Synthesis and biological evaluation of novel artemisone derivatives

Richard James Mabilika

25265679

Dissertation submitted in fulfilment of the requirements for the degree *Magister Scientiae* in Pharmaceutical Chemistry at the Potchefstroom Campus of the North-West University

Supervisor: Prof. D.D. N'Da

Co-Supervisor: Prof. R.K. Haynes

Co-supervisor: Dr. F.J. Smit

November 2015

Solemn Declaration

I, **RICHARD JAMES MABILIKA**,

herewith declare that the thesis entitled,

NORTH-WEST UNIVERSITY ®
YUNIBESITI YA BOKONE-BOPHIRIMA
NOORDWES-UNIVERSITEIT
POTCHEFSTROOM CAMPUS

SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL ARTEMISONE DERIVATIVES

which I herewith submit to the North-West University, Potchefstroom Campus, in compliance with the requirements set for the degree, **Magister Scientiae**, is my own work, has been language edited and has not already been submitted to any other university.

I understand and accept that the copies that are submitted for examination are the property of the University.

Signature of student: _____

University number: **25265679**

Signed at **Potchefstroom** this _____ day of _____ 2016

Declared before me on this _____ day of _____ 2016

Commissioner of Oaths: _____

Declaration by Supervisor / Promoter / Research Director

The undersigned declares:

1. that the student attended an approved module(s) of study for the relevant qualification and that the work for the course has been completed, or that work approved by the Senate has been done;
2. that the student has complied with the minimum duration of study as stated in the calendar;
3. the student is hereby granted permission to submit his/her mini-dissertation/dissertation or thesis;
4. that registration/amendment of the title has been approved;
5. that the appointment/amendment of examiners has been finalised;
6. that the student‟s work has been submitted to TurnItIn and a satisfactory report has been obtained; and
7. that all the procedures have been followed according to the Manual for Postgraduate Studies.

Signature Supervisor/Promoter: _____ Date: _____

Signature Research Director: _____ Date: _____

Preface

This thesis is submitted in an article format in accordance with the General Academic Rules (A.13.7.3) of the North-West University. Two articles in the form of a manuscript are included in this thesis:

Chapter 3: Article 1
Malaria drug discovery: Recent advances in antimalarial peroxides

Chapter 4: Article 2
Synthesis and biological evaluation of novel artemisone derivatives

The respective Journals of the submitted articles 1 and 2 grant the author the right to include these articles in a thesis. Permission for the Journals is given within their Author's Guides:

Permission from Elsevier: http://www.journals.elsevier.com/bioorganic-and-medicinal-chemistry/

Permission from ScienceDirect: http://www.sciencedirect.com/science/journal/00404020

ACKNOWLEDGEMENTS

To Prof. D.D. N'Da, my Supervisor for the guidance, courage and support throughout the study. Your un-ending support was always the driving force to continue pushing further, you taught me hard work and I will forever be grateful. Thank you very much Prof.

To Prof R.K. Haynes, my Co-supervisor, your extensive knowledge and experience with artemisinins antimalarials will forever be admired. You will always remain an inspiration to me.

To Dr. F.J. Smit, my Co-supervisor, for all the support enthusiasm throughout the study. You always made time for me whenever I needed assistance. I'm grateful for meeting and working with you. Thank you very much Dr. Smit.

To Dr. H.H. Wong, for the continued support during the sudy.

To A. Jourbert, for the NMR analysis.

To Dr. J. Jordaan, for the MS analysis.

To Prof. L. Birkholtz and Dr. D. Coertzen for the biological evaluation of my compounds.

To my parents James Mabilika Masunga and Margaret Thobias Manoni for their continued support and undying love for me and for knowledge. May the Almighty God give you many more years ahead.

To my wife Rehema and my daughter Mageni, thank you for the un-dying love and support that I have always earned from you sweethearts. The future ahead awaits and I'm glad and confident that with you in my side we will concur it and together cherish the best it has to offer.

To dear colleagues in the malaria group, Beteck, Christo, Nkuli, Morake and Rozanne; thank you very much for the cooperation I got. Indeed you made our group a family and home away from home.

North-West University, for financial support.

Medical Research Council of South Africa, for financial support.

ABSTRACT

Despite intensive international efforts to eradicate malaria, the disease, continues to inflict an enormous toll on human lives, especially in Africa. An estimated 198 million malaria cases and 584, 000 deaths were reported in 2013. The burden is heaviest in the African region accounting for 90% of all malaria deaths in 2013. The most affected are pregnant women and children aged less than 5 years, the latter group alone accounting for 78% of all deaths.

Artemisinins continue to remain the mainstay in the treatment of malaria more than 40 years after their discovery in the 1970s. They form the most important drug component in the so called „artemisinin combination therapies" (ACTs) used in the management of uncomplicated malaria in endemic countries today. Delayed clearance times of parasites treated with artemisinins were first reported in Cambodia and Thailand in Southeast Asia, and now that this phenomenon is definitively associated with resistance, a significant threat has emerged to the global initiative to control the disease. In 2011, the WHO issued the so called „Guideline for the global initiative for artemisinin resistance containment" which nevertheless has not been able to contain the threat. Furthermore, toxicity of the currently used artemisinins to laboratory animals continues to raise concerns on their safety to humans. Initiatives driven by the Medicines for Malaria Venture (MMV) in Geneva emphasized the urgent need for novel synthetic or semi-synthetic drugs safe and effective enough to replace the existing ones. Following its preparation, artemisone – a second generation semi-synthetic derivative of artemisinin - did not result into any measurable neurotoxicity in both *in vitro* and *in vivo* assays. The drug has a longer half-life (3.1h) compared to all other derivatives, namely artesunate (~50 min), artemether (1.3 h) and DHA (~ 45 min) and shows superior activity to artesunate against both CQ-resistant and -sensitive *P. falciparum* strains, making it a drug-like compound. However, with a measured Log *P* value of 2.49 which is lower than those of artesunate (2.77), artemether (3.98), and DHA (~2.6) and an aqueous solubility of 89 mg/L, artemisone is rated as a polar compound, and the question therefore arises as to whether enhancing the lipophilicity of artemisone may result in a better drug.

To address the polarity issue of artemisone, a series of novel lipophilic artemisone derivatives (**6** – **22**) were synthesized in low to moderate yields (11 - 38%) *via* acylation or alkylation of the α-sulfonyl carbanion dereived from treatment of artemisone with the strong non-nucleophilic base lithium N,N-diisoproplyamide in anhydrous tetrahydrofuran in an inert environment. The structures of the products were confirmed by means of nuclear magnetic resonance spectroscopy (NMR), infrared spectroscopy (IR) and mass spectrometry (MS).

The compounds were screened together with artemisone, artemether, and artesunate against NF54, K1 and W2 asexual stages of *P. falciparum* parasites as well as early stage gametocytes of the NF54 strain. Additionally, cytotoxicity was evaluated against the normal human fetal lung fibroblast WI-38 cell lines.

All the compounds except the derivatives **13** and **14** with long aliphatic chains were highly active with IC_{50} values in the range of 0.42-5.9, 0.50-3.26 and 1-6.7 nM against NF54, K1 and W2 strains of *P. falciprum* respectively. Compounds **6** - **11**, **17**, **19** and **21** were the most active against the NF54 strain, while compounds **6**, **7**, **9** and **20** were the most active against the K1 strain, with IC_{50} values less than 1 nM. Compounds **7** - **9** were equipotent with artemisone, 2-4 and 3-6 times more potent than artesunate and artemether respectively against both the NF54 and K1 parasite strains.

Compounds **13** and **14** were the least active in the series with IC_{50} values of 22 and >500 nM against the NF54 strain, 34.54 and >500 nM against K1 and 32.1 and >500 nM against the W2 strain, respectively.

Artemisone derivatives **6** - **10** and **21** were again the most potent against the W2 strain in the series with IC_{50} values <1.8 nM, with potency comparable to that of of artemether, while being 4 – 6 times more potent than artesunate.

The compounds were also very active against the early gametocyte stages of the NF54 strain. Compounds **6** - **12**, **17** - **19**, **21** and **22** were almost twice as active as artesunate and 5 - 6 times more active than artemether when tested against early stage gametocytes. Most compounds had impressive resistance indices (RIs) (RI = IC_{50} K1/ IC_{50} NF54) of less than 1.5, making them almost equally active towards the CQ sensitive and resistant parasites. In particular compounds **6** and **16** had RIs of less than one suggesting greater activity against the resistant K1 than the sensitive NF54 strain. Compound **6** was the only one with an RI (IC_{50} W2/IC_{50} NF54) of less than 1.5 and therefore the only non-cross resistant derivative against W2, making it the most active in the series with impressive RIs against all strains tested. Additionally most of the target compounds had excellent selectivity indices of more than 70,000 indicative of their selective antiparasitic effects resulting from intrinsic activity and not cytotoxicity. Compounds **6** and **10** were identified as the best candidates for further investigation as potential drugs in the search for new, effective and safe antimalarial drugs.

Keywords: Malaria, artemisinin, artemisone, Plasmodium, cytotoxicity

OPSOMMING

Ten spyte van internasionale pogings om malaria uit te wis, eis hierdie siekte ’n enorme aantal lewens, veral in Afrika. Daar word beraam dat 198 miljoen gevalle van malaria en 584, 000 sterftes in 2013 aangemeld is. Die las is die swaarste in Afrika met 90% van sterftes in 2013. Swanger vroue en kinders onder 5 jaar word die meeste geraak, met 78% van sterftes wat onder kinders voorkom.

