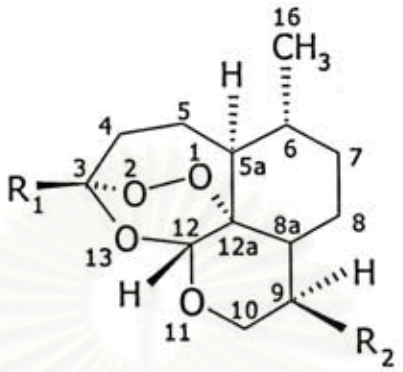

Figure 4.1 Structures of proposed artemisinin compounds number 69-71.

Table 4.1 Structures and biological data of 3 proposed artemisinin compounds.

No	R_1	R_2	O_1 charge	C_3 charge	log(D-6)	log(W-2)
69	C_6H_5	CH_3	-0.323	0.596	2.18	2.37
70	CH_3	F	-0.328	0.584	2.16	1.95
71	CH_3	$C{=}OCH_3$	-0.327	0.585	1.80	1.61

From Table 4.1, the antimalarial activities of all 3 proposed compounds were predicted using our models to be very high compared to the highest activities of 1.84 and 1.52 for log(D-6) and log(W-2) of compound number 60 in group 3. The $-C_6H_5$ group at the C_3 position influences the C_3 charge to be more positive, hence activities of compound 69 were much enhanced. On the other hand, the –F and $-C{=}OCH_3$ groups at the C_9 position induce the O_1 charge to be more negative. Therefore, the improvement in biological activities is evidently shown.

4.3 Suggestions for Further Works

It is very interesting to further study the QSAR. In order to find a better QSAR model, a non-linear statistical method is worth to apply. This is because the relationship between molecular properties and biological activity may not be a linear form.

REFERENCES

1. Malaria Unit, World Health Organization. Global malaria control. Bull. WHO 71 (1993): 281-284.
2. World Health Organization. The World Health Report 1996.
3. Butter, D.; Maurice, J.; and O'Brien, C. Time to put malaria control on the global agenda. Nature 386 (1997): 535-536.
4. กรองทอง ทิมาสาร. ความสูญเสียจากโรคมาลาเรีย. วารสารมาลาเรีย 33 (1998): 217-218.
5. Wyler D.J. Malaria: overview and update. Clin. Infect. Dis. 16 (1993): 449-458.
6. Butler, A.R.; and Wu, Y.-L. Artemisinin (Qinghaosu): A New Type of Antimalarial Drug. Chem. Soc. Rev. (1992): 85-90.
7. Bruce-Chwatt, L.J. (ed.), Chemotherapy of Malaria. Revised second edition, England: World Health Organization, 1986.
8. Curd, F.H.S.; Davey, D.G.; and Rose, F.L. Studies on synthetic antimalarial drugs. X. Some biguanide derivatives as new types of antimalarial substances with both therapeutic and causal prophylactic activity. Ann. Trop. Med. Parasitol. 39 (1945): 208-216.
9. Falco, E.A.; Goodwin, L.G.; Hitchings, G.H.; Rollo, I.M.; and Russell, P.B. 2,4-Diaminopyrimidines – a new series of antimalarials. Br. J. Pharmac. Chemother. 6 (1951): 185-200.
10. Moore, D.V.; and Lanier, J.E. Observations on two *Plasmodium falciparum* infections with an abnormal response to chloroquine. Am. J. Trop. Med. Hyg. 10 (1961): 5-9.
11. Canfield, C.J.; and Heiffer, M.H. The U.S. Army Drug Development Program. In M. Adolphe (ed.), Advances in Pharmacology and Therapeutics, Proceedings of the 7th International Congress of Pharmacology, Paris 1978, Volume 10 Chemotherapy, pp. 99-108. Paris, 1978.
12. Berrett, P.J.; Emmins, P.D.; Clarke, P.D.; and Bradley, D.J. Comparison of adverse events associated with use of mefloquine and combination of chloroquine and proguanil as antimalarial prophylaxis: Postal and telephone survey of travellers. Br. Med. J. 313 (1996): 525-528.

