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Part A: Antimalarial agents modified at the C-16 position of artemisinin; Part B: Lead optimization of falcipain-2 and falcipain-3 inhibitors

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PART A: ANTIMALARIAL AGENTS MODIFIED AT THE C-16
POSITION OF ARTEMISININ;

PART B: LEAD OPTIMIZATION OF FALCIPAIN-2 AND
FALCIPAIN-3 INHIBITORS

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by

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ABSTRACT

Part A: Antimalarial Agents modified at the C-16 position of Artemisinin.

Malaria is a widespread tropical and subtropical parasitic disease which is caused by malarial parasites and transmitted by the infected anopheles mosquitoe. The natural product artemisinin and its derivatives are currently considered the most effective drugs against drug resistant *plasmodium falciparum*. However, its undesired physicochemical proprieties have limited its usage. In order to improve its effectiveness, scientists around the world have developed novel methodology to synthesize artemisinin derivatives on different positions of the artemisinin skeleton. Previous work in our group has shown that many analogues modified at the C-16 of artemisinin had improved efficacy along with modified physicochemical proprieties. This work focuses on the synthesis of heteroatomic and heterocyclic derivatives of artemisinin with the emphasis on C-16 substituted triazole containing side-chains. Successful synthetic results and subsequent bioassay demonstrated that the compounds have modest antimalarial activity compared to artemisinin and improved water solubility. With these encouraging results in hand, further work is underway to tune the desired physicochemical properties so that plasma half-life and oral bioavailability will be improved.

Part B: Lead Optimization of Falcipain-2 and Falcipain-3 Inhibitors.

The expanding usage of artemisinin combination therapy casts concern about the potential development of drug resistance to this drug family, thus the search for new drug targets is always needed. Falcipain-2 (FP-II) and falcipain-3 (FP-III) are two cysteine proteases which malarial parasites utilize to degrade hemoglobin to obtain amino acids essential to the parasite. The inhibition of these two enzymes has been shown to have deadly effects on the protozoan life cycle. Recently published crystal structures of FP-II provided an outstanding opportunity for rational drug design and discovery. In the present study, structure-based optimization of virtual screening hits was carried out using scaffold hopping, docking and analogue synthesis. Unfortunately, the biological evaluation of the synthesized compounds against FP-II and FP-III indicated these compounds are inactive. However, the information gained from this exercise could aid further in optimization of this series of compounds.

DEDICATION

This dissertation is dedicated to my family

Without their support I would have given up long time ago.

LIST OF ABBREVIATIONS AND SYMBOLS

Ac   acetyl

aq.   aqueous

[Bmim]BF$_4$ 1-butyl-3-methy imidazolium tetrafluoroborate

cat.   catalytic

DBU   1,8-diazabicyclo[5.4.0]undec-7-ene

DCM   dichloromethane

DDQ   2,3-dichloro-5,6-dicyano-1,4-benzoquinone

DIBAL-H   diisobutylaluminum hydride

DMAP   *N*,*N*-4-dimethylaminopyridine

DMF   *N*,*N*-dimethylformamide

EDCI   1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride

Et   ethyl

HMBC   heteronuclear multiple bond connectivity quantum coherence

HMQC   heteronuclear multiple bond coherence correlation

KHMDS   potassium *bis*(trimethylsilyl)amide

LAH   lithium aluminum hydride

LHMDS   lithium *bis*(trimethylsilyl)amide

m-CPBA	*meta* chloroperbenzoic acid
Me	methyl
Ms	mesyl (methanesulfonyl)
NaHMDS	sodium *bis*(trimethylsilyl)amide
NMR	nuclear magnetic resonance
PDC	pyridinium dichromate
Ph	phenyl
PMB	*p*-methoxybenzyl
PPTS	pyridinium *p*-toluenesulfonate
r.t.	room temperature
TBAF	tetra-*n*-butylammonium bromide
TBS	*t*-butyldimethylsilyl
TBHP	*t*-butyl hydroperoxide
TEA	triethylamine
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TMS	trimethylsilyl

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Part A

Antimalarial Agents Modified at the C-16 Position of Artemisinin

CHAPTER 1

INTRODUCTION

1.1 Malaria

Malaria is a parasitic disease which is caused by malarial parasites and transmitted by infected anopheles mosquitoes. Despite a long history of the fight against malaria, this disease remains a leading cause of morbidity and mortality in developing countries (Figure 1-1). Out of the five parasitic species which can infect and be transmitted to humans, *Plasmodium falciparum* causes the most deaths compared to other species: *Plasmodium vivax; Plasmodium malariae; Plasmodium ovale; and Plasmodium knowlesi*[1]. According to WHO, 3.3 billion people are at risk of infection by this disease. In the year 2010, Malaria infected 216 million people and killed 655,000 people worldwide.
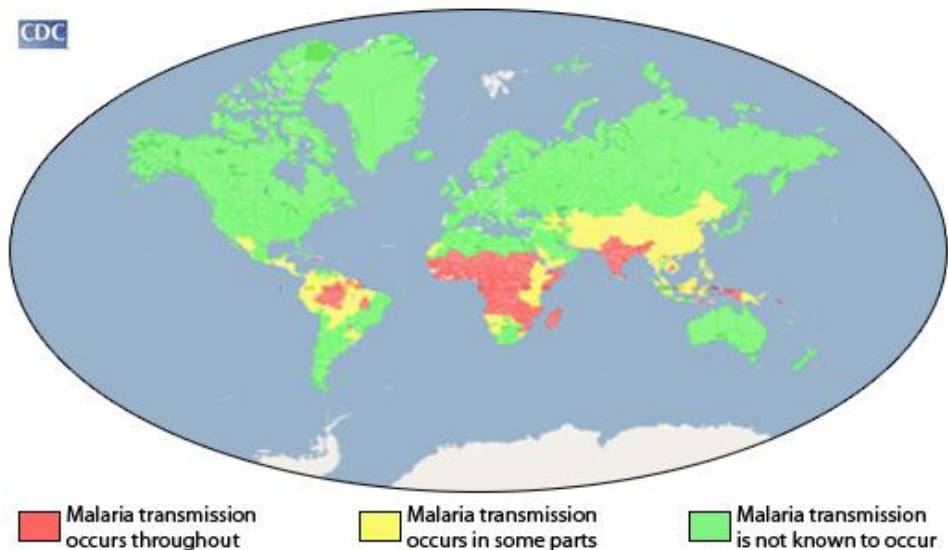
Figure 1-1 World malaria distribution (source: CDC)

Most of these cases and deaths occurred in Africa; 86% of deaths were in children under 5 years of age[2]. Not only is this deadly disease a threat to human health, it is also a huge economic burden for those developing countries in regions where it is endemic. It is estimated that at least 12 billion dollars, or 1.3% of the GDP, is lost in these countries each year as a result of malaria[3].

The prevention of malaria has relied on insecticide spraying or on insecticide-treated bed-nets. Attempts to develop a malaria vaccine have been fruitless so far. In the early 20th century, people used insecticides such as DDT (dichlorodiphenyltrichloroethane) to kill mosquitoes and thus prevent the spread of malaria. Insecticides have led to elimination of malaria in USA, Europe, the Caribbean, and parts of Asia and South-Central America[4]. However, this inexpensive and highly effective chemical was later banned by most of the western developed countries due to its environmental impact. Insecticide resistance was also acquired by mosquitoes. The insecticide-treated bed-nets are an effective way to reduce malaria though it has very poor compliance. A mathematical model suggested this disease could be eradicated if 75% of the endemic population were using bed-nets properly[5]. Vaccines are another highly desirable method to prevent the disease. The development of a vaccine is hampered by the complexity of the parasite species and low response towards the vaccine. The most advanced vaccine mosquirix (RTS,S) showed a 30% reduction rate in infants in phase III clinical trials[6].

The treatment of malaria thus mainly relies on the chemotherapy. Several well-known small molecules such as quinine (1.1), chloroquine (1.2) and pyrimethamine (1.3) have been utilized to treat malaria for several decades (Figure 1-2). These drugs have been replaced by artemisinin combination therapy (ACT) due to their development of resistance in parasites and/or significant side effects. Artemisinin combination therapy consists of a derivative of the natural

product artemisinin and another chemically unrelated antimalarial drug such as amodiaquine, mefloquine, lumefantrine, pyrimethamine, piperaquine etc[7].

| 1.1 Quinine | 1.2 Chloroquine | 1.3 Pyrimethamine |
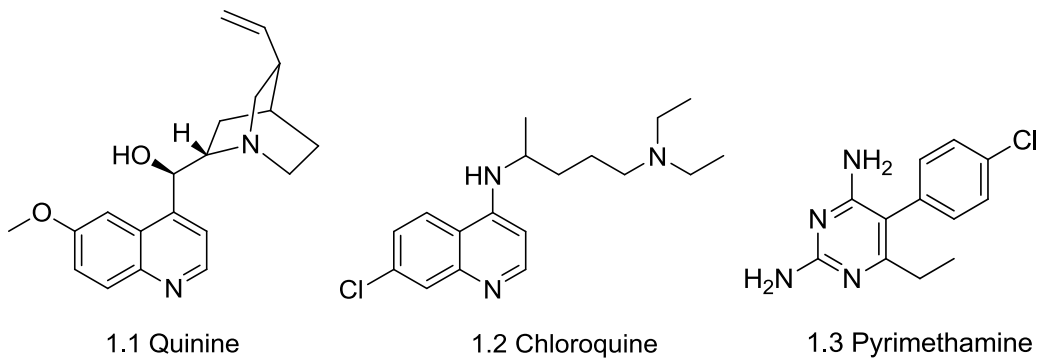
Figure 1-2 Some well-known antimalarial drugs that
have developed resistance

1.2 Artemisinin

The discovery of artemisinin was a huge milestone in the history of antimalarial development. Artemisinin **1** (Figure 1.3) is a sesquiterpene natural product which was first isolated from *Artemisia annua* by a team of Chinese scientists who, under direction of Youyou Tu began "Project 523" in 1972[8]. In 2011, she received the Lasker award for the discovery of artemisinin[9]. The structure of artemisinin was finally elucidated in 1979 through a combination of chemical reactions, spectral analysis and singal crystal X-ray diffraction analysis[10]. Surprisingly, artemisinin has a very rare but stable endoperoxide bridge which was found essential for its antimalarial activity and recently, anticancer[11], antifungal[12], and antiviral[13] activity. Artemisinin, like most natural products, has some pharmaceutical issues which need to be addressed before it was used in the clinic. These drawbacks include poor solubility in water and oil[14], a short plasma half-life[15], and a high recrudescence rate[16]. In addition to its endoperoxide functional group, there are also lactone, ketal and acetal groups presented in the

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structure, which makes it unstable towards reductive metal, acid and alkaline conditions (Figure 1-3)[17]. Despite these inherent synthetic challenges, new derivatives with optimized physicochemical properties are urgently needed to overcome these disadvantages.
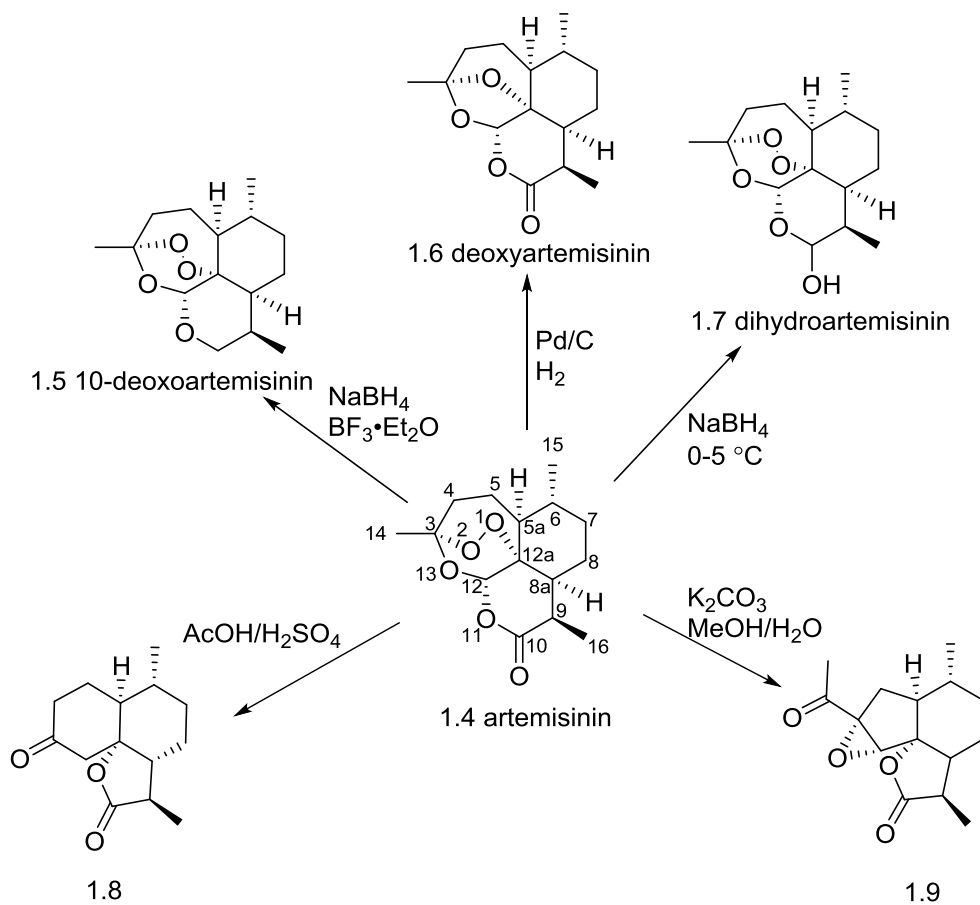
Figure 1-3 Artemisinin and its typical reactions[18]

1.3 Mode of action

As mentioned before, artemisinin is highly effective against drug resistant parasites which indicates that it has a different mechanism from previous antimalarial drugs. In order to improve the physicochemical properties of artemisinin and prepare more potent derivatives, it is

desirable to understand the mechanism by which artemisinin kills the parasites. Extensive studies have been carried out during the past three decades and many theories have been proposed.

It has become clear that artemisinin goes reductive activation to generate reactive intermediates and then these reactive intermediates exert a parasiticidal effect through alkylation, oxidation of lipid membranes, disruption of mitochondrial function or binding to parasite proteins. Up till now, there has been no consensus about what species activates artemisinin and what targets they are attacking. It has been suggested artemisinin could be activated by heme, iron or mitochondria[19]. The proposed targets including heme[20], plasmodium falciparum sarcoendoplasmic reticulum (SR) calcium transport ATPase (PfATP6)[21], translationally controlled tumor protein (TCTP)[22], Falcipain-2[23] etc. However, the most widely accepted theory that heme mediated C-centered radical formation followed by an alkylation process is untenable due to the short half-life of a C-centered radical (microseconds to milliseconds in nonviscous solvent)[24] (Scheme 1-1).

Artemisinin acts on the ring stage of malarial parasites within the red blood cell. At this stage, the parasites have limited ability to synthesize their own amino acids. When they invade red blood cells, they degrade the host's hemoglobin to produce globin and heme. Globin is hydrolyzed to amino acids for parasite protein synthesis. Heme is toxic to parasites but they can be detoxified by the parasite through a polymerization process to give hemazoin particles. Each heme consists of a porphyrin ring and a central iron (II) atom. Artemisinin can be activated by heme iron and thus the endoperoxide bond is reduced to generate oxygen free radicals. Due to the unsymmetrical nature of the peroxy bond, an O1 radical (1.13) or O2 radical (1.10) can be formed (Scheme 1-1). These oxygen free radicals are not stable and quickly rearrange to carbon centered radicals. O1 radical 1.13 can undergo a 1, 5-H shift to generate the secondary C-4

radical 1.14 whilst the O2 radical 1.10 can give a C-4 primary radical 1.11 by a C-C β-scission

process. These C-centered radicals can then alkylate heme[25] or other targets inside the parasite

such as cysteine residues in proteins and consequently either disrupt the heme polymerization

process or impair protein receptor function or ion-channel function, leading ultimately to parasite

death. The secondary C-centered radical 1.14 has been evidenced by electron spin resonance

(ESR) spin experiments from different groups[26, 27]. The primary C-centered radical was also

confirmed by heme-artemisinin adducts both *in vitro*[28] and *in vivo*[25].
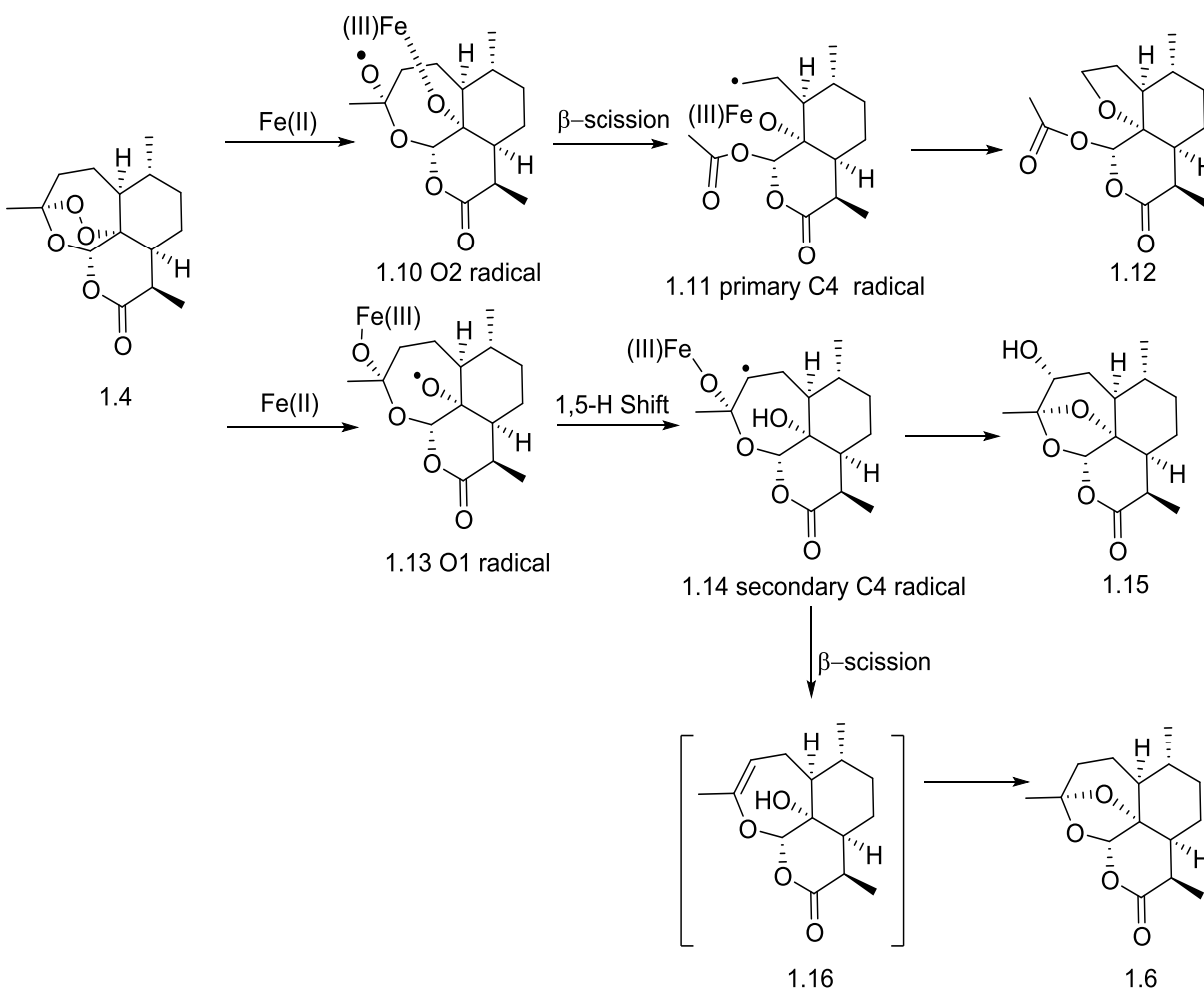
Scheme 1-1 Proposed C-centered radicals formation of artemisinin by Fe(II)

1.4 Artemisinin synthesis

Artemisinin is extracted from the plant and its production relies mainly on artificial cultivation. Its cultivation is affected by soil, region, weather, genetics and harvest time. From seed planting, harvesting to final product manufacturing, the whole production cycle usually takes at least 14 months which has resulted in a fluctuational supply and poorly controlled quality[29]. Also, the artemisinin price is much higher compared to other antimalarial drugs like chloroquine. Most patients occur in the poorest regions, thus this drawback is unacceptable. Scientists have been trying to find a commercially feasible synthetic route to artemisinin for the past three decades[30-32].

Artemisinin presents a synthetic challenge due to its condensed seven stereogenic centers and one endoperoxide bond which is attached to two tertiary carbons. A number of partial syntheses and total syntheses of artemisinin have been reported. Partial-syntheses of artemisinin from artemisinic acid 1.17, a crucial intermediate in the biosynthesis of artemisinin, have been reported[33-35]. Artemisinic acid can also be found in *Artemisia annua* with 10 times more quantity than artemisinin[36]. In 1983, Zhou[37] reported the first semi-synthesis of artemisinin from artemisinic acid in 8 steps. This route was improved by Acton[38] in 1989 to 3 steps: hydrogenation with $NaBH_4$ or NiCl, photooxdation, and air oxidation (Scheme 1-2 route A). The overall yield was also improved to 17-32%[39]. Recently, Seeberger[40] used a continuous flow synthesizer to produce artemisinin from artemisinic acid which is more suitable for industrial development (Scheme 1-2 route B).
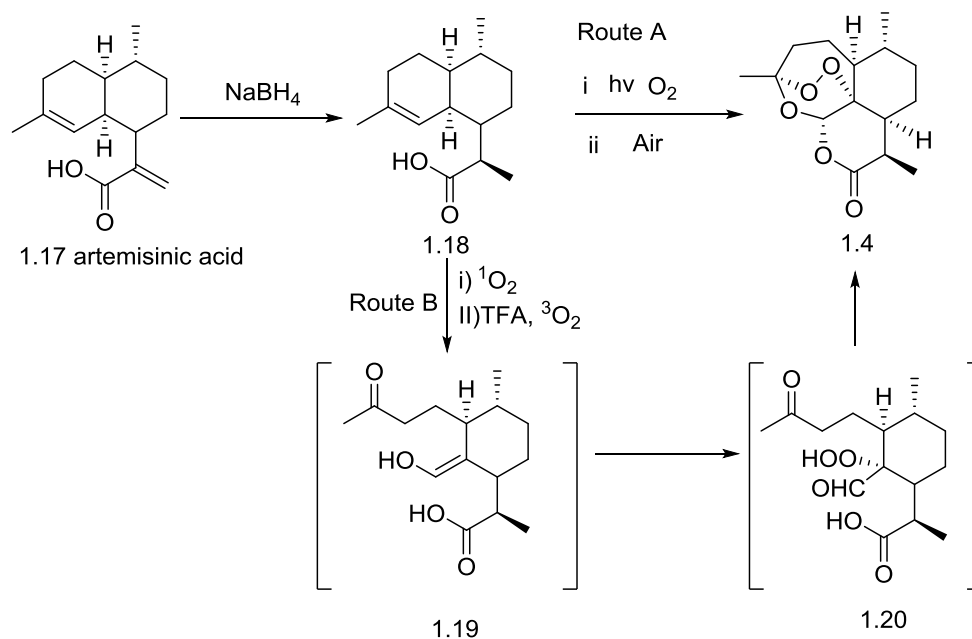
Scheme 1-2 Partial synthesis of artemisinin

The total synthesis of artemisinin has also been accomplished by several groups[41-46]. The key step is the introduction of the peroxy bond. Most of them use photooxidation (singlet oxygen) with one exception which uses an abnormal ozonolysis of a vinylsilane[47] to install the peroxy moiety (Scheme 1-3).
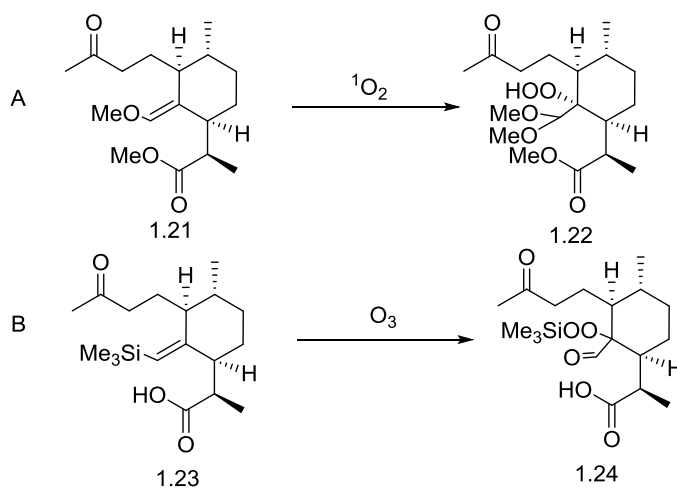
Scheme 1-3 General methods to install peroxy bond

Schmid[46] reported the first total synthesis of artemisinin in 1983. They used (-)-Isopulegol 1.25 as a starting material and furnished artemisinin in 13 steps, with an overall yield of 2.1% (Scheme 1-4). The US Army was unable to reproduce their findings[48]. Xu and coworkers[45] reported their total synthetic work starting from *R*-(+) citronellol, they first synthesized artemisinic acid and then finished artemisinin in a total 20 steps with an overall yield of 0.25%.
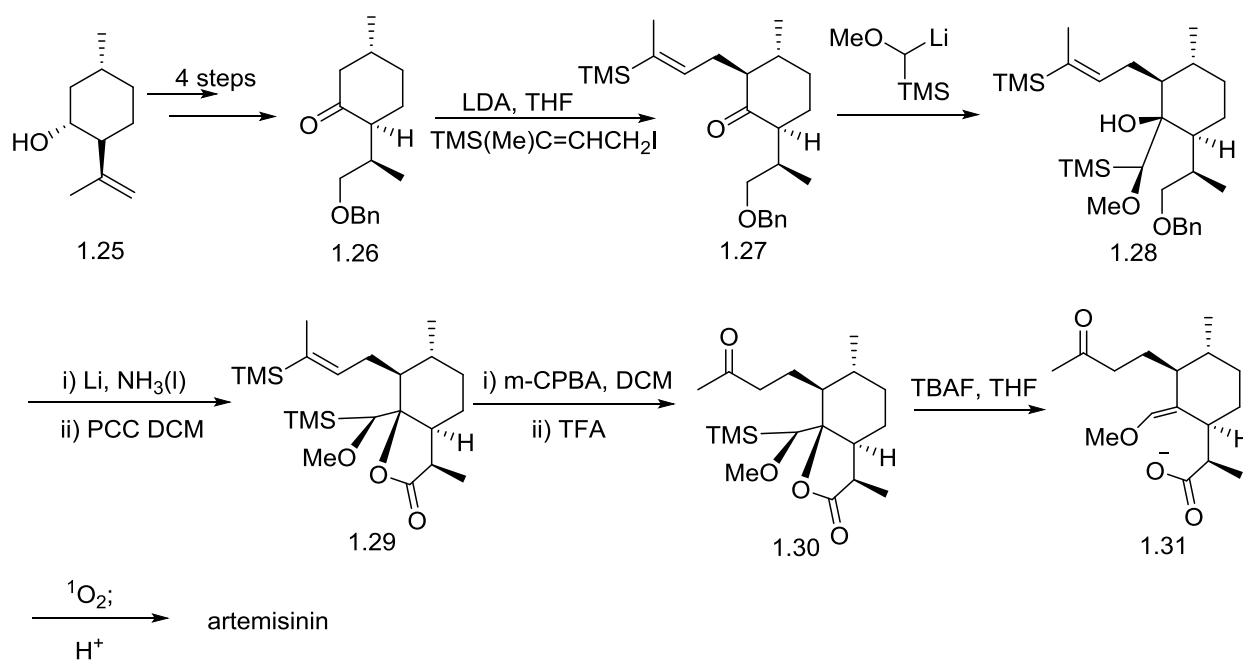
Scheme 1-4 Schmid's total synthesis of artemisnin

Avery's total synthesis of artemisinin[43] started with R-(+)-pulegone 1.32 and furnished *R*-(+)-artemisinin in 10 steps with an overall yield of 3.6% (Scheme 1-5).
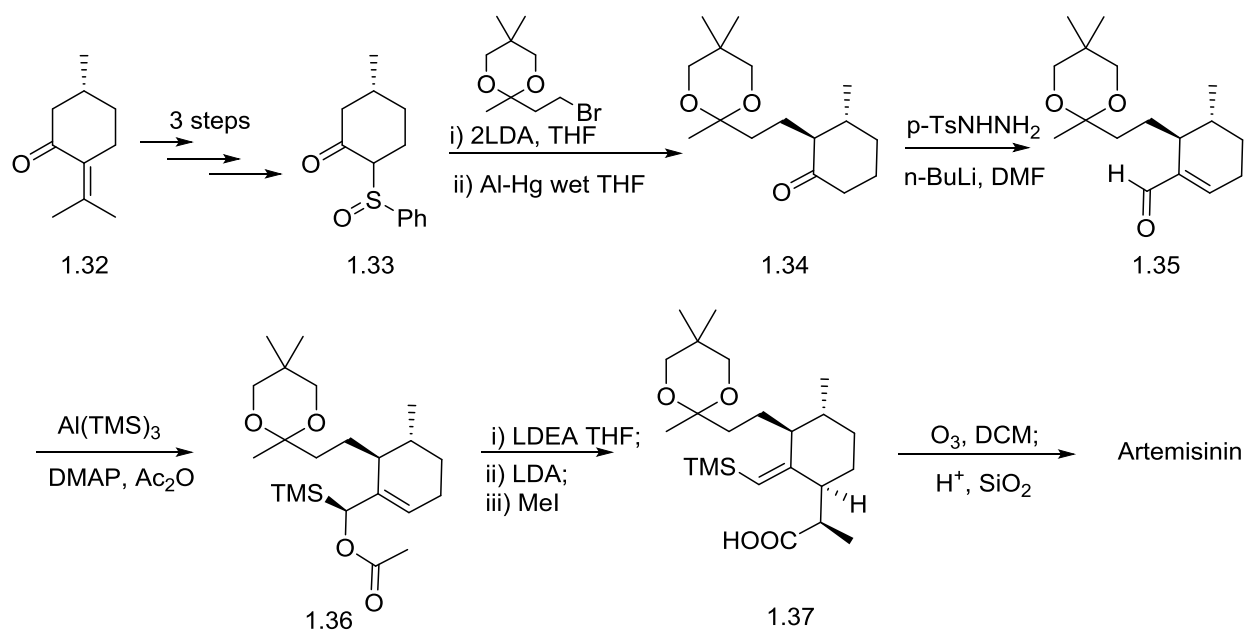
Scheme 1-5 Avery's total synthesis of Artemisinin.

