Synthesis and *in vitro* antimalarial activity of esters with truncated artemisinin scaffold

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PREFACE

This thesis is submitted in an article format in accordance with the General Academic Rules (A.13.7.33) of the North-West University. An article in the form of a manuscript is included in this thesis.

The article entitled "**Synthesis and biological evaluation of a series of non-hemiacetal ester derivatives of artemisinin"** was published in the European Journal of Medicinal Chemistry

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ABSTRACT

Malaria is an infectious disease caused by *Plasmodium* parasites, with *P. falciparum*, responsible for most cases of morbidity and mortality. In 2014, malaria killed 438 000 people, with most deaths occurring in sub-Saharan Africa. The emergence and spread of *P. falciparum* drug resistance drives the search for new drugs to combat the disease.

Currently, artemisinins remain the mainstay of antimalarial chemotherapy. They possess superior potency, rapid action, good tolerability as well as a broad spectrum of antiplasmodial activity against *P. falciparum*. Undesirably, the use of these drugs is impeded by chemical and thermal instabilities as well as human neurotoxicity at high doses. Additionally, artemisinins have short pharmacological half-lives which result in recrudescence when used in monotherapy and ultimately the development of parasite resistance. Consequently, the WHO recommends ACT (artemisinin combination therapy) which is the administration of an artemisinin drug in partnership with a longer acting drug from a different antimalarial class, in order to elicit efficient and curative antimalarial treatment while also avoiding parasite resistance to artemisinins. Despite this strategy, the emergence of *P. falciparum* resistance has been reported. Attempts to combat artemisinin resistance and the search for new drugs are, therefore, incumbent.

Most shortcomings of clinical artemisinins are due to the structural lability of the hemiacetal D-ring. During this study, an investigation of robust and stable non-hemiacetal esters of artemisinin was conducted. The purpose was to find derivatives that would trade on the benefits of the clinically used artesunate (ARS), but offer more stability, improved solubility and most importantly, the inability to metabolise into dihydroartemisinin (DHA) both *in vitro* and *in vivo*.

In this study, truncated non-hemiacetal artemisinin ester derivatives were synthesised through the reaction of acid anhydrides, or acid chlorides with an artemisinin derived alcohol. Their structures were confirmed by NMR, IR and MS. The truncated esters were screened for *in vitro* antimalarial activity alongside chloroquine (CQ), DHA, ARM (artemether) and ARS against CQ sensitive (NF54) and CQ resistant (Dd2) strains of *P. falciparum*. Furthermore, the compounds were screened *in vitro* for cytotoxicity using mammalian WI-38 (human) and CHO (animal) cell lines. *In vitro* anticancer activity of the compounds was tested in TK10 (renal), UACC62 (melanoma) and MCF7 (breast) cancer cells. The

compounds generally displayed poor anticancer activity therefore none of the compounds stood out as a potential anticancer candidate drug.

All synthesised esters were active against both strains of *P. falciparum*. The majority of the compounds were equipotent to ARS, with the exception of *p*-nitrobenzoate and furan-2-carboxylate, which had superior antimalarial activity against the resistant Dd2 parasites, however none showed superior activity to DHA. The derivatives had good safety profiles. Additionally, resistance index (RI<1) suggested that Dd2 parasites posed no resistance to the majority of the new derivatives. Most of the esters were found to be more stable than the clinical artemisinins. Ultimately, the *p*-nitrobenzoate **11** was identified as the best candidate for further investigation as a potential drug in the search for new, safe and effective antimalarial drugs, based on its efficacy, tolerability, safety profile, as well as thermal stability.

Keywords: *malaria, artemisinins, neurotoxicity, stability, truncation, resistance, Plasmodium falciparum*

UITTREKSEL

Malaria is ‘n aansteeklike siekte wat deur *Plasmodium*-parasiete veroorsaak word. *P. falciparum* is vir die meeste siekte- en sterfgevalle verantwoordelik. In 2014, was malaria die oorsaak van 438 000 sterfgevalle onder mense, waarvan die meeste in sub-Sahara Afrika voorgekom het. Die ontstaan en verspreiding van geneesmiddelweerstandige *P. falciparum* is die dryfveer vir die soeke na nuwe geneesmiddels om die siekte te beveg.

Die artemisiniene is nog steeds die belangrikste middels vir antimalariaterapie. Hulle beskik oor die hoogste potensie, vinnige werking, word goed verdra en beskik oor ‘n breë spektrum van plasmodiese aktiwiteit teen *P. falciparum*. Ongelukkig word die gebruik van hierdie geneesmiddels aan bande gelê deur chemiese en termiese onstabiliteit asook neurotoksisiteit by mense by hoë dosisse. Hierbenewens beskik artemisiniene oor kort farmakologiese halfleeftye wat opflikkering van simptome kan veroorsaak indien dit as monoterapie toegedien word en wat uiteindelik tot ontwikkeling van parasietweerstandigheid lei. Gevolglik beveel die WGO (Wêreldgesonheidsorganisasie) ACT (artemisinienkombinasieterapie) aan. Dit behels die toedien van ‘n artemisinien saam met ‘n langerwerkende middel van ‘n ander antimalariaklas, om sodoende effektiewe genesing van malaria te bewerkstellig en terselfdertyd parasietweerstandigheid teenoor die artemisiniene te voorkom. Ten spyte van hierdie benadering is *P. falciparum*-weerstandigheid reeds aangemeld. Pogings om weerstand teen artemisinien te beveg en die soeke na nuwe geneesmiddels is derhalwe noodsaaklik.

Die meeste van die tekortkomige van die kliniese artemisiniene is te wyte aan die strukturele onstabiliteit van die hemiasetale D-ring. In die loop van hierdie studie is robuuste en stabiele nie-hemiasetaalesters van artemesinien ondersoek. Die doelwit was om derivate te identifiseer wat die voordele van artesunaat (ARS), wat klinies aangewend word, te behou, maar wat groter stabiliteit en verhoogde oplosbaarheid sal bied en baie belangrik: nie *in vitro* en *in vivo* metabolisme na dihidroartemisinien (DHA) ondergaan nie.

In hierdie studie is verkorte, nie-hemiasetaal, artemisinienesterderivate gesintetiseer deur reaksie van suuranhidriede of suurchloriede met ‘n artemisinienafgeleide alkohol. Die strukture is met KMR, IR en MS bevestig. Die verkorte esters is vir *in vitro* antimalaria-aktiwiteit teen chlorokiensensitiewe NF54) en chlorokienweerstandige CQ (Dd2) rasse van *P. falciparum* getoets en vergelyk met chlorokien (CQ), DHA, ARM (artemeter) en ARS. Hierbenewens is die verbindings ook vir *in vitro* sitotoksisiteit teenoor soogdier WI-38

(menslike) en CHO (dierlikel) sellyne getoets. *In vitro* antikankeraktiwiteit van die verbindings is teenoor TK10 (renale), UACC62 (melanoom-) en MCF7 (bors-) kankerselle getoets. Die verbindings het swak antikankeraktiwiteit en nie een van die verbindings toon belofte as potensiële kandidaat vir geneesmiddelbehandeling van kanker nie.

Al die gesintetiseerde esters was aktief teen beide *P. falciparum*-rasse. Die meeste van die verbindings was net so potent soos ARS, met die uitsondering van *p*-nitrobensoaat en furaan-2-karboksilaat, wat beter antimalaria-aktiwiteit teen die weerstandige Dd2-parasiet getoon het. Geen verbinding het egter die aktiwiteit van DHA oortref nie. Die verbindings het goeie veiligheidsprofiele. Boonop dui hul weerstandigheidsindekse (RI<1), daarop dat Dd2-parasiete nie weerstandigheid teenoor die meeste van hierdie nuwe derivate toon nie. Die meeste van die esters was meer stabiel as die kliniese artemesiniene. Verbinding **11**, *p*-nitrobensoaat, is, vanweë sy effektiwiteit, verdraagbaarheid, veiligheidsprofiel en termiese stabiliteit, as die belowendste kandidaat vir die soektog na nuwe, veilige en effektiewe antimalariageneesmiddels geïdentifiseer.

Sleutelwoorde: *malaria, artemisiniene, neurotoksisiteit, stabiliteit, verkleining, weerstand, Plasmodium falciparum*

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LIST OF SCHEMES

LIST OF ABBREVIATIONS

ACT	Artemisinin based combination therapy
AIDS	Acquired Immune Deficiency Syndrome
AL	Artemether-lumefantrine
ARM	Artemether
ARS	Artesunate
AS/AQ	Artesunate-amodiaquine
AS/CD	Artesunate-chlorproguanil-dapsone
AS/MQ	Artesunate-mefloquine
AS/SP	Artesunate-sulphadoxine-pyrimethamine
AP	Atovaquone-proguanil
AQ	Amodiaquine
$BF_3 \cdot Et_2O$	Boron trifluoride diethyl etherate
CCl_4	Carbon tetrachloride
^{13}C NMR	Carbon NMR
CQ	Chloroquine
CQR	Chloroquine resistant
CQS	Chloroquine sensitive
DCM	Dichloromethane
DEE	Diethyl ether
DHA	Dihydroartemisinin
DHFR	Dihydrofolate reductase
DHPS	Dihydropteroate synthase
DMAP	4-dimethylaminopyridine
EDG	Electron-donating groups
EWG	Electron-withdrawing groups
^{1}H NMR	Proton NMR
HCl	Hydrochloric acid
HIV	Human Immunodeficiency virus
HRMS	High resolution mass spectrometry
IC_{50}	50% inhibitory concentration
IPTi	Intermittent preventive treatment for infants
IPTp	Intermittent preventive treatment in pregnancy
IRS	Indoor residual spraying
IR	Infrared spectroscopy

ITNs	Insecticide-treated mosquito nets
IPTi	Intermittent preventative treatment
MeOH	Methanol
$MgSO_4$	Magnesium sulphate
Mp	Melting point
$NaBH_4$	Sodium borohydride
$NaHCO_3$	Sodium bicarbonate
NH_4Cl	Ammonium chloride
NMR	Nuclear magnetic resonance
PTD	Parthenolide
RI	Resistance index
ROS	Reactive oxygen species
SI	Selectivity index
SMC	Seasonal malaria chemoprevention
SP	Sulfadoxine-pyrimethamine
SRB	Sulforhodamine B
TEA	Triethylamine
TGA	Thermogravimetric analysis
TLC	Thin layer chromatography
WHO	World Health Organization

CHAPTER 1
INTRODUCTION AND PROBLEM STATEMENT

1.1. Background

Malaria is a vector borne parasitic disease that is transmitted to humans through the bite of an infected female *Anopheles* mosquito. The disease is caused by five species of the genus *Plasmodium* (*P.)*, namely *P. ovale*, *P. malariae*, *P. knowlesi*, *P. vivax* and *P. falciparum*, with the latter two being the most virulent (Pawluk *et al.*, 2013; WHO, 2015b). *P. vivax* can develop and survive in the human host through the hypnozoites, even at cooler climates. Most malaria incidents and deaths are caused by *P. falciparum*, which is the most prevalent in sub-Saharan Africa (WHO, 2015b).

Along with human immunodeficiency virus (HIV) and tuberculosis (TB), malaria is one of the main causes of human death worldwide (Murray *et al.*, 2014), which mainly affects Sub-Saharan Africa, South-East Asia and the Eastern Mediterranean (Figure 1.1). In 2014, malaria was responsible for 438 000 deaths globally, 308 000 of which occurred among children under the age of 5. However, in the past 15 years, great strides have been made towards its reduction, resulting in an 18% decline in malaria cases, 37% in incidences, 48% in deaths and an overall 60% decrease in the malaria mortality rate (WHO, 2015b). These reductions have been attributed to a combination of various factors, including the implementation of efficient vector control measures, as well as the expanded use of both preventive and curative chemotherapies. The former plays a critical role in reducing parasite transmission from human to mosquito, in order to contain the spread of infection to humans. This is achieved through the use of insecticide-treated mosquito nets (ITNs) and indoor residual spraying (IRS).

Preventive chemotherapies, which are measures put in place to suppress blood-stage infection in humans have two main objectives namely to protect high risk groups (pregnant women, infants and children) and to minimise *Plasmodium* parasite transmission during peak malaria transmission seasons. These objectives are achieved through intermittent preventive treatment in pregnancy (IPTp) and intermittent preventive treatment for infants (IPTi) programs through the administration of sulfadoxine-pyrimethamine (SP), as well as the seasonal malaria chemoprevention (SMC) program, using sulfadoxine-pyrimethamine and amodiaquine (SP + AQ) (WHO, 2015b).

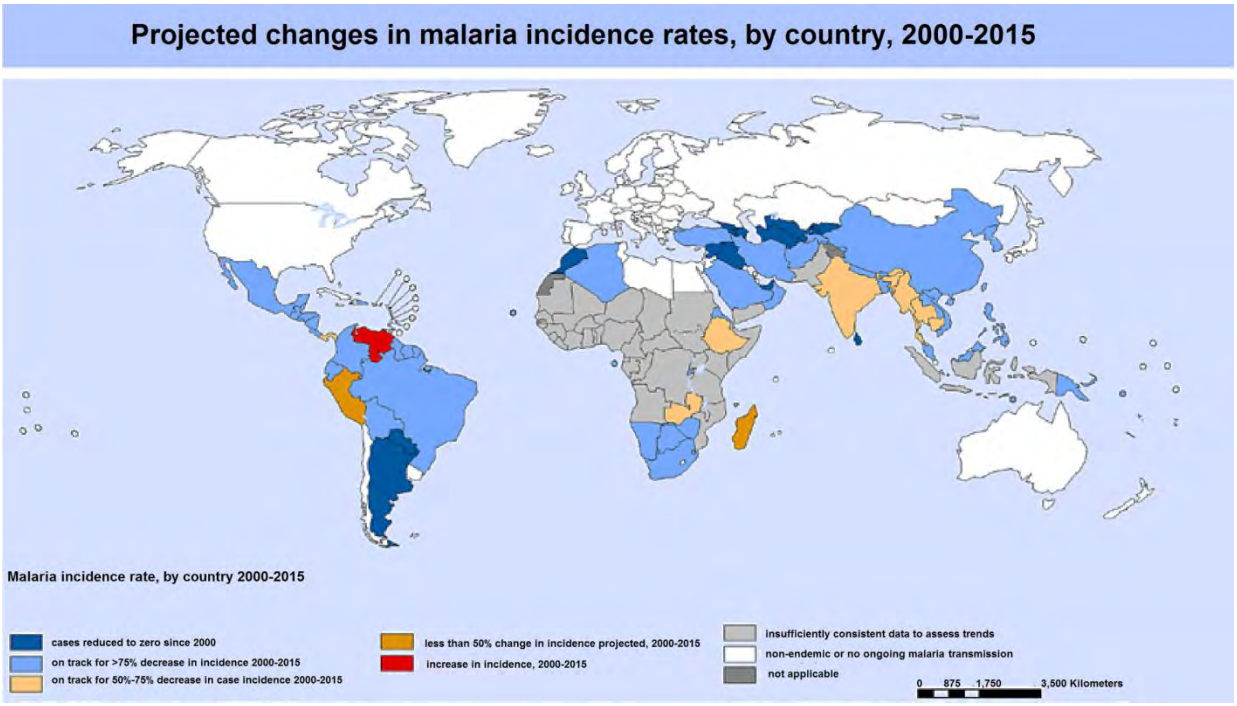
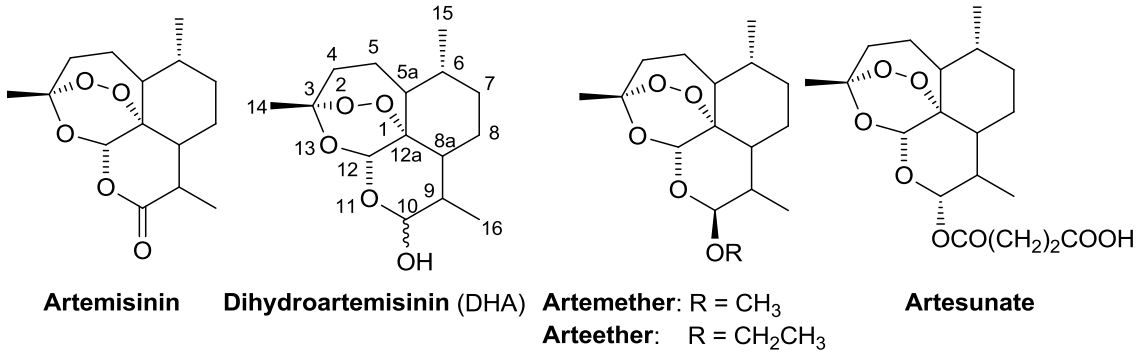


Figure 1.1: Global malaria prevalence map. The map shows the sparsity of global malaria prevalence as well as current progress towards malaria reduction in order to achieve the Millennium Development Goal (MDG) 6 which is "to have halted and begun to reverse the incidence of malaria" (Target 6C) (WHO, 2015b).

Currently, artemisinins (Figure 1.2) are the cornerstone of curative malaria chemotherapy. They are quick acting antimalarial drugs, whose efficacy is exerted at low concentrations, and they are effective against all asexual stages of the parasite with the ability to rapidly reduce the parasite burden thereby offering relief from symptoms (Haynes and Krishna, 2004, Krishna *et al.*, 2004, Woodrow *et al.*, 2005, Cheng *et al.*, 2012).



Artemisinin **Dihydroartemisinin** (DHA) **Artemether**: R = CH_3 **Artesunate**
Arteether: R = CH_2CH_3

Figure 1.2: Chemical structures of artemisinin and its clinically used derivatives

Artemisinin-based combination therapy (ACT) is widely used as first line treatment for uncomplicated malaria, as a means to address parasite recrudescence, which commonly occurs with artemisinins monotherapy as a consequence of their short pharmacological half-lives (Das *et al.*, 2013, Haynes and Krishna, 2004). An ACT encompasses an artemisinin and a longer-acting antimalarial drug from another class. ACT administration ensures that continued chemotherapy is carried out by the partner drug, once the artemisinin concentration falls below therapeutic levels (Haynes *et al.*, 2007a). Current clinical ACT regimens include artemether-lumefantrine (AL), artesunate-amodiaquine (AS/AQ), artesunate-mefloquine (AS/MQ), artesunate-chlorproguanil-dapsone (AS/CD) and artesunate-sulphadoxine-pyrimethamine (AS/SP) (Yakasai *et al.*, 2015). ACT also aids in reducing the risk of drug tolerance (Woodrow *et al.*, 2005), as well as in delaying the development of drug resistance (Dondorp & Ringwald, 2013; Klein, 2013; WHO, 2013). This approach furthermore ensures early and effective treatment of uncomplicated malaria, in order to prevent serious complications and death, as consequences of severe malaria. This method of treatment also plays a role in the malaria control and elimination programs (Cheng *et al.*, 2012).

However, the emergence of parasite resistance to artemisinins puts strain on recent progress, as well as on the malaria elimination goals. While resistance has only been reported in Greater Mekong Sub-region (WHO, 2015a), its threat is amplified by the lack of better or alternative antimalarial drugs, the instability and neurotoxicity of artemisinins (Franco-Paredes *et al.*, 2005, Starzengruber *et al.*, 2012, Van Neck *et al.*, 2007). Instability is instigated by the hemiacetal nature of clinical artemisinins, in the form of alkyl acetals and an ester, which is necessary since it offers improvement in drug plasma levels and drug efficacies (Kamchonwongpaisan and Meshnick, 1996). However, the derivatives undergo enzymatic oxidative dealkylation *in vivo* and are easily metabolised to DHA (Haynes *et al.*, 2002, Singh *et al.*, 2012). DHA is associated with neurotoxicity (Schmuck and Haynes, 2000), which consequentially renders the clinical derivatives neurotoxic as well.

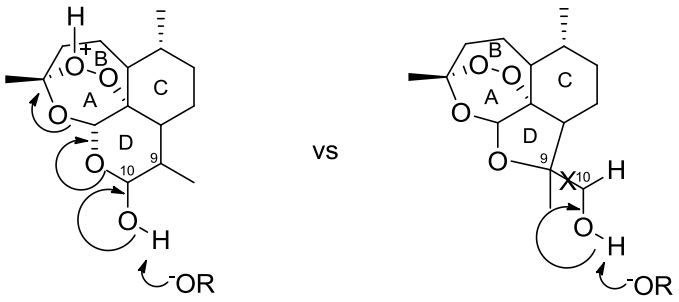
Most reports on artemisinins induced neurotoxicity stem from studies on laboratory animals, exposed to the drugs for extended periods of time and at high dosages (Efferth and Kaina, 2010, Gordi and Lepist, 2004). However, in the wake of resistance, the issue of neurotoxicity in humans becomes a concern, since strategies to maintain artemisinin efficacy may involve increased artemisinin concentrations, or increased dosages (Das *et al.*, 2013).

Although current clinical derivatives are either hydrolytically or metabolically unstable, and together with the principal metabolite, DHA, elicit neurotoxicity, artemisinins are still the

drugs of choice for the treatment of malaria, so long as there are no alternatives. Additionally, thermal instability of artemisinins is an important parameter to consider, since malaria is endemic in tropical areas. Thermal stability plays a crucial role in drug dosage, as the decomposition of active ingredients leads to the administration of sub-therapeutic regimens and thus contributes towards the development of drug resistance (Haynes *et al.*, 2007b).

Moreover, artemisinins have also been explored as anticancer agents *in vitro* and in animal studies. Much like their antimalarial therapy, the endoperoxide is believed to be responsible for the anticancer activity of artemisinins. This results from the formation of cytotoxic free radicals, which are generated upon reaction of the endoperoxide with intracellular ferrous iron. The free radicals then cause growth inhibition through oxidative stress and ultimately apoptosis of cancer cells (Das, 2015, Lai *et al.*, 2013).

In summary, artemisinins are effective and efficacious antimalarial drugs. However, instability, neurotoxicity, as well as resistance place urgency on the need to develop new and better drugs with similar, or improved antimalarial activity, which are safe and well-tolerable for human use. These drugs should also be stable enough to elicit therapeutic concentrations. A strategy towards the development of such compounds includes the truncation of the 6-membered D-ring (Figure 1.3) of the artemisinin scaffold into a more robust 5-membered tetrahydrofuran ring. This will enable the replacement of the labile hemiacetal and ultimately lead to derivatives that would not be metabolised to DHA *in vivo*, consequently addressing the neurotoxicity concern of current artemisinins.



1 **2**

Figure 1.3: Ring opening of DHA *versus* the truncated DHA that cannot undergo ring opening (Haynes *et al.*, 2007a)

1.2. Aim and Objectives of the study

The aim of this study was to investigate novel, non-hemiacetal ester derivatives of artemisinin, with the ultimate goal of delivering efficacious antimalarial compounds with improved stability and safety profile.

In order to achieve this aim, the following objectives were set:

- Synthesis of novel, non-hemiacetal esters with truncated artemisinin scaffolds and their characterisation, using IR (infrared spectroscopy), NMR (nuclear magnetic resonance spectroscopy) and MS (mass spectrometry).
- Evaluate *in vitro* antimalarial activity of the synthesised esters against chloroquine sensitive (CQS) and chloroquine resistant (CQR) strains of intraerythrocytic *P. falciparum* parasites.
- Evaluate *in vitro* cytotoxicity of the synthesised compounds against mammalian cell lines.
- Evaluate *in vitro* anticancer activity of the esters against TK10 (renal), UACC62 (melanoma) and MCF7 (breast) cancer cells.

REFERENCES

Cheng, Q., Kyle, D. E. & Gatton, M. L., 2012. Artemisinin resistance in Plasmodium falciparum: A process linked to dormancy? *International Journal for Parasitology: Drugs and Drug Resistance*, 2, 249-255.

Das, A. K., 2015. Anticancer Effect of AntiMalarial Artemisinin Compounds. Annals of Medical and Health Sciences Research, 5, 93-102.

Das, D., Tripura, R., Phyo, A. P., Lwin, K. M., Tarning, J., Lee, S. J., Hanpithakpong, W., Stepniewska, K., Menard, D., Ringwald, P., Silamut, K., Imwong, M., Chotivanich, K., Yi, P., Day, N. P., Lindegardh, N., Socheat, D., Nguon, C., White, N. J., Nosten, F. & Dondorp, A. M., 2013. Effect of high-dose or split-dose artesunate on parasite clearance in artemisinin-resistant falciparum malaria. *Clinical Infectious Diseases*, 56, e48-58.

Dondorp, A. M. & Ringwald, P., 2013. Artemisinin resistance is a clear and present danger. *Trends in Parasitology*, 29, 359-60.

Efferth, T. & Kaina, B., 2010. Toxicity of the antimalarial artemisinin and its dervatives. *Critical Reviews in Toxicology*, 40, 405-21.

Franco-Paredes, C., Dismukes, R., Nicolls, D. & Kozarsky, P. E., 2005. Neurotoxicity due to antimalarial therapy associated with misdiagnosis of malaria. *Clinical Infectious Diseases*, 40, 1710-1.

Gordi, T. & Lepist, E. I., 2004. Artemisinin derivatives: toxic for laboratory animals, safe for humans? *Toxicology Letters*, 147, 99-107.

Haynes, Richard K., Chan, H.-W., Cheung, M.-K., Lam, W.-L., Soo, M.-K., Tsang, H.-W., Voerste, A. & Williams, Ian D., 2002. C-10 Ester and Ether Derivatives of Dihydroartemisinin − 10-α Artesunate, Preparation of Authentic 10-β Artesunate, and of Other Ester and Ether Derivatives Bearing Potential Aromatic Intercalating Groups at C-10. *European Journal of Organic Chemistry*, 2002, 113-132.

Haynes, R. K., Chan, H. W., Lung, C. M., Ng, N. C., Wong, H. N., Shek, L. Y., Williams, I. D., Cartwright, A. & Gomes, M. F., 2007a. Artesunate and dihydroartemisinin (DHA): unusual decomposition products formed under mild conditions and comments on the fitness of DHA as an antimalarial drug. *ChemMedChem*, 2, 1448-63.

Haynes, R. K. & Krishna, S., 2004. Artemisinins: activities and actions. *Microbes and Infection*, 6, 1339-46.

Haynes, R. K., Wong, H. N., Lee, K. W., Lung, C. M., Shek, L. Y., Williams, I. D., Croft, S. L., Vivas, L., Rattray, L., Stewart, L., Wong, V. K. & Ko, B. C., 2007b. Preparation of N-sulfonyl- and N-carbonyl-11-azaartemisinins with greatly enhanced thermal stabilities: in vitro antimalarial activities. *ChemMedChem,* 2, 1464-79.

Kamchonwongpaisan, S. & Meshnick, S. R., 1996. The mode of action of the antimalarial artemisinin and its derivatives. *General Pharmacology: The Vascular System*, 27, 587-592.

Klein, E. Y., 2013. Antimalarial drug resistance: a review of the biology and strategies to delay emergence and spread. *International Journal of Antimicrobial Agents*, 41, 311-7.

Krishna, S., Uhlemann, A. C. & Haynes, R. K., 2004. Artemisinins: mechanisms of action and potential for resistance. *Drug Resistance Updates*, 7, 233-44.

Lai, H. C., Singh, N. P. & Sasaki, T., 2013. Development of artemisinin compounds for cancer treatment. Invest New Drugs, 31, 230-46.

Murray, C. J. L., Ortblad, K. F., Guinovart, C., Lim, S. S., Wolock, T. M., Roberts, D. A., Dansereau, E. A., Graetz, N., Barber, R. M., Brown, J. C., Wang, H., Duber, H. C., Naghavi, M., Dicker, D., *et al.*, 2014. Global, regional, and national incidence and mortality for HIV, tuberculosis, and malaria during 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013. *The Lancet*, 384, 1005-1070.

Pawluk, S. A., Wilby, K. J. & Ensom, M. H., 2013. Pharmacokinetic profile of artemisinin derivatives and companion drugs used in artemisinin-based combination therapies for the treatment of Plasmodium falciparum malaria in children. *Clinical Pharmacokinetics*, 52, 153-67.

Schmuck, G. & Haynes, R. K., 2000. Establishment of an in vitro screening model for neurodegeneration induced by antimalarial drugs of the artemisinin-type. *Neurotoxicity Research*, 2, 37-49.

Singh, C., Kanchan, R., Chaudhary, S. & Puri, S. K., 2012. Linker-based hemisuccinate derivatives of artemisinin: synthesis and antimalarial assessment against multidrug-resistant Plasmodium yoelii nigeriensis in mice. *Journal of Medicinal Chemistry*, 55, 1117-26.

Starzengruber, P., Swoboda, P., Fuehrer, H. P., Khan, W. A., Hofecker, V., Siedl, A., Fally, M., Graf, O., Teja-Isavadharm, P., Haque, R., Ringwald, P. & Noedl, H., 2012. Current status of artemisinin-resistant falciparum malaria in South Asia: a randomized controlled artesunate monotherapy trial in Bangladesh. *PLoS One*, 7, e52236.

Van Neck, T., Van Mierloo, S. & Dehaen, W., 2007. Functionalisation of artemisinin and its ring-contracted derivatives. *Molecules*, 12, 395-405.

World Health Organisation, 2013. Emergency Response to Artemisinin Resistance in the Greater Mekong Sub Region. Regional Framework For Action 2013-2015. Geneva Switzerland.

www.who.int/iris/bitstream/10665/79940/1/9789241505321_eng.pdf?ua=1 (access date: 28 August 2015).

World Health Organisation, 2015a. Strategy for Malaria Elimination in the Greater Mekong Subregion (2015-2030). http://iris.wpro.who.int/bitstream/handle/10665.1/10945/9789290617181_eng.pdf?sequence=1 (accessed date 30 January 2016).

World Health Organisation, 2015b. World Malaria Report 2015. apps.who.int/iris/bitstream/10665/200018/1/9789241565158_eng.pdf?ua=1 (accessed date 30 January 2016).

Woodrow, C. J., Haynes, R. K. & Krishna, S., 2005. Artemisinins. *Postgraduate Medical Journal*, 81, 71-8.

Yakasai, A. M., Hamza, M., Dalhat, M. M., Bello, M., Gadanya, M. A., Yaqub, Z. M., Ibrahim, D. A. & Hassan-Hanga, F., 2015. Adherence to Artemisinin-Based Combination Therapy for the Treatment of Uncomplicated Malaria: A Systematic Review and Meta-Analysis. *Journal of Tropical Medicine*, 2015, 189232.

CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

Malaria continues to plague our modern society with high rates of morbidity and mortality. The disease remains endemic in 96 countries and mainly occurs in Sub-Saharan Africa, South-East Asia and the Eastern Mediterranean. In 2014, an estimated 214 million cases of malaria, with 438 000 deaths (WHO, 2015a) were reported worldwide. Malaria control and its eradication remain the critical and ultimate goals. The importance of these goals is imposed by the fact that, while malaria is a health issue, it also greatly influences social development on a global scale, since it mostly affects the poorest communities with limited access to resources, and as such its elimination would be an immense achievement towards addressing seven of the Millennium Development Goals (MDGs). These MDGs include halting and reversing malaria incidences, reducing the mortality rate of children under the age of five and improving maternal health (WHO, 2015a).

Significant progress has been made in malaria control and elimination, as evidenced by a 60% global decline in deaths, as well as a 37% and 42% decline in malaria cases globally and in Africa, respectively. This progress can be attributed to collaborative efforts on vector control, preventive and curative chemotherapeutic measures (WHO, 2015a). While current statistics could be hailed as progressive success, the *Plasmodium* (*P.*) pathogen, particularly *P. falciparum*, has always seemed to find a way to hinder efforts towards its elimination through the development of resistance to antimalarial drugs.

2.2 Life cycle and pathogenesis

The eukaryotic *Plasmodium* parasite has a very complex life cycle (Figure 2.1), comprising of an asexual phase that occurs in the vertebrate (human) and a sexual phase that takes place in the female *Anopheles* mosquito (Opsenica and Šolaja, 2012, WHO, 2012b). Malaria infection of the human host is initiated by an infected female *Anopheles* mosquito during its blood meal. The mosquito injects sporozoites, into the human host's bloodstream through its saliva, in a process known as the pre-erythrocytic, or exoerythrocytic stage. The sporozoites are transferred into the liver and invade the hepatocytes, where they multiply and become enlarged to form schizonts. Each schizont fragments into a number of smaller cells, called

the merozoites, which continue to multiply in the hepatocyte until it ruptures, releasing the merozoites into the bloodstream, where they invade the erythrocytes to signal the start of the erythrocytic schizogony stage (Cowman and Crabb, 2006, Sherman, 1979). Once in the erythrocytes, the parasite encapsulates itself within the parasitophorous vacuole, known as the ring stage (Cowman and Crabb, 2006). In the ring stage, the parasites develop into trophozoites that catabolise the red blood cells' cytoplasm, which contains about 95% of haemoglobin (Francis *et al.*, 1997, Goldberg *et al.*, 1990). Haemoglobin catabolism is a process during which amino acids are generated, and oxygen radicals and haem are released. The process is synonymous with parasite growth, that is, as the parasite matures, there is an increased demand for and consumption of haemoglobin (Dhangadamajhi *et al.*, 2010, Foley and Tilley, 1998). Some of the immature ring stage trophozoites, instead of continuing the asexual cycle of the parasite, initiate the sexual cycle by developing into male (microgametocyte) and female (macrogametocyte) gametocytes that circulate in the human host's bloodstream until they are ingested by a mosquito when it takes a blood meal. The sexual stage continues within the mosquito when the human blood cells rupture and release the gametocytes. Gametocytes develop into male or female gametes, which fuse to form diploid zygotes that develop into ookinetes, which then develop into oocysts in the mosquito's midgut, where they grow and develop into sporozoites that will be injected into a human host when the infected mosquito feeds on a human, starting the cycle anew (Wells and Poll, 2010).

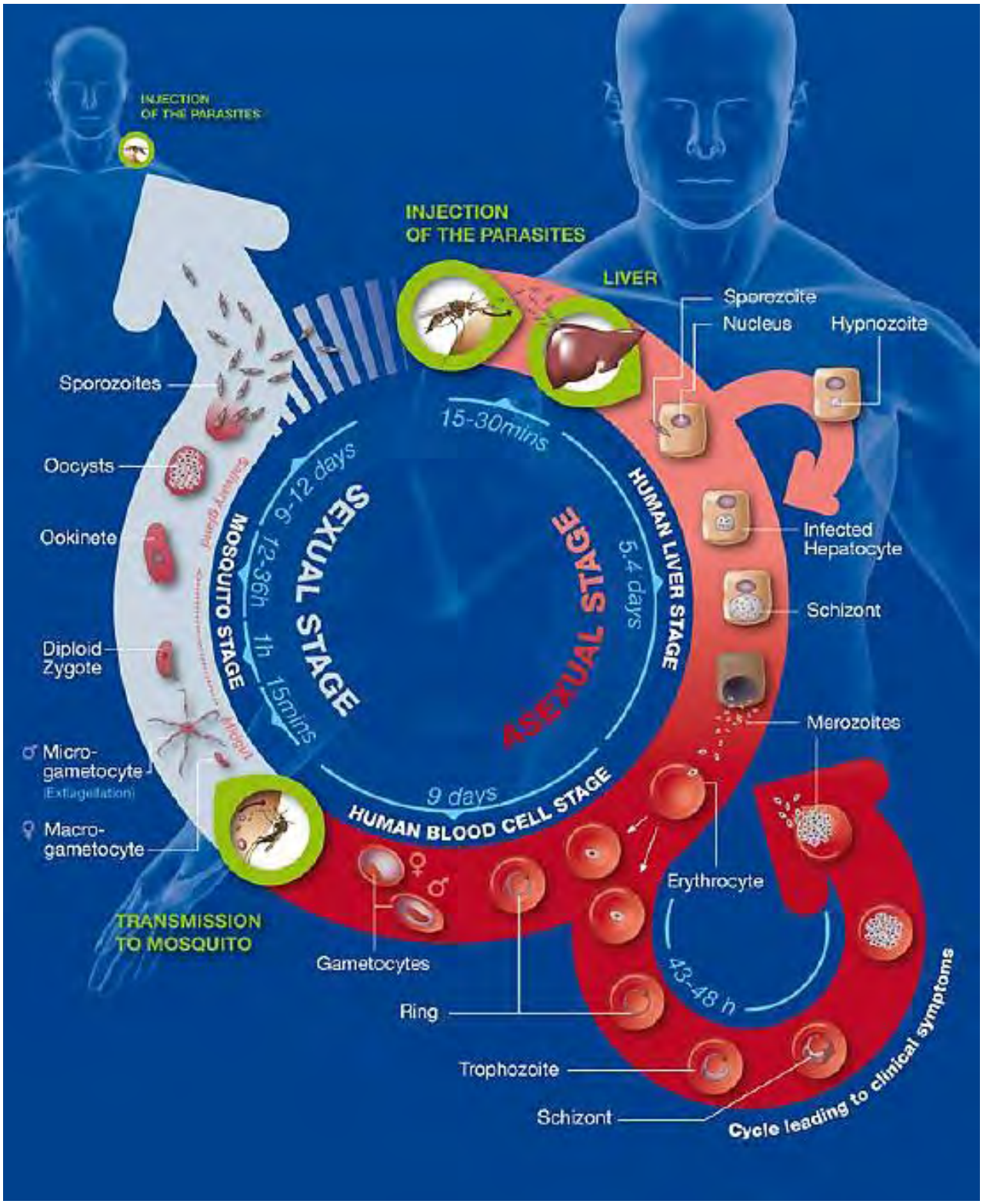


Figure 2.1: Full life cycle of the Plasmodium parasite, showing both the cycle that occurs in the mosquito and the cycle that occurs in the human body (Wells and Poll, 2010).

P. falciparum infections can be divided into two categories, namely uncomplicated malaria and complicated, or severe malaria. Uncomplicated malaria is not life threatening, but it must be treated and cured in order to avoid further complications, or progress into severe malaria

(Dalrymple, 2012). Progression from uncomplicated to complicated malaria may be the result of missed or delayed diagnosis (Pasvol, 2005). The main contributing factor to this is that the symptoms of uncomplicated malaria are similar to those of a wide variety of diseases. The catastrophic results therefore not only end in the progression of the disease, but also contribute towards other problems, such as over diagnosis and careless/unnecessary treatments, which may ultimately lead to problems, such as drug resistance, which will be discussed at length later in this chapter (Dalrymple, 2012).

The host responds to malaria infection by augmenting splenic immune function, thus parasite clearance (White *et al.*, 2014; del Portillo *et al.*, 2012). The spleen has phagocytic and cellular immune functions. It actively removes infected erythrocyte by-products that result from the rupture of the schizonts and those opsonised by immunoglobulin. Moreover, the spleen has the ability to extract *Plasmodium* parasites from young infected erythrocytes through pitting (Urban *et al.*, 2005, White *et al.*, 2014). *P. falciparum* has the ability to evade the immune system's pathogen destruction mechanisms, such as spleen-dependent immune mechanisms (Carvalho *et al.*, 2013, Ho and White, 1999, Magowan *et al.*, 1988). This occurs in cases of complicated malaria, where the parasite promotes its own survival through cytoadherence, also known as erythrocyte sequestration. It should be noted that erythrocyte sequestration is associated with high fatality, since it enables the parasite riddled erythrocytes to adhere onto the endothelium cells, thus protecting the parasite from being identified as a pathogen and being removed by the spleen (Carvalho *et al.*, 2013, Ho and White, 1999, Magowan *et al.*, 1988). This results in the disturbance of microcirculation, leading to complications, such as oxygen deficiency in the tissues, metabolic disturbances and multiple organ failure. The distribution of erythrocyte sequestration is indicated as a symptom of key clinical illness. In comatose patients, suffering from cerebral malaria, high erythrocyte sequestration is present in the cerebrum (Carvalho *et al.*, 2013, Ho and White, 1999).

Human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) and tuberculosis (TB) may also facilitate malaria complication, because victims have a compromised immune system (Sanyaolu *et al.*, 2013, Ter Kuile *et al.*, 2004). HIV-malaria co-infections can cause an increase in clinical attacks of malaria and higher parasite densities. Moreover, acute malaria infection can increase the HIV burden in HIV positive individuals. There is also evidence that non-immune malaria patients, co-infected with HIV, have a higher risk of severe malaria and malaria related mortality (Hogan, 2009).

2.3 Signs and symptoms

The first symptoms of malaria are non-specific and may be representative of a wide number of diseases. These symptoms include fever, diarrhoea, headaches and chills, to name a few (White *et al.*, 2014). Some affected areas of the body, as well as the manifestations thereof are highlighted in Figure 2.2.

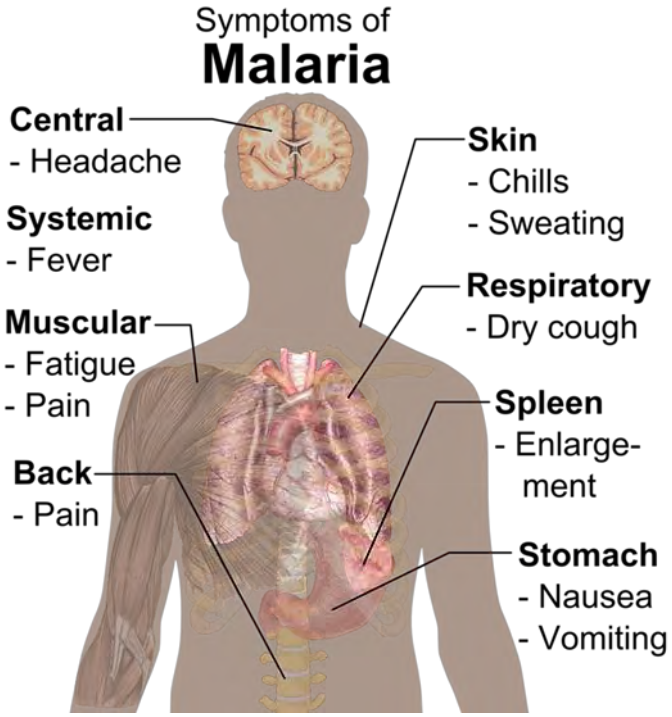


Figure 2.2: Symptoms of malaria and the affected areas of the body (Bouc, 2013)

Complicated malaria symptoms include drifting in and out of consciousness, convulsions, decreased urinary output, respiratory distress and abnormal bleeding. Severe complications may include coma, convulsions, metabolic acidosis, hypoglycaemia, renal failure, secondary infections, bleeding disorders and anaemia. Acute anaemia, in highly malaria endemic areas, occurs in children under 2 years of age and has the potential to develop into cerebral malaria later on (Pasvol, 2005). Cerebral infection is the most severe and deadliest form of malaria. Therefore, its onset should be monitored and anticipated, especially among patients presenting complicated malaria-like symptoms (Pasvol, 2005). As a result, the WHO recommends that all forms of severe, or complicated malaria be treated through parenteral administration of medicines, because patients with such symptoms may be unable to take oral drugs and oral administration may not give the required dose, since nausea and vomiting may occur (Pasvol, 2005).

2.4 Diagnosis

Prompt and accurate diagnosis of malaria is very crucial in ensuring timeous treatment to effectively prevent further spread of infection in the community. Malaria infection can be suspected, based on the patient's travel history and symptoms, but confirmation must be obtained through correct diagnosis. As a way of ensuring accurate diagnosis, it is important to obtain a complete blood count and a routine chemistry panel. This information is vital in determining whether the patient has complicated or uncomplicated malaria infection, which in turn will inform of the proper method of treatment. The importance of routine chemistry panel tests cannot be overstated, since these tests can detect renal failure, hypoglycaemia, severe anaemia, hyperbilirubinemia and acid-base disturbances, which are all consequences of malaria infection (Tangpukdee *et al.*, 2009).

Malaria diagnostic methods include microscopic and molecular diagnoses, as well as antigen detection. Microscopic diagnosis is achieved by smearing a drop of a patient's blood, stained with Giemsa, on a slide, which is then examined. Antigen detection tests, also known as Rapid Diagnostic Tests (RDTs), are designed to provide results in about 15 minutes. This diagnostic method provides a faster alternative to microscopic diagnosis, but it is costly and requires some improvement in accuracy (Tangpukdee *et al.*, 2009). Molecular diagnosis on the other hand, involves polymerase chain reaction (PCR) to detect the parasites' nucleic acids. This method is more sensitive than the other methods and is used to confirm the *Plasmodium* species (Tangpukdee *et al.*, 2009).

2.5 Prevention and control

In the bid to ultimately eliminate malaria, preventive and curative measures are employed. Vector control, barriers to transmission and chemoprevention are used as preventive measures (AlKadi, 2007, WHO, 2014). Vector control, which is facilitated by the use of pesticides, seeks to reduce/disrupt mosquito breeding. Indoor residual spraying (IRS), which involves coating the walls and other surfaces of the house with residual pesticides, is an example of an effective vector control measure. The pesticide kills mosquitos that come in contact with these surfaces. While this method does not prevent mosquito bites and infection, it prevents transmission (White *et al.*, 2014). Barriers to transmission include the use of insecticide-treated bed nets (ITNs) and the application of mosquito repellent on the skin (Dalrymple, 2012). These interventions provide protection, are cost effective, easy to use and require less technical outlay to implement (Binka and Akweongo, 2006). ITNs offer a crucial and effective method of controlling transmission (WHO, 2015a). Chemoprevention,

through intermittent preventative treatment (IPT) and seasonal malaria chemoprevention (SMC), is aimed at addressing causal effects, as well as taking advantage of the therapeutic effects of prophylactic drugs. Intermittent preventive treatment in pregnancy (IPTp) is the administration of sulfadoxine-pyrimethamine (SP) during the second and third trimester of pregnancy. SMC is the administration of amodiaquine (AQ) and sulfadoxine-pyrimethamine (SP) to children, aged 3 - 59 months, during high transmission seasons, thus ensuring therapeutic levels of the antimalarial drug in the body during peak transmission seasons. The result is a reduction in both mortality and morbidity cases (WHO, 2015a).

2.6 Chemotherapy

Chemotherapy is vital in the efforts aimed at malaria eradication. While no ideal drug for the treatment of this disease currently exists, there are three classes of drugs used for curative treatment, namely quinolines, antifolates and artemisinins, which offer varying efficacy and safety profiles (Dalrymple, 2012).

2.6.1 Quinolines and related drugs

Quinolines are amongst the oldest antimalarials to date. They occur as both natural and synthetic compounds which can be grouped/sub-divided into 4-aminoquinolines, 8-aminoquinolines and aryl aminoalcohols, also known as cinchona alkaloids or quinolines (Kakkilaya, 2015). These drugs are known to mostly act during the blood stages of the parasite's life cycle (Dalrymple, 2012, Kaur *et al.*, 2010). Additionally, some quinolines such as quinine and chloroquine, are considered safe for use during pregnancy (Foley and Tilley, 1998, Achan *et al.*, 2011). Most of these quinolines continue to be used as partner drugs in ACT (artemisinin combination therapy) for malaria treatment despite the widespread parasite resistance against them.

2.6.1.1 4-Aminoquinolines

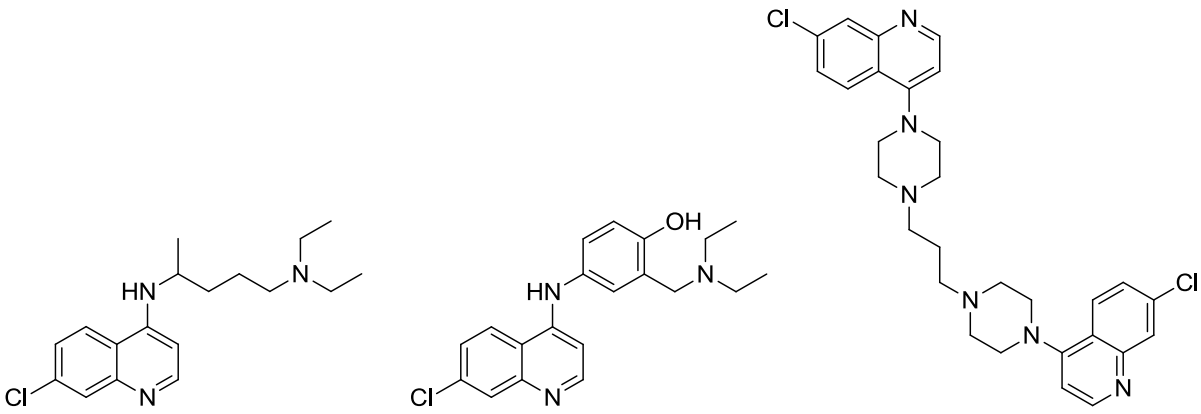
4-Aminoquinolines are selective antimalarial drugs that accumulate in the parasite's digestive vacuole to interfere with haemoglobin metabolism. They act by inhibiting the parasite from detoxifying haem, a haemoglobin by-product, which is toxic to the parasite (O'Neill *et al.*, 1998). The parasite polymerises haem into hemozoin, an insoluble crystalline pigment, thereby avoiding the toxicity of haem (Pandey *et al.*, 2003, Goldberg *et al.*, 1991). However, because these drugs have a high affinity for melanin rich cells and tissues, they

are associated with ocular toxicity at high cumulative concentrations, which may result in retinopathy (Foley and Tilley, 1998, O'Neill *et al.*, 2003). Additionally, these drugs are contraindicated for individuals who have liver and kidney dysfunction, since they accumulate in these tissues and may result in toxicity (O'Neill *et al.*, 2003). Most important drugs in this group include chloroquine, amodiaquine and piperaquine.

Chloroquine (CQ) is a synthetic 4-aminoquinoline which replaced quinine, because of its affordability and efficacy, as well as its safety profile, making it ideal for use during pregnancy (Petersen *et al.*, 2011). Additionally, CQ (**1**) has a 70 - 80% oral bioavailability, however its elimination half-life of 30 - 60 days (Krishna and White, 1996 , Petersen *et al.*, 2011), initially an advantage, especially in prophylaxis, led to parasite resistance. Indeed, as a result of this long half-life, the parasite remained exposed to the drug even when concentrations fell below therapeutic levels ultimately rendering the drug redundant (Foley and Tilley, 1997). Furthermore, the bitter taste played a role towards patients' non-compliance during malaria treatment, which consequently contributed to the development of CQ resistance (Foley and Tilley, 1998). The side effects following CQ administration include vomiting, rashes and itching (Foley and Tilley, 1998). The mechanism of action of CQ is not well-understood, however it is anticipated to target the metabolic processes involved in the uptake, or digestion of haemoglobin (Foley and Tilley, 1997, Foley and Tilley, 1998).

Amodiaquine (**2**) is another 4-aminoquinoline currently in clinical use. However, unlike chloroquine, it is more palatable and is associated with less itching, but has reduced activity (Foley and Tilley, 1998). Amodiaquine is active against blood schizonts and some chloroquine resistant plasmodia (O'Neill *et al.*, 1998). It is metabolised to the antiplasmodial monodesethylamodiaquine, which boosts the overall half-life of amodiaquine from 3 - 5 hours to 18 - 19 days. This phenomenon is responsible for the slow development of resistance to the drug (Petersen *et al.*, 2011). In fact, amodiaquine continues to be used in antimalarial treatment as a longer acting partner drug in ACT with artesunate (Yakasai *et al.*, 2015), as well as in chemoprevention (SMC) (WHO, 2015a). Additionally, amodiaquine has a good safety profile, although side effects, such as visual and gastro-intestinal disturbances have been reported. Furthermore, its prolonged use is associated with hepatotoxicity and agranulocytosis (Foley and Tilley, 1998, Glick, 1957, O'Neill *et al.*, 1998). Much like other quinolines, the mechanism of action is poorly understood, although amodiaquine accumulates in the digestive vacuole and inhibits haem polymerisation (Petersen *et al.*, 2011).

Piperaquine (**3**) is a bis-4-aminoquinoline with structural similarities to chloroquine, which gives piperaquine a mode of action similar to that of chloroquine. Moreover, its bulky nature is thought to be the reason for its efficacy in CQR (chloroquine resistant) strains and that it is necessary for antimalarial activity. However, the emergence of parasite resistance in China, coupled with the use of artemisinin derivatives led to the decline in its use (Davis *et al.*, 2005). However its long half-life of 5 weeks and the emergence of CQ resistance lead to its rediscovery for use as a longer acting partner drug in ACT (Petersen *et al.*, 2011, Davis *et al.*, 2005). Moreover, piperaquine is less toxic, has a better therapeutic index than CQ and is effective against *P. vivax* and CQR *P. falciparum* parasites (Davis *et al.*, 2005). As a result the drug has been recommended for use in ACT as a partner drug to DHA (dihydroartemisinin) (Kakuru *et al.*, 2016).