13. Olliaro, P.L.; and Trigg, P.I. Status of antimalarial drugs under development. Bull. WHO 73 (1995): 565-571.

14. Mockenhaupt, F.P. Mefloquine resistance in *Plasmodium falciparum*. Parasitol. Today 11 (1995): 248.

15. Boudreau, E.F.; Webster, H.K.; Pavanand, K.; and Thosingha, L. Type II mefloquine resistance in Thailand. Correspondence. Lancet 2 (1982): 1335.

16. Qinghaosu Antimalaria Coordinating Research Group. Antimalaria Studies on Qinghaosu. Chin. Med. J. 92 (1979): 811-816.

17. China Cooperative Research Group on Qinghaosu and Its Derivatives as Antimalarials. Chemical studies on qinghaosu (artemisinin), J. Tradit. Chin. Med. 2 (1982): 3-8.

18. Luo, X.-D.; Yeh, H.J.C.; Brossi, A.; Flippen-Anderson, J.L.; and Gilardi, R. The Chemistry of Drugs: Configurations of Antimalarials Derived from Qinghaosu: Dihydroqinghaosu, Artemether, and Artesunic Acid. Helv. Chim. Acta 67 (1984): 1515-1522.

19. Leban, I.; and Golic, L. Crystal and molecular structure of Qinghaosu: a redetermination. Acta Pharm. Jugosl. 38 (1988): 71-77.

20. Klayman, D.L.; Lin, A.J.; Acton, N.; Scovill, J.P.; Hock, J.M.; Milhous, W.K.; Theoharides, A.D.; and Dobek, A.S. Isolation of artemisinin (qinghaosu) from *Artemisia annua* growing in the United States. J. Nat. Prod. 47 (1984): 715-717.

21. Jiang, J.-B.; Li, G.-Q.; Guo, X.-B.; Kong, Y.C.; and Arnold, K. Antimalarial Activity of Mefloquine and Qinghaosu. Lancet 8 (1982): 285-288.

22. Klayman, D.L. Qinghaosu (Artemisinin): An Antimalarial Drug from China. Science 228 (1985): 1049-1055.

23. China Cooperative Research Group on Qinghaosu and Its Derivatives as Antimalarials. The Chemistry and Synthesis of Qinghaosu Derivatives, J. Tradit. Chin. Med. 2 (1982): 9-16.

24. China Cooperative Research Group on Qinghaosu and Its Derivatives as Antimalarials. Clinical studies on the treatment of malaria with quighaosu and its derivatives. J. Tradit. Chin. Med. 2 (1982): 45-50.

25. Looareesuwan, S.; Viravan, C.; Vanijanonta, S.; Wilairatana, P.; Suntharasamai, P.; Charoenlarp, P.; Arnold, K.; Kyle, D.; Canfield, C.; and Webster, H.K. A randomised trial of mefloquine, artesunate, and artesunate followed by mefloquine in acute uncomplicated falciparum malaria. Lancet 339 (1992): 821-824.

26. White, N.J.; Waller, D.; Crawley, J.; Nosten, F.; Chapman, D.; Brewster, D.; and Greenwood, B.M. Comparison of artemether and chloroquine for severe malaria in Gambian children. Lancet 339 (1992): 317-321.

27. White, N.J. Artemisinin: current status. Trans. Royal Soc. Trop. Med. Hyg. 88 (Suppplement 1, 1994): 3-4.

28. Win, K.; Than, M.; and Thwe, Y. Comparison of combinations of parenteral artemisinin derivatives plus oral mefloquine with intravenous quinine plus oral tetracycline for treating cerebral malaria. Bull. WHO 70 (1992): 777-782.

29. Shwe, T.; and Hla, K.K. The effect of artemether plus mefloquine on Myanmar patients with complicated falciparum malaria. Southeast Asia J. Trop. Med. Pub. Hlth. 23 (Supplement 4, 1992): 117-121.