Yadav et al[42] developed a protecting group free route with an overall yield of 3.2% in 10 steps. All these total syntheses utilize optically active natural products as starting material. Although the production rate of artemisinin from these total synthesis is higher than the production rate of extraction from the plant (0.4%-1%), they suffer from cost effectiveness. Recently, Zhu and Cook[41] published a short total synthesis using cyclohexenone as starting material and synthesized artemisinin in 8 steps (Scheme 1-6).
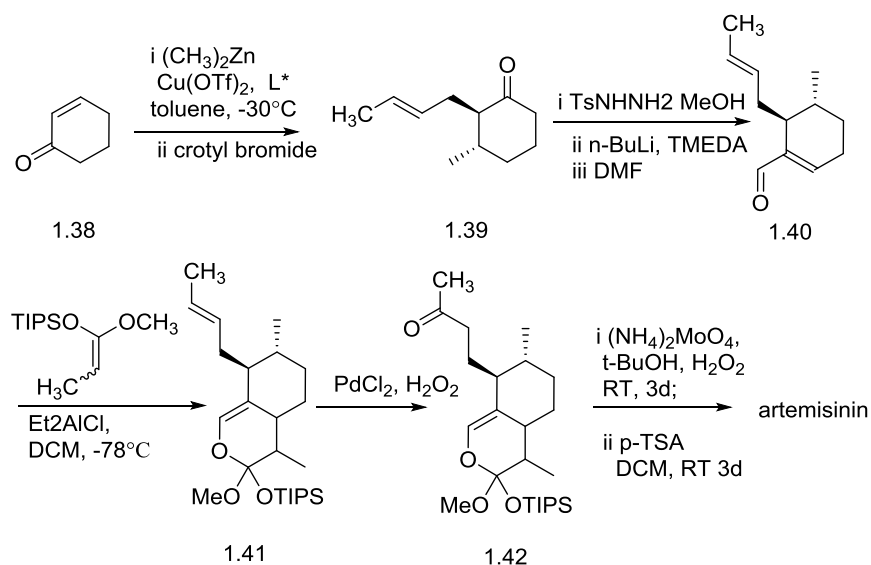
Scheme 1-6 Cook's total synthesis of artemisinin

Besides chemical synthesis, researchers also employed synthetic biology technology to produce artemisinin. Dr. Keasling and his team from the University of California, Berkeley genetically engineered a yeast to produce artemisinic acid from glucose[49]. Partnered with Sanofi, a French pharmaceutical company, they launched a semi-synthetic artemisinin production line and announced production of 35 tons of artemisinic acid in 2013[29]. They are hoping to stabilize the artemisinin world supply.

1.5 Artemisinin derivatives

1.5.1 First generation of artemisinin derivatives

During the structure elucidation of artemisinin, scientists found that its lactone moiety could be reduced by $NaBH_4$ in methanol at 0-5 ℃ to give a lactol, dihydroartemisinin 1.7 (DHA, Figure 1-4). This is interesting because many lactones do not respond to these conditions[18]. DHA is twice as active as artemisinin, but it has a relatively high degree of neurotoxicity at large doses in animal models[50]. Nonetheless, DHA provides an active hydroxyl group from which to prepare

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the first generation of artemisinins which include artemether 1.43[51], arteether 1.44[52], and artesunate 1.45[53] (Figure 1-4).
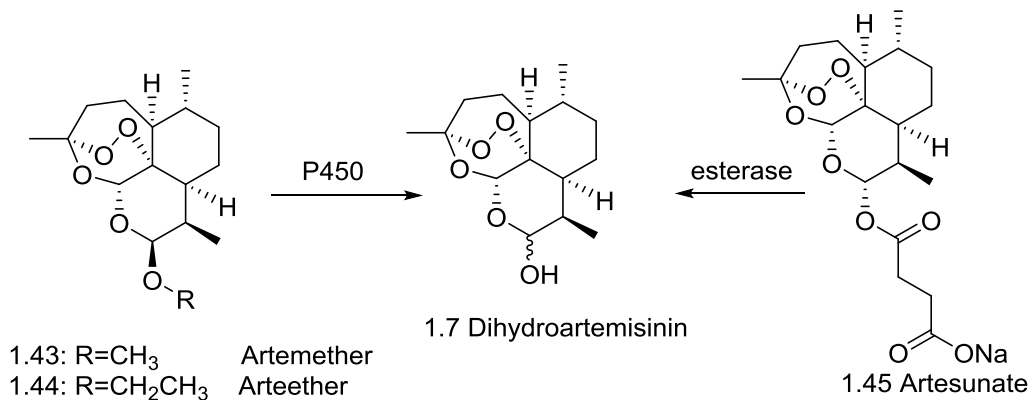
Figure 1-4 First generation of artemisinin derivatives and their metabolism pathway

Among these, DHA, artemether 1.43 and artesunate 1.45 have been used in ACT as recommended by WHO to treat uncomplicated malaria (Table 1-1)[7]. All of these derivatives are simple ether or ester analogues of DHA. They could metabolize to DHA either through cytochrome P-450 oxidation or esterase cleavage *in vivo*, thus have potential to be neurotoxic. In addition, these derivatives still showed short plasma half-lives. Efforts to prolong the half-life and lower the potential for neurotoxicity of artemisinin derivatives are ongoing. These new analogues are designated as the second generation of artemisinins.

Table 1-1 ACTs recommended by WHO*

Name	Artemisinins	Partner	$t_{1/2}$ of Artemisinins[54]	$t_{1/2}$ of partner drug[55]
Coarsucam	Artesunate	amodiaquine	0.39-0.65hr	~10 d
Artequin	Artesunate	mefloquine	0.39-0.65hr	2-3 week
Coartem	Artemether	lumefantrine	2-3.1hr	3-4 d
Ariplus	Artesunate	sulfadoxine pyrimethamine	0.39-0.65hr	3-7 d
Duocotecxin Artekin	Dihydroartemisinin	piperaquine	1.57-1.63hr	4-5 week

* ACT= artemisinin combination therapie;

1.5.2 Second generation of artemisinin derivatives

To overcome the drawback of the first generation of artemisinin derivatives, researchers have focused on the development of more robust and metabolically stable derivatives of artemisinin. Different strategies have been applied which include blocking the metabolic position of the ether side chain, changing the acetal functional group to non-acetal group, etc.

Since the ether of DHA is metabolized by CYP 450, a practical approach to prolong the half life is to design a derivative which is a poor substrate for CYP 450. Artelinic acid 1.46[56], developed by the Walter Reed Army Institute, was water soluble and hydrolytically stable.

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Further modification of artelinic acid has yielded compound 1.47[57] which showed 20 and 40 times more activity than artemisinin and artelinic acid respectively. O'Neill[58] changed alkylether to arylether to prevent the CYP450 oxidation, compound 1.48 exhibited 3 more times active than artemether when tested *in vivo*. Fluorine substitution is well known to slow metabolism of many lead molecules[59]. Bégué[60] prepared some fluoroalkyl ethers of dihydroartemisinin which are represented by compound 1.49. These compounds have IC$_{50}$ values between 27nM and 72 nM. However, low water solubility rendered them useless for further development (compound 1.49 log *P*=6.1).
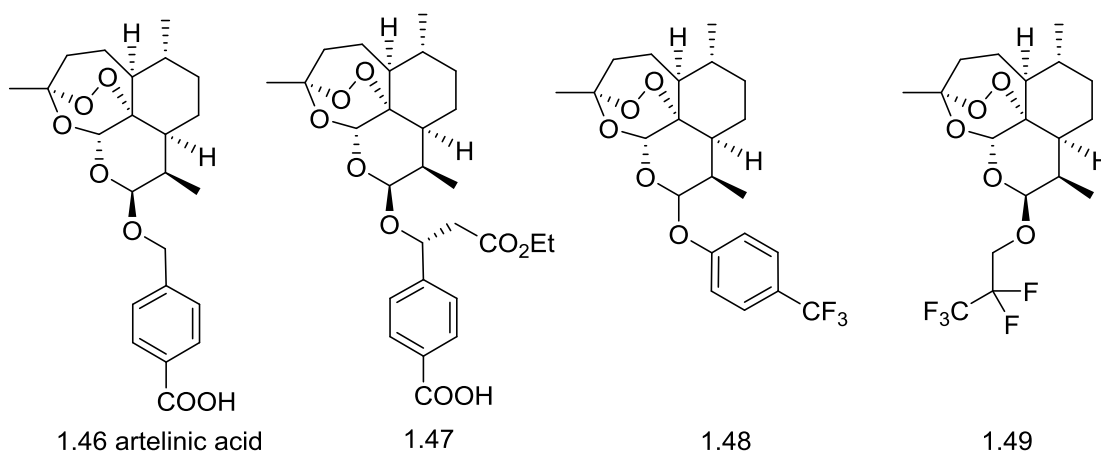
Figure 1-5 Examples of C-10 ether derivatives of artemisinin

Another approach toward this purpose is to replace the O, O-acetal with an O, N-acetal. By changing O-11 to N-11, a more stable lactam was obtained which are termed 11-azaartemisinins. Torok et al[61] reacted artemisinin with alkylamines using H_2SO_4/SiO_2 as a catalyst to obtain N-subsituted 11-azaartemisinins which is exemplified by compound 1.50. *In vitro* bioassay indicated that this series of derivatives were more active than artemisinin. Avery's group[62] took advantage of their total synthesis route to prepare a series of N-Alkyl-11-aza-9-

desmethylartemisinins. *In vitro* assay showed that short alkyl or phenyl terminus were more active than artemisinin. Particularly, compound 1.51 showed 7 times more activity than artemisinin towards the W-2(chloroquine-resistant and mefloquine-sensitive) strain. Following these reports, Haynes[63], Mekonnen[64] and Singh[65], also studied many different substitutions at N-11 by Michael addition or acylation of the amide nitrogen; all these compounds were found to be active.

1.50   1.51   1.52 R=SO$_2$R$_1$, COR$_2$
1.53 R=CH$_2$EWG
1.54 R=NH$_2$ NCH$_2$OH
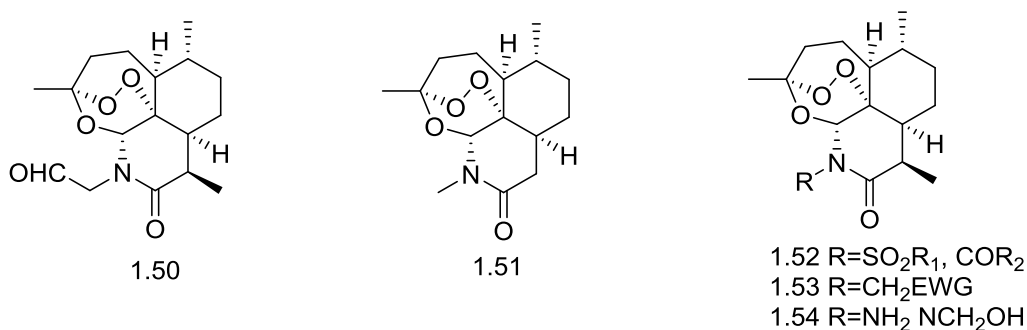
Figure 1-6 Examples of 11-azaartemisinins

Replacement of the C-10 oxygen atom by nitrogen was also explored to achieve more metabolically stable derivatives. Many aryl amino derivatives were prepared. Compound 1.55[66] was reported to be several fold more active than artemisinin (IC$_{50}$ ≤0.16ng/mL), however, *in vivo* activity toward *P. berghei* was not significant. On the contrary, compound 1.56[67] was more active than artemisinin *in vivo*. Haynes synthesized several 10-alkylamino artemisinins from which they identified artemisone (1.57)[68]. This compound is 10 times more potent than artesunate *in vitro* and was not metabolized to DHA *in vivo*. It has been further developed by Bayer and Medicines for Malaria Venture (MWV) and is in phase II clinical trial[69]. Cho et al[70] prepared a series of 10-subsititued triazolyl artemisinins by Huisgen 1,3-dipolar cycloaddition

(1.58). These compounds were tested against several cancer lines instead of malaria parasite and found active.
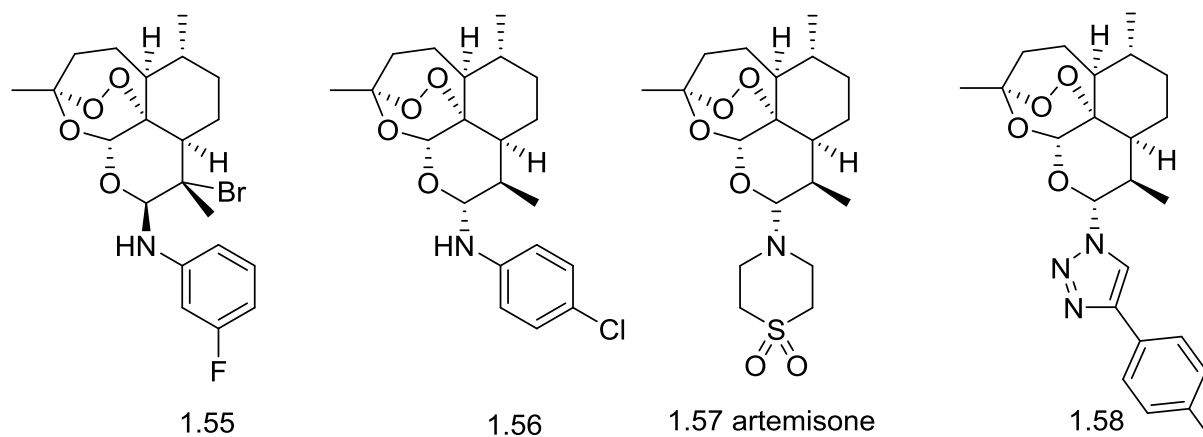
Figure 1-7 C-10 amino derivatives of artemisinin

Replacement of the C-10 oxygen of DHA with carbon led to 10-carbartemisinin. Since they are no longer acetals, they cannot be metabolized to DHA *in vivo*, thus may not be neurotoxic. This strategy has been the focus of artemisinin derivatives synthesis during the last 20 years and a huge number of artemisinin derivatives have been reported. Reaction between DHA, allytrimethylsilane and boron trifluoride etherate afforded compound 1.59[71] which served as an intermediate for synthesis of compounds 1.60 and 1.61. Compound 1.60 demonstrated comparable activity with artemether, but was less active than DHA[72]. Compound 1.61 exhibited superior activity to artemether and artesunate *in vitro* and *in vivo*[73]. The C-10 aryl derivative 1.62 was prepared by Lewis acid catalyzed arylation of the benzoate of DHA with benzene and it showed an IC_{50} value of 0.31 and 0.37 ng/mL against W2 and D6 strains, respectively[74]. Treating the 10α-acetate of DHA with 2-naphthol in the presence of boron trifluoride etherate yielded a 1:1 mixture of 1.63 and 1.64[75]. The C-9 methyl configuration in 1.64 was epimerized which was

found detrimental to the activity. These C-10 carba derivatives usually are much less labile in acidic conditions[76].

Another set of interesting derivatives are fluorinated artemisinins. By introducing a trifluoromethyl group at C-10, fluorinated artemether 1.65[77] was found to be 60 times more stable than artemether under simulated stomach conditions and had an IC_{50} value of 0.8nM compared to artemether (3.5nM) in *in vitro* assay against the *P. falciparum* FCB1 strain.
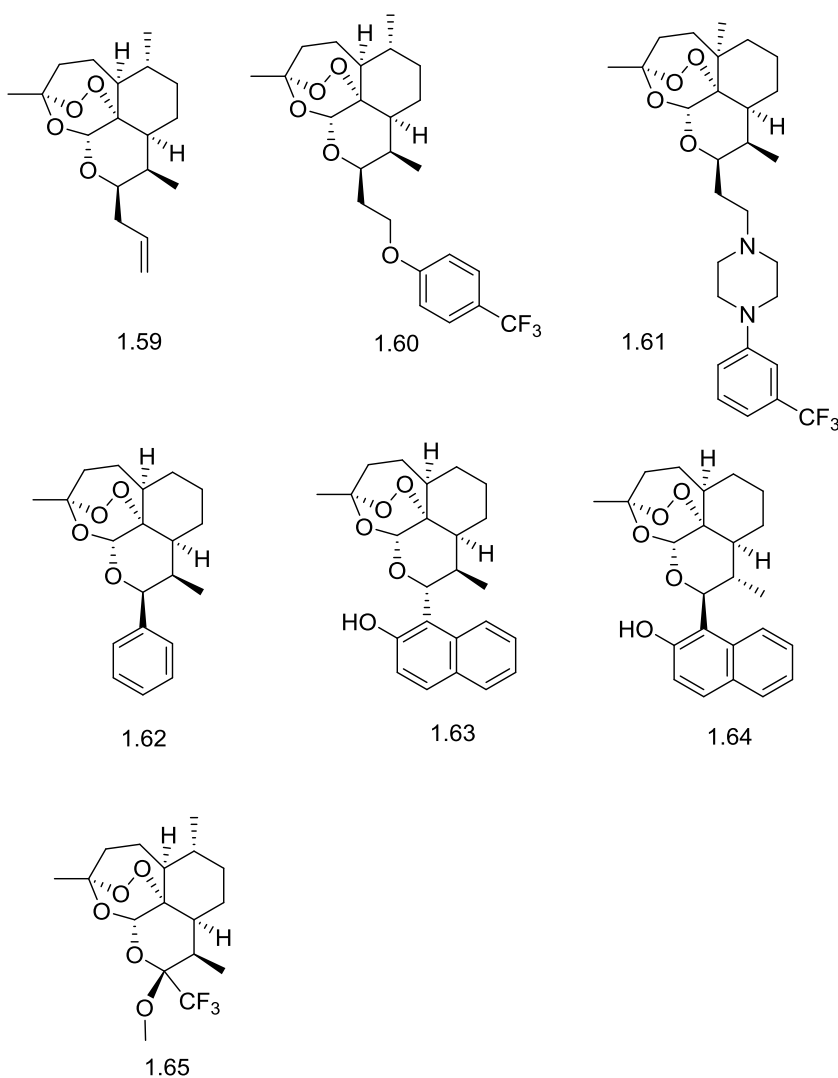
Figure 1-8 Representatives of C-10 carbaartemisinins

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Besides finding more metabolically stable derivatives of artemisinins, researchers also explored other positions of artemisinin to prepare derivatives with the aim of finding more potent compounds, investigation of the SAR and as a probe for the mode of action. These derivatives are discussed in the next section in detail.

1.5.2 C-3 derivatives of artemisinin

C-3 is near the endoperoxide bond, so substitution at this position might have a large effect on its activity. 1.66 and 1.67 are the first analogues to appear in the literature[78], however, no activity was reported. Avery et al synthesized an array of the C-3 and C-9 substituted artemisinin[79] (1.68 and 1.69) and 10-deoxoartemisinin analogues[80] 1.70 and evaluated their *in vitro* antimalarial activity. The results showed that many of these derivatives were more potent than artemisinin.
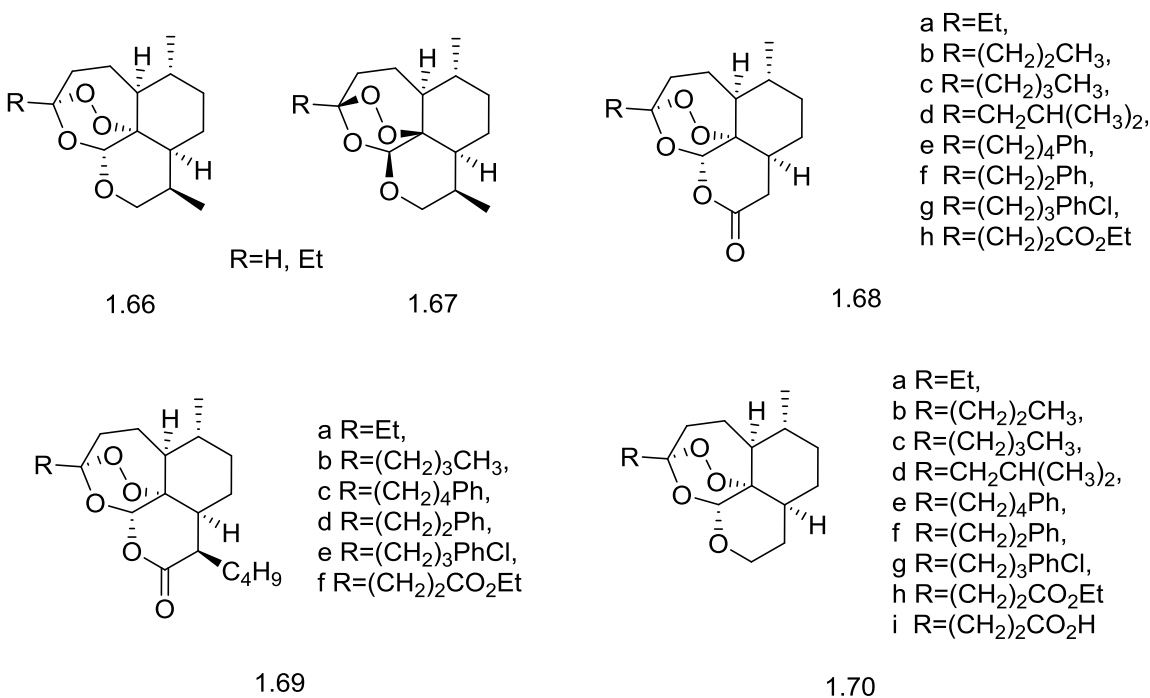
Figure 1-9 Examples of C-3 derivative of artemisinin

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1.5.3 C-16 derivatives of artemisinin

C-16 derivatives are less populous than derivatives at C-10. Artemisitene 1.71 is a minor component from the same plant and it has an exomethylene lactone which can serve as Michael acceptor. Compounds 1.72a-e were synthesized from 1.71 following Mukiyama conditions[81]. The heterocycles 1,2,4-triazole, benzotriaole, or benzimidazole can also react with artemisitene under neutral or basic conditions to afford compounds 1.72f-h[82]. Artemisitene can also react with diverse nucleophiles such as organolithiums or Grignard reagents by 1.4-addition to produce sets of C-16 derivatives. Among these, 1.73 was the product of lithium enolate of artemisinin addition to artemisitene and showed 10 times more activity than artemisinin[83].
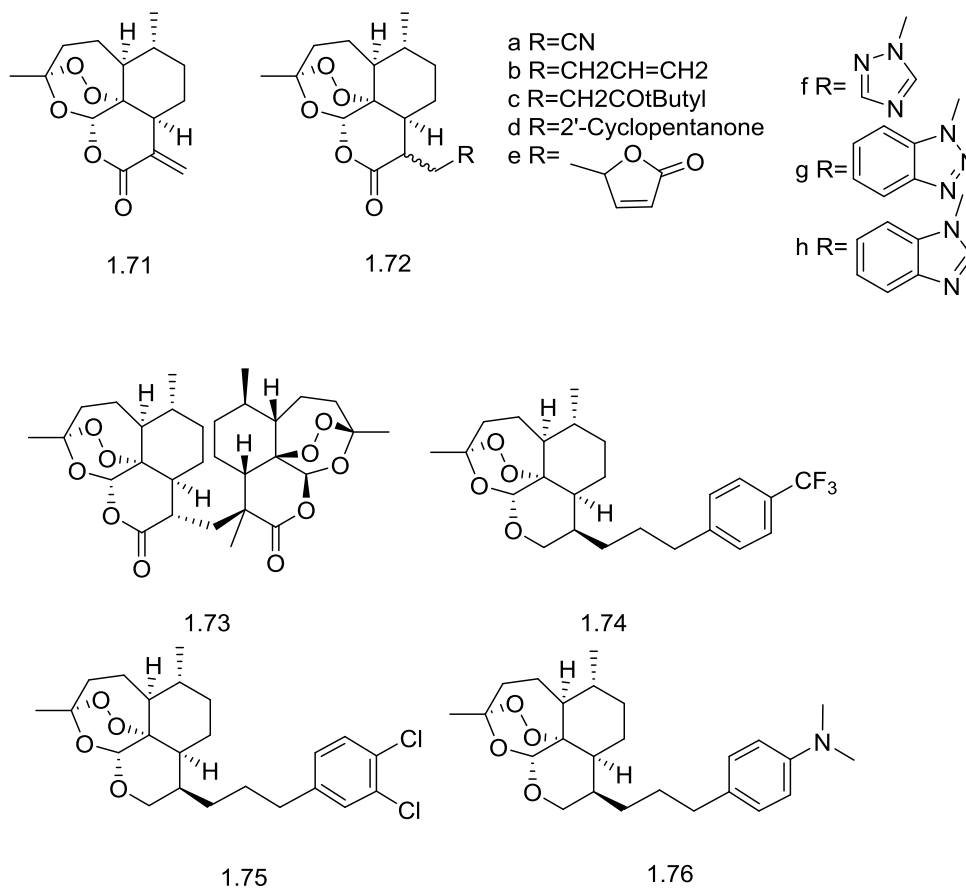
Figure 1-10 Examples of C-16 derivatives of artemisinin

Another useful reaction developed by Avery et al[84] involving the free radical induced Michael addition of alkyl or arylhalides to artemisitene. This reaction generates α and β isomers in a 1:1 ratio. The α-isomer can be epimerized to the more potent β-isomer by refluxing with DBU in THF for 12 h. Compounds 1.74 and 1.75 exhibited superior activity *in vitro*. Their low water solubility needs to be addressed in the future; the HCl salt of 1.76 is water soluble.