Chloroquine (CQ), **1** Amodiaquine, **2** Piperaquine, **3**

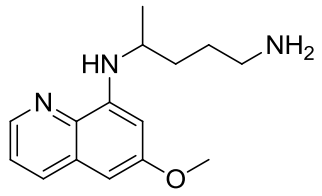
Figure 2.3: Structures of 4-aminoquinolines

2.6.1.2 8-Aminoquinolines

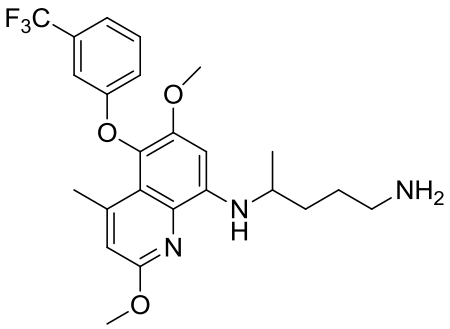
8-Aminoquinolines are a class of quinolines that are active against hypnozoites, hence their use in treatment of *P. vivax* and *P. ovale* (Foley and Tilley, 1998). Although they possess prophylactic properties, their toxicity and the high doses required make them unsuitable for this purpose (Grewal, 1981). Additionally, this class of compounds is contraindicated for G6PD deficient individuals since they cause haemolysis (Recht *et al.*, 2014). The drugs of note in this class of antimalarials include primaquine and tafenoquine. The former is used in the treatment of *P. vivax* hypnozoite liver stages (Petersen *et al.*, 2011), and the latter is currently in clinical trials for treatment of *P. falciparum* and *P. vivax* malaria (Recht *et al.*, 2014).

Primaquine (**4**) is mainly used for the treatment of *P. vivax* and *P. ovale* malaria, because of its ability to prevent relapse, commonly associated with *P. vivax.* Primaquine is a potent gametocytocide for *P. falciparum* parasites, when co-administered with a drug that has good activity against the asexual stage, which therefore blocks parasite transmission (Ashley *et al.*, 2014). The generally acceptable dose of primaquine is 15 mg over 14 days, which is then concentrated in the liver, brain, heart, lungs and skeletal muscle and can also cross the placental barrier. It has a half-life of 4 - 9 hours, with rapid absorption in the gastro-intestinal tract. Its effectiveness is increasingly noted when used in conjunction with blood schizonticides, such as quinine and chloroquine (Ashley *et al.*, 2014). Unfortunately, *P. vivax* shows tolerance against primaquine in South-East Asia and Ocenia (Baird and Hoffman, 2004). Its mechanisms of action and metabolism are poorly understood, however 8-aminoquinolines, such as primaquine, kill mature *Plasmodium* gametocytes of all species in the liver and the dormant hypnozoites of *P. vivax* and *P. ovale*. However, the drug causes haemolysis in patients with G6DP deficiency, which, in severe cases may lead to life threatening anaemia and haemoglobinuric renal failure (Ashley *et al.*, 2014, Baird and Hoffman, 2004).

Tafenoquine (**5**) is a derivative of primaquine, which possesses improved antimalarial efficacy and reduced toxicity in comparison. This 8-aminoquinoline is also active against the blood stages of the parasite. Its 14 days half-life is an additional improvement to the 6 hours of primaquine, making it an ideal drug for use in prophylaxis. Tafenoquine has both gametocytocidal and sporontocidal activities, which make it a good transmission blocking agent (Recht *et al.*, 2014). This drug is well tolerated by G6PD normal individuals, however, it may cause haemolysis in G6PD deficient patients (Deshpande, 2016, Prashar and Paul, 2009, Recht *et al.*, 2014).



Primaquine, **4**



Tafenoquine, **5**

Figure 2.4: Structures of 8-aminoquinoline antimalarials

2.6.1.3 Aryl aminoalcohols

Alkaloids consist of both natural and synthetic quinolines. The former include quinine, cinchonine, quinidine and cinchondine, which are isolated from the bark of the cinchona tree (Achan et al., 2011, Foley and Tilley, 1998), whereas the synthetic alkaloids include mefloquine, lumefantrine and halofantrine. These drugs are potent antiplasmodial agents, with quinine being the most effective and primarily used.

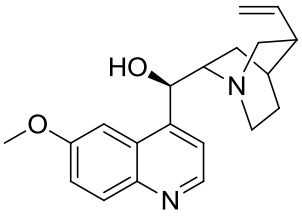
Quinine (**6**), which is mainly prepared as a salt with hydrochlorides, dihydrochlorides (the most commonly used), sulphates, bisulphates and gluconates, is active against the schizont stage of the malaria parasite as well as the gametocyte stages of *P. vivax* and *P. malariae*. Unfortunately, it does not affect the gametocyte stages of *P. falciparum* (Achan *et al.*, 2011, Foley and Tilley, 1997, Foley and Tilley, 1998). It has a half-life of between 11 - 18 hours and can cross the placental barrier. Thus, it's contraindicated in pregnancy, but remains the primary option for treatment in the case of severe malaria (Adam *et al.*, 2004). Quinine accumulates in the parasites digestive vacuole and plays a role in the detoxification of haem; its mechanism of action, however, remains poorly understood (Achan *et al.*, 2011, Petersen *et al.*, 2011). The use of quinine, initially hampered by cost and its complicated synthesis led to the use of chloroquine, which interestingly, was soon reversed as a result of chloroquine resistance. Quinine continues to be used as a second-line treatment in Africa for the treatment of uncomplicated *falciparum* malaria and as an injection in some cases of severe malaria, as well as in combination with antibiotics for the treatment of resistant malaria (Petersen *et al.*, 2011). The effectiveness of this drug is accompanied by a variety of side effects, namely tinnitus, slight hearing impairment, headaches and nausea, collectively

termed, cinchonism. The more severe side effects include diarrhoea, vomiting, abdominal pain, vertigo, loss of vision, auditory loss, venous thrombosis in intravenous administration and hypoglycaemia. Though less frequent in their occurrence, other side effects may include asthma, psychosis, thrombocytopenia, hepatic injury and skin eruptions (Achan *et al.*, 2011, Foley and Tilley, 1997, Foley and Tilley, 1998). There are only sparse reports of parasite resistance to this drug (Dalrymple, 2012, WHO, 2012a) and often refer to reduced or delayed activity and low grade quinine (Achan *et al.*, 2011).

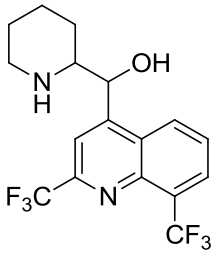
Mefloquine (**7**) is a synthetic, lipophilic aryl aminoalcohol, which was introduced to treat CQR malaria (Foley and Tilley, 1997). While it is better tolerated and has superior antimalarial activity compared to quinine, mefloquine exerts neuropsychiatric side effects, such as anxiety, seizures, depression, acute psychosis on top of cinchonism (a general side effect of quinolines, whose symptoms include blurred vision and tinnitus) (Foley and Tilley, 1998). Its ability to bind high-density lipoproteins to both infected and uninfected erythrocytes has been linked to its long half-life (Foley and Tilley, 1998). Furthermore, its long half-life (20 - 30 days) is thought to be the reason for parasite resistance against this drug, since it remains present in the blood for months at concentrations below therapeutic levels. Mefloquine is associated with intrinsic resistance in areas where it hasn't been used before, which is thought to be a result of existing quinine resistance (Foley and Tilley, 1997, Foley and Tilley, 1998). Intrinsic resistance poses a problem in that mefloquine is one of the longer acting drugs used in ACT (Na-Bangchang *et al.*, 2013). Much like other quinoline drugs, mefloquine inhibits haem detoxification. Furthermore, it inhibits the import of solutes into the parasite's digestive vacuole by interfering with *Pf*MDR1 transport (Petersen *et al.*, 2011, Foley and Tilley, 1998).

Lumefantrine (**8**) is a hydrophobic aryl aminoalcohol, with a half-life of 3 - 5 days. Its bioavailability varies between individuals. Its efficacy however, can be improved by co-ingestion with a high-fat meal (Petersen *et al.*, 2011). Lumefantrine acts by binding to ferriprotoporphyrin IX to disrupt the synthesis of hemazoin (Warhurst, 2001). This potent gametocytocidal (Makanga and Krudsood, 2009) and blood schizontocidal (Warhurst, 2001) drug plays a role in blocking transmission and is currently used as a co-drug with artemether in ACT (Makanga, 2014, Petersen *et al.*, 2011). This ACT regimen is administered for the treatment of acute uncomplicated *P. falciparum* malaria in both adults and children (Makanga and Krudsood, 2009).

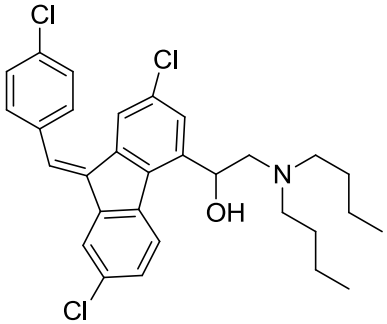
Halofantrine (**9**) is an orally administered blood schizonticide (Andersen *et al.*, 1995) that has an elimination half-life of 1 - 5 days and 3 - 7 days for its active metabolite, N-desbutylhalofantrine (Siriez *et al.*, 2012). Although the drug shows good efficacy and rapid action, adverse reactions, such as cardiotoxicity, which could potentially be fatal, hence its use only permissible under rare circumstances and after careful consideration of its contraindications (Bouchaud *et al.*, 2009). Parasite drug resistance, associated with halofantrine, has been seen in both CQR and CQS (chloroquine sensitive) *P. falciparum* strains. Intriguingly, halofantrine resistance seems to relieve CQ resistance in previously resistant parasites (Nateghpour *et al.*, 1993) through transporter mutations (Cui *et al.*, 2015, Ritchie *et al.*, 1996). These mutations alter the transport and accumulation of drugs into the parasite's digestive vacuole (Cui *et al.*, 2015, Duraisingh and Cowman, 2005, Ritchie *et al.*, 1996).



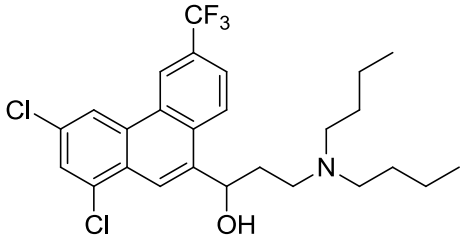
Quinine, **6**



Mefloquine, **7**



Lumefantrine, **8**



Halofantrine, **9**

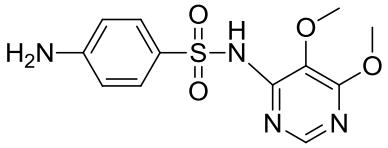
Figure 2.5: Structures of aryl aminoquinolines

2.6.2 Antifolates

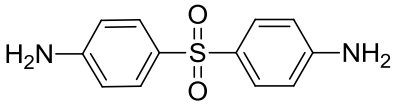
Antifolates are synthetic antimalarial drugs that exert their antimalarial activity by disrupting *de novo* folate synthesis. The disruption of folate synthesis results in reduced levels of tetrahydrofolate, which consequently results in the disruption of DNA replication (Gregson and Plowe, 2005, Hyde, 2005). These drugs can be housed under two sub-classes, namely sub-class I antifolates which are inhibitors of dihydropteroate synthase (DHPS) and sub-class II antifolates, which are inhibitors of dihydrofolate reductase (DHFR) (Nzila, 2006). Both sub-classes are used in combination in order to take advantage of their synergistic activity (Gregson and Plowe, 2005, Nzila, 2006). Sulfadoxine-pyrimethamine (SP), for example, is currently used as the mainstay of preventive chemotherapy (WHO, 2015a) and in ACT as a long acting partner drug with artesunate (ARS) (Yakasai *et al.*, 2015). However, problems of low efficacy, high toxicity, resistance and side effects, such blood dyscrasias, respiratory disorders, hepatic disorders, gastrointestinal reactions and skin lesions (Bjorkman and Phillips-Howard, 1991) limit the use of antifolates.

2.6.2.1 Sub-class I antifolates

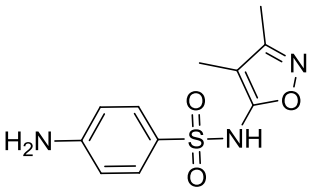
Sub-class I antifolates disrupt *de novo* folate synthesis by competing with p-aminobenzoic acid (PABA) for binding to the enzyme, dihydropteroate synthase (DHPS) (Ferone, 1977, Gregson and Plowe, 2005, Hyde, 2005, Nzila, 2006) (Figure 2.5). This class of antimalarials include sulfa drugs, with both prophylactic and therapeutic properties against malaria parasites (Bjorkman and Phillips-Howard, 1991, Nzila, 2006). Sulfa drugs of note are sulfadoxine (**10**) and dapsone (**11**), which are known for their long elimination half-lives of 200 hours and 28 hours, respectively (Dalrymple, 2012, Bjorkman and Phillips-Howard, 1991). The long half-lives of these two first generation antimalarial sulfa drugs were suspected to contribute to the development of parasite resistance, which led to the discovery and development of shorter half-life analogues, such as sulfafurazole (**12**) and sulfamethoxazole (**13**), with 6 hours and 10 hours half-lives, respectively (Bjorkman and Phillips-Howard, 1991).



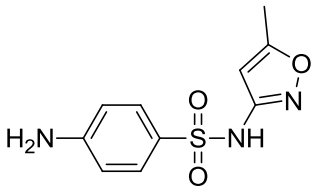
Sulfadoxine, **10**



Dapsone, **11**



Sulfafurazole, **12**



Sulfamethoxazole, **13**

Figure 2.6: Sub-class I antifolates

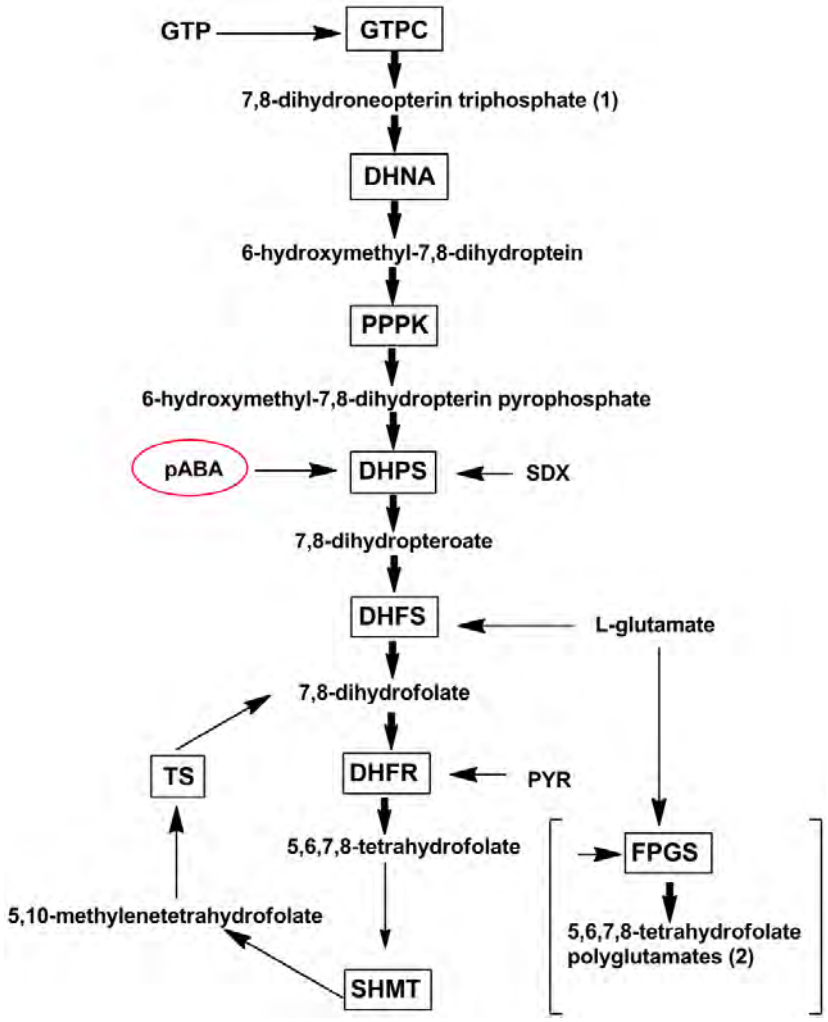
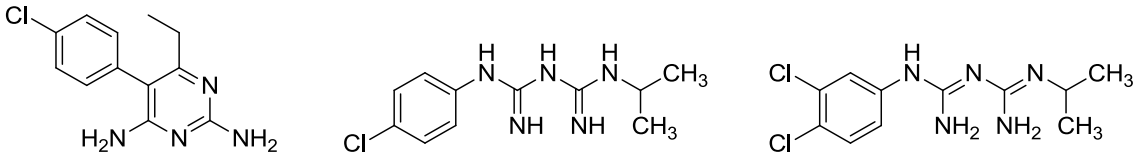


Figure 2.7: Folate metabolism pathway involving dihydropteroate synthase (DHPS) (Hyde, 2005)

2.6.2.2 Sub-class II antifolates

Sub-class II antifolates inhibit the activity of DHFR consequently disrupting both DNA replication and protein synthesis (Hastings and Sibley, 2002). Important sub-class II antifolates include pyrimethamine (**14**), proguanil (**15**) and chlorproguanil (**16**).



Pyrimethamine, **14** Proguanil, **15** Chlorproguanil, **16**

Figure 2.8: Sub-class II antifolates

Pyrimethamine (**14**) is a 2,4-diaminopyrimidine derivative that has both prophylactic and therapeutic effects with schizontocidal activity. Although, it was introduced to combat parasite drug resistance to chloroquine, the development of parasite resistance to pyrimethamine, when the drug is used in monotherapy, started soon after its introduction (Gregson and Plowe, 2005, Nzila, 2006). Therefore, its combination with sulfadoxine was introduced in order to take advantage of the synergistic effects of these two drugs (Watkins *et al.*, 1997). This combination is well tolerated and has a good safety profile, hence its use in malaria chemoprevention, with positive results, as evidenced by the decreased rates of malaria associated maternal anaemia and low birth weight (Peters *et al.*, 2007).

Proguanil (**15**) is a highly protein-bound synthetic antifolate that is metabolised in the liver into its potent dihydrofolate reductase inhibitor, chlorcycloguanil (**16**) (Baggish and Hill, 2002). The drug is then concentrated in the erythrocytes and has a half-life of 12 - 21 hours (Baggish and Hill, 2002). Proguanil on its own has weak antiplasmodial activity, hence its prophylactic use in combination with atovaquone (Kain, 2003, Looareesuwan *et al.*, 1999, Nzila, 2006, Baggish and Hill, 2002). Atovaquone acts by disrupting the normal mitochondrial function of the parasite by interfering with the electron transport chain (Cordel *et al.*, 2013). The atovaquone-proguanil (AP) combination is used for the treatment of uncomplicated *P. falciparum* in travellers (Baggish and Hill, 2002, Cordel *et al.*, 2013) and as an alternative treatment in areas where artemisinin resistance occurs (Baggish and Hill, 2002, Khositnithikul *et al.*, 2008). Adverse reactions reported for this combination include

nausea, vomiting, skin disorders, headaches and in some cases confusion (Cordel *et al.*, 2013). Additionally, AP treatment failure, mainly reported in Africa, is linked to resistance to atovaquone, which is associated with the *P. falciparum* mitochondrial *cytb* gene (Khositnithikul *et al.*, 2008). This would make the targeting of this gene a rationale strategy for the development of new atovaquone derivatives.

2.6.3 Artemisinins

Artemisinin (**17**) is a sesquiterpene lactone peroxide, extracted from sweet wormwood (*Artemisia annua L*. or *A. annua*) of the genus *Artemisia* (Petersen *et al.*, 2011). Since the rediscovery of artemisinin as an antimalarial drug in 1971 (Dalrymple, 2012, Meshnick *et al.*, 1996, Woodrow *et al.*, 2005), a variety of lipophilic and hydrophilic, semi-synthetic artemisinin derivatives have been synthesised, mainly due to its poor solubility in both oil and water (Petersen *et al.*, 2011). The insolubility of artemisinin leads to problems with administration and absorption of the drug, which ultimately impacts on its bioavailability. The oil-soluble artemisinin antimalarials, namely DHA (**18**), artemether (**19**) and arteether (**20**), show improved potency to the parent drug and are administered as oral, parenteral and rectal formulations (de Vries and Dien, 1996, White *et al.*, 2014). Artesunate (**21**), a hydrophilic hemisuccinate artemisinin derivative, is formulated as a sodium salt. It is the most versatile of all artemisinin derivatives in clinical use, since it is used for the treatment of both uncomplicated and severe malaria and can be administered intravenously, orally, intramuscularly and rectally (Morris *et al.*, 2011).

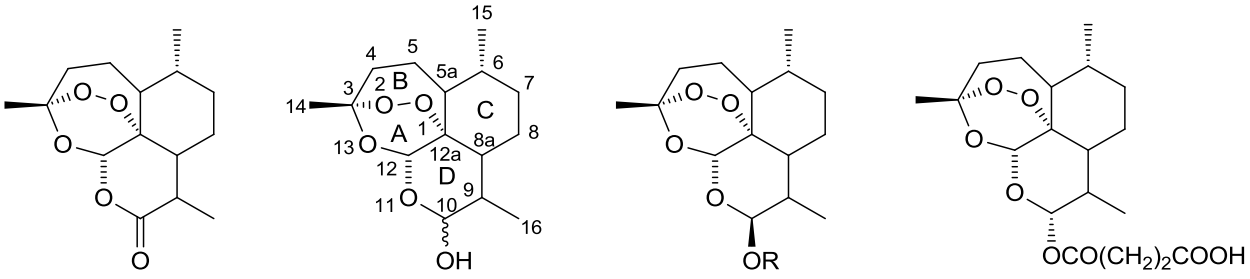


Figure 2.9: Artemisinin (**17**) and its clinically used derivatives.

Artemisinins rapidly clear parasite burden at nanomolar concentrations making their quick action notably better than that of other antimalarial drugs (Krishna *et al.*, 2004, Meshnick *et al.*, 1996). They kill all *Plasmodium* parasites that infect humans by exerting their

chemotherapeutic effect against a wide range of the parasite life cycle stages. Artemisinins are highly active against asexual and late ring stages of the parasite, trophozoites, as well as gametocytes. Unfortunately, their activity does not affect the liver stages of *P. vivax* and *P. falciparum* (Meshnick *et al.*, 1996, Woodrow *et al.*, 2005). Their activity also arrests the early stages of parasite development in erythrocytes (Haynes and Krishna, 2004, Krishna *et al.*, 2004, Woodrow *et al.*, 2005). Artemisinins arrest gametocyte development and remove ring stage parasites from erythrocytes, thus decreasing circulating parasite burden at a faster pace. Advantageously, this ability potentially reduces transmission and contributes towards malaria control programmes (Dalrymple, 2012, Krishna *et al.*, 2004).

Considering erythrocyte sequestration, artemisinins are currently the only antimalarials with the ability to effectively inhibit cytoadherence (Ho and White, 1999, Krishna *et al.*, 2004). Erythrocyte sequestration, which is characteristic of *P. falciparum* infections, is the adherence of infected erythrocytes containing trophozoites and schizonts to the endothelium of capillaries and venules. Cytoadherence enables the parasite to evade spleen-dependent immune responses. Additionally, in cerebral malaria, it has the ability to obstruct blood flow (MacPherson *et al.*, 1985, Magowan *et al.*, 1988, Storm and Craig, 2014). Therefore inhibition of cytoadherence is a lifesaving phenomenon (Magowan *et al.*, 1988).

There are still ongoing debates about the mechanism of action of artemisinins, however, what is clearly known is that the endoperoxide bridge is responsible for their antimalarial activity. This is supported by the lack of antimalarial activity in deoxyartemisinins (Wang *et al.*, 2010) and the potency of other peroxide containing compounds that are structurally different from artemisinins (Charman *et al.*, 2011). A number of hypotheses have been suggested for the mechanism of action of artemisinins. These are either widely accepted or justified by consequences of the use of artemisinins with the consensus about the formation of reactive oxygen species (ROS).

The first suggestion is that artemisinins target *Plasmodium* mitochondria. The peroxide bridge is activated by the electron transport chain to generate free radicals, which then cause damage to the mitochondrial membrane through swelling, thus disrupting the normal function of the mitochondria. Additionally, oxidative stress renders the outer mitochondrial membrane permeable, which results in the release of reactive oxygen species into the cytoplasm, ultimately causing cell death (Schmuck *et al.*, 2002). In fact, this hypothesis has been linked to neurotoxicity of artemisinins (Schmuck *et al.*, 2002).

The second hypothesis is the interaction between artemisinin and ferrous ion, which leads to conformational changes in *Pf*ATP6, causing inaccessibility of the calcium binding site and hence the loss of function of the parasite's Ca^{2+} pump and its subsequent death (Eckstein-Ludwig *et al.*, 2003).

A third suggestion is the reaction of artemisinins with haem ferrous ion (Fe^{2+}) to form highly reactive free radicals, which are subsequently rearranged to more stable, carbon centred radicals. The free radicals work to modify and inhibit the parasite's metabolism, thus killing the parasite. This hypothesis may appear plausible, since the *Plasmodium* parasite is rich in haem iron, derived from the breakdown of the host cells' haemoglobin (Posner *et al.*, 1995, Postma *et al.*, 1996).

The fourth hypothesis is the oxidative formation of the hydroperoxide model, as motivated by the effect of oxidants and antioxidants on the antimalarial activity of artemisinins. In this hypothesis, haem iron is not essential, since the hydroperoxide could either act as an oxidant, or form reactive oxygen species. The endoperoxide bridge is opened between the O-2 and the C-3 through protonation, forming a hydroperoxide which can be converted into peroxy radicals and other reactive oxygen species or transfer oxygen to substrates that can be oxidized (Olliaro *et al.*, 2001).

The formation of free radicals by haem iron and the hydroperoxide formation hypotheses may both be further motivated by the toxicity of artemisinins. The association of artemisinins with neurotoxicity supports the mechanism of action that involves the inhibition of mitochondrial function and the induction of oxidative stress, as evidenced by increases in reactive oxygen species and lipid peroxidation (Clark *et al.*, 2011).

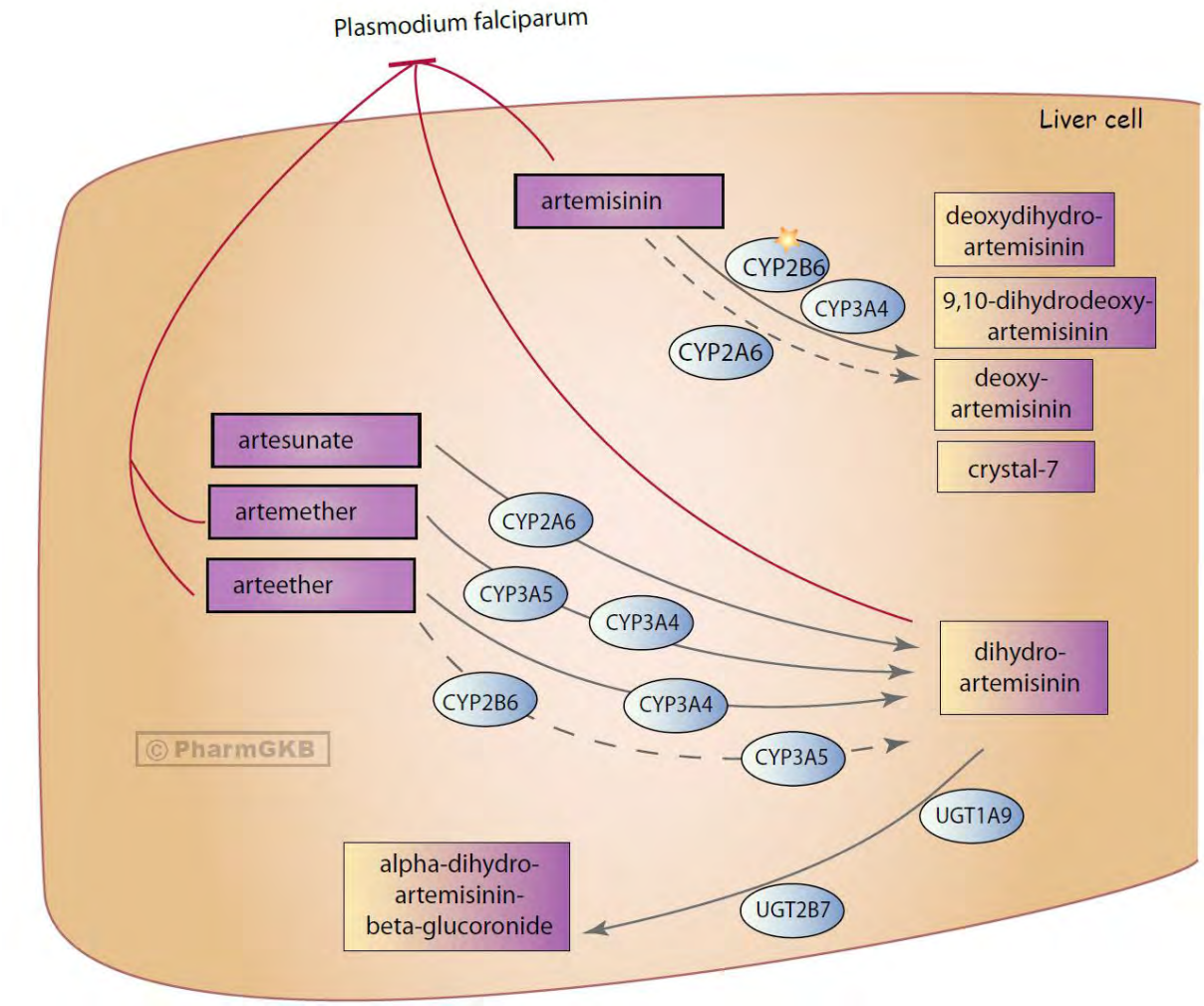


Figure 2.10: Semisynthetic artemisinin derivatives as DHA prodrugs. Cytochrome 450 plays a role in the metabolism of artemisinins into DHA. Cytochrome CYP3A4 is responsible for the conversion of both ether derivatives by dealkylation to DHA. Cytochrome CYP2A6 is responsible for the conversion of artesunate to DHA. Glucuronidation by UGT1A9 and UGT2B7 metabolise DHA to simple inactive metabolites, glucoronides, which are eliminated in the bile (Sangkuhl, 2010).

The preservation of the efficacy and efficiency of artemisinins as highly effective antimalarial drugs is motivated by numerous reasons, as mentioned above. Moreover, there is no alternative antimalarial medicine currently available that offers the same level of efficacy and tolerability, as artemisinins, nor efficient treatment as offered by ACTs (WHO, 2013). This makes the seriousness of the shortcomings of artemisinins a priority. These limitations include short pharmacological half-lives, the formation of DHA as a metabolite of all clinical artemisinins (Figure 2.8), chemical and thermal instabilities. The former has consequences such as recrudescence and treatment failure (Cheng *et al.*, 2012, Teuscher *et al.*, 2010) in

monotherapy and it has been cited as a contributing factor to the development of parasite resistance (Cheng *et al.*, 2012). As a contingency measure to augment efficacy and to suppress the rate and emergence of drug resistance, artemisinins are administered together with a longer acting antimalarial drug in ACT. The rationale behind ACT is that the artemisinin will quickly reduce the parasite burden and when its concentration falls below therapeutic levels, the longer acting antimalarial will ensure continued treatment. ACT regimens, as recommended by the WHO, include artemether-lumefantrine (AL), artesunate-amodiaquine (AS/AQ), artesunate-mefloquine (AS/MQ) and artesunate-sulphadoxine-pyrimethamine (AS/SP) (Yakasai *et al.*, 2015). Additionally, dihydroartemisinin-piperaquine (DA/PQ) is currently undergoing clinical trials, because of parasite resistance to sulphadoxine-pyrimethamine (Kakuru *et al.*, 2016).

Most shortcomings of current clinical artemisinins are due to the structural lability of the hemiacetal nature of the D-ring (Figure 2.7). One strategy to remedy these shortcomings involves the truncation of the D-ring, which seeks to remove the hemiacetal functionality from the ring, thereby improving stability and avoiding DHA upon metabolism. Venugopalan and co-workers synthesised truncated alcohol (**21**), aldehyde (**22**), thiocarbamate (**23**), ether (**24**) and ester derivatives of artemisinin, which were tested for antimalarial activity in mice, infected with CQS *Plasmodium berghei*. Derivatives **21** and **22** produced total parasite clearance when subcutaneously administered in mice, infected with drug sensitive *P. berghei* (K-173) and moderately CQ sensitive *P. berghei* (NS) (Venugopalan *et al.*, 1993). Thiocarbamate (**23**) and ether (**24**) derivatives showed comparable activity to arteether (Venugopalan *et al.*, 1995). Grellepois and colleagues also successfully synthesised a series of D-ring-contracted artemisinin trifluoromethyl ketones (**25**), however, to date there is no biological data available to accompany this work (Grellepois *et al.*, 2002).

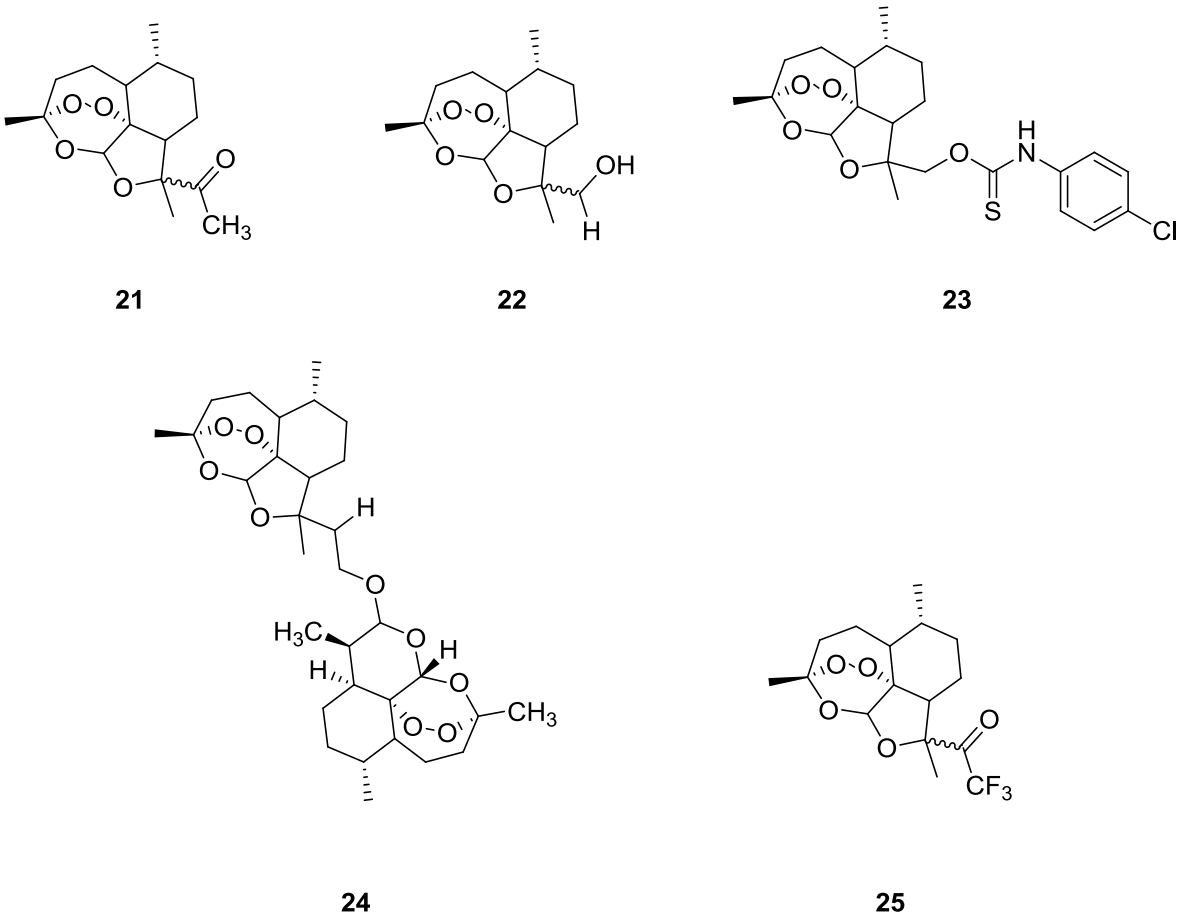


Figure 2.11: Truncated artemisinin derivatives. **21** and **22** act as bridging intermediates between conventional DHA and truncated derivatives of artemisinin.

2.7 Summary

Malaria continues to be a health problem. There is a variety of drugs at disposal for the treatment of this disease however, these are accompanied by hindrances. Problems such adverse reactions, the emergence of resistance and the selective activity of antimalarial drugs make it difficult to sustainably treat the disease, let alone achieve its eradication. These problems continuously inspire the search for better drugs. Most antimalarial drugs possess activity on one, or a few stages of the *Plasmodium* life cycle and ultimately, the parasite develops drug resistance. However, with the existence of drugs, such as artemisinins, it becomes incumbent to preserve and/or improve them. Hence, efforts such as the use of ACT, the improvement of artemisinins' solubility and consequently bioavailability through the use of artesunate and artemether, are put in place. Artemisinins have antimalarial activity at low concentrations and over a broad spectrum of the pathogen's life cycle, as well as across all human *Plasmodium* parasites. Additionally, they are well

tolerated and have good therapeutic indices. However, shortcomings, such as chemical instability, insolubility, thermal instability, drug resistance and metabolism to DHA remain stumbling blocks to the efficiency of artemisinins. Chapter 3 discusses at length the synthesis and biological activity evaluation of a series of non-hemi-acetal ester derivatives of artemisinin in an attempt to improve thermal and chemical stabilities, as well as the efficacy of current artemisinins.

REFERENCES

Achan, J., Talisuna, A. O., Erhart, A., Yeka, A., Tibenderana, J. K., Baliraine, F. N., Rosenthal, P. J. & D'Alessandro, U., 2011. Quinine, an old anti-malarial drug in a modern world: role in the treatment of malaria. *Malarial Journal*, 10, 144.

Adam, I., Mirghani, O. A., Saed, O. K., Ahmed, S. M., Mohamadani, A. A., Ahmed, H. M., Mackenzie, C. D., Homeida, M. M. & Elbashir, M. I., 2004. Quinine therapy in severe Plasmodium falciparum malaria during pregnancy in Sudan. *Eastern Mediterranean Health Journal*, 10, 159-66.

AlKadi, H. O., 2007. Antimalarial drug toxicity: a review. *Chemotherapy*, 53, 385-91.

Andersen, S. L., Ager, A., McGreevy, P., Schuster, B. G., Wesche, D., Kuschner, R., Ohrt, C., Ellis, W., Rossan, R. & Berman, J., 1995. Activity of azithromycin as a blood schizonticide against rodent and human plasmodia in vivo. *American Journal of Tropical Medicine and Hygiene*, 52, 159-61.

Ashley, E., Recht, J. & White, N., 2014. Primaquine: the risks and the benefits. *Malaria Journal*, 13, 418.

Baggish, A. L. & Hill, D. R., 2002. Antiparasitic Agent Atovaquone. *Antimicrobial Agents and Chemotherapy*, 46, 1163-1173.

Baird, J. K. & Hoffman, S. L., 2004. Primaquine therapy for malaria. *Clinical Infectious Diseases*, 39, 1336-45.

Binka, F. & Akweongo, P., 2006. Prevention of malaria using ITNs: potential for achieving the millennium development goals. *Current Molecular Medicine*, 6, 261-7.

Bjorkman, A. & Phillips-Howard, P. A., 1991. Adverse reactions to sulfa drugs: implications for malaria chemotherapy. *Bulletin of the World Health Organization*, 69, 297-304.

Bouc, A. (2013) malaria in the US: Symptoms and Prevention in malaria, S. o. (Ed., Guardianlv.com.

Bouchaud, O., Imbert, P., Touze, J. E., Dodoo, A. N., Danis, M. & Legros, F., 2009. Fatal cardiotoxicity related to halofantrine: a review based on a worldwide safety data base. *Malaria Journal*, 8, 1-8.

Carvalho, P. A., Diez-Silva, M., Chen, H., Dao, M. & Suresh, S., 2013. Cytoadherence of erythrocytes invaded by Plasmodium falciparum: quantitative contact-probing of a human malaria receptor. *Acta Biomaterialia,* 9, 6349-59.

Charman, S. A., Arbe-Barnes, S., Bathurst, I. C., Brun, R., Campbell, M., Charman, W. N., Chiu, F. C., Chollet, J., Craft, J. C., Creek, D. J., Dong, Y., Matile, H., Maurer, M., Morizzi, J., Nguyen, T., Papastogiannidis, P., Scheurer, C., Shackleford, D. M., Sriraghavan, K., Stingelin, L., Tang, Y., Urwyler, H., Wang, X., White, K. L., Wittlin, S., Zhou, L. & Vennerstrom, J. L., 2011. Synthetic ozonide drug candidate OZ439

offers new hope for a single-dose cure of uncomplicated malaria. *Proceedings of the National Academy of Sciences of the United States of America*, 108, 4400-5.

Cheng, Q., Kyle, D. E. & Gatton, M. L., 2012. Artemisinin resistance in Plasmodium falciparum: A process linked to dormancy? *International Journal for Parasitology, Drugs and Drug Resistance*, 2, 249-255.

Clark, R. L., Brannen, K. C., Sanders, J. E. & Hoberman, A. M., 2011. Artesunate and artelinic acid: association of embryotoxicity, reticulocytopenia, and delayed stimulation of hematopoiesis in pregnant rats. *Birth defects research. Part B, Developmental and reproductive toxicology*, 92, 52-68.

Cordel, H., Cailhol, J., Matheron, S., Bloch, M., Godineau, N., Consigny, P.-H., Gros, H., Campa, P., Bourée, P., Fain, O., Ralaimazava, P. & Bouchaud, O., 2013. Atovaquone-proguanil in the treatment of imported uncomplicated Plasmodium falciparum malaria: a prospective observational study of 553 cases. *Malaria Journal*, 12, 1-9.

Cowman, A. F. & Crabb, B. S., 2006. Invasion of Red Blood Cells by Malaria Parasites. *Cell*, 124, 755-766.

Cui, L., Mharakurwa, S., Ndiaye, D., Rathod, P. K. & Rosenthal, P. J., 2015. Antimalarial Drug resistance: literature review and activities and findings of the ICEMR network. *The American Journal of Tropical Medicine and Hygiene*, 93, 57-68.

Dalrymple, D. G., 2012. *Artemisia annua*, Artemisinin, ACTs & Malaria Control in Africa. *Tradition, Science and Public Policy,* Washington D.C, POLITICS & PROSE BOOKSTORE.

Davis, T. M., Hung, T. Y., Sim, I. K., Karunajeewa, H. A. & Ilett, K. F., 2005. Piperaquine: a resurgent antimalarial drug. *Drugs*, 65, 75-87.

de Vries, P. J. & Dien, T. K., 1996. Clinical pharmacology and therapeutic potential of artemisinin and its derivatives in the treatment of malaria. *Drugs*, 52, 818-36.

del Portillo, H. A., Ferrer, M., Brugat, T., Martin-Jaular, L., Langhorne, J. & Lacerda, M. V. G., 2012. The role of the spleen in malaria. *Cellular Microbiology*, 14, 343-355.

Deshpande, S. K., Bhimanna., 2016. 4-aminoquinolines: An Overview of Antimalarial Chemotherapy. *Medicinal chemistry*, 6, 001-011.

Dhangadamajhi, G., Kar, S. K. & Ranjit, M., 2010. The survival strategies of malaria parasite in the red blood cell and host cell polymorphisms. *Malaria Research and Treatment*, 2010, 973094.

Duraisingh, M. T. & Cowman, A. F., 2005. Contribution of the pfmdr1 gene to antimalarial drug-resistance. *Acta Tropica*, 94, 181-90.

Eckstein-Ludwig, U., Webb, R. J., Van Goethem, I. D., East, J. M., Lee, A. G., Kimura, M., O'Neill, P. M., Bray, P. G., Ward, S. A. & Krishna, S., 2003. Artemisinins target the SERCA of Plasmodium falciparum. *Nature*, 424, 957-61.

Ferone, R., 1977. Folate metabolism in malaria. *Bulletin of the World Health Organization*, 55, 291-298.

Foley, M. & Tilley, L., 1997. Quinoline antimalarials: mechanisms of action and resistance. *International Journal for Parasitology*, 27, 231-40.

Foley, M. & Tilley, L., 1998. Quinoline antimalarials: mechanisms of action and resistance and prospects for new agents. *Pharmacology & Therapeutics*, 79, 55-87.

Francis, S. E., Sullivan, D. J., Jr. & Goldberg, D. E., 1997. Hemoglobin metabolism in the malaria parasite Plasmodium falciparum. *Annual Review of Microbiology*, 51, 97-123.

Glick, L., 1957. Fatal agranulocytosis during treatment with a amodiaquine. *British Medical Journal*, 1, 932.

Goldberg, D. E., Slater, A. F., Beavis, R., Chait, B., Cerami, A. & Henderson, G. B., 1991. Hemoglobin degradation in the human malaria pathogen Plasmodium falciparum: a catabolic pathway initiated by a specific aspartic protease. *Journal of Experimental Medicine*, 173, 961-9.

Goldberg, D. E., Slater, A. F., Cerami, A. & Henderson, G. B., 1990. Hemoglobin degradation in the malaria parasite Plasmodium falciparum: an ordered process in a unique organelle. *Proceedings of the National Academy of Sciences of the United States of America*, 87, 2931-5.

Gregson, A. & Plowe, C. V., 2005. Mechanisms of resistance of malaria parasites to antifolates. *Pharmacological Reviews*, 57, 117-45.

Grellepois, F., Chorki, F., Crousse, B., Ourevitch, M., Bonnet-Delpon, D. & Begue, J. P., 2002. Anhydrodihydroartemisinin and its 10-trifluoromethyl analogue: access to novel d-ring-contracted artemisinin trifluoromethyl ketones. *The Journal of Organic Chemistry*, 67, 1253-60.

Grewal, R. S., 1981. Pharmacology of 8-aminoquinolines. *Bulletin of the World Health Organization*, 59, 397-406.

Hastings, M. D. & Sibley, C. H., 2002. Pyrimethamine and WR99210 exert opposing selection on dihydrofolate reductase from Plasmodium vivax. *Proceedings of the National Academy of Sciences of the United States of America*, 99, 13137-41.

Haynes, R. K. & Krishna, S., 2004. Artemisinins: activities and actions. *Microbes and Infection*, 6, 1339-46.

Ho, M. & White, N. J., 1999. Molecular mechanisms of cytoadherence in malaria. *American Journal of Physiology*, 276, C1231-42.

Hogan, B., 2009. Guidelines for the treatment of malaria in South Africa. http://www.kznhealth.gov.za/medicine/2009malariaguideline.pdf (access date: 25 September 2015)

Hyde, J. E., 2005. Exploring the folate pathway in Plasmodium falciparum. *Acta tropica*, 94, 191-206.

Kain, K. C., 2003. Current status and replies to frequently posed questions on atovaquone plus proguanil (Malarone) for the prevention of malaria. *BioDrugs*, 17, Suppl. 1, 23-8.

Kakkilaya, B. S., 2015 Antimalarial Drugs. http://www.malariasite.com/malaria-drugs/ (access date: 12 June 2015)

Kakuru, A., Jagannathan, P., Muhindo, M. K., Natureeba, P., Awori, P., Nakalembe, M., Opira, B., Olwoch, P., Ategeka, J., Nayebare, P., Clark, T. D., Feeney, M. E., Charlebois, E. D., Rizzuto, G., Muehlenbachs, A., Havlir, D. V., Kamya, M. R. & Dorsey, G., 2016. Dihydroartemisinin–Piperaquine for the Prevention of Malaria in Pregnancy. *New England Journal of Medicine*, 374, 928-939.

Kaur, K., Jain, M., Reddy, R. P. & Jain, R., 2010. Quinolines and structurally related heterocycles as antimalarials. *European Journal of Medicinal Chemistry*, 45, 3245-3264.

Khositnithikul, R., Tan-ariya, P. & Mungthin, M., 2008. In vitro atovaquone/proguanil susceptibility and characterization of the cytochrome b gene of Plasmodium falciparum from different endemic regions of Thailand. *Malaria Journal*, 7, 1-5.

Krishna, S., Uhlemann, A. C. & Haynes, R. K., 2004. Artemisinins: mechanisms of action and potential for resistance. *Drug Resistance Updates*, 7, 233-44.

Krishna, S. & White, N. J., 1996 Pharmacokinetic s of quinine, chloroquine and amodiaquine. Clinical implications. *Clinical Pharmacokinetics*, 263 - 299.

Looareesuwan, S., Chulay, J. D., Canfield, C. J. & Hutchinson, D. B., 1999. Malarone (atovaquone and proguanil hydrochloride): a review of its clinical development for treatment of malaria. Malarone Clinical Trials Study Group. *The American Journal of Tropical Medicine and Hygiene*, 60, 533-41.

MacPherson, G. G., Warrell, M. J., White, N. J., Looareesuwan, S. & Warrell, D. A., 1985. Human cerebral malaria. A quantitative ultrastructural analysis of parasitized erythrocyte sequestration. *The American Journal of Pathology*, 119, 385-401.

Magowan, C., Wollish, W., Anderson, L. & Leech, J., 1988. Cytoadherence by Plasmodium falciparum-infected erythrocytes is correlated with the expression of a family of variable proteins on infected erythrocytes. *The Journal of Experimental Medicine*, 168, 1307-1320.

Makanga, M., 2014. A review of the effects of artemether-lumefantrine on gametocyte carriage and disease transmission. *Malaria Journal*, 13, 1-15.

Makanga, M. & Krudsood, S., 2009. The clinical efficacy of artemether/lumefantrine (Coartem(®)). *Malaria Journal*, 8, S5-S5.

Meshnick, S. R., Taylor, T. E. & Kamchonwongpaisan, S., 1996. Artemisinin and the antimalarial endoperoxides: from herbal remedy to targeted chemotherapy. *Microbiological Reviews*, 60, 301-15.

Morris, C. A., Duparc, S., Borghini-Fuhrer, I., Jung, D., Shin, C.-S. & Fleckenstein, L., 2011. Review of the clinical pharmacokinetics of artesunate and its active metabolite dihydroartemisinin following intravenous, intramuscular, oral or rectal administration. *Malaria Journal*, 10, 263-263.

Na-Bangchang, K., Muhamad, P., Ruaengweerayut, R., Chaijaroenkul, W. & Karbwang, J., 2013. Identification of resistance of Plasmodium falciparum to artesunate-mefloquine combination in an area along the Thai-Myanmar border: integration of clinico-parasitological response, systemic drug exposure, and in vitro parasite sensitivity. *Malaria Journal*, 12, 263.

Nateghpour, M., Ward, S. A. & Howells, R. E., 1993. Development of halofantrine resistance and determination of cross-resistance patterns in Plasmodium falciparum. *Antimicrobial Agents and Chemotherapy*, 37, 2337-2343.

Nzila, A., 2006. The past, present and future of antifolates in the treatment of Plasmodium falciparum infection. *Journal of Antimicrobial Chemotherapy*, 57, 1043-54.

O'Neill, P. M., Bray, P. G., Hawley, S. R., Ward, S. A. & Park, B. K., 1998. 4-Aminoquinolines--past, present, and future: a chemical perspective. *Pharmacology & Therapeutics*, 77, 29-58.

O'Neill, P. M., Mukhtar, A., Stocks, P. A., Randle, L. E., Hindley, S., Ward, S. A., Storr, R. C., Bickley, J. F., O'Neil, I. A., Maggs, J. L., Hughes, R. H., Winstanley, P. A., Bray, P. G. & Park, B. K., 2003. Isoquine and Related Amodiaquine Analogues: A New Generation of Improved 4-Aminoquinoline Antimalarials. *Journal of Medicinal Chemistry*, 46, 4933-4945.

Olliaro, P. L., Haynes, R. K., Meunier, B. & Yuthavong, Y., 2001. Possible modes of action of the artemisinin-type compounds. *Trends in Parasitololgy*, 17, 122-6.

Opsenica, D. M. & Šolaja, B. A., 2012. Artemisinins and synthetic peroxides as highly efficient antimalarials. *Macedonian Journal of Chemistry and Chemical Engineering*, 31, No. 2, pp. 137–182.

Pandey, A. V., Babbarwal, V. K., Okoyeh, J. N., Joshi, R. M., Puri, S. K., Singh, R. L. & Chauhan, V. S., 2003. Hemozoin formation in malaria: a two-step process involving histidine-rich proteins and lipids. *Biochemical and Biophysical Research Communications*, 308, 736-743.

Pasvol, G., 2005. The treatment of complicated and severe malaria. *British Medical Bulletin*, 75-76, 29-47.

Peters, P. J., Thigpen, M. C., Parise, M. E. & Newman, R. D., 2007. Safety and toxicity of sulfadoxine/pyrimethamine: implications for malaria prevention in pregnancy using intermittent preventive treatment. *Drug Safety*, 30, 481-501.