30. Nosten, F.; Luxemburger, C.; ter Kuile, F.O.; Woodrow, C., Pa Eh, J.; Chongsuphajaisiddhi, T.; and White, N.J. Treatment of Multidrug-Resistant *Plasmodium falciparum* Malaria with 3-Day Artesunate-Mefloquine Combination. J. Infect. Dis. 171 (1994): 971-977.

31. White, N.J. Preventing antimalarial drug resistance through combinations. Drug Resistance Updates 1 (1998): 3-9.

32. Avery, M.A.; Gao, F.; Chong, W.K.M.; Mehrotra, S.; and Milhous, W.K. Structure-Activity Relationships of the Antimalarial Agent Artemisinin. 1. Synthesis and Comparative Molecular Field Analysis of C-9 Analogs of Artemisinin and 10-Deoxoartemisinin. J. Med. Chem. 36 (1993): 4264-4275.

33. Meshnick, S.R.; Taylor, T.E.; and Kamchonwongpaisan, S. Artemisinin and the Antimalarial Endoperoxides: from Herbal Remedy to Targeted Chemotherapy. Microbiol. Rev. 60 (1996): 301-315.

34. Meshnick, S.R.; Thomas, A.; Ranz, A.; Xu, C.-M.; and Pan, H.-Z. Artemisinin (qinghaosu): the role of intracellular hemin in its mechanism of antimalarial action. Mol. Biochem. Parasitol. 49 (1991): 181-190.

35. Meshnick, S.R.; Yang, Y.-Z.; Lima, V.; Kuypers, F.; Kamchonwongpaisan, S.; and Yuthavong, Y. Iron-Dependent Free Radical Generation from the Antimalarial Agent Artemisinin (Qinghaosu). Antimicrob. Agents Chemother. 37 (1993): 1108-1114.

36. Padmanaban, G.; and Rangarajan, P.N. Heme Metabolism of Plasmodium Is a Major Antimalarial Target. Biochem. Biophys. Res. Comm. 268 (2000): 665-668.

37. Meshnick, S.R.; Tsang, T.W.; Lin F.-B.; Pan, H.-Z.; Chang, C.-N.; Kuypers, F.; Chiu, D.; and Lubin, B. Activated oxygen mediates the antimalarial activity of qinghaosu. Prog. Clin. Biol. Res. 313 (1989): 95-104.

38. Posner, G.H.; Cummings, J.N.; Ploypradith, P.; and Oh. C.O. Evidence for Fe(IV)=O in the Molecular Mechanism of Action of the Trioxane Antimalarial Artemisinin. J. Am. Chem. Soc. 117 (1995): 5885-5886.

39. Jefford, C.W., Vicente, M.G.H., Jacquier, Y., Favarger, F., Mareda, J., MillassonSchmidt, P., Brunner, G., Burger, U. The Deoxygenation and Isomerization of Artemisinin and Artemether and Their Relevance to Antimalarial Action. Helv. Chim. Acta 79 (1996): 1475-1487.

40. Gaby, T.; Krugliak, M.; Shalmiev, G.; and Ginsburg, H. Inhibition by antimalarial drugs of haemoglobin denaturation and iron release in acidified red blood cells lysates-a possible mechanism of their antimalarial effect? Parasitology 108 (1994): 371-381.

41. Pandey, A.V.; Tekwani, B.L.; Singh, R.L.; and Chauhan, V.S. Artemisinin, an Endoperoxide Antimalarial, Disrupts the Hemoglobin Catabolism and Heme Detoxification Systems in Malarial Parasite. J. Biol. Chem. 274 (1999): 19383-19388.

42. Peter, W.; Li, Z.L.; Robinson, B.L.; and Warhurst, D.C. The chemotherapy of rodent malaria, XL. The action of artemisinin and related sesquiterpenes. Ann. Trop. Med. Parasitol. 80 (1986): 483-489.