1.5.4 Derivatives of other positions

Derivatives from C-5, C-6 and C-7 are rare due to the absence of accessible functional groups at these positions. However, it was found that these positions can be hydroxylated when incubating with microorganisms. Compound 1.77 and 1.78 were synthesized from 7α-hydroxylartether and 15-hydroxylartether respectively which in turn were produced by fungal incubation[85, 86]. Staring with 7β-hydroxyartemisinin[87], many novel derivatives have been synthesized and evaluated *in vitro*. The most promising compound 1.79 showed >96% suppression in parasitemia at a dose of 3.3mg/kg which is comparable to artesunate[88]. Though this compound is promising, the access to starting material is limited by fermentation scale-up difficulties.
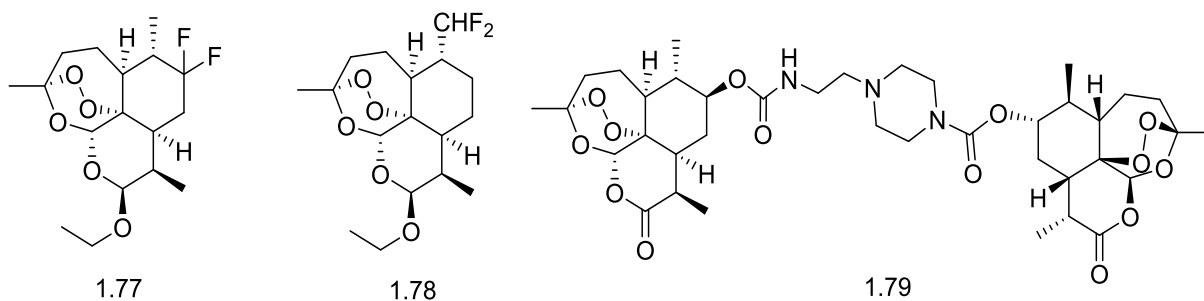
Figure 1-11 C-6, C-7 derivatives of artemisinin

1.5.5 Carbaartemisinins

To explore the effect of oxygen atoms on antimalarial activities, several carbaartemisinins in which the oxygen atom is replaced by a methylene group were synthesized (Figure 1-12). Compound 1.80-1.82[89-91] showed much lower antimalarial activity *in vitro*. Compound 1.83, two oxygen atoms were missing and showed lower antimalarial activity. Since they all lost the peroxide bond, this result confirmed that the peroxy bond was crucial for the activity. Compound 1.84[91], however, was reported to be 8 times more active than artemisinin. C-13 carbaartemisinin1.85-1.87[92] were synthesized to investigate the effect of the nonperoxidic trioxane oxygen atom of artemisinin. Compound 1.85 maintained 4% activity while deoxo analogue 1.86 held 16% of the activity. More interestingly, the isomeric peroxide 1.87 was found to possess about 60% antimalarial activity. These findings suggested that the nonperoxidic oxygen was required for optimal activity.
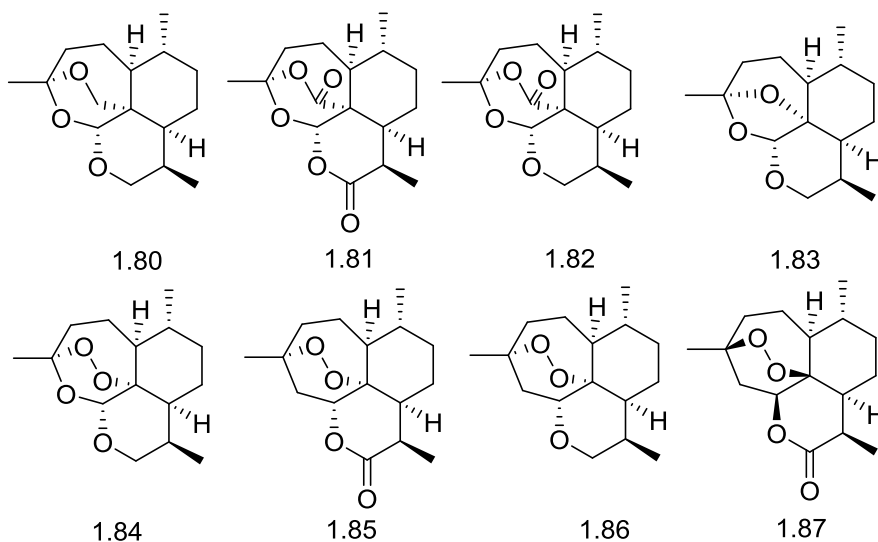
Figure 1-12 Examples of carbaartemisinins

1.5.6 Seco artemisinins

It is of interest to find out if a simplified structure of artemisinin can preserve the activity. If so, it would cut down the synthetic cost. Since the 1,2,4-trioxane was essential for the activity, Avery's group[93],[94] and Posner's group[95] [96]have synthesized some seco artemisinins lacking the A ring or D ring. These compounds showed variable activity. Some typical compounds are shown in Figure 1-13. Compound 1.88 retained the activity while compound 1.89 is more active than artemisinin which demonstrated that A ring and D ring were not critical for the activity. This opened a new door for the search of structurally simplified, easily prepared 1,2,4-trioxanes to combat malaria.
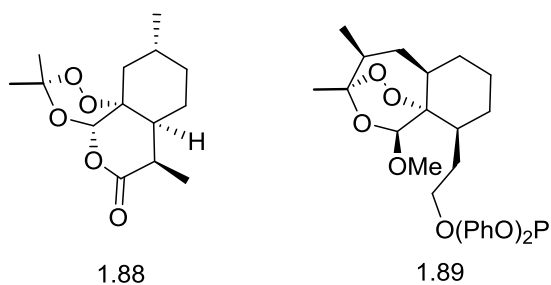
Figure 1-13 Examples of seco artemisinins

1.6 Purpose of this study

A great number of derivatives/analogues of artemisinin have appeared in the literature. Extensive research has also found an array of reaction conditions that are compatible with the endoperoxide bond. The efforts to prolong the half-life and improve potency are ongoing. This project is a continued effort to further explore the C-16 position of artemisinin with heterocycles and heteroatoms substitutions with the aim to find an optimal physicochemical combination for

the *in vivo* antimalarial activity. New derivatives will fill the chemical spaces thus providing insights to the structure-activity relationships.

CHAPTER 2

2.1 Development of C-16 derivatives of artemisinin

Artemisinin has attracted numerous scientists' attention all over the world. Our group's interest in this fantastic molecule began with a successful asymmetric total synthesis[43]. This route greatly facilitates subsequent analogue synthesis afterwards. Using this route our group has synthesized more than 200 analogues of artemisinin, including C-13 carbon analogues[92], N-11 derivatives[62], C-3 alkyl[79], and C-16 derivatives[80]. Many of these compounds are more active than artemisinin and have contributed valuable information towards QSAR[79, 97-99].

In particular, some C-16 derivatives have shown very promising results (Figure 2-1). Compound 2.1[99], the propyl side chain, was first identified in a series of linear alkyl, branched alkyl, aryl and arylalkyl substituted analogues at C-16 of artemisinin. It showed 12 and 6 times more activity than artemisinin in the W-2 and D-6 clone (choroquine-sensitive and mefloquine-resistant), respectively. Since removal of the lactone carbonyl (the product termed as 10-deoxoartemisinin) is more potent and less toxic than artemisinin[100], a new series of C-16 modified 10-deoxoartemisinin derivatives was prepared[80]. *In vitro* antimalarial assay indicated that compound 2.2 was 58 and 20 times more active than artemisinin in D-6 and W-2 clones, respectively. Compound 2.2 also demonstrated an IC_{100}=8mg/kg/day *in vivo* conducted on *P berghei* infected mice by subcutaneous administration (SC)[101]. However, oral administration (PO) of compound 2.2 showed 0% survival rate even at 128mg/kg/day dose. Compound 2.3 and

2.4 also showed very potent *in vitro* activity, being 25-70 times more active than artemisinin. Exploration of different functionality on the phenyl ring yielded compound 2.5[84]. This compound had an ED_{50} of 0.4ng/mL for SC and ED_{50} of 1.25ng/mL for PO. The positive control sodium artesunate showed an ED_{50} 0.8(SC) and 2.4(PO), respectively. Preliminary PK studies revealed that compound 2.5 had a half-life of 73 minutes and a clearance value of 20-30 µl/min/kg. However, it still had very low aqueous solubility (0.1 µg/ml at Ph=6.5 phosphate buffer) and therefore low bioavailability. To address this issue, different polar substituents (amine, sulfur, hydroxyl) on the phenyl ring were explored[97]. All of these compounds showed *in vitro* antimalarial activity against *P. falciparum*. Compound 2.6, with a para-hydroxy was chosen to conduct a SAR study at this position. A set of diverse substituents including ester, sulfonates, carbamates, phosphates were attached to the hydroxyl group[102]. Compound 2.7 showed 8 times more activity than artemisinin in *in vitro* antimalarial assay. In *in vivo* studies performed in *P. berghei* infected mice, it showed 80% reduction in parasitemia on intravenous administration (iv) while the oral activity was only 40% compared to arteether which is 100% and 60.5%, respectively.

The development of potent and yet orally active antimalarial agents based on artemisinin is ongoing. At this point, we felt the C-16 phenylethyl analogue could be changed to a heterocyclic ring which is more polar and metabolically stable. This modification might be able to increase the water solubility and thus increase the oral bioavailability. The 1, 2, 3-triazole ring came to our attention due to its intriguing physiochemical property, polarity and stability.
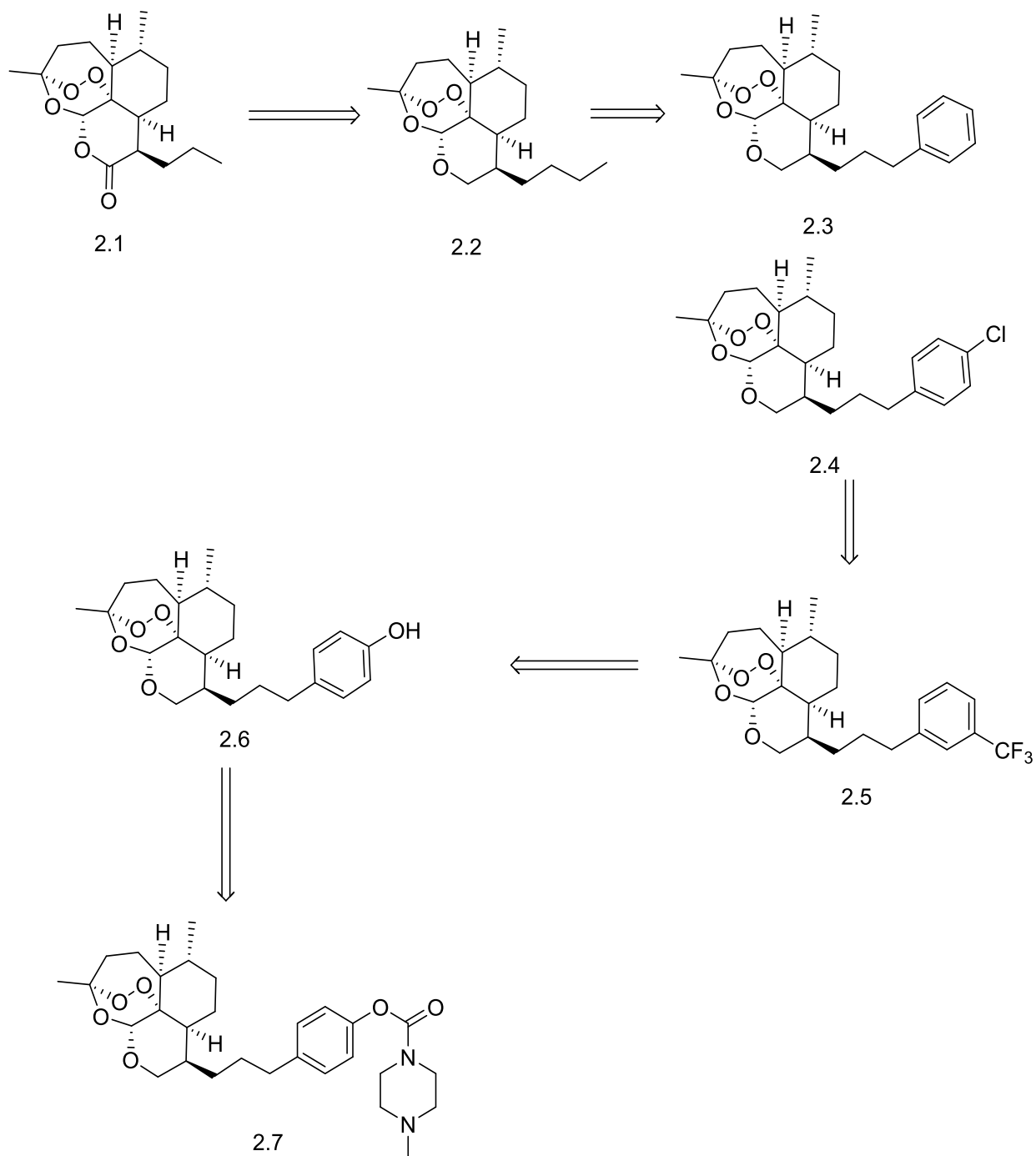
Figure 2-1 Development of 10-deoxo-16 substituted artemisinin derivatives

2.2 Click chemistry

The 1,2,3-triazole ring system did not gain much attention until recently due to the quickly growing interest in click chemistry. Click chemistry as a concept was first introduced by Sharpless in 2001[103]. They defined click chemistry as a set of chemical reactions capable of modular synthesis, mild conditions, stereospecificity, high yield and simple work-up. Four types of reactions satisfied this criteria: a) nucleophilic ring opening reactions; b) non-aldol type carbonyl reactions; c) C-C multiple bonds addition and d) cycloaddition reactions[103]. Among these reactions, copper catalyzed azide-alkyne cycloaddition (CuACC) to give the 1, 2, 3-triazole become the most popular one in the last decade which is often referred as the 'click reaction'.

The azide-alkyne cycloaddition was originally developed by Huisgen in 1963[104]. The reactions were usually carried out by heating the alkyne and azide to more than 100°C and required several hours or even days to complete. In addition, this reaction produced a mixture of triazole with 1, 4 and 1, 5-regioisomer when using unsymmetrical alkynes. These drawbacks have limited its application in organic synthesis for over 40 years. The breakthrough came from two independent studies conducted by Meldal[105] and Sharpless[106] in 2002. A copper catalyzed azide-alkyne cycloaddition can be carried out in aqueous media and at room temperature. Additionally, in contrast to the original reaction it only affords the 1, 4-regioisomer (Scheme 2-1). Later, the regiospecific synthesis of the other 1,5-regioisomer was achieved by ruthenium catalysis[107].
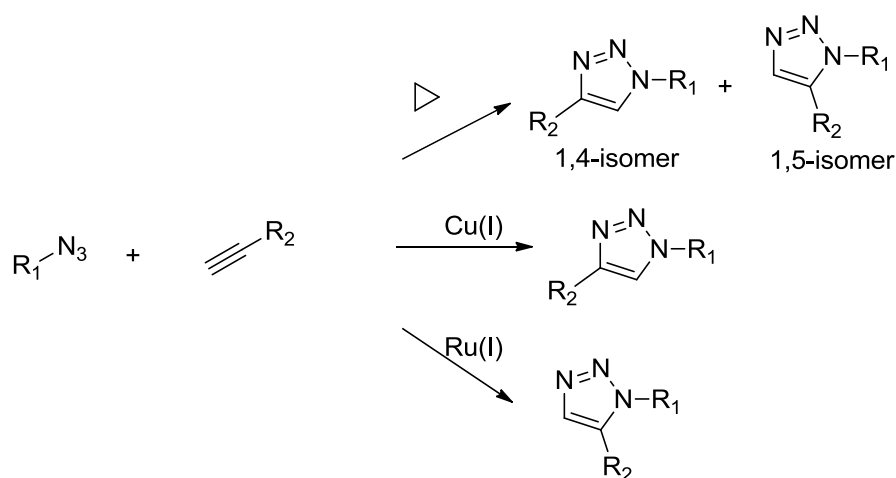
Scheme 2-1 1,2,3-triazole formation under different conditions

2.3 Proprieties of the 1,2,3-triazole

The 1,2,3-triazole as a building block has a wide application in medicinal chemistry[108-110]. It has many intriguing features that are favored in drug discovery. The 1,2,3-triazole is a five membered aromatic heterocycle. Due to its aromatic stabilization, it is far less reactive to acidic and basic hydrolysis and stable under reductive and oxidative conditions. It is also stable to metabolic transformation[111]. In addition, this compound is water soluble with a high dipole moment (calculated value 5D). Finally, a 1,2,3-triazole can also participate in hydrogen bonding, and π-π stacking interactions[112]. The 1,2,3-triazoles are not found in nature, however, several drugs containing these heterocylces include tazobactam[113], cefatrizine[114], and carboxyamidotriazole[115] have been approved by the FDA. By using a 1,2,3-triazole group, one may be able to optimize the physicochemical properties[116].

2.4 C-16 modified artemisinin with 1,2,3-triazole

Recently, our group synthesized a series of 1,2,3-triazoles at the C-16 position with the hope of improved potency relative to artemisinin[117]. Although these derivatives were less potent than artemisinin in *in vitro* antimalarial assay, preliminary *in vivo* studies involving compound 2.8 (Figure 2-2) confirmed that it did have a longer half-life and better solubility. This result implied that the heterocyclic ring could increase the hydrophilicity of the compound and thus enable optimization of the pharmacokinetic properties.

2.8
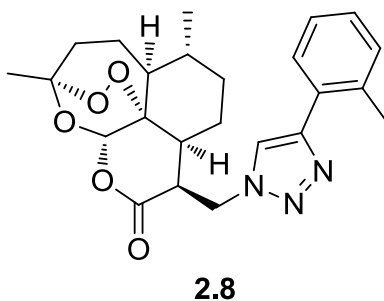
Figure 2-2 C-16 triazole modified artemisinin

2.5 Aims of study

Guided by previous studies in our group, we decided to investigate further the role of the aromatic substituent in the lead structures 2.4-2.6. SAR analysis from this work indicated that proper spacer length needed for optimal activity. As continued efforts to develop orally active antimalarial agents based on artemisinin, the present study aims to synthesize artemisinin C-16 derivatives with heterocyclic and heteroatomic side chains and to perform antimalarial biological evaluation on the synthetic products.

2.5.1 The 1, 2, 3-triazole side chain substituted at C-16

Based on the information gained from previous studies in our group, compound 2.9 was designed to restore the potency to the 1,2,3-triazole substituted artemisinins. Compound 2.9 combines all optimized features derived from SAR studies. Like compound 2.5, it has a three-carbon chain for optimal activity; the triazole was introduced for better pharmacokinetic and pharmacodynamic properties as shown in compound 2.8. For the substitution at the 4' position of the triazole ring, a tertiary amine was chosen for several reasons. First, this amine group might provide additional H-bonding that could enhance antimalarial activity. Also, the amine substituent could facilitate transport of the compound to the food vacuole of the parasite since the food vacuole is slightly acidic (pH~4-6)[118] and thus would be expected to be trapped inside the food vacuole and thus concentrate the compound over time. In addition, the protonated amine group will further increase the polarity of the compound to obtain better water solubility.

2.5.2 Heteroatoms substitution at C-16

The Avery group is also interested in continued development of our QSAR models to improve reliability and predictivity. For a QSAR model to be extrapolatively predictive, a certain chemical space needs to be covered[119]. As can be seen from chapter 1, artemisinin derivatives modified at C-16 are relatively less populous in comparison to the number of C-10 derivatives in the literature. New derivatives with heteroatom substitution at this position would thus expand the blind spot in the current chemical space and make contributions to an updated QSAR model.

2.5

IC$_{50}$=19.2nM (D6)

IC$_{50}$=68.8nM (W2)

2.8

IC$_{50}$=137nM (D6)

IC$_{50}$=132 nM (W2)

Different substituents for SAR and solubility in water

Triazole for better PK

2.9

Three-carbon chain is optimal for activity
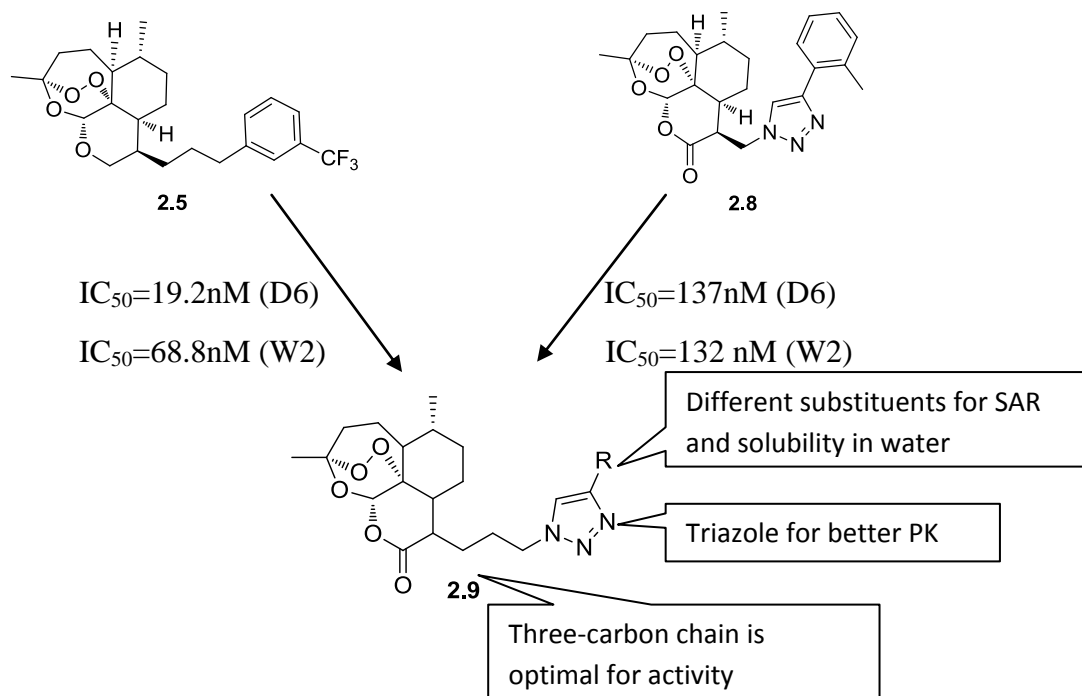
Figure 2-3 Designed compound 2.9

CHAPTER 3

3.1 Synthesis of artemisinin derivatives

3.1.1 Synthesis of artemisitene

Artemisitene coexists in *Artemisia annua* along with artemisinin as a low percentage component. In order for researchers to investigate the C-16 substituent effect on the artemisinin scaffold, large quantities of artemisitene is needed and thus it has been prepared from artemisinin by several routes. El-Feraly[120], the first to convert artemisinin to artemisitene, employed a four-step sequences; Pitayatat et al[121] improved the route to a two pot method by using a selenoxide elimination reaction. To avoid using toxic selenium, this route was further improved in our group[84] using a sulfoxide elimination reaction (Scheme 3-1). Typically, artemisinin was treated with LDA at low temperature followed by $PhSS(O_2)Ph$ which gave the C9β-sulfide of artemisinin, this phenylsulfide was then oxidized to the sulfoxide with m-CPBA at -78 ℃. The resultant sulfoxide spontaneously underwent a Cope-type elimination at room temperature to provide the target molecule *in situ*. This route provided a major improvement. However, to obtain the best results, the m-CPBA needed to be recrystallized before use and the solvent DCM needed to be anhydrous. In addition, the reaction temperature needed to be maintained at -78 ℃ for a long time to avoid over oxidization of sulfide to a sulfone.
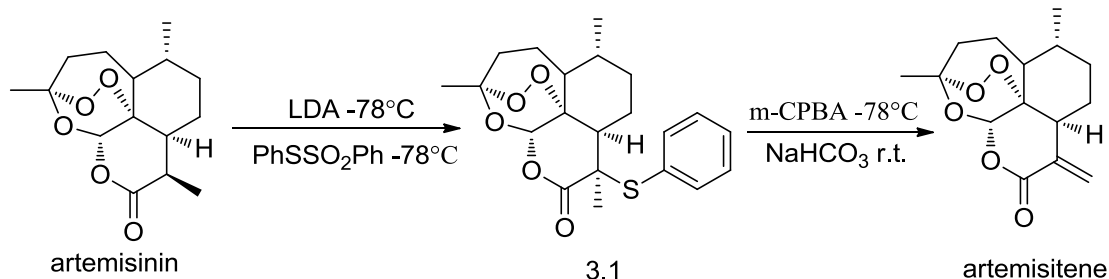
Scheme 3-1 Artemisitene synthesis

To simplify the operation, we tried to use hydrogen peroxide together with TMS-Cl which is reported to selectively oxidize sulfides to sulfoxides[122]. The active species is formed *in situ* (Scheme 3-2). Thus, intermediate 3.1 was dissolved in acetonitrile and treated with TMS-Cl and hydrogen peroxide at room temperature. After separation by flash chromatography, artemisitene was afforded in 75% yield. This improvement employed room temperature conditions, the by-product trimethylsilylanol was volatile on rotary evaporation, and the yield was higher or comparable to the method using m-CPBA at low temperature.
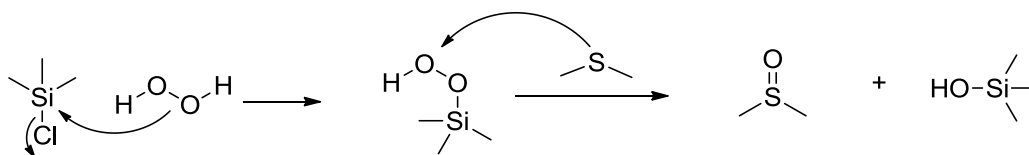
Scheme 3-2 Sulfoxide formation by TMS-Cl activated H_2O_2

3.1.2 Synthetic strategy

With this crucial intermediate in hand, we planned our synthetic routes as follows (Scheme 3-3). Retrosynthesis of the designed analogues suggested that these compounds could

be synthesized from two routes (Scheme 3-3): In route A, an azide functional group was introduced into artemisitene first followed by reaction with different alkynes by click reaction to furnish the final target compounds. This route has the advantage of quickly accessing target libraries using alkyne synthons available commercially. In route B, the triazole side chain could be built first and then incorporated into artemisitene by a radical induced 1, 4-addition.
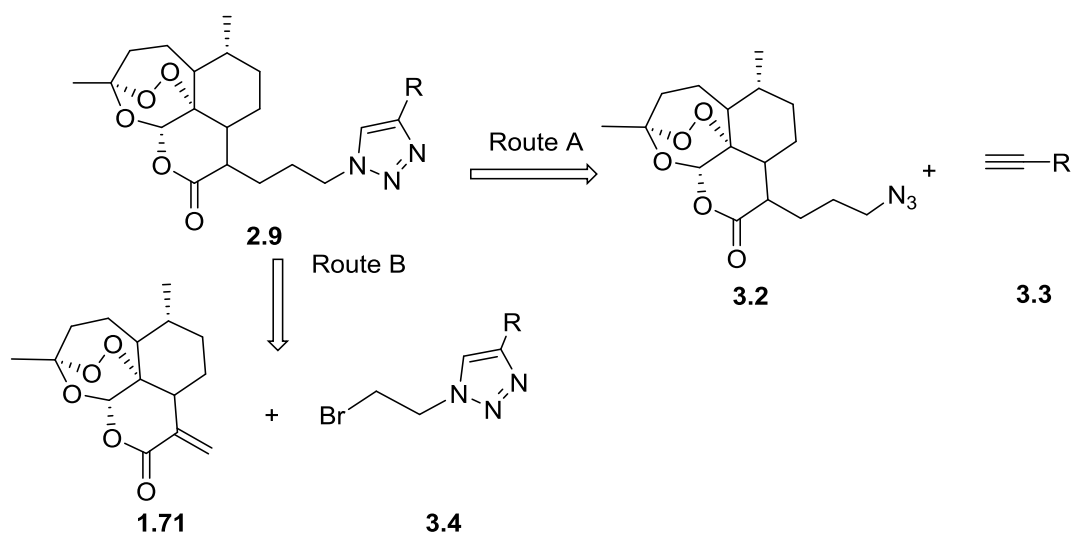
Scheme 3-3 Retrosynthesis of the target compound 2.9

3.2 Proof of concept

To generate a proof of concept, that the three-carbon chain between artemisinin and the aromatic 1,2,3-triazole ring in 2.4 enhances antimalarial activity, we first investigated the synthesis of a model molecule **3.9** (Scheme 3-4).
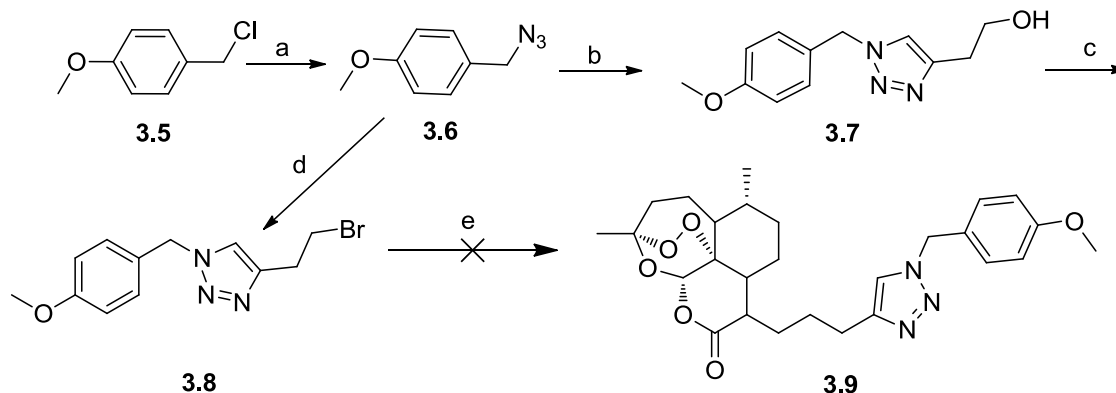
Scheme 3-4 Synthesis of model compound 3.9

Reagents and conditions: a). NaN$_3$, DMF, 105 °C, 96%; b). but-3-yn-1-ol，CuSO$_4$, sodium ascorbate, water: t-BuOH, 80% c)Ph$_3$P$^+$-Br Br$^-$, pyridine, 80%; d) 4-bromo-1-butyne, CuSO$_4$, sodium ascorbate, water: t-BuOH (1:1), 75%; e).n-Bu$_3$SnH, AIBN. PhH, artemisitene, or Tris(trimethylsilyl)silane, AIBN, PhH, sealed tube.