Veertig jaar na hul ontdekking in die 1970s, is artemisinienne steeds die belangrikste behandeling vir malaria. Hulle is die belangrikste komponente van artemisinien kombinasieterapie (AKT) wat tans vir die behandeling van ongekompliseerde malaria in endemiese gebiede gebruik word. Vertraagde opruiming van parasiete na behandeling met artemisinienne is eerste in Kambodja en Thailand in Suidoos-Asië aangemeld. Hierdie verskynsel word met weerstand geassosieer en verteenwoordig ’n groot bedreiging vir die globale inisiatief om malaria te bekamp. Alhoewel die WGO in 2011 riglyne bekend gestel het vir die voorkoming van weerstand teen artemisinienne, bly dit steeds ’n bedreiging. Verder is die toksisiteit van die huidige artemisinienne in eksperimentele diere kommerwekkend. Inisiatiewe deur die Geneesmiddels vir Malaria Onderneming (GMO) in Genève, beklemtoon die noodsaaklikheid vir nuwe sintetiese en semisintetiese geneesmiddels wat veilig en effektief genoeg is om die huidige middels te vervang. Na sy sintese het artemisoon – ’n tweedegenerasie semisintetiese derivaat van artemisinien – geen meetbare neurotoksisiteit in *in vitro* en *in vivo* eksperimente getoon nie. Dié geneesmiddel het ’n langer halfleeftyd (3.1 h) as ander derivate soos artesunaat (~50 min), artemeter (1.3 h) en DHA (~ 45 min), en toon beter aktiwiteit as artesunaat teen chlorokienweerstandige en –sensitiewe *P. falciparum* lyne. Hierdie verbinding kan dus as geneesmiddel-soortig beskou word. Nogtans is die Log P waarde 2.49, wat laer is as dié van artesunaat (2.77), artemeter (3.98) en DHA (~2.6), en met ’n wateroplosbaarheid van 89 mg/L, kan artemisoon as ’n polêre verbinding geklassifiseer word. Die vraag ontstaan dus of artemisoon ’n beter geneesmiddel sal wees indien die lipofilisiteit daarvan verhoog word.

Die polariteit van artemisoon is ondersoek deur ’n reeks nuwe lipofiele artemisoonderivate (**6** – **22**) te sintetiseer in lae tot gemiddelde opbrengste (11 - 38%). Hierdie reeks is gesintetiseer deur asilering of alkilering van die α-sulfonielkarbanioon wat ontstaan na behandeling van artemisoon met die sterk nie-nukleofiliese basis, litium N,N-diisopropielamied, in anhidriese tetrahidrofuraan in ’n inerte atmosfeer. Die strukture van die produkte is bevestig met kernmagnetieseresonansiespektroskopie (KMR), infrarooispektroskopie (IR) en massaspektroskopie (MS).

Die biologiese aktiwiteit van die verskillende verbindings sowel as artemisoon, artemeter en artesunaat teen NF54, K1 en W2 aseksuele stadiums van *P. falciparum* parasiete en die vroeë stadium gametosiete is ondersoek. Daar is ook vir sitotoksisiteit teen die normale menslike fetale longfibroblast WI-38-sellyn getoets.

Al die verbindings behalwe die lang alifatiese ketting derivate nommer **13** en **14** was aktief met IC_{50} waardes wat wissel van 0.42-5.9, 0.50-3.26 en 1-6.7 nM teen NF54-, K1- en W2-lyne van *P. falciparum* onderskeidelik. Die verbindings **6**-**11**, **17**, **19** en **21** het die meeste aktiwiteit teen die NF54-lyn getoon, terwyl verbinding **6**, **7**, **9** en **20** aktief was teen die K1-lyn, met IC_{50} waardes laer as 1 nM. Verbindings **7**-**9** het dieselfde aktiwiteit as artemisoon getoon en was 2-4 en 3-6 keer meer aktief teen die NF54 en K1 parasietlyne as artesunaat en artemeter. Verbindings **13** en **14** het die minste aktiwiteit getoon met IC_{50} waardes van 22 en >500 nM teen die NF54-lyn, 34.54 en >500 nM teen die K1-lyn en 32.1 en >500 teen die W2-lyn onderskeidelik.

Die artemisoon derivate **6**-**10** en **21** was baie aktief teen die W2-lyn met IC_{50} waardes van <1.8 nM, en het aktiwiteit gelykstaande aan dié van artemeter, maar 4-6 keer meer as artesunaat getoon. Die verbindings was ook baie aktief teen die vroeë gametosiet stadiums van die NF54-lyn. Die verbindings **6**-**12**, **17**-**19**, **21** en **22** was twee keer meer aktief as artesunaat en 5-6 keer meer aktief as artemeter teen vroeë stadium gametosiete. Die meeste verbindings het indrukwekkende weerstandindekse (RIs) (RI = IC_{50} K1/IC_{50} NF54) van minder as 1.5 wat 'n aanduiding is van aktiwiteit teen CQ sensitiewe en weerstandige parasiete. Veral verbinding **6** en **16** het weerstandindekse van minder as een wat dui op beter aktiwiteit teen die weerstandige K1-lyn as teen die sensitiewe NF54-lyn. Verbinding **6** was die enigste verbinding met 'n weerstandindeks (IC_{50} W2/IC_{50} NF54) van minder as 1.5 en is derhalwe die enigste weerstandbiedende derivaat teen die W2-lyn, wat daarop dui dat dit die meeste aktiwiteit teen al die lyne getoon het.

Die meeste van die verbindings toon selektiwiteitsindekse van meer as 70 000, wat 'n aanduiding is van selektiewe antiparasitiese effekte as gevolg van intrinsieke aktiwiteit en nie sitotoksisiteit nie. Verbindings **6** en **10** is geïdentifiseer as die beste kandidate vir verdere ondersoek as potensiële geneesmiddels teen malaria.

Sleutelwoorde: Malaria, artemisinien, artemisoon, Plasmodium, sitotoksisiteit

TABLE OF CONTENTS

LIST OF TABLES, FIGURES AND SCHEMES

LIST OF ABBREVIATIONS

ACT	Artemisinin-based combination therapy
ADME	Absorption, distribution, metabolism and excretion
AIDS	Acquired immune-deficiency syndrome
APCI	Atmospheric pressure chemical ionization
ATS	Artemisone
ARM	Artemether
ARS	Artesunate
ALI	Acute lung injury
AO	Acridine orange
ARDS	Acute Respiratory Distress Syndrome
BBB	Blood brain barrier
BCP	Benzothiocarboxypurine
CDC	Centre for Disease Control
CDRI	Central Drug Research Institute
CQ	Chloroquine
CQR	Chloroquine resitance
CQS	Chloroquine sensitive
DCM	Dichloromethane
DDT	Dichlorodiphenyltrichloroethane
DEET	*N*,*N*-diethyl-3-methylbenzamide
dTMP	2‟-deoxythymidine-5‟-monophosphate
DHPS	Dihydropteroate synthase
DHFR	Dihydrofolate reductase
DHPS	Dihydropteroate synthase
DHFR	Dihydrofolate reductase
DMSO	Dimethyl sulfoxide
ESI	Electrospray ionization

Fe(III)PPIX	Iron (III) protoporphyrin IX
GPARC	Global Plan for Artemisinin Resistance Containment
G6PD	Glucose-6-phospate dehydrogenase
GPI	Glycosylphosphatidylinositol
HIV	Human immunodeficiency virus
HRP2	Histidine rich protein 2
HPPK	Hydroxymethylpterin pyrophosphokinase
HFLF	Human fetal lung fibroblast
ITNs	Insecticidal treated nets
ICH	International Conference of Harmonization
IPT	Intermittent preventive therapy
IRS	Insecticide residual sprays
IR	Infrared
LDA	Lithium diisopropylamine
LDH	Lactate dehydrogenase
MDGs	Millennium Development Goals
MMV	Medicines for Malaria Venture
MS	Mass spectroscopy
NMR	Nuclear Magnetic Resonance
*Pf*CRT	*P. falciparum* chloroquine resistant transporter
*Pf*MDR	*P. falciparum* multidrug resistance
*Pf*MDT	*P. falciparum* metabolite drug transporter
*P*AB	*p*-aminobenzoate
PCR	Polymerase chain reaction
PRBCs	Parasitized red blood cells
PI3P	Phosphatidylinositol-3-phospate
PK	Pharmacokinetics
RDT	Rapid diagnostic tests

RBM	Roll Back Malaria
RBCs	Red blood cells
ROS	Reactive oxygen species
RI	Resistance index
SERCA	Sarcoplasmic endoplasmic reticulum Ca^{2+}-ATPase
SI	Selectivity index
SAR	Structure-activity relationships
SNPs	Single-nucleotide polymorphisms
SP	Sulfadoxine-pyrimethamine
SRB	Sulforhodamine B
TLC	Thin layer chromatography
THF	Tetrahydrofolate
TS	Thymidylate synthase
THF	Tetrahydrofolate
TS	Thymidylate synthase
UV	Ultra violet
WHO	World Health Organisation

CHAPTER 1

INTRODUCTION AND PROBLEM STATEMENT

1.1 Introduction

Malaria is an infectious protozoan disease, which, together with acquired immunodeficiency syndrome (AIDS) and tuberculosis (TB), are the three main causes of morbidity and mortality worldwide (WHO, 2009). Malaria is a significant global public health threat in more than one hundred countries, inhabited by roughly 3.4 billion people, which represent approximately 40% of the world"s total population (WHO, 2014). Five species of the *Plasmodium* genus, i.e. *P. falciparum, P. vivax, P. ovale, P. malariae* and *P. knowlesi* cause malaria infections in humans (Kantele and Jokiranta, 2011). Most infections are, however, attributed to the most lethal species, *P. falciparum,* and secondly to *P. vivax* (White et al., 2014). The female *Anopheline* mosquito is the only known vector of the disease (WHO, 2005).

In 2013, an estimated 198 million malaria cases and 584,000 related deaths were reported (WHO, 2014). The burden was heaviest in the African region, where an estimated 90% of all malaria deaths in 2013 occurred, of which children under the age of 5 years accounted for 78% of all these fatalities (WHO, 2014). The past decade has, however, witnessed a significant progress in the fight against malaria, since malaria mortality rates had decreased by 47% worldwide and by 54% in the African region, between 2000 and 2013. These successes resulted from a combination of factors, including the expanded use of chemotherapy, insecticide treated nets (ITNs) and indoor residual sprays (IRS) (WHO, 2014).

The treatment of malaria has for long relied on chemotherapeutic agents and this is expected to continue into the foreseeable future, since an effective vaccine against the disease is not yet available. The search for a vaccine has been continuing since the 1960s (WHO, 2013b). A number of potential vaccines were subjected to clinical trials, among which the most advanced candidate, RTS, S/AS01, has completed Phase III trials, with a protection efficacy of only 46% among children (2-12 years) and 27% among infants (<24 months) (WHO, 2014).