43. Posner, G.H.; Oh, C.H.; Wang, D.; Gerena, L.; Milhous, W.K.; Meshnick, S.R.; and Asawamahasakda, W. Mechanism-Based Design, Synthesis, and *in Vitro* Antimalarial Testing of New 4-Methylated Trioxanes Structurally Related to Artemisinin: The Importance of a Carbon-Centered Radical for Antimalarial Activity. J. Med. Chem. 37 (1994): 1256-1258.

44. Posner, G.H.; Wang, D.; Cumming, J.N.; Oh, C.H.; French, A.N.; Bodley, A.L.; and Shapiro, T.A. Further Evidence Supporting the Importance of and the Restrictions on a Carbon-Centered Radical for High Antimalarial Activity of 1,2,4-Trioxanes Like Artemisinin. J. Med. Chem. 38 (1995): 2273-2275.

45. Asawamahasakda, W.; Ittarat, I.; Pu, Y.-M.; Ziffer, H.; and Meshnick, S.R. Reactions of antimalarial endoperoxides with specific parasite proteins. Antimicrob. Agents. Chemother. 38 (1994): 1854-1858.

46. Haynes, R.K.; and Vonwiller, S.C. The Behavior of Qinghaosu (Artemisinin) in the Presence of Non-heme Iron(II) and (III). Tetrahedron Lett. 37 (1996): 257-260.

47. Posner, G.H.; and Oh, C.H. A Regiospecifically Oxygen-18 Labeled 1,2,4-Trioxane: A Simple Chemical Model System to Probe the Mechanism(s) for the Antimalarial Activity of Artemisinin (Qinghaosu). J. Am. Chem. Soc. 114 (1992): 8328-8329.

48. Butler, A.R.; Gilbert, B.C.; Hulme, P.; Irvine, L.R.; Renton, L.; and Whitwood, A.C. EPR Evidence for the Involvement of Free Radicals in the Iron-Catalysed Decomposition of Qinghaosu (Artemisinin) and Some Derivatives; Antimalarial Action of Some Polycyclic Endoperoxides. Free Rad. Res. 28 (1998): 471-476.

49. Yang, Y.-Z.; Asawamahasakda, W.; and Meshnick, S.R. Alkylation of human albumin by the antimalarial artemisinin. Biochem. Pharmacol. 46 (1993): 336-339.

50. Yang, Y.-Z.; Little, B.; and Meshnick, S.R. Alkylation of proteins by artemisinin. Effects of heme, pH, and drug structure. Biochem. Pharmacol. 48 (1994): 569-573.

51. Meshnick, S.R.; Thomas, A.; Ranz, A.; Xu, C.-M.; and Pan, H.-Z. Artemisinin (qinghaosu): the role of intracellular hemin in its mechanism of antimalarial action. Mol. Biochem. Parasitol. 49 (1991): 181-189.

52. Hong, Y.-L.; Yang, Y.-Z.; and Meshnick, S.R. The interaction of artemisinin with malarial hemozoin. Mol. Biochem. Prarsitol. 63 (1994): 121-128.

53. Kleinberg, M. L.; and Wanke, L. A. New approaches and technologies in drug design and discovery. Am. J. Health-Syst. Pharm. 52 (1995): 1323-1336.

54. Kubinyi, H. Methods and Principles in Medicinal Chemistry. Vol. 1 QSAR: Hansch Analysis and Related Approaches. New York: VCH Publishers, 1993.

55. Trinajstic, N. Chemical Graph Theory. Vols. I, II. Florida: CRC Press, 1983.

56. Wiener, H. Correlation of heats of isomerization, and difference in heats of vaporization of isomers, among the paraffin hydrocarbons. J. Am. Chem. Soc. 69 (1947): 2636.

57. Hall, L. H.; and Kier, L. B. The Molecular Connectivity Chi Indexes and Kappa Shape Indexes in Structure-Property Modeling. In K.B. Lipkowitz and D.B. Boyd (eds.), Reviews in Computational Chemistry, Volume II, pp. 367-422. New York: VCH Publishers, 1991.