Petersen, I., Eastman, R. & Lanzer, M., 2011. Drug-resistant malaria: molecular mechanisms and implications for public health. *FEBS Letters*, 585, 1551-62.

Posner, G. H., Cumming, J. N., Ploypradith, P. & Oh, C. H., 1995. Evidence for Fe(IV):O in the Molecular mechanism of action of the trioxane antimalarial artemisinin. *Journal of the American Chemical Society*, 117, 5885-5886.

Postma, N. S., Zuidema, J., Mommérs, E. C. & Eling, W. M. C., 1996. Oxidative stress in malaria; implications for prevention and therapy. *Pharmacy World and Science*, 18, 121-129.

Prashar, L. & Paul, R., 2009. Tafenoquine: A New 8- Aminoquinoline. *Medical Journal of Zambia*, 36, 4, pp 187-190.

Recht, J., Ashley, E. & White, N., 2014. Safety of 8-aminoquinoline antimalarial medicines. World Health Organisation, www.who.int/iris/bitstream/10665/112735/1/9789241506977_eng.pdf (access date: 25 September 2015).

Ritchie, G. Y., Mungthin, M., Green, J. E., Bray, P. G., Hawley, S. R. & Ward, S. A., 1996. In vitro selection of halofantrine resistance in Plasmodium falciparum is not associated with increased expression of Pgh1. *Molecular and Biochemical Parasitology*, 83, 35-46.

Sangkuhl, K. (2010) Artemisinin and Derivatives Pathway, Pharmacokinetics. https://www.pharmgkb.org/pathway/PA165378192 (access date: 8 October 2015).

Sanyaolu, A. O., Fagbenro-Beyioku, A. F., Oyibo, W. A., Badaru, O. S., Onyeabor, O. S. & Nnaemeka, C. I., 2013. Malaria and HIV co-infection and their effect on haemoglobin levels from three health-care institutions in Lagos, southwest Nigeria. *African Health Sciences*, 13, 295-300.

Schmuck, G., Roehrdanz, E., Haynes, R. K. & Kahl, R., 2002. Neurotoxic mode of action of artemisinin. *Journal of Antimicrobial Chemotherapy*, 46, 821-7.

Sherman, I. W., 1979. Biochemistry of Plasmodium (malarial parasites). *Microbiological Reviews*, 43, 453-95.

Siriez, J.-Y., Lupoglazoff, J.-M., Bouchy-Bagros, M.-L., Pull, L. & Denjoy, I., 2012. Effect of halofantrine on QT interval in children. *Pathogens and Global Health*, 106, 124-125.

Storm, J. & Craig, A. G., 2014. Pathogenesis of cerebral malaria—inflammation and cytoadherence. *Frontiers in Cellular and Infection Microbiology*, 4, 100.

Tangpukdee, N., Duangdee, C., Wilairatana, P. & Krudsood, S., 2009. Malaria Diagnosis: A Brief Review. *The Korean Journal of Parasitology*, 47, 93-102.

Ter Kuile, F. O., Parise, M. E., Verhoeff, F. H., Udhayakumar, V., Newman, R. D., Van Eijk, A. M., Rogerson, S. J. & Steketee, R. W., 2004. The Burden of co-infection with human immunodeficiency virus Type 1 and malaria in pregnant women in sub-Saharan Africa. *The American Journal of Tropical Medicine and Hygiene*, 71, 41-54.

Teuscher, F., Gatton, M. L., Chen, N., Peters, J., Kyle, D. E. & Cheng, Q., 2010. Artemisinin induced dormancy in Plasmodium falciparum: Duration, recovery rates and implications in treatment failure. *The Journal of infectious diseases*, 202, 1362-1368.

Urban, B. C., Hien, T. T., Day, N. P., Phu, N. H., Roberts, R., Pongponratn, E., Jones, M., Mai, N. T., Bethell, D., Turner, G. D., Ferguson, D., White, N. J. & Roberts, D. J., 2005. Fatal Plasmodium falciparum malaria causes specific patterns of splenic architectural disorganization. *Infection and Immunity*, 73, 1986-94.

Venugopalan, B., Bapat, C. P., Karnik, P. J., Chatterjee, D. K., Iyer, N. & Lepcha, D., 1995. Antimalarial Activity of Novel ring-contracted artemisinin derivatives. *Journal of Medicinal Chemistry*, 38, 1922-1927.

Venugopalan, B., Bapat, C. P., Karnik, P. J., Lal, B., Chatterjee, D. K., Iyer, S. N. & Blumbach, J. (1993) 9-substituted compounds of 3α, 11α-epoxy-3,4,5,5aα,6,7,8,8a,9,11,11a-undecahydro-3β,6.alpha.,9-trimethylfurano[3,4-j][1,2]benzodioxepin, processes for their preparation and their use as antiprotozoal and antiviral agents. (Patent: US 5246930).

Wang, J., Huang, L., Li, J., Fan, Q., Long, Y., Li, Y. & Zhou, B., 2010. Artemisinin directly targets malarial mitochondria through its specific mitochondrial activation. *PLoS One*, 5, e9582.

Warhurst, D. A., IS; Beck, HP; Duraisingh, MT; Kirby, GC; von Seidlein, L; Wright, CW. 2001. Mode of action of artemether lumefantrine (COARTEM): The sole, fixed, oral ADCC and its role in combatting multidrug resistance. *Southeast Asian journal of tropical medicine and public health*, 32, Suppl.1, 4-8.

Watkins, W. M., Mberu, E. K., Winstanley, P. A. & Plowe, C. V., 1997. The efficacy of antifolate antimalarial combinations in Africa: a predictive model based on pharmacodynamic and pharmacokinetic analyses. *Parasitology Today*, 13, 459-64.

Wells, T. N. & Poll, E. M., 2010. When is enough enough? The need for a robust pipeline of high-quality antimalarials. *Discovery Medicine*, 9, 389-98.

White, N. J., Pukrittayakamee, S., Hien, T. T., Faiz, M. A., Mokuolu, O. A. & Dondorp, A. M., 2014. Malaria. *The Lancet*, 383, 723-35.

World Health Organisation, 2012a. Management of severe malaria – A practical handbook, http://apps.who.int/iris/bitstream/10665/79317/1/9789241548526_eng.pdf?ua=1 (access date: 25 September 2015).

World Health Organisation, 2012b. World Malaria Report 2012. www.who.int/malaria/...malaria_report_2012/wmr2012_full_report.pdf (access date: 18 June 2013)

World Health Organisation, 2013. Emergency Response to Artemisinin Resistance in the Greater Mekong Sub Region. Regional Framework For Action 2013-2015. www.who.int/iris/bitstream/10665/79940/1/9789241505321_eng.pdf?ua=1 (access date: 15 July 2015).

World Health Organisation, 2014. World Malaria Report 2014. apps.who.int/iris/bitstream/10665/144852/2/9789241564830_eng.pdf (access date: 15 July 2015).

WHO. 2015a. World Malaria Report 2015, http://www.who.int/malaria/publications/world-malaria-report-2015/report/en/ (access date: 30 January 2016).

Woodrow, C. J., Haynes, R. K. & Krishna, S., 2005. Artemisinins. *Postgraduate Medical Journal*, 81, 71-78.

Yakasai, A. M., Hamza, M., Dalhat, M. M., Bello, M., Gadanya, M. A., Yaqub, Z. M., Ibrahim, D. A. & Hassan-Hanga, F., 2015. Adherence to Artemisinin-Based combination therapy for the treatment of uncomplicated malaria: A Systematic review and meta-analysis. *Journal of Tropical Medicine*, 2015, 189232.

CHAPTER 3
PUBLISHED ARTICLE

Chapter 3 comprises the article entitled "**Synthesis and biological evaluation of a series of non-hemiacetal ester derivatives of artemisinin"** which has been published in the European Journal of Medicinal Chemistry. The article presents the Introduction, Results, Discussion, Conclusion and Materials and methods of the synthesised antimalarial compounds of this study.

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Research paper

Synthesis and biological evaluation of a series of non-hemiacetal ester derivatives of artemisinin

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ABSTRACT

In an attempt to improve the efficacy and stability of current, clinically used artemisinins, a series non-hemiacetal ester derivatives of artemisinin were synthesized and evaluated for their *in vitro* antiplasmodial and anticancer activities as well as cytotoxicities. These esters were synthesized through the reaction of acid anhydrides, or acid chlorides with artemisinin derived alcohol. *In vitro* antiplasmodial activity assessments were conducted against intraerythrocytic NF54 and Dd2 *Plasmodium falciparum* strains. Cytotoxicities were assessed, using normal human fetal lung fibroblast (WI-38) and Chinese hamster ovarian (CHO) mammalian cell lines, while anticancer activities were tested by using panels with three cell lines, consisting of renal (TK10), melanoma (UACC62) and breast (MCF7) cancer cells. Most compounds were found active against the breast cancer cell line. Since antiplasmodial activities for most compounds were found comparable only to that of artesunate, this study did not yield any esters with significantly improved antimalarial efficacies, nor did it deliver any promising antitumor hits. However, from the outcomes of this study, compounds with good safety profiles and increased thermal stabilities, compared to the clinically used artemisinins, were identified. The benzoate derivative **11** was found to have antimalarial activity, comparable to that of dihydroartemisinin and was it subsequently identified as a candidate for further investigation in the urgent search for new, safe and effective antimalarial drugs.

1. Introduction

Malaria is an infectious, treatable and manageable disease that is transmitted to humans through the bite of an infected female *Anopheles* mosquito [1,2]. The disease is prevalent in tropical and sub-tropical areas, specifically in sub-Saharan Africa, South-East Asia and the Eastern Mediterranean region. In 2014 alone, 214 million malaria cases were reported, the majority of which (88%) were in the World Health Organization (WHO) African region. Ninety percent (90%) of the 438 000 malaria related deaths had occurred in the WHO African Region, with most of these fatalities having been children under the age of five. The WHO reported a 48% decline in malaria mortalities between 2000 and 2015, of which 90% still were among African children. Despite this decrease in malaria fatalities, children under 5 years of age remain the group that is the most vulnerable to malaria [2].

Five species of the genus, *Plasmodium* (*P.*), namely, *Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae, Plasmodium ovale and Plasmodium knowlesi* are responsible for malaria infections in humans [2]. *P. falciparum* accounts for high cases of morbidity and mortality, while *P. vivax* infections, although rarely life threatening, has consequential morbidity implications, since it has the ability to develop in the *Anopheles* mosquito at low temperatures and to develop into a dormant liver stage [2–4]. As evidenced by the statistics, the life threatening effects of malaria can be dire, especially if the infection is caused by *P. falciparum*, which may either be uncomplicated, or severe. The importance of treating uncomplicated *P. falciparum* malaria is necessitated by its risk of developing into life threatening, severe malaria.

The recent progress being made in reducing malaria infections and fatalities can be attributed to effective prevention and treatment interventions. The key prevention interventions include vector control and chemoprevention. Insecticide treated mosquito nets (ITNs) and indoor residual spraying (IRS) are used as vector control methods, aimed at reducing the transmission of parasites

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from humans to mosquitoes and then back to humans. Chemoprevention is particularly effective in pregnant women, infants and children, as it diminishes blood-stage infection in humans. Other chemoprevention programs include intermittent preventive treatments during pregnancy (IPTp), intermittent preventive treatments for infants (IPTi) and seasonal malaria chemo-prevention (SMC), which is achieved by the administration of either sulfadoxine-pyrimethamine alone, or a combination of amodiaquine and sulfadoxine-pyrimethamine [2]. The artemisinins currently are the lifeline of antimalarial treatments, as these clinically used drugs have the highest efficacies against the malaria parasites. They remain the mainstay of antimalarial chemotherapies, mainly because they are active against all *Plasmodium* species that infect humans. The artemisinins are effective against a wide range of the life stages of the parasites, including the asexual blood and gametocyte stages of *Plasmodium* [5–7]. In addition to their potency, rapid action, as well as their broad antiplasmodial spectrum against the malarial parasite, the artemisinins also show good tolerability and no serious adverse drug reactions [8].

The WHO recommended artemisinin-based combination therapies (ACTs), which involve the administration of the artemisinins in combination with other, longer acting antimalarial drugs, aimed at ensuring constantly persistent antimalarial action in the course of treatment of uncomplicated malaria. This strategy had been necessitated to prevent parasite recrudescence, which occurs as a consequence of the short pharmacological half-lives of the artemisinins, when used in monotherapy [5,9,10]. Owing to the ACTs, artemisinins have continued to elude *P. falciparum* drug resistance until recently, when the emergence of resistance at the boarder of Thai-Cambodia was reported [11]. Since the parasite is continually evolving and adapting to its environment, it ultimately develops drug resistance and are the artemisinins no exception. The emergence of resistance against the artemisinins and the spread thereof pose a serious global health threat and risk to the ultimate goal of eradicating malaria [2,12]. This growing emergence of drug resistance has led to the inception of counter measures to try and ensure that the artemisinins, especially, remain effective against malaria [10,13]. Increased dosages of artemisinin regimens have been suggested as a means to combat the reduced sensitivity of the parasite, by either increasing the dosage frequency of the drugs, or by increasing the concentration of the artemisinins in ACTs [10]. An increase in the dosage of the artimisinins, however, is a cause of great concern, since literature reports on animal studies suggest that neurotoxicity is one of the consequences of administering artemisinin at high doses [14,15]. Neurotoxicity of artemisinins in humans remains a grey area, with only a few isolated cases being reported [16]. However, it is well documented that DHA, the principal metabolite of current, clinically used artemisinins (Fig. 1), is linked to their neurotoxicity [17,18]. This necessitates finding ways to ensure that new artemisinins remain the effective mainstay in malaria chemotherapies, but also that the progress made is not marred by any adverse side effects of the new derivatives.

A crucial starting point in the strategy to develop new derivatives is to maintain the endoperoxide bridge (O-1 – O-2), which is responsible for the antimalarial activity of artemisinin, notwithstanding the fact that the effectiveness of artemisinin is hindered by its poor solubility in both oil and water, as well as its poor efficacy upon oral administration [19]. Although the water soluble ester derivative of artemisinin, i.e. artesunate, currently is the golden standard among all antimalarial drugs in terms of its efficacy, it quickly metabolizes into DHA, following oral administration [20]. New ester derivatives, with truncated artemisinin scaffold, therefore are worth investigating as potential alternatives.

During this research, new ester derivatives were synthesized based upon the hypothesis that the truncation of an artemisinin scaffold would preserve the endoperoxide pharmacophore so that there is no loss in antimalarial activity, but rather a possible improvement in efficacy. Since all current, clinically used artemisinins are metabolized into DHA as a result of the D-ring, the contraction of this six-membered ring into a five-membered ring, with the C-10 carbon being rendered exocyclic, might eliminate the formation of DHA upon metabolism. Such truncation may thus also result in derivatives with improved stability, because of the elimination of the hemiacetal in the DHA structure. The synthesis and *in vitro* biological activities outcomes of the newly prepared esters are reported on.

2. Results

2.1. Chemistry

Adopting the method reported by Lin et al. [21], the synthesis of glycal **2** was achieved in a good yield (95%). The conversion of **2** into the halohydrin **3** also accomplished a good yield (90%), when using a literature adapted method [19]. Compounds **4** and **5** were successfully synthesized in good yields (91% and 72%, respectively) by applying and slightly modifying methods from the literature [22]. These compounds were isolated as epimeric mixtures, following purification through silica gel column chromatography. The esters **6**–**18** were prepared in moderate to good yields (43–78%) through the reaction of **5** with either an acid anhydride, or an acid chloride. Purification through silica gel column chromatography and/or recrystallization afforded the target compounds as epimeric mixtures. Attempts to isolate the crystals through recrystallization for single isomer identification, using X-ray diffraction (XRD), proved futile.

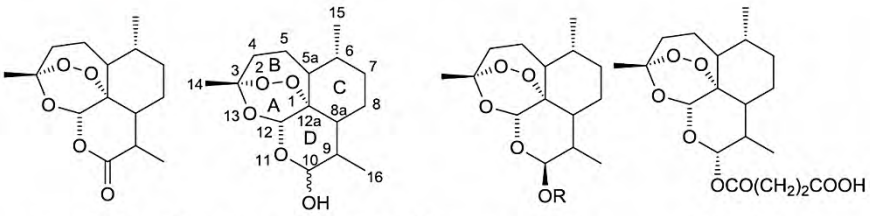


Fig. 1. Structures of artemisinin and its clinically used derivatives The artemisinins class comprises artemisinin and its oil-soluble semi-synthetic derivatives dihydroartemisinin, artemether and arteether, and water-soluble, artesunate.

2.2. Physicochemical properties

Since malaria is endemic in tropical countries, with high average temperatures, and since the disease largely occurs during the hot seasons [23], TGA and differential scanning calorimetry (DSC) analyses were employed to determine the thermal stability, as well as the physical state and phase transitions of the synthesized compounds. The TGA thermograms provided information about the thermal stability of each compound, as they represented the measured sample weight loss at increased temperatures. The results are summarised in Table 1. Most of the esters were found stable between 160 and 205 °C. Ester **13** was the least thermally stable, with a decomposition onset temperature of 97.3 °C. DSC analyses showed no endothermic phase transitions for esters **10**, **14**–**17**. However, esters **11** and **18** both had the highest glass transition temperatures of 110 °C and 116 °C, respectively, while **6** had an endotherm at 90 °C.

Furthermore, clog*P* values of the synthesized compounds were found to be in the 2–5 range.

2.3. In vitro biological activities

2.3.1. Antimalarial activity and cytotoxicity

The synthesized esters with truncated artemisinin scaffolds, intermediates **4** and **5** and the subsequent esters **6**–**18**, were screened *in vitro* alongside the standard drugs, dihydroartemisinin (DHA), artemether (ARM), artesunate (ARS) and chloroquine (CQ), against the chloroquine sensitive (CQS) NF54 and chloroquine resistant (CQR) Dd2 strains of *P. falciparum*. The IC_{50} values, as measured fluorometrically over a period of 96 h, are presented in Table 2.

According to the outcomes, the synthesized esters could be grouped into three sub-series, i.e. the alkanoates (**6**–**10**), benzoates (**10**–**13**) and heterocyclic esters (**14**–**18**). The alkanoates (except **9**) were the least active against both *P. falciparum* strains, with IC_{50} values ranging from 11 nM to 60 nM. The benzoates, however, were equipotent to ARM and ARS, with IC_{50} values in the 3–7 nM range, and were they hence moderately less active than DHA, (1–2 nM potency against both strains). The heterocyclic esters were also found to be active against both strains, with IC_{50} values in the 4–15 nM range. The activities within this latter sub-series were more pronounced against Dd2, than against NF54, with potencies equal to that of ARS for NF54, but up to four-fold less than DHA against Dd2.

Furthermore, emetine, an antiprotozoal drug, known for its high cytotoxicity, was used as a reference drug in the cytotoxicity studies. All synthesized compounds were less cytotoxic than emetine against the WI-38 and CHO cell lines, with IC_{50} values above 100 μM, compared to the 0.05 μM of emetine. Even the pyrrolidine ester **18**, which had IC_{50} values of 31.6 and 97.1 μM against the WI-38 and CHO cells, respectively, was up to six-hundred-fold less cytotoxic than emetine. Additionally, all the target compounds showed exceptional selectivity by only targeting the intra-erythrocytic *P. falciparum* parasites in the presence of the mammalian cells, as indicated by the high selectivity index values of above 4000.

2.3.2. Anticancer activity

The synthesized esters were screened *in vitro* against a panel of three cancer cell lines, consisting of renal (TK-10), melanoma (UACC-62) and breast (MCF-7) cell lines, alongside parthenolide (PTD) as reference. The IC_{50} values are summarised in Table 3. Generally, the synthesized compounds were more active against the melanoma cells, than against the breast cells, while they were totally inactive against the renal cells, with only four compounds, i.e. **4**, **10**, **12** and **18** showing activity against all three cell lines.

The compounds were less active than PTD against the renal cells, with all of the alkanoates and heterocyclic esters (except **18**) showing no activity against that cell line. Contrary, different activity profiles were observed against the melanoma and breast cell lines. Indeed, most of the alkanoates (except **9** and **10**), the benzoates (except **11**) and the heterocyclic esters (except **16**) showed activity against the melanoma cells, with ester **18** being the best performer by displaying a remarkable five-fold higher potency than the reference cells. Comparable activity profiles were observed against the breast cell line, with the best performers being **10**, **12** and **18**, all being equipotent to parthenolide. With the selectivity index values below 50, all compounds were found non-selective with regards to their anticancer activities in the presence of the mammalian cells. However, the compounds were more selective with regards to their antiparasitic actions in the presence of cancer cells (Table 3).

Table 1
Physical properties of compounds. DSC served to determine the both the physical state and transition phases while TGA was used to determine the thermal stability. ACD/ChemSketch 4.54 served to calculate the predicted log*P* values.

Compd	Melting point[a] (°C)	Onset of decomposition temperature[b] (°C)	clog*P*[c]
DHA **1**	160.0	136.7	3.1
4	174.9	141.3	2.4
5	160.9	144.0	2.3
6	171.2	164.0	2.8
7	nd*	nd*	3.9
8	nd*	nd*	4.9
9	nd*	nd*	4.9
10	173.8	225.3	6.5
11	172.4	220.0	4.5
12	171.9	201.3	4.9
13	175.2	177.3	4.1
14	170.8	206.7	4.5
15	nd*	nd*	4.9
16	177.3	217.3	3.7
17	172.1	98.7	5.2
18	172.2	126.0	4.2
ARM	186.0	131.8	2.6
ARS	134.7	148.9	2.9

[a] Melting point obtained by TGA.
[b] Onset of decomposition temperature obtained by TGA.
[c] calculated using ACD/Chemsketch v 4.5, * nd (not determined) for compounds are oils, DHA (dihydroartemisinin), ARM (artemether), ARS (artesunate).

Table 2
IC_{50} values of compounds tested *in vitro* for antiplasmodial activity against NF54 and Dd2 strains of *Plasmodium falciparum* and cytotoxicity their cytotoxicity against WI-38 HFLF and CHO cell lines. Parasites were incubated with compounds at various concentrations of compounds for 48 h thereafter the antimalarial activity was determined using parasite lactate dehydrogenase (pLDH) assay. Cytotoxicity was evaluated against WI-38 and CHO cells using SRB and MTT assays, respectively.

Compd	Antiplasmodial activity IC_{50} nM (ng/ml)		Resistance Index	Cytotoxicity IC_{50} (μM)[b]		Selectivity Index	
	NF54	Dd2	RI[a]	HFLF WI-38[b]	CHO	SI_1[c]	SI_2[d]
DHA **1**	2.2 (0.63 ± 0.09)	1.2 (0.35 ± 0.14)	0.6	nd	>100 (35.0 ± 3.97)	nd	>45 455
4	6.9 (1.96 ± 0.12)	4.1 (1.16 ± 0.34)	0.6	>100	>100 (73.7 ± 18.78).	>14 493	>14 493
5	4.1 (1.16 ± 0.34)	7.4 (2.10 ± 0.36)	1.8	>100	>100	>24 390	>24 390
6	11.2 (3.64 ± 1.17)	12.6 (4.11 ± 2.22)	1.1	>100	>100	>8923	>8923
7	16.9 (5.99 ± 0.49)	6.9 (2.46 ± 0.51)	0.4	>100	>100	>5917	>5917
8	60.1 (23.0 ± 0.29)	43.2 (16.53 ± 2.73)	0.7	>100	>100	>1664	>1664
9	7.4 (3.09 ± 0.15)	11.5 (4.8 ± 1.60)	1.6	>100	>100	>13 514	>13 514
10	7.3 (3.40 ± 0.84)	6.5 (3.04 ± 0.13)	0.9	>100	>100	>13 699	>13 699
11	6.7 (2.91 ± 0.10)	2.6 (1.13 ± 0.11)	0.4	>100	>100 (87.1 ± 3.03)	>14 925	>14 925
12	3.7 (1.50 ± 0.44)	4.4 (1.79 ± 0.60)	1.2	>100	>100	>27 027	27 027
13	2.7 (1.20 ± 0.25)	4.3 (1.92 ± 0.08)	1.6	>100	>100	>37 037	>37 037
14	4.8 (1.88 ± 0.53)	5.9 (2.34 ± 0.23)	1.2	>100	>100	>20 833	>20 833
15	7.2 (2.95 ± 0.22)	7.1 (2.88 ± 0.05)	1.0	>100	>100	>13 889	>13 889
16	9.2 (3.49 ± 0.51)	3.7 (1.39 ± 0.24)	0.4	>100	>100	>10 870	>10 870
17	14.6 (6.25 ± 0.50)	7.1 (3.05 ± 0.37)	0.5	>100	>100	>6849	>6849
18	7.9 (3.78 ± 0.85)	7.8 (3.74 ± 0.86)	1.0	31.6	97.1 (46.6 ± 2.8)	4000	12 291
ARM	3.8 (1.12 ± 0.34)	2.3 (0.69 ± 0.20)	0.6	nd	>100	nd	>26 316
ARS	8.0 (3.06 ± 0.14)	7.5 (2.88 ± 0.05)	0.9	>100	>100	>12 500	>12 500
CQ	7.2 (2.31 ± 0.27)	112.5 (35.97 ± 8.09)	15.6	nd	nd	nd	nd
EM	nd	nd	nd	0.05	0.29 (0.141 ± 0.02)	nd	nd

[a] Resistance Index (RI) = IC_{50} Dd2/IC_{50} NF54.
[b] WI-38 cell line of normal human fetal lung fibroblast.
[c] Selectivity Index (SI_1) = IC_{50} WI-38-HFLF/IC_{50} NF54 (nM).
[d] Selectivity Index (SI_2) = IC_{50} CHO/IC_{50} NF54 (nM); ARM (artemether), ARS (artesunate), CQ (chloroquine), EM (emetine); nd (not determined).

3. Discussion

3.1. Chemistry

Dehydration of DHA was carried out in an acidic medium, by adding a Lewis acid, typically boron trifluoride-diethyl ether (i), to produce the glycal in high yields. The subsequent halohydrogenation (ii) resulted in halohydrin **3** in high yields, as a mixture of isomers. This mixture could be purified, but it could not be isolated, because of its instability at room temperature, and was it hence used as such in the subsequent reaction. It is noteworthy that previous reports suggest that the prepared compound **3** that had been obtained was a single compound, when carbon tetrachloride (CCl_4) was used as solvent [24]. During this study, however, this was not the case, since no reaction occurred in that solvent. Because carbon tetrachloride is carcinogenic [25] and its commercial supply discontinued, many relatively safer solvents were used, including acetone, chloroform, dichloromethane and combinations thereof. The reaction in chloroform was very slow and inefficient, while poor yields were obtained in methanol and acetone. The polar acetone-water (4:1) mixture, however, stood out as the best combination, as it resulted in the highest halohydrin yield.

The opening of the tetrahydro-2H pyran D-ring of the halohydrin compound **3** and its re-arrangement into a tetrahydrofuran ring, with dehalogenation in the basic medium (provided by triethylamine), afforded aldehyde **4** in a high yield, which was also isolated as a mixture of epimers that was further reduced into an alcohol mixture **5**. The presence of the hydroxyl functional group was confirmed by the broad O-H stretching, *ca.* 3456 cm^{-1} on the IR spectra. The reaction of selected acid anhydride and acyl chloride with **5** produced the esters, **6**–**18**, through nucleophilic addition, or through the elimination mechanism. Evidence of ester functionalities in the structures are visible through the C=O stretching *ca.* 1780 - 1685 cm^{-1} on the IR spectra (Supplementary information) and was the peak, resulting from the resonance of carbonyl carbon (C-1′), found *ca.* 170 ppm on the ^{13}C spectra.

Table 3
In vitro anticaner activity. The growth inhibitory effects of compounds were tested SRB assay in a 3-cell lines panel consisting of TK-10, UACC-62 and MCF-7. Cells were inoculated in a 96-well microtiter plates at plating densities of 10 000 cells/well and incubated for 24 h. Thereafter, solution of the compounds were applied and the IC_{50} values were determined by non-linear regression.

Compd	Anticancer activity IC_{50} (μM)[a]			Selectivity index					
	TK-10[b]	UACC-62[c]	MCF-7[d]	SI_1[e]	SI_2[f]	SI_3[g]	SI_4[h]	SI_5[i]	SI_6[j]
4	36.6	7.8	6.4	>3	>13	6	nd	nd	nd
5	>100	>100	>100	nd	nd	nd	nd	nd	nd
6	>100	>100	>100	–	–	–	–	–	–
7	>100	>100	53.6	–	–	>2	–	–	>2
8	>100	>100	47.47	–	–	>2	–	–	>2
9	>100	69.3	34.2	–	>1.4	>3	–	>1.4	>3
10	41.7	12.0	2.51	>2	>8	>40	>2	>8	>40
11	56.0	>100	44.4	>2	nd	>2	>2	nd	>2
12	41.7	12.0	2.5	>2	>8	>40	>2	>8	>40
13	>100	19.0	10.5	nd	nd	>10	nd	nd	>10
14	>100	39.3	46.6	–	–	>2	–	–	>2
15	>100	13.9	6.8	–	–	>15	–	–	>15
16	>100	>100	>100	–	–	nd	–	–	nd
17	>100	78.7	18.2	–	–	>6	–	–	>6
18	13.0	3.0	2.9	2	11	11	7.5	32.4	33.5
PTD	6.4	15.0	5.8	nd	nd	nd			

[a] Minimum concentration of compound inducing 50% cells growth inhibition.
[b] TK-10 (renal).
[c] UACC-62 (melanoma).
[d] MCF-7 (breast); Selectivity indexes.
[e] SI_1 = IC_{50}WI-38-HFLF/IC_{50} TK-10.
[f] SI_2 = IC_{50} WI-38-HFLF/IC_{50} UACC-62.
[g] SI_3 = IC_{50} WI-38-HFLF/IC_{50} MCF-7.
[h] SI_4 = IC_{50} CHO/IC_{50} TK-10.
[i] SI_5 = IC_{50} CHO/IC_{50} UACC-62.
[j] SI_6 = IC_{50} CHO/IC_{50} MCF-7, PTD (parthenolide); nd (not determined).

Truncation of the D-ring of DHA and the subsequent reduction of the aldehyde gave alcohol **5**, in which carbon C-10 was exocyclic, while carbon C-9 remained cyclic and chiral. Consequently, the methylene protons H-10 were non-equivalent, and thus appeared

as two doublets of doublets in the 4.8–4.0 ppm region on the ^{1}H NMR spectrum.

The esters were usually obtained as epimeric mixtures. Attempts to separate them through silica gel flash chromatography and/or recrystallization were unsuccessful and were these mixtures characterized through spectroscopic methods. In general, two overlapping singlets, due to H-12 appeared in the 5.7–5.5 ppm regions of the ^{1}H NMR spectra for the majority of the synthesized compounds, which was indicative of the presence of the two epimers, designated as A and B. The ratio of the integrals of those peaks corresponded to the ratio of each isomer in the mixture. By arbitrarily assigning isomer A to the highest integral and isomer B to lowest, revealed that all derivatives predominantly contained isomer A in the 60–80% range (Supplementary information). Compound **10** was the only prepared ester that was a pure enantiomer. The crystals were, however, unsuitable for X-ray structural determination. Consequently, the biological activities of mixtures of the target compounds were screened, instead.

3.2. Physicochemical properties

The stability of drugs plays an important role in determining optimal dosages, since compromised drugs may lead to the administration of sub-therapeutic concentrations, which would contribute towards treatment failures and ultimately in the development of drug resistance [26]. The thermal stability of antimalarial drugs is therefore fundamental, since malaria is mostly endemic in tropical areas and largely occurs during the hot seasons [23]. The onset of decomposition of the synthesized esters ranged between 160 and 205 °C, which suggested improved thermal stabilities, when compared to the clinically used artemisinins, ARM, DHA and ARS, which decompose at 131.8 °C, 136.7 °C and 148.9 °C, respectively (Fig. 2). Ester **13** was an exception, having the lowest decomposition onset temperature of 97.3 °C, which may have been attributed to the lability of the second ester functionality at carbon C-7′.

It was interesting to note that only one decomposition onset temperature was recorded for each compound, despite the esters having been epimeric mixtures. This suggested that the epimers all had the same crystalline structures. Again, ester **13** was the exception by having two decomposition onset temperatures, as was evident from the additional indent at 177.30 °C. This may, however, have been caused by the resultant compound following the C-7′ ester breakage, instead of the two epimers having two distinctly different onset temperatures.

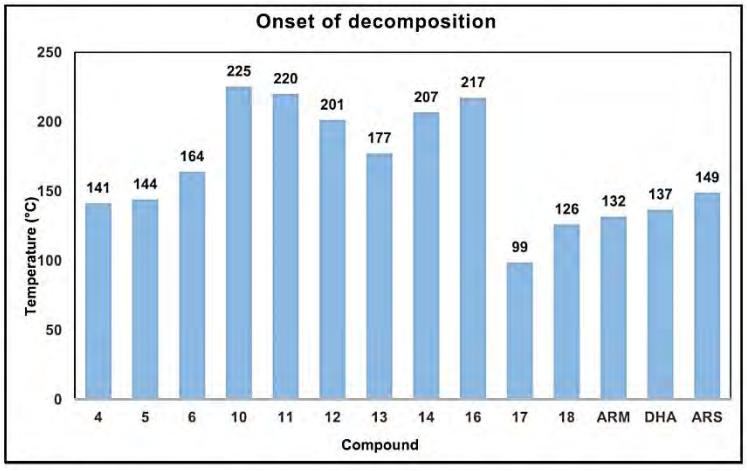
A drug must possess a balanced aqueous solubility and be neither too lipophilic, nor too hydrophilic, in order to permeate through biological membranes. Lipophilic drugs show poor aqueous solubility and tend to be taken up in fatty globules in the intestine, while hydrophilic drugs may either be excreted directly by the kidneys, or could they succeed in penetrating a cell membrane, but then become entrapped in the intracellular aqueous media. An ideal drug must therefore possess balanced lipophilic-hydrophilic properties to be able to both permeate biological membranes and to be taken up in the systemic circulation. The *n*-octanol/water partition coefficient (log*P*) offers a reliable measure of this balance, with values between 1 and 5 being targeted, and values between 1 and 3 being ideal [27,28]. Calculated Log *P* (clog*P*) values are theoretical only and do they not necessarily portray the true properties of the compounds. However, such calculated values give some indication of a compound's ability to be drug-like.

Since most of the synthesized, non-hemiacetal esters possessed clog*P* values in the target range, they could be viewed as drug-like. Esters **10** and **17** were outside of this range, with clog*P* values of 5.2 and 6.51, respectively. Interestingly, these two esters were the bulkiest. The esters had clog*P* values similar to those of DHA and ARS, suggesting that they would cross cell membranes in a similar fashion as these clinical artemisinins.

3.3. Biological evaluation

3.3.1. Antimalarial activity and cytotoxicity

The synthesized compounds were screened against the *P. falciparum* strains, NF54 and Dd2 to determine their *in vitro* antiplasmodial activities. NF54 is of African origin and is it susceptible to all known antimalarial drugs, including CQ. It is often used during *in vitro* antigametocyte activity tests, presumably because of the high gametocytemia count obtained with this strain in cell cultures. In contrast, the Dd2 strain is collected from Indochina/Laos and is it a multi-drug resistant strain (CQ-, quinine-,



7-9 and **15** were oils

Fig. 2. Comparative thermal stabilities of compounds provided by thermogravimetric analysis (TGA).

pyrimethamine- and sulfadoxine resistant) that delivers poor levels of gametocytemia in cultures [29].

Artemisinins currently are the most efficacious antimalarial drugs that are clinically used. However, their short pharmacological half-lives, poor oral availability, poor solubility and rapid first pass metabolism into inactive metabolites limit their efficiency [20,30]. The widespread uses of the ether (artemether and arteether) and ester (artesunate) derivatives are associated with improved drug plasma levels and drug efficacies [20]. These clinical derivatives, however, undergo enzymatic, oxidative de-alkylation and are easily hydrolysed into DHA [30,31]. The instability of these derivatives is facilitated by the hemi-acetal D-ring in their structures. New derivatives, i.e. the esters being investigated during this study, resulting from the modification on the D-ring, may act as new entities, which would trade on the benefits of the clinically used artesunate, but offer more stability, improved solubility and most importantly, the inability to metabolize into DHA.

Since the truncation of the D-ring makes the synthesized derivatives structurally different from the current artemisinins, it is expected that they would not be metabolized into DHA, thereby avoiding neurotoxicity in animals that is associated with high artemisinin dosages during treatment [17,18].

Because the synthesized compounds could not be isolated as pure compounds, they were evaluated as epimeric mixtures. Fortunately, these mixtures did not prevent the antimalarial activities of the newly prepared drugs, which corroborated previous findings that enantiomers of artemisinin-type compounds had not displayed any difference in antiparasitic activities and that the core structures of endoperoxide drugs could vary dramatically, without affecting their antimalarial efficacies [32].

Since the synthesized compounds were esters, their antimalarial activities were compared to that of artesunate. Moreover, since ARS is a pro-drug of DHA, and also its antimalarial active metabolite, DHA was included in the screens as a justified reference drug.

All of the new compounds were active against both strains of *P. falciparum* (indicating that the endoperoxide pharmacophore had survived the chemical transformations), with no toxicity (IC_{50} > 0.05) towards mammalian cells, and demonstrating very high selectivity in their antiparasitic actions (SI > 3000). However, the hexanoate **8** significantly was the least active, while *p*-nitro benzoate ester **11** stood as the most active compound against resistant parasites, similarly to artemether. With the exception of **7**, **8** and **17**, the majority of the prepared esters were equipotent against the NF54 strain. Equipotency was also observed against the CQR Dd2 strain, as most of the synthesized compounds (except **6** and **8**) possessed comparable activities. No significant loss of activity occurred against the resistant strain (RIs <2), since the Dd2 strain posed lower to no resistance to the majority of the new derivatives. The relatively most active compound **11** (RI = 0.5) would therefore probably not experience cross-resistance with chloroquine, quinine, pyrimethamine and sulfodoxine.

Furthermore, although the three sub-series were relatively narrow to allow for the drawing of realistic and definitive conclusions, the structure-activity relationships (SARs) suggested that within the alkanoate sub-series, the antiparasitic activities decreased as the *n*-alkyl chain lengthened, while no significant differences in activities were observed within the benzoate sub-series in relation to the electronic effect of the substituent being attached to the benzene ring. Indeed, esters **11** and **12** had electron withdrawing groups (EWG) (in the para position), i.e. nitro and fluoro, respectively, whereas **10** and **13** possessed electron donating groups (EDG), i.e. phenyl and acetyloxy, respectively, and yet, all four esters possessed comparable activities, regardless of the *P. falciparum* considered. Similarly, there were no significant differences in activities among the heterocyclic esters sub-series.

In summary, the artemisinin D-ring truncation strategy delivered non-hemiacetal esters, with good antimalarial activities (<10 nM), comparable to that of artesunate, but equally potent to DHA and artemether.

3.3.2. Anticancer activity

The synthesized compounds generally displayed poor anticancer activities, with only 25% (4 out of 15) of the compounds, i.e. **4**, **10**, **12** and **18** showing activities against all three anticancer cell lines. The breast cells were the most susceptible, whereas the renal cells were the most resistant to the anticancer actions of these esters. The anticancer activities were intrinsic, since the compounds showed no toxicity towards mammalian cells. Structure-activity relationships could not indicate clear trends within the three sub-series of compounds. The most active compound against all cell lines was the pyrrolidine ester **18**, with moderate and comparable potencies towards the parthenolide reference, against the melanoma and breast cell lines, respectively. This activity might have been attributed to the trifluoromethyl group at C-7′, which could have additionally been responsible for the increase in cytotoxicity, since this derivative appeared unsafe, with the ability to indistinctively inhibit the growth of both cancer and healthy mammalians cells (SI = 2–11) (Table 4), which disqualified it as a promising anticancer drug candidate.

Overall, the truncation of the artemisinin scaffold strategy had not yielded any viable, effective and safe antitumor derivative.

4. Conclusions

The synthesis of esters with truncated artemisinin scaffold had been motivated by the need to find candidate compounds that would possess similar, or improved antimalarial efficacies, but that would not metabolize into the cytotoxic DHA, like the currently used clinical artemisinins, because of the lability of the hemiacetal D-ring of the artemisinin scaffold. Overall, the majority of the synthesized esters showed comparable antimalarial activities to those of artesunate and artemether, more so against the chloroquine resistant parasites, but with no evidence of cytotoxicity against human cell lines. The strategy of truncating the artemisinin scaffold therefore also resulted in compounds with good selectively towards the two

Table 4
Selective *in vitro* antiplasmodial activity versus *in vitro* anticancer activity of synthesized compounds. Data indicate how selective the synthesized amides are in their activity against parasitic cells in the presence of cancerous cells.

Compd	Selectivity Index		
	SI_1[a]	SI_2[b]	SI_3[c]
4	8927	1900	1560
5			
6			
7			7768
8			1100
9		6026	2974
10	6415	1846	385
11	21 539	17 077	
12	6415	1846	385
13		4419	2442
14		6661	7898
15		1958	958
16			
17		11 085	2563
18	1667	385	372

[a] Selectivity index (SI_1) = IC_{50}TK-10/IC_{50} Dd2.
[b] Selectivity index (SI_2) = IC_{50} UACC-62/IC_{50} Dd2.
[c] Selectivity index (SI_3) = IC_{50}MCF-7/IC_{50} Dd2.

Plasmodium parasites being tested, and resulted in compounds with improved thermal stabilities, compared to the clinically used artemisinins. However, no significantly improved antiplasmodial efficacies were achieved. Further *in vivo* metabolic studies would be required to ascertain whether, or not, the esters would be metabolized into DHA. The *para*-nitrobenzoate **11** stood as the better drug candidate on accounts of its equipotency to DHA, an improved resistance index and better thermal stability. None of the synthesized compounds stood out as a potential antitumor candidate.

5. Material and methods

5.1. Materials

Dihydroartemisinin (mixture of 10-α and 10-β epimers) was purchased from Changzhou Kaixuan Chemical Co (Chunjiang, China). Boron trifluoride diethyl etherate ($BF_3{\cdot}Et_2O$), 4-dimethylaminopyridine (DMAP), biphenyl-4-carbonyl chloride, 3-methylthiophene-2-carbonyl chloride, 2-thiophenecarbonyl chloride, benzofuran-2-carbonyl chloride, 3-(2-chloro-6-fluorophenyl)-5-methylisoxazole-4-carbonyl chloride, 2,5-dimethylfuran-3-carbonyl chloride, 3-phenyl propionyl chloride, (S)-(−)-N-(trifluoroacetyl)pyrrolidine-2-carbonyl chloride, 2-furoyl chloride, 4-nitrobenzoyl chloride, 4-fluorobenzoyl chloride, acetysalicyloyl chloride, butyric anhydride and hexanoic anhydride were purchased from Sigma–Aldrich (Johannesburg, South Africa). Methanol (MeOH), ammonium chloride (NH_4Cl), sodium bicarbonate ($NaHCO_3$), magnesium sulphate ($MgSO_4$), diethyl ether (DEE), dichloromethane (DCM) and ethyl acetate (EtOAc) were purchased from ACE chemicals (Johannesburg, South Africa). All the chemicals and reagents were of analytical grade. Chemicals were used without further purification.

5.2. General procedures

The ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Advance™ III 600 spectrometer at a frequency of 600 MHz and 150.913 MHz, respectively, in DMSO-d_6 or $CDCl_3$-*d*. Chemical shifts are reported in parts per million δ (ppm), with the residual protons of the solvent as reference. The splitting pattern abbreviations are as follows: singlet (s), doublet (d), doublet of doublet (dd), doublet of doublet of doublets (ddd), doublet of triplets (dt), doublet of quartets (dq), triplet (t), triplet of doublets (td), triplet of triplets (tt), quartet of doublets (qd) and multiplet (m).

High resolution mass spectrometry (HRMS) was recorded on a Bruker MicroTOF Q II mass spectrometer, equipped with an APCI or an ESI source, set at 200 °C or 180 °C, respectively, using Bruker Compass DataAnalysis 4.0 software. A full scan from 50 to 1500 *m/z* was performed at a capillary voltage of 4500 V, an end plate offset voltage of −500 V, with the nebulizer set at 1.6 Bar and 0.4 Bar, respectively, and a collision cell RF voltage of 100 Vpp.

Mass spectra (MS) were recorded in positive mode on a Thermo Electron LXQ™ ion trap mass spectrometer, equipped with Xcalibur 2.2 data acquisition and analysis software. The MS had an APCI source set at 300 °C, and was direct infusion with a Harvard syringe pump utilized at a flow rate of 10 μL/min. A full scan from 100 to 1200 amu was achieved in 1 s, with a capillary voltage of 7 V, while the corona discharge was 10 μA.

Infrared (IR) spectra were recorded on a Bruker Alpha-P FTIR instrument. Thin layer chromatography (TLC) was performed, using silica gel plates ($60F_{254}$), obtained from Merck (Johannesburg, South Africa). Column chromatography was performed, using MN silica gel 60, 70–230 mesh ASTM, supplied by Macherey-Nagel (Germany).

Thermogravimetric analysis (TGA) was performed on a Shimadzu DTG-60, equipped with software. Two empty aluminium crimp cells were placed on the furnace of the TGA and the microbalance manually zeroed. Data was collected for the quantity of sample that was transferred into the aluminium cell, after placing the sample on the right hand side of the furnace and closing the system. The system parameters were programmed as follows: the starting temperature was 25 °C, the maximum temperature was 300 °C, the heating rate was 10 °C/min and the nitrogen gas flow rate was 35 ml/min. The sample was automatically weighed, before starting the thermal process. A thermogram was generated and analysed, using the Schimadzu TGA software. The results are summarised in Table 1, while the thermograms are illustrated in the Supplementary Information.

5.3. Syntheses

5.3.1. Anhydrodihydroartemisinin, 2

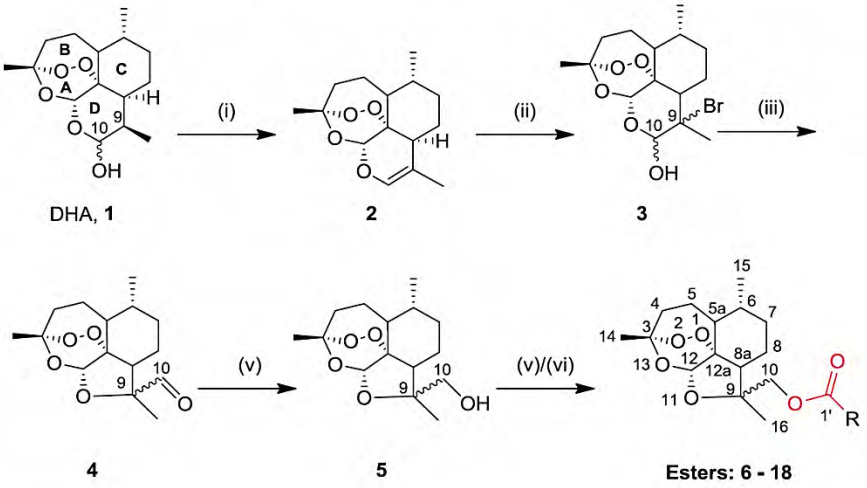
DHA **1** (3.62 mmol, 10.3 g) was suspended in 1000 ml of diethyl ether and cooled to 0 °C (in an ice bath), with continuous stirring for 15 min. $BF_3{\cdot}Et_2O$ (24.3 mmol, 30 ml, 6.7 eq) was added to the suspension (Scheme 1), which resulted in the cloudy suspension turning clear and colorless. Stirring was continued for another 10 min. The reaction flask was then removed from the ice bath, wrapped in aluminium foil and allowed to stand at room temperature for 24–48 h. The reaction mixture was washed with 10% of $NaHCO_3$ (3 × 200 ml), brine (3 × 200 ml) and finally with water (3 × 200 ml). The organic layer was dried overnight using anhydrous $MgSO_4$, filtered and then concentrated *in vacuo*. Purification through column chromatography, eluting with hexane:ethyl acetate (4:1, v/v), afforded **2** as light yellow crystals, 9.2 g (95%). IR (ATR) ν_{max}/cm^{-1}: 2953, 2922, 2865, 1684, 1110. ^1H NMR (600 MHz, $CDCl_3$) δ (ppm): 6.16 (d, J = 2.5 Hz, 1H, H-10), 5.52 (s, 1H, H-12), 2.46–2.34 (m, 1H, H-8a), 2.05–2.00 (m, 1H, 4α), 1.90–1.81 (m, 2H, H-8α, H-6), 1.72 (m, 1H, 4β), 1.60 (s, 3H, H-14), 1.59–1.47 (m, 5H, H-16, H-5β, H-7α), 1.47–1.01 (m, 2H, H-5α, H-5a), 0.96 (d, J = 5.9 Hz, 3H, H-15). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 134.95 (C-10), 108.10 (C-9), 104.51 (C-3), 89.66 (C-12), 78.94 (C-12a), 51.40 (C-5a), 44.41 (C-8a), 37.45 (C-4), 36.20 (C-7), 34.28 (C-6), 29.96 (C-5), 25.38 (C-8), 20.27 (C-14), 19.97 (C-15), 16.17 (C-16).

5.3.2. 9-Bromodihydroartemisinin, 3

Anhydrodihydroartemisinin, **2** (34.5 mmol, 9.2 g) was dissolved in 300 ml of acetone. H_2O (10 ml), followed by Br_2 (50 mmol, 2.7 ml, 1.5 eq), were added to this solution. The mixture was stirred at room temperature (r.t.), until a white, fluffy precipitate formed. The precipitate was filtered off and washed successively with water and acetone. The precipitation and filtration processes were repeated once more, resulting in 11.5 g (90%) of crystals. The crystals were kept at −5 °C, until being used in the next reaction, without further purification.

5.3.3. (3S, 6R, 12aR)-3,6,9-trimethyl-1,2,11,13-tetraoxatetracyclo-[9.5.3.$0^{5a-12a}0^{8a-12a}$]pentadecan-2-carbaldehyde, 4

Compound **3** (6.9 mmol, 2.5 g) was dissolved in 150 ml of DCM and was in triethylamine (TEA) (17.2 mmol, 2.5 ml, 2.5 eq) added to the solution. The reaction mixture was stirred at room temperature and monitored through TLC (eluent, hexane:EtOAc, 3:2, v/v), until completion of the reaction. The mixture was then treated with HCl (0.6 N) to neutralize any excess TEA. The organic solution was washed with H_2O (3 × 100 ml), dried over $MgSO_4$, filtered and was the solvent then removed *in vacuo*, which resulted in white crystals (2.1 g). Purification through column chromatography, eluting with DCM:MeOH (99:1, v/v), afforded **4** as fine, cream-white crystals, 1.8 g (91%), representing a mixture of isomers, with an isomeric ratio of 2:1, m.p.: 174.9 °C. IR (ATR) ν_{max}/cm^{-1}: 2926, 2871, 1730, 1446, 1375. ^1H NMR (600 MHz, $CDCl_3$) δ (ppm): 9.98 (s, 1H, H-10B),



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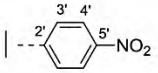
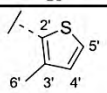
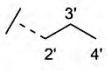
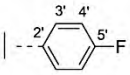
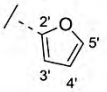
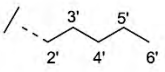
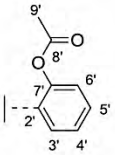
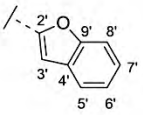
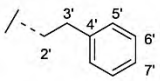
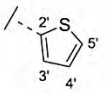
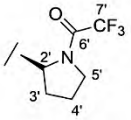
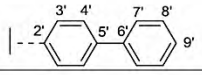
6		11		15	
7		12		16	
8		13		17	
9		14		18	
10					

Scheme 1. Multi-step synthesis of target ester derivatives. Reagents and conditions: (i): $BF_3{\cdot}EtO_2$ (6.7 eq), diethyl ether, 0 °C – r.t, 24–48 h; (ii): Br_2 (1.5 eq), acetone, H_2O, 3 h, r.t; (iii): TEA (2.5 eq), DCM,, 3 h, r.t; (iv): $NaBH_4$ (5 eq), anhydrous MeOH, 2 h, 0 °C – r.t; (v): **6**–**8**: acid anhydride (1.2 eq), TEA (1.2 eq), DMAP (0.1 eq), dry DCM, 0 °C; (vi): **9**–**18**: acid chloride (1.8), TEA (1.8 eq.), dry DCM, 0 °C, 1 h then r.t, 18 h.