Several drugs have been used throughout the past century for the treatment of malaria. Chloroquine (CQ), an inexpensive, fully synthetic quinolone was one of the most significant antimalarial drugs being developed during the twentieth century. Unfortunately, the unwise use of this drug had resulted in the rapid emergence and spread of CQ resistant (CQR) strains of *P. falciparum* parasites (Foley and Tilley, 1998). The effectiveness of CQ against *P. vivax* parasites also progressively declined across *P. vivax* malaria endemic areas. Subsequently, towards the end of the 1970s, it became apparent that new drugs were urgently required to counter the emerging resistance of the malaria parasites against these once effective antimalarial drugs and against the combination drug, Fansidar (sulfadoxine & pyrimethamine) (Marfurt et al., 2012).

Artemisinin (**1**) and its derivatives (**2-4**) (Figure 1.1), referred to as the "*artemisinins*", are another class of antimalarial drugs that were introduced in the 1970s, as replacement for CQ. Artemisinin is a structurally unique endoperoxide compound that is obtained from the indigenous Chinese herbal plant, *Artemisia annua* (Haynes et al., 2013). The isolation of artemisinin and its characterisation as peroxide in the 1970s and 1980s, together with the preparations of its derivatives, such as dihydroartemisinin (DHA) (**2**), artemether (**3**) and artesunate (**4**) by Chinese scientists as part of Project 523, collectively represented one of the significant achievements in medicine in the late twentieth century (Haynes et al., 2013).

The three artemisinins, dihydroartemisinin, artemether and artesunate are currently clinically used. These drugs are highly potent, well tolerated during all phases of the disease and reduce parasitaemia more rapidly than any other antimalarial drug. They, however, have several serious disadvantages, such as low solubility, thermal instability and short pharmacological half-lives. These may lead to incomplete clearance of the parasites and result in recrudescence when used in monotherapy, which inevitably contribute towards the development of parasite resistance (Gautam et al., 2009, WHO, 2013a).

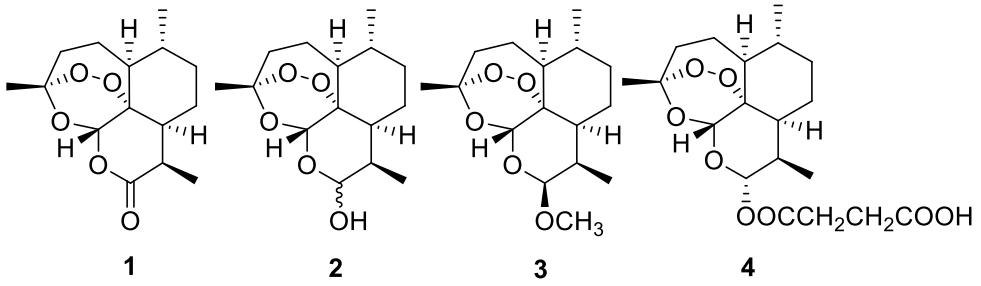
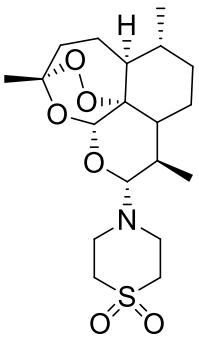


Figure 1.1: Chemical structures of artemisinin (**1**), dihydroartemisinin (**2**), artemether (**3**) and artesunate (**4**).

To prevent the development of resistance towards these drugs, the World Health Organization (WHO) recommends that they be employed in combination, rather than in monotherapies (WHO, 2010). Artemisinin based combination therapies (ACTs), used for the treatment of uncomplicated malaria, have now been introduced and adopted in almost all CQR endemic regions (WHO, 2014). ACTs comprise of an artemisinin derivative, having a longer half-life, in combination with either a quinoline or an arylmethanol (Nosten and White, 2007, Klein, 2013).

Because the use of artemisinin is hampered by its poor solubility in both oil and water, it was converted into the first generation of semi-synthetic derivatives, i.e. the oil soluble derivatives **2** and **3**, and the water soluble derivative **4**. All artemisinins in clinical use hence are structurally either an alkyl acetal, or an ester acetal. The other important shortcoming of these compounds is their short pharmacological half-lives, which result from their acid lability and facile metabolism into DHA, their active metabolite. DHA is associated with neurotoxicity in laboratory animals, rendering artemether and artesunate neurotoxic as well (Schmuck and Haynes, 2000). Additionally, increased tolerance to ACTs by the parasites now confirmed as emerging parasite resistance to the artemisinins, has been reported in South-East Asia (Dondorp et al., 2010, WHO, 2013c).

To counteract the potential neurotoxicity of the artemisinins in humans, in the late 1990s, the Medicines for malaria venture (MMV) prioritised the development of new derivatives that would not metabolise into DHA on screens and *in vivo*. This led to the development of artemisone (**5**) (Figure 1.2), a second generation, semi-synthetic derivative of artemisinin, found to have no measurable neurotoxicity in both *in vitro* and *in vivo* assays. Artemisone has a longer half-life (3.1 h), compared to all other derivatives, namely artesunate (~50 min), artemether (1.3 h) and DHA (~ 45 min) (Pooley et al., 2010). The measured Log *P* value of artemisone of 2.49 is also lower than those of artesunate (2.77), artemether (3.98) and DHA (~2.6). Furthermore, artemisone shows superior activity than artesunate towards both CQR and CQS *P. falciparum* strains. It shows substantially higher *in vivo* activity in rodent *P. berghei* and *P. falciparum* in primate models (Nagelschmitz et al., 2008), which warrants it a "drug like" compound. It also has an aqueous solubility of 89 mg/L, which exceeds the limiting requirement for a drug to permeate biological membranes (Haynes et al., 2006).



5

Figure 1.2: Chemical structure of artemisone (**5**).

The challenges of countering the development of resistance by the malaria parasite towards both the conventional antimalarial drugs of the quinoline class and towards the current clinical artemisinins are substantial. New antimalarial drugs are urgently required. If these are to be based upon the artemisinin class, or a synthetic peroxide analogue, development must be mindful of the potential neurotoxicity associated with the current artemisinins. Overall, safe, effective and longer acting antimalarial drugs are needed and can these be achieved with regards to the artemisinins by improving their physical and chemical properties.

Artemisone does not form DHA upon metabolism (Haynes et al., 2006), the principal metabolite of all other artemisinin derivatives that has for long been associated with neurotoxicity (Schmuck and Haynes, 2000). As a result, artemisone is a far safer antimalarial drug of the artemisinin class. However, the relative polarity of this drug raises a question regarding its ability to rapidly permeate biological membranes. Structural modifications through incorporating selected lipophilic groups into the 10-(4'-S,S-dioxo-4'-thiomorpholin-1'-yl) group may result in enhanced permeation through very lipophilic membranes. It should, however, also be established whether the enhancement of its lipophilicity would not also render the drug more neurotoxic and/or cytotoxic.

1.2 Aims and objectives of the study

The aims of this study were to investigate a series of substituted artemisone derivatives with enhanced lipid solubility profiles and metabolic stability, compared to those of artemisone, as well as to assess their *in vitro* antimalarial activities against both CQ sensitive (CQS) and CQ resistant (CQR) strains of *P. falciparum*, and finally, to measure their cytotoxicity profiles against mammalian cells.

To achieve the stated aims, the following objectives were set:

- To synthesise and characterise a series of lipophilic derivatives of artemisone.
- To assess the *in vitro* antimalarial activities of these lipophilic artemisone derivatives against both CQR and CQS strains of *P. falciparum*.
- To assess the cytotoxicity profiles of these lipophilic artemisone derivatives towards *ex vivo* animal and human cells.

Bibliography

Dondorp, A. M., Yeung, S., White, L., Nguon, C., Day, N. P., Socheat, D. & von Seidlein, L., 2010. Artemisinin resistance: current status and scenarios for containment. *Nature Reviews Microbiology*, 8, 272-80.

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

Gautam, A., Ahmed, T., Batra, V. & Paliwal, J., 2009. Pharmacokinetics and pharmacodynamics of endoperoxide antimalarials. *Current Drug Metabolism*, 10, 289-306.

Haynes, R. K., Cheu, K. W., N'Da, D., Coghi, P. & Monti, D., 2013. Considerations on the mechanism of action of artemisinin antimalarials: part 1--the 'carbon radical' and 'heme' hypotheses. Infectious *Disorders Drug Targets*, 13, 217 - 77.

Haynes, R. K., Fugmann, B., Stetter, J., Rieckmann, K., Heilmann, H.-D., Chan, H.-W., Cheung, M.-K., Lam, W.-L., Wong, H.-N., Croft, S. L., Vivas, L., Rattray, L., Stewart, L., Peters, W., Robinson, B. L., Edstein, M. D., Kotecka, B., Kyle, D. E., Beckermann, B., Gerisch, M., Radtke, M., Schmuck, G., Steinke, W., Wollborn, U., Schmeer, K. & Römer, A., 2006. Artemisone-a highly active antimalarial drug of the artemisinin class. *Angewandte Chemie International Edition* 45, 1989-1989.

Kantele, A. & Jokiranta, T. S., 2011. Review of cases with the emerging fifth human malaria parasite, *Plasmodium knowlesi*. Clinical Infectious Diseases, 52, 1356-62.

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, 311-317.

Marfurt, J., Chalfein, F., Prayoga, P., Wabiser, F., Wirjanata, G., Sebayang, B., Piera, K. A., Wittlin, S., Haynes, R. K., Mohrle, J. J., Anstey, N. M., Kenangalem, E. & Price, R. N., 2012. Comparative ex vivo activity of novel endoperoxides in multidrug-resistant *Plasmodium falciparum* and *P. vivax. Antimicrobial Agents and Chemotherapy*, 56, 5258-63.

Nagelschmitz, J., Voith, B., Wensing, G., Roemer, A., Fugmann, B., Haynes, R. K., Kotecka, B. M., Rieckmann, K. H. & Edstein, M. D., 2008. First assessment in humans of the safety,tolerability,pharmacokinetics, and ex vivo pharmacodynamic antimalarial activity of the new artemisinin derivative artemisone. *Antimicrobial Agents and Chemotherapy*, 52, 3085-3091.