58. Oxford Molecular Groups. TSAR 3.2 [Computer Software]. Oregon: Oxford Molecular Groups Inc., 1998.

59. Van de Waterbeemd, H. Quantitative Approaches to Structure-Activity Relationships. In C.G. Wermuth (ed.), The Practice of Medicinal Chemistry, pp. 367-389. London: Academic Press, 1996.

60. Stone, M. Cross-validatory choice and assessment of statistical prediction. J. Royal Stat. Soc. B 36 (1974): 111-133.

61. Clementi, S.; and Wold, S. How to choose the proper statistical method. In H. Van der Waterbeemd (ed.), Chemometric Methods in Molecular Design, pp.319-338. New York: VCH Publishers, 1995.

62. Acton, N.; Karle, J.M.; and Miller, R.E. Synthesis and Antimalarial Activity of Some 9-Substituted Artemisinin Derivatives. J. Med. Chem. 36 (1993): 2552-2557.

63. Avery, M.A.; Mehrotra, S.; Bonk, J.D.; Vroman, J.A.; Goins, D.K.; and Miller, R. Structure-Activity Relationships of the Antimalarial Agent Artemisinin. 4. Effect of Substitution at C-3. J. Med. Chem. 39 (1996): 2900-2906.

64. Lin, A.J.; Klayman, D.L.; and Milhous, W.K. Antimalarial Activity of New Water-Soluble Dihydroartemisinin Derivatives. J. Med. Chem. 30 (1987): 2147-2150.

65. Lin, A.J.; and Miller, R.E. Antimalarial Activity of New Dihydroartemisinin Derivatives. 6. α-Alkylbenzylic Ethers. J. Med. Chem. 38 (1995): 764-770.

66. Lin, A.J.; Li, L.-Q.; Andersen, S.L.; and Klayman, D.L. Antimalarial Activity of New Dihydroartemisinin Derivatives. 5. Sugar Analogues. J. Med. Chem. 35 (1992): 1639-1642.

67. Pu, Y.M.; Torok, D.S.; Ziffer, H.; Pan, X.-Q.; and Meshnick, S.R. Synthesis and Antimalarial Activities of Several Fluorinated Artemisinin Derivatives. J. Med. Chem. 38 (1995): 4120-4124.

68. Avery, M.A.; Mehrotra, S.; Johnson, T.L.; Bonk, J.D.; Vroman, J.A.; and Miller, R. Structure-Activity Relationships of the Antimalarial Agent Artemisinin. 5. Analogs of 10-Deoxoartemisinin Substituted at C-3 and C-9. J. Med. Chem. 39 (1996): 4149-4155.

69. Frisch, M.J.; Trucks, G.W.; Schlegel, H.B.; Scuseria, G.E.; Robb, M.A.; Cheeseman, J.R.; Zakrzewski, V.G.; Montgomery, Jr., J.A.; Stratmann, R.E.; Burant, J.C.; Dapprich, S.; Millam, J.M.; Daniels, A.D.; Kudin, K.N.; Strain, M.C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G.A.; Ayala, P.Y.; Cui, Q.; Morokuma, K.; Malick, D.K.; Rabuck, A.D.; Raghavachari, K.; Foresman, J.B.; Cioslowski, J.; Ortiz, J.V.; Baboul, A.G.; Stefanov, B.B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R.L.; Fox, D.J.; Keith, T.; Al-Laham, M.A.; Peng, C.Y.; Nanayakkara, A.; Gonzalez, C.; Challacombe, M.; Gill, P.M.W.; Johnson, B.; Chen, W.; Wong, M.W.; Andres, J.L.; Gonzalez, C.; Head-Gordon, M.; Replogle, E.S.; and Pople, J. A. Gaussian 98, Revision A.7 [Computer Software]. Pennsylvania: Gaussian Inc., 1998.