9.72 (s, 1H, H-10A), 5.82 (s, 1H, H-12A), 5.75 (s, 1H, H-12B). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 204.88 (C-10), 103.85 (C-3), 98.03 (C-12), 89.95 (C-12a), 86.50 (C-9), 52.33 (C-5a), 51.90 (C-8a), 47.26 (C-4), 36.71 (C-7), 32.40 (C-6), 25.21 (C-5), 24.79 (C-16), 22.25 (C-14), 22.70 (C-15), 19.75 (C-8). HRMS *m/z* $[M+H]^+$: 283.1518 (Calculated for $C_{15}H_{22}O_5$ 283.1545).

5.3.4. (3S, 6R, 12aR)-3,6,9-trimethyl-1,2,11,13-tetraoxatetracyclo-[9.5.3.0^{5a-12a}0^{8a-12a}] pentadecan-9-yl methanol, ***5***

Aldehyde **4** (7 mmol, 2.0 g) was dissolved in anhydrous MeOH (30 ml). $NaBH_4$ (14 mmol, 0.53 g, 5 eq) was added and the solution stirred at r.t. for 3 h. The reaction was quenched with HCl (0.6 N) and water, followed by extraction with DCM. The organic phase was dried over $MgSO_4$ and then evaporated under reduced pressure, affording **5** as a fine white powder 1.44 g (72%), representing an isomeric mixture, m.p.: 160.9 °C. IR (ATR) ν_{max}/cm^{-1}: 3456, 2996, 2849, 1445, 1395, 1375. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 5.61 (s, 1H, H-12A), 5.58 (s, 1H, H-12B), 3.97 (d, J = 10.9 Hz, 1H, H-10), 3.64 (s, 1H, OH), 3.50 (dd, J = 19.0, 11.1 Hz, 1H, H-10). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 103.57 (C-3), 97.02 (C-12), 86.93 (C-12a), 84.71 (C-

9), 67.50 (C-10), 51.68 (C-5a), 49.11 (C-8a), 37.21 (C-4), 36.74 (C-7), 32.57 (C-6), 26.02 (C-16), 24.50 (C-5), 24.18 (C-14), 20.07 (C-15), 19.93 (C-8). APCI-HRMS *m/z* $[M+H]^+$: 285.1659 (Calculated for $C_{15}H_{24}O_5$ 285.1623).

*5.3.5. Syntheses of esters **6–8***

Esters **6–8** were prepared in accordance with the general procedure, as illustrated by Scheme 1 and described as follows:

Alcohol **5** (2.7 mmol, 0.76 g) was dissolved in dry DCM (15 ml) and the solution cooled to 0 °C (in an ice bath), with continuous stirring for 15 min. Acid anhydride (3.2 mmol, 1.2 eq), TEA (4.1 mmol, 572 μl, 1.2 eq) and DMAP (0.27 mmol, 33 mg, 0.1 eq) were added successively. The reaction mixture was stirred at 0 °C for 30 min and quenched with 1% of HCl (50 ml), after which it was washed with 1% of HCl (2 × 50 ml) and brine (2 × 50 ml). The organic layer was dried over $MgSO_4$, filtered and was the solvent removed *in vacuo*, which resulted in a viscous oil. Purification through column chromatography, eluting with hexane:EtOAc (7:3, v/v), afforded each ester as a mixture of isomers.

*5.3.5.1. (3S, 6R, 12aR)-3,6,9-trimethyl-1,2,11,13-tetraoxatetracyclo-[9.5.3.$0^{5a-12a}0^{8a-12a}$] pentadecan-9-yl methyl acetate, **6**.* The reaction of **5** with acetic anhydride (3.2 mmol, 302 μl, 1.2 eq) yielded ester **6** as a light yellow oil, 0.63 g (72%), with an isomeric ratio of 3:2, m.p.: 171.2 °C. IR (ATR) ν_{max}/cm^{-1}: 2979, 2862, 1826, 1745, 1428, 1375. ^1H NMR (600 MHz, $CDCl_3$) δ (ppm): 5.59 (s, 1H, H-12B), 5.57 (s, 1H, H-12A), 4.03 (d, J = 11.2 Hz, 2H, H-10B), 3.97 (d, J = 11.1 Hz, 2H, H-10A), 2.29–2.15 (m, 4H, H-4, H-2′). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 170.73 (C-1′), 103.52 (C-3), 96.75 (C-12), 86.55 (C-12a), 83.67 (C-9), 70.02 (C-10), 68.18 (C-5a), 51.45 (C-8a), 38.59 (C-4), 36.69 (C-7), 32.32 (C-2′), 29.58 (C-6), 25.98 (C-16), 24.64 (C-5), 22.86 (C-14), 20.50 (C-15), 19.78 (C-8). APCI-HRMS *m/z* $[M+H]^+$: 327.1792 (Calculated for $C_{17}H_{26}O_6$ 327.1729).

*5.3.5.2. (3S, 6R, 12aR)-3,6,9-trimethyl-1,2,11,13-tetraoxatetracyclo-[9.5.3.$0^{5a-12a}0^{8a-12a}$] pentadecan-9-yl methyl butanoate, **7**.* The reaction of **5** with butyric anhydride (3.4 mmol, 560 μl, 1.2 eq) yielded ester **7** as a viscous, clear oil, 0.70 g (71%), with an isomeric ratio of 3:2. IR (ATR) ν_{max}/cm^{-1}: 2967, 2876, 1733, 1452, 1384. ^1H NMR (600 MHz, $CDCl_3$) δ (ppm): 5.57 (s, 1H, H-12B), 5.55 (s, 1H, H-12A), 4.31 (dd, J = 105.5, 10.7 Hz, 2H, H-10B), 3.99 (dd, J = 35.6, 8.9 Hz, 2H, H-10A), 2.36 (t, J = ??, 7.3 Hz, 2H, H-2′), 1.91–1.87 (m, 2H, H-3′), 1.01–0.89 (m, 10H, H-14, H-15, H-7, H-4′). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 169.27 (C-1′), 103.44 (C-3), 96.70 (C-12), 86.53 (C-12a), 83.64 (C-9), 69.70 (C-10), 53.34 (C-5a), 51.39 (C-8a), 37.06 (C-4), 35.99 (C-2′), 35.34 (C-7), 25.95 (C-16), 24.19 (C-5), 22.81 (C-14), 20.56 (C-15), 19.73 (C-8), 17.59 (C-3′), 13.24 (C-4′). APCI-HRMS *m/z* $[M+H]^+$: 355.2095 (Calculated for $C_{19}H_{30}O_6$ 355.2042).

*5.3.5.3. (3S, 6R, 12aR)-3,6,9-trimethyl-1,2,11,13-tetraoxatetracyclo-[9.5.3.$0^{5a-12a}0^{8a-12a}$] pentadecan-9-yl methyl hexanoate, **8**.* The reaction of **5** with hexanoic anhydride (3.2 mmol, 740 μl, 1.2 eq) yielded ester **8** as a light colored, clear oil, 0.58 g (68%), with an isomeric ratio of 3:2. IR (ATR) ν_{max}/cm^{-1}: 2955, 2930, 2872, 1737, 1708, 1458, 1377. ^1H NMR (600 MHz, $CDCl_3$) δ (ppm): 5.61 (s, 1H, H-12B), 5.59 (s, 1H, H-12A), 4.35 (dd, J = 108.5, 10.7 Hz, 2H, H-10B), 4.04 (dd, J = 54.7, 12.7 Hz, 2H, H-10A), 2.41 (t, J = 7.5 Hz, 2H, H-2′), 1.93 (m, 4H, H-8, H-5, H-3′), 1.43–1.14 (m, 3H, H-4′, H-6), 1.04–0.93 (m, 8H, H-14, H-15, H-5′), 0.90–0.83 (m, 3H, H-6′). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 173.58 (C-1′), 103.60 (C-3), 96.81 (C-12), 86.64 (C-12a), 83.81 (C-9), 69.84 (C-10), 51.51 (C-5a), 48.94 (C-8a), 37.17 (C-4), 34.23 (C-7), 33.90 (C-2′), 32.60 (C-6), 32.40 (C-4′), 28.87 (C-16), 26.07 (C-3′), 24.76 (C-5), 22.41 (C-14), 22.20 (C-5′), 20.66 (C-15), 19.85 (C-8), 13.78 (C-6′). APCI-HRMS *m/z* $[M+H]^+$: 383.2450 (Calculated for $C_{21}H_{34}O_6$ 383.2433).

*5.3.6. Syntheses of esters **9–18***

These compounds were prepared in accordance with Scheme 1.

A solution of **5** (2.7 mmol, 0.76 g) and TEA (4.9 mmol, 680 μl, 1.8 eq) in dry DCM (10 ml) was cooled to 0 °C (in an ice bath), with continuous stirring for 15 min. Acyl/aryl chloride (4.9 mmol, 1.8 eq) was added to this solution. The resultant solution was stirred and held at 0 °C for 1 h and then at r.t. for 18 h. The solution was then poured into saturated NH_4Cl (100 ml), after which it was extracted with DCM (3 × 50 ml). The organic layer was washed with water, dried with $MgSO_4$, filtered and the solvent removed *in vacuo* to give a crude product. Purification through column chromatography, eluting with hexane:ethyl acetate (7:3, v/v), afforded the target ester as a mixture of isomers.

*5.3.6.1. (3S, 6R, 12aR)-3,6,9-trimethyl-1,2,11,13-tetraoxatetracyclo-[9.5.3.$0^{5a-12a}0^{8a-12a}$] pentadecan-9-yl methyl-3-phenylpropanoate, **9**.* The reaction of **5** with 3-phenylpropanoyl chloride (4.9 mmol, 730 μl 1.8 eq) yielded ester **9** as a brownish yellow viscous oil, 0.82 g (74%), with an isomeric ratio of 3:2. IR (ATR) ν_{max}/cm^{-1}: 2926, 2872, 1733, 1453, 1375. ^1H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.29–7.10 (m, 5H), 5.60 (s, 1H, H-12B), 5.59 (s, 1H, H-12A), 4.36 (dd, J = 121.8, 10.7 Hz, 2H, H-10B), 4.02 (dd, J = 66.1, 13.1 Hz, 2H, H-10A), 2.94 (t, J = 7.9 Hz, 2H, H-2′), 2.66 (t, J = 21.7, 20.3 Hz, 2H, H-3′). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 172.60 (C-1′), 140.52 (C-4′), 128.52 (C-5′, C-9′), 128.25 (C-6′, C-8′), 126.28 (C-7′), 103.61 (C-3), 96.82 (C-12), 86.65 (C-12a), 83.76 (C-9), 70.06 (C-10), 68.18 (C-5a), 51.47 (C-8a), 37.19 (C-4), 36.78 (C-7), 32.58 (C-6), 30.95 (C-2′), 29.67 (C-3′), 26.05 (C-16), 25.25 (C-5), 24.23 (C-14), 20.63 (C-15), 19.87 (C-8). APCI-HRMS *m/z* $[M+H]^+$: 417.2254 (Calculated for $C_{24}H_{32}O_6$ 417.2277).

*5.3.6.2. (3S, 6R, 12aR)-3,6,9-trimethyl-1,2,11,13-tetraoxatetracyclo-[9.5.3.$0^{5a-12a}0^{8a-12a}$] pentadecan-9-yl methyl-4-phenylbenzoate, **10**.* The reaction of **5** with biphenyl-4-carbonyl chloride (4.9 mmol, 1.1 g, 1.8 eq) yielded ester **10** as white crystals, 0.54 g (43%), m.p.: 173.8 °C. IR (ATR) ν_{max}/cm^{-1}: 2958, 2875, 1711, 1450, 1376. ^1H NMR (600 MHz, $CDCl_3$) δ (ppm): 8.10–8.05 (m, 2H, H-3′), 7.66–7.64 (m, 2H, H-4′), 7.60 (d, J = 7.5 Hz, 2H, H-7′), 7.45 (t, J = 7.6 Hz, 2H, H-8′), 7.40–7.37 (m, 1H, H-9′), 5.68 (s, 1H, H-12), 4.26 (dd, J = 11.1 Hz, 2H, H-10). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 166.06 (C-1′), 145.85 (C-5′), 139.92 (C-8′), 130.15 (C-2′), 128.94 (C-9′), 128.63 (C-8′), 128.18 (C-3′), 127.27 (C-7′), 127.11 (C-4′), 103.68 (C-3), 96.94 (C-12), 86.77 (C-12a), 83.36 (C-9), 76.79 (C-10), 68.59 (C-5a), 51.63 (C-8a), 37.22 (C-4), 36.90 (C-7), 32.47 (C-6), 25.72 (C-16), 25.35 (C-5), 24.97 (C-14), 24.28 (C-15), 19.90 (C-8). APCI-HRMS *m/z* $[M+H]^+$: 465.2253 (Calculated for $C_{28}H_{32}O_6$ 465.2277).

*5.3.6.3. (3S, 6R, 12aR)-3,6,9-trimethyl-1,2,11,13-tetraoxatetracyclo-[9.5.3.$0^{5a-12a}0^{8a-12a}$] pentadecan-9-yl methyl-4-nitrobenzoate, **11**.* The reaction of **5** with 4-nitrobenzoyl chloride (4.7 mmol, 1.8 eq) yielded ester **11** as white crystals, 0.87 g (78%), with an isomeric ratio of 3:1, m.p.: 172.4 °C. IR (ATR) ν_{max}/cm^{-1}: 2945, 2926, 2875, 2861, 2840, 1718, 1432, 1381. ^1H NMR (600 MHz, $CDCl_3$) δ (ppm): 8.27 (d, J = 8.8 Hz, 2H, H-4′), 8.19 (d, J = 8.7 Hz, 2H, H-3′), 5.67 (s, 1H, H-12B), 5.66 (s, 1H, H-12A), 4.67 (dd, J = 10.6, 7.6 Hz, 2H, H-10B), 4.34 (dd, J = 23.9, 2.2 Hz, 2H, H-10A). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm):164.39 (C-1′), 150.61 (C-5′), 135.70 (C-2′), 130.78 (C-3′), 123.60 (C-4′), 103.74 (C-3), 96.98 (C-12), 86.62 (C-12a), 83.09 (C-9), 69.48 (C-10), 51.64 (C-5a), 48.92 (C-8a), 37.17 (C-4), 36.86 (C-7), 32.39 (C-6), 26.11 (C-16), 25.44 (C-5), 24.23 (C-14), 20.82 (C-15), 19.87 (C-8). HRMS *m/z* $[M+H]^+$: 434.1857 (Calculated for $C_{22}H_{27}NO_8$ 434.1815).

*5.3.6.4. (3S, 6R, 12aR)-3,6,9-trimethyl-1,2,11,13-tetraoxatetracyclo-[9.5.3.$0^{5a-12a}0^{8a-12a}$] pentadecan-9-yl methyl-4-fluorobenzoate, **12**.* The reaction of **5** with 4-fluorobenzoyl chloride (4.9 mmol, 1.8 eq)

yielded ester **12** as white crystals, 0.85 g (78%), with an isomeric ratio of 3:2, m.p.: 171.9 °C. IR (ATR) ν_{max}/cm^{-1}: 3076, 2989, 2919, 1714, 1454, 1374. ^1H NMR (600 MHz, $CDCl_3$) δ (ppm): 8.03 (m, 2H, H-3′), 7.09 (m, 2H, H-4′), 5.66 (s, 1H, H-12B), 5.65 (s, 1H, H-12A), 4.61 (dd, J = 146.2, 10.7 Hz, 2H, H-10B), 4.28 (dd, J = 56.9, 17.6 Hz, 2H, H-10A). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 166.67 (C-1′), 164.98 (C-5′), 132.77 (C-3′, 7′), 129.01 (C-2′), 115.66 (C-4′, 6′), 103.68 (C-3), 96.92 (C-12), 86.70 (C-12a), 83.89 (C-9), 70.59 (C-10), 53.41 (C-5a), 51.61 (C-8a), 37.19 (C-4), 36.79 (C-7), 32.63 (C-6), 29.63 (C-16), 26.16 (C-5), 25.32 (C-14), 24.91 (C-15), 24.25 (C-8). APCI-HRMS *m/z* $[M+H]^+$: 407.1854 (Calculated for $C_{22}H_{27}FO_6$ 407.1869).

5.3.6.5. (3S, 6R, 12aR)-3,6,9-trimethyl-1,2,11,13-tetraoxatetracyclo-[9.5.3.$0^{5a-12a}0^{8a-12a}$] pentadecan-9-yl methyl-2-(acetyloxy) benzoate, ***13****.* The reaction of **5** with 4-methoxybenzoyl chloride (4.9 mmol, 775 μl, 1.8 eq) afforded ester **13** as a sticky, light brownish-yellow oil, 0.52 g (43%), with an isomeric ratio of 3:2, m.p.: 175.2 °C. IR (ATR) ν_{max}/cm^{-1}: 2928, 2874, 1768, 1722, 1451, 1370. ^1H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.97 (ddd, J = 9.4, 7.8, 1.7 Hz, 1H, H-3′), 7.58–7.50 (d, J = 7.3 Hz, 1H, H-6′), 7.31–7.29 (m, 1H, H-4′), 7.09 (t, J = 11.4, 9.5 Hz, 1H, H-5′), 5.64 (s, 1H, H-12B), 5.64 (s, 1H, H-12A), 4.58 (dd, J = 124.2, 10.7 Hz, 2H, H-10B), 4.23 (dd, J = 59.8, 11.1 Hz, 2H, H-10A), 2.36 (s, 3H, H-9′). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 169.88 (C-8′), 164.00 (C-1′), 150.85 (C-7′), 133.97 (C-5′), 131.67 (C-3′), 126.00 (C-4′), 123.91 (C-6′), 122.97 (C-2′), 103.63 (C-3), 96.89 (C-12), 86.71 (C-12a), 83.87 (C-9), 70.52 (C-10), 51.62 (C-5a), 48.94 (C-8a), 37.18 (C-4), 36.86 (C-7), 32.61 (C-6), 29.67 (C-16), 25.52 (C-5), 24.25 (C-14), 22.66 (C-15), 20.73 (C-9′), 19.86 (C-8). APCI-HRMS *m/z* $[M+H]^+$: 447.2068 (Calculated for $C_{24}H_{30}O_8$ 447.2019).

5.3.6.6. (3S, 6R, 12aR)-3,6,9-trimethyl-1,2,11,13-tetraoxatetracyclo-[9.5.3.$0^{5a-12a}0^{8a-12a}$] pentadecan-9-yl methyl-thiophene-2-carboxylate, ***14****.* The reaction of **5** with 2-thiophenecarbonyl chloride (4.9 mmol, 525 μl, 1.8 eq) yielded ester **14** as cream-white crystals, 0.58 g (54%), with an isomeric ratio of 3:2, m.p.: 170.80 °C. IR (ATR) ν_{max}/cm^{-1}: 2922, 2869, 2849, 1776, 1451, 1379. ^1H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.79 (d, J = 3.7 Hz, 1H, H-3′), 7.53 (d, J = 14.3 Hz, 1H, H-5′), 7.08 (d, J = 8.2 Hz, 1H, H-4′), 5.65 (s, 1H, H-12B), 5.65 (s, 1H, H-12A), 4.44 (dd, J = 193.0, 10.6 Hz, 2H, H-10B), 4.26 (dd, J = 67.8, 44.2 Hz, 2H, H-10A). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 161.71 (C-1′), 133.88 (C-2′), 132.56 (C-3′), 128.44 (C-5′), 127.84 (C-4′), 103.64 (C-3), 96.94 (C-12), 86.75 (C-12a), 83.83 (C-9), 70.55 (C-10), 68.52 (C-5a), 51.58 (C-9), 37.20 (C-4), 36.80 (C-7), 29.67 (C-6), 26.17 (C-16), 25.68 (C-5), 25.27 (C-14), 24.23 (C-15), 21.00 (C-8). ESI-HRMS *m/z* $[M+H]^+$: 395.1485 (Calculated for $C_{20}H_{26}SO_6$ 395.1528).

5.3.6.7. (3S, 6R, 12aR)-3,6,9-trimethyl-1,2,11,13-tetraoxatetracyclo-[9.5.3.$0^{5a-12a}0^{8a-12a}$] pentadecan-9-yl methyl 3-methylthiophene-2-carboxylate, ***15****.* The reaction of **5** with 3-methylthiophenecarbonyl chloride (4.9 mmol, 600 μl, 1.8 eq) yielded ester **15** as a light colored, yellow oil, 0.49 g (45%), with an isomeric ratio of 3:2. IR (ATR) ν_{max}/cm^{-1}: 2927, 2873, 1771, 1450, 1377. ^1H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.36 (d, J = 13.9 Hz, 1H, H-5′), 6.94 (d, J = 13.7 Hz, 1H, H-4′), 5.66 (s, 1H, H-12B), 5.64 (s, 1H, H-12A), 4.48 (dd, J = 264.3, 10.4 Hz, 2H, H-10B), 4.21 (dd, J = 11.5, 3.3 Hz, 2H, H-10A), 2.54 (s, 3H, H-6′). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 162.18 (C-1′), 146.50 (C-3′), 132.34 (C-5′), 131.72 (C-2′), 103.63 (C-3), 96.92 (C-12), 86.63 (C-12a), 83.63 (C-9), 70.15 (C-10), 53.41, (C-5a), 49.01 (C-8a), 37.20 (C-4), 32.67 (C-7), 29.67 (C-6), 26.19 (C-16), 25.32 (C-5), 24.99 (C-14), 24.36 (C-15), 24.25 (C-8), 21.33 (C-6′). ESI-HRMS *m/z* $[M+H]^+$: 409.1668 (Calculated for $C_{21}H_{28}SO_6$ 409.1685).

5.3.6.8. (3S, 6R, 12aR)-3,6,9-trimethyl-1,2,11,13-tetraoxatetracyclo-[9.5.3.$0^{5a-12a}0^{8a-12a}$] pentadecan-9-yl methyl furan-2-carboxylate, ***16****.* The reaction of **5** with 2-furoyl chloride (4.9 mmol, 485 μl, 1.8 eq) yielded ester **16** as pink crystals, 0.56 g (55%), m.p.: 177.3 °C. IR (ATR) ν_{max}/cm^{-1}: 2989, 2945, 2868, 2849, 1719, 1430, 1386, 1290. ^1H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.58–7.53 (d, J = 8.6 Hz, 1H, H-5′), 7.16 (d, J = 3.5 Hz, 1H, H-3′), 6.48 (t, 3.7 Hz, 1H, H-4′), 5.64 (s, 1H, H-12), 4.30 (dd, J = 70.4, 11.2 Hz, 2H, H-10). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 158.32 (C-1′), 146.49 (C-6′), 144.39 (C-2′), 118.13 (C-3′), 111.82 (C-4′), 103.64 (C-3), 96.92 (C-12), 86.71 (C-12a), 83.84 (C-9), 68.30 (C-10), 51.57 (C-5a), 48.47 (C-8a), 37.20 (C-4), 36.79 (C-7), 32.44 (C-6), 25.49 (C-16), 25.32 (C-5), 24.85 (C-14), 24.23 (C-15), 19.89 (C-8). APCI-HRMS *m/z* $[M+H]^+$: 379.1748 (Calculated for $C_{20}H_{26}O_7$ 379.1756).

5.3.6.9. [(3S, 6R, 12aR)-3,6,9-trimethyl-1,2,11,13-tetraoxatetracyclo-[9.5.3.$0^{5a-12a}0^{8a-12a}$] pentadecan-9-yl] methyl 1-benzofuran-2-carboxylate, ***17****.* The reaction of **5** with benzofuran-2-carbonyl chloride (4.9 mmol, 885 μg, 1.8 eq) yielded ester **17** as cream-white crystals, 0.62 g (55%), with an isomeric ratio of 2:1, m.p.: 172.1 °C. IR (ATR) ν_{max}/cm^{-1}: 2921, 2869, 1734, 1448, 1375, 1208. ^1H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.66 (d, J = 7.0 Hz, 1H, H-5′), 7.57 (d, J = 8.4 Hz, 1H, H-8′), 7.52 (s, 1H, H-3′), 7.47–7.39 (m, 1H, H-7′), 7.32–7.22 (m, 1H, H-6′), 5.67 (s, 1H, H-12B), 5.66 (s, 1H, H-12A), 4.57 (dd, J = 129.3, 10.7 Hz, 2H, H-10B), 4.32 (dd, J = 71.5, 9.4 Hz, 2H, H-10A). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 159.23 (C-1′), 155.51 (C-9′), 145.54 (C-2′), 127.71 (C-4′), 126.96 (C-7′), 123.82 (C-6′), 122.83 (C-5′), 114.12 (C-8′), 112.40 (C-3′), 103.68 (C-3), 96.6 (C-12), 86.71 (C-12a), 83.84 (C- 9), 70.71 (C-10), 68.79 (C-5a), 51.61 (C-8a), 48.57 (C-4), 37.14 (C-7), 32.62 (C- 6), 29.68 (C-16), 26.14 (C-5), 25.27 (C-14), 24.24 (C-15), 20.79 (C-8). APCI-HRMS *m/z* $[M+H]^+$: 429.1904 (Calculated for $C_{24}H_{28}O_7$ 429.1913).

5.3.6.10. [(3S, 6R, 12aR)-3,6,9-trimethyl-1,2,11,13-tetraoxatetracyclo-[9.5.3.$0^{5a-12a}0^{8a-12a}$] pentadecan-9-yl] methyl 1-(2,2,2-trifluoroacetyl) pyrrolidine-2-carboxylate, ***18****.* The reaction of **5** with (S)-(−)-N-(trifluoroacetyl)pyrrolidine-2-carbonyl chloride (4.9 mmol, 860 μl, 1.8 eq) yielded ester **18** as cream-white crystals, 0.93 g (72%), with an isomeric ratio of 3:2, m.p.: 172.2 °C. IR (ATR) ν_{max}/cm^{-1}: 2992, 2902, 2849, 1731, 1691, 1451, 1375, ^1H NMR (600 MHz, $CDCl_3$), δ (ppm): 5.61 (s, 1H, H-12B), 5.60 (s, 1H, H-12A) 4.33 (dd, J = 169.3, 10.6 Hz, 2H, H-10B), 4.18 (dd, J = 74.2, 11.1 Hz, 2H, H-10A), 4.05–3.93 (m, 2H, H-5′), 2.30–2.25 (m, 2H, H-3′) 2.27–1.83 (m, 4H, H-3′), 2.16–1.83 (m, 2H, H-4′). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 183.43 (C-1′), 177.12 (C-7′), 103.12 (C-8′), 96.83 (C-3), 86.78 (C-12), 83.93 (C-12a), 83.39 (C-9), 69.61 (C-10), 67.72 (C-2′), 51.47 (C-5a), 48.97 (C-8a), 48.30 (C-5′), 38.79 (C-4), 36.42 (C-7), 32.69 (C-6), 29.67 (C-3′), 27.78 (C-16), 25.63 (C-4′), 25.26 (C-5), 24.27 (C-14), 20.98 (C-15), 19.88 (C-8). APCI-HRMS *m/z* $[M+H]^+$: 478.2462 (Calculated for $C_{22}H_{30}NO_7$ 478.2052).

5.4. In vitro biological evaluation

5.4.1. Antimalarial assay

The screening of samples was done in triplicate to determine *in vitro* antiplasmodial activity against chloroquine sensitive (CQS) strain of *P. falciparum* (NF54) and against the chloroquine resistant (CQR) strain of *P. falciparum* (Dd2). Continuous *in vitro* cultures of asexual erythrocyte stages of *P. falciparum* were maintained using a modified method of Trager and Jensen [33]. Quantitative assessment of antiplasmodial activity *in vitro* was determined *via* the parasite lactate dehydrogenase assay using a modified method as described by Makler and co-workers [34]. The test samples were prepared to a 20 mg/ml stock solution in 100% DMSO and sonicated to enhance solubility. Samples were tested as a suspension if not

completely dissolved. Stock solutions were stored at −20 °C. Further dilutions were prepared on the day of the experiment. Sodium artesunate and chloroquine (CQ) were used as the reference drugs in all experiments. A full dose-response was performed for all compounds to determine the concentration inhibiting 50% of parasite growth (IC_{50} value). Samples were tested at a starting concentration of 100 ng/ml, which was then serially diluted 2-fold in complete medium to give 10 concentrations; with the lowest concentration being 0.2 ng/ml. The same dilution technique was used for all samples. The highest concentration of solvent to which the parasites were exposed to had no measurable effect on the parasite viability (data not shown). The IC_{50} values were obtained using a nonlinear dose-response curve fitting analysis via Graph Pad Prism v.4.0 software.

5.4.2. Cytotoxicity assays

WI-38 Human fetal lung fibroblast (WI-38 HFLF) cell line. The samples were screened for *in vitro* cytotoxicity using the sulforhodamine B assay on a mammalian cell line, WI-38 HFLF according to the method described by Skehan and co-workers [35]. The method measures drug induced cytotoxicity and cell proliferation. The WI-38 cell was routinely maintained as a monolayer cell culture at 37 °C, 5% CO_2, 95% air and 100% relative humidity in EMEM containing 10% fetal bovine serum, 2 mM L-glutamine and 50 μg/ml gentamicin. For screening experiment, the cells (21–50 passages) were inoculated in a 96-well microtiter plates at plating densities of 10 000 cells/well and were incubated for 24 h. After 24 h the cells were treated with the experimental drugs which were previously dissolved in DMSO and diluted in medium to produce 5 concentrations. Cells without drug addition served as control. The blank contains complete medium without cells. Emetine was used as a standard. The plates were incubated for 48 h after addition of the compounds. Viable cells were fixed to the bottom of each well with cold 50% trichloroacetic acid, washed, dried and dyed by SRB. Unbound dye was removed and protein-bound dye was extracted with 10 mM Tris base for optical density determination at the wavelength 540 nm using a multiwell spectrophotometer. Data analysis was performed using GraphPad Prism software. Fifty percent (50%) of cell growth inhibition (IC_{50}) was determined by non-linear regression.

Chinese Hamster Ovarian (CHO) cell line. The compounds tested for *in vitro* cytotoxicity against a mammalian cell-line, CHO using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT)-assay. This assay is used as a colorimetric assay for cellular growth and survival, and compares well with other available assays [36,37]. The tetrazolium salt MTT was used to measure all growth and chemosensitivity. The test samples were tested in triplicate on one occasion. The test samples were prepared to a 2 mg/ml stock solution in 10% methanol or 10% DMSO and were tested as a suspension if not properly dissolved. Test compounds were stored at −20 °C until use. Emetine was used as the reference drug in all experiments. The initial concentration of emetine was 100 μg/ml, which was serially diluted in complete medium with 10-fold dilutions to give 6 concentrations, the lowest being 0.001 μg/ml. The same dilution technique was applied to the all test samples. The highest concentration of solvent to which the cells were exposed to had no measurable effect on the cell viability (data not shown).

The 50% inhibitory concentration (IC_{50}) values were obtained from full dose-response curves, using a non-linear dose-response curve fitting analysis via GraphPad Prism v.4 software.

The results against both cell lines are summarised in Table 2.

5.4.3. Anticancer assay

The growth inhibitory effects of the compounds were tested in the 3-cell line panel consisting of TK10 (renal), UACC62 (melanoma) and MCF7 (breast) cancer cells by sulforhodamine B (SRB) assay. The SRB assay was developed by Skehan and colleagues to measure drug-induced cytotoxicity and cell proliferation. Its principle is based on the ability of the protein dye sulforhodamine B (Acid Red 52) to bind electrostatically in a pH-dependent manner to protein basic amino acid residues of trichloroacetic acid-fixed cells. Under mild acidic conditions it binds to the fixed cellular protein, while under mild basic conditions it can be extracted from cells and solubilized for measurement [35]. Cell lines were routinely maintained as a monolayer cell culture at 37 °C, 5% CO_2, 95% air and 100% relative humidity in RPMI containing 5% fetal bovine serum, 2 mM L-glutamine and 50 μg/ml gentamicin. For screening experiment, the cells (3–19 passages) were inoculated in a 96-well microtiter plates at plating densities of 7–10 000 cells/well and were incubated for 24 h. After 24 h the cells were treated with the experimental drugs which were previously dissolved in DMSO and diluted in medium to produce 5 concentrations. Cells without drug addition served as control. The blank contains complete medium without cells. Parthenolide was used as a standard. The plates were incubated for 48 h after addition of the compounds. Viable cells were fixed to the bottom of each well with cold 50% trichloroacetic acid, washed, dried and dyed by SRB. Unbound dye was removed and protein-bound dye was extracted with 10 mM Tris base for optical density determination at the wavelength 540 nm using a multiwell spectrophotometer. Data analysis was performed using GraphPad Prism software. 50% of cell growth inhibition (IC_{50}) was determined by non-linear regression.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2016.07.027.

References

[1] D.M. Opsenica, B.A. Šolaja, Maced. J. Chem. Chem. Eng. 31 (2012).
[2] WHO, World Malaria Report 2015, 2015. http://www.who.int/malaria/publications/world-malaria-report-2015/report/en/ (access date: 30 January 2016).
[3] J.K. Baird, S.L. Hoffman, Clin. Infect. Dis. 39 (2004) 1336–1345.
[4] R. Harmse, H.N. Wong, F. Smit, R.K. Haynes, D.D. N'Da, Curr. Med. Chem. 22 (2015) 3607–3630.
[5] R.K. Haynes, S. Krishna, Microbes Infect. 6 (2004) 1339–1346.
[6] S. Krishna, A.C. Uhlemann, R.K. Haynes, Drug Resist Updat 7 (2004) 233–244.
[7] C.J. Woodrow, R.K. Haynes, S. Krishna, Postgrad. Med. J. 81 (2005) 71–78.
[8] W.R. Taylor, N.J. White, Drug Saf. 27 (2004) 25–61.
[9] R.K. Haynes, H.W. Chan, C.M. Lung, N.C. Ng, H.N. Wong, L.Y. Shek, I.D. Williams, A. Cartwright, M.F. Gomes, ChemMedChem 2 (2007) 1448–1463.
[10] D. Das, R. Tripura, A.P. Phyo, K.M. Lwin, J. Tarning, S.J. Lee, W. Hanpithakpong, K. Stepniewska, D. Menard, P. Ringwald, K. Silamut, M. Imwong, K. Chotivanich, P. Yi, N.P. Day, N. Lindegardh, D. Socheat, C. Nguon, N.J. White, F. Nosten, A.M. Dondorp, Clin. Infect. Dis. 56 (2013) e48–e58.

[11] WHO, Strategy for Malaria Elimination in the Greater Mekong Subregion (2015-2030), http://iris.wpro.who.int/bitstream/handle/10665.1/10945/9789290617181_eng.pdf?seque, (access date: 06 May 2016).
[12] E.A. Ashley, M. Dhorda, R.M. Fairhurst, C. Amaratunga, P. Lim, S. Suon, S. Sreng, J.M. Anderson, S. Mao, B. Sam, C. Sopha, C.M. Chuor, C. Nguon, S. Sovannaroth, S. Pukrittayakamee, P. Jittamala, K. Chotivanich, K. Chutasmit, C. Suchatsoonthorn, R. Runcharoen, T.T. Hien, N.T. Thuy-Nhien, N.V. Thanh, N.H. Phu, Y. Htut, K.T. Han, K.H. Aye, O.A. Mokuolu, R.R. Olaosebikan, O.O. Folaranmi, M. Mayxay, M. Khanthavong, B. Hongvanthong, P.N. Newton, M.A. Onyamboko, C.I. Fanello, A.K. Tshefu, N. Mishra, N. Valecha, A.P. Phyo, F. Nosten, P. Yi, R. Tripura, S. Borrmann, M. Bashraheil, J. Peshu, M.A. Faiz, A. Ghose, M.A. Hossain, R. Samad, M.R. Rahman, M.M. Hasan, A. Islam, O. Miotto, R. Amato, B. MacInnis, J. Stalker, D.P. Kwiatkowski, Z. Bozdech, A. Jeeyapant, P.Y. Cheah, T. Sakulthaew, J. Chalk, B. Intharabut, K. Silamut, S.J. Lee, B. Vihokhern, C. Kunasol, M. Imwong, J. Tarning, W.J. Taylor, S. Yeung, C.J. Woodrow, J.A. Flegg, D. Das, J. Smith, M. Venkatesan, C.V. Plowe, K. Stepniewska, P.J. Guerin, A.M. Dondorp, N.P. Day, N.J. White, N. Engl. J. Med. 371 (2014) 411–423.
[13] A. Mbengue, S. Bhattacharjee, T. Pandharkar, H. Liu, G. Estiu, R.V. Stahelin, S.S. Rizk, D.L. Njimoh, Y. Ryan, K. Chotivanich, C. Nguon, M. Ghorbal, J.J. Lopez-Rubio, M. Pfrender, S. Emrich, N. Mohandas, A.M. Dondorp, O. Wiest, K. Haldar, Nature 520 (2015) 683–687.
[14] G. Schmuck, E. Roehrdanz, R.K. Haynes, R. Kahl, J. Antimicrob. Chemother. 46 (2002) 821–827.
[15] T. Efferth, B. Kaina, Crit. Rev. Toxicol. 40 (2010) 405–421.
[16] C. Franco-Paredes, R. Dismukes, D. Nicolls, P.E. Kozarsky, Clin. Infect. Dis. 40 (2005) 1710–1711.
[17] S. Toovey, Toxicol. Lett. 166 (2006) 95–104.
[18] D.L. Wesche, M.A. DeCoster, F.C. Tortella, T.G. Brewer, Antimicrob. Agents Chemother. 38 (1994) 1813–1819.
[19] B. Venugopalan, C.P. Bapat, P.J. Karnik, D.K. Chatterjee, N. Iyer, D. Lepcha, J. Med. Chem. 38 (1995) 1922–1927.
[20] S. Kamchonwongpaisan, S.R. Meshnick, Gen. Pharmacol. 27 (1996) 587–592.
[21] A.J. Lin, M. Lee, D.L. Klayman, J. Med. Chem. 32 (1989) 1249–1252.
[22] B. Venugopalan, C.P. Bapat, P.J. Karnik, B. Lal, D.K. Chatterjee, S.N. Iyer, J. Blumbach, US 5246930 (1993).
[23] B. Autino, A. Noris, R. Russo, F. Castelli, Mediterr. J. Hematol. Infect. Dis. 4 (2012) e2012060.
[24] F. Grellepois, D. Bonnet-Delpon, J.-P. Bégué, Tetrahedron Lett. 42 (2001) 2125–2127.
[25] M.K. Manibusan, M. Odin, D.A. Eastmond, J. Environ. Sci. Health C 25 (2007) 185–209.
[26] Q. Cheng, D.E. Kyle, M.L. Gatton, Int. J. Parasitol. Drugs Drug Resist 2 (2012) 249–255.
[27] C.A. Lipinski, F. Lombardo, B.W. Dominy, P.J. Feeney, Adv. Drug Deliv. Rev. 46 (2001) 3–26.
[28] J. Owens, Drug Discov. Today 8 (2003) 12–16.
[29] M. Roncales, J. Vidal-Mas, D. Leroy, E. Herreros, J. Parasitol. Res. 2012 (2012) 927148.
[30] C. Singh, R. Kanchan, S. Chaudhary, S.K. Puri, J. Med. Chem. 55 (2012) 1117–1126.
[31] R.K. Haynes, H.-W. Chan, M.-K. Cheung, W.-L. Lam, M.-K. Soo, H.-W. Tsang, A. Voerste, Ian D. Williams, Eur. J. Org. Chem. 2002 (2002) 113–132.
[32] J. Wang, L. Huang, J. Li, Q. Fan, Y. Long, Y. Li, B. Zhou, PLoS One 5 (2010) e9582.
[33] W. Trager, J.B. Jensen, J. Parasitol. 91 (2005) 484–486.
[34] M.T. Makler, J.M. Ries, J.A. Williams, J.E. Bancroft, R.C. Piper, B.L. Gibbins, D.J. Hinrichs, Am. J. Trop. Med. Hyg. 48 (1993) 739–741.
[35] P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J.T. Warren, H. Bokesch, S. Kenney, M.R. Boyd, J. Natl. Cancer Inst. 82 (1990) 1107–1112.
[36] T. Mosmann, J. Immun. Methods 65 (1983) 55–63.
[37] L.V. Rubinstein, R.H. Shoemaker, K.D. Paull, R.M. Simon, S. Tosini, P. Skehan, D.A. Scudiero, A. Monks, M.R. Boyd, J. Natl. Cancer Inst. 82 (1990) 1113–1117.

CHAPTER 4
SUMMARY AND CONCLUSION

Malaria continues to inflict high rates of morbidity and mortality on a global scale. While there are five *Plasmodium* (*P.*) species that can infect humans, only *P. falciparum* and *P. vivax* have serious health implications. *P. vivax* hibernates in a dormant state and causes relapse, while *P. falciparum* is responsible for high mortality rates, which are amplified by its development into severe malaria. Proper diagnosis and early treatment are, therefore, fundamental in containing the disease. Recent progress made in the reduction of malaria infections and fatalities can be attributed to combination parameters, which include effective prevention through vector control and chemoprevention, as well as curative treatment, achieved through the use of artemisinin combination therapy (ACT). The WHO recommends ACTs as a means to elicit effective curative treatment of uncomplicated malaria, to avoid parasite recrudescence in monotherapy and to ultimately prevent parasite resistance against artemisinins (Lin *et al.*, 2010). ACTs essentially combine an artemisinin derivative with a longer acting partner drug from another class of antimalarials (WHO, 2015a). In these combinations, the artemisinin, due to its fast acting ability, swiftly reduces the parasite burden, and the longer acting partner drug continues to elicit antimalarial therapy, once the artemisinin concentration falls below therapeutic levels. Despite this precautionary measure, artemisinin resistance has been reported in the Greater Mekong Sub-region (WHO, 2015b). However, artemisinins are still used and are currently the pillar of antimalarial curative chemotherapy mainly because of their activity against all *Plasmodium* species and their efficacy against a wide range of the parasite's life cycle stages. They are active against the asexual blood and early gametocyte stages of the parasite (Krishna *et al.*, 2004, Meshnick *et al.*, 1996). Additionally, artemisinins rapidly reduce the parasite burden and are well tolerated by humans, although they are afflicted with numerous shortcomings, namely short pharmacological half-lives, metabolism to DHA, solubility problems (Petersen *et al.*, 2011), chemical and thermal instabilities (Autino *et al.*, 2012). Most of these shortcomings are consequences of the lability of the hemiacetal/acetal nature of their six-membered D-ring.

Artesunate (ARS), a water soluble acetal ester derivative of artemisinin, although not exempted from the shortcomings, remains the most versatile of all clinical artemisinins. It is used for both uncomplicated and severe malaria treatment and can be administered intravenously, orally, intramuscularly and rectally (Morris *et al.*, 2011). Non-hemiacetal esters of artemisinin were investigated, based on the hypothesis that the truncation of the D-ring

would result in more robust and stable ester derivatives that would trade on the benefits of the clinically used artesunate, but offer more stability, improved solubility and most importantly, the inability to metabolise into DHA.

The aim of this study was to investigate novel, non-hemiacetal ester derivatives of artemisinin, with the ultimate goal of delivering efficacious antimalarial compounds, with improved stability and safety profile.

In order to achieve this aim, the following objectives were set:

- Synthesis of novel, non-hemiacetal esters with truncated artemisinin scaffold and their characterisation, using IR, NMR and MS.
- Evaluate *in vitro* antimalarial activity of the synthesised esters against chloroquine sensitive (CQS) and chloroquine resistant (CQR) strains of intraerythrocytic *P. falciparum* parasites.
- Evaluate *in vitro* cytotoxicity of the synthesised compounds against mammalian cell lines.
- Evaluate *in vitro* anticancer activity of the esters against TK10 (renal), UACC62 (melanoma) and MCF7 (breast) cancer cells.

The synthesis of non-hemiacetal esters was carried out through the dehydration of DHA with $BF_3.Et_2O$ to form anhydrodihydroartemisinin, which was subjected to halohydration, using Br_2. Subjecting the resulting halohydrin to a base led to the elimination of HBr and rendered C-10 exocyclic, subsequently forming an epimeric mixture of the truncated aldehyde. The reduction of the aldehyde with $NaBH_4$ afforded the alcohol intermediate. The non-hemiacetal esters were then synthesised via nucleophilic substitution reaction between the alcohol and an acyl chloride, or acid anhydride. After purification by column chromatography, the resulting esters were isolated as epimeric mixtures. The structures of the synthesised esters were confirmed by ^{1}H and ^{13}C NMR, HR-MS and IR. Thermal stability of the compounds was determined using TGA.

There are no stereoselective differences in antiparasitic activity between enantiomers of several artemisinin derivatives (Wang *et al.*, 2010). Furthermore, artemisinin derivatives such as DHA, which is an epimeric mixture, are clinically used for antimalarial treatment (Haynes *et al*, 2007) therefore, the epimers were tested as mixtures. The esters were

subjected to *in vitro* antimalarial activity testing against Dd2 (CQR) and NF54 (CQS) *P. falciparum* parasites. The results were compared to those of ARS and DHA. The latter was included in the screening as it is an active metabolite of ARS.

All of the synthesised compounds were active against both strains, with no toxicity towards mammalian cells and very high selectivity in their antiparasitic action. However, the hexanoate **8** was the least active and the *p*-nitro benzoate derivative **11** was the most active against the CQR strain. With the exception of the butanoate **7**, hexanoate **8** and benzofuran ester **17**, the majority of the esters were equipotent to ARS against the NF54 (CQS). Equipotency was also observed against the Dd2 (CQR), since most esters possessed comparable activities. No significant loss of activity occurred against the resistant strain. Overall, the *p*-nitrobenzoate **11** was found to have antimalarial activity, comparable to that of DHA and was identified as the best candidate for further investigation as a potential drug in search for new, safe and effective antimalarial drugs, based on efficacy, tolerability, safety profile and thermal stability. In addition, its favourable resistance index (RI = 0.5) indicates that this derivative would probably not experience cross-resistance with chloroquine, quinine, pyrimethamine and sulfodoxine.

Ultimately, the artemisinin D-ring truncation strategy delivered esters with good antimalarial activities (<10 nM), comparable to that of artesunate, although some compounds were less potent than DHA and artemether. Thus, the esters in this study, resulting from the modification on the D-ring may act as new entities, which would trade on the benefits of the clinically used artesunate, but offer more stability, improved solubility and most importantly, the inability to metabolise to DHA. The ester functionality is susceptible to hydrolysis, therefore while it is anticipated that hydrolysis would likely occur, the resulting metabolite would not be DHA. Specific *in vivo* tests will, however, need to be conducted to substantiate this statement.

The synthesised esters were also tested for antitumor activity using breast, renal and melanoma cell lines and they displayed in general poor anticancer activity. The pyrrolidine ring, containing ester **18**, was the most active compound against all cell lines, with moderate and comparable potencies to the reference drug (parthenolide) against melanoma and breast cell lines, respectively. However, it appeared unsafe, with the ability to indistinctively inhibit the growth of both cancer and healthy mammalians cells, which consequently disqualified it as a potential anticancer drug candidate.

In summary, the synthesis of structurally modified artemisinins of similar efficacy as current artemisinins was successfully achieved. Additionally, the compounds synthesised during this study have improved thermal stability and good tolerability by mammalian cells. However, this strategy did not yield a significant improvement in antiplasmodial activity. None of the compounds stood out as an antitumor hit.

REFERENCES

Autino, B., Noris, A., Russo, R. & Castelli, F., 2012. Epidemiology of Malaria in Endemic Areas. *Mediterranean Journal of Hematology and Infectious Diseases*, 4, e2012060.

Haynes, R. K., Chan, H. W., Lung, C. M., Ng, N. C., Wong, H. N., Shek, L. Y., Williams, I. D., Cartwright, A. & Gomes, M. F., 2007a. Artesunate and dihydroartemisinin (DHA): unusual decomposition products formed under mild conditions and comments on the fitness of DHA as an antimalarial drug. *ChemMedChem*, 2, 1448-63.

Krishna, S., Uhlemann, A. C. & Haynes, R. K., 2004. Artemisinins: mechanisms of action and potential for resistance. *Drug Resistance Updates*, 7, 233-244.

Lin, J. T., Juliano, J. J. & Wongsrichanalai, C., 2010. Drug-Resistant Malaria: The Era of ACT. *Current infectious disease reports*, 12, 165-173.

Meshnick, S. R., Taylor, T. E. & Kamchonwongpaisan, S., 1996. Artemisinin and the antimalarial endoperoxides: from herbal remedy to targeted chemotherapy. *Microbiology Reviews*, 60, 301-315.

Morris, C. A., Duparc, S., Borghini-Fuhrer, I., Jung, D., Shin, C.-S. & Fleckenstein, L., 2011. Review of the clinical pharmacokinetics of artesunate and its active metabolite dihydroartemisinin following intravenous, intramuscular, oral or rectal administration. *Malaria Journal*, 10, 263-263.

Petersen, I., Eastman, R. & Lanzer, M., 2011. Drug-resistant malaria: molecular mechanisms and implications for public health. *FEBS Letters*, 585, 1551-1562.

Wang, J., Huang, L., Li, J., Fan, Q., Long, Y., Li, Y. & Zhou, B., 2010. Artemisinin directly targets malarial mitochondria through its specific mitochondrial activation. *PLoS One*, 5, e9582.

WHO. 2015a. World Malaria Report 2015, http://www.who.int/malaria/publications/world-malaria-report-2015/report/en/ (access date: 30 January 2016).

WHO (2015b) Strategy for Malaria Elimination in the Greater Mekong Subregion (2015-2030), http://iris.wpro.who.int/bitstream/handle/10665.1/10945/9789290617181_eng.pdf?seque, (access date: 06 May 2016).

APPENDIX A: SPECTRA

Table 1: Percentage abundance of isomers A and B, calculated from the ratio of H-12 integral heights of each isomer.

Compd	H-12 integral heights (cm)		Epimeric ratio: A/B	A%	B%
	A[a]	B[b]			
4	4	2	2/1	67	33
5					
6	3	2	3/2	60	40
7	1.8	1.2	3/2	60	40
8	3.9	2.4	3/2	60	40
9	4.2	2.7	3/2	60	40
10			1		
11	4.3	1.5	3/1	75	25
12	4.5	3	3/2	60	40
13	4.6	3	3/2	60	40
14	5.3	3.6	3/2	60	40
15	2.9	2	3/2	60	40
16	-	-	1	-	-
17	4.5	2.1	2/1	67	33
18	3.6	2.3	3/2	60	40

A[a] height of the highest integral, B[b] height of the lowest integral, A% -abundance of isomer with highest integral isomer, B% –abundance of isomer with lowest integral.