Nosten, F. & White, N. J., 2007. Artemisinin-based combination treatment of *falciparum* malaria. *The American Journal of Tropical Medicine and Hygiene*, 77, 181-92.

Pooley, S., Fatih, F. A., Krishna, S., Gerisch, M., Haynes, R. K., Wong, H.-N. & Staines, H. M., 2010. Artemisone uptake in *Plasmodium falciparum*-infected erythrocytes. *Antimicrobial Agents and Chemotherapy*, 55, 550-556.

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.

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

WHO. 2005. World malaria report;http://apps.who.int/iris/bitstream/10665/43213/1/9241593199_eng.pdf; [Date of access: 09 Sep. 2015]

WHO. 2009. World health statistics report; [WEB]:http://www.who.int/gho/publications/world_health_statistics/EN_WHS09_Full.pdf; [Date of access: 15 Sep. 2015]

WHO. 2010. Guidelines for the treatment of malaria 3rd ed; [WEB]: http://apps.who.int/iris/bitstream/10665/162441/1/9789241549127_eng.pdf; [Date of access: 10 Sep.2015]

WHO. 2013a. Emergency response to artemisinin resistance in the Greater Mekong sub region: regional framework for action 2013-2015; [WEB]: http://www.who.int/malaria/publications/atoz/9789241505321/en/; [Date of access: 20 Feb. 2015]

WHO. 2013b. World malaria report; [WEB]: http://www.who.int/malaria/publications/world_malaria_report_2013/en; [Date of access: 25 Feb. 2014]

WHO. 2013c. World malaria [WEB]: report;http://www.who.int/malaria/publications/world_malaria_report_2013/en/: [Date of access : 2 Feb.2014]

WHO. 2014. World malaria report; [WEB]: http://www.who.int/malaria/publications/world_malaria_report_2014/report/en/; [Date of access: 12 Feb. 2015]

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Malaria is a global public health threat, with nearly half of the world"s population at risk. It is a leading cause of morbidity and mortality in the low and lower-middle income countries (WHO, 2014). Approximately 198 million malaria cases and 584,000 related deaths were reported worldwide in 2013, of which most were in sub-Saharan Africa (WHO, 2014). The disease is caused by five species of the protozoan parasite belonging to the genus, *Plasmodium* (WHO, 2013e). Four of these species, i.e. *P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale* are malaria species that infect humans and are they spread by female mosquitoes of the genus, *Anopheles* (Ashley et al., 2006). Recently, however, human malaria cases have also been reported as having been caused by *P. knowlesi*, species known to cause malaria among monkeys in certain forested areas of South-East Asia (WHO, 2014). Malaria that originates from *P. falciparum* is the most deadly form of the disease (WHO, 2013e).

2.1.1 Geographical distribution of malaria

The distribution of the main human pathogenic *Plasmodium* species shows high prevalence of *P. falciparum* in tropical Africa, while *P. vivax* prevails over *P. falciparum* in the Americas, South-East Asia and Western Pacific (WHO, 2012c). Although *P. malariae* may occur in all malaria affected areas, its prevalence is generally very low, with the exception of tropical Africa, where it frequently overlaps with *P. falciparum* (Wernsdorfer, 2012). Tropical Africa is also the only known region where endemic *P. ovale* exists (Wernsdorfer, 2012, Lysenko and Beljaev, 1969).

Most people at risk of malaria live in areas of stable transmission, where population immunity is sufficient to prevent large epidemics from occurring. In such areas, transmission is limited by rainfall, or lower temperatures (climate dependent), resulting in strong seasonal patterns and occasional major epidemics (Kakkilaya, 2004). Of the 106 countries with continuous malaria transmission, 64 countries are meeting the Millennium Development

Goals (MDGs) target of reversing the incidence of malaria, and of these, 55 countries are on track to meet the Roll Back Malaria (RBM) and World Health Assembly (WHA) target of reducing malaria incidence rates with 75% by the end of this year *i.e.*, 2015 (WHO, 2014). More than 80% of all estimated malaria deaths occur in just seventeen countries, and 80% of all estimated cases occur in only 18 countries, with the Democratic Republic of Congo and Nigeria together accounting for 40% of the total estimated malaria cases (WHO, 2014).

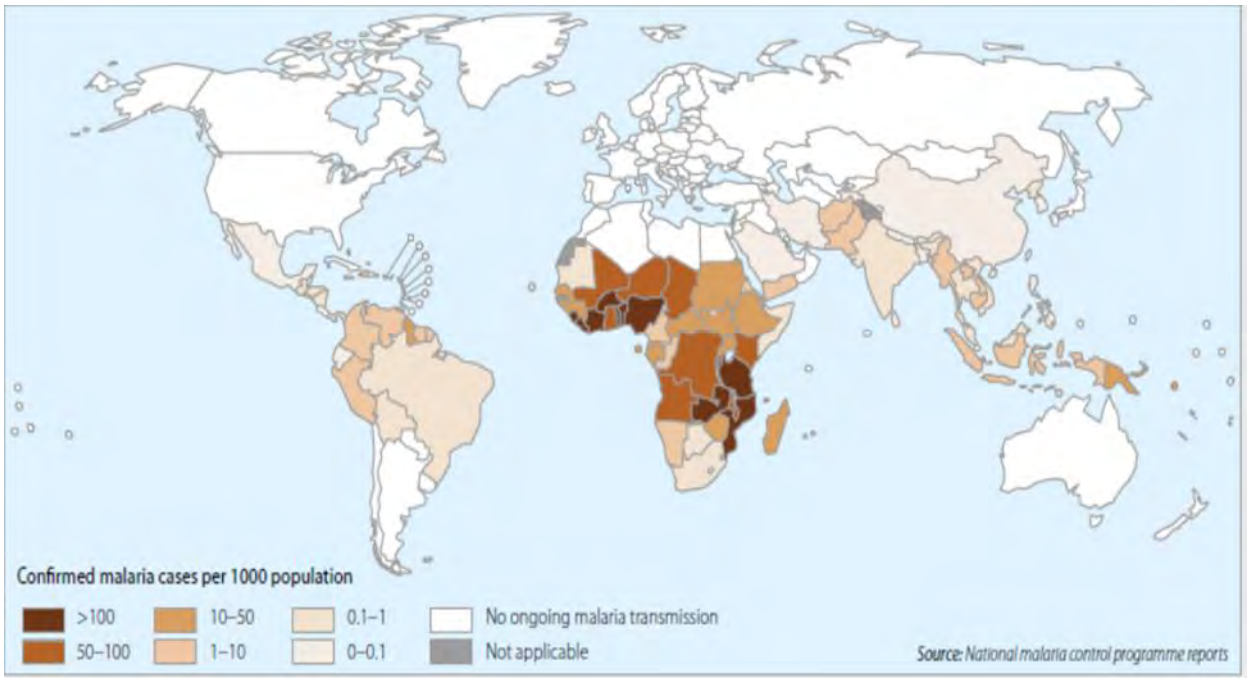


Figure 2.1: World map showing countries with continuous malaria transmission (WHO, 2014).

In endemic areas, the distribution of malaria varies among population groups and are infants, children under the age of five, pregnant women, patients with human immunodeficiency virus / acquired immunodeficiency syndrome (HIV/AIDS), and non-immune migrants higher at risk of contracting the disease (WHO, 2013e).

Malaria during pregnancy is a major cause of maternal deaths, low birth weights and still births and do the clinical features of infection during pregnancy vary according to the degree of immunity that women have acquired by the time they become pregnant and will they thus depend upon the epidemiological setting (Desai et al., 2007). In high transmission areas, first-time pregnant women (primigravida) are at higher risk of infection, whereas the risk is low among multi-time pregnant women (multigravida) in low transmission areas, and absent in areas with epidemic malaria (Kochar et al., 1998). Younger maternal age (especially adolescence) is also an independent factor to malaria during pregnancy, both among

primigravida and multigravida (Ogbodo et al., 2009), which suggests that in addition to the parity-specific immunity being acquired through consecutive pregnancies, age associated immunity also plays an important role in controlling the infection during pregnancy in areas of high and stable transmission (Desai et al., 2007).

2.2 Life cycle of malaria parasites

The malaria parasite that infects humans has a complex life cycle that occurs within two living hosts, the insect vector (female anopheles mosquito species) and the human host. The parasite passes through several stages of development, as described next and as depicted in Figure 2.2.

2.2.1 Sporogony

The transmission of malaria relies upon the successful development of *Plasmodium* parasites within mosquitoes, a process that is referred to as sporogony. It is a complex event, involving several morphologically distinct life stages and begins when mosquitoes ingest blood that contains both male and female gametocytes (Kakkilaya, 2004). Within the mosquito vector, the development of the parasite progresses through various stages, i.e. gametogenesis and fertilisation, zygote transformation into ookinetes, ookinete motility through the blood meal and peritophic matrix, penetration across midgut epithelia, and encystment beneath the mid-gut basal lamina to form oocysts (Zollner et al., 2006). In the basal lamina, oocysts first enlarge in size and later undergo multiple rounds of mitosis to form a syncytium, followed by differentiation to form several thousands of daughter cells, called sporozoites (Aly et al., 2009). These then migrate to the salivary glands of the mosquito (Zollner et al., 2006, Dhangadamajhi et al., 2010).

2.2.2 Ex-erythrocytic phase

The sporozoites are injected from the salivary glands of the mosquito through the skin of the host to enter the blood circulation and are they transported to the liver, where the parasites undergo nuclear replication (Vaughan et al., 2008). Malaria parasites have evolved strategies that suppress the host"s immune system, which ensure that at least a fraction of the thousands of injected sporozoites survive the hostile blood environment and successfully invade the hepatocytes (Florens et al., 2002, Zheng et al., 2014).