70. Oxford Molecular Groups. TSAR 3.2 [Computer Software]. Oregon: Oxford Molecular Groups Inc., 1998.

71. Balaban, A.T. Highly discriminating distance-based topological index. Chem. Phys. Lett. 89 (1982): 399-404.

72. Tonmunphean, S.; Parasuk, V.; and Kokpol, S. QSAR Study of Antimalarial Activities and Artemisinin-Heme Binding Properties Obtained from Docking Calculations. Quant. Struct.-Act. Rel. 19 (2000): 475-483.

73. Tonmunphean, S.; Parasuk, V.; and Kokpol, S. Automated docking calculations of artemisinin to heme. J. Mol. Model. 7 (2001): 26-33.

74. Wu, J.-A.; and Ji, R.-Y. A quantitative structure-activity study on artemisinin analogues. Acta Pharmacol. Sinica 3 (1982): 55-60.

75. Tang, Y.; Jiang, H. L.; Chen, K. X.; and Ji, R. Y. QSAR study of artemisinin (Qinghaosu) derivatives using neural network method. Indian J. Chem. 35B (1996): 325-332.

76. Jiang, H. L.; Chen, K. X.; Tang, Y.; Chen, J. Z.; Li, Y.; Wang, Q. M.; Ji, R. Y.; and Zhuang, Q. K. Theoretical and cyclic voltammetry studies on antimalarial mechanism of artemisinin (Qinghaosu) derivatives. Indian J. Chem. 36B (1997): 154-160.

77. Nguyen-Cong, Y.; Dang, G. V.; and Rode, B. M. Using multivariate adaptive regression splines to QSAR studies of dihydroartemisinin derivatives. Eur. J. Med. Chem. 31(1996): 797-803.

78. Avery, M. A.; Alvim-Gaston, M.; and Woolfrey, J. R. Synthesis and Structure-Activity Relationships of Peroxidic Antimalarials based on Artemisinin. Adv. Med. Chem. 4 (1999): 125-217.

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

CURRICULUM VITAE

Name Mrs. Sirirat Kokpol

Education

Degree/area	Year	University/Country
Ph.D. (Physical Chemistry)	1979	Villanova University, USA
M.Sc. (Physical Chemistry)	1973	Chulalongkorn University, Thailand
B.Sc. (Chemistry)	1971	Chulalongkorn University, Thailand

Present Position Associate Professor, Chemistry Department,
Faculty of Science, Chulalongkorn University

List of Publications:

1. **Articles**

1. Dhabanandana, S. and Ho-Ampawanwong, S., "A study of Redox Reactions in Non-Aqueous Media and Related Works: I. Conductivities and Ionic Interactions of TI(I)/TI(II) System in Glacial Acetic acid", Report on Scientific Research, Faculty of Science, Chulalongkorn University, 1980, 7, 47.
2. Dhabanandana,S. and Ho-Ampawanwong,S., "A Study of Redox Reactions in Non-Aqueous Media and Related Works: I. Conductivities and Ionic Interactions of TI(I)/TI(II) System in Glacial Acetic acid", Report on Scientific Research, Faculty of Science, Chulalongkorn University, 1980, 5, 56.
3. Ho-Ampawanwong,S., "Optimized Gaussion Expansion of Atomic Orbitals", J.Sci Soc.Thailand, 1980, 6, 178.
4. Ho-Ampawanwong, S., "The Modification of the Slater-type Orbital Using a Longarithmic Term", Report on Scientific Research, Faculty of Science, Chulalongkorn University, 1980, 5, 29.
5. Sirirat Ho-Ampawanwong, "Mr. Inorganic Chemistry", Science, 1981 Year 35, Vol.5, page 409-410 (in Thai).