The synthesis of compound 3.9 commences from a commercially available compound p-methoxybenzenylchloride 3.5. This compound was converted to azide 3.6 through a nucleophilic substitution by treating it with sodium azide. Then click additions between azide 3.6 and but-3-yn-1-ol afforded the triazole 3.7. This triazole 3.7 was transformed into bromide 3.8 by the Appel reaction using Ph$_3$PBr$_2$ in the presence of pyridine. Also, bromide 3.8 could be obtained by reacting the azide with 4-bromo-1-butyne. However, the subsequent radical-induced 1, 4-addition to artemisitene was somewhat problematic. Different radical sources such as tributyltin hydride or tris(trimethylsilyl)silane were tried, however, the desired product was not detected. We postulated that the benzylic protons might be the cause of the failure of this radical-induced Michael addition, so it would be reasonable to use a precursor without benzylic hydrogens (Scheme 3-5).
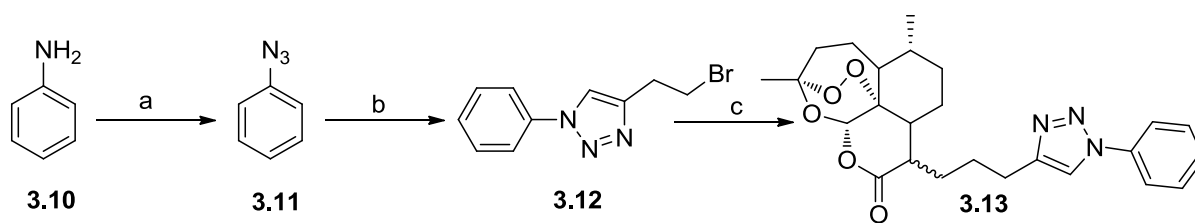
Scheme 3-5 Synthesis of compound 3.13

Reagents and conditions: a): HCl, NaNO$_2$, NaN$_3$, 86%; b): 4-Bromo-1-butyne, sodium ascorbate, CuSO$_4$, water: t-BuOH(1:1) , 67%; c): Artemisitene**,** n-Bu$_3$SnH**,** AIBN*,* PhH, 80 °C, 8hr, KF. 10-30%

To this end, aniline was converted to phenylazide through diazo transfer and azide displacement to give 3.11. A click reaction between phenyl azide and 4-bromo-1-butyne provided the triazole compound 3.12. Radical conjugate addition to artemisitene then occurred smoothly to give both C9α and C9β isomers 3.13. Separation of this mixture by flash column chromatography with a carefully controlled EtOAc/hexanes gradient afforded the pure diastereomers 3.13α and 3.13β (Figure 3-1).
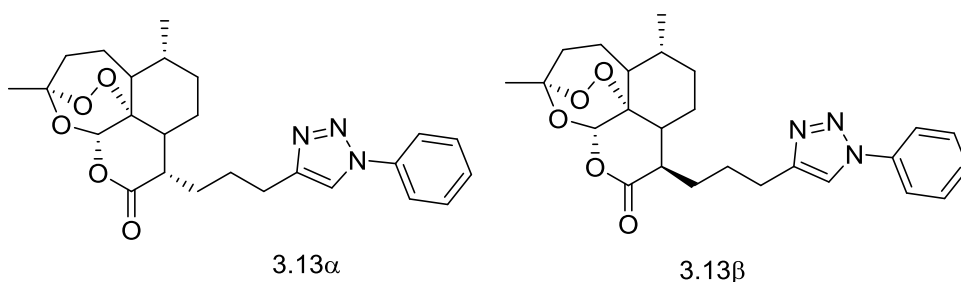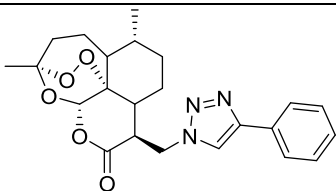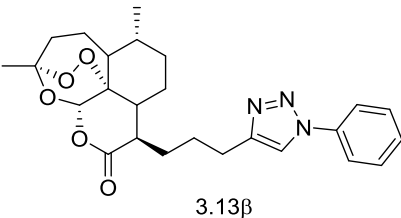
Figure 3-1 Structure of compound 3.13α and 3.13β

The *in vitro* biological results (Table 3-1) were encouraging. Compound 3.13β showed 2 times more activity than the homologous compound with one carbon space linker. This demonstrates that the hypothesis that a three-carbon chain between artemisinin and the aromatic

37

ring is optimal for activity. Encouraged by these results, we decided to embark on synthesis of a triazole library.

Table 3-1 *In vitro* activity of selective compounds

Structure	P. falciparum IC$_{50}$ (nM)	
	D6	W2
	152.8	135
3.13β	70.6	48.54
artemisinin	17.73	23.21

3.3 Synthesis approach through route A

Route A was explored first by trying to introduce an azide functional group at C-16 with an appropriate linker length so that we could easily build a small library using different commercially available substituted alkynes (Scheme 3-6). We envisioned that this azide could be obtained from an amine through diazo formation and azide displacement[123]. Thus, 2-bromoethylamine was first protected as a PMB carbamate (Moz) and then incorporated into

artemisitene by a radical addition. Deprotection of PMB group by trifluoroacetic acid gave amine 3.17. This compound amine 3.17 was detected by TLC, but was not stable enough to undergo column chromatographic purification.
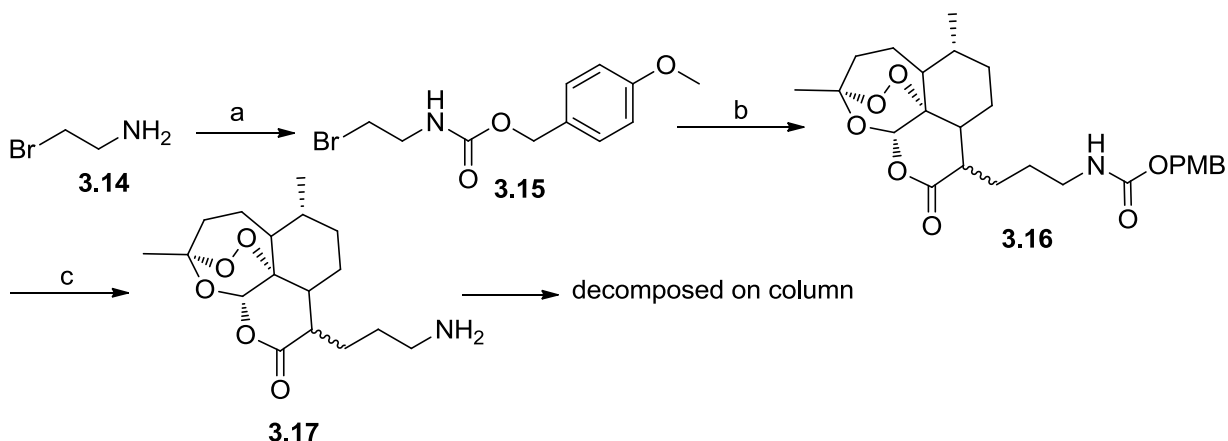
Scheme 3-6 Synthetic approach A

Reagents and conditions: a) CDI, PMB-OH, DIPEA, 50%; b) Artemisitene, n-Bu$_3$SnH, AIBN, PhH. 80 ℃, 10%-30%; c) TFA, DCM.

3.4 Synthesis derivative through route B

As a result, we refocused our attention on route B (Scheme 3-7). In this route, the 1,2,3-triazole side chain would be built first, and then incorporated into artemisitene **3.3** by a radical-induced Michael addition. Our first choice for substituents on the 1,2,3-triazoles was a tertiary amine which are frequently found in antimalarial drugs. These amine substituents are believed to have several functions. First, they can decrease the lipophilicity of the compound, thus they might increase oral bioavailability, which is a major issue for the compounds that our group previously synthesized. Second, they can block the metabolism on the side chain. Third, they can help to localize the compound to the parasite[124]. Fourth, they can also provide an additional nucleophilic position for derivatization.
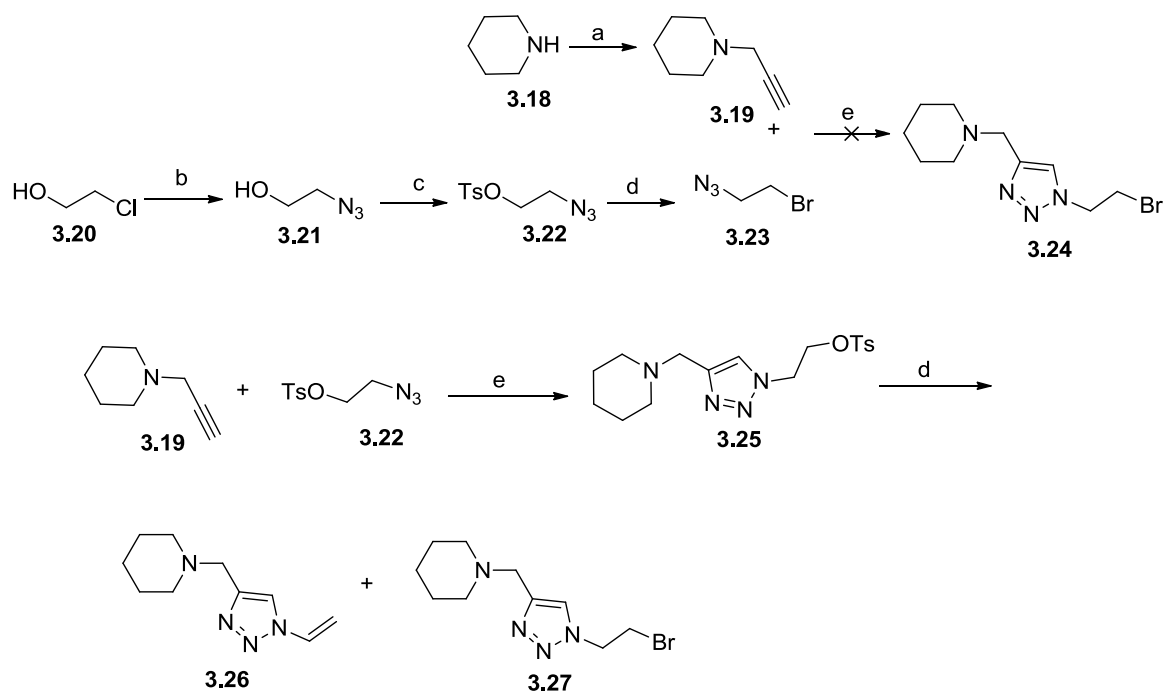
Scheme 3-7 Synthetic approach B
Reagents and conditions: a) Cs_2CO_3, propargyl bromide, acetone, 50%; b) NaN_3, NEt_3, DMF, 92%; c) TsCl, pyridine, 60% ; d) LiBr, acetone reflux, 30%; e) sodium ascorbate, $CuSO_4$, water: t-BuOH(1:1)

Azide synthon 3.23 was prepared by following a three-step procedure in the literature[125] while alkyne synthon 3.19 was prepared by reacting piperidine with propargyl bromide. Surprisingly, copper catalyzed azide-alkyne cycloaddition between these two synthons did not give the desired product. When we tried reacting alkyne 3.19 with the tosylated azide 3.22, triazole 3.25 was obtained. Nucleophilic bromination of compound 3.25 with LiBr in acetone gave a mixture of products 3.26 and 3.27 which resulted from tosylate elimination (E2) and bromo substitution (Sn2), respectively. The separation of these two compounds by column chromatography proved difficult due to their similar polarity. We also tried to separate them using Sephadex LH-20. However, the difference in molecular weight was not great enough to

improve separation. HPLC was used to separate these two compounds and was successful. However, given the fact that we needed large quantities of the compound 3.27 to carry out free radical-induced Michael addition with artemisitene, we thought it would be wise to find an alternative way to prepare this intermediate. In addition, azide compounds are usually considered to be explosive. As a rule of thumb, the compound should have a (C+O)/N ratio of more than 3 to be safe[103]. To lower the risk, we needed a way to synthesize compound 3.23 without separation.
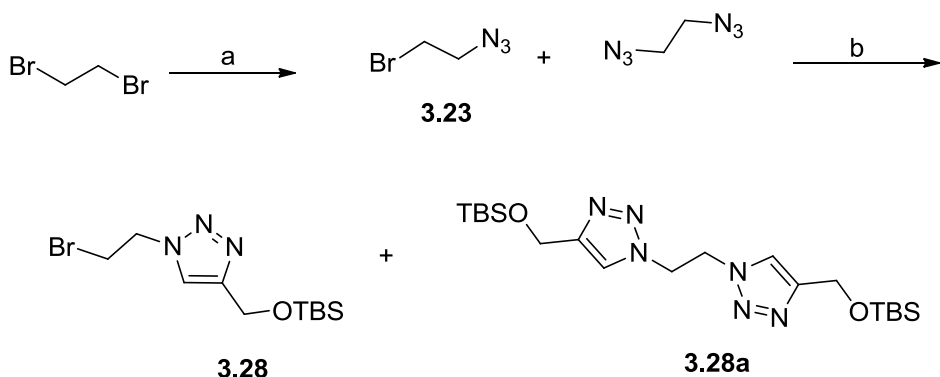
Scheme 3-8 Synthesis of side chain
Reagents and conditions: a) NaN$_3$, MeOH:H$_2$O (10:1) 45 °C, overnight; b) TBS protected propargyl alcohol, sodium ascorbate, CuSO$_4$, 40% over two steps.

Thus, as outlined in Scheme 3-8, 1, 2-dibromoethane was treated with a 0.5 equivalents of sodium azide in methanol and water (10:1) as solvent to give mono- and di- substituted azides as a mixture. The by-product sodium bromide was removed by water extraction and the organic layer was used without further purification. It was treated with tert-butyldimethyl (prop-2-yn-1-yloxy) silane directly to give a mixture of compounds 3.28 and 3.28a. These two compounds were separated by flash silica gel chromatography.
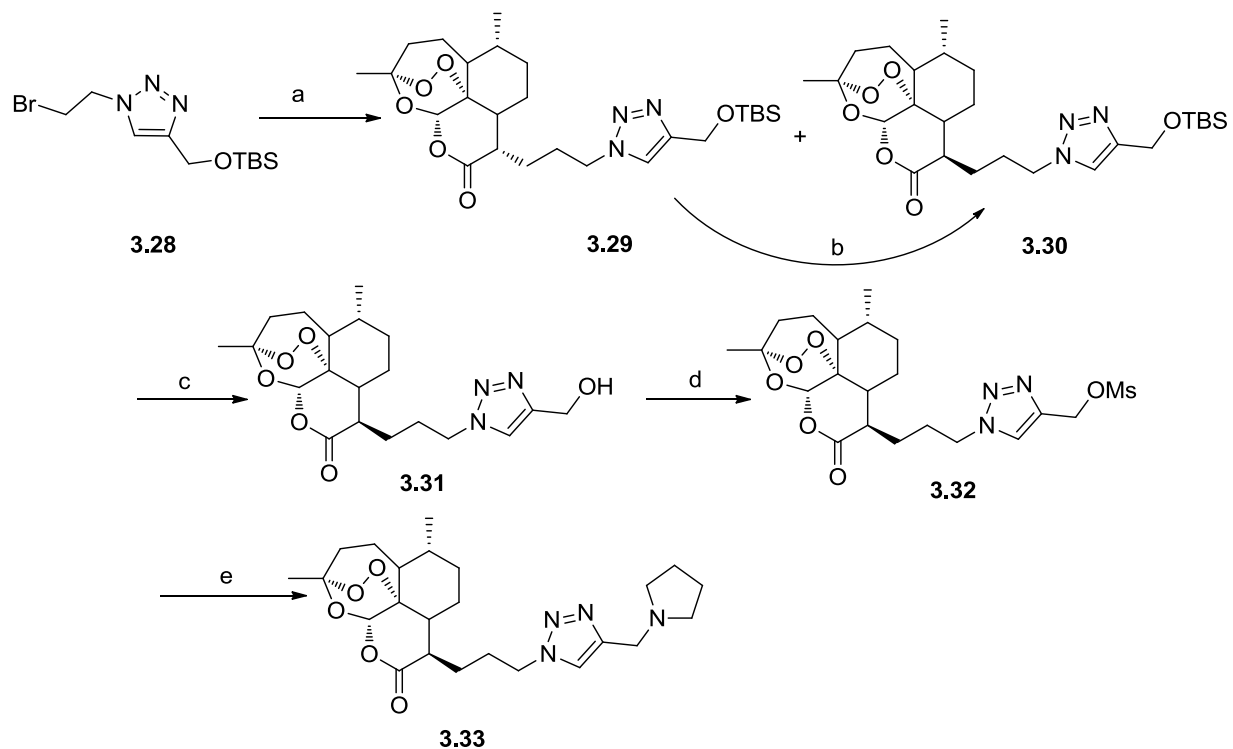
Scheme 3-9 Synthesis of target compound

Reagents and conditions: a) Artemisitene, n-Bu$_3$SnH, AIBN. PhH. 80 °C 10%-30%; b) DBU, THF, reflux, overnight 45%; c) TBAF, THF 80%; d) MsCl, NEt$_3$, DCM 90%; e) pyrrolidine, NEt$_3$, DCM, 55%

Once the intermediate bromide 3.28 was obtained, it was incorporated into artemisitene by radical addition, which gave two diastereomers C9-α 3.29 and C9-β 3.30 as a mixture. Given that the C-9β derivatives are usually more potent than the C-9α isomers, compound 3.29 was epimerized to the C9-β congener by treating it with DBU in refluxing THF overnight. Since artemisinin is sensitive to basic conditions, and fluoride anion is sufficiently basic, different fluoride ions were explored in the deprotection of the TBS group such as CsF, TBAF, TBAF/HOAc. Among the fluoride resources tried, TBAF was found to be the most efficient at removing the TBDMS-protecting group. Deprotected alcohol 3.31 thus was transferred to mesylate 3.32, which reacted with pyrrolidine to give the final compound 3.33.

3.5 Other derivatives synthesized from artemisitene

Besides 1,2,3-triazoles, we also explored other heteroatoms like phosphorus and sulfur as nucleophilic species in order to introduce new functional groups at the C-16 position of artemisinin (Scheme 3-10-Scheme 3-12). These new derivatives were designed to help fill the chemical space and were hoped to provide insights into Quantitative Structure-Activity Relationship (QSAR) studies.

Thus, artemisitene reacted with sodium dimethyl phosphonate or sodium diethyl phosphonate at room temperature to give phosphonate substituted analogues 3.34 or 3.35, respectively (Scheme 3-10). Synthesis of dithiocarbamates analogues were achieved by employing a three-component reaction of artemisitene, secondary amines, and carbon disulfide catalyzed by an ionic liquid [Bmim] BF_4 under room temperature (Scheme 3-11). Lipoic acid is a naturally occurring compound which has shown neuroprotection effect in Alzheime's patients[126]. Thus, lipoic acid was reduced to dihydrolipoic acid 3.38 by sodium borohydride in a solution of sodium bicarbonate (0.25M) at 0 °C. The resultant dithiol then reacted with artemisitene in the presence of triethylamine to give dimer 3.39 (Scheme 3-12).
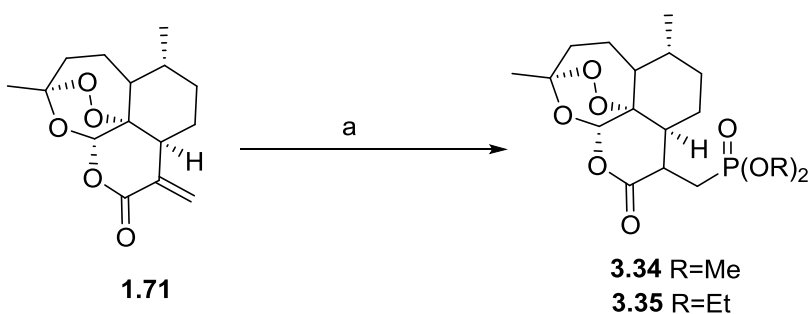
Scheme 3-10 Phosphonate substitution at C-16
Reagents and conditions: a). Na, HP(=O)(OR)$_2$

1.71

3.36 R= (pyrrolidine)
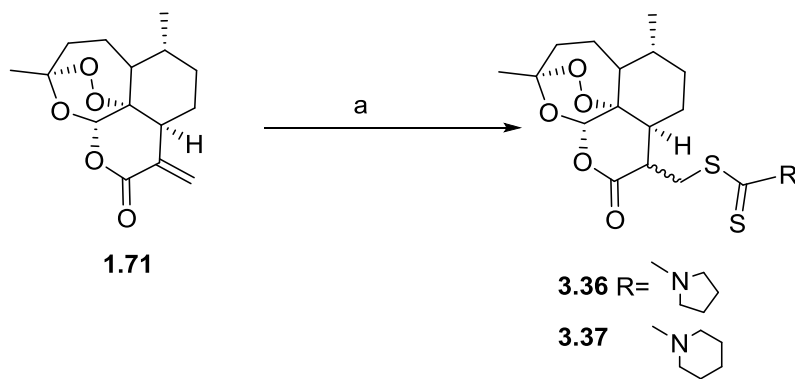
3.37 (piperidine)

Scheme 3-11 Dithiocarbamate substitution at C-16
Reagents and conditions: a). CS_2, [Bmim]BF_4, pyrrolidine or piperidine

lipoic acid

3.38

3.39 R=artemisinin
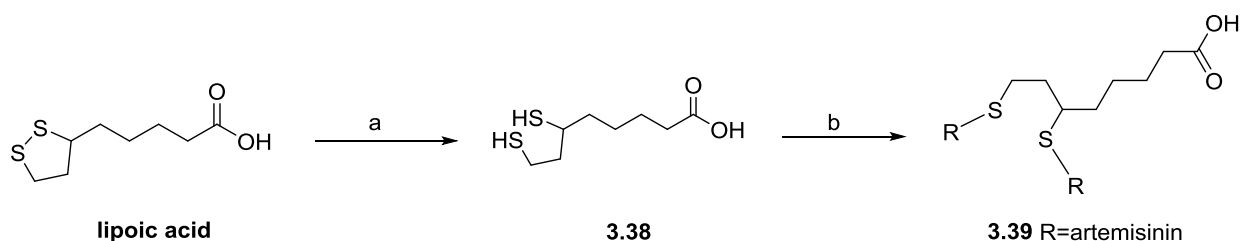
Scheme 3-12 Sulfur substitution at C-16
Reagents and conditions: a). $NaBH_4$, $NaHCO_3$, H_2O, 0 ℃ b) NEt_3, THF, 37%

3.6 Activities and conclusions
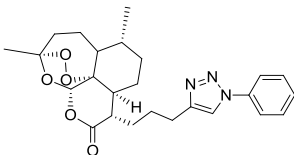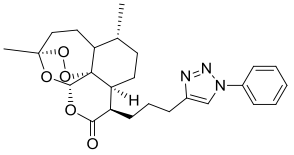
We have tested all of the compounds prepared in the schemes for antimalarial activity *in vitro* either at the University of California, San Francisco *(*UCSF) or The National Center for Natural Products Research (NCNPR), or both. The bioassays at these two sites were slightly different. UCSF used parasite cells while the NCNPR used red blood cell infected with the parasite. The results are shown in Table 3-2. When tested at both sites, the compounds' activity correlates well except for one compound, 3.7. The results of our current study confirm the hypothesis that compounds with a three-carbon chain between artemisinin and the aromatic ring have optimal activity. Radical-induced 1, 4-Michael addition on artemisitene provided two diastereoisomers, namely, 9α and 9β derivatives. As far as *in vitro* activity, 9β substituted

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derivatives usually showed better activity than 9α derivatives as can be seen in compound 3.13a and 3.13b, 3.16a and 3.16b, 3.36a and 3.36b. These trends are consistent with previous findings by our group and by others[81, 127]. It is interesting to note that the difference in activity between two isomers, like 3.13a and 3.13b, is much less than that of other isomer pairs. Compounds 3.13, 3.16b and 3.31 displayed similar IC_{50} values. Compound 3.16b has a carbamate, while 3.13 and 3.31 has a triazole instead, which indicates that a triazole ring could be a good bioisostere for carbamates. By introducing a 1,2,3-triazole side chain with a three carbon-chain as a linker, the activity was improved. Different substitutions on the 4' of the triazole ring have an influence on activity. For example, when changing from 3.13b (phenyl) to 3.31 (hydroxyl), though the polarity of two compounds is expected to be different, their activity is in the same range. However, when changing from 3.31(hydroxyl) to 3.33 (amine), the activity against D6 clone did improve twofold. 1,2,3-triazole moiety has been used to improve the oral absorption of a cephalosporin drug[116]. Poor water solubility and low oral absorption has been a big issue in the development of C-16 modified artemisinin derivatives in our group. Since compound 3.33 showed improvements in its *in vitro* activity, we are eager to see if it shows improvement *in vivo* as well. It has been enriched to the 200mg scale and *in vivo* PK studies are forthcoming.
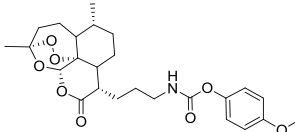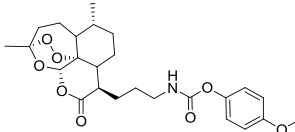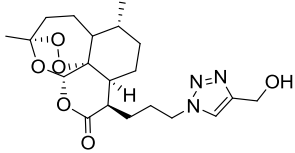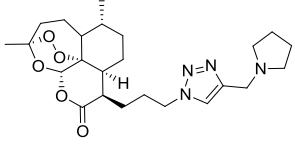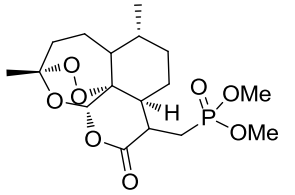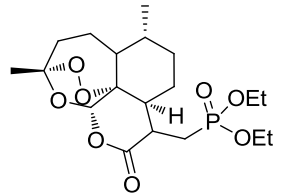
Different heteroatoms were also introduced to artemisitene as nucleophiles. The derivatives synthesized in this study are designed to fill the chemical space for SAR studies. *In vitro* activity showed that they are less active than artemisinin, which indicates the proper space linker is needed for optimal activity. It was noticed that a small change in structure could change activity dramatically. For instance, compound 3.34 and 3.35 only changed from methyl to ethyl, attenuating activity by half. Another example is compound 3.36b (pyrrolidine) to 3.37 (piperidine). From a five-membered ring to a six-membered ring, activity is significantly

different, with an almost nine-fold reduction. Compound 3.38 is the only dimer made from this work. Artemisinin dimers linked at C-10 have shown potent antimalarial activity[128, 129]. Artemisinin dimer linked at C-16 from others[83] and compound 3.8, on the other hand, showed modest activity. In conclusion, although their biological activity is not as good as artemisinin, they could provide insights into structure-activity relationships.
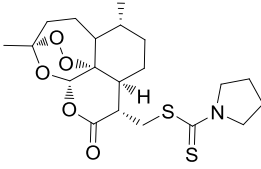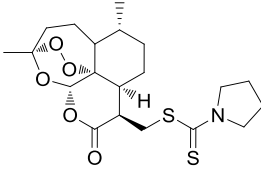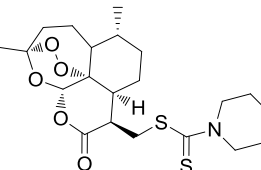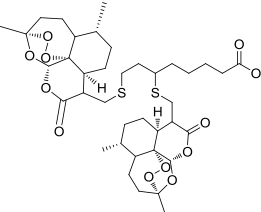
Table 3-2 *In vitro* Antimalarial activity of new derivatives*

Code	Structure	IC$_{50}$(nM)		
		D6	W2	W2
3.13a		86	75	
3.13b		70.6	48.5	

3.16a		449.4	326.8	
3.16b		77.6	51.1	
3.31		83.5	63.8	
3.33		47.8	56.5	
3.34		187.1	123	157.65
3.35		71.7	71.7	68.585

3.36a		110	86.6	40.16
3.36b		93.6	58.5	48.6
3.37		838.7	453.4	14.345
3.38		49.5	54.7	9.481
	Artemisinin	17.9	15.4	
	Chloroquine	51.6	468.9	21.74

*No compounds are cytotoxic at the tested concentrations.