COMPOUND 4

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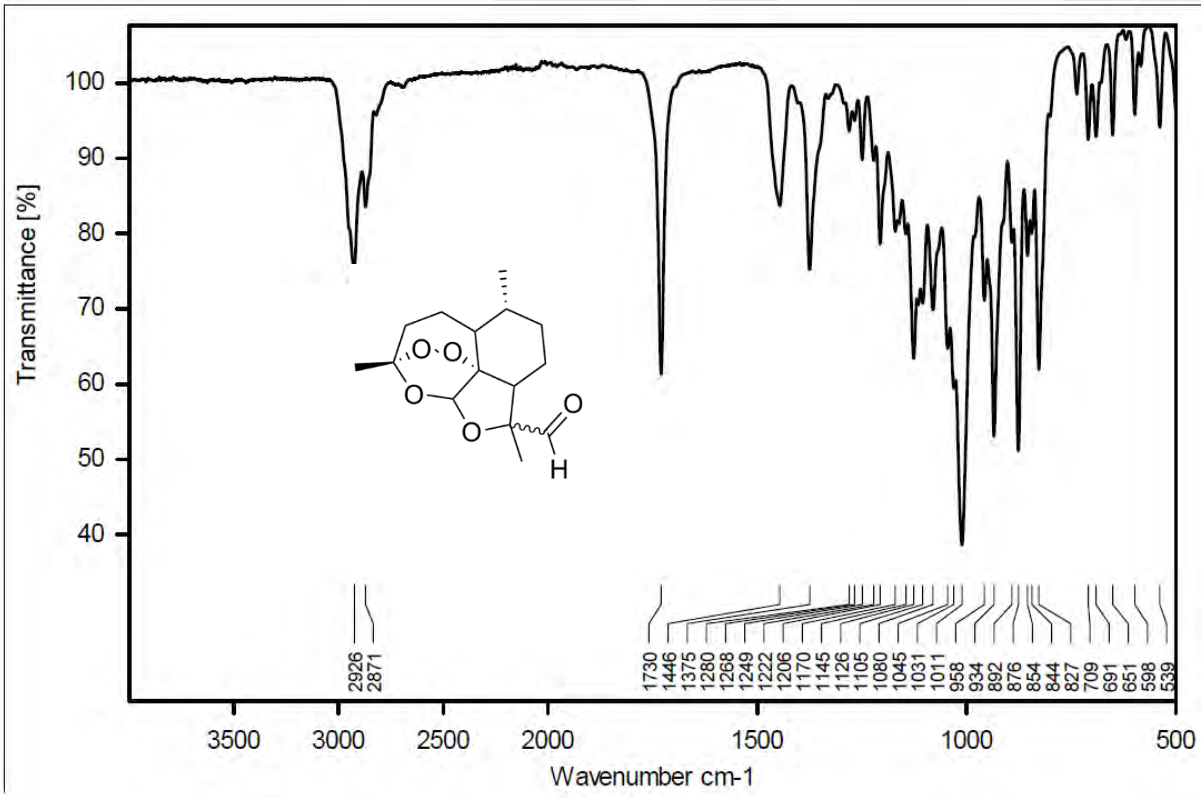

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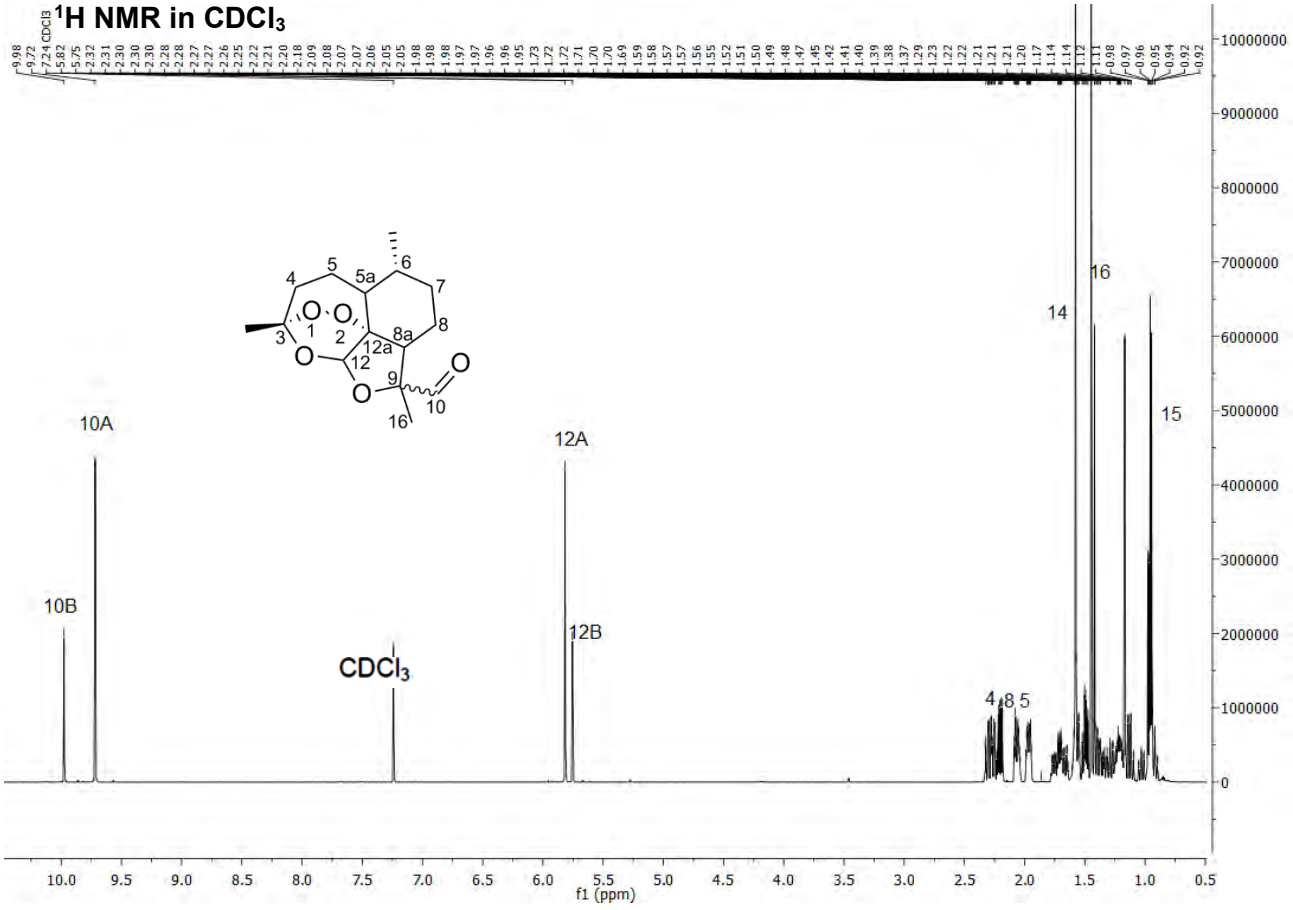

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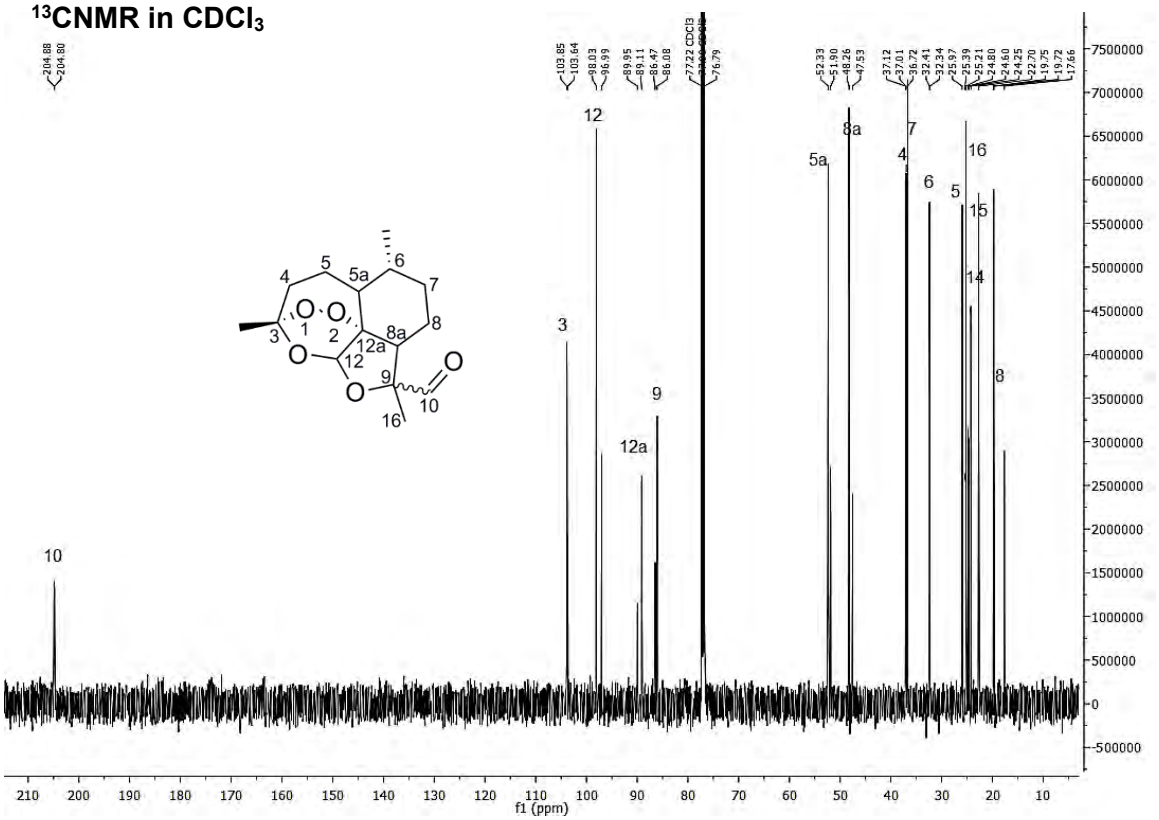

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Acquisition Parameter

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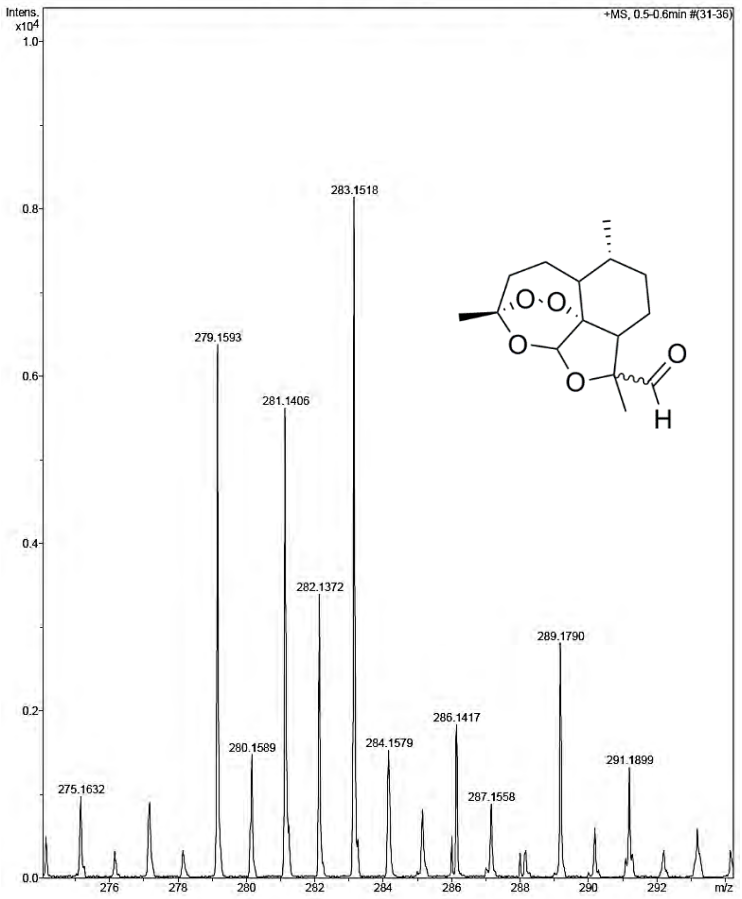

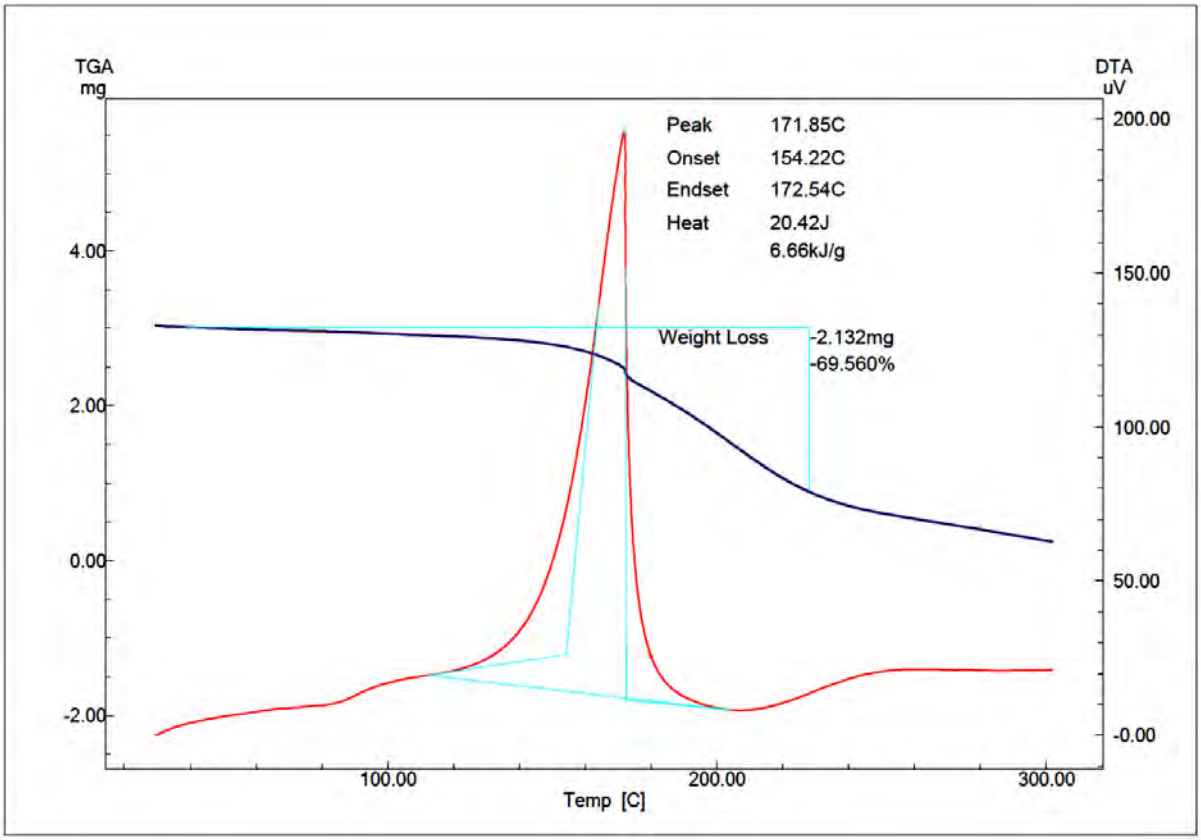
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Endset 172.54C
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-69.560%
4.00
2.00
0.00
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Temp [C]

COMPOUND 5

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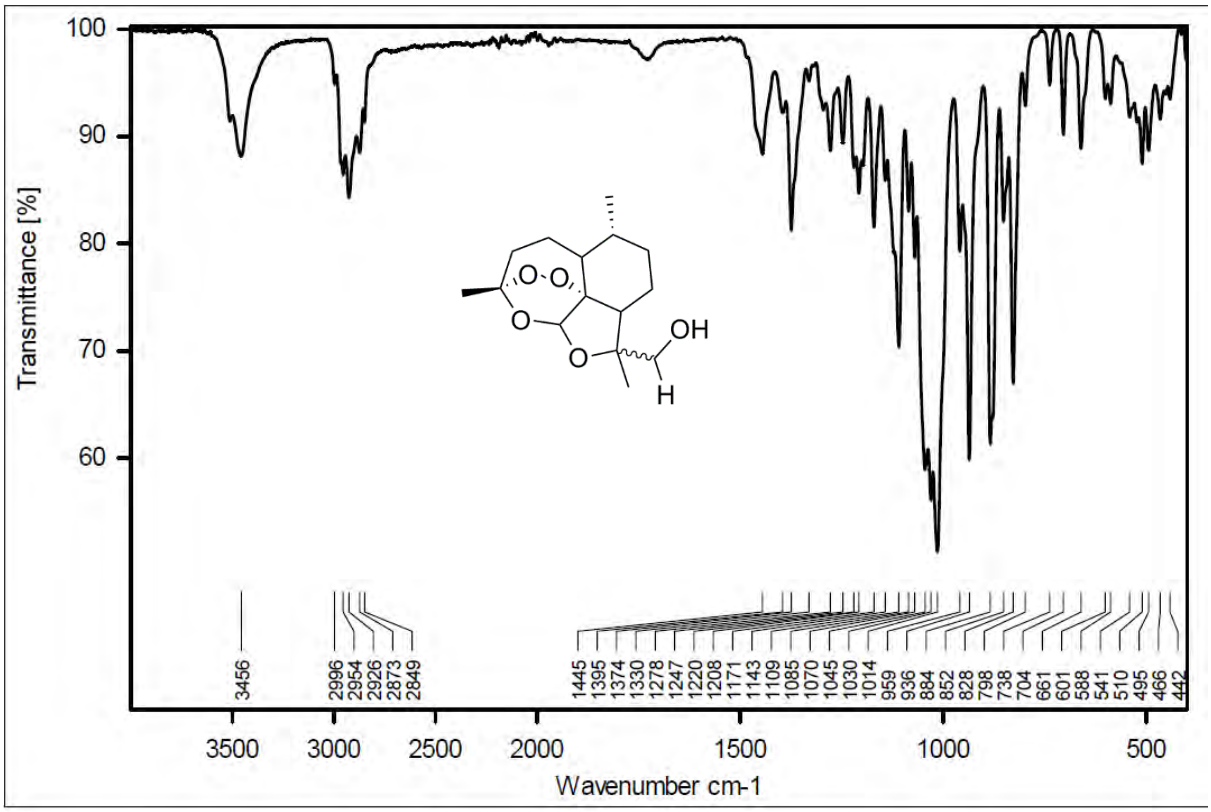

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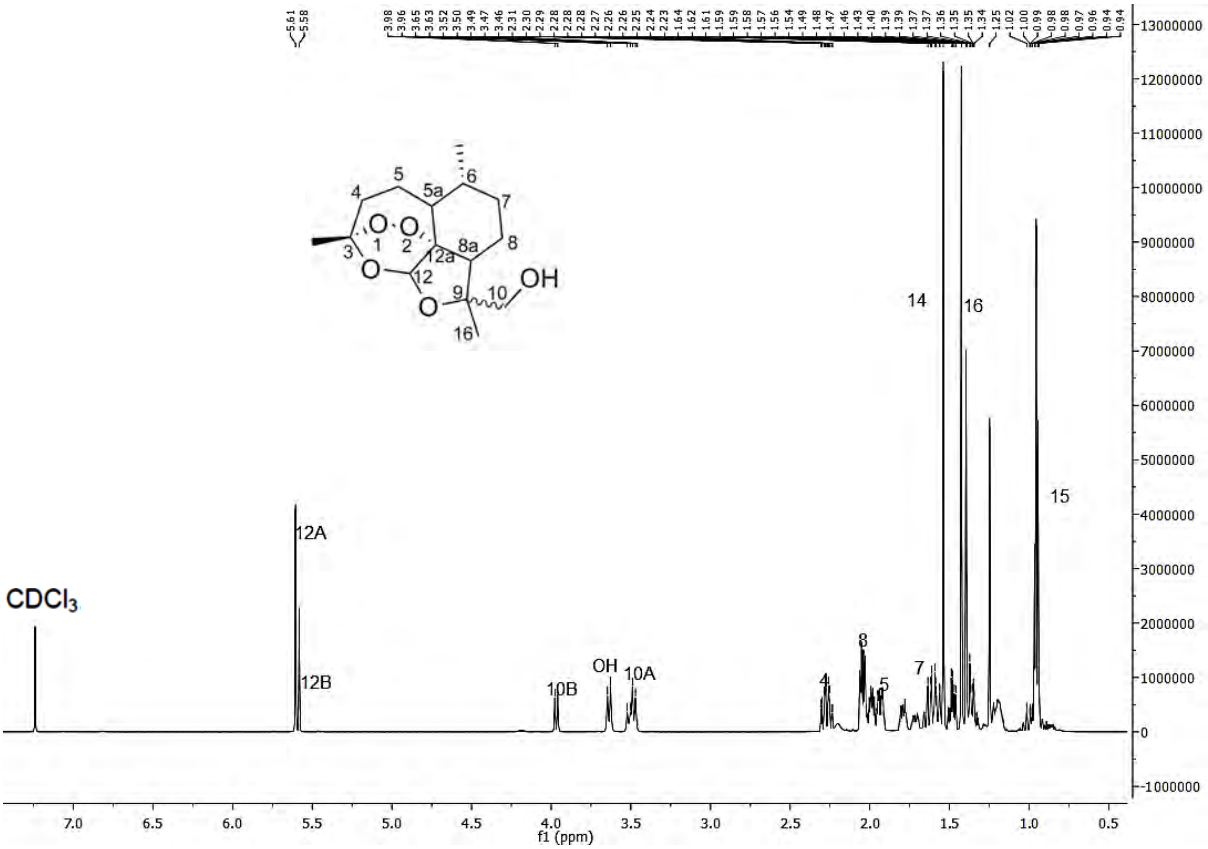

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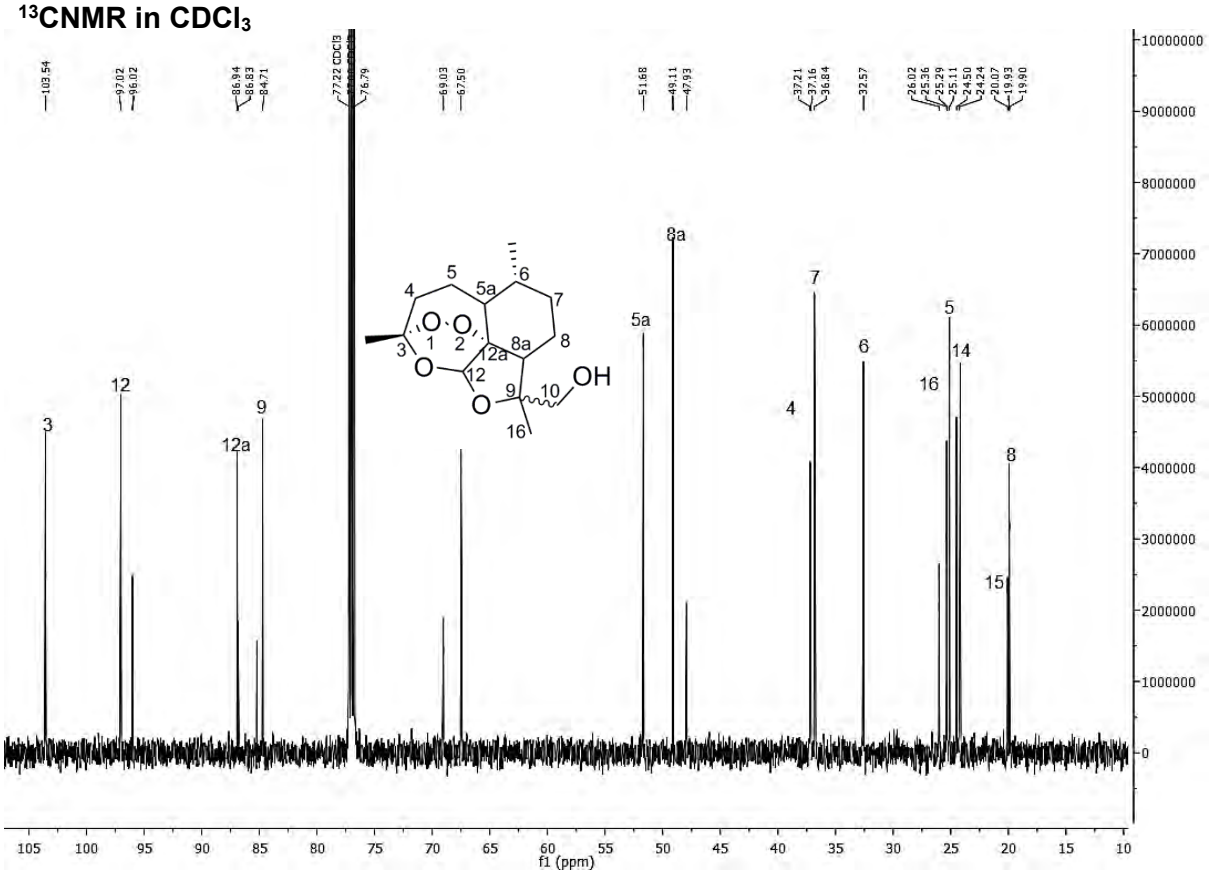

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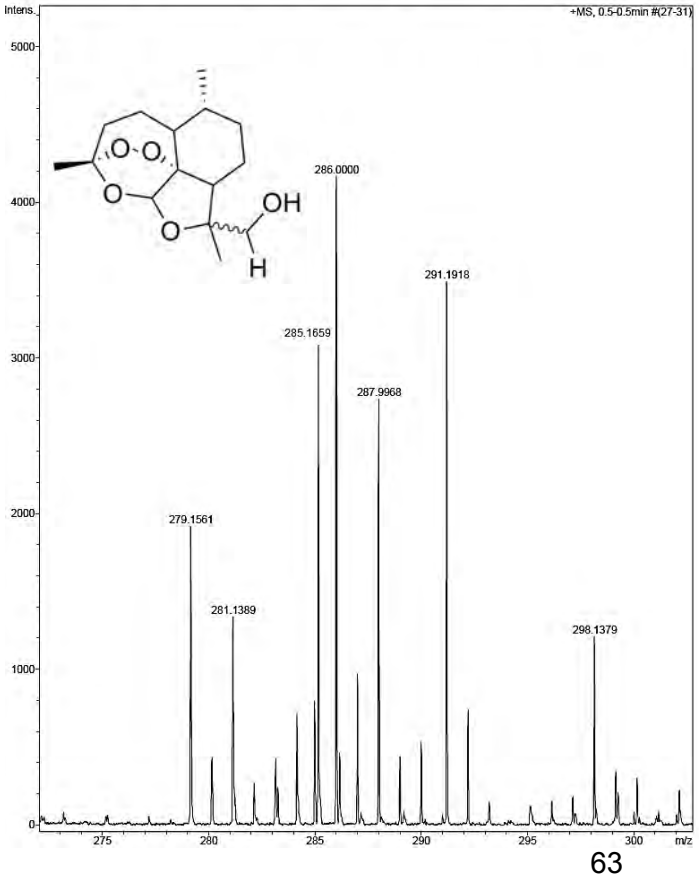

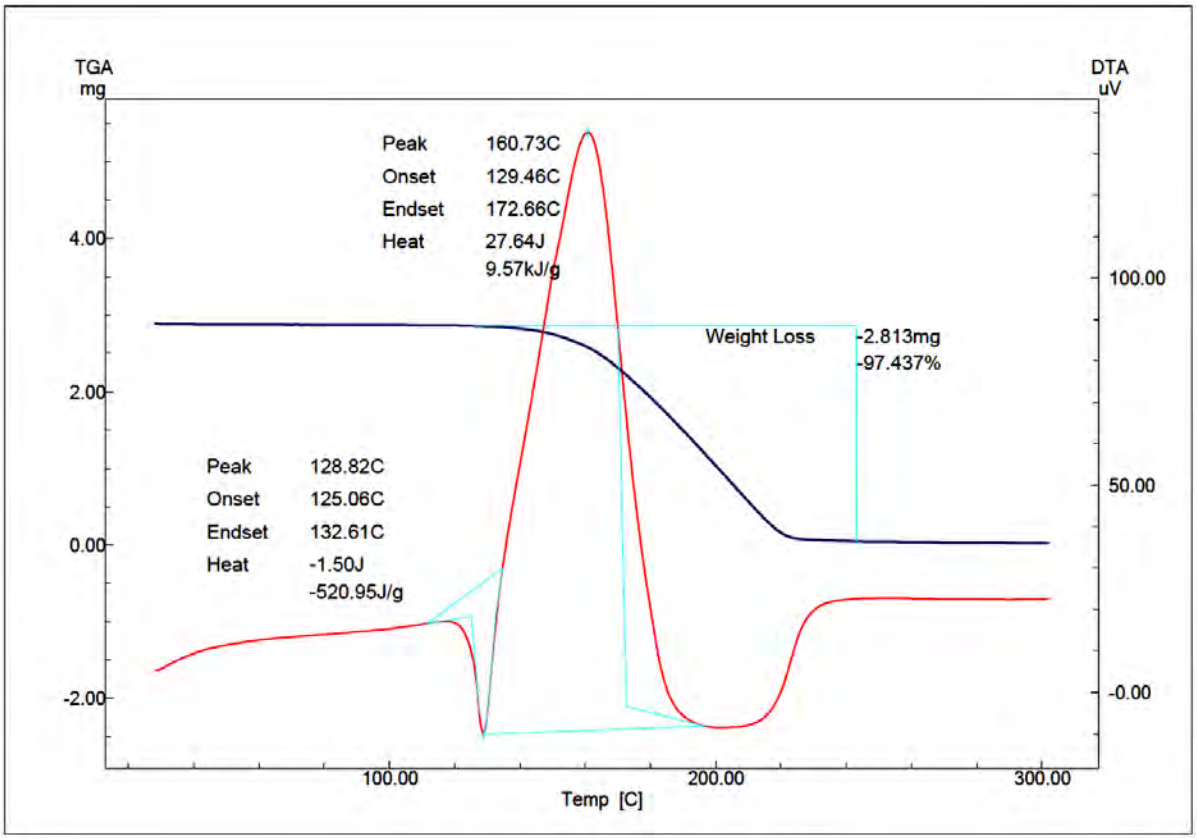
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mg
DTA
uV
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Endset 172.66C
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Weight Loss -2.813mg
-97.437%
Peak 128.82C
Onset 125.06C
Endset 132.61C
Heat -1.50J
-520.95J/g
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Temp [C]

COMPOUND 6

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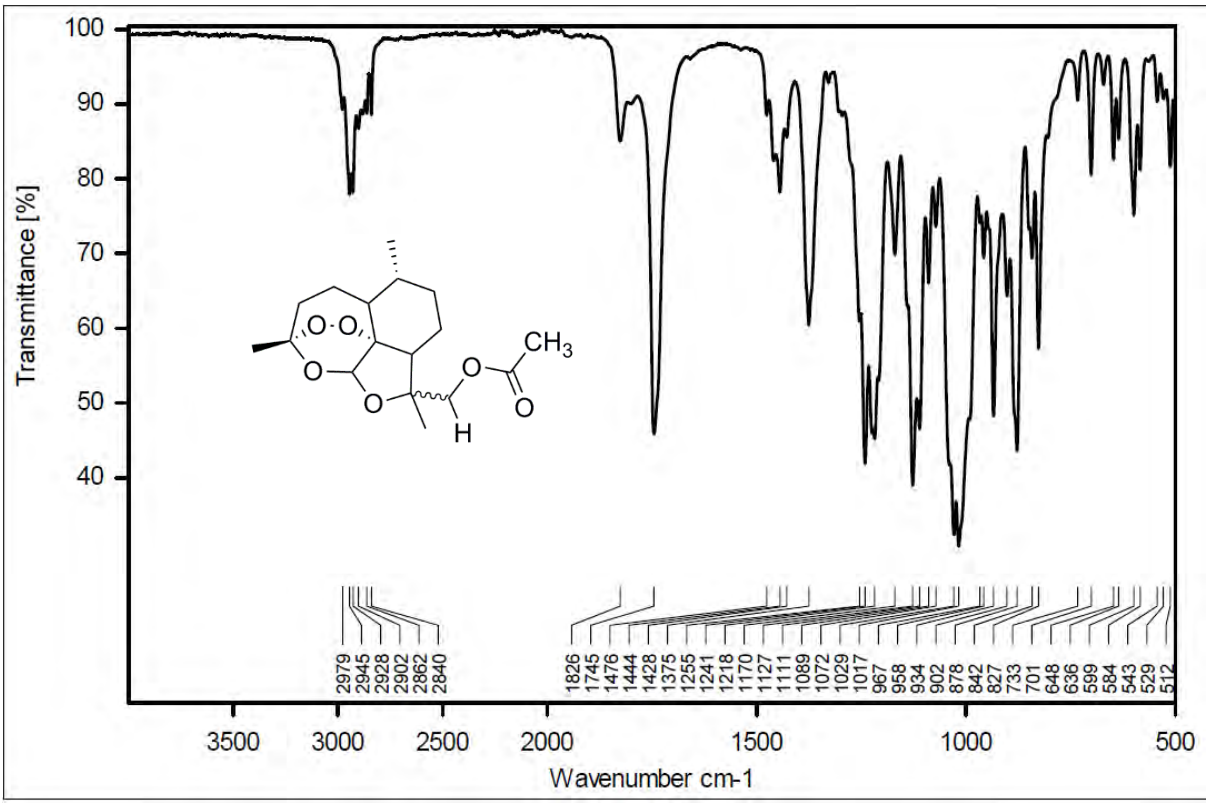

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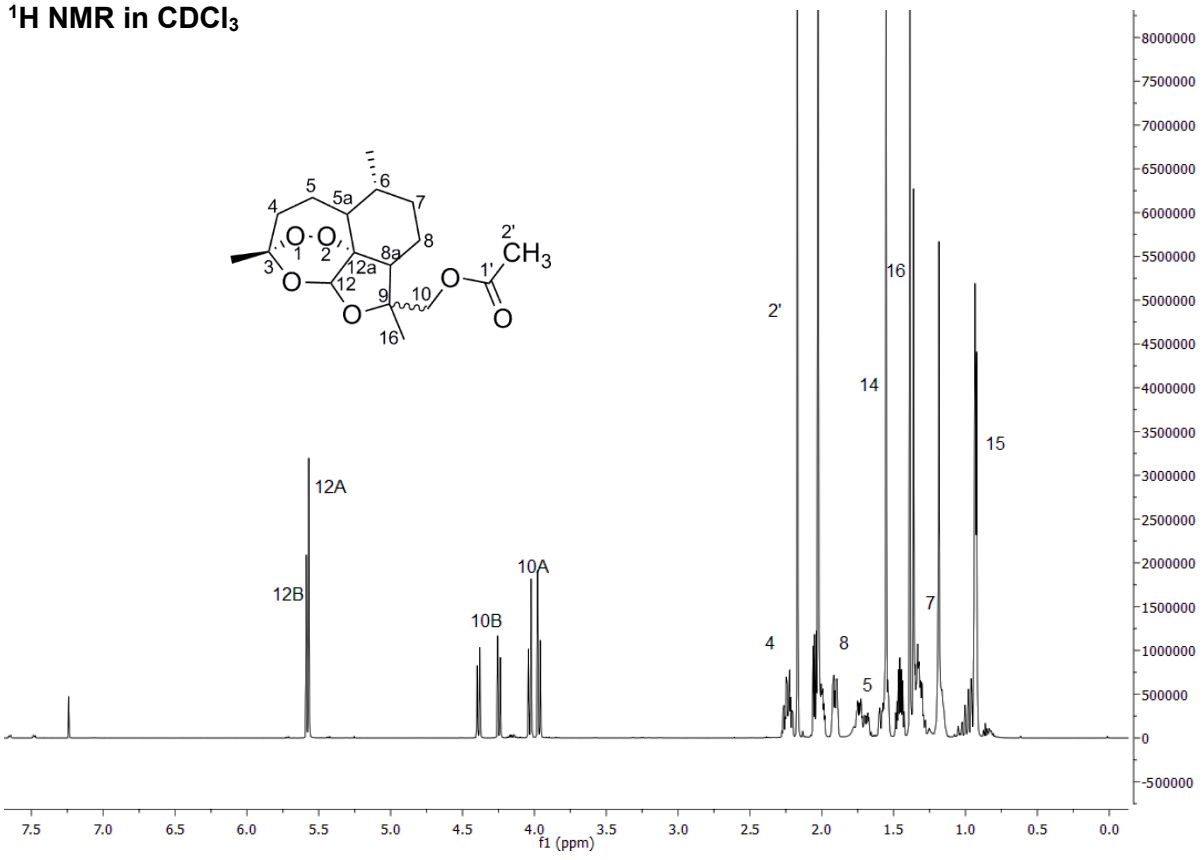

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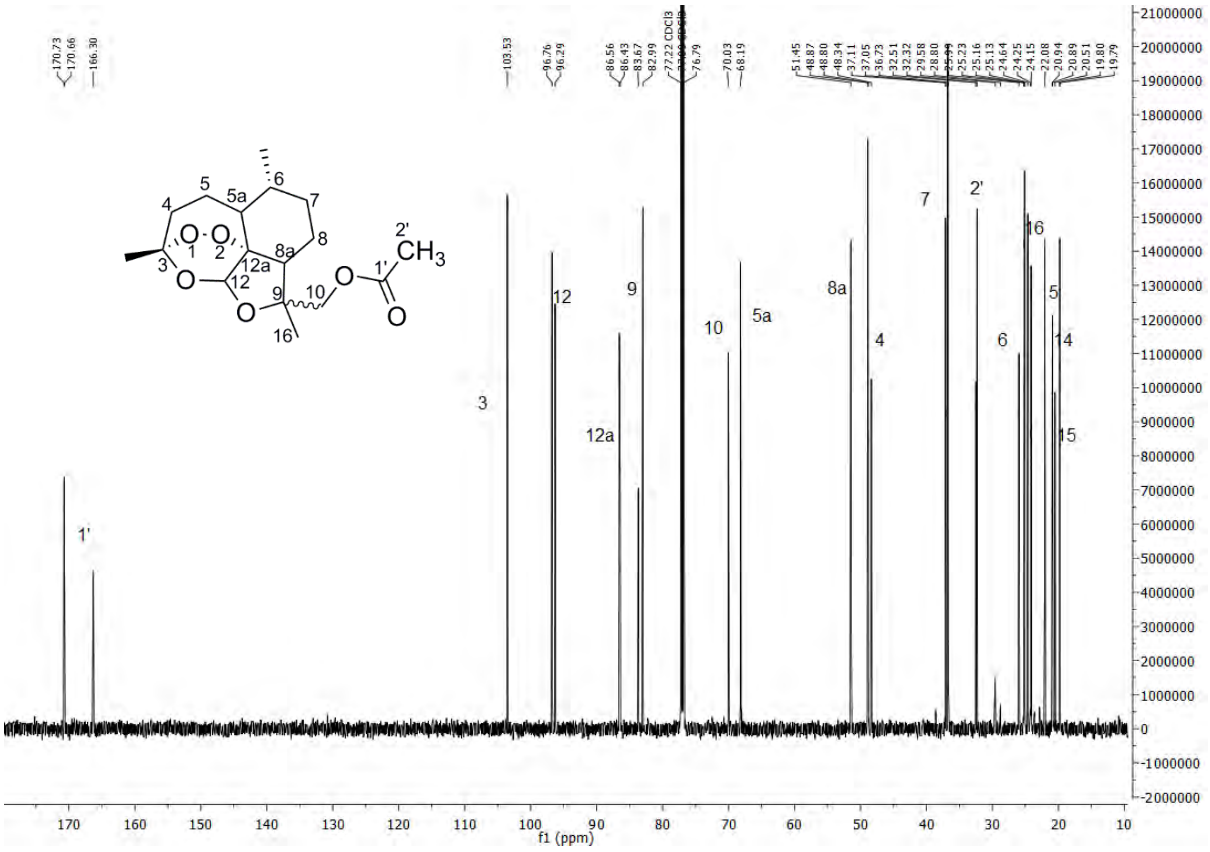

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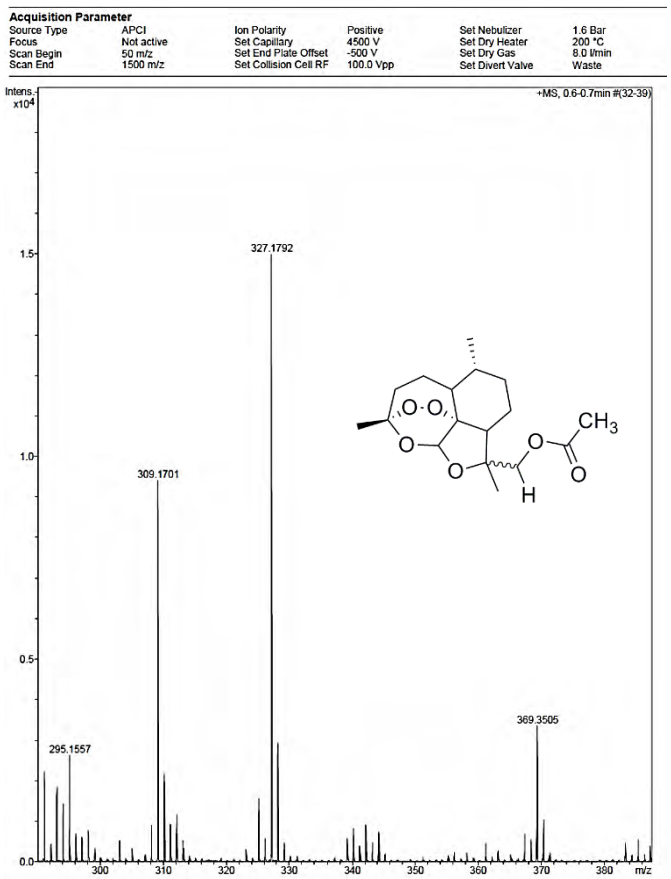

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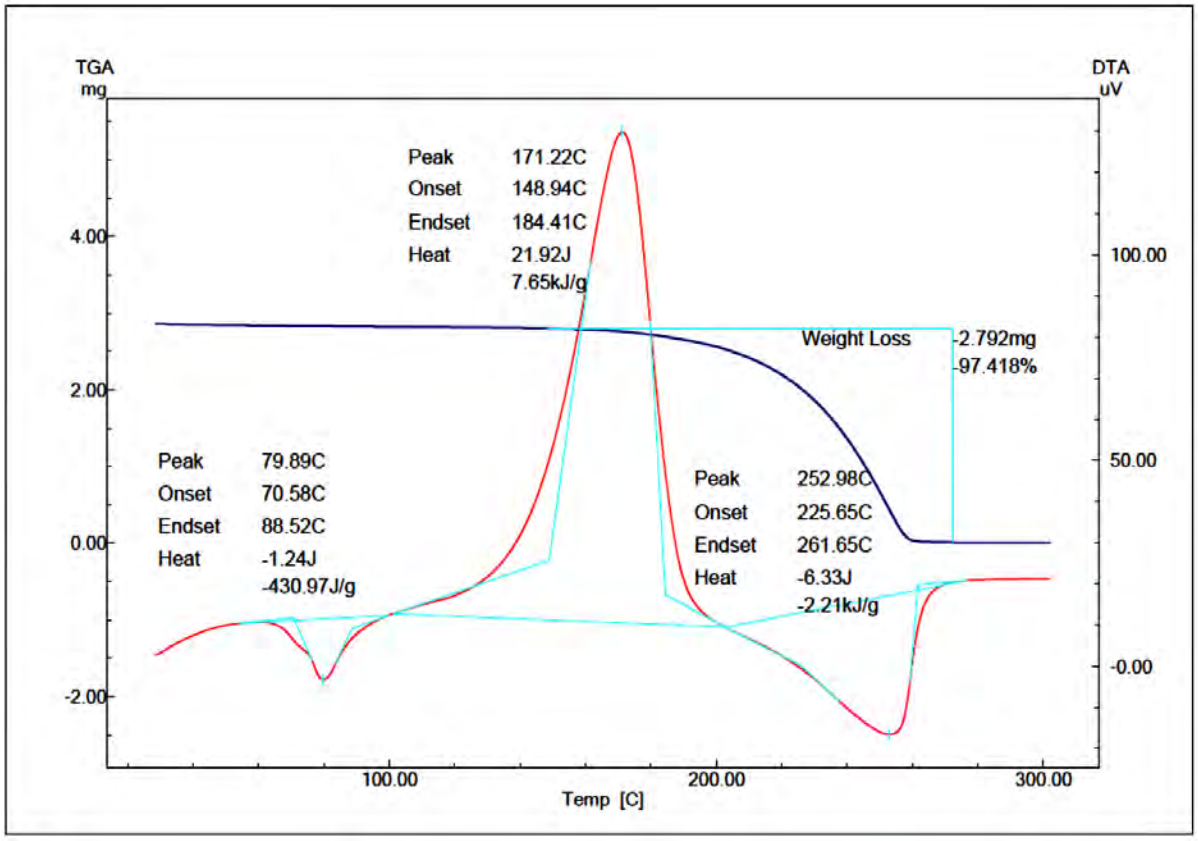
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mg
DTA
uV
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-97.418%
Peak 79.89C
Onset 70.58C
Endset 88.52C
Heat -1.24J
-430.97J/g
Peak 252.98C
Onset 225.65C
Endset 261.65C
Heat -6.33J
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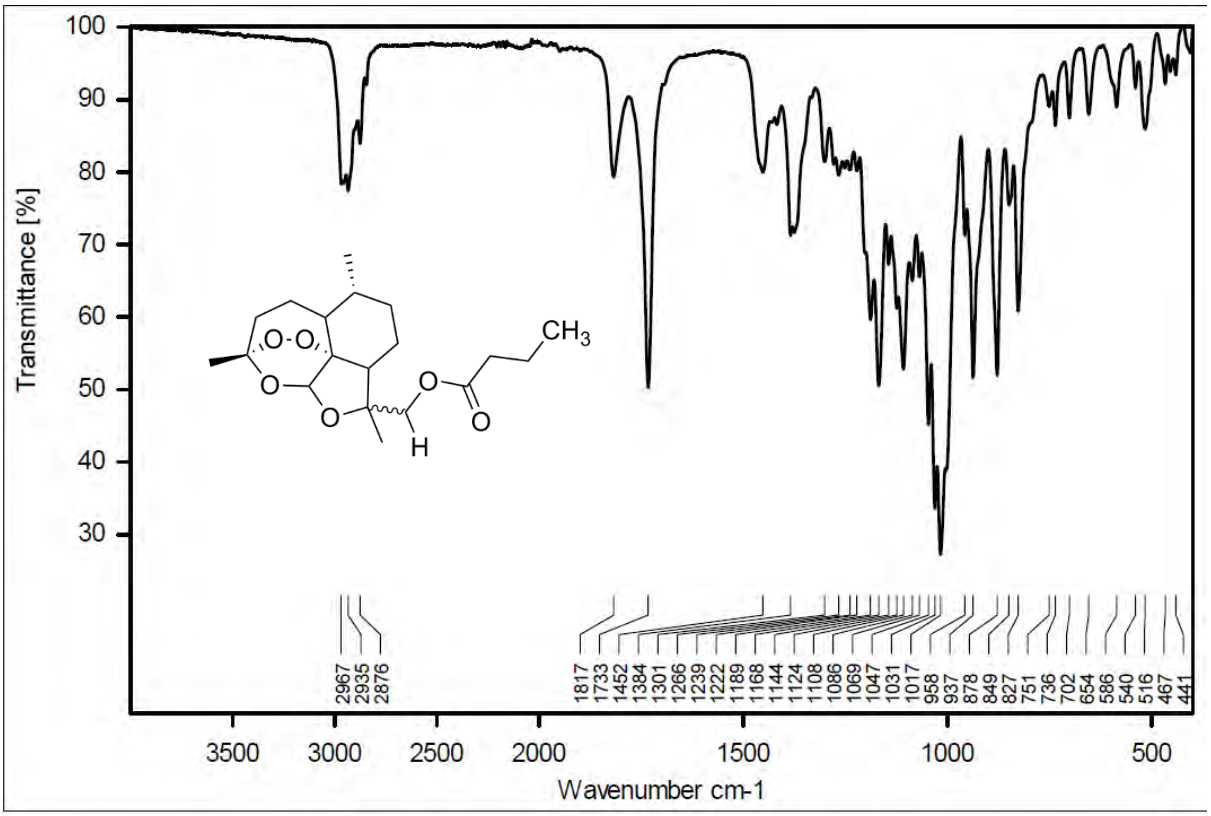

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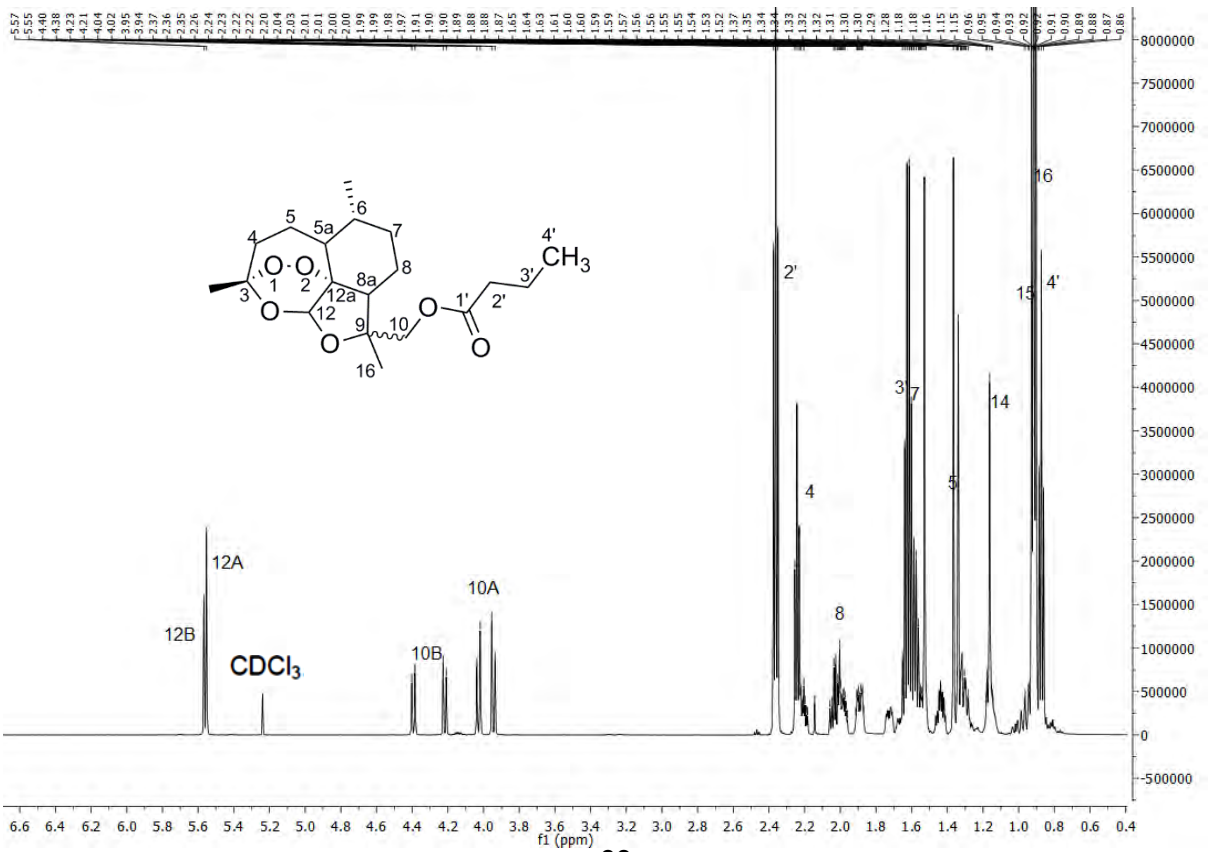

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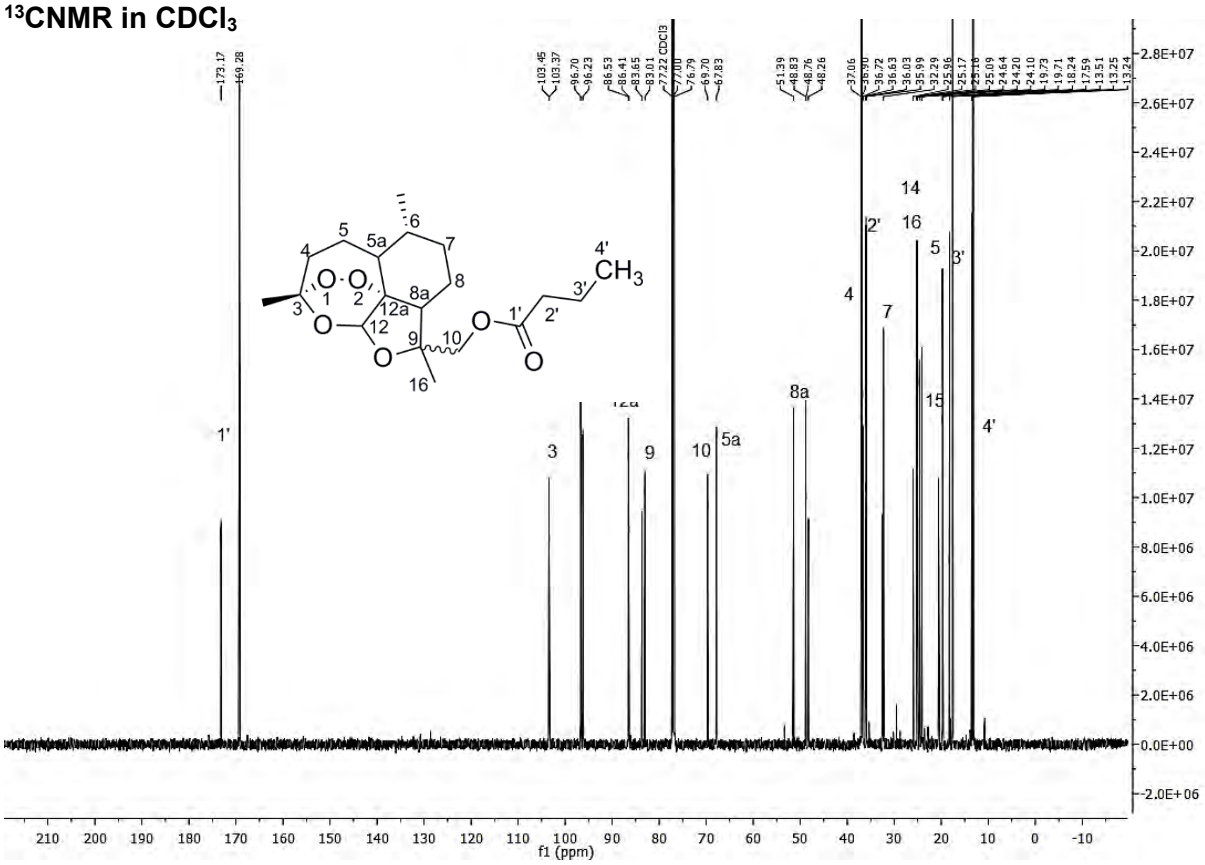