6. Chaiyasith. P.; Kokpol, S.; and Sa-Yakanit., V., "A simplified Approach to Impurity-Band Tails in Heavily Doped Semi-Concuctors", Physics Letters, 1983, 98A (5-6), 273.
7. P.Doungdee, S.U.Kokpol and B.M.Rode, "A Comparative Study of the Hydration of Aliphatic Amino acid by CNDO/2 and Ab Initio Molecular Orbital Calculations", J.Sci.Soc.Thailand, 1985, 11, 113.
8. J.Limtrakul., S.Kokpol and B.M.Rode, "Quantum Chemical Investigations on Ion-Dipeptide Complex Formation", J.Sci.Soc.Thailand, 1985, 11, 129.
9. S.U.Kokpol., S.V.Hannongbua, B.M.Rode and J.P.Limtrakul, "Investigation of Metal Ion Induced Conformational Change in N-Acetyl Alanine Methyl Ester", Inorg.Chim.Acta., 1986, 125, 107.
10. P.Doungdee, S.U.Kokpol and B.M.Rode, "The Influence of Hydration on the Rotation Barriers of Glycine", Manatshefte fuer Chemie, 1987, 118, 691.
11. J.P.Limtrakul, S.V.Hannongbua, S.U.Kokpol and B.M.Rode, "Cation Binding Effect on Hydrogen Bonded Dimer of Imidazole and Water", Inorg.Chim.Acta, 1987, 138, 131.
12. S.V.hannongbua, S.U.Kokpol., S.Kheawsrekul, S.Polman and B.M.Rode, "Intermolecular Potential Function for Ammonia-Lithium Ion Based on Ab-Initio Calculations", Z.Naturforsch, 1988, 34a, 143.
13. S.U.Kokpol, P.B.Doungkee, S.V.Hannongbua, J.P.Limtrakul and B.M.Rode, "An Study Ab-Initio on the Hydration of Glycine Zwitterion", J.Chem.Soc.Faraday Trans.II., 1988, 84, 1789.
14. S.U.Kokpol, S.V.Hannonghua, N.Thongrit, S.Polman, B.M.Rode and M.G.Schwendinger, "Quantum Pharmacological Studies on PrimaquineAntimalarial Activity", Analytical Science,1988, 4, 565.
15. S.V.Hannongbua, S.U.Kokpol and J.P.Limtrakul, "An Analysis of the Pathways for Magnesium Ion in the Field of a Macrocyclic Ligand", Can.J.Chem.,1989, 67, 1298.
16. S.Kheawsrikul, S.V.Hannongbua, S.U.Kokpol and B.M.Rode, "A Monte Carlo Study on Preferentail Solvation of Lithium(I)in Aqueous Ammonia", J.Chem.Soc.Faraday Trans.II., 1989, 85, 643.