Once within the hepatocytes, the sporozoites differentiate and develop into liver trophozoites, which replicate rapidly (Vaughan et al., 2008) to produce the daughter parasites, called merozoites (Khan and Waters, 2004). The merozoites are contained within host cell derived vesicles, called merosomes that protect them from phagocytosis, until shortly before they are released into the blood stream (Silvie et al., 2008). Cysteine proteases are thought to mediate the release of the merozoites from the hepatocytes through a process, known as egress and, unlike the erythrocytic phase that occurs repeatedly, the ex-erythrocytic phase occurs in a single cycle (Kakkilaya, 2004).

The liver stages of the two most important species, *P. falciparum* and *P. vivax*, differ significantly. Unlike *P. falciparum*, *P. vivax* has a dormant liver stage, known as a hypnozoite that enables it to survive for long periods as a potential reservoir of infection (WHO, 2014). Hypnozoites can activate months, or even years later to cause relapse (Dembele et al., 2014).

2.2.3 Erythrocytic phase

The blood stages of the malaria infection include asexual forms of the parasite that undergo repeated cycles of multiplication, whilst the male and female sexual forms (gametocytes) have to be ingested by mosquitoes to continue their development in the insect host (Greenwood et al., 2008).

Following their release from the liver, the asexual merozoites enter the blood cells through multiple, receptor-ligand interactions in as little as 60 seconds (Kakkilaya, 2004). This quick disappearance from the circulation into the red blood cells (RBCs) minimises exposure of the antigens on the surface of the parasites, which protects them from the host"s immune system (Cowman et al., 2012). Within the RBCs, the parasite numbers increase rapidly, with cycles occurring every 24 h in the case of *P. knowlesi*, every 48 h for *P. falciparum*, *P. vivax* and *P. ovale,* and every 72 h for *P. malariae*. During each cycle, each merozoite grows and divides within the vacuole into 8-32 fresh merozoites, through the stages of ring trophozoite and schizont (Greenwood et al., 2008, Kakkilaya, 2004). At the end of the cycle, the infected RBCs rupture and release new merozoites that in turn infect more RBCs (Lee and Fidock, 2008).

A small portion of these parasites differentiate into the sexual male and female gametocytes stage, which are ingested by the female *Anopheles* mosquitoes during their blood meal,

where the parasites continue the sexual phase of their life cycle (Cowman and Crabb, 2006). The gametocytes represent the only stage within the life cycle of the malaria parasites during which they are able to mediate the transition from the human host into the insect host. They are formed, depending on the *Plasmodium* species, approximately 7-15 days after the parasites‟ appearance in the human blood (Kuehn and Pradel, 2010). Immature gametocytes are sequestered out of the circulation to avoid immune clearance in the spleen and are they only released when mature to be ingested by the mosquito vector during its blood meal (Bousema and Drakeley, 2011). It is the mature gametocytes that are solely responsible for parasite transmission from the human host to the mosquito (Tiburcio et al., 2015), which makes them a logical target for transmission blocking by antimalarial drugs (Delves, 2013).

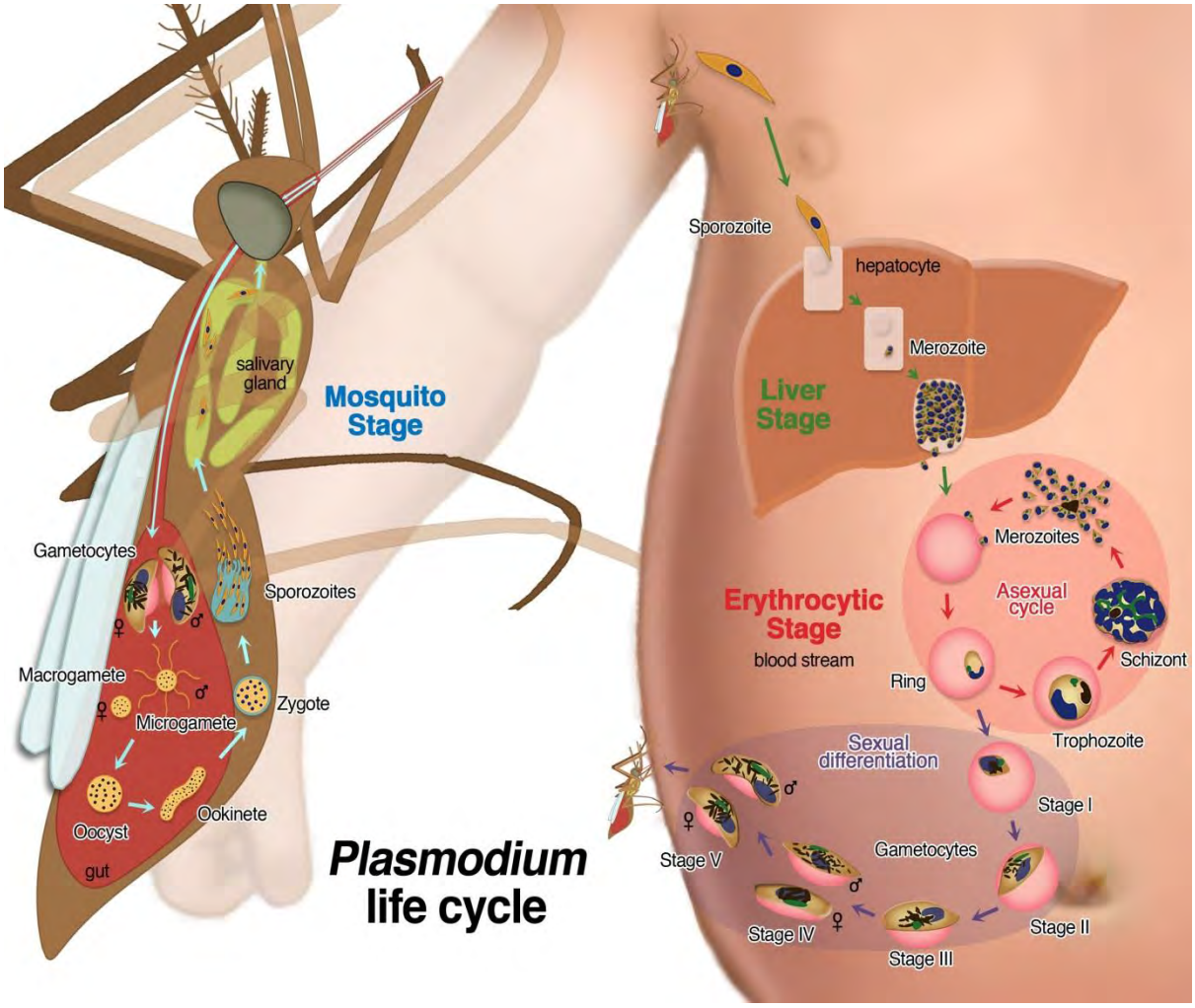


Figure 2.2: Life cycle of the malaria parasite (Wikipedia, 2015).

2.3 Malaria symptoms

The common symptoms of this deadly, but preventable and curable disease include headaches, lassitude, fatigue, abdominal discomfort, myalgia and arthralgia, as well as fever, chills and rigors, perspiration, anorexia and vomiting. The symptoms are non-specific and commonly associated with other viral and bacterial illnesses (Peter et al., 2011, Sowunmi et al., 2000). The possibility of malaria infection should, however, be considered in all patients who present with fevers in endemic areas (Janneck et al., 2011).

2.4 Malaria pathophysiology

2.4.1 Systemic manifestation of malaria

High morbidity and mortality, resulting from *P. falciparum* malaria is highly associated with the expression of cell surface proteins by the parasite on red blood cell membranes. These surface proteins mediate adhesion of the erythrocytes to platelets and endovascular cells, causing vessel occlusion and downstream ischemia (Janneck et al., 2011). Adhesion in the cerebral circulation can cause cerebral malaria, leading to encephalopathy and seizures. Adhesion in placental vasculatures can cause miscarriage, whereas adhesion in other organs can lead to organ damage (Janneck et al., 2011).

When newly formed merozoites are released from the ruptured RBCs, numerous known and unknown waste substances, such as red blood cell membrane products, haemozoin pigments and other toxic factors, such as glycosylphosphatidylinositol (GPI), are also released into the blood (Kakkilaya, 2004). These products, particularly the GPI, activate macrophages and endothelial cells that secrete cytokines and inflammatory mediators, such as tumour necrosis factor, interferon, interleukin-1, lymphotoxin and nitric oxide (Kakkilaya, 2004). The systemic manifestations of malaria, such as headaches, fever and rigors, nausea and vomiting, diarrhoea, anorexia, tiredness, aching joints and muscles, thrombocytopenia, immunosuppression, coagulopathy and central nervous system symptoms have largely been attributed to the various cytokines that are being released in response to these parasites and red cell membrane products (Kakkilaya, 2004). Individuals, who cannot effectively clear erythrocytic stage infection, are at high risk of progressing into severe malaria, which is common among young children and pregnant women (Chua et al., 2013).

2.4.2 Cerebral malaria

Cerebral malaria is a severe complication of *P. falciparum* infection, with a mortality rate of up to 30% among patients who develop this condition. Post mortem results from the brain tissues of cerebral malaria patients have revealed that small blood vessels are often packed with parasitised RBCs, a phenomenon referred to as sequestration (Adams et al., 2002). Increased permeability of the blood brain barrier (BBB) has been found in several types of neurological diseases, including infectious, ischemic neurodegenerative conditions and brain tumours. With cerebral malaria, however, the parasitised RBCs contribute towards the BBB dysfunction through adhesion mediated effects during sequestration (Adams et al., 2002).

Reduced levels of consciousness in a patient is not necessarily accounted for by cerebral malaria. Indeed, decreased levels of consciousness may result from various metabolic and haemodynamic complications. This term should therefore be restricted to patients presenting with sustained impairment of consciousness (inability to localise pain), even after correction of hypoglycaemia and hypovolaemia levels (decreased volume of circulating blood) (Maitland et al., 2003). Characteristically, manifestation of cerebral malaria includes a 1-4 day history of fever and convulsions. Coma is frequently precipitated by a prolonged seizure and the most common types of seizures are focal, motor, or generalised tonic-clonic convulsions (Maitland et al., 2003).