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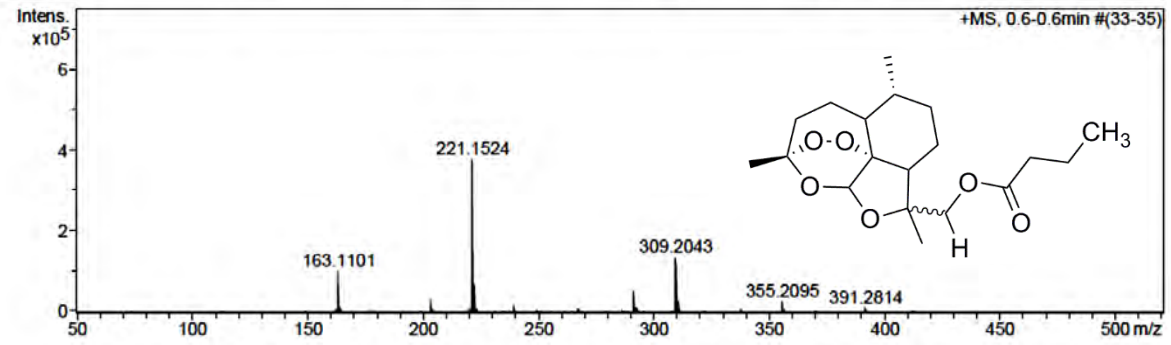

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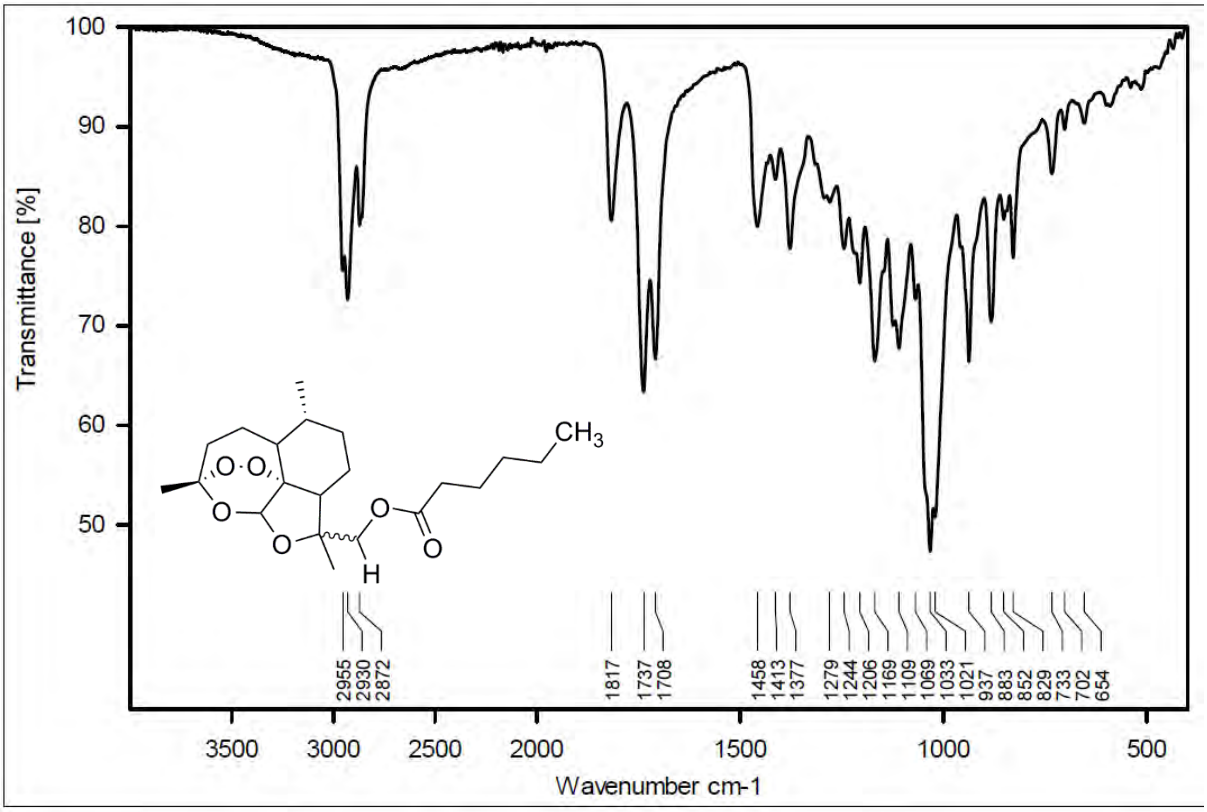

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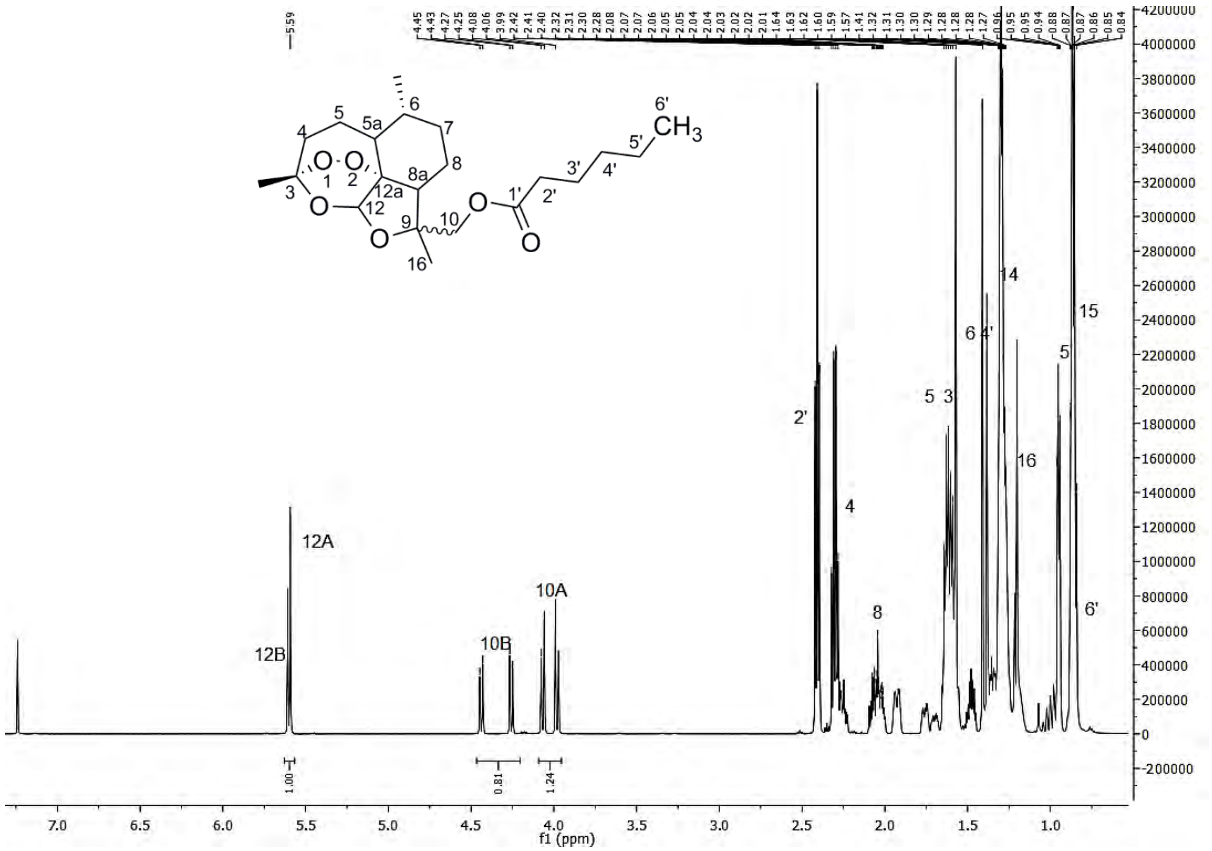

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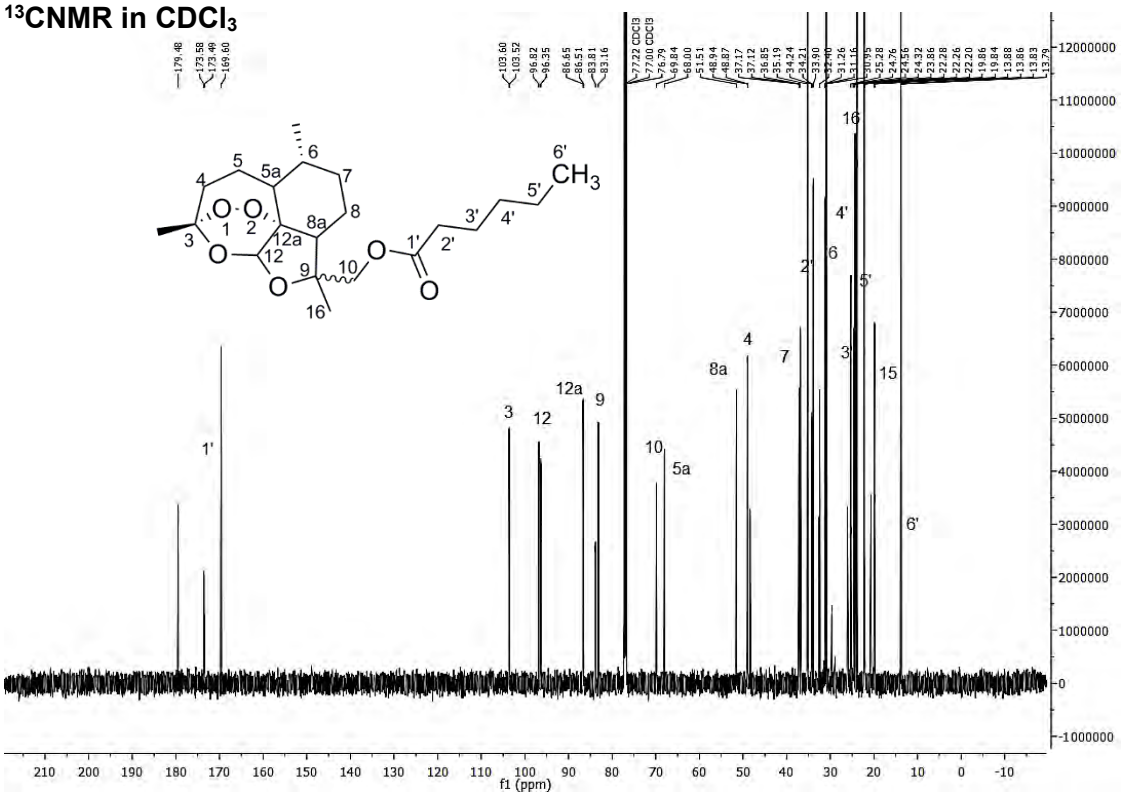

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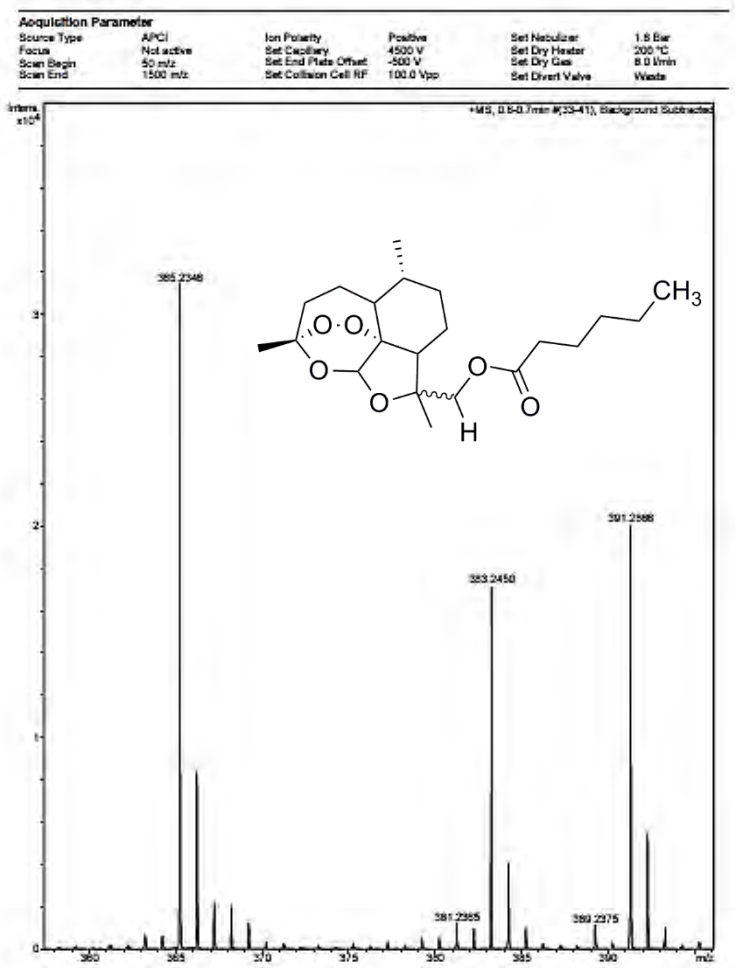

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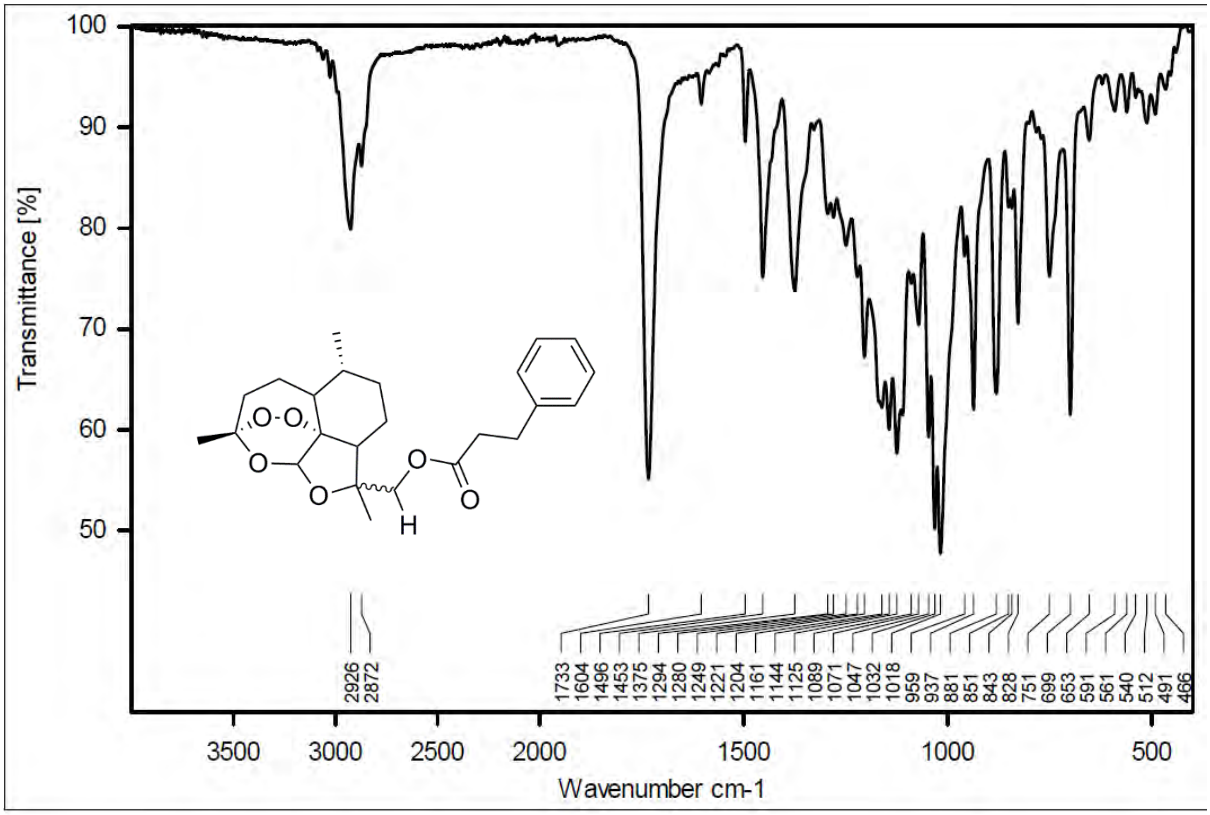

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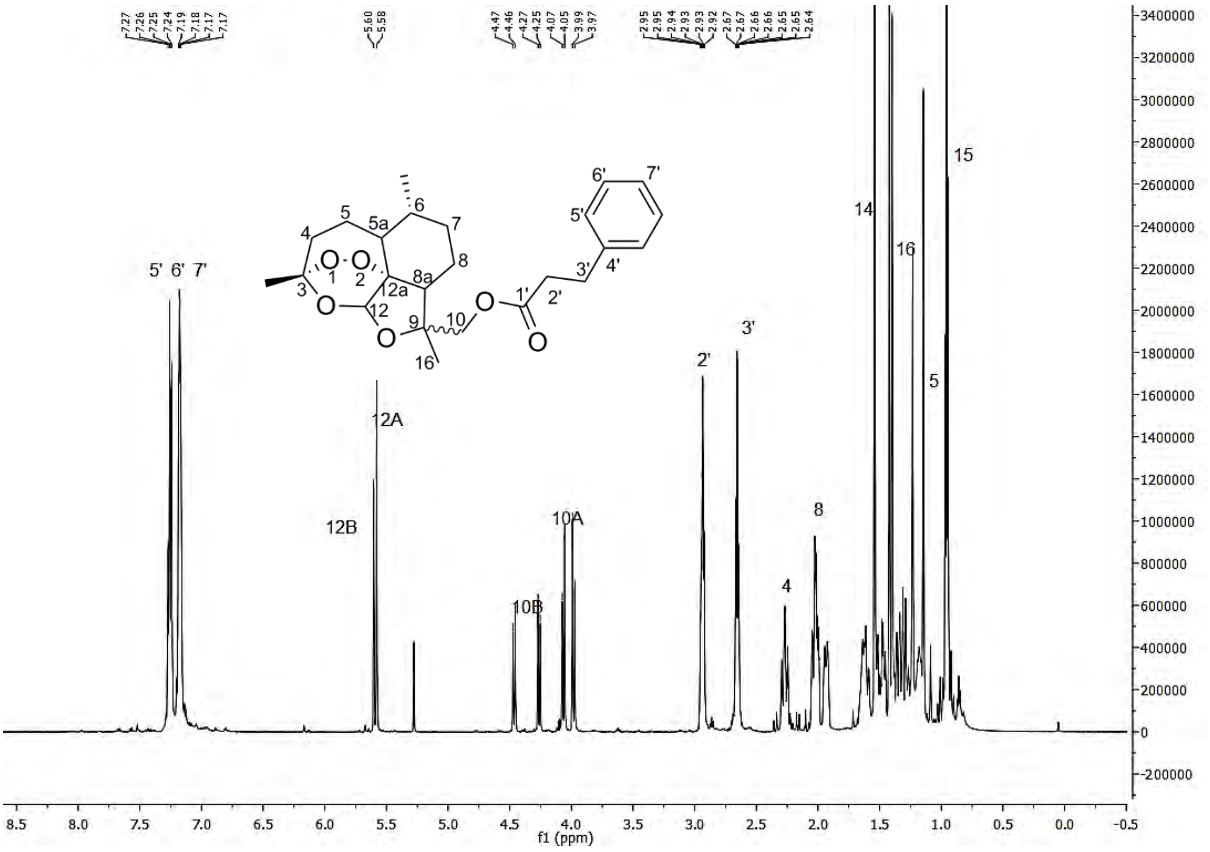

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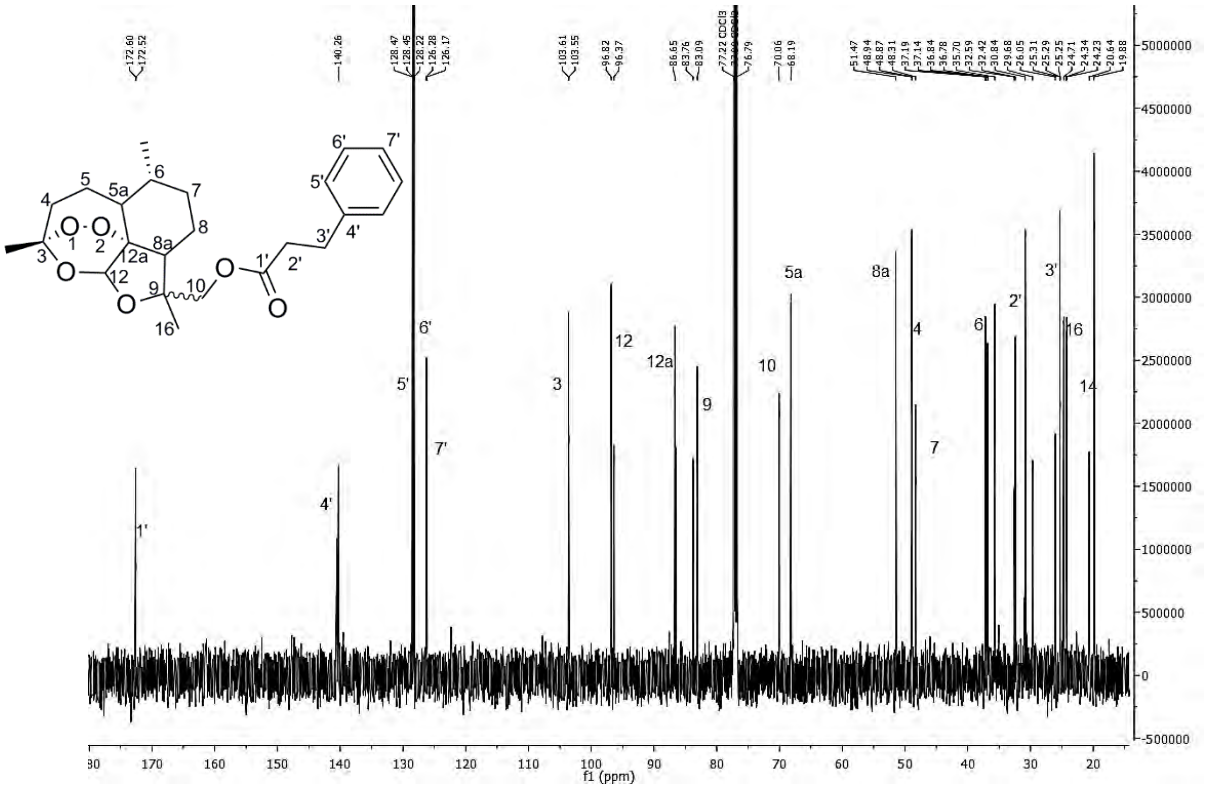

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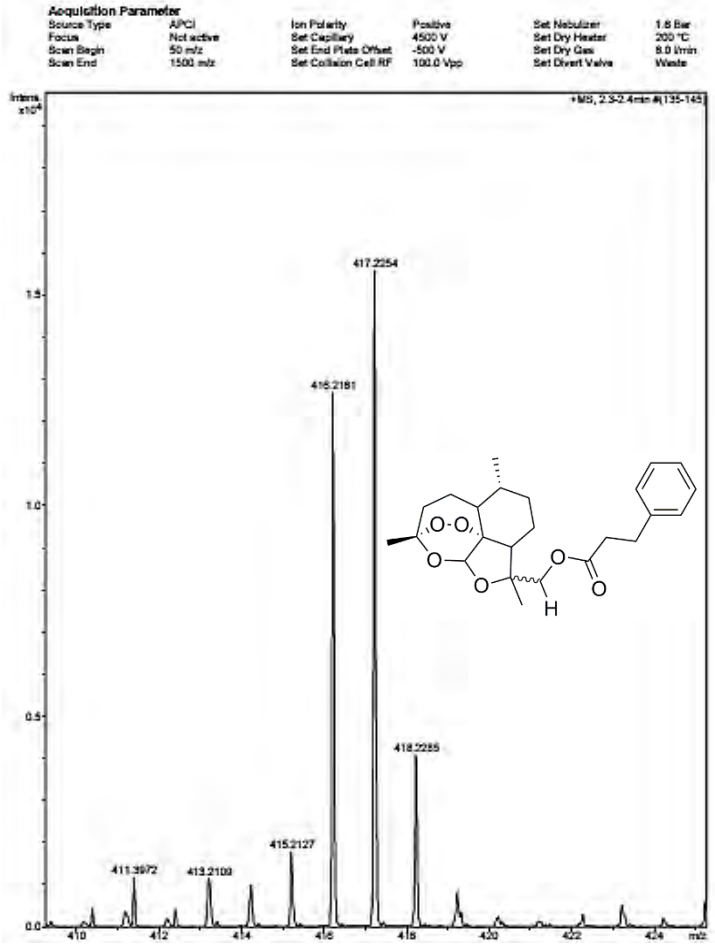

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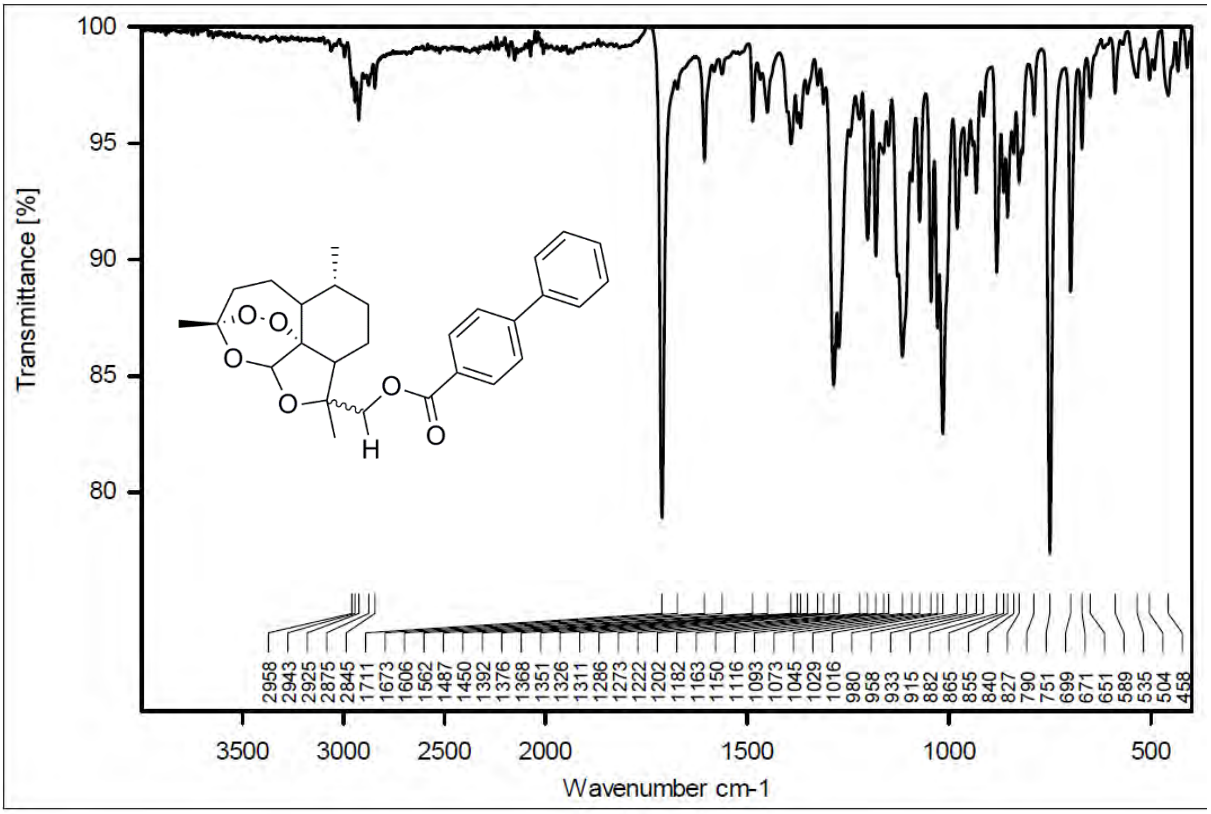

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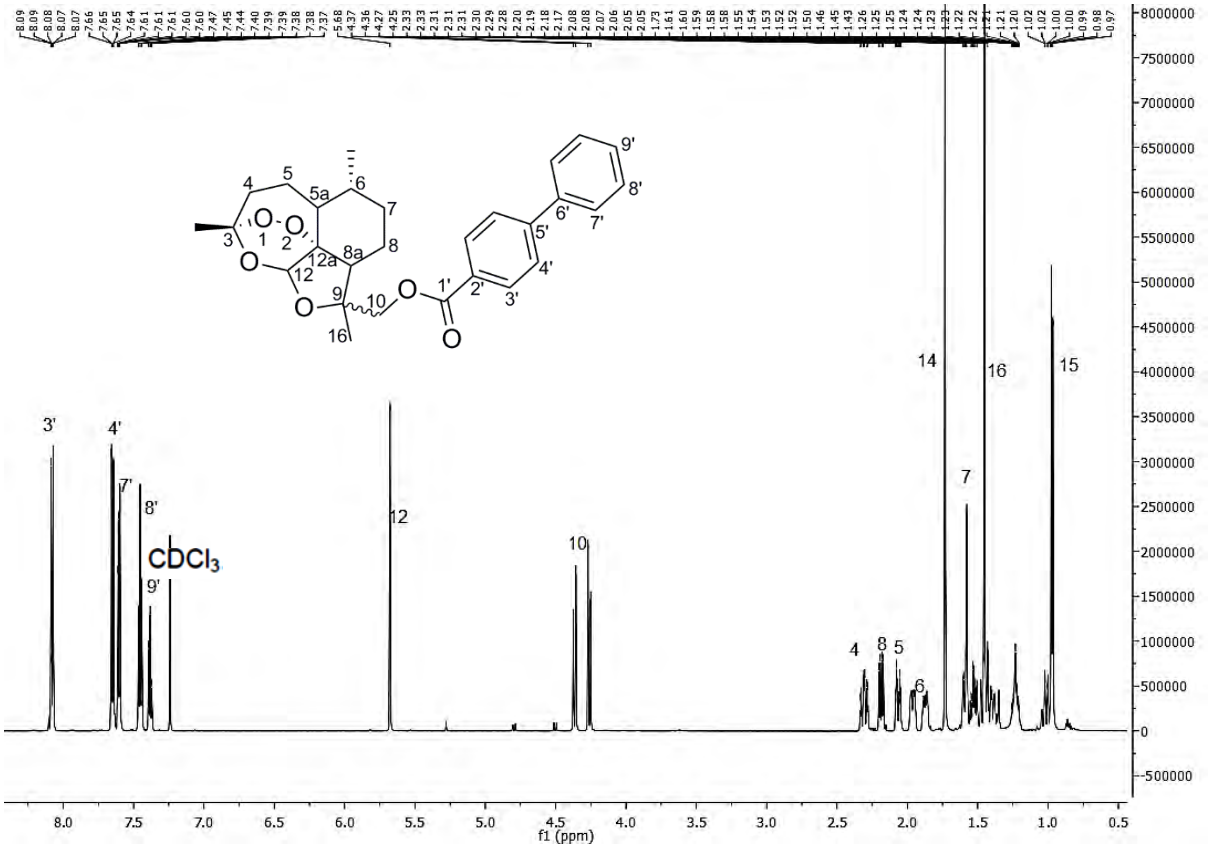
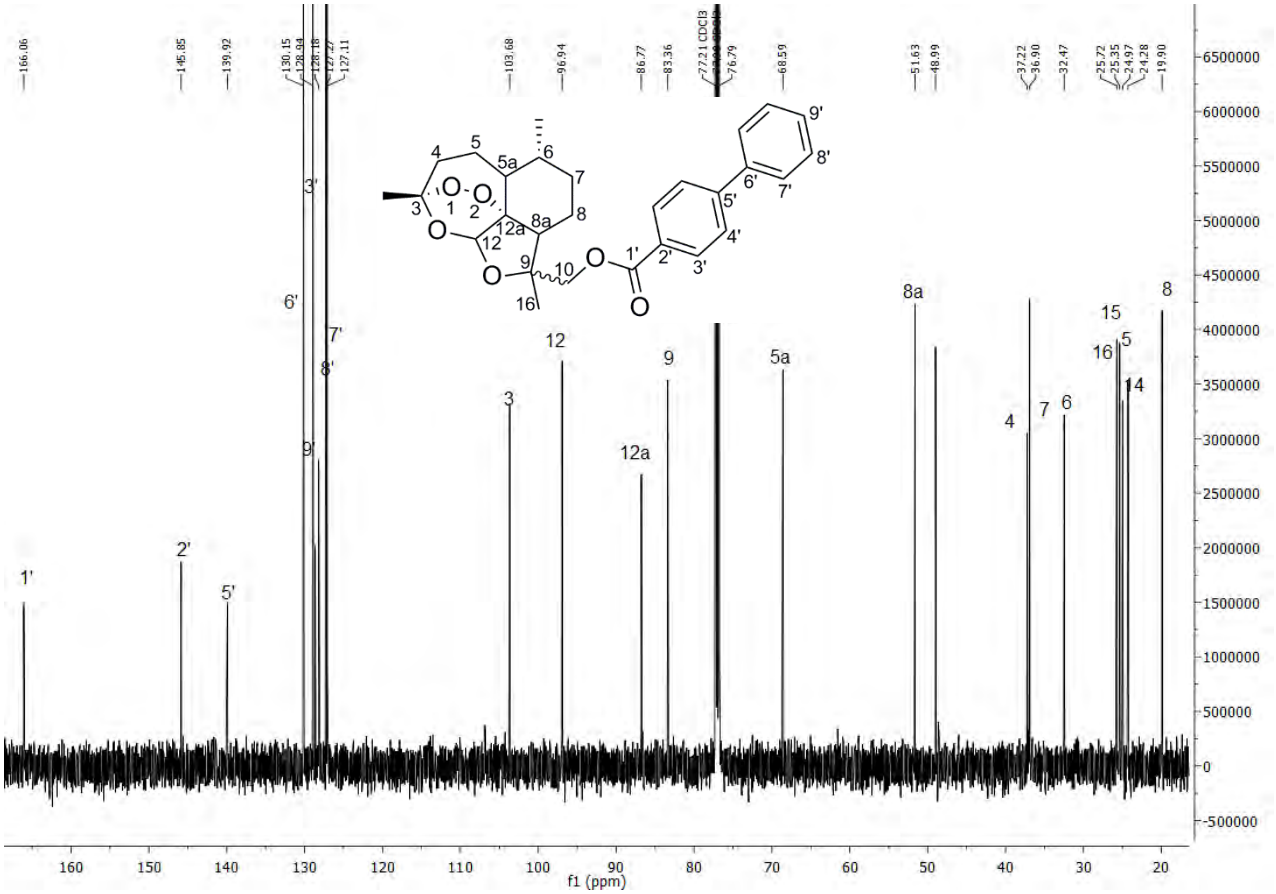


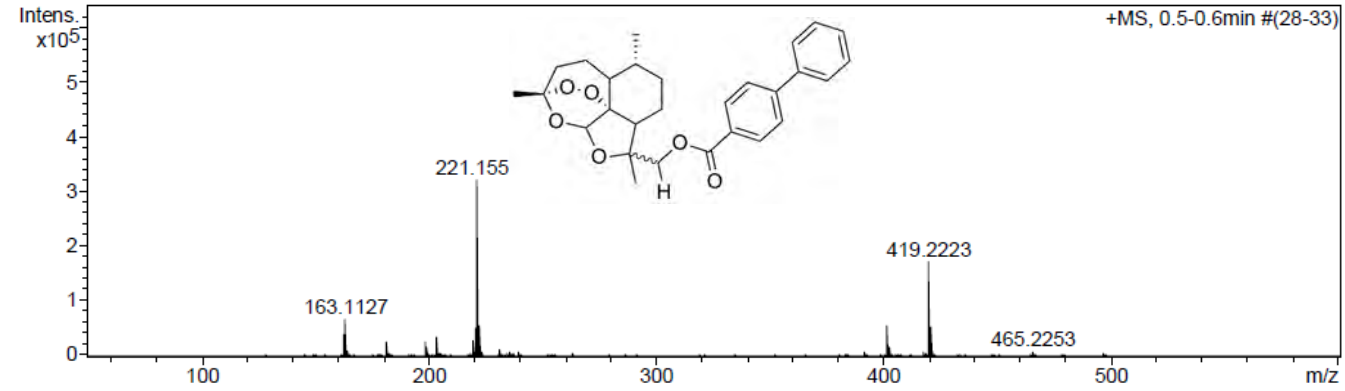
^{13}CNMR in $CDCl_3$

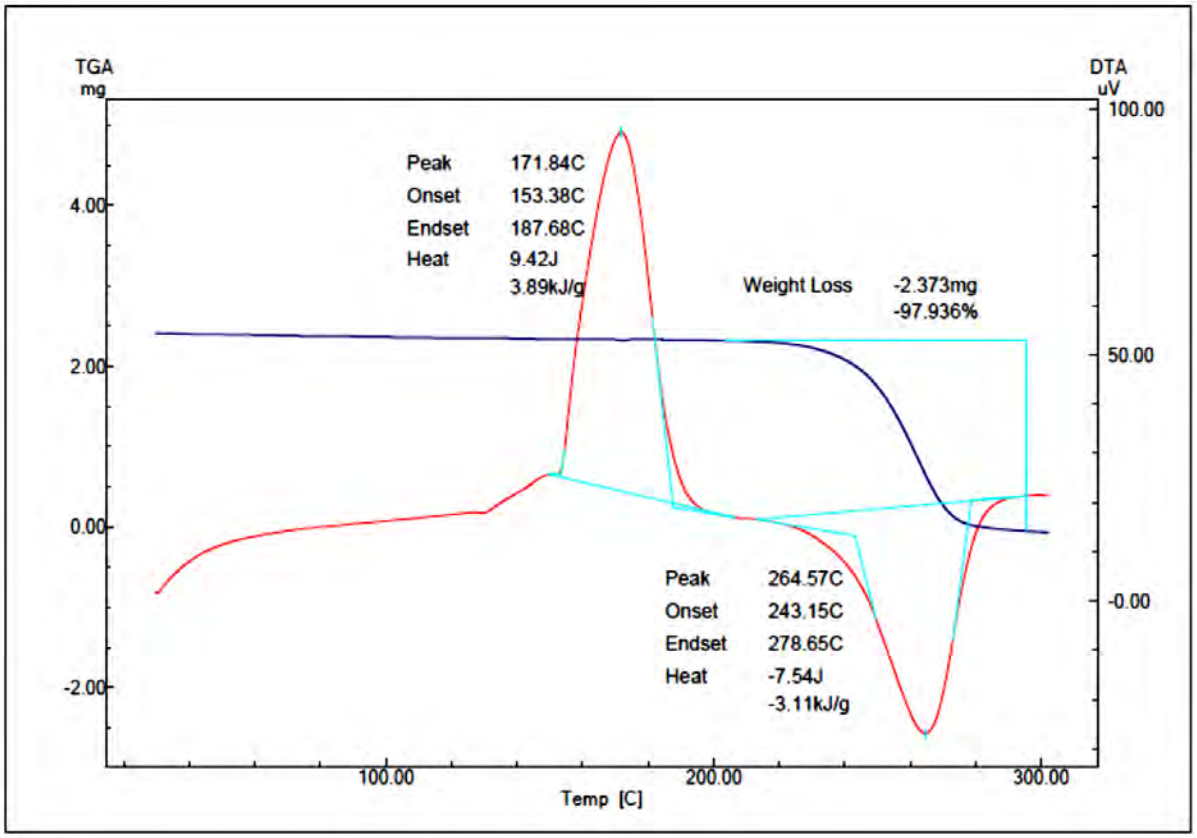


HRMS

Acquisition Parameter

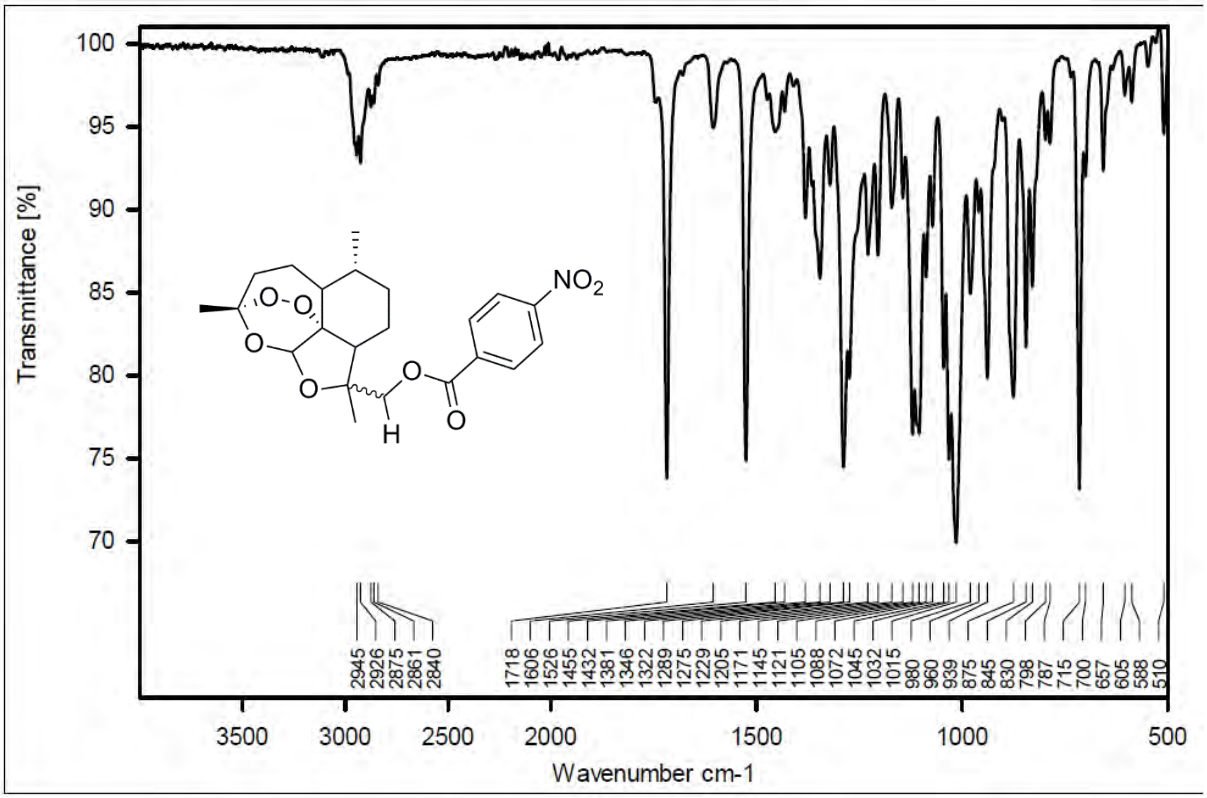
Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	1500 m/z	Set Collision Cell RF	100.0 Vpp	Set Divert Valve	Waste



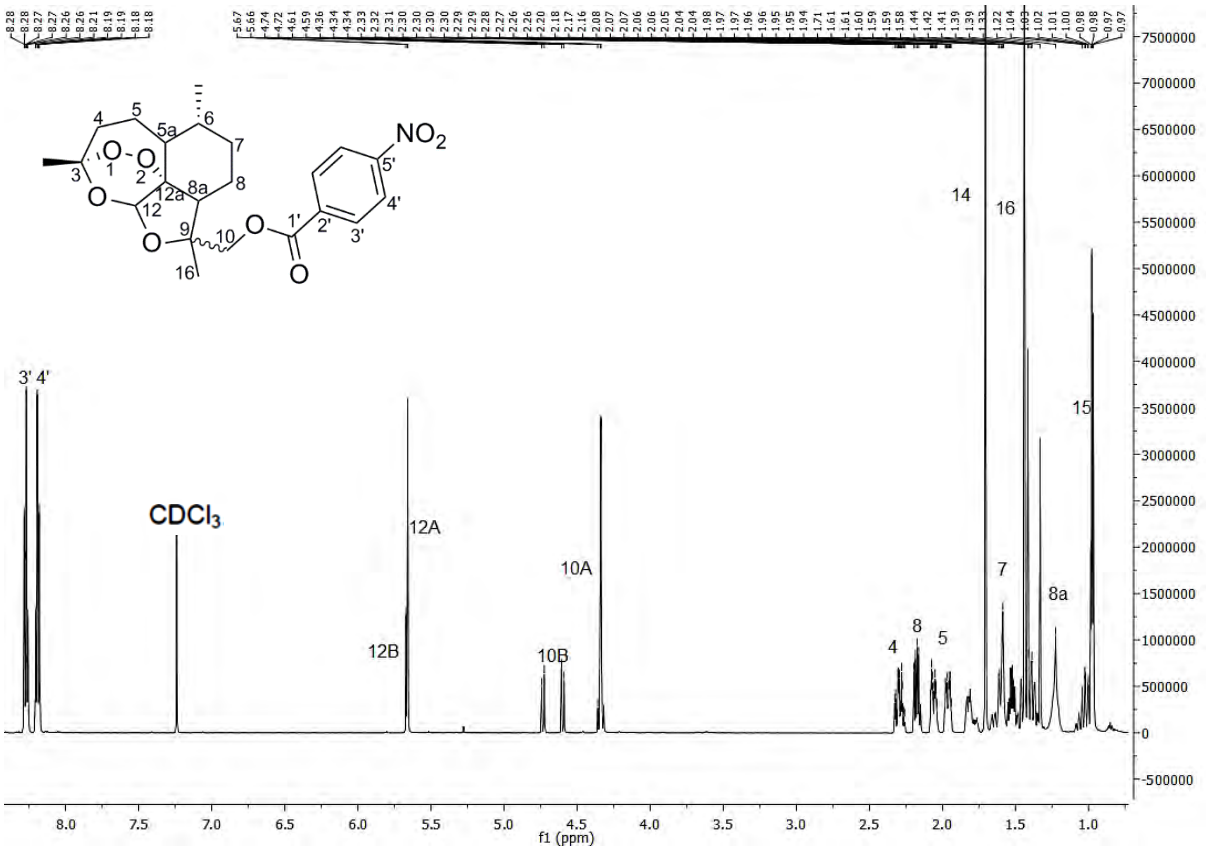

TGA
mg
DTA
uV
100.00
4.00
2.00
0.00
-2.00
50.00
-0.00
Peak 171.84C
Onset 153.38C
Endset 187.68C
Heat 9.42J
3.89kJ/g
Weight Loss -2.373mg
-97.936%
Peak 264.57C
Onset 243.15C
Endset 278.65C
Heat -7.54J
-3.11kJ/g
100.00
200.00
300.00
Temp [C]

COMPOUND 11

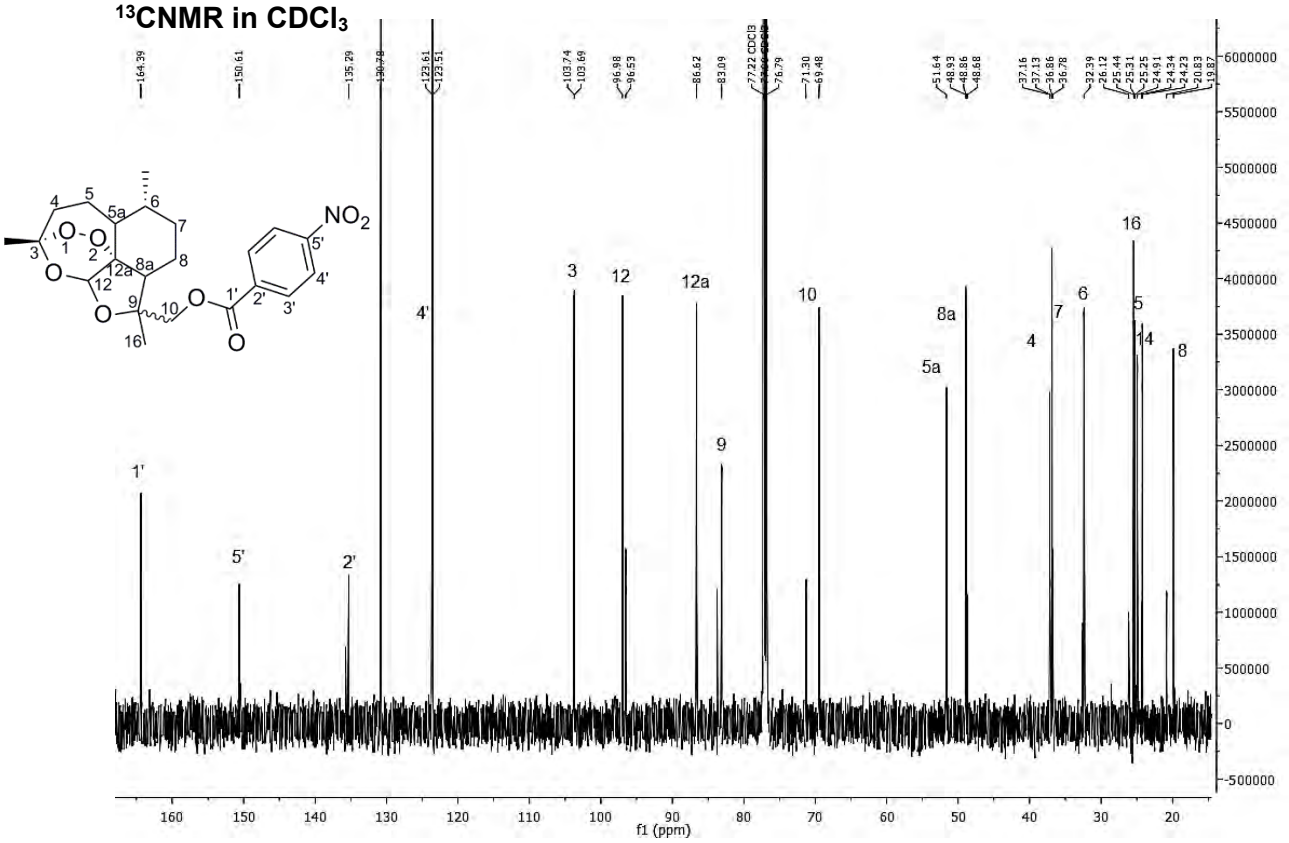
IR



^{1}H NMR in $CDCl_3$



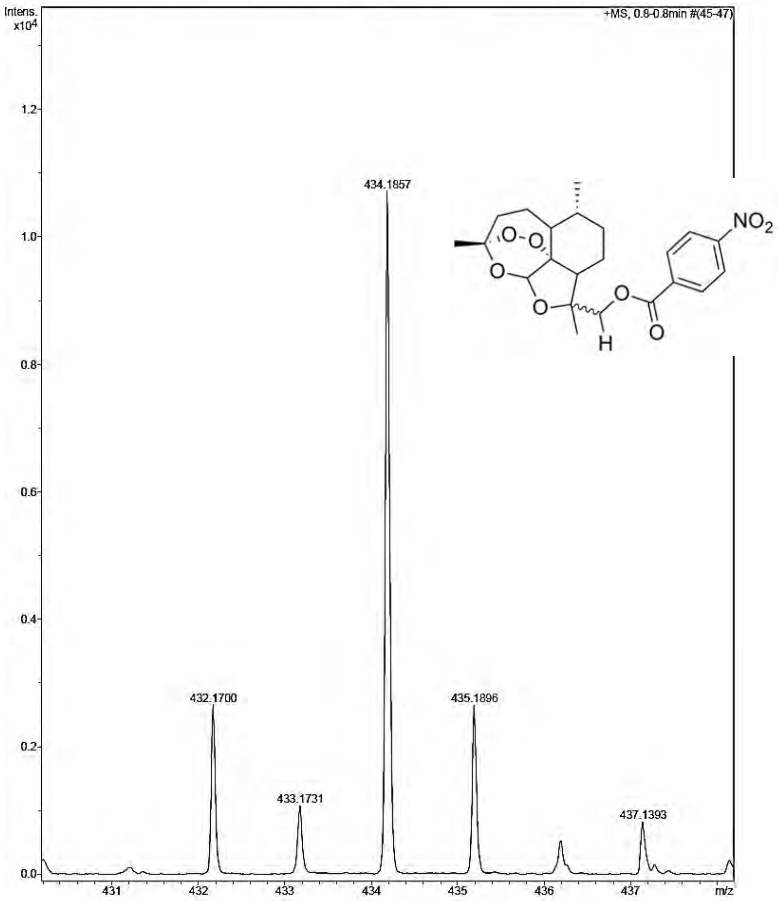
^{13}CNMR in $CDCl_3$

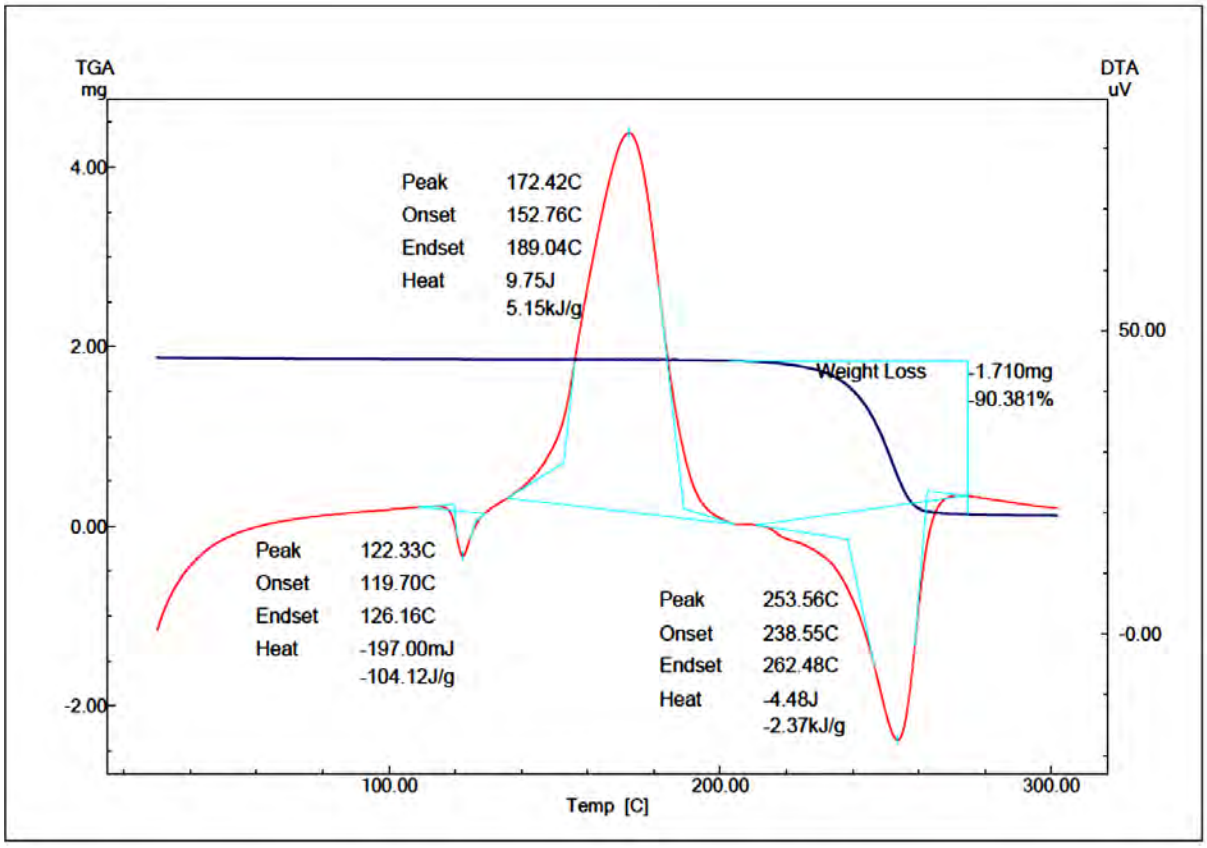


HRMS

Acquisition Parameter

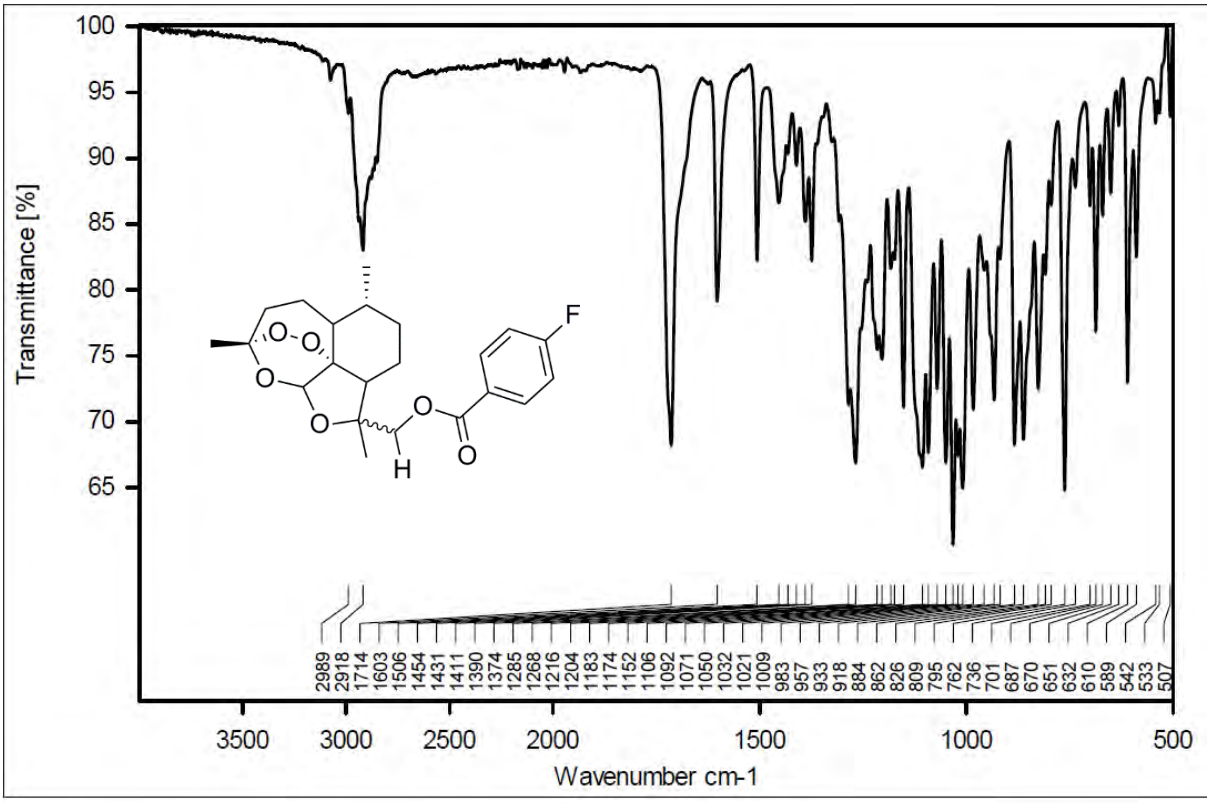
Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	1500 m/z	Set Collision Cell RF	100.0 Vpp	Set Divert Valve	Waste