17. S.U.Kokpol, S.V.Hannongbua, W.Yentongchai and B.M.Rode, "Conformational Analysis of Be(II)and Mg(II)Complexes of N-Acetyl Alanine Methyl Ester", Inorg.Chim.Acta, 1989, 166, 123.
18. B.M.Rode, M.G.Schewendinger, S.U.Kokpol, S.V.Hannongbua and S.Polman, "Quantum Pharmacological Studies on Antimalarial Drugs", Manatshefie fuer Chemie, 1989 120, 913.
19. S.Polman, S.U.Kokpol, S.V.Hannongbua and B.M.Rode, "Quantum Pharmacological Analysis of Structure-Activity Relationship for Mafloquine Antimalarial Drugs", Analytical Science, 1989, 5, 641.
20. S.U.Kokpol, S.V.Hannongbua, J.P.Limtrakul and B.M.Rode, "The Influence of Na(I)on Neghbouring Hydrogen Bonds of Aliphatic Amino Acid", Monatshefie fuer Chemie, 1989, 120, 491.
21. S.U.Kokpol, S.V.Hannongbua and T.Sangsoontorn, "Conformational Analysis of Metal Complexes of N-Acetyl Alanine Methyl Ester by CNDO/2 method", J.Sci.Soc.Thailand, 1989, 15, 215.
22. Yongyos P.Yongyai, Sirirat Kokpol and B.M.Rode, "Zinc Ion in Water: Interaction Potential with Approximate 3-Body Correction and Monte Carlo Simulation", Chemical Physics, 1991, 156, 403.
23. Sirirat Kokpol, Prayong Doungdee, Supot Hannongbua and Naiyana Chuankrerkkul, "The Rotational Barrier of the Hydrated Glycine Zwitterion", J.Sci Soc.Thailand, 1992, 18, 175.
24. Yongyos P.Yongyai, Sirirat Kokpol and Bernd M.Rode, "Microstructure and Species Distribution of Aqueous Zinc Chloride Solutions: Results from Monte Carlo", Faraday Trans, 1992, 11, 1537.
25. Pornthep Sompornpisut, Sirirat Kokpol and B.M.Rode, "Li+ in Liquid Hydroxylamine: Intermolecular Potential Function and Monte Carlo Simulation", Chemical Physics, 1993, 172/1, 8.
26. Pornthep Sompornpisut, Sirirat Kokpol and B.M.Rode, "Chloride Ion in Liquid Hydroxylamine Pair Potential Function and Monte Carlo Simulation", Z.Naturforsch, 1994, 49a, 797-801.
27. S.Hannongbua, S.Kokpol, Z.Gurskii, and K.Heizinger, "Cluster Formation in a Concentrated Lithium-Liquid Ammonia Solution. A Monte Carlo Study", Z.Naturforsch, 1997, 52a, 828-834.

28. Tonmunphean, S.; Kokpol, S.; Parasuk, V.; Wolschann, P.; Winger, R.H.; Liedl, K.R.; Rode, B.M., "Comparative Molecular Field Analysis (CoMFA) of Artemisinin Derivatives: Ab initio vs. semiempirical optimized structures", J.Comput.-Aided Mol.Design, 1998, 12, 397-409.
29. Tonmunphean, S.; Irle, S.; Kokpol, S.; Parasuk, V. "Ab initio and Density Functional Study on Singlet and Triplet States of Artemisinin", J.Mol.Struct.(THEOCHEM), 1998, 454, 87-90.
30. Somsak Tonmunphean, Vudhichai Parasuk, and Sirirat Kokpol, "QSAR Study of Antimalarial Activities and Artemisinin-Heme Binding Properties Obtained from Docking Calculations", Quantitative Structure Activity Relationships (QSAR), 2000, 19, 475-483.
31. Somsak Tonmunphean, Vudhichai Parasuk, and Sirirat Kokpol, "Automated docking calculations of artemisinin to heme", Journal of Molecular Modeling, 2001, 7, 26-33.

2. Books

1. Sophon Roengsumran, Sirirat Ho-Ampawanwong, Walaipan Luangdilok and Thepjumnong Sangsoontorn, "Experimental Chemistry for Nursing Students", Chemistry Department, Faculty of Science, Chulalongkorn University, 1981, 103 pages (in Thai).
2. Sophon Roengsumran, Sirirat Ho-Ampawanwong , Walaipan Luangdilok and Thepjumnong Sangsoontorn, "Chemistry for Nursing Students", Graphic Art Publisher, 1982, 326 pages (in Thai).
3. Sirirat Kokpol, "Computational Quantum Chemistry", Chemistry Department, Faculty of Science, Chulalongkorn University, 1992, 455 pages (in Thai).
4. Sophon Roengsumran, Sirirat Kokpol, Walaipan Luangdilok and Thipjumnong Sangsoontorn, "General Chemistry for the Life Science and Nursing", Chulalongkorn University Press, Bangkok, 1992, 361 pages (in Thai).

Mailing Address Department of Chemistry, Faculty of Science
Chulalongkorn University
Phyathai Rd., Patumwan, Bangkok 10330, Thailand.
Tel. (662)2185222 Fax (662)2521730
email: sirirat@atc.atccu.chula.ac.th

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จุฬาลงกรณ์มหาวิทยาลัย

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