2.4.3 Anaemia

Anaemia is a common and potentially serious condition that is encountered in many countries of sub-Saharan Africa. About three quarters of children less than five years of age in this region suffer from anaemia, defined as haemoglobin concentrations of less than 11 g/dL in blood. Although malaria accounts for both acute and chronic anaemia, malnutrition and micronutrient deficiencies are also involved in the pathogenesis of severe anaemia and severe malaria (Akhwale et al., 2004). The pathogenesis of anaemia, due to severe malaria, is multifactorial and involves the increased destruction of erythrocytes (infected and uninfected), suppressed erythropoiesis and dyserythropoiesis. Monocytes/macrophages are directly, or indirectly involved in each of these mechanisms (Chua et al., 2013).

Together with cerebral malaria, severe anaemia is a leading cause of morbidity and mortality among malaria victims. It can occur in individuals with chronic infections and low parasitaemia as well as in those with acute *P. falciparum* malaria having high parasitaemia

(Chang and Stevenson, 2004). The population at greatest risk are children under the age of five and pregnant women in areas where *P. falciparum* is endemic, especially in sub-Saharan Africa (Chang and Stevenson, 2004).

2.4.4 Acute renal failure

Acute renal failure (ARF) is a rare, but serious complication of *P. falciparum* malaria among malaria naïve visitors to endemic regions (Das, 2008). The incidence of ARF is absent, or very low in Sub-Saharan Africa, particularly in areas of intense malaria transmission (Doolan et al., 2009, Meremo et al., 2014).

2.4.5 Pulmonary oedema

Respiratory complications in patients with acute *P. falciparum* malaria vary from minor symptoms, consistent with bronchitis, to full blown respiratory failure and even fatal pulmonary apoplexy (Taylor et al., 2006). The overall incidence of respiratory complications during this infection ranges from 3% to 10% (Safdar et al., 1999). Pulmonary complications during the course of malaria bear high mortality rates. In a series of twelve cases for example, the mortality rate was 75%, with more than half the number of deaths occurring during the first 24 h (Safdar et al., 1999).

Autopsies on patients who had died from malaria associated, acute respiratory distress syndrome (ARDS), revealed pulmonary oedema, congested pulmonary capillaries, thickened alveolar septa, hyaline membrane formation and intra-alveolar haemorrhage (Hee et al., 2011). ARDS is at the most severe end of the spectrum of lung manifestations resulting from malaria and fortunately occurs infrequently (Mohan et al., 2008). Mild respiratory signs include coughing, impairment in gas transfer and increased pulmonary phagocytic activity, with a much higher frequency among uncomplicated *P. falciparum*, *P. vivax* and *P. ovale* malaria. Studies on non-immune adults with severe malaria found that 21 - 23% of cases developed pulmonary oedema (Hee et al., 2011).

Hyperventilation is a common type of respiratory distress among children and adult malaria patients in Africa, which results from metabolic acidosis, mainly due to accumulated lactate/lactic acid being produced by the parasite, or by peripheral oxygen deficient tissues (Van den Steen et al., 2013). Malaria associated ARDS and acute lung injury (ALI) have been associated with the disruption of alveolar membrane integrity, leading to leakage of

plasma fluid into the interstitium and the alveoli. Damage to the endothelial barrier presumably occurs first and results in interstitial oedema, and when the epithelial barrier has been compromised, oedema fluids leak into the alveoli as well. As a consequence, eosinophilic hyaline membranes are formed, which cover the alveolar walls and sometimes fill the alveoli with fibrin containing proteinaceous depositions (Van den Steen et al., 2013).

2.4.6 Hypoglycaemia

Hypoglycaemia (blood glucose of <2.2 mmol/L) has been identified as an independent risk of death in children, who present with severe malaria, along with coma, repeated convulsions, shock and hyperparasitaemia (Ogetii et al., 2010, Osonuga et al., 2011). Generally, 8% of adults, 30% of children and 50% of pregnant women in late stages develop malaria induced hypoglycaemia (Osonuga et al., 2011). Sudden, unexplained deterioration of a patient with severe *P. falciparum* malaria is common, due to hypoglycaemia. Such condition is, however, clinically easily overlooked, because the manifestations may be similar to those of cerebral malaria (White et al., 1983, Osonuga et al., 2011).

Although the aetiology of hypoglycaemia is incompletely understood, it does appear to be multifactorial, with the implication of depleted glucose stores, due to starvation, the parasites‟ utilisation of glucose and the cytokine induced impairment of gluconeogenesis. Hyperinsulinemia, resulting from quinine therapy, has also been identified as an iatrogenic cause and it is well established in adults (Ogetii et al., 2010).

2.5 Diagnosis of malaria

Early and accurate diagnosis of cases is the cornerstone in the management of malaria (WHO, 2013e). In malaria endemic areas, this is accomplished either through laboratory facilities at health centres and hospitals, rapid diagnostic tests (RDT) at peripheral facilities where microscopy is unavailable, or through patient clinical presentations (Sharew et al., 2009). Microscopic examination of giemsa stained thick and thin blood films has remained the gold standard technique in the diagnosis of malaria (Wongsrichanalai et al., 2007). Despite this reliable technique, it is, however, difficult to make microscopy services available at peripheral health care services where most patients are treated (Sharew et al., 2009).

Microscopy requires expensive equipment and requires a high level of skill to achieve acceptable sensitivity (Tietje et al., 2014). Malaria RDTs are easier to use and can they

detect specific *Plasmodium* parasites‟ antigens by using one or more of the three target antigens, i.e. histidine rich protein 2 (HRP2), lactate dehydrogenase (LDH) and aldolase. HRP2 is expressed only by *P. falciparum* and is it the most widely used target antigen for malaria RDTs (Maltha et al., 2013). LDH and aldolase are expressed by all *Plasmodium* species, but tend to yield lower diagnostic accuracy in commercially available RDTs (Tietje et al., 2014).

The advantages of RDT techniques, such as obtaining results in half an hour, even with unskilled technicians, are tempered by a few limitations (Getnet et al., 2015). These methods do not offer better sensitivity than microscopy, and the test sensitivity does decrease, if the parasitaemia level is below 100 parasites/μL (Jelinek et al., 1999). False positive results are particularly observed after treatment, as the parasite antigens can remain in the circulation following parasite clearance (Moody, 2002). Moreover, the majority of RDTs detect HRP2, an antigen expressed only by *P. falciparum* and not by other species, thus rendering the RDT technique *P. falciparum* malaria specific (Surabattula et al., 2013).

In an attempt to enhance the detection of malaria parasites in blood films, alternative methods have been introduced. Certain fluorescent dyes have an affinity for the nucleic acids in the parasite‟s nucleus. These attach to the nuclei and on excitation by ultraviolet (UV) light at an appropriate wavelength, cause the nucleus to strongly fluoresce (Adeoye and Nga, 2007). Two fluorochromes have frequently been used for this purpose, namely acridine orange (AO) and benzothiocarboxypurine (BCP), which exhibit green to yellow fluorescence on excitation at 490 nm (Makler et al., 1991, Adeoye and Nga, 2007). Rhodamine-123 is also useful in assessing the viable state of the parasite, since its uptake relies upon an intact parasitic membrane (Adeoye and Nga, 2007).

Several polymerase chain reaction (PCR) based assays have also been developed for the detection and identification of malaria parasites. Although the PCR and real-time PCR are more sensitive and can detect 1-5 parasites/μL of blood in some instances, these methods are very expensive, require sophisticated instruments and are feasible only in well-established laboratories, with proper technical expertise (Mohon et al., 2014).

2.6 Malaria prevention

2.6.1 Insecticide treated nets and indoor residual spraying

The major vector control interventions in highly endemic malaria regions include indoor residual sprays (IRS) and the use of insecticide treated nets (ITNs). The choice for ITNs, or IRS is made, based upon a number of entomological, epidemiological and operational factors, such as seasonality of transmission, housing density and distribution, and insecticide susceptibility of *Anopheles* vectors (WHO, 2013e). In areas where both ITN and IRS vector control interventions were used, the prevalence was significantly reduced, when compared to areas where each of these interventions was used in isolation, suggesting an additive effect of the two techniques (Ulrich et al., 2013).

The successes being achieved with pyrethroid insecticide treated nets have revitalised interest in vector control as a viable means to reduce the malaria burden, even in parts of sub-Saharan Africa, where high transmission levels result in extremely stable prevalence, incidence and clinical burden (Killeen and Smith, 2007). The efficacy of the pyrethroid based insecticide has, however, significantly decreased in many malaria endemic areas, due to the formation of resistance (Gnankine et al., 2013).

Dichlorodiphenyltrichloroethane (DDT) remains among the recommended insecticides for IRS and until equally cost effective alternatives to DDT are developed, the use of this environmentally unfriendly chemical is expected to continue in the foreseeable future (WHO, 2011a). DDT has several characteristics that are of particular relevance to malaria vector control. It is the insecticide with the longest residual efficacy when sprayed onto walls and ceilings (WHO, 2011a). It also has a spatial repellence and has an irritant effect on malaria vectors that strongly limit vector-human contact. Despite the debate on its environmental impact, DDT has continued to be used in IRS in malaria endemic countries, even long after this compound was banned in the United States (US) and many other developed countries (Corbel et al., 2012, WHO, 2014).

2.6.2 Biological vector control

In the most intensely endemic parts of Africa and the Pacific, current vector control methods, based upon ITN and IRS, may not significantly halt parasite transmission. Alternative techniques, including microbial application in malaria transmission control, have hence

attracted global attention (Kamareddine, 2012). *Bacillus* based biolarvicides have been used to control mosquito larvae in different breeding habitats (Mittal, 2003), while entomopathogenic fungi have also shown promise as effective and evolution proof agents against adult mosquitoes (Lynch et al., 2012). Nevertheless, the deployment and possible use in integrated vector management programmes of this set of techniques still require investigation (Abdul-Ghani et al., 2012).

2.6.3 Skin based insect repellents

Skin based insect repellents (IR), along with protective clothing, continue to be the mainstay in the prevention of mosquito bites in- and outdoors (Wilson et al., 2014). Many formulations of IR are available on the market today, including aerosols, pump sprays, lotions, creams, suntan oils, powders, grease sticks and cloth-impregnating laundry emulsions. The currently available active ingredients in these formulations include *N*,*N*-diethyl-3-methylbenzamide (DEET), lemon eucalyptus oil, citronella and picaridin (Katz et al., 2008). DEET, however, remains the most commonly and widely used insect repellent on the market today (Wilson et al., 2014).