D6: Sierra Leone D6 (chloroquine-sensitive)

W2: Indochina clone chloroquine resistant

CHAPTER 4
EXPERIMENTAL

General Experimental:

Proton (^{1}H NMR) and carbon (^{13}C NMR) nuclear magnetic resonance spectra were recorded in CDCl$_3$ or DMSO on Bruker DRX-400 spectrometers at 400 MHz and 100 MHz, respectively, or on Bruker DPX-500 spectrometers at 500 MHz and 125 MHz if specified. Chemical shifts are given in parts per million (ppm) on the delta (δ) scale. The solvent peak or the internal standard tetramethylsilane were used as reference values. For ^{1}H NMR: CDCl$_3$=7.27, TMS=0.00. For ^{13}C NMR: CDCl$_3$=77.2, TMS=0.0. Infrared (IR) spectra were recorded on a PerkinElmer Spectrum 100FT-IR Spectrometer. High resolution mass spectra (HRMS) were obtained on a Waters Micromass Q-TOF micro mass spectrometer. Analytical thin layer chromatography (TLC) was performed on EMD Chemical INC 25 TLC aluminum sheets, silica gel 60 F$_{254}$ or whatman precoated silica gel G or GP Analtech TLC plates. Melting points were measured on an OptiMelt® V.1.061 (Stanford Research systems) instrument and were uncorrected. Flash column chromatography was performed using silica gel (Whatman 60Å, 230-400 mesh). Reagent grade ethyl acetate, hexanes, and diethyl ether were purchased from fisher scientific Inc. and used as is for chromatography. All reagents and dry solvents were purchased from Sigma/Aldrich, Fluka, or Fisher. All reactions were conducted under argon atmosphere, unless otherwise specified.

Bioassay for antimalarial activity:

The antimalarial assays were performed at the University of California, San Francisco *(*UCSF) or The National Center for Natural Products Research (NCNPR).

In vitro antimalarial activities of various analogues were determined by incubating different concentrations of samples with chloroquine-resistant W2-Indochina strains of *P. falciparum* for 48 hours, beginning at the ring stage, counting new ring forms by fluorescence-activated cell sorting (FACS) analysis, and comparing parasitemias with those of untreated controls. Parasitemia was determined from dot plots (forward scatter *vs*. Fluorescence FL-1) acquired on a FACSort[TM] flow cytometer (Beckton Dickinson) using CellQuest software (Beckton Dickinson). IC_{50} values for growth inhibition were determined from plots of percent control parasitemia over inhibitor concentration using Prism v5.0 software (GraphPad).

The *in vitro* antimalarial assay procedure utilized at the NCNPR, University of Mississippi, is adapted from a pLDH assay developed by Makler et al[130]. A Microdilution protocol was used with a *P. falciparum* clone [Sierra Leone D6 (chloroquine-sensitive)]. The antimalarial agents chloroquine and artemisinin were used as controls, while DMSO was the solvent control. The procedure is described as follows: Prepare a suspension of red blood cells with a 2% parasitemia and 2% hematocrit in malaria complete medium (approximately 20 ml per 96-well plate). Dispense 200 μl aliquots of this suspension into each well of a 96-well, flat-bottomed microtiter plate. Next, add 10 μl volumes of the drugs to be tested in duplicate to the appropriate wells. Place the plates into the humidified chamber and flush the cultures with gas mixture 90% N_2, 5% O_2, 5% CO_2. Place chamber containing the plates into a 37 ℃ incubator for approximately 48h. After 48h, add 100 μl aliquots of the Malstat reagent to each well of a new 96-well microtiter

plate. Resuspend the cultures from the assay plate by mixing each well up and down several times. Remove 20 μl from each well of the resuspended culture and add to the plate containing the Malstat reagent. Incubate the plates at room temperature for 30 min. After 30 min, add to each well 20 μl of a NBT/PES (1:1) solution (2 mg/ml and 0.1 mg/ml, respectively). Incubate plates in the dark for 1h. At the end of the 1 h incubation, the reaction is stopped by the addition of approximately 100 μl of a 5% acetic acid solution. The plate is then read at an endpoint of approximately 650 nm[131].

4-methoxybenzyl azide (3.6):

To a solution of 4-Methoxybenzyl chloride (1.089 ml, 8 mmol) in dry DMF (5 ml) was charged with sodium azide (0.572 g, 8.80 mmol), the mixture was heated at 105 °C for 7hrs. The reaction mixture was formed as a suspension. KI was added and the reaction turned to brown. After heated for 17 hrs, it was cooled to r. t. Water and ether were added to the mixture, the organic layer was separated, and the water layer was extracted with ether (2x20ml). The organic layers were combined and washed with water, brine and dried over Na_2SO_4. They were then filtered and evaporated to give the crude product (1.30g), which was used without further purification. IR (neat) ν_{max}: 2091(N_3)

4-(2-bromoethyl)-1-(4-methoxybenzyl)-1H-1,2,3-triazole(3.8):

To a solution of 4-methoxybenzyl azide (163 mg, 1 mmol) and 4-bromobut-1-yne (94 μl, 1.001 mmol) in 1:1 water/t-BuOH (2 mL), Sodium ascorbate (39.6 mg, 0.200 mmol) was added

followed by a copper sulfate solution (100 μl, 0.020 mmol). The reaction turned to light yellow and became blurring. After 3 days, white precipitate appeared. The reaction was quenched with saturated NH$_4$Cl solution (1ml) and extracted with EtOAc (3x10ml). The combined organic layer was washed with water, brine and dried over Na$_2$SO$_4$. The solvent was evaporated and NMR confirmed the structure.^{1}H NMR (400 MHz, CDCl$_3$): 3.22 (t, J=7.03 Hz, 13 H) 3.59 (t, J=7.03 Hz, 13 H) 3.77 (s, 20 H) 5.41 (s, 13 H) 6.86 (d, J=8.53 Hz, 12 H) 7.19 (d, J=8.53 Hz, 12 H) 7.33 (s, 7 H). ^{13}C NMR (101 MHz, CDCl$_3$): 29.32, 31.32, 53.43, 55.16, 114.26, 121.26, 126.53, 129.38, 144.96, 159.69. IR (neat) ν_{max} : 3066, 2965, 1611, 1512, 1458, 1301, 1247, 1175, 1052, 1033, 880, 767.

Phenylazide (3.11):

 Aniline (1.77g) was suspended in hydrochloric acid at r.t. and ethanol was added to make a clear solution. The solution was cooled to 0 ℃ and NaNO$_2$ was added. After stirring at 0 ℃ for half an hour, NaN$_3$ (1.49g, 1.2 eq) was added and the reaction mixture was stirred for 3 hours while being slowly warmed to r.t. The reaction mixture was extracted with ether (3x50ml). and the combined organic layer was washed with sat. NaHCO$_3$, brine, and dried over MgSO$_4$. The solvent was evaporated *in vacuo* to give phenylazide which was used for the next step without further purification. IR(neat) ν_{max}: 2089(N$_3$).

4-(2-bromoethyl)-1-phenyl-1H-1,2,3-triazole(3.12):

Phenylazide (170mg, 1.427 mmol), 1-bromo-butyne (0.147 ml, 1.570 mmol), and sodium ascorbate (28.3 mg, 0.143 mmol) were suspended in water (0.5 ml) and t-butanol (0.500 ml). 1M Copper sulfate (0.071 ml, 0.071 mmol) was added and the reaction mixture was stirred for 2 days. The reaction mixture was diluted with EtOAc and extracted further by EtOAc. The combined organic layer was washed with water, brine, and dried over Na_2SO_4. Flash chromatography with hexane :EtOAc ranging from 4:1 to 3:1 to 1:1 as an eluent system gave the title compound (320mg) as a clear oil. ^{1}H NMR (400 MHz, CDCl$_3$): 3.39 (t, *J*=6.78 Hz, 2 H) 3.73 (t, *J*=6.78 Hz, 2 H) 7.38 - 7.48 (m, 1 H) 7.49 - 7.58 (m, 2 H) 7.69 - 7.79 (m, 2 H) 7.91 (s, 1 H). ^{13}C NMR (101 MHz, CDCl$_3$): 29.41, 31.36, 119.90, 120.47, 128.66, 129.71, 137.05, 145.50. IR (neat) ν_{max}: 3126, 3070, 1595, 1497, 1464, 1364, 1217, 1174, 1049, 993, 907, 827, 757, 689, 680.

(3*R*,5a*S*,6*R*,8a*S*,9*S*, 12*S*,12a*R*)-3,6-dimethyl-9-(3-(1-phenyl-1H-1,2,3-triazol-4-yl)propyl)octa-hydro-3H-3,12-epoxy[1,2]dioxepino[4,3-i]isochromen-10(12H)-one(3.13a) and (3*R*,5a*S*,6*R*,8a*S*,9*R*,12*S*,12a*R*)-3,6-dimethyl-9-(3-(1-phenyl-1H-1,2,3-triazol-4-yl)propyl) octa-hydro-3H-3,12-epoxy[1,2]dioxepino[4,3-i]isochromen-10(12H)-one (3.13b)

To a 250 mL round-bottomed flask was placed artemisitene (280 mg, 0.999 mmol), AIBN (8.20 mg, 0.050 mmol), and 4-(2-bromoethyl)-1-phenyl-1*H*-1,2,3-triazole (277 mg, 1.099 mmol) in benzene (100 ml) which gave a yellow solution. The solution was heated to reflux and tributyltin hydride (0.293 ml, 1.099 mmol) in 30ml benzene was added by syringe pump over a period of 8 hours. The reaction mixture was refluxed for an additional 2 hours and cooled to ambient temperature. The reaction mixture was evaporated to dryness. The residue was taken up

by ether (20ml) and saturated KF solution (10ml) was added and stirred for 10 hours. The reaction mixture was filtered. The filtrate was washed with water, brine, dried over Na_2SO_4. Removal of the solvent by rotary evaporation gave the crude product which was purified by silica gel column chromatography using 15% EtOAc/hexanes as eluent to give the 9α and 9β isomers, 3.13a and 3.13b.

3.13a: ^{1}H NMR (400 MHz, CDCl$_3$): 0.99 (d, *J*=5.52 Hz, 3 H) 1.16 (t, *J*=11.29 Hz, 1 H) 1.24 - 1.31 (m, 1 H) 1.39 (br., 1 H) 1.45 (s, 3 H) 1.47 - 1.52 (m, 1 H) 1.68 (d, *J*=2.26 Hz, 1 H) 1.73 (td, *J*=6.09, 3.14 Hz, 1 H) 1.77 (s, 1 H) 1.79 - 1.85 (m, 1 H) 1.85 - 1.92 (m, 2 H) 1.95 (d, *J*=5.52 Hz, 1 H) 2.02 - 2.06 (m, 1 H) 2.06 - 2.10 (m, 1 H) 2.17 (br., 1 H) 2.18 (d, *J*=3.51 Hz, 1 H) 2.33 - 2.43 (m, 1 H) 2.80 - 2.90 (m, 2 H) 5.92 (s, 1 H) 7.39 - 7.45 (m, 1 H) 7.51 (t, *J*=7.78 Hz, 2 H) 7.74 (d, *J*=7.78 Hz, 2 H) 7.80 (s, 1 H). ^{13}C NMR (101 MHz, CDCl$_3$) 19.87, 24.69, 25.43, 25.45, 27.06, 31.62, 33.61, 33.93, 35.90, 37.55, 42.81, 44.86, 50.47, 80.29, 93.79, 105.27, 119.16, 120.41, 128.41, 129.63, 137.23, 148.33, 171.83. IR (neat) ν_{max}: 2926, 2868, 1730, 1598, 1501, 1376, 1211, 1103, 1031, 997, 831, 759.

3.13b: ^{1}H NMR (400 MHz, CDCl$_3$): 0.97 - 1.03 (m, 3 H) 1.08 (d, *J*=10.37 Hz, 1 H) 1.23 - 1.32 (m, 1 H) 1.44 (br., 4 H) 1.62 (br., 1 H) 1.72 - 1.90 (m, 5 H) 1.93 (br., 1 H) 1.96 - 2.16 (m, 4 H) 2.37 - 2.49 (m, 1 H) 2.81 - 2.93 (m, 2 H) 3.22 - 3.32 (m, 1 H) 5.85 (s, 1 H) 7.39 - 7.47 (m, 1 H) 7.51 (t, *J*=7.73 Hz, 2 H) 7.69 - 7.78 (m, 2 H) 7.81 (s, 1 H). ^{13}C NMR (101 MHz, CDCl$_3$): 19.74, 23.28, 24.79, 25.10, 25.46, 26.40, 26.77, 33.48, 35.84, 37.48, 37.69, 43.01, 49.98, 79.16, 93.46, 105.32, 119.11, 120.32, 128.39, 129.61, 137.15, 148.22, 171.50. IR (neat) ν_{max}: 2934, 2867, 1740, 1599, 1501, 1376, 1224, 1181, 1110, 1033, 994, 883, 760.

4-methoxybenzyl (2-bromoethyl) carbamate (3.15):

To a solution 4-Methoxybenzyl alcohol (1.494 ml, 12.00 mmol) in dry CH_2Cl_2 (40 ml) was charged triphosgene (1.187 g, 4.00 mmol) at 0 °C followed by the dropwise addition of diisopropylethyl amine (2.230 ml, 12.80 mmol). The reaction mixture was allowed to warm to r.t. and stirred for 1 hour to obtain chloroformate solution in CH_2Cl_2. Then, the prepared chloroformate was added dropwisely to a solution of bromoethyl amine hydrogen bromide (1.639 g, 8 mmol) and triethylamine (2.474 ml, 17.60 mmol) in dry CH_2Cl_2 (40 ml) at 18 °C. After being stirred for 16 hours, the reaction was quenched by adding water and extracted with EtOAc (3x50ml) and the combined organic extracts were washed with brine and dried over Na_2SO_4. Filtration and rotary evaporation gave a crude residue which was purified by flash column chromatography (20% EtOAc in Hexanes) to afford compound 3.15 as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): 7.33 (d, J = 8.55 Hz, 2H), 6.91 (d, J = 8.55 Hz, 2H), 5.19 (br., 1H), 5.07 (br., 2H), 3.83 (s, 3H), 3.62 (d, J = 5.56 Hz, 2H), 3.43 - 3.53 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) 159.6, 130.0, 128.3, 113.9, 66.8, 55.3, 42.7, 32.4.

4-methoxybenzyl (3-((3R,6R,8aS,9R,12S,12aR)-3,6-dimethyl-10-oxodecahydro-3H-3,12-epoxy[1,2]dioxepino[4,3-i]isochromen-9-yl)propyl)carbamate(3.16):

To a 250 mL round-bottomed flask was placed artemisitene (0.140g, 0.5mmol), 4-methoxybenzyl (2-bromoethyl) carbamate (0.173 g, 0.600 mmol), and AIBN (8.21 mg, 0.050 mmol) in Benzene (60 ml) which gave a colorless solution. The reaction mixture was heated for reflux and tributyltin hydride (0.174 ml, 0.650 mmol) in benzene (24 ml) was added by syringe pump over a period of 8 hours. After the addition was over, the reaction mixture was refluxed for

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an additional 2 hours. The solvents were removed *in vacuo*, and diether ether (20ml) followed by a saturated KF solution (6ml) was added. The solution was stirred for 10 hours at room temperature and then was filtered, washed with water, brine, and dried over Na_2SO_4. Filtration and rotary evaporation gave the crude product which was purified on silica gel (EtOAc in hexanes gradient from 10 to 25%) to give 3.16a (9α isomer) and 3.16b (9β isomers) as light yellow viscous liquid.

3.16a: ^{1}H NMR (400 MHz, CDCl$_3$) 0.81 - 0.90 (m, 1 H) 0.93 (d, *J*=2.01 Hz, 3 H) 1.06 (d, *J*=11.54 Hz, 1 H) 1.15 - 1.23 (m, 3 H) 1.23 - 1.34 (m, 2 H) 1.37 (d, *J*=2.76 Hz, 3 H) 1.51 - 1.77 (m, 5 H) 1.82 - 1.93 (m, 1 H) 1.94 - 2.03 (m, 4 H) 2.07 (br., 1 H) 2.23 - 2.38 (m, 1 H) 3.13 (br., 1 H) 3.73 (d, *J*=3.01 Hz, 2 H) 4.02 - 4.13 (m, 2 H) 4.96 (br., 1 H) 5.85 (s, 1 H) 6.78 - 6.88 (m, 1 H) 7.19 - 7.26 (m, 1 H). ^{13}C NMR (101 MHz, CDCl$_3$) 19.65, 24.48, 25.23, 26.61, 27.63, 30.97, 31.31, 33.72, 35.70, 37.26, 42.80, 44.29, 50.24, 55.00, 66.04, 80.09, 93.58, 105.01, 113.63, 128.63, 129.62, 156.34, 159.24, 170.86. IR (neat) ν$_{max}$: 3347, 2929, 1712, 1514, 1241, 1104, 1030, 998, 828.

3.16b: ^{1}H NMR (400 MHz, CDCl$_3$) 0.92 (t, *J*=7.28 Hz, 1 H) 0.98 (d, *J*=5.77 Hz, 3 H) 1.04 (br., 2 H) 1.19 - 1.39 (m, 4 H) 1.43 (s, 3 H) 1.52 - 1.68 (m, 2 H) 1.76 (br., 3 H) 1.91 - 2.13 (m, 3 H) 2.35 - 2.50 (m, 1 H) 3.19 (br., 2 H) 3.79 (s, 3 H) 4.93 (br., 1 H) 5.01 (br., 2 H) 5.83 (s, 1 H) 6.87 (d, *J*=8.78 Hz, 2 H) 7.28 (d, 2 H). ^{13}C NMR (101 MHz, CDCl$_3$) 19.71, 23.23, 24.14, 24.74, 25.06, 27.74, 33.45, 35.81, 37.41, 37.65, 40.78, 43.20, 49.92, 55.17, 66.30, 79.09, 93.45, 105.29, 113.79, 128.66, 129.82, 156.42, 159.40, 171.33. IR (neat) ν$_{max}$: 3353, 2931, 1716, 1631, 1514, 1241, 1110, 1031, 999, 821.

(3*R*,6*R*,12*S*,12*aR*)-9-(3-aminopropyl)-3,6-dimethyloctahydro-3H-3,12-epoxy[1,2]dioxepino[4,3-i]isochromen-10(12H)-one(3.17)

To a solution of 4-methoxybenzyl (3-((3R,6R,8aS,9R,12S,12aR)-3,6-dimethyl-10-oxodeca-hydro-3H-3,12-epoxy[1,2]dioxepino[4,3-i]isochromen-9-yl)propyl)carbamate (200mg, 0.409 mmol) in DCM (1.8 ml) was added TFA (0.2 ml, 2.60 mmol) and the reaction mixture was stirred at r.t. for 2 hours. Purple color appeared before the reaction was neutralized by saturated NaHCO$_3$ solution. The combined organic layer was washed with water, brine and dried over MgSO$_4$. The crude was loaded on a silica gel column and it decomposed on the silica gel column.

1-(prop-2-yn-1-yl) piperidine (3.19):

To a stirred mixture of piperidine (851 mg, 10.0 mmol), cesium carbonate (3.26 g, 10.0 mmol) and acetone(20 mL) was added 3-bromoprop-1-yne (1.19 g, 10.0 mmol) at r.t.. The white suspension was stirred for 18 hours at r.t. and then was filtered. The filtrate was concentrated. The resulting residue was triturated with diethyl ether (10 mL), filtered, and the filtrate was concentrated to obtain the product as an colorless oil (0.70 g, 56%).^{1}H NMR (400 MHz, CDCl$_3$): 1.27 (br., 2 H) 1.35 - 1.54 (m, 4 H) 2.04 - 2.15 (m, 1 H) 2.33 (br., 4 H) 3.03 - 3.17 (m, 2 H). ^{13}C NMR (101 MHz, CDCl$_3$): 23.51, 25.51, 47.20, 52.71, 72.52, 78.81 . IR (neat) ν_{max}: 3302, 2965, 2910, 2789, 1632, 1461, 1348, 1325, 1200, 1125, 899, 877.

2-azido ethanol (3.21):

A mixture of 2-chloroethanol (3g) and NaN_3 (3.39 g, 1.4eq) was heated at 70 ℃ for 12 hours and then poured into a mixture of ethyl ether and water (50 mL, 1:1). The organic layer was separated and the aqueous layer was extracted with ethyl ether (2 x 15 mL). The combined organic layer was washed with water (30 mL), dried and carefully reduced in volume and used for the next step without further purification. IR (neat) ν_{max}: 3377(OH), 2095(N_3).

2-azidoethyl 4-methylbenzenesulfonate (3.22):

To a solution of 2-azido ethanol (3g, 34.5mmol) in DCM was added triethylamine (5.26ml, 37.9mmol) at 0 ℃ under argon followed by slow addition of TsCl (7.2g, 37.9mmol). The reaction mixture was stirred for 3 hours while the temperature was allowed to warm to room temperature slowly and then diluted with DCM, washed with aq HCl (1N, 50ml), water and brine. The organic phase was dried over Na_2SO_4 and concentrated to give the compound 3.22 as an colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): 2.36 (s, 3 H) 3.41 (t, J=4.89 Hz, 2 H) 3.94 - 4.22 (m, 2 H) 7.30 (m, J=8.03 Hz, 2 H) 7.73 (m, J=8.28 Hz, 2 H). ^{13}C NMR (101 MHz, $CDCl_3$): 21.52, 49.53, 68.50, 127.84, 130.04, 132.41, 145.37. IR (neat) ν_{max}: 2957, 2109, 1735, 1363, 1175, 912.

2-(4-(piperidin-1-ylmethyl)-1H-1,2,3-triazol-1-yl)ethyl 4-methylbenzenesulfonate(3.25):

This compound was synthesized by similarly method to the compound 3.12. ^{1}H NMR (400 MHz, $CDCl_3$): 1.35 (d, J=4.27 Hz, 2 H) 1.45 - 1.58 (m, 5 H) 2.37 (s, 7 H) 3.54 (s, 2 H) 4.33 (t, J=4.89

Hz, 2 H) 4.53 - 4.57 (m, 1 H) 7.25 (d, *J*=8.03 Hz, 2 H) 7.51 (s, 1 H) 7.61 (d, *J*=8.28 Hz, 2 H). [13]C

NMR (101 MHz, CDCl$_3$): 21.42, 23.85, 25.56, 48.67, 53.61, 53.93, 67.56, 123.59, 127.57,

129.82, 131.81, 144.60, 145.23.

1-((1-(2-bromoethyl)-1H-1,2,3-triazol-4-yl)methyl)piperidine and 1-((1-vinyl-1H-1,2,3-triazol-4-yl)methyl)piperidine (3.26 and 3.27)

2-(4-(piperidin-1-ylmethyl)-1H-1,2,3-triazol-1-yl)ethyl 4-methylbenzenesulfonate (290mg, 0.796

mmol) was dissolved in DMF (5 ml) to make a light yellow solution. Lithium bromide (207 mg,

2.387 mmol) was added to the solution. The reaction mixture was stirred at 70 ℃ overnight and

then was diluted with water and extracted with EtOAc (3x25ml). The combined organic layers

were washed with water, brine and dried over Na$_2$SO$_4$. Removal of the organic solvent under

reduced pressure gave the crude product as a light yellow oil. The crude product consisted of

compound 3.26 and 3.27 as a mixture.

1-((1-(2-bromoethyl)-1H-1,2,3-triazol-4-yl)methyl)piperidine (3.26):

^{1}H NMR (400 MHz, CDCl3): 1.28 - 1.37 (m, 2H) 1.48 (dt, *J*=11.04, 5.52 Hz, 4H) 2.37 (br., 4 H)

3.58 (s, 2 H) 3.67 (t, *J*=6.15 Hz, 2 H) 4.65 (t, *J*=6.15 Hz, 2 H) 7.63 (s, 1 H). ^{13}C NMR (101 MHz,

CDCl3): 23.65, 25.26, 29.29, 51.12, 53.41, 53.76, 123.63, 143.73.