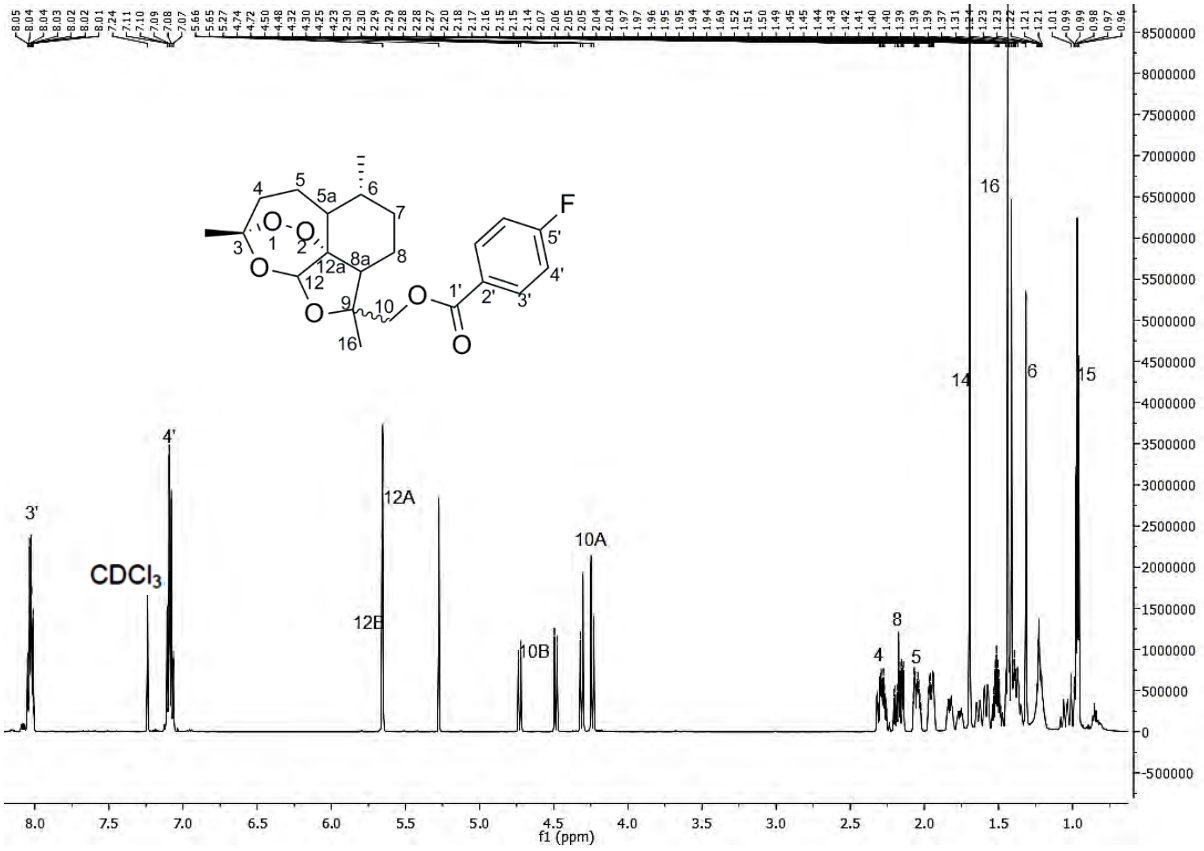

TGA
mg
DTA
uV
4.00
2.00
0.00
-2.00
50.00
-0.00
Peak 172.42C
Onset 152.76C
Endset 189.04C
Heat 9.75J
5.15kJ/g
Weight Loss -1.710mg
-90.381%
Peak 122.33C
Onset 119.70C
Endset 126.16C
Heat -197.00mJ
-104.12J/g
Peak 253.56C
Onset 238.55C
Endset 262.48C
Heat -4.48J
-2.37kJ/g
100.00
200.00
300.00
Temp [C]

COMPOUND 12

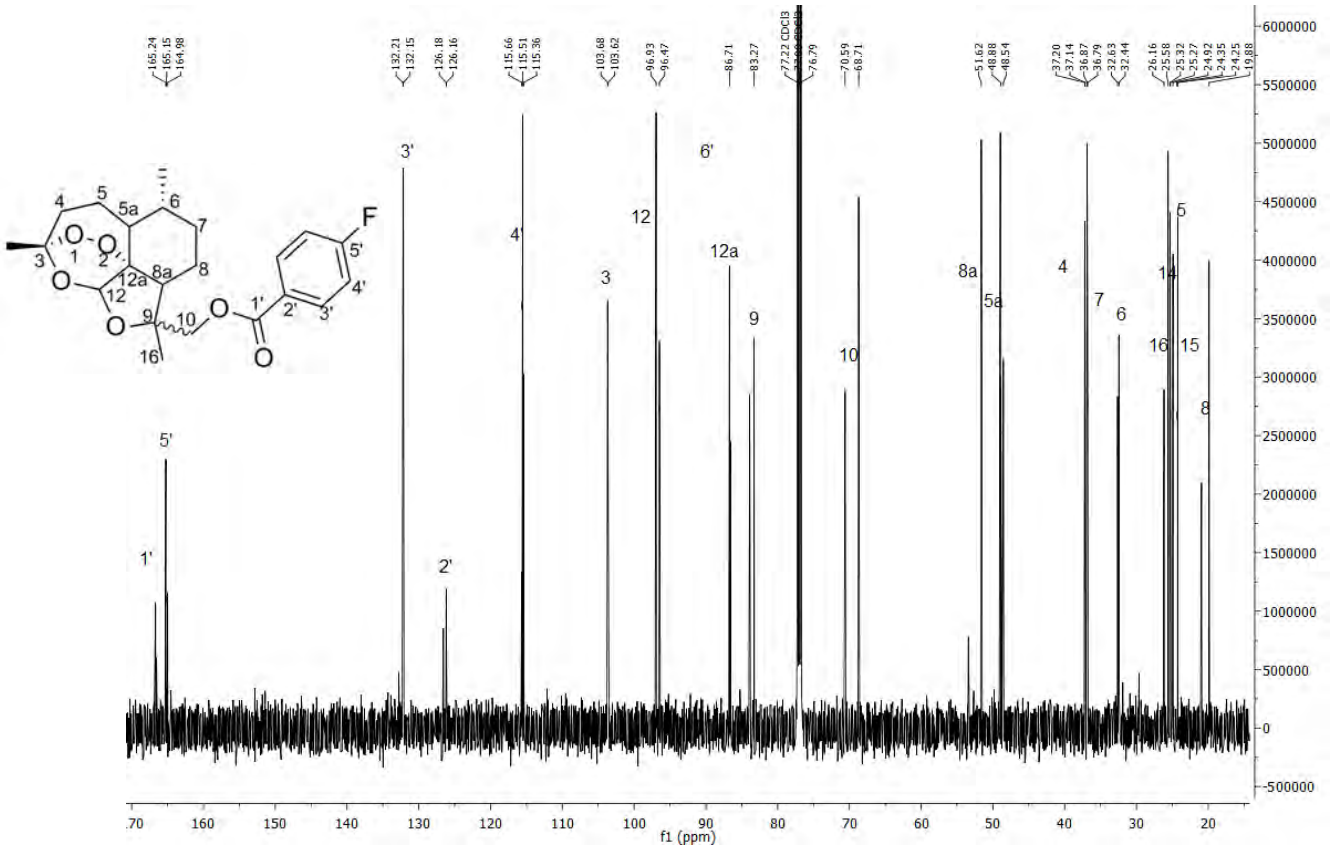
IR



^{1}H NMR in $CDCl_3$



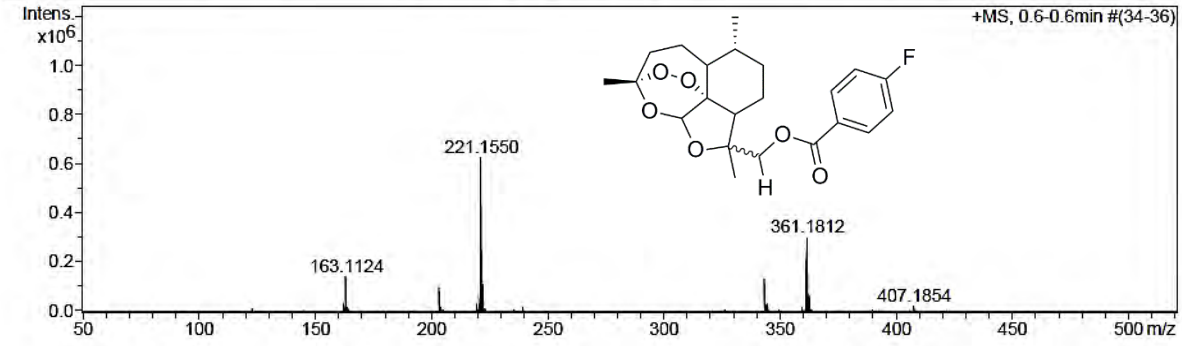
^{13}CNMR in $CDCl_3$

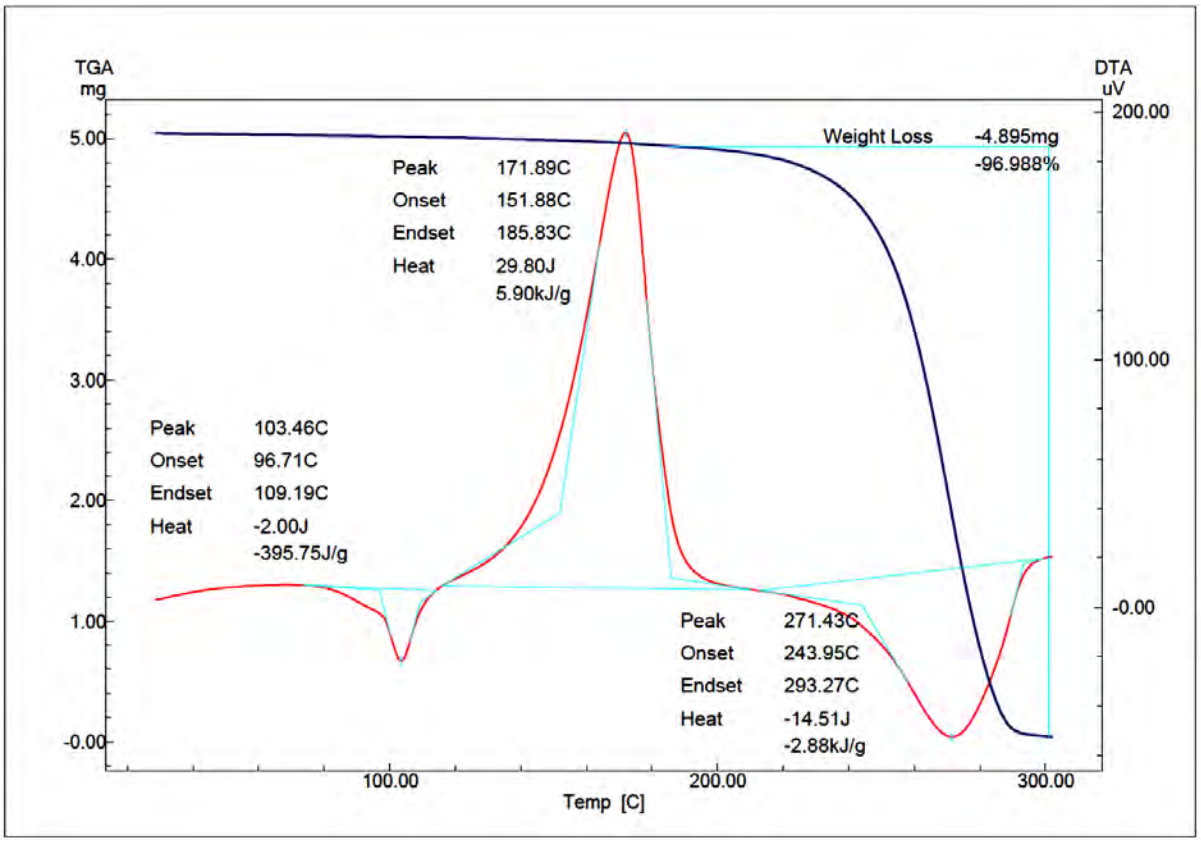


HRMS

Acquisition Parameter

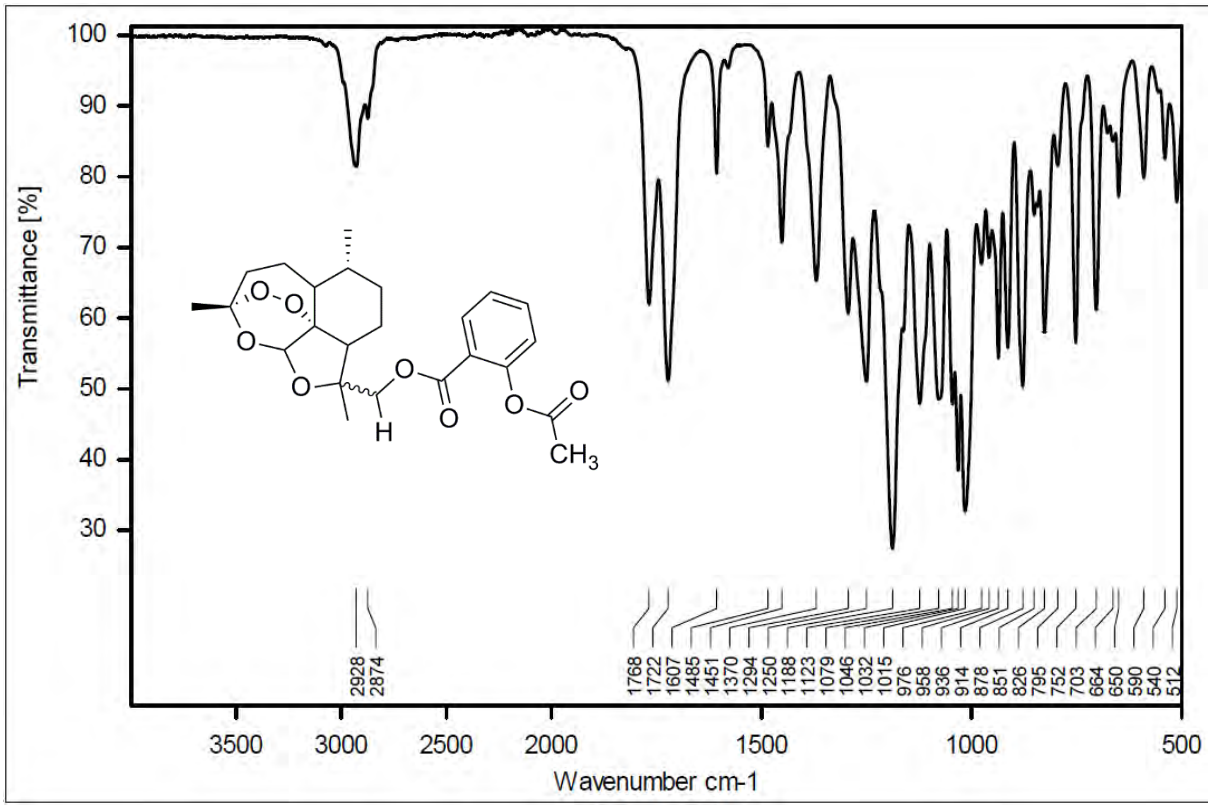
Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	1500 m/z	Set Collision Cell RF	100.0 Vpp	Set Divert Valve	Waste



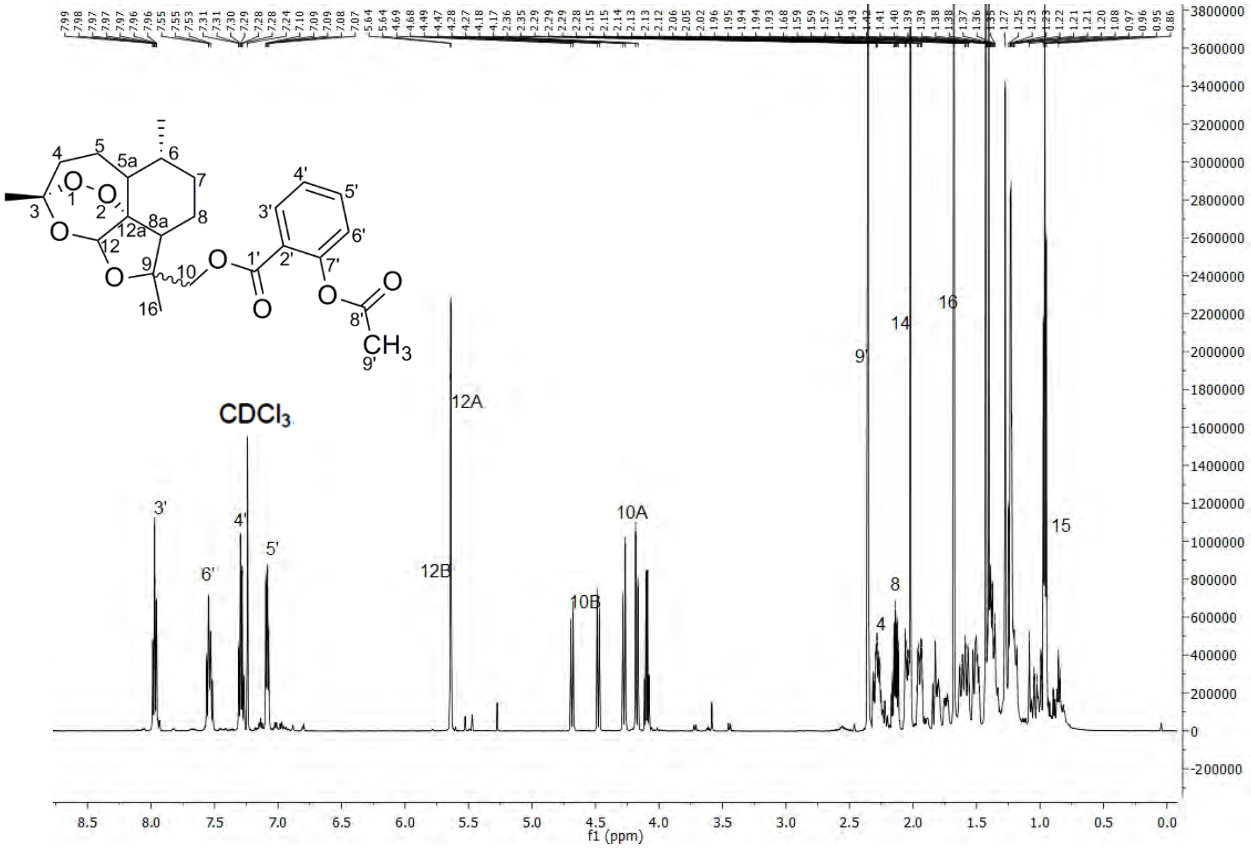

TGA
mg
DTA
uV
5.00
4.00
3.00
2.00
1.00
-0.00
200.00
100.00
-0.00
Weight Loss -4.895mg
-96.988%
Peak 171.89C
Onset 151.88C
Endset 185.83C
Heat 29.80J
5.90kJ/g
Peak 103.46C
Onset 96.71C
Endset 109.19C
Heat -2.00J
-395.75J/g
Peak 271.43C
Onset 243.95C
Endset 293.27C
Heat -14.51J
-2.88kJ/g
100.00
200.00
300.00
Temp [C]

COMPOUND 13

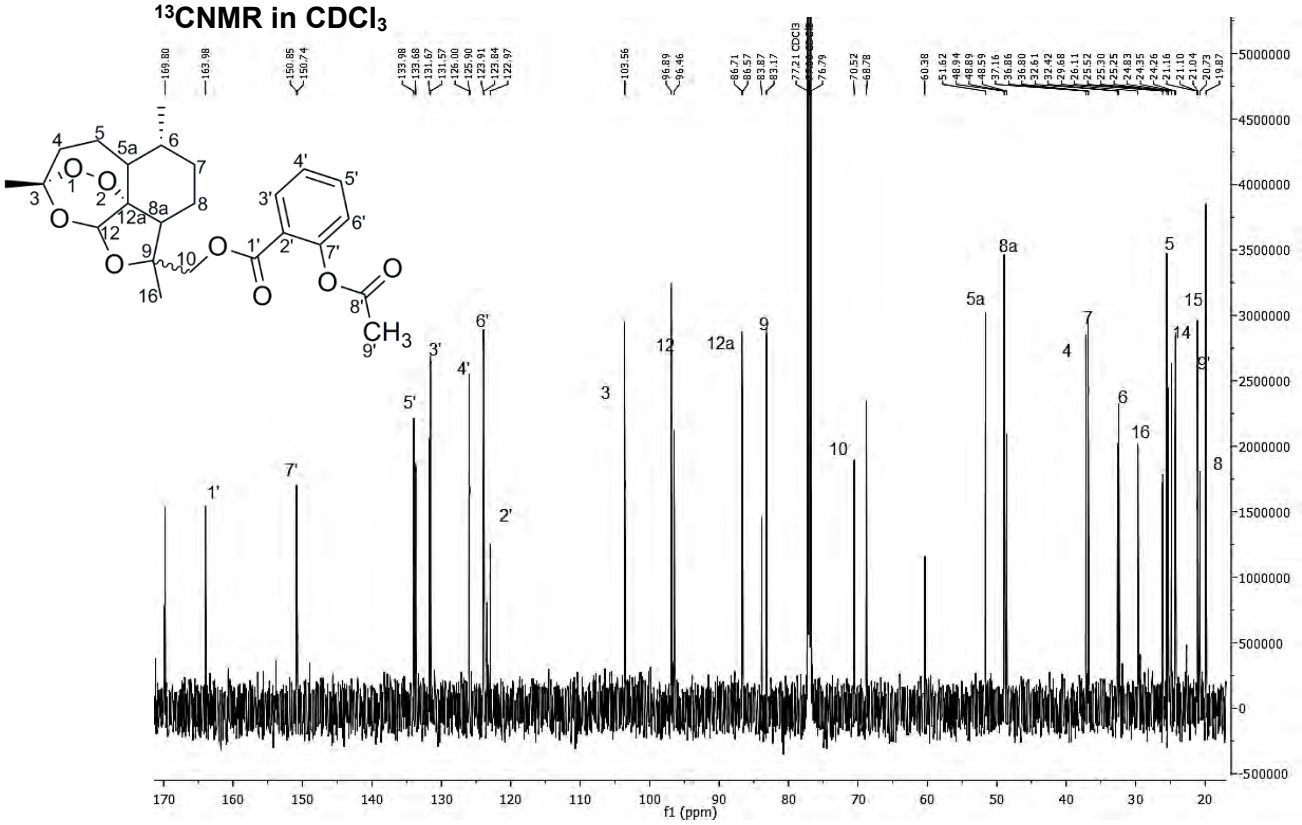
IR



^{1}H NMR in $CDCl_3$



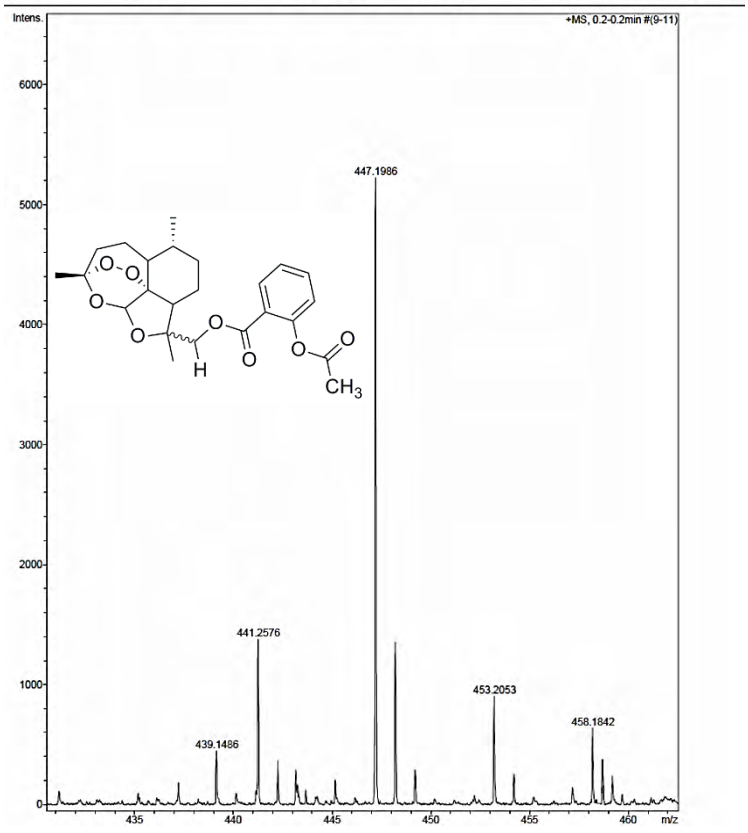
^{13}CNMR in $CDCl_3$



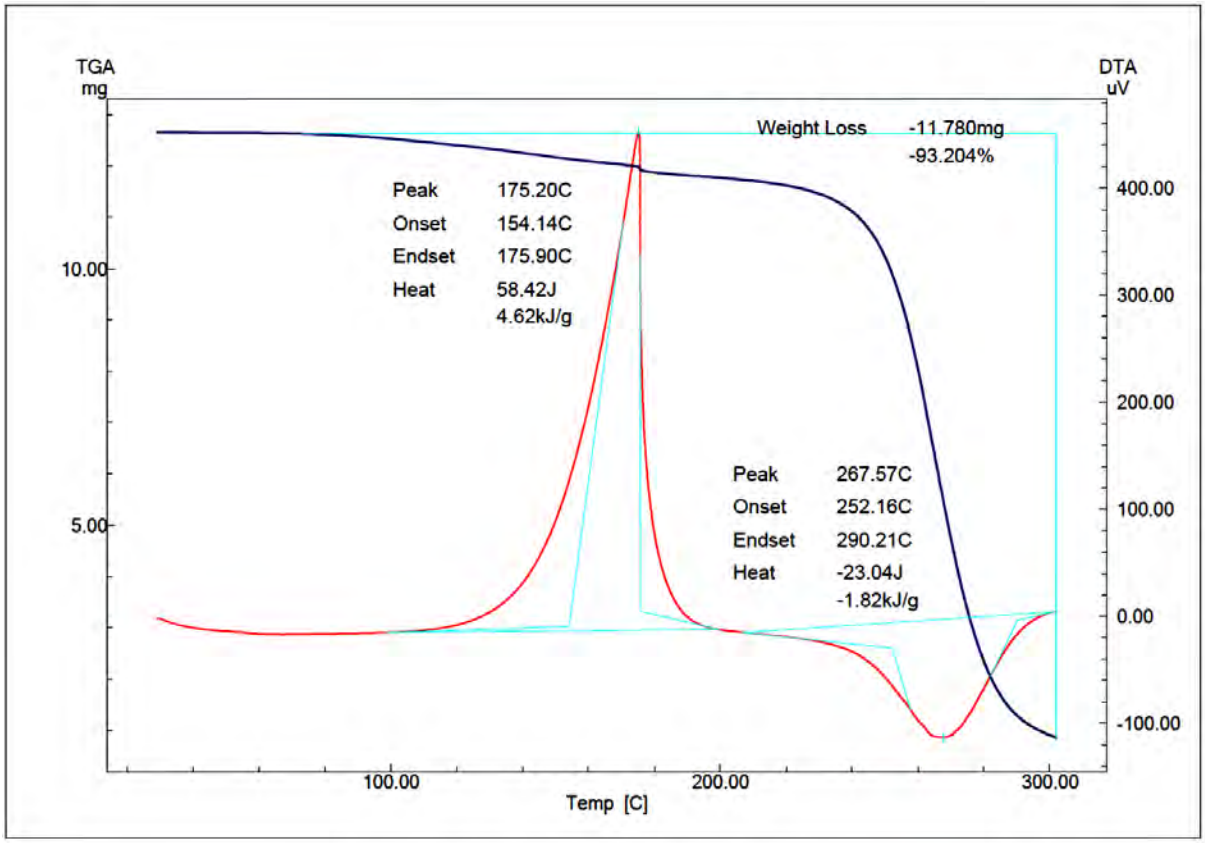
HRMS

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1500 m/z	Set Collision Cell RF	100.0 Vpp	Set Divert Valve	Waste

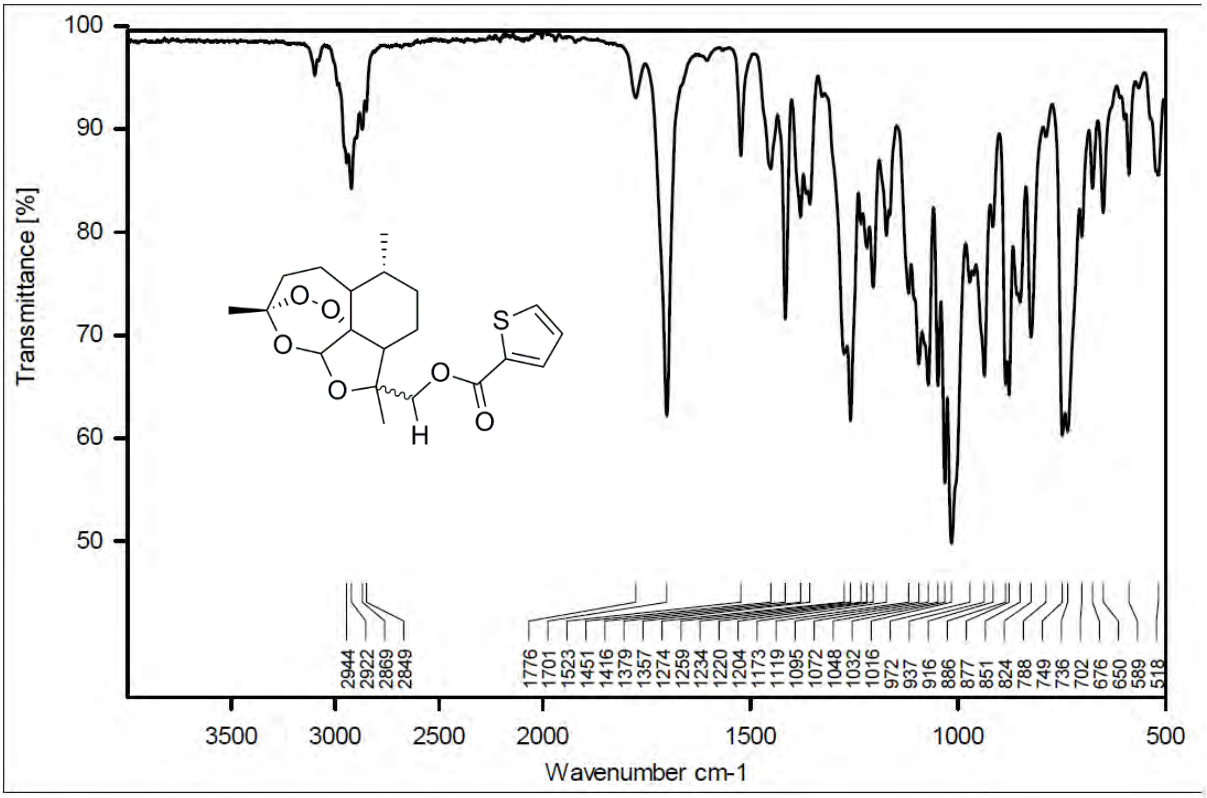


DSC/ TGA

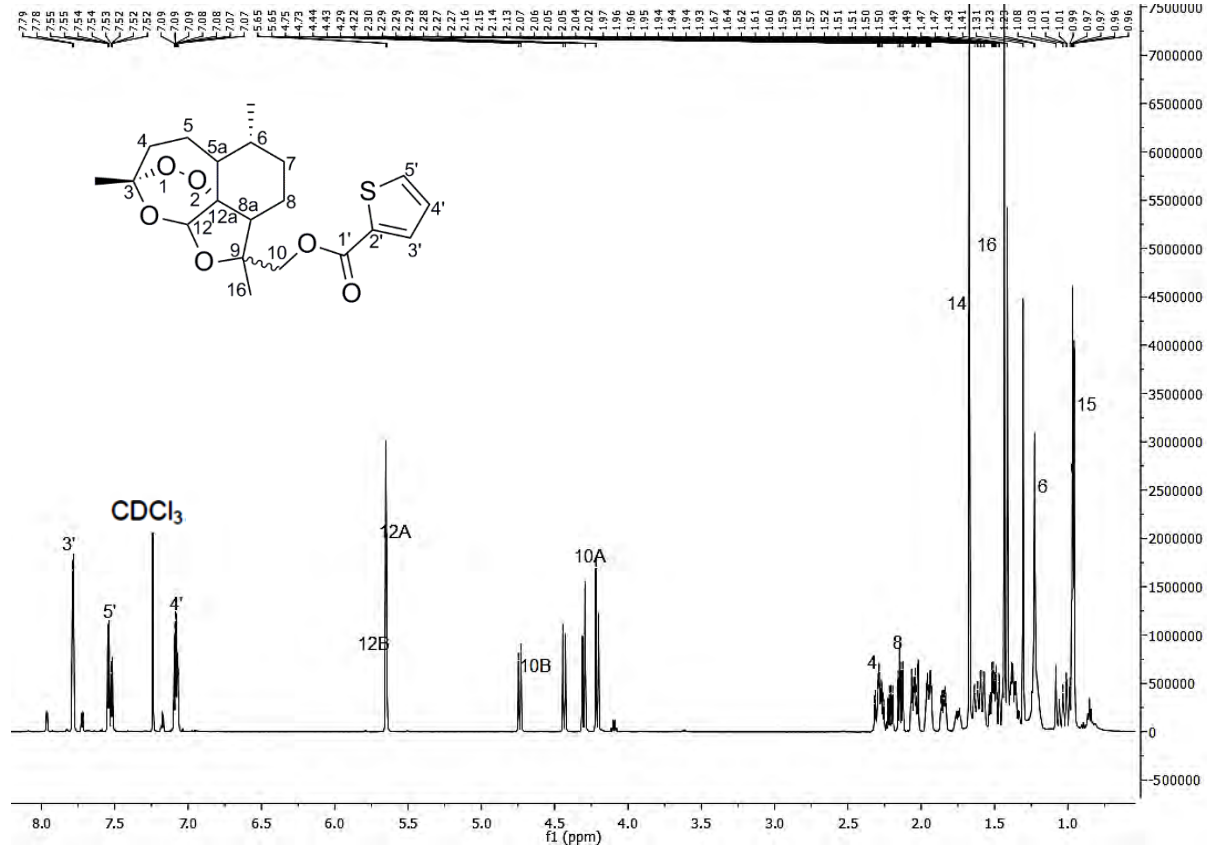

TGA
mg
DTA
uV
Weight Loss -11.780mg
-93.204%
Peak 175.20C
Onset 154.14C
Endset 175.90C
Heat 58.42J
4.62kJ/g
Peak 267.57C
Onset 252.16C
Endset 290.21C
Heat -23.04J
-1.82kJ/g
10.00
5.00
400.00
300.00
200.00
100.00
0.00
-100.00
100.00
200.00
300.00
Temp [C]