Although oil of citronella is approved for use on humans as insect repellent, with little or no known toxicity, it is known to trigger hypersensitivity associated dermatological reactions (Shapiro, 2012). Clove oil, blended with geranium oil, or thyme oil, is also highly efficacious, but has the potential of causing skin irritations (Maia and Moore, 2011, Cortés-Rojas et al., 2014).

Picaridin is a new generation, customised active ingredient, designed to repel a variety of arthropods (EWG, 2013). Alone, or in combination with oil of lemon eucalyptus, or DEET (WHO, 2012a), it offers up to 14 h of protection against insect bites and does it not significantly attack household materials, including plastics, coatings, foils and varnishes, which makes it user friendly (Ajwa et al., 2010).

2.6.4 Preventive chemotherapy

2.6.4.1 Intermittent preventive treatments for children and pregnant women

Pregnant women and children are the most vulnerable groups to contract malaria and to develop serious complications from the disease (Takem et al., 2009). An intermittent

preventive therapy (IPT) comprises the administration of a full therapeutic course of an antimalarial drug to risk groups at specified time intervals, whether or not the parasites are present (Ndiaye et al., 2013). Because of its low cost, safety and prolonged post-treatment prophylactic effects, sulfadoxine-pyrimethamine (SP) is recommended as preventive therapy for malaria vulnerable populations (WHO, 2005b). Two doses of SP are administered to pregnant women in malaria endemic areas, beginning in the second trimester of pregnancy (WHO, 2013e). More recently, it has been established that continuous chemoprophylaxis in infants reduces malaria related morbidity and mortality (WHO, 2013e, Aponte et al., 2009).

2.6.4.2 Antimalarial chemoprophylaxis for non-/semi-immune travellers

An estimated 80 to 90 million travellers visit malaria endemic areas annually. Not all travellers, however, have a similar risk for contracting malaria, as it depends upon a number of factors, including the type and intensity of malaria transmission at the travel destination, the duration and style of travel, the prevention measures being employed and various individual characteristics (Schlagenhauf and Petersen, 2008). The prevention of malaria infections among healthy, non-immune, or semi-immune individuals, who travel to malaria risk areas, is therefore critical (Franco-Paredes and Santos-Preciado, 2006).

Among the drugs being recommended for prophylaxis by travellers in malaria endemic areas, atovaquone-proguanil (Malarone), primaquine and doxycycline are advised for short term travellers (daily dosing), while mefloquine and chloroquine are recommended for long term travellers, because of the long interval of up to one week in between doses (Boggild, 2008). Chemo-prophylactic treatment regimens must be started prior to arrival in the risk area, be taken for the duration of the travel period and continued for one to four weeks post-travel, depending on the specific regimen (Boggild, 2008). The decision by travellers to use chemoprophylaxis, however, depends upon an individual risk-benefit analysis of weighing the risk of contracting malaria, against the possible adverse effects of taking the medication, including, among many others, haemolysis of RBCs in glucose-6-phospate dehydrogenase deficient (G6PD) people with primaquine, gastro-intestinal symptoms (nausea and vomiting), as well as hypersensitivity reactions (Steinhardt et al., 2011, Fernando et al., 2011).

2.7 Malaria treatment

In the absence of any effective antimalarial vaccine to date, antimalarial chemotherapeutic agents remain the only treatment option (WHO, 2014). In 2014, four malaria vaccines were

subjected to field trials, three candidate vaccines had reached phase 2B clinical trials and one (RTS, S/AS01) had completed a phase III trial. In an 18-month follow-up Phase III clinical trial for RTS, S/AS01, a 46% decrease in severe malaria incidences among children, and a 27% decline among infants, who received the vaccine, were reported. A decision regarding a policy recommendation for use of the vaccine was yet to be issued by the WHO in 2014 (WHO, 2014).

Antimalarial drugs exert selective toxicity against the malaria parasites through various mechanisms that target different life cycle stages of the parasite, namely:

- **Tissue schizonticides**: These drugs act on hypnozoites in the liver to prevent relapse and reactivation of malaria symptoms. The 8-aminoquinoline primaquine is the prototype agent in this class (Delves et al., 2012).
- **Blood schizonticides**: These antimalarial drugs act on the blood forms of the parasite and help to alleviate the clinical symptoms of malaria. Most of the currently recommended drugs are in this category. They include, but are not limited to the artemisinins, chloroquine, quinine, mefloquine, pyrimethamine, sulfadoxine and tetracyclines (Kakkilaya, 2004, Foley and Tilley, 1998).
- **Gametocytocides**: These drugs destroy the sexual forms of the malaria parasites in the blood and thereby prevent transmission of infections to the mosquito. Chloroquine and quinine have gametocytocidal activity against *P. vivax* and *P. malariae*, but not against *P. falciparum* (Delves et al., 2012, Lopez-Antunano, 1999). Contrary, primaquine is active against the mature gametocytes of all of the *Plasmodium* species (Wilairatana et al., 2010).
- **Sporontocides**: These drugs prevent the development of oocysts in the mosquito and thereby interrupt the transmission cycle. The prototype drug is primaquine (Kakkilaya, 2004, Rieckmann et al., 1968).

2.8 Antimalarial drugs

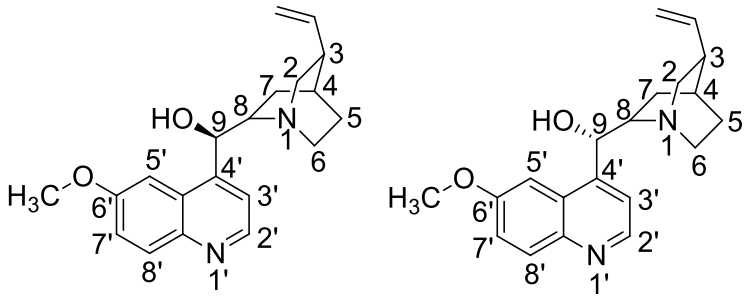
Several chemotherapeutic agents exist that have been employed in the treatment of malaria. The structural classes of antimalarial agents, some of which are no longer clinically used, due to widespread parasite resistance, are discussed next.

2.8.1 Quinoline scaffold based antimalarial

2.8.1.1 Cinchona alkaloids

The first antimalarial drugs were cinchona alkaloids that were extracted from the cinchona tree, a plant named after the countess of Cinchon, who, according to legend, had been cured of malaria in 1630 with a powder, made from its bark. The crystalline cinchona alkaloids, quinine (**6**) and cinchonine had been isolated from the tree"s bark by Runge in 1819 and by Pelletier *et. al.* in 1820 (Hofheinz and Merkli, 1984). Cinchona alkaloids, such as quinine (**6**) and quinidine (**7**) consist of two relatively rigid entities, an aromatic quinoline ring and an aliphatic quinuclidine ring that are attached by a methylene group and which differ in configuration at C-8 and C-9 (Cheng et al., 2013).

Quinine, a blood schizonticide, is a prototype antimalarial agent. It has a low therapeutic index and has the potential of causing serious adverse effects among patients (Achan et al., 2011). The side effects that are commonly experienced at therapeutic concentrations are referred to as cinchonism, of which mild forms include tinnitus, headaches, nausea and slight hearing impairment, with the latter usually being drug concentration dependent and reversible (WHO, 1990). Severe adverse events include vertigo, permanent auditory loss, drug induced hypoglycaemia and vision loss (Achan et al., 2011).



6 **7**

Figure 2.3: Chemical structures of quinine (**6**) and quinidine (**7**).

a. Mechanism of action

Although the parasite"s digestive food vacuole appears to be the site of action of cinchona alkaloids, their specific mechanism of action is still unclear (Karle and Bhattacharjee, 1999). It has, however, been suggested that following a blood meal, the parasites degrade haemoglobin in an acidic food vacuole to produce iron (III) protoporphyrin IX (Fe(III) PPIX) and reactive oxygen species as toxic by-products (Asghari-Khiavi et al., 2011). The toxic side effects are detoxified by the parasite through a series of anti-oxidant mechanisms

(Foley and Tilley, 1998), while Fe(III) PPIX is converted into a non-toxic, hydrogen-bonded, ladder like complex, called haemozoin, which is formed through the aggregation of Fe(III) PPIX monomers (de Villiers et al., 2008b). Quinine accumulates several thousand-fold in the parasite food vacuole and interferes with the aggregation through complex formation with Fe(III) PPIX (Foley and Tilley, 1998). The interaction of cinchona alkaloids with Fe(III) PPIX involves three point interactions, comprising of intra-molecular hydrogen bonds, π-stacking and coordination between the propionate side chain of Fe(III) PPIX and the protonated quinuclidine nitrogen of the cinchona alkaloids (de Villiers et al., 2012; de Villiers et al., 2008a). All of these reactions result in the accumulation of toxic metabolites that subsequently kill the parasite.

b. Resistance

Since the 1980s, a significant decline in the effectiveness of quinine against CQR malaria parasites in South-East Asia had been reported (Farooq and Mahajan, 2004). This had been related to the corresponding decrease in the innate susceptibility of *P. falciparum* to quinine (Sabchareon et al., 1988). The high sensitivity of malaria parasites to quinine in Africa had also decreased, while *in vitro* studies revealed the presence of resistant parasites in East Africa (Aminake and Pradel, 2013). Although resistance could not at the time be demonstrated *in vivo*, the fact that CQR parasites were less susceptible to quinine, was an ominous indication that the development of wide-spread quinine resistance would occur (Bjorkman et al., 1991). Mutations on the *Plasmodium falciparum* multidrug resistant (PfMDR) gene 1 is said to have accounted for the reduced susceptibility of the *Plasmodium* parasites to quinine (Farooq and Mahajan, 2004).