1-((1-vinyl-1H-1,2,3-triazol-4-yl)methyl)piperidine (3.27):

^{1}H NMR (400 MHz, CDCl3): 1.28 - 1.37 (m, 2H) 1.48 (dt, *J*=11.04, 5.52 Hz, 4H) 2.37 (br., 4H)

3.57 (br., 2 H) 5.03 (dd, *J*=9.03, 1.76 Hz, 1 H) 5.56 (dd, *J*=16.06, 1.76 Hz, 1 H) 7.23 (dd,

J=16.06, 9.03 Hz, 1 H) 7.76 (s, 1 H). ^{13}C NMR (101 MHz, CDCl3): 22.62, 25.32, 53.86, 104.34, 119.59, 129.96, 144.42.

tert-butyldimethyl (prop-2-yn-1-yloxy)silane:

TBS-Cl (3.62 g, 24.00 mmol) in DCM (5ml) was slowly added into the solution of propargyl alcohol (1.193 ml, 20 mmol) and triethylamine (3.76 ml, 27.0 mmol) in DCM (25ml) at 0 ℃. The mixture was slowly warmed to room temperature and stirred for 15 hours and was added 30 ml of water. The water layer was extracted with hexanes: EtOAc (1:1)(2x 25ml). The combined organic layers were washed with brine and dried over MgSO$_4$. The crude material was distilled by vacuum distillation to give the title compound as colorless oil (2.02g). ^{1}H NMR (400 MHz, CDCl$_3$) : 0.13 (s, 6 H) 0.91 (s, 9 H) 2.37 - 2.41 (m, 1 H) 4.31 (d, *J*=2.26 Hz, 2 H). ^{13}C NMR (101 MHz, CDCl$_3$): -5.24, 18.25, 25.76 (s, 3 C) 51.47, 72.80, 82.39. IR (neat) v_{max}: 3313, 2956, 2931, 2859, 1472, 1365, 1254, 1092, 1005, 833, 777.

1-(2-bromoethyl)-4-((((tert-butyldimethylsilyl)oxy)methyl)-1H-1,2,3-triazole(3.28):

1,2-dibromoethane (1.619 ml, 18.79 mmol) and sodium azide (0.611 g, 9.39 mmol) was dissolved in MeOH:Water (10:1 10ml). The reaction mixture was stirred at 50 ℃ overnight and extracted with ether. (Danger: toxic and explosive 1, 2-diazoethane was produced. Do not allow to dry.) The organic layer was concentrated to give the crude which was added MeOH. tert-butyldimethyl(prop-2-yn-1-yloxy)silane (1.6 g, 9.39 mmol), sodium ascorbate (1.879 ml, 1.879 mmol) and 1M solution of copper(II) sulfate (0.376 ml, 0.376 mmol) was added The mixture was

stirred overnight and diluted with sat. NaHCO$_3$ solution (15ml) and extracted with EtOAc (3x50ml). The combined organic layers were washed with water, brine and dried over MgSO$_4$. The crude product was purified on a silica gel column (Hexanes: EtOAc=2:1) to give compound 3.28 as a colorless oil and compound 3.29 as white solid. ^{1}H NMR (400 MHz, CDCl$_3$): -0.04 - 0.08 (m, 6 H) 0.81 - 0.90 (m, 9 H) 3.70 (t, *J*=6.27 Hz, 2 H) 4.69 (t, *J*=6.27 Hz, 2 H) 4.79 (br., 2 H) 7.58 (s, 1 H). ^{13}C NMR (101 MHz, CDCl$_3$): -5.29, 18.25, 25.83 (s, 3 C) 29.50, 51.43, 57.76, 122.43, 148.43. IR (neat) ν_{max}: 2930, 2857, 1463, 1362, 1255, 1084, 1047, 834, 776.

1,2-bis(4-((((tert-butyldimethylsilyl)oxy)methyl)-1H-1,2,3-triazol-1-yl)ethane(3.29)

^{1}H NMR (400 MHz, CDCl$_3$): 0.04 - 0.10 (m, 12 H) 0.86 - 0.91 (m, 18 H) 4.77 (s, 4 H) 4.90 (s, 4 H) 7.22 (s, 2 H). ^{13}C NMR (101 MHz, CDCl$_3$): -5.37, 18.23, 25.80, 49.45, 57.62, 122.65, 148.91. IR (neat) ν_{max} : 3146, 2951, 2929, 2857, 1462, 1337, 1253, 1119, 1085, 1047, 832, 773.

(3*R*,6*R*,8a*S*,9*R*,12*S*,12a*R*)-9-(3-(4-((((tert-butyldimethylsilyl)oxy)methyl)-1H-1,2,3-triazol-1-yl)propyl)-3,6-dimethyloctahydro-3H-3,12-epoxy[1,2]dioxepino[4,3-i]isochromen-10(12H)-one (3.29)and (3*R*,6*R*,8aS,9*S*,12*S*,12a*R*)-9-(3-(4-((((tert-butyldimethylsilyl)oxy)methyl)-1H-1,2,3-triazol-1-yl)propyl)-3,6-dimethyloctahydro-3H-3,12-epoxy[1,2]dioxepino[4,3-i]isochromen-10(12H)-one (3.30)

The reaction was performed similarly as a previous radical-induced Michael addition. The crude product was added to a silica gel column and was eluted with Hexanes/EtOAc (4:1) to hexanes/EtOAc (2:1) to give two diastereoisomers 3.29 and 3.30.

9β isomer 3.29: ^{1}H NMR (400 MHz, CDCl$_3$): 0.08 (s, 6 H) 0.89 (s, 9 H) 0.97 (d, J=5.77 Hz, 3 H) 1.20 - 1.25 (m, 2 H) 1.28 (d, J=8.03 Hz, 1 H) 1.35 (dd, J=10.04, 5.27 Hz, 2 H) 1.42 (s, 3 H) 1.56 - 1.66 (m, 2 H) 1.67 - 1.78 (m, 2 H) 1.92 - 1.99 (m, 2 H) 2.02 (s, 2 H) 2.10 - 2.23 (m, 1 H) 2.32 - 2.48 (m, 1 H) 3.18 (d, J=5.27 Hz, 1 H) 4.30 - 4.44 (m, 2 H) 4.82 (s, 2 H) 5.82 (s, 1 H) 7.47 - 7.52 (m, 1 H). ^{13}C NMR (101 MHz, CDCl$_3$): -5.36, 13.52, 18.24, 19.67, 24.41, 24.69, 25.04, 25.80, 26.73, 27.74, 28.39, 33.33, 35.75, 37.53, 43.61, 49.84, 57.82, 79.06, 93.54, 105.34, 121.52, 139.07, 171.07. IR (neat) ν$_{max}$: 2928, 2856, 1736, 1462, 1378, 1252, 1110, 1000, 834, 776.

9α isomer 3.30: ^{1}H NMR (400 MHz, CDCl$_3$) : 0.06 (s, 6 H) 0.83 - 0.91 (m, 9 H) 0.94 (d, J=5.52 Hz, 3 H) 1.21 (t, J=7.15 Hz, 1 H) 1.31 - 1.37 (m, 2 H) 1.40 (s, 4 H) 1.42 - 1.49 (m, 1 H) 1.60 (d, J=3.01 Hz, 1 H) 1.64 (br., 1 H) 1.65 - 1.74 (m, 2 H) 1.87 - 1.95 (m, 1 H) 1.99 (s, 2 H) 2.02 - 2.07 (m, 2 H) 2.12 (t, J=5.90 Hz, 1 H) 2.27 - 2.38 (m, 1 H) 4.32 (dq, J=14.49, 7.05 Hz, 2 H) 4.77 - 4.83 (m, 2 H) 5.87 (s, 1 H) 7.47 (s, 1 H). ^{13}C NMR (101 MHz, CDCl$_3$): -5.41, 18.18, 19.72, 24.50, 25.30, 25.75, 28.04, 30.80, 31.32, 33.71, 35.73, 37.33, 43.24, 44.04, 50.26, 57.76, 60.20, 80.02, 93.74, 105.15, 121.55, 148.36, 171.15. IR (neat) ν$_{max}$: 2928, 2856, 1735, 1462, 1244, 1103, 1001, 834, 777.

(3R,6R,8aS,9R,12S,12aR)-9-(3-(4-(hydroxymethyl)-1H-1,2,3-triazol-1-yl)propyl)-3,6-dimethyloctahydro-3H-3,12-epoxy[1,2]dioxepino[4,3-i]isochromen-10(12H)-one(3.31):

TBAF (1M, 0.575 ml, 0.575 mmol) in THF was added to the solution of (3R,6R,8aS,9R,12S,12aR)-9-(3-(4-(((tert-butyldimethylsilyl)oxy)methyl)-1H-1,2,3-triazol-1-yl)propyl)-3,6-dimethyl-octahydro-3H-3,12-epoxy[1,2]dioxepino[4,3-i]isochromen-10(12H)-one

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(150mg, 0.288 mmol) in THF (4 ml) at room temperature and stirred for 1 hour. The THF was evaporated and the residue was taken up with EtOAc (10ml). The organic layer was washed with water, brine, dried over MgSO$_4$, filtered and evaporated *in vacuo*. The crude product was added to a silica gel column and was eluted with EtOAc to give the compound 3.31 as a viscous liquid. ^{1}H NMR (400 MHz, CDCl$_3$) 0.93 (d, *J*=5.27 Hz, 3 H) 0.96 - 1.05 (m, 1 H) 1.26 (d, *J*=7.53 Hz, 1 H): 1.28 - 1.35 (m, 2 H) 1.37 (s, 3 H) 1.39 - 1.48 (m, 1 H) 1.62 - 1.76 (m, 3 H) 1.87 - 1.95 (m, 2 H) 1.96 - 2.00 (m, 2 H) 2.01 (d, *J*=3.76 Hz, 1 H) 2.07 - 2.18 (m, 1 H) 2.30 - 2.41 (m, 1 H) 3.06 - 3.16 (m, 1 H) 4.25 - 4.40 (m, 2 H) 4.70 (s, 2 H) 5.79 (s, 1 H) 7.60 (s, 1 H). ^{13}C NMR (101 MHz, CDCl$_3$): 19.57, 23.12, 24.19, 24.57, 24.94, 28.11, 33.17, 35.63, 37.23, 37.44, 43.31, 49.69, 49.94, 55.91, 79.01, 93.48, 105.24, 121.88, 147.82, 171.14. IR (neat) ν$_{max}$: 3357, 2928, 2874, 1731, 1111, 1032, 997, 727.

(1-(3-((3*R*,5a*S*,6*R*,8a*S*,9*R*,12*S*,12a*R*)-3,6-dimethyl-10-oxodecahydro-3H-3,12-epoxy[1,2] dioxepino[4,3-i]isochromen-9-yl)propyl)-1H-1,2,3-triazol-4-yl)methyl methanesulfonate (3.32):

(3*R*,6*R*,8a*S*,9*R*,12*S*,12a*R*)-9-(3-(4-(hydroxymethyl)-1H-1,2,3-triazol-1-yl)propyl)-3,6-dimethyl-octahydro-3H-3,12-epoxy[1,2]dioxepino[4,3-i]isochromen-10(12H)-one (70 mg, 0.172 mmol) was dissolved in CH$_2$Cl$_2$ (4 ml) and was cooled to 0 ℃. TEA (35.8 µl, 0.257 mmol) was slowly added followed by Ms-Cl (16 µl, 0.205 mmol) to the solution and the reaction mixture was stirred for 20 minutes before cold water (20ml) was added. The organic layer was washed with cold 0.1N HCl (5ml), sat. NaHCO$_3$, brine and dried over MgSO$_4$. Removal of the solvent by filtration and rotary evaporation gave the crude product which was purified by a flash column with hexanes/EtOAc as eluent to give 3.32 as a light yellow oil. ^{1}H NMR (400 MHz, CDCl$_3$): 0.93 (s, 1 H) 0.99 (d, *J*=5.52 Hz, 2 H) 1.02 - 1.16 (m, 2 H) 1.19 - 1.33 (m, 2 H) 1.34 - 1.53 (m, 6

H) 1.56 - 1.86 (m, 3 H) 1.88 - 2.13 (m, 4 H) 2.20 (d, J=6.02 Hz, 1 H) 2.34 - 2.57 (m, 1 H) 3.04 (s, 2 H) 3.19 (d, J=5.52 Hz, 1 H) 4.35 - 4.53 (m, 2 H) 5.37 (s, 1 H) 5.84 (s, 1 H) 7.78 (s, 1 H). ^{13}C NMR (101 MHz, CDCl$_3$): 19.70, 23.35, 24.48, 24.74, 25.08, 28.36, 33.37, 35.81, 37.42, 37.60, 38.30, 43.79, 49.91, 50.34, 62.44, 79.13, 93.65, 105.43, 124.39, 136.35, 171.12. IR (neat) ν_{max}: 2927, 1729, 1455, 1361, 1175, 1112, 1033, 996, 963, 726.

(3*R*,5a*S*,6*R*,8a*S*,9*R*,12*S*,12a*R*)-3,6-dimethyl-9-(3-(4-(pyrrolidin-1-ylmethyl)-1H-1,2,3-triazol-1-yl)propyl)octahydro-3H-3,12-epoxy[1,2]dioxepino[4,3-i]isochromen-10(12H)-one(3.33)

(1-(3-((3*R*,5a*S*,6*R*,8a*S*,9*R*,12*S*,12a*R*)-3,6-dimethyl-10-oxodecahydro-3H-3,12-epoxy[1,2]dioxe-pino[4,3-i]isochromen-9-yl)propyl)-1H-1,2,3-triazol-4-yl)methyl methanesulfonate (35 mg, 0.072 mmol) was dissolved in DMF (1 ml) to give colorless solution and was cooled to 0 ℃. DIPEA (0.063 ml, 0.36 mmol) was added followed by addition of pyrrolidine (0.03ml, 0.36 mmol). The reaction mixture was heated to 55 ℃ for 30 minutes and was added water, extracted with EtOAC (3 x 20ml). The combined organic layer was washed with H$_2$O, brine and dried over MgSO$_4$, filtered and concentrated to give the crude product which was purified by silica gel column chromatography (CH$_2$Cl$_2$/MeOH 95:5). This compound was further purified by prep HPLC. (Condition: (ACN in water from 20% to 100% in 20 minutes.) ^{1}H NMR (400 MHz, CDCl$_3$): 0.82 - 0.95 (m, 1 H) 0.99 (d, J=5.77 Hz, 3 H) 1.17 - 1.32 (m, 2 H) 1.34 - 1.46 (m, 5 H) 1.49 (br., 2 H) 1.61 (d, J=15.06 Hz, 1 H) 1.66 - 1.78 (m, 2 H) 1.83 (br., 4 H) 1.93 - 2.09 (m, 4 H) 2.12 (br., 1 H) 2.37 - 2.47 (m, 1 H) 2.68 (br., 3 H) 3.16 - 3.25 (m, 1 H) 3.87 (s, 2 H) 4.30 - 4.48 (m, 2 H) 5.84 (s, 1 H) 7.60 (s, 1 H). ^{13}C NMR (101 MHz, CDCl$_3$): 19.73, 23.34, 23.43, 24.46,

24.78, 25.10, 28.41, 33.41, 35.84, 37.46, 37.61, 43.66, 49.94, 50.08, 50.26, 53.60, 79.12, 93.60, 105.41, 122.76, 144.56, 171.07 . IR (neat) ν_{max}: 2928, 1735, 1459, 1378, 1200, 1112, 1033, 1000.

Dimethyl (((3R,6R,8aS,12S,12aR)-3,6-dimethyl-10-oxodecahydro-3H-3,12-epoxy[1,2]dioxe-pino [4,3-I]isochromen-9-yl)methyl)phosphonate(3.34)

This compound was prepared analogously with compound 3.35. ^{1}H NMR (400 MHz, CDCl$_3$) 0.97 (d, J=6.02 Hz, 3 H) 1.13 - 1.19 (m, 1 H) 1.20 - 1.25 (m, 1 H) 1.37 - 1.40 (m, 1 H) 1.42 (s, 3 H) 1.46 (br., 1 H) 1.49 (br., 1 H) 1.69 (dd, J=13.55, 3.01 Hz, 1 H) 1.89 - 1.97 (m, 2 H) 2.04 (d, J=15.81 Hz, 1 H) 2.11 - 2.19 (m, 1 H) 2.20 - 2.26 (m, 1 H) 2.32 - 2.41 (m, 1 H) 2.44 - 2.53 (m, 1 H) 2.60 - 2.73 (m, 1 H) 3.74 (d, J=1.51 Hz, 3 H) 3.72 (d, J=1.76 Hz, 3 H) 5.95 (s, 1 H). ^{13}C NMR (101 MHz, CDCl$_3$) 19.73, 24.67, 25.40, 28.25, 29.67, 30.68, 33.83, 35.78, 37.60, 39.45, 42.95, 50.19, 52.39, 80.89, 94.18, 105.34, 171.09 HRMS (ESI-TOF) m/z: [M+H]$^+$ Calcd for C$_{17}$H$_{28}$O$_8$P 391.1522; Found 391.1541.

Diethyl (((3R,6R,8aS,12S,12aR)-3,6-dimethyl-10-oxodecahydro-3H-3,12-epoxy[1,2]diox-epino [4,3 -I]isochromen-9-yl)methyl)phosphonate(3.35)

Sodium (5.8mg) was dissolved in 0.37ml of diethyl phosphite and cooled to 0 ℃. Artemisitene (53mg) in 0.25ml of diethyl phosphite was added slowly. The reaction mixture was stirred for 5 hours at room temperature before water was added and then was extracted with EtOAc. The combined organic layer was washed with NaOH, brine, dried over Na$_2$SO$_4$, filtered and evaporated *in vacuo.* The crude was purified by flash column chromatography with hexanes:

EtOAc from 9:1 to 1:1 to 1:3 as eluent system to give product (26mg) as viscous liquid.^{1}H NMR (400 MHz, CDCl$_3$): 0.99 (d, J=6.02 Hz, 3 H) 1.14 - 1.21 (m, 1 H) 1.25 (s, 1 H) 1.33 (t, J=7.03 Hz, 6 H) 1.39 - 1.43 (m, 1 H) 1.45 (s, 3 H) 1.48 (br., 1 H) 1.50 (br., 1 H) 1.71 (dd, J=13.43, 2.89 Hz, 1 H) 1.91 - 2.01 (m, 2 H) 2.02 - 2.10 (m, 1 H) 2.17 - 2.27 (m, 2 H) 2.34 - 2.44 (m, 1 H) 2.51 (t, J=13.30 Hz, 1 H) 2.61 - 2.74 (m, 1 H) 4.03 - 4.14 (m, 4 H) 5.97 (s, 1 H). ^{13}C NMR (101 MHz, CDCl$_3$): 16.43, 19.80, 24.75, 25.48, 29.33, 30.77, 33.91, 35.86, 37.69, 39.67, 42.86, 50.31, 61.76, 80.98, 94.21, 105.38, 171.30. IR (neat) v_{max}: 2930, 2872, 1734, 1445, 1377, 1246, 1204, 1161, 1110, 1048, 1025, 1006, 968, 938, 824. HRMS (ESI-TOF) m/z: [M+H]$^+$ Calcd for C$_{19}$H$_{32}$O$_8$P 419.1835; Found 419.1827.

6,8-dimercaptooctanoic acid(3.39)

This compound was prepared according to the published procedure[132]: Lipoic acid (1.22g, 5.9mmol) was dissolved in 0.25M NaHCO$_3$ (25 ml) and the solution was cooled to 0 °C. NaBH$_4$ (0.9g, 23.8mmol) was added slowly to the solution and the temperature was kept below 4 °C. The reaction mixture was stirred for an additional 30 minutes before it was purged with argon for 25 minutes. The reaction mixture was acidified with 6N HCl to pH=1 and extracted with toluene (3x10ml). The combined organic layer was washed with water, brine, dried over MgSO$_4$, and filtered. The solvent was evaporated to give a clear oil.   ^{1}H NMR (400 MHz, CDCl$_3$): 1.29 (d, J=7.78 Hz, 1 H) 1.34 (t, J=8.03 Hz, 1 H) 1.40 - 1.66 (m, 5 H) 1.67 - 1.73 (m, 1 H) 1.79 - 1.93 (m, 1 H) 2.33 (t, J=7.28 Hz, 2 H) 2.56 - 2.74 (m, 2 H) 2.82 - 2.94 (m, 1 H) 9.70 (br., 1 H) ^{13}C NMR (101 MHz, CDCl$_3$) : 22.28, 24.27, 26.41, 33.93, 38.62, 39.27, 42.71, 179.89.

6,8-bis((((3*R*,6*R*,8a*S*,12*S*,12a*R*)-3,6-dimethyl-10-oxodecahydro-3H-3,12-epoxy[1,2]diox-epino [4,3-i]isochromen-9-yl)methyl)thio)octanoic acid(3.38)

6,8-dimercaptooctanoic acid (20.8mg) was dissolved in THF(10ml) and cooled to 0 ℃ in an ice bath. TEA was added dropwisely and the mixture was stirred for 15 minutes before artemisitene (28mg) in THF (10ml) was transferred in the reaction mixture by a cannula. The reaction mixture was stirred for 12 hours at r.t. and the solvent was evaporated off. The residue was taken up in Sat. NH_4Cl and extracted with DCM (4x 10ml). The combined organic layer was washed with water and brine, dried over MgSO4 and filtered. DCM was removed *in vacuo* to give crude product which was purified on a silica gel column to give product (28.7mg) as a white solid. mp. 63.2-64.5. ^{1}H NMR (400 MHz, CDCl$_3$): 1.01 (d, *J*=5.02 Hz, 3 H) 1.12 - 1.25 (m, 2 H) 1.26 (br., 1 H) 1.41 - 1.47 (m, 4 H) 1.57 (s, 1 H) 1.79 (d, *J*=10.54 Hz, 1 H) 1.93 (br., 1 H) 1.95 - 2.03 (m, 3 H) 2.03 - 2.11 (m, 3 H) 2.15 - 2.25 (m, 1 H) 2.44 (t, *J*=12.67 Hz, 1 H) 3.59 (d, *J*=6.53 Hz, 2 H) 3.62 - 3.73 (m, 2 H) 3.73 - 3.81 (m, 1 H) 3.93 (t, *J*=6.90 Hz, 2 H) 5.88 (s, 1 H). ^{13}C NMR (101 MHz, CDCl$_3$): 19.78, 24.25, 24.83, 25.13, 26.05, 27.16, 31.96, 33.39, 34.20, 35.85, 37.53, 39.63, 44.04, 49.88, 50.58, 79.33, 94.00, 105.41, 170.86, 192.15. IR (neat) ν$_{max}$: 2923, 2868, 1731, 1469, 1436, 1331, 1202, 1183, 1113, 1036, 996, 969, 884, 835. HRMS (ESI-TOF) m/z: [M+H][+] Calcd for $C_{38}H_{57}O_{12}S_2$ 769.3291; Found 769.3298.

((3*R*,6*R*,8a*S*,9*R*,12*S*,12a*R*)-3,6-dimethyl-10-oxodecahydro-3H-3,12-epoxy[1,2]dioxepino[4,3 -i]isochromen-9-yl)methyl pyrrolidine-1-carbodithioate (3.36b) and ((3*R*,6*R*,8a*S*,9*R*,12*S*, 12a*R*)-3,6-dimethyl-10-oxodecahydro-3H-3,12-epoxy[1,2]dioxepino[4,3-i]isochromen-9-yl)methyl pyrrolidine-1-carbodithioate (3.36a)

Carbon disulfide (31μl, 39mg) was added into the solution of pyrrolidine (18mg) and 1-Butyl-3-methylimidazolium tetrafluoroborate ([Bmim]BF$_4$)(20%) in THF(0.5ml) at 0 ℃. White solid formed instantly, the reaction mixture was stirred for 5 minutes and artemisitene (89mg) in THF (0.6ml) was added. The reaction mixture was allowed to warm to r.t. slowly and stirred overnight. After removal of THF under reduced pressure, the residue was taken up with water and extracted with EtOAc (3x25ml). The combined organic layer was washed with water, brine and dried over Na$_2$SO$_4$. The organic solvents were removed *in vacuo* to give crude product which was purified by flash column with 10% EtOAc/Hexanes as the eluent system to give compound 3.36a (33mg) and 3.3.6b (27mg) as white solids.

3.36a: Mp 151-152.3 ℃. ^{1}H NMR (400 MHz, CDCl$_3$): 1.29 (d, *J*=7.78 Hz, 1 H) 1.34 (t, *J*=8.03 Hz, 1 H) 1.40 - 1.66 (m, 3 H) 1.67 - 1.73 (m, 1 H) 1.79 - 1.93 (m, 1 H) 2.33 (t, *J*=7.28 Hz, 2 H) 2.56 - 2.74 (m, 2 H) 2.82 - 2.94 (m, 1 H) 9.70 (br., 1 H). ^{13}C NMR (101 MHz, CDCl$_3$): 22.28, 24.27, 26.41, 33.93, 38.62, 39.27, 42.71, 179.89. IR (neat) ν$_{max}$: 2923, 2868, 1731, 1469, 1436, 1331, 1183, 1113, 996, 969, 884, 835. HRMS (ESI-TOF) m/z: [M+H]$^+$ Calcd for C$_{38}$H$_{57}$O$_{12}$S$_2$ 428.1565; Found 428.1529.

3.36b: Mp 151.5-152.9 ℃. ^{1}H NMR (400 MHz, CDCl$_3$): 0.99 (d, *J*=6.02 Hz, 2 H) 1.10 - 1.23 (m, 1 H) 1.34 - 1.43 (m, 2 H) 1.44 (s, 3 H) 1.45 - 1.52 (m, 3 H) 1.69 (dd, *J*=13.30, 3.01 Hz, 1 H) 1.89 - 2.02 (m, 4 H) 2.02 - 2.13 (m, 4 H) 2.33 - 2.55 (m, 1 H) 2.81 (t, *J*=6.65 Hz, 1 H) 3.59 - 3.74 (m, 3 H) 3.82 - 3.87 (m, 1 H) 3.90 (t, *J*=6.90 Hz, 2 H) 5.92 (s, 1 H). ^{13}C NMR (101 MHz, CDCl$_3$): 19.86, 24.25, 24.69, 25.51, 26.05, 30.97, 33.88, 35.94, 37.62, 40.21, 43.78, 44.48, 50.36, 50.67, 54.99, 80.48, 94.10, 105.29, 170.75, 192.32. IR (neat) ν$_{max}$: 2923, 2868, 1731,

1469, 1436, 1331, 1183, 1113, 996, 969, 884, 835. HRMS (ESI-TOF) m/z: [M+H]$^+$ Calcd for

$C_{20}H_{29}NO_5S_2$ 428.1565; Found 428.1391.

((3R,6R,8aS,9R,12S,12aR)-3,6-dimethyl-10-oxodecahydro-3H-3,12-epoxy[1,2]dioxepino

[4,3-i]isochromen-9-yl)methyl piperidine-1-carbodithioate(3.37)

This compound was prepared similarly to compound 3.36 with the exception that piperidine was

used as the nucleophile. Mp 125-126 ℃. ^{1}H NMR (400 MHz, CDCl$_3$): 0.88 (t, J=6.15 Hz, 1 H)

1.00 (d, J=5.77 Hz, 3 H) 1.23 - 1.29 (m, 1 H) 1.41 (br., 1 H) 1.46 (s, 3 H) 1.47 - 1.51 (m, 1 H)

1.63 (br., 1 H) 1.70 (br., 7 H) 1.94 (d, J=15.31 Hz, 2 H) 2.00 - 2.11 (m, 2 H) 2.39 (br., 1 H) 2.84

(t, J=6.65 Hz, 1 H) 3.65 (dd, J=14.31, 6.53 Hz, 1 H) 3.90 (dd, J=13.93, 7.15 Hz, 2 H) 4.12 (d,

J=7.28 Hz, 1 H) 4.27 (br., 2 H) 5.93 (s, 1 H). ^{13}C NMR (101 MHz, CDCl$_3$): 19.86, 24.25, 24.71,

25.52, 31.00, 33.91, 35.95, 37.64, 43.82, 44.41, 50.38, 76.70, 77.03, 77.34, 80.49, 94.09, 105.31,

170.82, 195.22. IR (neat) ν$_{max}$: 2940, 2856, 2099, 1732, 1483, 1448, 1436, 1378, 1279, 1243,

1232, 1202, 1106, 1035, 994, 969, 880, 834. HRMS (ESI-TOF) m/z: [M+H]$^+$ Calcd for

$C_{21}H_{32}NO_5S_2$ 442.1722; Found 442.1745.