COMPOUND 14

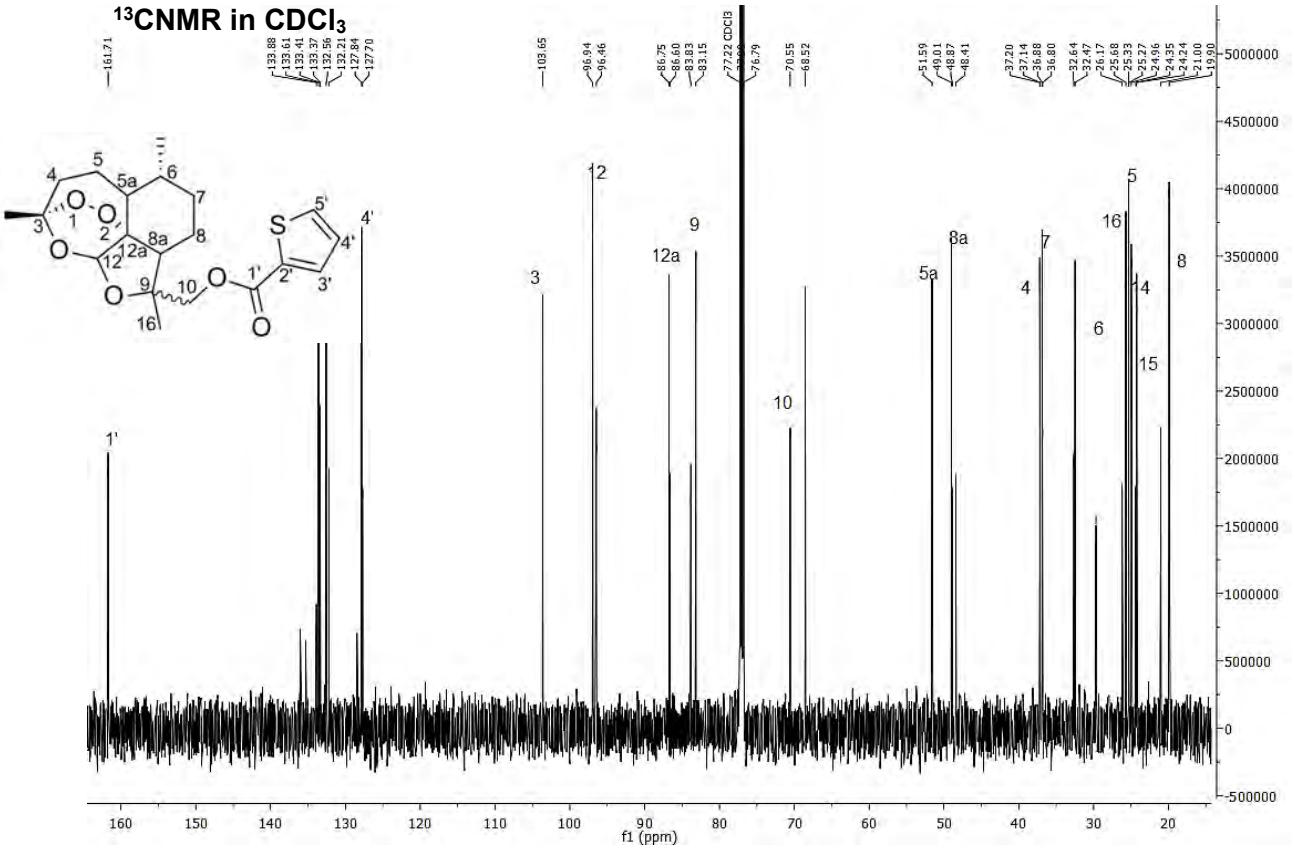
IR



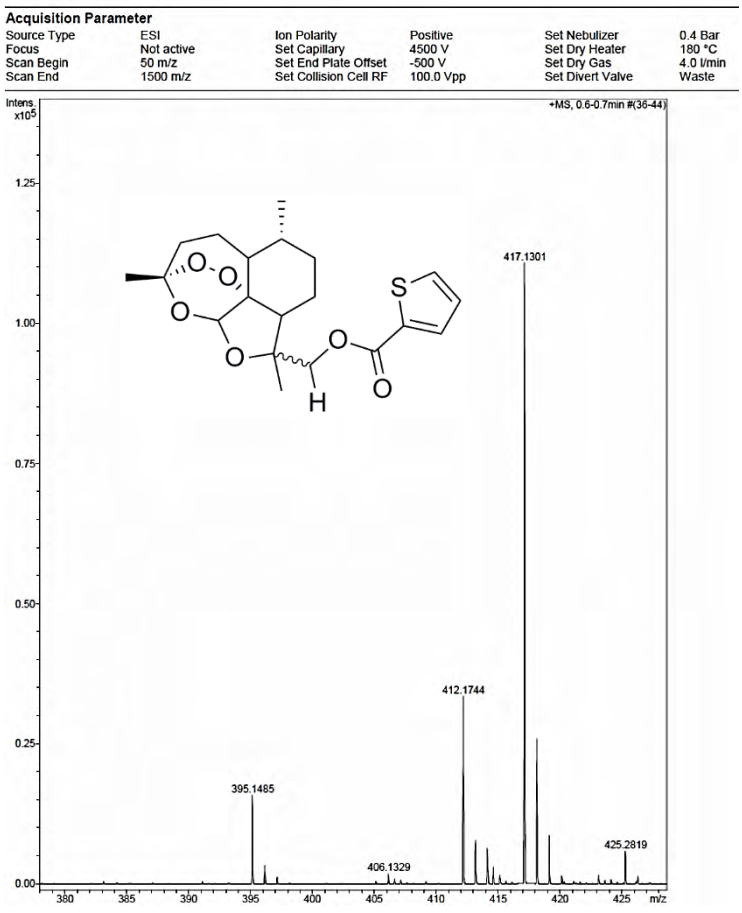
^{1}H NMR in $CDCl_3$



^{13}CNMR in $CDCl_3$

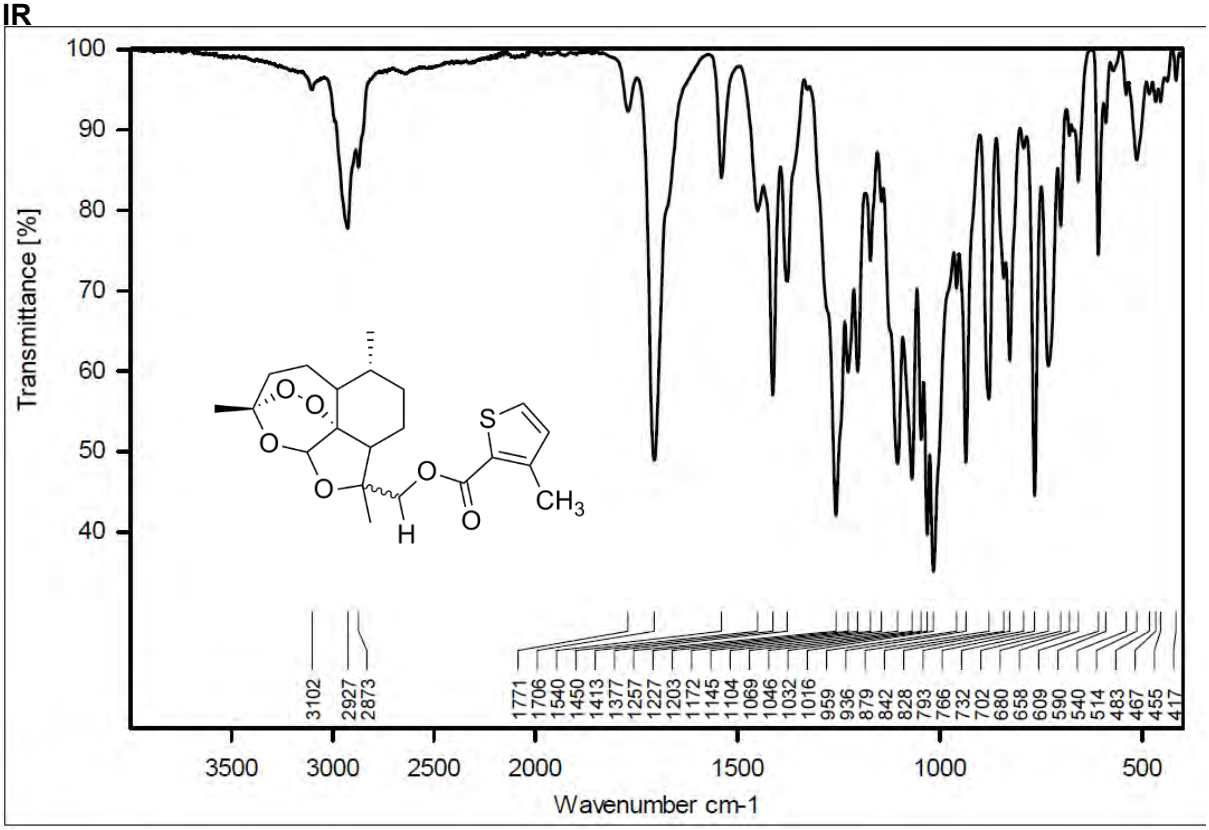


HRMS

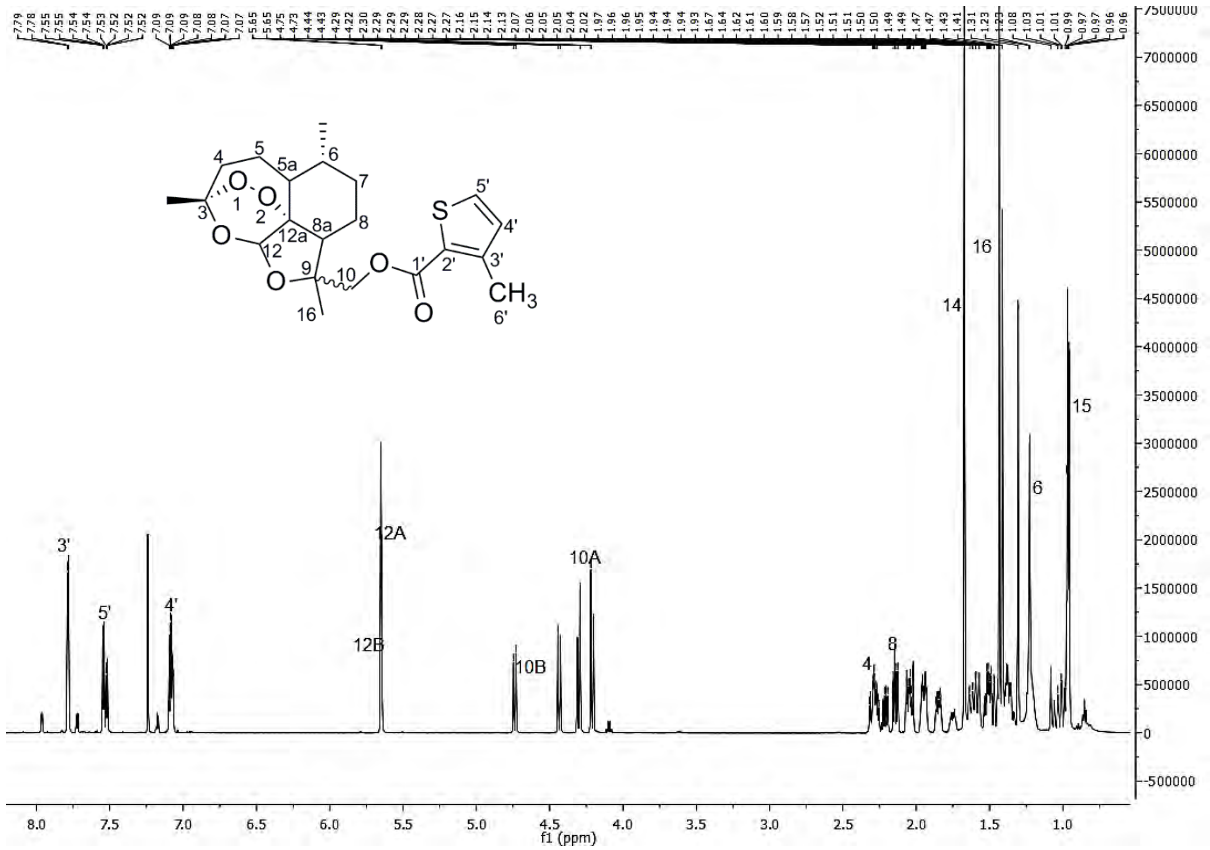


COMPOUND 15

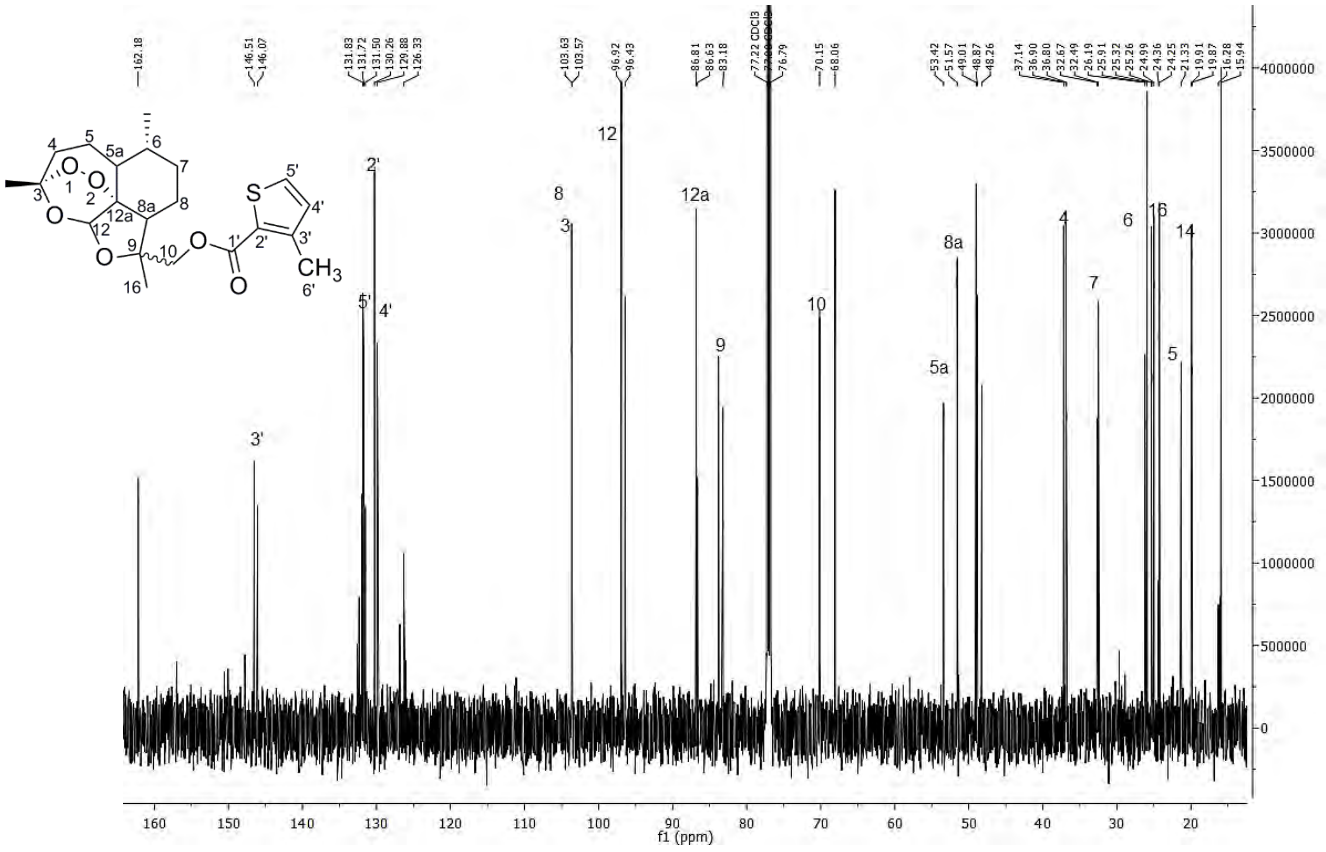
IR



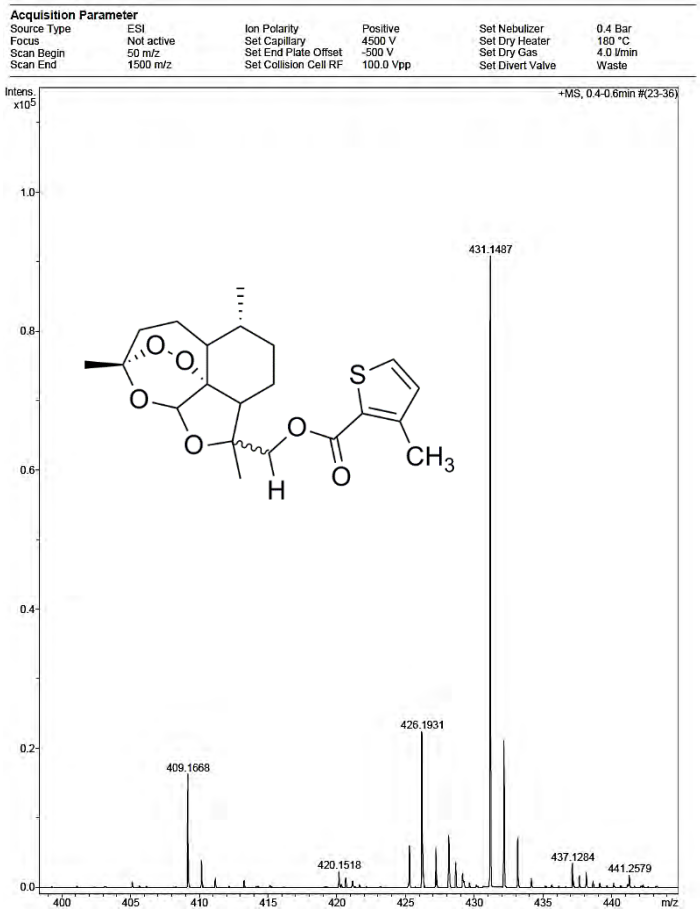
^{1}H NMR in $CDCl_3$



^{13}CNMR in $CDCl_3$

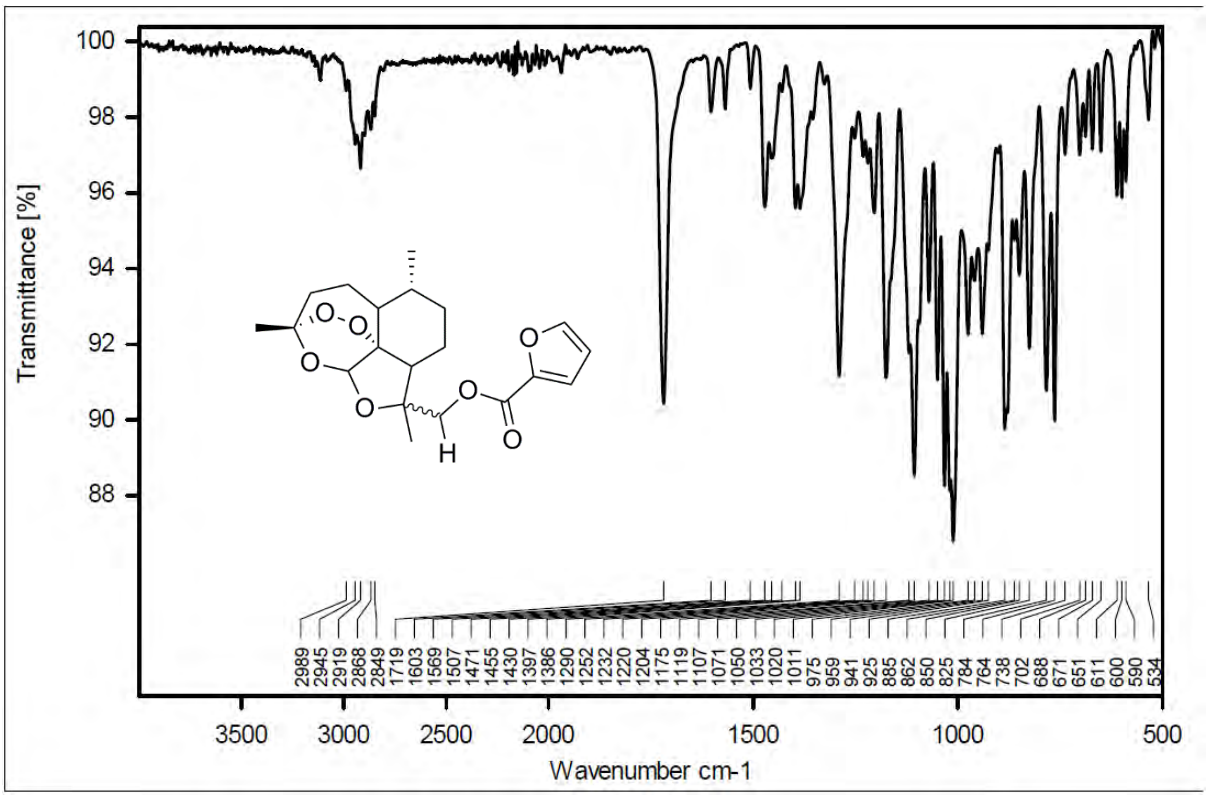


HRMS

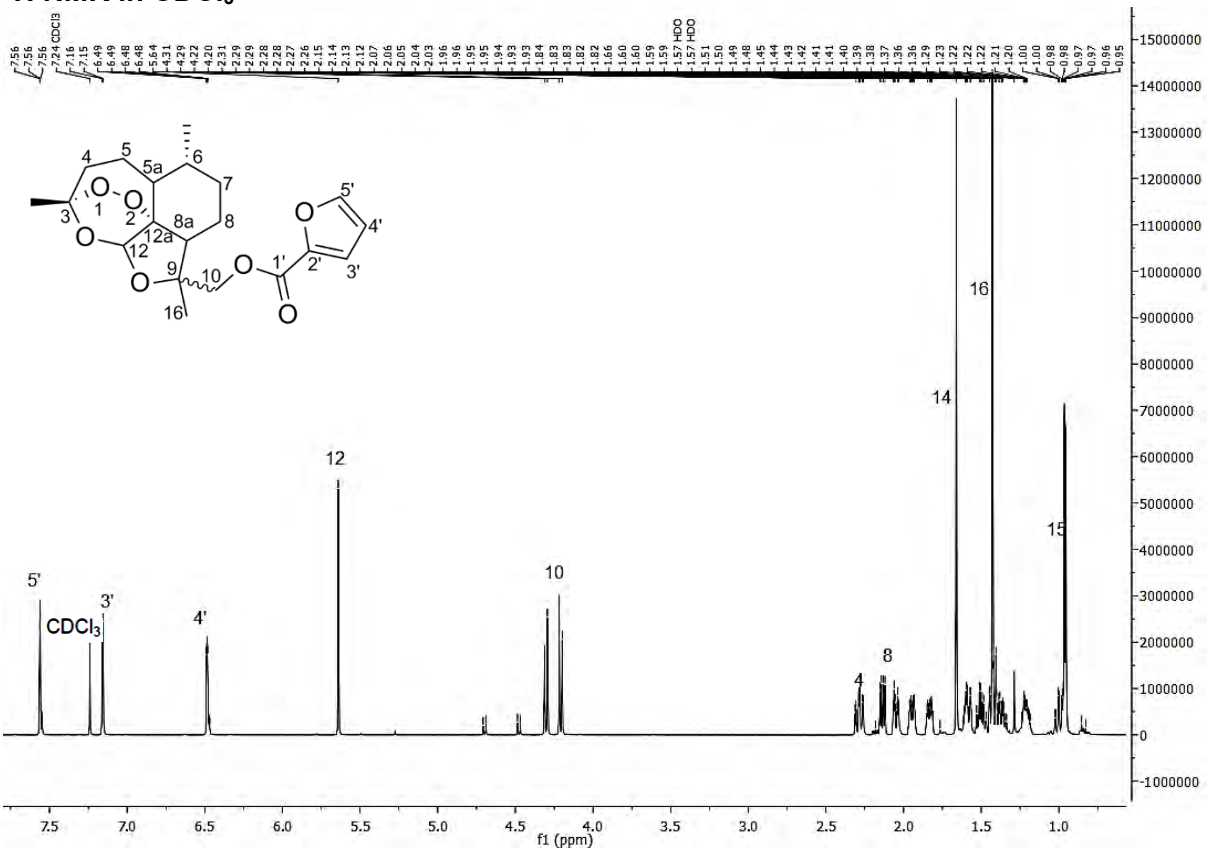


COMPOUND 16

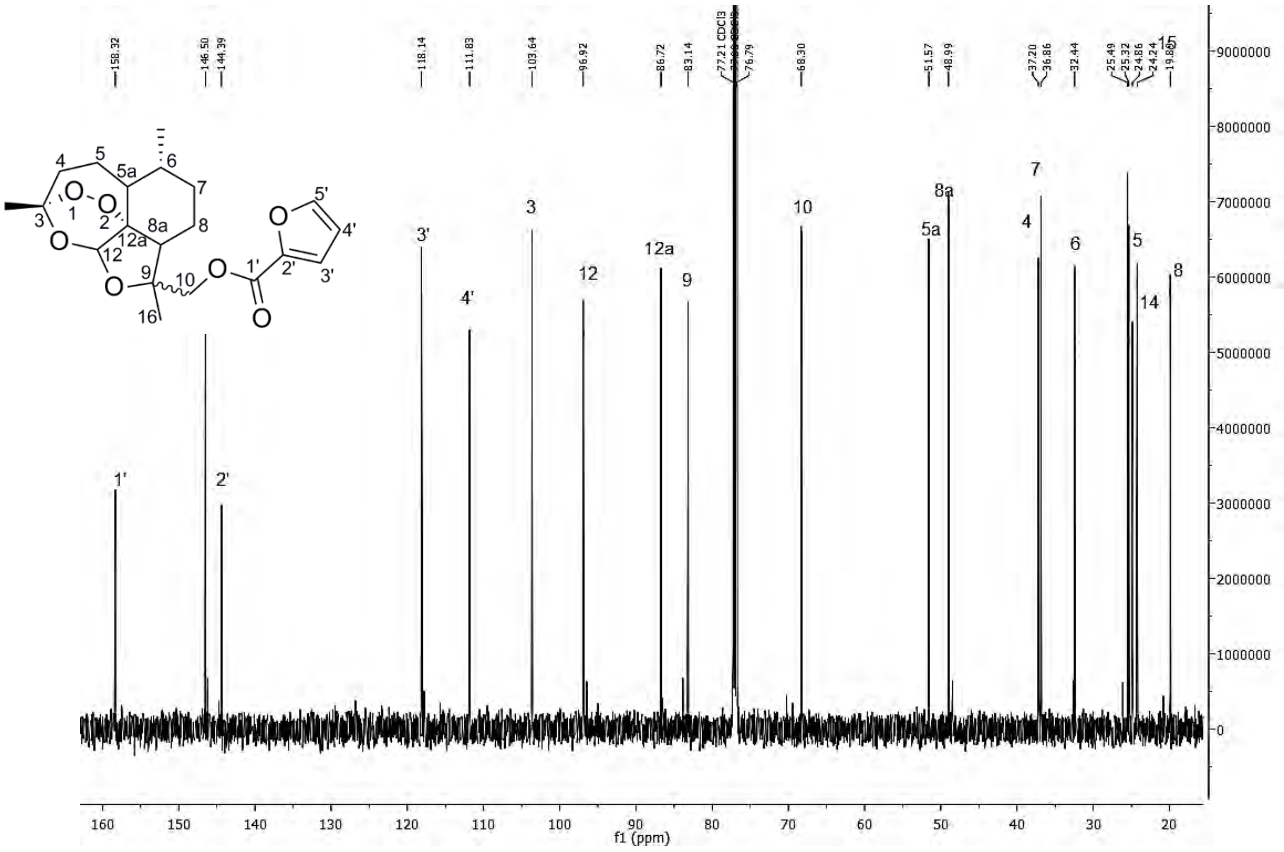
IR



^{1}H NMR in $CDCl_3$



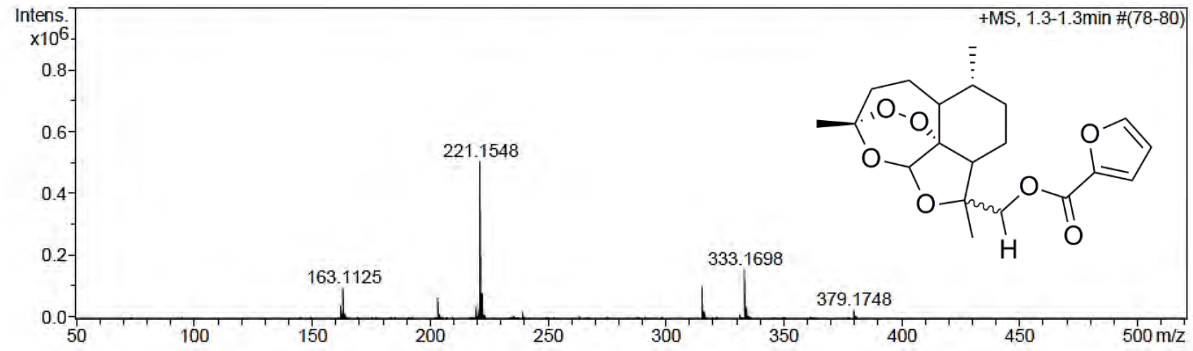
^{13}CNMR in $CDCl_3$



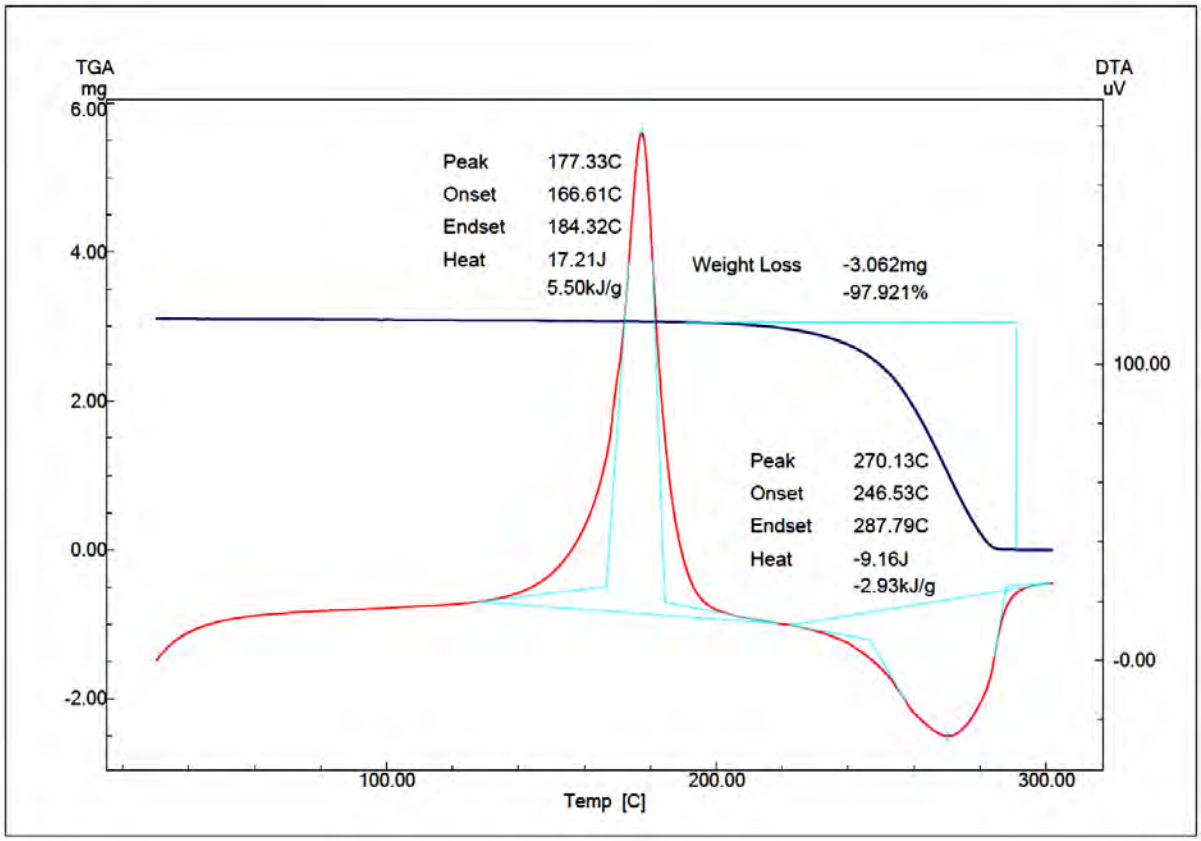
HRMS

Acquisition Parameter

Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	1500 m/z	Set Collision Cell RF	100.0 Vpp	Set Divert Valve	Waste

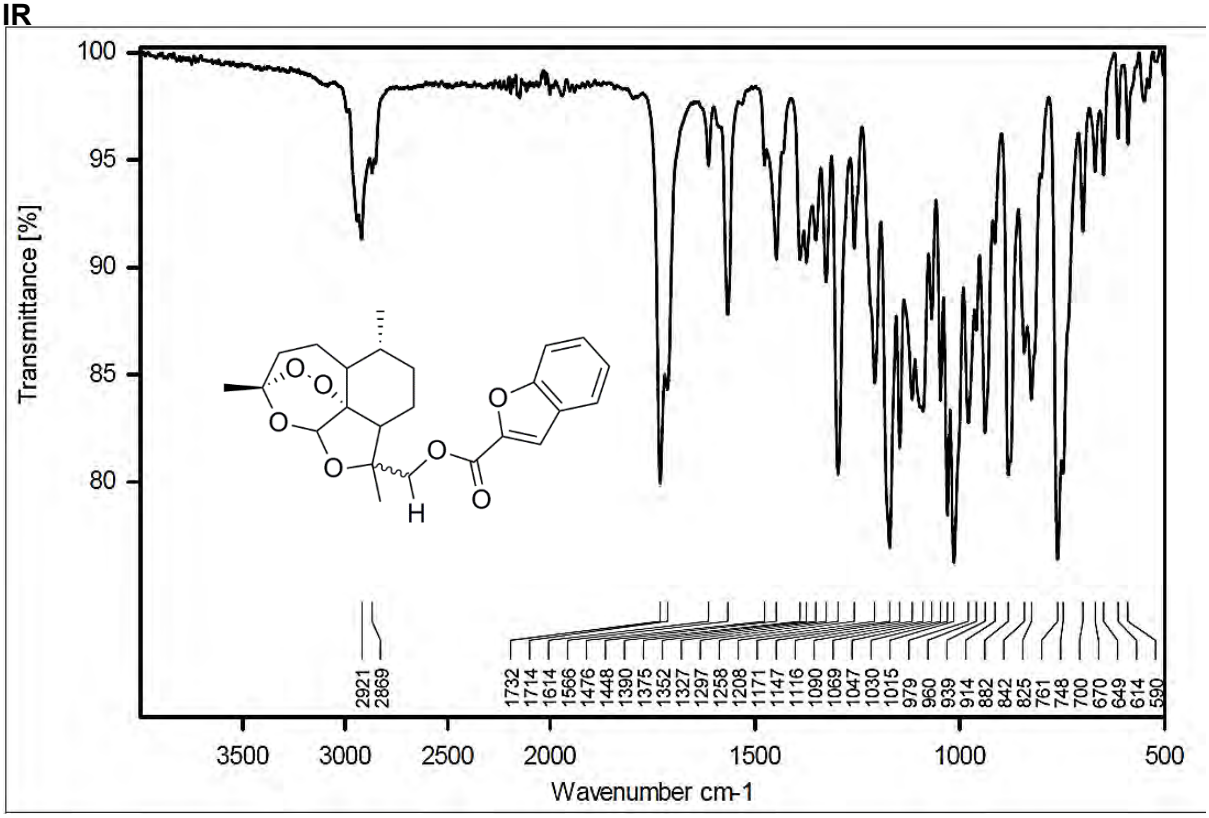


DSC/TGA

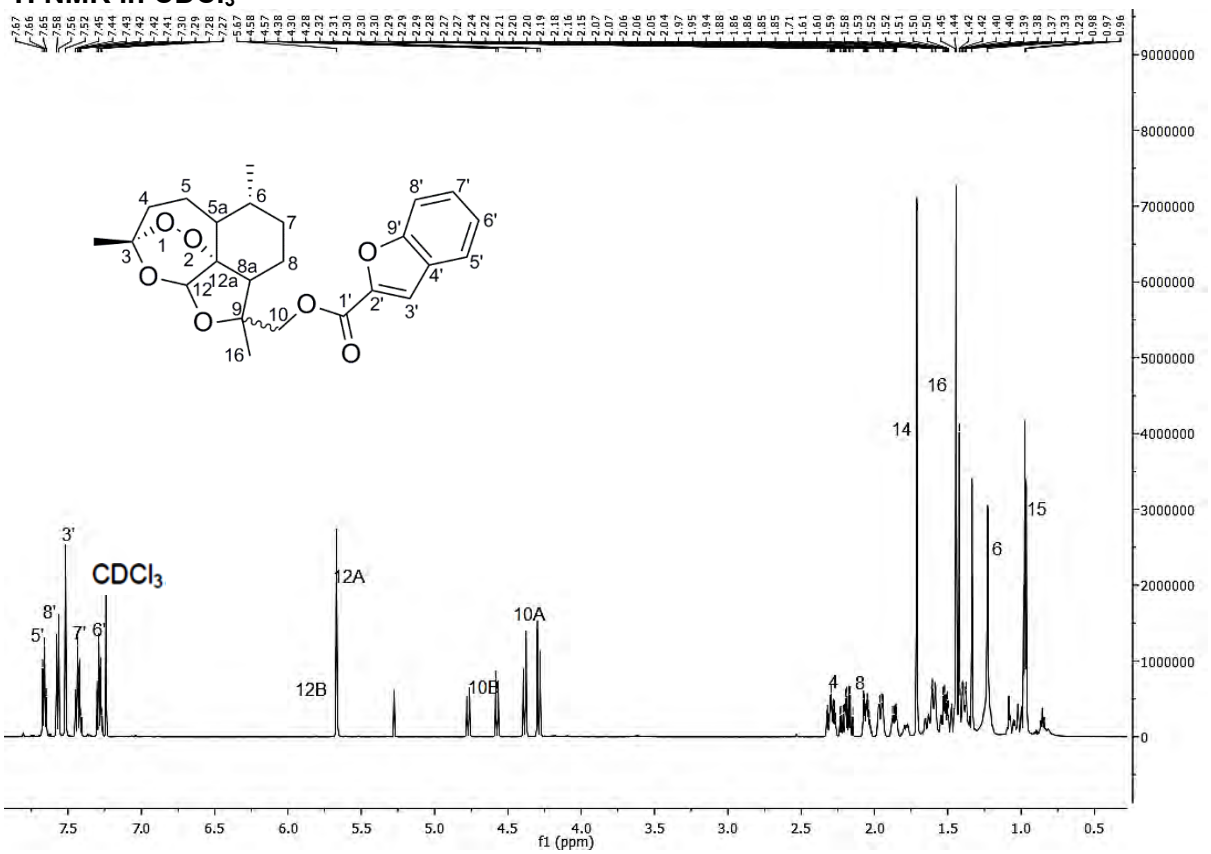

TGA
mg
DTA
uV
6.00
4.00
2.00
0.00
-2.00
100.00
-0.00
Peak 177.33C
Onset 166.61C
Endset 184.32C
Heat 17.21J
5.50kJ/g
Weight Loss -3.062mg
-97.921%
Peak 270.13C
Onset 246.53C
Endset 287.79C
Heat -9.16J
-2.93kJ/g
100.00
200.00
300.00
Temp [C]

COMPOUND 17

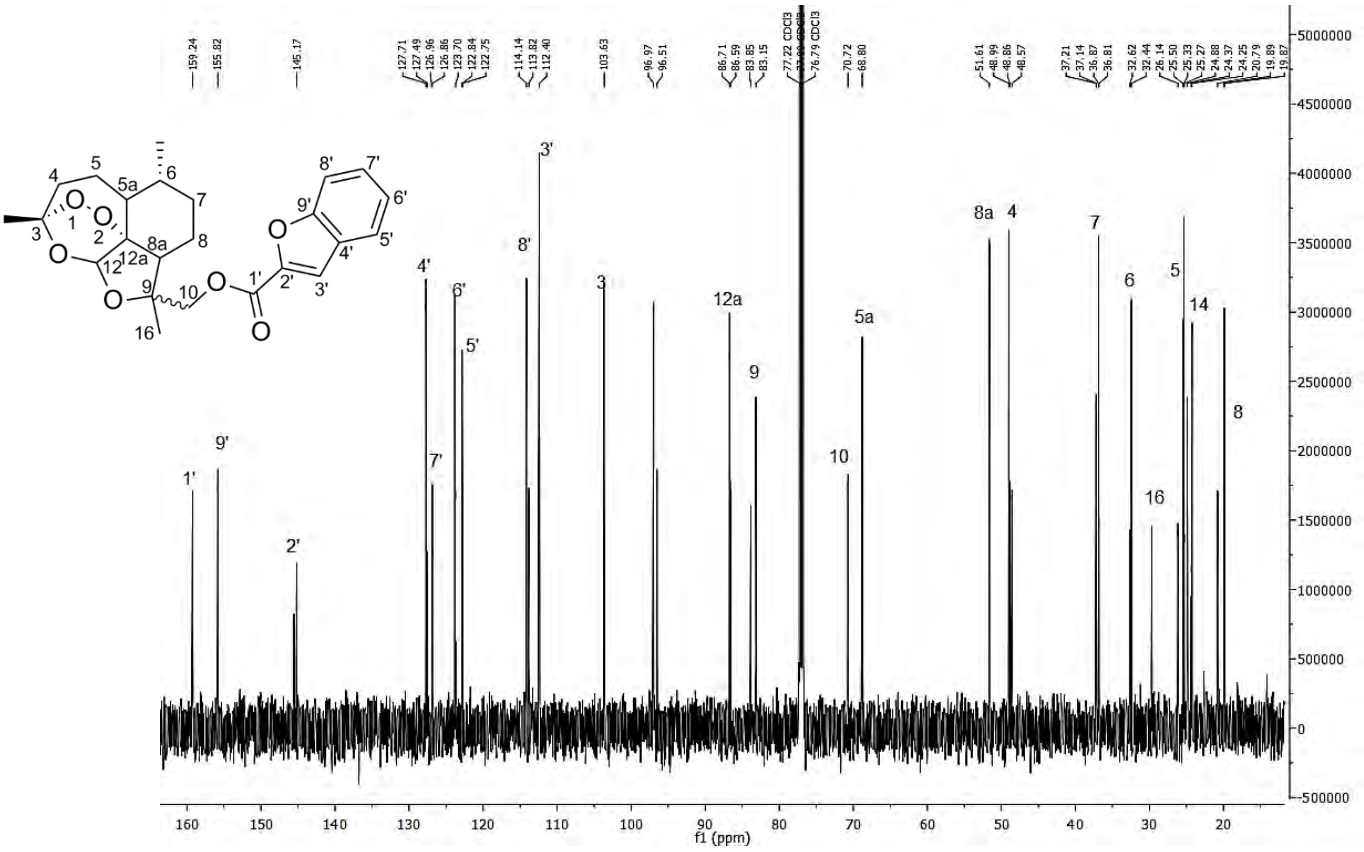
IR



^{1}H NMR in $CDCl_3$



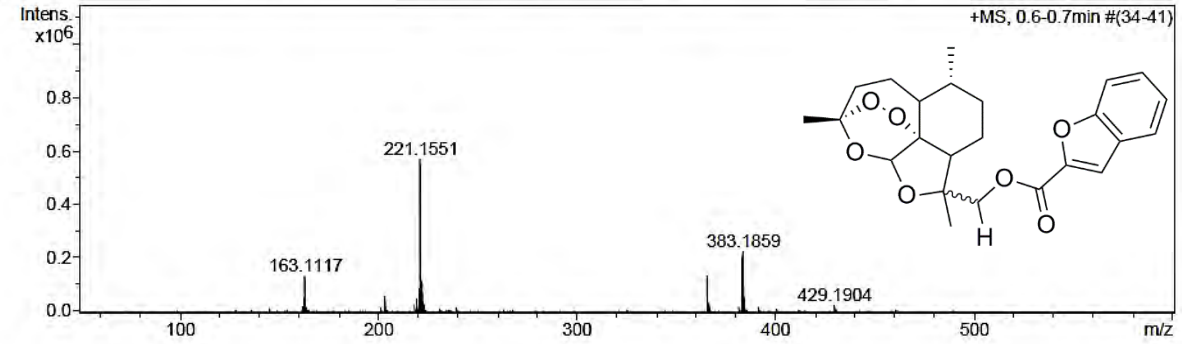
^{13}CNMR in $CDCl_3$



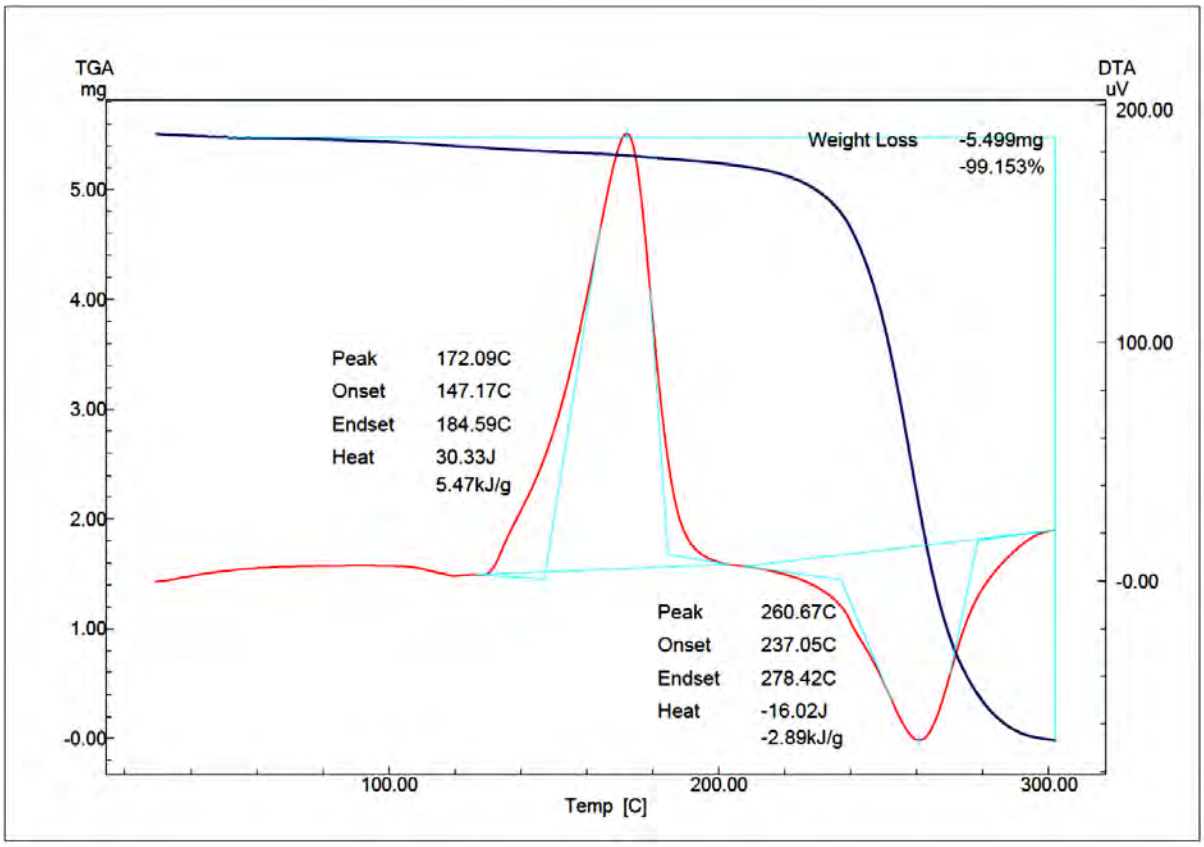
HRMS

Acquisition Parameter

Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	1500 m/z	Set Collision Cell RF	100.0 Vpp	Set Divert Valve	Waste

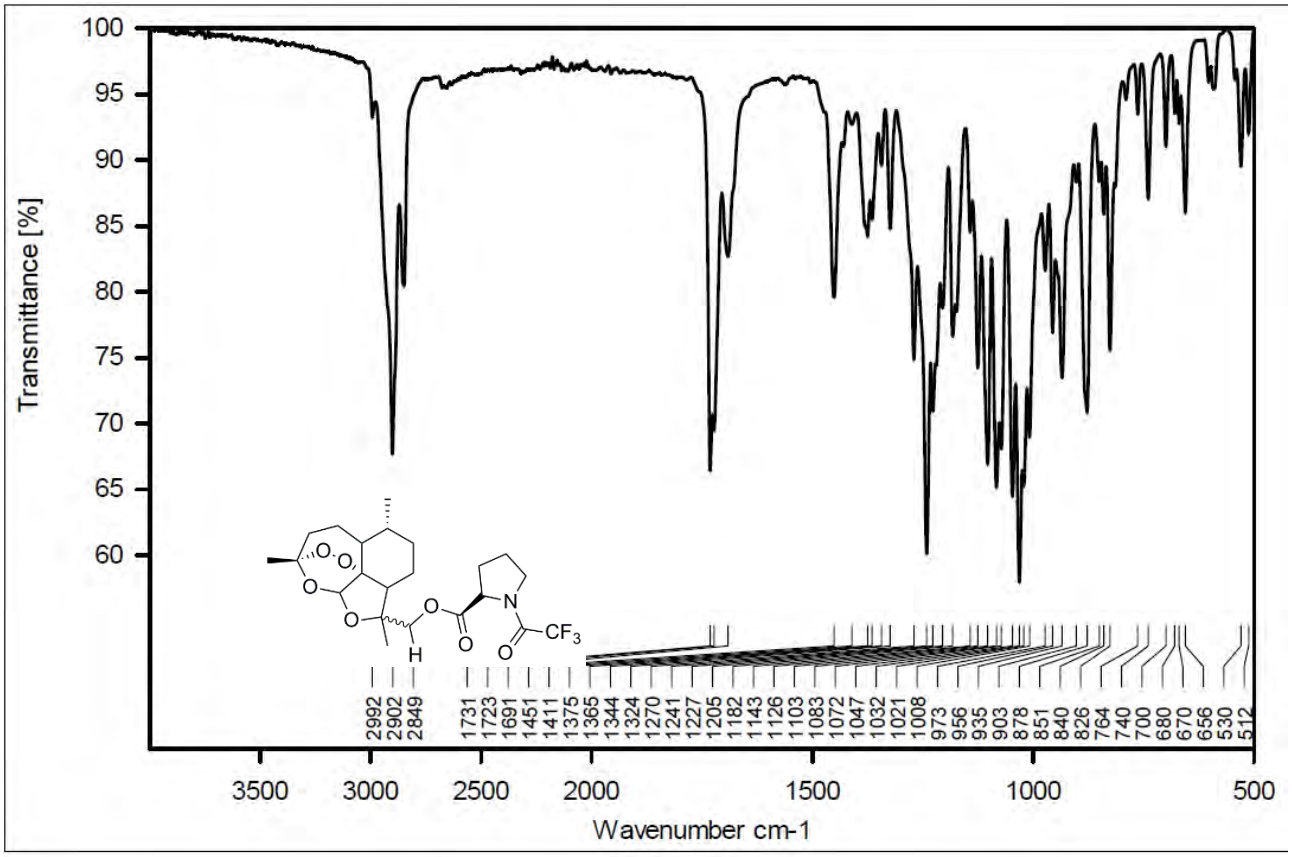


DSC/TGA

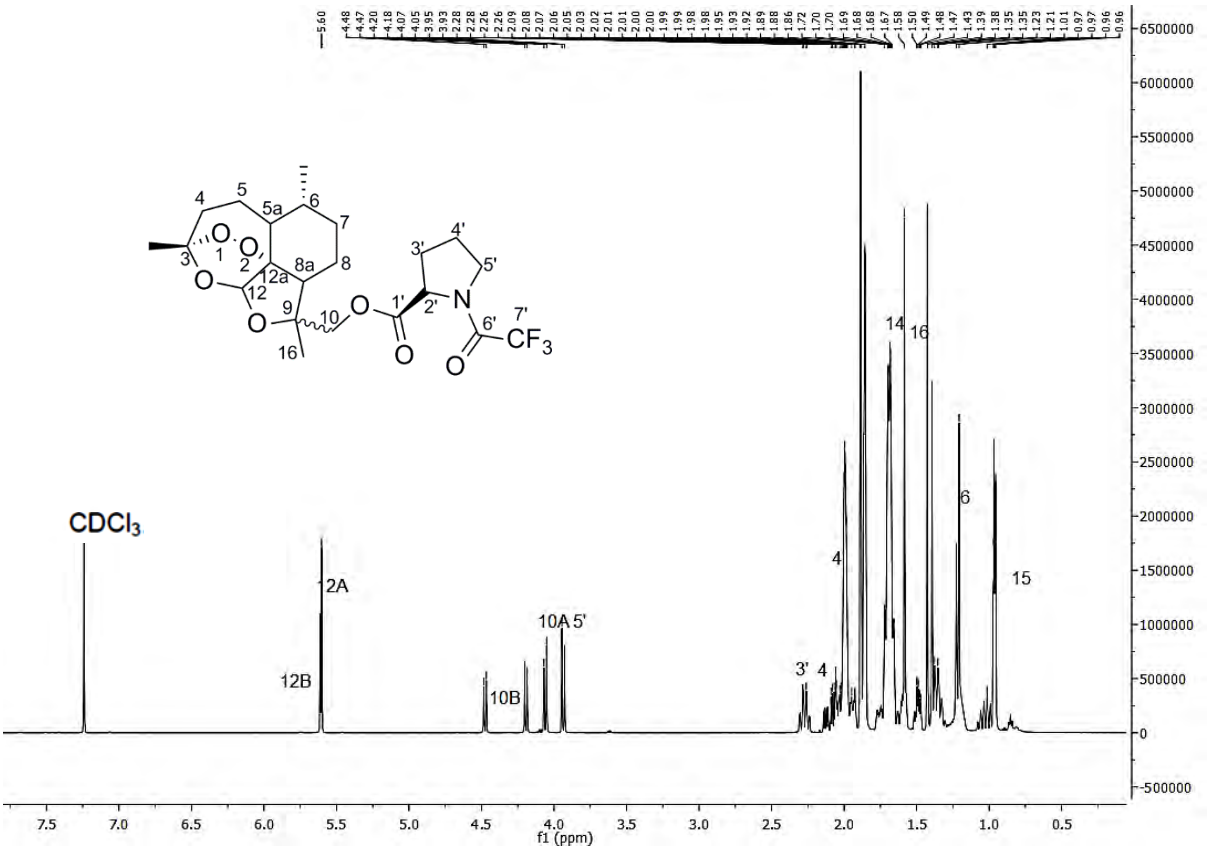

TGA
mg
DTA
uV
5.00
4.00
3.00
2.00
1.00
-0.00
200.00
100.00
-0.00
Weight Loss -5.499mg
-99.153%
Peak 172.09C
Onset 147.17C
Endset 184.59C
Heat 30.33J
5.47kJ/g
Peak 260.67C
Onset 237.05C
Endset 278.42C
Heat -16.02J
-2.89kJ/g
100.00
200.00
300.00
Temp [C]

COMPOUND 18

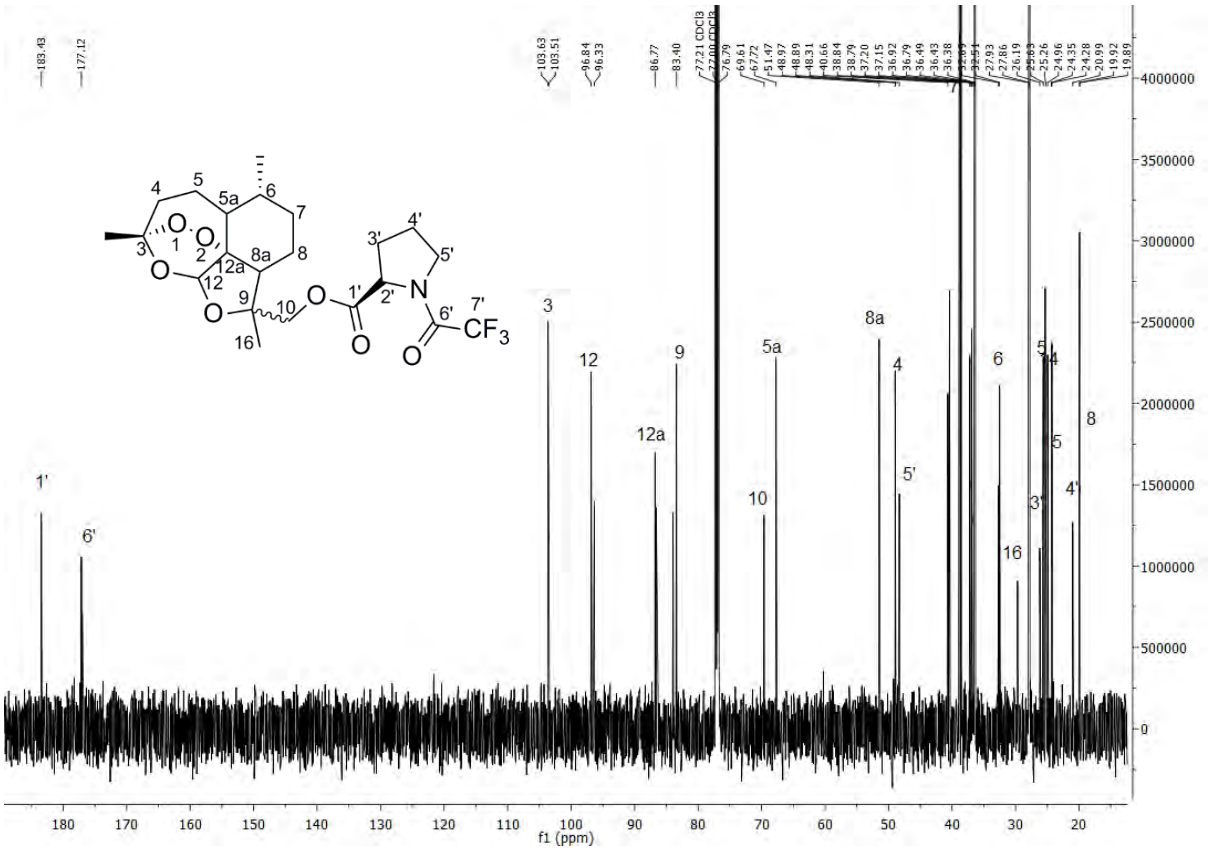
IR



^{1}H NMR in $CDCl_3$



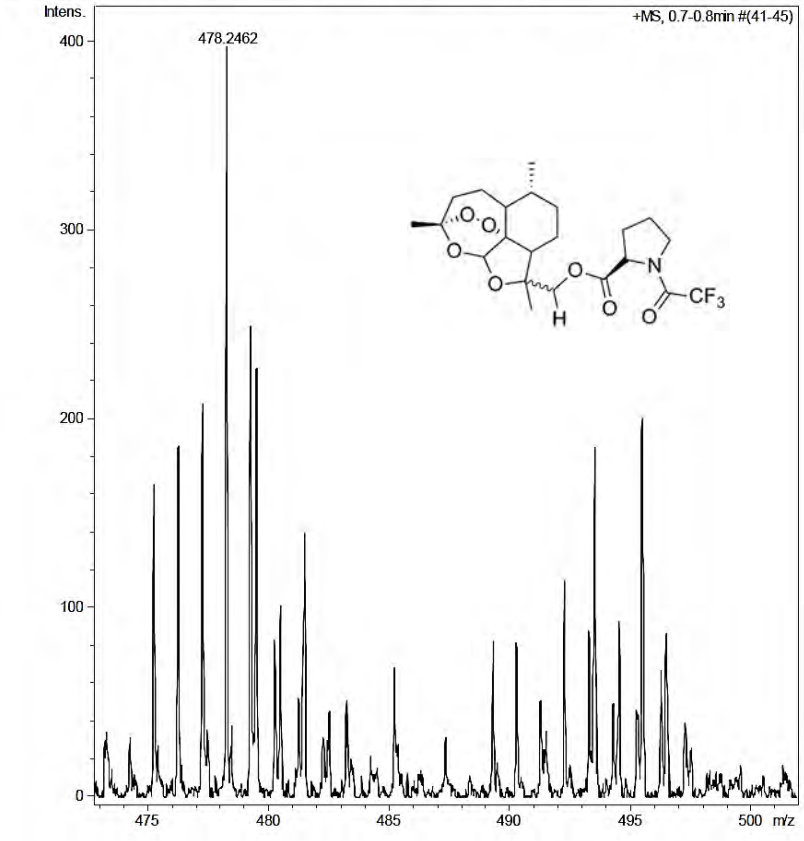
^{13}CNMR in $CDCl_3$



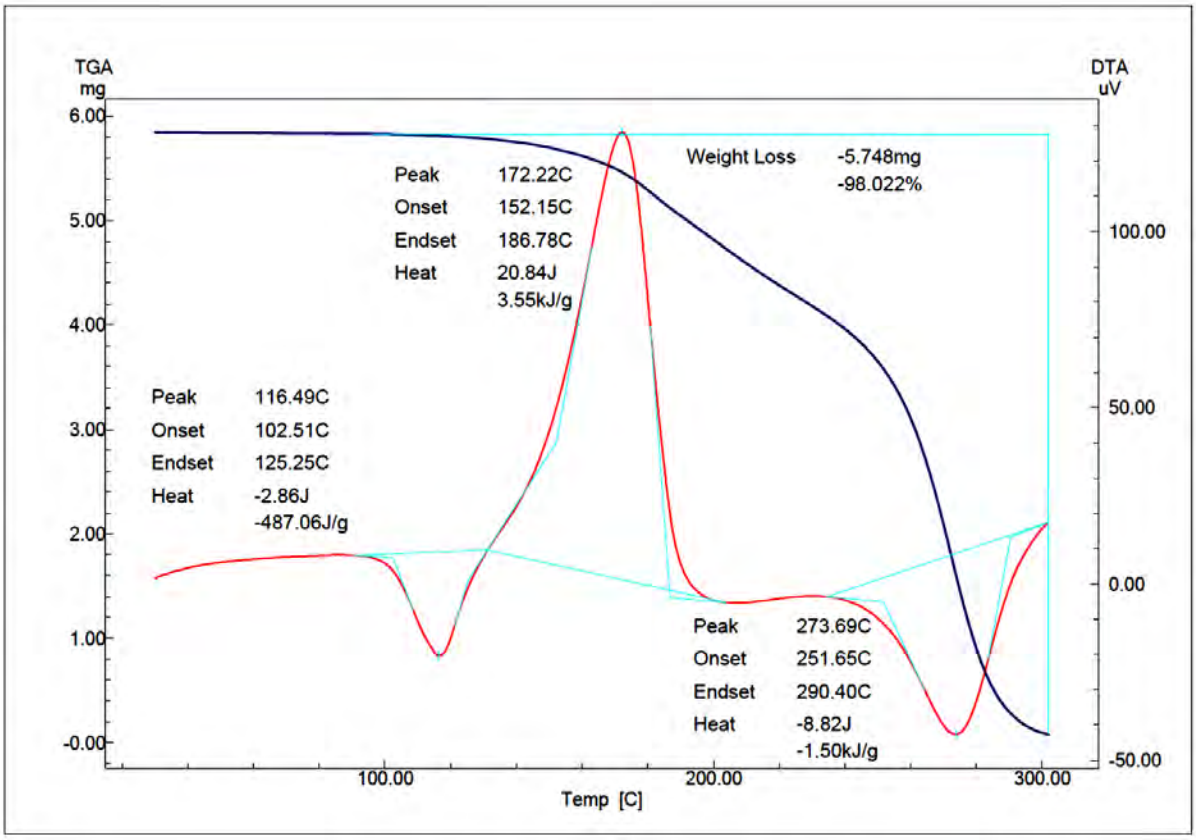
HRMS

Acquisition Parameter

Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	1500 m/z	Set Collision Cell RF	100.0 Vpp	Set Divert Valve	Waste



DSC/TGA


TGA
mg
DTA
uV
6.00
5.00
4.00
3.00
2.00
1.00
-0.00
100.00
50.00
0.00
-50.00
100.00
200.00
300.00
Temp [C]
Weight Loss -5.748mg
-98.022%
Peak 172.22C
Onset 152.15C
Endset 186.78C
Heat 20.84J
3.55kJ/g
Peak 116.49C
Onset 102.51C
Endset 125.25C
Heat -2.86J
-487.06J/g
Peak 273.69C
Onset 251.65C
Endset 290.40C
Heat -8.82J
-1.50kJ/g