Part B
Lead optimization of Falcipain-2 and falcipain-3 inhibitors

CHAPTER 5

5.1 Introduction

Artemisinins are currently considered to be the most efficacious antimalarial agents. WHO has recommended artemisinin combination therapy (ACT), which consists of an artemisinin analogue together with an agent from another class of antimalarials having a long half-life, as a first-line treatment for uncomplicated malaria in 2003 [133]. Widespread usage of artemisinin derivatives for treatment of malaria has raised concern over the possible emergence of resistance to this group of drugs. In fact, a recent report revealed the parasite has shown slow response to artemisinin therapy near the Thai-Cambodian border[134]. Luckily, there is no evidence this resistance has spread to other regions so far[135, 136]. However, the development of resistance is thought to be just matter of time. Therefore, there is an urgent need for discovering and developing new antimalarial drugs as novel targets.

Among the emerging targets for the antimalarial chemotherapy, cysteine proteases of the malaria parasite are necessary for infectivity and are validated targets[137]. Falcipain-2 and falcipain-3 are two cysteine proteases which play an important role in the life cycle of malarial parasites by degrading the hemoglobin to provide essential amino acids for parasites survival. Inhibitors of falcipain-2 and falcipain-3 are of interest in antimalarial drug research as well. Until now, most inhibitors in the literature are peptides or peptidomimetics which are not selective.

Recently the X-ray structure of FP-2 and FP-3 together with small molecule inhibitors(co-crystals) have been solved and deposited to the Protein Data Bank[138]. Our group is interested in developing non-peptidic small molecules inhibitors of falcipain-2 and falcipain-3. During virtual screening based on the crystal structure of FP-2, compound 5.1 emerged as an active lead. Biological evaluation showed that the compound 5.1 had an IC_{50} value in the micromolar range. By using scaffold hopping[139] we designed a series of 1,2,3-triazole compounds with the expectation of enhancing the potency of the lead compound 5.1 by exploring the SAR of this lead.

5.1
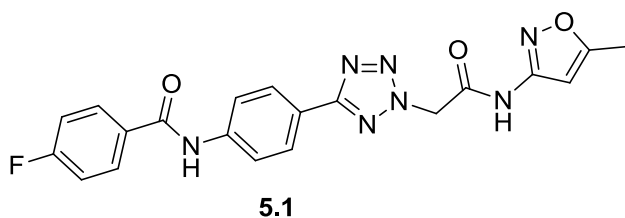
FP-2 IC_{50}=2.20 µM

FP-3 IC_{50}=4.95 µM

Figure 5-1 Structure of virtue screening hit

5.2 Research design and methods

5.2.1 Docking studies

Docking studies of 5.1 (Fig. 5.2 a) indicated that the tetrazole core could be replaced by a 1,2,3-triazole ring without loss of the key interaction with Gly83, thus optimization of 5.1 could be enhanced by click chemistry via classic Huisgen cycloaddition. The isoxazole ring is retained because it showed extensive hydrogen bond interactions with important residues in the S1-S1' pockets of the FP-2 active site (residues in Fig 5-2a: C42, Q36, W206, N173). The alkyl or aryl groups with varying hydrophobic and other stereoelectronic properties were thus appended only

at the fourth position of the 1,2,3-triazole moiety. In this triazole series, our aim was to investigate the effects of different substitutions on the activity of the fourth position of triazole that would be specifically interacting with the residues of the critical S2 pocket.
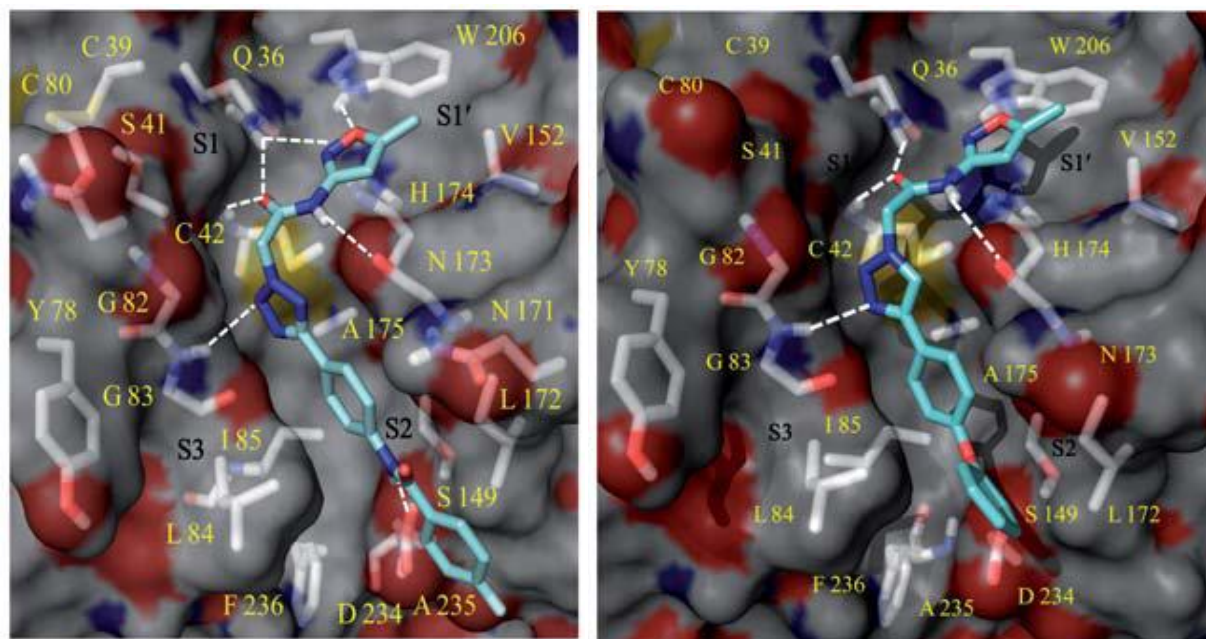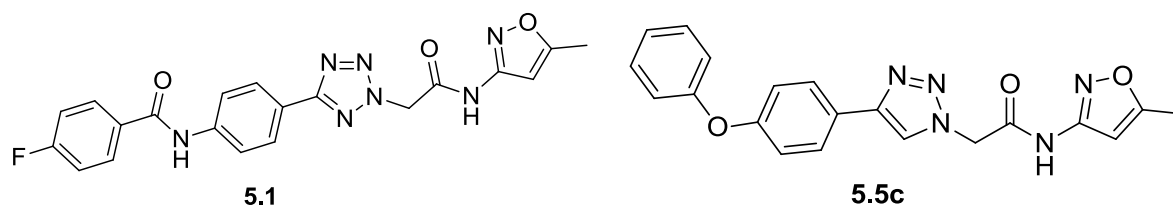

a   b

Figure 5-2 Docking pose of compound **5.1**(a) and designed compound **5.5c** (b)

5.2.2 Synthetic chemistry

The synthesis strategy of the 1,2,3-triazole compounds are showed in Scheme 5.1. The acyl chloride intermediate 5.3 was readily synthesized by coupling commercially available 5-methylisoxazol-3-amine (5.2) with chloroacetyl chloride, which, upon substitution reaction with

sodium azide in acetone, gave an azide intermediate 5.4. The 1,2,3,-triazole derivatives were then obtained through classic Huisgen 1,3-dipolar cycloaddition between the organic azide 5.4 and a series of terminal alkynes (Table 5.1).
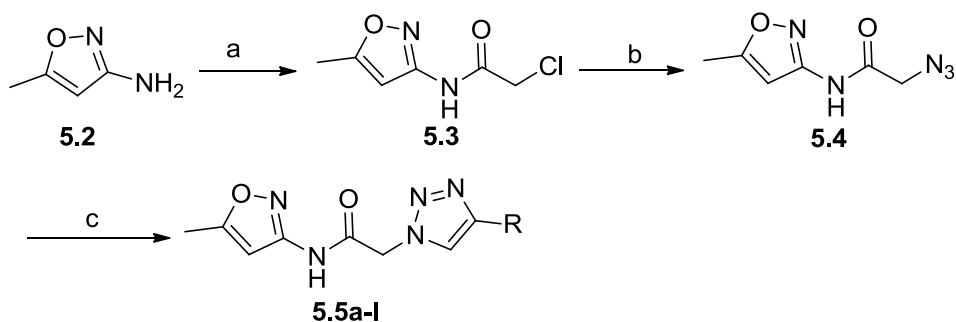
Scheme 5-1 General synthetic route

Reagents and conditions: a) chloroacetyl chloride, triethylamine 0℃; 88% b) sodium azide, reflux 12h, 80%; c) acetylene, Copper(II) sulfate, sodium ascorbate, water/t-BuOH (1:1) r.t to 55 ℃, 70-90%. (see table 5-1 for Structure of different R substituents)
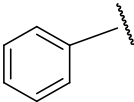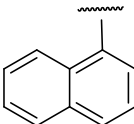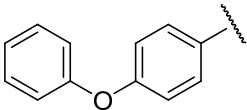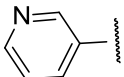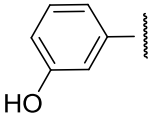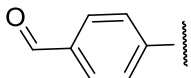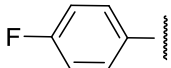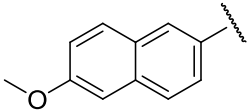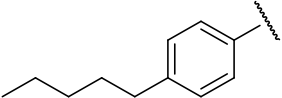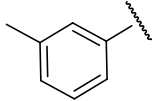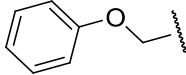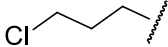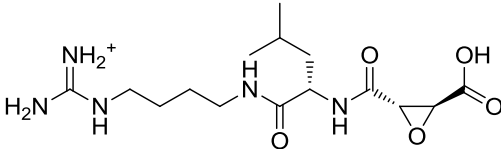
5.2.3 Biological evaluation

The synthesized compounds were evaluated for inhibition of recombinant FP-2 and FP-3 (Table 5-1).

Table 5-1 Structure and *in vitro* activity of triazole compounds*

5.5a-l
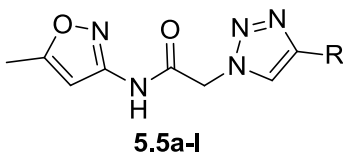
Compounds	structure	IC$_{50}$

No.	R=	μM			
		FP2	SD	FP3	SD
5.5a		**>50**	NA	**>50**	NA
5.5b		**55.190**	±22.27	**>50**	NA
5.5c		**19.875**	±1.63	**39.735**	±9.99
5.5d		**>50**	NA	**>50**	NA
5.5e		**>50**	NA	**>50**	NA
5.5f		**>50**	NA	**>50**	NA
5.5g		**>50**	NA	**>50**	NA
5.5h		**38.4**	±9.991	**>50**	NA
5.5i		**38.96**	±21.213203	**>50**	NA

5.5j		**>50**	NA	**>50**	NA
5.5k		**>50**	NA	**>50**	NA
5.5l		**>50**	NA	**>50**	NA
E64		**0.06**		**0.187**	

* NA=not available; SD=standard deviation

5.3 Results and Discussions

The docking study showed compounds 5.5a–l adopted similar binding modes as shown for the parent compound 5.1 (Fig. 5-2). However, very few compounds displayed inhibition of FP-2 in *in vitro* test. These active compounds have common features as they all have bulkier substituents at the phenyl ring compared to the other analogues. For example, compound 5.5c, 5.5h and 5.5i afforded modest activity against FP-2. Additional hydrophobic interactions with Leu172 which is buried deep in the S2 pocket of FP-2 might account for the modest activity of these compounds against FP-2. The inactivity of other 1,2,3-triazole analogues might be due to unsuitable or shorter hydrophobic R groups as compared to the parent compound 5.1. However, the molecular weight of these compounds is relatively low. Most of them have a MW around 300, which gives them adequate room for improvement in accordance of Lipinski's Rule of Five[140]. From this point of view, compound 5.5f and 5.5e which have aldehyde and hydroxyl functionalities, respectively are two good starting points for continued study.

5.4 Experimental

The selected compounds were tested for inhibition of FP-2, FP-3 and against chloroquine resistant (W2 strain) *P.falciparum* parasites. To determine IC_{50} values, recombinant FP-2 and FP-3 were incubated for 30 minutes at room temperature in 100 mM sodium acetate, pH 5.5, and 10 mM dithiothreitol with different concentrations of inhibitors prepared from stocks in DMSO (maximum concentration of DMSO in the assay was 1%). After 30 minutes, the substrate Z-Leu-Arg-AMC (benzoxycarbonyl-Leu-Arg-7-amino-4-methyl-coumarin) in the same buffer was added to a final concentration of 25 μM. Fluorescence was monitored for 15 minutes at room temperature in a Lab systems Fluoroskan Ascent spectrofluorometer. IC_{50} values were determined from plots of percent activity over compound concentration using Prism v5.0 software (GraphPad).

2-chloro-*N*-(5-methylisoxazol-3-yl)acetamide (5.3):

 In a 5 mL round bottom flask was Reactant 2 (0.196 g, 2 mmol) in DCM (2 ml) to give a light yellow solution. Chloroacetyl chloride (0.176 ml, 2.200 mmol) was added. The reaction mixture became murky and stirred for 2 hours. Then saturated $NaHCO_3$ solution was added to quench the reaction. The reaction mixture was poured into a separatory funnel and extracted with DCM (3x50ml). The combined organic layers were washed with water, brine and dried over Na_2SO_4. Crude product was used without further purification. ^{1}H NMR (400 MHz, DMSO-d_6): 10.32 (br., 1H), 5.66 (s, 1H), 3.34 (s, 2H), 1.42 (s, 3H); ^{13}C NMR (100MHz, DMSO-d_6): 170.3, 165.3, 158.0, 96.5, 43.2, 12.4

2-azido-*N*-(5-methylisoxazol-3-yl)acetamide (5.4) :

In a 10 mL round-bottomed flask was added 2-chloro-*N*-(5-methylisoxazol-3-yl) acetamide (50mg, 0.286 mmol) in acetone (5 ml) to give a yellow solution. Sodium azide (28mg) and sodium iodide (4.29 mg, 0.029 mmol) were added. The reaction vessel was heated to 60 ℃ for 4 hr. The reaction turned to brown. The solvent was evaporated under reduce pressure and the residue was taken up with water and extracted with EtOAc (3X10ml). The organic layer was combined and washed with water, brine, dried over Na_2SO_4, filtered and evaporated *in vacuo*. The crude was purified with flash column to give the product as white solid. ^{1}H NMR (400 MHz, CDCl$_3$): 9.46 (br., 1H), 6.73 (s, 1H), 4.16 (s, 2H), 2.43 (s, 3H); ^{13}C NMR (101 MHz, CDCl$_3$) : 170.4, 165.1, 136.4, 96.3, 52.6, 12.7.

General procedure for preparation of triazole compounds:

To a suspension of azide(45mg, 0.248 mmol) and selected alkyne (0.071 ml, 0.745 mmol) in water (1 ml) and t-BuOH(1 ml) was added sodium ascorbate (0.025 ml, 0.025 mmol) followed by Copper(II) sulfate pentahydrate (0.025 ml, 4.97 μmol). The suspension was vigorously stirred for 16 hrs, and then more catalyst was added if needed, or heated at 55 ℃ for 4 hrs. The reaction mixture was filtered and washed with diethyl ether to yield products.

***N*-(5-methylisoxazol-3-yl)-2-(4-phenyl-1H-1,2,3-triazol-1-yl)acetamide (5.5a)**

MP: 186-187 ℃; ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 8.56 (s, 1H), 7.86 (d, J = 7.43 Hz, 2H), 7.46 (t, J = 7.53 Hz, 2H), 7.29 - 7.39 (m, 1H), 6.58 (br., 1H), 5.41 (br., 2H), 2.36 (s, 3H); ^{13}C NMR

(126 MHz, DMSO-d$_6$) δ 170.0, 157.6, 146.2, 130.6, 128.9, 127.9, 125.1, 123.0, 96.1, 51.9, 12.1; IR (neat) (cm-1): 3090, 3036, 1723, 1630, 1483, 1432, 1270, 1207, 923, 821, 766, 695; HRMS (ESI-TOF) m/z: [M + Na] calcd for C$_{14}$H$_{13}$N$_5$O$_2$Na, 306.0967; found, 306.0960.

N-(5-methylisoxazol-3-yl)-2-(4-(naphthalen-1-yl)-1H-1,2,3-triazol-1-yl)acetamide (5.5b)

White solid, m.p. 187-188 ℃. ^{1}H NMR (500 MHz, CDCl$_3$) δ 11.52 (br., 1H), 8.60 (s, 1H), 8.47 (d, J = 4.27 Hz, 1H), 7.94 - 8.12 (m, 2H), 7.73 - 7.85 (m, 1H), 7.52 - 7.72 (m, 3H), 6.63 (br., 1H), 5.51 (s, 2H), 3.31 (s, 1H), 2.39 (s, 3H); ^{13}C NMR (126 MHz, DMSO-d$_6$) δ 170.0, 157.6, 145.3, 133.5, 130.2, 128.5, 128.5, 127.8, 126.8, 126.6, 126.1, 125.7, 125.6, 125.2, 96.2, 51.9, 12.1; 3225, 3043, 2982, IR (neat) ν$_{max}$: 1727, 1631, 1487, 1435, 1271, 1211, 1057, 1031, 925, 798, 771, 700. HRMS (ESI-TOF) m/z: [M + Na] calcd for C$_{18}$H$_{15}$N$_5$O$_2$Na, 356.1123; found, 356.1126.

N-(5-methylisoxazol-3-yl)-2-(4-(4-phenoxyphenyl)-1H-1,2,3-triazol-1-yl)acetamide (5.5c)

Light yellow solid, m.p. 186-187 ℃. ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 11.49 (br., 1H), 8.53 (s, 1H), 7.87 (d, J = 8.28 Hz, 2H), 7.42 (t, J = 7.53 Hz, 2H), 7.17 (t, J = 7.28 Hz, 1H), 7.08 (t, J = 7.78 Hz, 4H), 6.59 (br., 1H), 5.43 (br., 2H), 2.38 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d$_6$) δ 170.0, 157.6, 156.4,156.4, 145.8, 130.1, 126.9, 126.0, 123.7, 122.7, 118.9, 118.8, 96.1, 51.9, 12.1; IR (neat) ν$_{max}$: 3223, 3098, 3038, 2979, 1728, 1632, 1486, 1433, 1247, 750. HRMS (ESI-TOF) m/z: [M + Na] calcd for C$_{20}$H$_{17}$N$_5$O$_3$Na, 398.1229; found, 398.1237.

N-(5-methylisoxazol-3-yl)-2-(4-(pyridin-3-yl)-1H-1,2,3-triazol-1-yl)acetamide (5.5d)

Light yellow solid, m.p. 192.1-192.5 ℃. ^{1}H NMR (500 MHz, DMSO-d$_6$) δ 11.51 (br., 1H), 9.08 (br., 1H), 8.72 (s, 1H), 8.56 (br., 1H), 8.24 (d, J = 8.12 Hz, 1H), 7.50 (dd, J = 5.13, 6.84 Hz, 1H), 6.59 (s, 1H), 5.47 (s, 2H), 2.26 - 2.46 (m, 3H); ^{13}C NMR (126 MHz, DMSO-d$_6$) δ 170.0, 164.6, 157.6, 148.9, 146.4, 143.5, 132.4, 124.0, 123.8, 96.1, 52.0, 12.1; IR (neat) ν_{max} : 3070, 3029, 1710, 1574, 1508, 1453, 1224, 1039, 900, 827, 805, 703. HRMS (ESI-TOF) m/z: [M + Na] calcd for C$_{13}$H$_{12}$N$_6$O$_2$Na, 307.0919; found, 307.0931.

2-(4-(3-hydroxyphenyl)-1H-1,2,3-triazol-1-yl)-N-(5-methylisoxazol-3-yl)acetamide (5.5e)

Gray solid, m.p. 190-192 ℃. ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 11.49 (br., 1H), 9.55 (s, 1H), 8.50 (s, 1H), 7.21 - 7.46 (m, 3H), 6.70 - 6.87 (m, 1H), 6.59 (br., 1H), 5.41 (br., 2H), 2.37 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d$_6$) δ 170.0, 164.7, 157.8, 157.6, 146.3, 131.8, 130.0, 123.0, 116.1, 115.0, 111.9, 96.2, 51.9, 12.1; IR (neat) ν_{max} : 3263, 3154, 1716, 1632, 1576, 1474, 1438, 1346, 1245, 1217, 1153, 1082, 1051, 924, 807, 714. HRMS (ESI-TOF) m/z: [M + Na] calcd for C$_{15}$H$_{13}$N$_5$O$_3$Na, 322.0916; found, 322.0937.

2-(4-(4-formylphenyl)-1H-1,2,3-triazol-1-yl)-N-(5-methylisoxazol-3-yl)acetamide (5.5f)

White solid m.p. 187-188 ℃. ^{1}H NMR (500 MHz, DMSO-d$_6$) δ 11.52 (br., 1H), 10.02 (s, 1H), 8.78 (s, 1H), 8.11 (d, J = 7.27 Hz, 2H), 8.00 (d, J = 7.27 Hz, 2H), 6.59 (br., 1H), 5.47 (br., 2H), 2.38 (br., 3H); ^{13}C NMR (126 MHz, DMSO-d$_6$) δ 195.7, 173.7, 149.6, 137.3, 136.2, 130.3, 125.5, 124.6, 118.1, 96.1, 52.0, 38.7, 37.5, 12.1; IR (neat) ν_{max} : 3237, 3180, 3089, 1711, 1694,

1579, 1510, 1351, 1302, 1230, 1204, 1171, 1041, 958, 901, 822, 809. HRMS (ESI-TOF) m/z: [M + Na] calcd for $C_{15}H_{13}N_5O_3Na$, 334.0916; found, 334.0938.

2-(4-(4-fluorophenyl)-1H-1,2,3-triazol-1-yl)-N-(5-methylisoxazol-3-yl)acetamide (5.5g)

Light yellow solid, m.p. 217-219 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.48 (br., 1H), 8.56 (s, 1H), 7.90 (dd, J = 5.65, 8.16 Hz, 2H), 7.29 (t, J = 8.66 Hz, 2H), 6.58 (s, 1H), 5.42 (s, 2H), 2.37 (s, 3H); 13C NMR (101 MHz, DMSO-d_6) δ 170.0, 157.6, 145.4, 127.2, 127.1, 123.0, 120.0, 116.0, 115.8, 96.1, 51.9, 12.1 .IR (neat) ν_{max} : 3222, 3089, 3036, 2979, 1723, 1632, 1567, 1484, 1434, 1270, 1228, 1207, 1053, 1029, 925, 837, 814, 702. HRMS (ESI-TOF) m/z: [M + Na] calcd for $C_{14}H_{12}FN_5O_2Na$, 324.0873; found, 324.0882.

2-(4-(6-methoxynaphthalen-2-yl)-1H-1,2,3-triazol-1-yl)-N-(5-methylisoxazol-3-yl)acetamide (5.5h)

White solid, m.p. 202-204 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.51 (br., 1H), 8.63 (s, 1H), 8.35 (s, 1H), 7.93 - 7.99 (m, J = 8.53 Hz, 1H), 7.89 (d, J = 8.53 Hz, 1H), 7.34 (s, 1H), 7.17 - 7.23 (m, 1H), 6.60 (s, 1H), 5.46 (s, 2H), 3.89 (s, 3H), 2.38 (s, 3H); 13C NMR (101 MHz, DMSO-d_6) δ 170.0, 157.5, 146.5, 133.9, 129.6, 129.3, 128.7, 128.6, 127.4, 125.9, 124.1, 123.5, 122.9, 119.4, 119.1, 106.0, 96.2, 55.2, 12.1; IR (neat) ν_{max} : 3263, 3140, 2975, 1724, 1629, 1478, 1437, 1268, 1262, 1218, 1165, 1025, 925, 864, 827, 799, 734. HRMS (ESI-TOF) m/z: [M + Na] calcd for $C_{19}H_{17}N_5O_3Na$, 386.1229; found, 386.1218.

N-(5-methylisoxazol-3-yl)-2-(4-(4-pentylphenyl)-1H-1,2,3-triazol-1-yl)acetamide (5.5i)

White solid, m.p. 209-210 ℃. ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 11.49 (br., 1H), 8.51 (s, 1H), 7.75 (d, J = 7.78 Hz, 2H), 7.27 (d, J = 8.03 Hz, 2H), 6.59 (s, 1H), 5.41 (s, 2H), 2.59 (t, J = 7.65 Hz, 2H), 2.38 (s, 3H), 1.52 - 1.65 (m, 2H), 1.28 - 1.37 (m, 4H), 0.86 (t, J = 6.78 Hz, 3H); ^{13}C NMR (101 MHz, DMSO-d$_6$) δ 170.0, 164.8, 157.6, 146.3, 142.2, 128.8, 128.1, 125.1, 122.7, 96.2, 51.9, 34.9, 30.9, 30.5, 22.0, 13.9, 12.1; IR (neat) ν_{max} : 3225, 3088, 3036, 2926, 1729, 1632, 1574, 1486, 1435, 1271, 1214, 1051, 1031, 926, 823, 701. HRMS (ESI-TOF) m/z: [M + Na] calcd for C$_{19}$H$_{23}$N$_5$O$_2$Na, 376.1749; found, 376.1756.

N-(5-methylisoxazol-3-yl)-2-(4-(m-tolyl)-1H-1,2,3-triazol-1-yl)acetamide (5.5j)

White solid, m.p. 185-186 ℃. ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 11.29 (br., 1H), 8.01 (s, 1H), 7.60 (s, 1H), 7.50 - 7.57 (m, J = 7.53 Hz, 1H), 7.47 (s, 1H), 7.22 (t, J = 7.53 Hz, 1H), 7.01 - 7.11 (m, J = 7.78 Hz, 1H), 6.52 (s, 1H), 5.26 (s, 2H), 2.83 (s, 2H), 2.31 (s, 3H); ^{13}C NMR (101 MHz, CDCl$_3$) δ 168.9, 157.2, 137.6, 135.7, 129.8, 128.1, 128.0, 125.6, 122.1, 121.1, 95.7, 62.9, 20.7, 11.9; IR (neat) ν_{max} : 3294, 3255, 3188, 3106, 2942, 1707, 1563, 1514, 1224, 1094, 1055, 1035, 1008, 960, 894, 808, 790, 699. HRMS (ESI-TOF) m/z: [M + Na] calcd for C1$_5$H$_{15}$N$_5$O$_2$Na, 320.1123; found, 320.1141.

N-(5-methylisoxazol-3-yl)-2-(4-(phenoxymethyl)-1H-1,2,3-triazol-1-yl)acetamide (5.5k)

White solid, m.p. 183-185 ℃. ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 11.46 (br., 1H), 8.24 (s, 1H), 7.31 (t, J = 7.91 Hz, 2H), 7.05 (d, J = 8.28 Hz, 2H), 6.95 (t, J = 7.28 Hz, 1H), 6.58 (s, 1H), 5.40

(s, 2H), 5.17 (s, 2H), 2.37 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.0, 164.7, 158.1, 157.6, 142.6, 129.5, 126.2, 120.8, 114.6, 96.1, 60.9, 51.8, 12.1; IR (neat) ν_{max} : 3263, 3220, 3078, 2983, 1722, 1625, 1564, 1484, 1432, 1269, 1232, 1206, 1041, 925, 820, 755, 690. HRMS (ESI-TOF) m/z: [M + Na] calcd for $C_{15}H_{15}N_5O_3Na$, 336.1073; found, 336.1091.

2-(4-(3-chloropropyl)-1H-1,2,3-triazol-1-yl)-N-(5-methylisoxazol-3-yl)acetamide (5.5l)

White solid, m.p. 166-168 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.42 (br., 1H), 7.91 (s, 1H), 6.57 (br., 1H), 5.32 (s, 2H), 3.63 - 3.88 (m, 2H), 2.78 (t, J = 6.78 Hz, 2H), 2.37 (s, 3H), 2.00 - 2.13 (m, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.9, 164.8, 157.6, 145.4, 123.8, 96.1, 51.7, 44.6, 31.8, 22.2, 12.1; IR (neat) ν_{max}: 3273, 3222, 3057, 2979, 1725, 1629, 1484, 1435, 1355, 1270, 1213, 1068, 1031, 927, 824, 767, 711. HRMS (ESI-TOF) m/z: [M + Na] calcd for $C_{11}H_{14}ClN_5O_2Na$, 306.0734; found, 306.0705.

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Appendix
^{1}H-NMR and ^{13}C-NMR

3.8

3.8

3.12

3.12

CHLOROFORM-d

3.13a

7.80, 7.74, 7.73, 7.53, 7.52, 7.50, 7.44, 7.42, 7.40, 7.27
5.92
2.87, 2.85, 2.83, 2.19, 2.18, 2.17, 2.05, 1.94, 1.90, 1.89, 1.77, 1.48, 1.47, 1.45, 1.26, 1.00, 0.99

Chemical Shift (ppm)

CHLOROFORM-d

3.13a

171.83
148.33
137.23
129.63, 128.41
120.41, 119.16
105.27
93.79
80.29
50.47, 44.86, 42.81, 37.55, 35.90, 33.93, 33.61, 31.62, 27.06, 25.45, 24.69, 19.87

Chemical Shift (ppm)

3.13a

129.67
128.45
120.45
119.21
93.83
50.50
44.90
42.84
37.59
35.94
33.97
33.65
31.66
27.10
25.50
25.46
24.74
19.91

Chemical Shift (ppm)

3.13a

F1 Chemical Shift (ppm)

F2 Chemical Shift (ppm)

3.13b

3.13b

3.13b

129.67
128.46
120.39
119.17
93.52
50.03
43.07
37.74
37.54
35.90
33.53
26.82
26.46
25.15
24.85
23.33
19.79

Chemical Shift (ppm)

3.13b

F1 Chemical Shift (ppm)

F2 Chemical Shift (ppm)

3.15

3.15

CHLOROFORM-d

7.24
7.23
7.22
6.82
6.82
6.80
5.85
5.25
5.24
5.08
4.96
4.08
4.08
4.07
4.06
4.05
4.04
4.03
3.74
3.73
3.13
2.28
2.01
1.98
1.97
1.64
1.38
1.37
1.24
1.21
1.21
1.20
1.19
1.18
0.93
0.85

3.16a

1.81 1.93   0.94   1.90   2.902.92   1.79   1.106.28 7.04 4.78 1.69

Chemical Shift (ppm)

CHLOROFORM-d

EtOAc

EtOAc

EtOAc

EtOAc

EtOAc

170.86
159.24
156.34
129.62
128.63
113.63
105.01
93.58
80.09
66.04
55.00
50.24
44.29
42.80
40.44
37.26
35.70
33.72
31.31
30.97
27.63
26.61
25.23
24.48
19.65
13.96

3.16a

Chemical Shift (ppm)

3.16b

3.16b

CHLOROFORM-d

3.16b

129.82
113.79
93.45
66.28
55.17
49.92
43.20
37.41
35.81
33.45
25.06
24.75
23.23
19.70

Chemical Shift (ppm)

113

3.19

3.19

3.22

3.22

3.25

130.01
127.75
123.77
67.74
54.12
53.80
48.86
25.74
24.03
21.61

Chemical Shift (ppm)

3.27

3.26

7.76
7.63
7.60
7.58
7.44
7.43
7.26
7.24
7.22
7.20
5.58
5.58
5.54
5.54
5.04
5.04
5.02
4.67
4.65
4.64
4.10
3.81
3.68
3.67
3.65
3.58
3.57
2.37
1.51
1.49
1.48
1.47
1.45
1.33
1.32
1.30
0.81
0.79
0.78

Chemical Shift (ppm)

13C peaks: 167.66

144.66, 143.96, 132.30, 130.85, 130.20, 128.67, 123.86, 119.82

104.58

77.56, 77.24, 76.92, 68.02

54.10, 54.00, 53.65, 51.36

38.60, 30.24, 29.52, 28.80, 25.50, 23.88, 23.63, 22.85, 13.96, 10.87

3.27

3.26

Chemical Shift (ppm)

1H peaks: 4.31, 4.31, 2.39, 2.39, 0.91, -0.13

H3C, CH3, H3C, CH3, Si, O, CH3, CH3, HC

2.13   1.00   9.49   6.12

Chemical Shift (ppm)

M05(s)
M09(s)
M04(s)
M02(s)
M03(s)
M01(s)

82.39
77.32
77.00
76.68
72.80
51.47
25.76
18.25
-5.24

3.00
1.65

Chemical Shift (ppm)

7.58
7.54
4.79
4.71
4.69
4.68
3.72
3.70
3.68
0.90
0.88
0.85
0.08
0.07
0.04

3.28

0.94
1.84
1.99
9.00
5.69

Chemical Shift (ppm)

3.28

3.28

3.28a

3.28a

3.29

^{1}H NMR peaks: 7.47, 7.27, 5.87, 4.80, 4.77, 4.35, 4.33, 4.31, 4.29, 4.10, 4.08, 4.06, 4.04, 4.01, 2.32, 2.12, 2.05, 2.04, 2.03, 1.99, 1.64, 1.42, 1.40, 1.36, 1.23, 1.21, 0.95, 0.94, 0.87, 0.06

Integrations: 0.89, 0.83, 1.80, 1.81, 0.96, 1.00, 0.71, 1.43, 9.75, 5.48

3.29

^{13}C NMR peaks: 171., 148.36, 121.55, 105.15, 93.74, 80.02, 60.20, 57.76, 50.26, 44.04, 43.24, 37.33, 35.73, 33.71, 31.32, 30.80, 28.04, 25.75, 25.30, 24.50, 19.72, 18.18

3.30

3.30

121.62
93.63
57.90
50.09
49.92
43.69
37.62
37.46
35.83
33.41
28.48
25.89
25.12
24.78
24.49
23.34
19.76
-5.28

220   200   180   160   140   120   100   80   60   40   20   0   -20
Chemical Shift (ppm)

3.30

8   7   6   5   4   3   2   1   0   -1
F2 Chemical Shift (ppm)

0
20
40
60
80
100
120
F1 Chemical Shift (ppm)

126

56.08
50.11
49.85
43.47
37.60
37.39
35.80
33.35
28.28
25.10
24.73
24.36
23.28
19.73

122.05

93.64

3.31

Chemical Shift (ppm)

F2 Chemical Shift (ppm)

F1 Chemical Shift (ppm)

127

3.32

7.78
5.84
5.37
4.46
4.44
4.42
4.40
3.20
3.19
3.04
2.71
2.41
2.21
2.07
2.06
2.04
2.03
1.98
1.77
1.74
1.43
1.40
1.39
1.06
0.99
0.98

0.99 0.38 1.22 2.20 2.90 3.46 0.53 1.94 6.69 2.61 0.82

10 9 8 7 6 5 4 3 2 1 0

Chemical Shift (ppm)

3.32

171.
136.35
124.39
105.43
93.65
79.13
62.44
50.34
49.91
43.79
38.30
37.60
37.42
35.81
33.37
28.36
25.08
24.74
24.48
23.35
19.70

200 180 160 140 120 100 80 60 40 20 0

Chemical Shift (ppm)

128

3.32

—124.43

—93.69

—62.48
—50.38
—49.95
—43.83
—38.34
—37.64
—37.46
—35.85
—33.41
—25.12
—24.78
—24.52
—23.39
—19.75

Chemical Shift (ppm)

F2 Chemical Shift (ppm)

F1 Chemical Shift (ppm)

129

3.33

CHLOROFORM-d

—7.60

—5.84

4.43
4.41
4.40
4.36
4.34
4.06
3.87
3.22
3.21
3.19
2.68
2.42
2.12
2.04
2.02
2.01
1.99
1.97
1.83
1.49
1.44
1.41
1.39
1.25
0.99
0.98

1.28 0.59   1.00   2.67 2.75   1.00   0.83 7.32 5.18 3.33 1.62

10   9   8   7   6   5   4   3   2   1   0
Chemical Shift (ppm)

3.33

CHLOROFORM-d

—171.07

—144.56

—122.76

—105.41

—93.60

—79.12

53.60
50.26
50.08
49.94
43.66
37.61
37.46
35.84
33.41
28.41
25.10
24.78
24.46
23.43
19.73

67.21   2.76   1.16

200   180   160   140   120   100   80   60   40   20   0
Chemical Shift (ppm)

130

3.33

3.35

94.22
77.21
61.83
50.30
42.87
39.67
37.69
35.87
33.91
30.77
29.33
25.49
24.76
19.81
16.38

220 200 180 160 140 120 100 80 60 40 20 0 -20
Chemical Shift (ppm)

3.36a

3.36a

3.36a

94.10
54.99
50.67
44.49
43.78
40.21
35.94
33.88
30.97
26.05
25.51
24.69
24.25
19.86

220 200 180 160 140 120 100 80 60 40 20 0 -20

Chemical Shift (ppm)

3.36a

3.36b

3.36b

192.15
170.86
105.41
94.00
79.33
50.58
49.88
44.04
39.63
37.53
35.85
34.20
33.39
27.16
26.05
25.13
24.83
24.25
19.78

Chemical Shift (ppm)

3.36b

94.01
55.09
50.59
49.89
44.04
39.63
37.54
34.21
33.39
26.05
25.14
24.84
24.25
23.93
19.78

Chemical Shift (ppm)

CHLOROFORM-d

5.93

4.27
4.11
3.92
3.90
3.89
3.87
3.67
3.66
3.64
3.62
2.86
2.84
2.04
2.02
1.96
1.70
1.63
1.49
1.47
1.46
1.44
1.41
1.26
1.00
0.99

3.37

0.99

0.66 1.17

0.99 1.12 2.100.85 1.62 0.83

Chemical Shift (ppm)

3.37

3.38

3.38

141

3.38

3.39

3.39

5.3

DMSO

10.32
5.66
3.34
1.42

1.08
1.07
2.00
3.07

10 9 8 7 6 5 4 3 2 1 0
Chemical Shift (ppm)

5.3

170.05
165.10
157.83
96.29
42.98
39.93
39.72
39.51
39.09
38.89
12.19

200 180 160 140 120 100 80 60 40 20 0
Chemical Shift (ppm)

144

5.5a

5.5a

5.5b

5.5b

DMSO-d6

5.5c

DMSO-d6

169.97
157.62
156.45
156.40
145.78
130.11
126.91
125.96
122.67
118.90
118.84
96.14
51.93
12.12

0.87

Chemical Shift (ppm)

water
DMSO-d6

9.08
8.72
8.56
8.25
8.23
7.51
7.50
7.49
7.48
6.59
5.47
3.33
2.45
2.38

5.5d

0.94 1.00 0.89 0.98 0.88 2.03 3.72 3.00

Chemical Shift (ppm)

Spectrum for compound **5.5d** (¹³C NMR):

Peaks labeled: 169.99, 164.61, 157.60, 148.91, 146.35, 143.46, 132.41, 124.03, 123.76, 96.13, 52.03, DMSO-d6, 12.12

Integration: 1.00

X-axis: Chemical Shift (ppm), from 200 to 0

Spectrum for compound **5.5e** (¹H NMR):

Peaks labeled: 9.55, 8.50, 7.30, 7.25, 7.23, 7.21, 6.75, 6.75, 6.74, 6.74, 6.73, 6.59, 5.41, 3.37 (water), 2.37 (DMSO-d6)

Integrations: 1.11, 1.14, 3.67, 0.94, 2.25, 3.00

X-axis: Chemical Shift (ppm), from 10 to 0

13C NMR spectrum of compound 5.5e with peaks at 169.98, 164.75, 157.78, 157.64, 146.34, 131.85, 130.00, 123.00, 116.06, 114.96, 111.87, 96.16, 51.93, DMSO-d6, 12.13.

1H NMR spectrum of compound 5.5f with peaks at 10.02, 8.78, 8.11, 8.10, 8.01, 7.99, 6.59, 5.47, 3.32 (water), 2.38 (DMSO-d6). Integrations: 1.01, 1.02, 2.00, 1.01, 0.09, 3.66, 2.90.

5.5f

5.5g

5.5g

169.98
157.61
145.37
127.21
127.12
122.96
120.04
115.97
115.75
96.13
51.95
DMSO-d6
12.12

Chemical Shift (ppm)

5.5h

5.5h

154

5.5i

1H NMR peaks: 8.51, 7.76, 7.75, 7.28, 7.26, 6.59, 5.41, 4.07, 2.61, 2.59, 2.57, 2.54, 2.38, 1.63, 1.61, 1.59, 1.57, 1.30, 1.29, 1.26, 0.88, 0.86, 0.84

Water

Integrations: 1.32, 2.60, 2.59, 1.47, 2.61, 4.40, 2.54, 1.19, 3.84

5.5i

13C NMR peaks: 170.00, 164.77, 157.64, 146.34, 142.17, 128.84, 128.12, 125.13, 122.67, 96.16, 51.93, 34.87, 30.88, 30.53, 21.96, 13.93, 12.14

5.5j

5.5j

5.5k

5.5k

157

5.5I

¹H NMR peaks: 7.91, 6.57, 5.32, 3.69, 3.68, 3.66, 3.35, 2.79, 2.78, 2.76, 2.37, 2.09, 2.07, 2.06, 2.04, 2.02

water

Integrations: 1.09, 1.02, 2.26, 2.21, 1.12, 0.00, 2.00

Chemical Shift (ppm)

5.5I

¹³C NMR peaks: 169.92, 164.85, 157.64, 145.37, 123.84, 96.14, 51.70, 44.64, 31.77, 22.25, 12.11

Chemical Shift (ppm)

VITA

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EDUCATION

Ph. D. Medicinal Chemistry, University of Mississippi, MS, USA 2012
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RESEARCH EXPERIENCE

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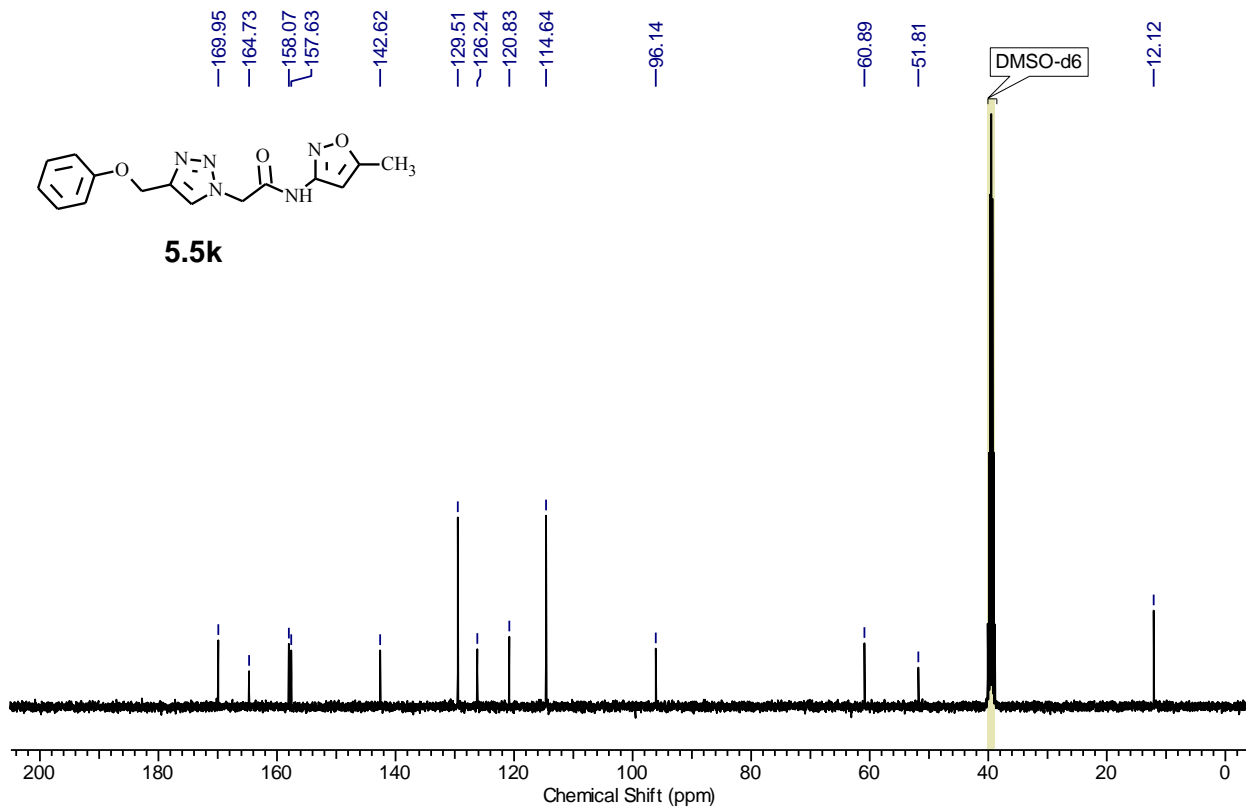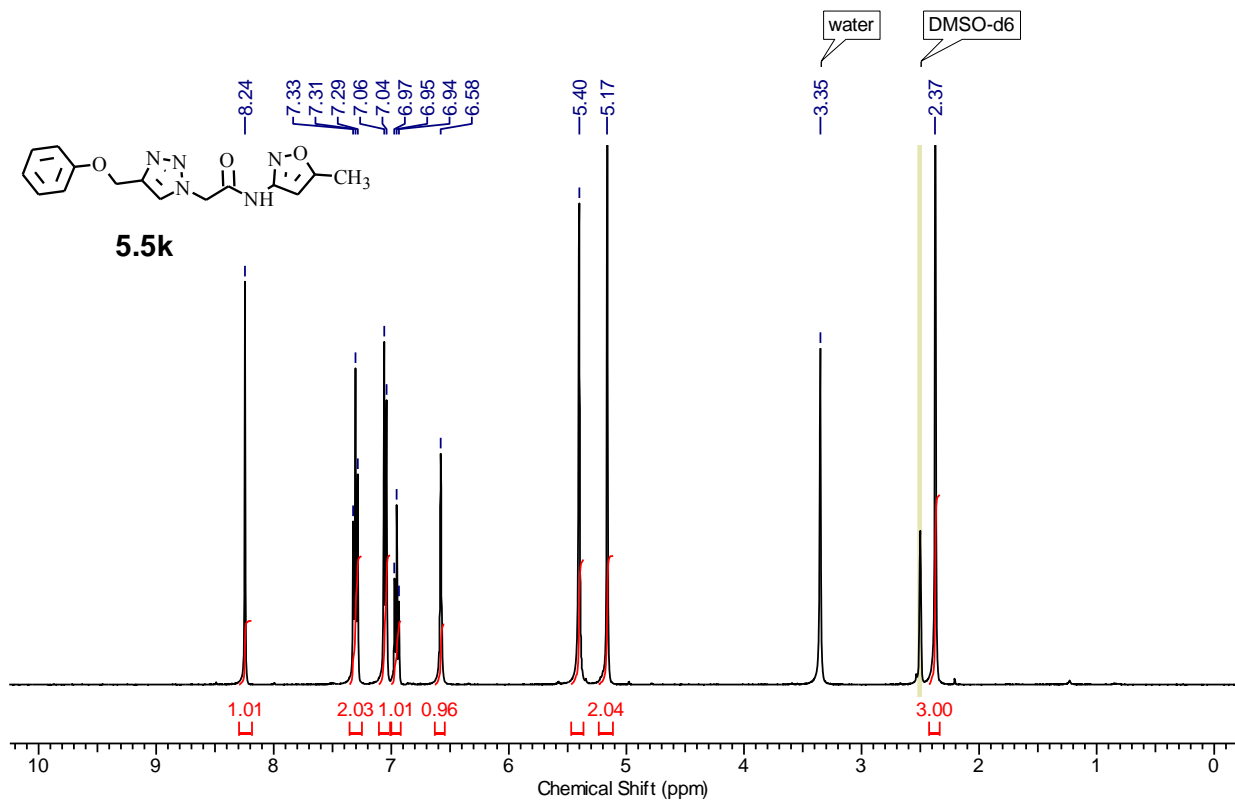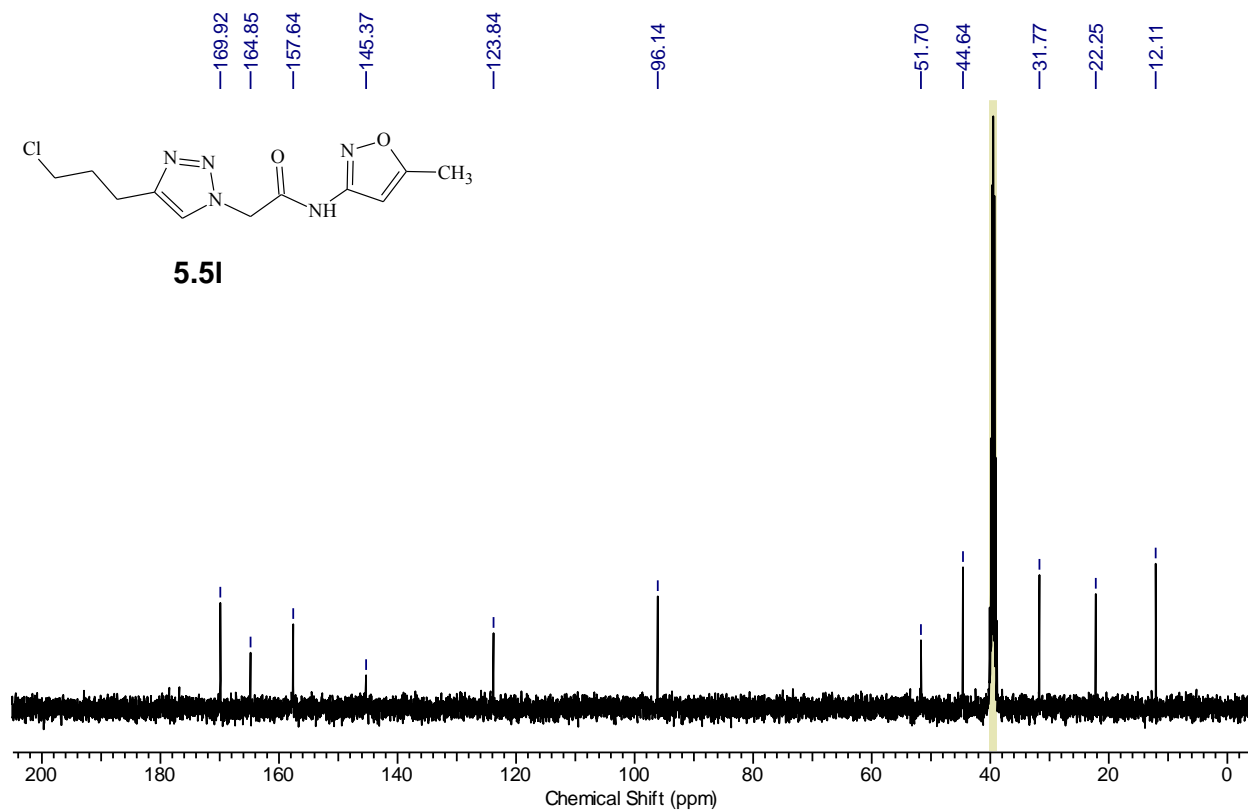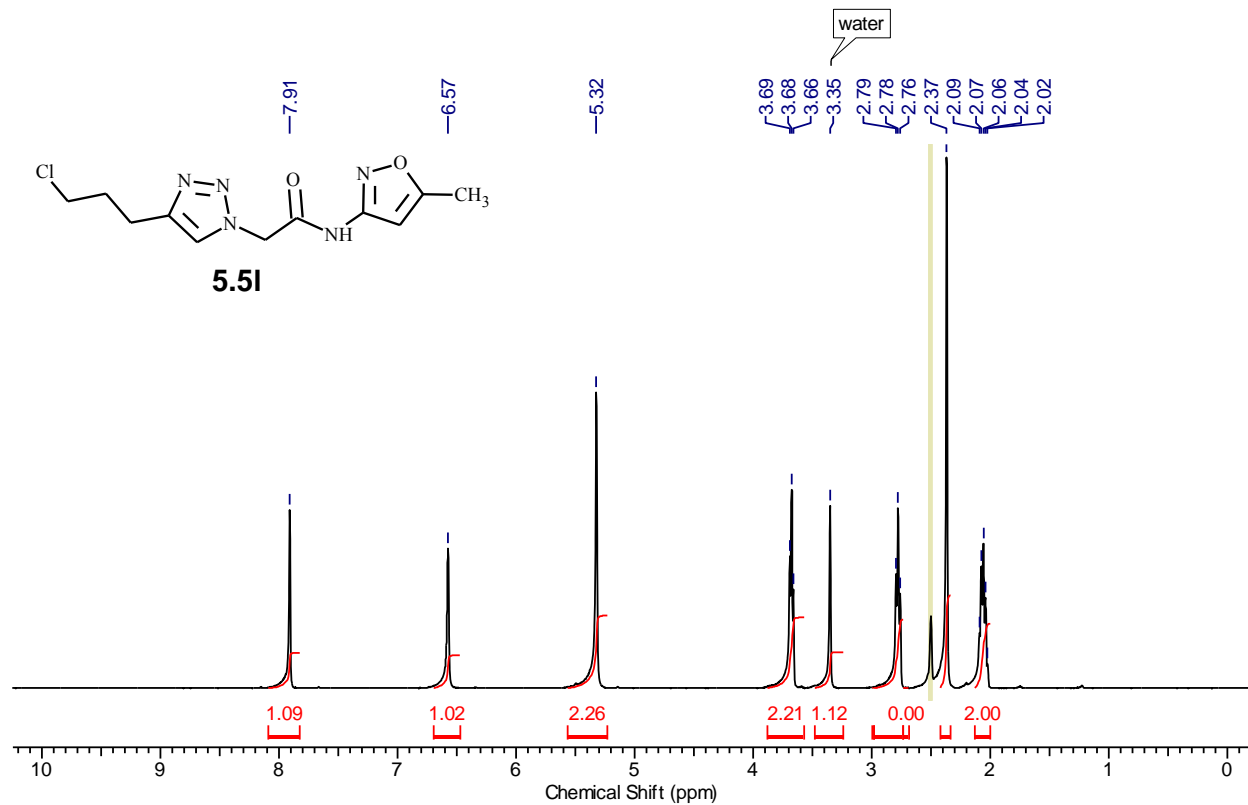Postdoctoral Research Associate Ablitech Inc.Balitmore, MD 2012
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WORKING EXPERIENCE

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ACADEMIC HONORS

1998-1999: Xinchang Scholarship at Beijing Medical University
2004: Outstanding employee at the Institute of Materia Medica
2005: Prize "Outstanding Experimental Record at Institute of Materia Medica"
2010: NIH Predoctoral Fellow 2011, the Center of Research Excellence in Natural Products Neuroscience (CORE-NPN)
2011: Phi Kappa Phi: Honor Society Membership

PUBLICATIONS

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