2.8.1.2 Synthetic quinolines

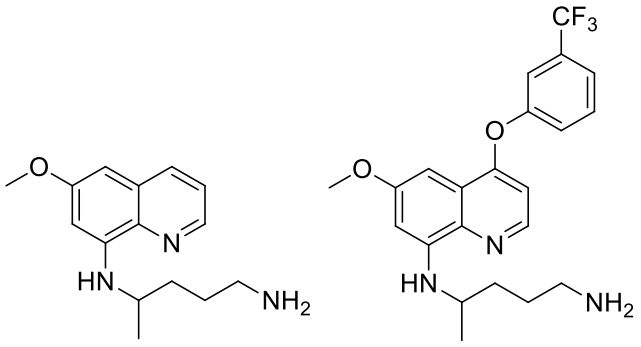
The increased demands of the quinoline antimalarial drugs during World War II led to the decision by British and German scientists to develop synthetic alternatives. A series of 8- and 4-aminoquinolines were first synthesised by researchers in the Bayer laboratories in Wuppertal, Germany (Foley and Tilley, 1998).

i. The 8-aminoquinoline antimalarials

Primaquine (**8**), the only antimalarial that is used for both the liver stage and for relapsing malaria, acts on latent, or hypnozoite forms of *P. ovale* and *P. vivax* in the liver of the host (Aminake and Pradel, 2013). In combination with a blood schizonticide (artemisinins, halofantrine, or pyrimethamine), it can completely cure latent and relapsing malaria (WHO,

2012b). Furthermore, primaquine has been long known for possessing potent antimalarial activities against *P. falciparum* mature gametocytes (Aminake and Pradel, 2013). The major shortcoming of this drug, however, is its haemotoxicity, i.e. methaemoglobinaemia and haemolysis in individuals who suffer from glucose-6-phosphate dehydrogenase deficiency (G6PD). As a result, its use in malaria vulnerable groups, such as in pregnant women, is prohibited (Dutta et al., 2006, Fernando et al., 2011), especially so, because the foetal G6PD status may not have been identified.

Tafenoquine (**9**) is another 8-aminoquinoline derivative that had initially been developed as an alternative to primaquine in the management and prevention of relapsing *P. vivax* malaria (Puri and Dutta, 2003). The drug, however, did not offer significant benefits as a tissue schizonticide, compared to those of primaquine, but did it rather present potent blood schizonticidal activities against multidrug resistant *P. falciparum* parasites (Peters, 1999), a characteristic that is recently being connected to its more enhanced accumulation in RBCs, compared to primaquine (Rajapakse et al., 2015). Tafenoquine is slowly absorbed and it is in contrast to primaquine (half-life of 4-9 h), slowly metabolised, with a much longer half-life of up to 14 days (Brueckner et al., 1998). In addition to its higher efficacy *in vitro* and *in vivo,* tafenoquine is less toxic than primaquine (Peters, 1999). However, to date, no extensive safety data exists for its use during pregnancy, in children and in individuals with G6PD deficiency (Rajapakse et al., 2015).



8 **9**

Figure 2.4: Chemical structures of primaquine (**8**) and tafenoquine (**9**).

a. Mechanism of action

The mechanisms of action of tafenoquine and primaquine are still unclear. However, these drugs are believed to severely disrupt the metabolic processes of the plasmodium mitochondria, by interfering with ubiquinone, an electron carrier in the respiratory chain (Hill

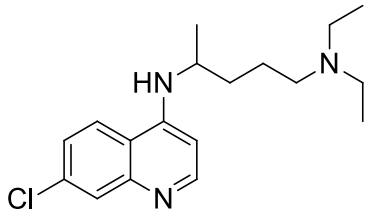
et al., 2006). Another potential mechanism involves the formation of free radical metabolites that increase oxidative stress, which subsequently kills the parasite (La-scalea, 2006).

b. Resistance

Resistance of *Plasmodium* parasites to primaquine is difficult to ascertain, because most of the so called resistant cases have been averted with the use of higher doses of the drug (Fernando et al., 2011). The validity of its reported resistance is also likely to be influenced by errors in trial design, the risk of re-infection, inadequate dosing and unsupervised treatments (Collins and Jeffery, 1996). Primaquine furthermore does not act in isolation and must resistance towards it if any must be defined in relation to the concurrently administered blood schizonticide (Fernando et al., 2011, Hill et al., 2006).

ii. The 4-aminoquinoline antimalarials

Chloroquine (**10**) was first synthesised in 1934 and was it introduced as the drug of choice in the treatment of uncomplicated malaria after World War II (Wellems and Plowe, 2001). It is the safest and most effective blood schizonticidal 4-aminoquinoline derivative ever used in the fight against malaria. However, its appearance in Asia in the 1960s and the subsequent spread of CQR strains of *P. falciparum*, were major setbacks to global efforts to try and eliminate malaria (van Schalkwyk and Egan, 2006, Wang et al., 2014).



10

Figure 2.5: Chemical structure of chloroquine (**10**).

a. Mechanism of action

Following its blood meal, the malaria parasite digests a major portion of the RBC haemoglobin to release a toxic haemoglobin derived Fe(III)PPIX (Foley and Tilley, 1998), which is then detoxified into haemozoin in the parasitic digestive food vacuole (Ziegler et al., 2001). Haemozoin formation is a bio-crystallisation process that is exploited by the malaria parasite to rid itself off toxic Fe(III)PPIX, which is released during digestion (Gildenhuys et al., 2015). The mechanism of action of chloroquine therefore involves the inhibition of the polymerisation of IX (Fe(III)PPIX) into haemozoin (Egan et al., 1997), a phenomenon that is believed to result from π-π complexation between the drug and Fe(III)PPIX (Egan, 2006). Furthermore, studies have demonstrated the critical role being played by the quinoline amino

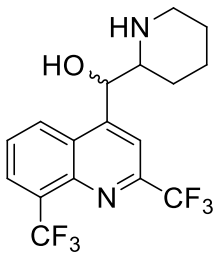
groups in the formation of drug-Fe(III)PPIX complexes through the π-π interactions (Rafiee et al., 2004), resulting in the accumulation of toxic metabolic by-products that subsequently kill the parasite.

b. Resistance

Resistance to chloroquine is now prevalent in nearly all malaria-endemic countries and has this limited the use of this safe and cost-effective antimalarial (WHO, 2014). The mechanism of resistance against this drug remains uncertain. The reduced accumulation of chloroquine in resistant parasites, compared to sensitive parasites, fuelled the assumption that resistance is actually based upon the restricted access of the drug to the parasite food vacuole (Chinappi et al., 2010, Bray et al., 2005). Recent studies have associated the reduced accumulation in resistant parasites with point mutations in the gene, encoding for the *P. falciparum* CQR transporter (PfCRT) protein (Aminake and Pradel, 2013). Studies have consistently shown that *PfCRT* is responsible for the efflux of chloroquine from the digestive food vacuole (Bray et al., 2005).

iii. The quinoline methanol antimalarials

Mefloquine (**11**) was developed in the 1970s by the US army in an effort to counteract the development of resistance to chloroquine (Schmidt et al., 1978). It offers an effective treatment against drug resistant *P. falciparum* and maintains high concentrations in the blood for several weeks (Milatovic et al., 2011). With a half-life permitting weekly dosing, mefloquine is prescribed as a drug of choice for both chemoprophylaxis and malaria therapy (Schlagenhauf et al., 2010). Its clinical use has, however, been questioned by reports of its adverse side effects, including nausea, headaches, insomnia, anxiety and depression (Milatovic et al., 2011), with the more severe events being hallucinations, amnesia and psychosis during both prophylaxis and treatment (Hood et al., 2010).



11

Figure 2.6: Chemical structure of mefloquine (**11**).

a. Mechanism of action

Like the 4-aminoquinolines, mefloquine primarily acts on the intra-erythrocytic asexual stages of the parasite (Foley and Tilley, 1998). Its mechanism of action is, however, not completely understood. Fe(III) PPIX, the toxic metabolite, as discussed above, has been widely considered as the target of all of the clinically active quinolines through the formation of a drug-Fe(III) PPIX complex toxic to the parasite (Egan et al., 1997). A recent study with crystal X-ray diffraction of the coordination complexes being formed between Fe(III) PPIX and mefloquine, has shown that interaction within the complex takes place through the deprotonated benzylic alcohol (alkoxide) functional groups of mefloquine and the metal iron (Gildenhuys et al., 2015). In addition to ligand-metal coordination, π-stacking and hydrogen bonding between the propionate side chain and the diprotic piperidinium nitrogen atom of mefloquine, have been associated with mefloquine-Fe(III) PPIX complex formation (Gildenhuys et al., 2015). It is the accumulation of this highly toxic complex that ultimately kills the malaria parasites.

b. Resistance

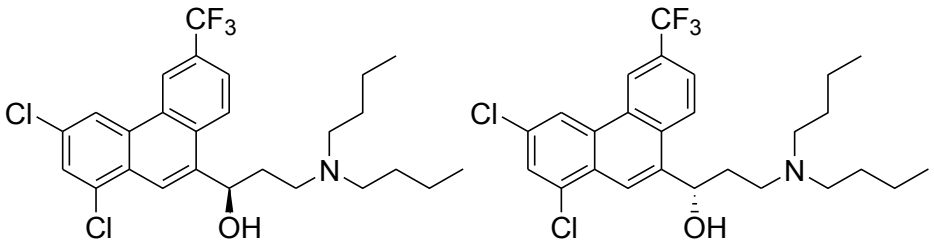
Mefloquine resistance is now common in some areas of South-East Asia, and it has also been reported in the Amazon region of South America and sporadically in Africa (Aminake and Pradel, 2013). The long half-life (2-4 weeks) of this drug is thought to increase the likelihood of resistance, due to the prolonged elimination periods (Bloland, 2001). Studies have associated parasite resistance towards mefloquine to the amplification of the *P. falciparum* multidrug resistant gene 1 (P*f*MDR*)* (Wurtz et al., 2014) and with an over expression of its protein product, *Plasmodium* glycoprotein gene homologue (Pgh-1) (Peel et al., 1994, Cowman et al., 1994). Increased mefloquine resistance in an *in vitro* line of *P. falciparum* resulted in increased resistance to halofantrine and quinine, suggesting a common mechanism of resistance against the three drugs (Peel et al., 1994, Lim et al., 1996).

2.8.2 The aryl-amino alcohol antimalarials

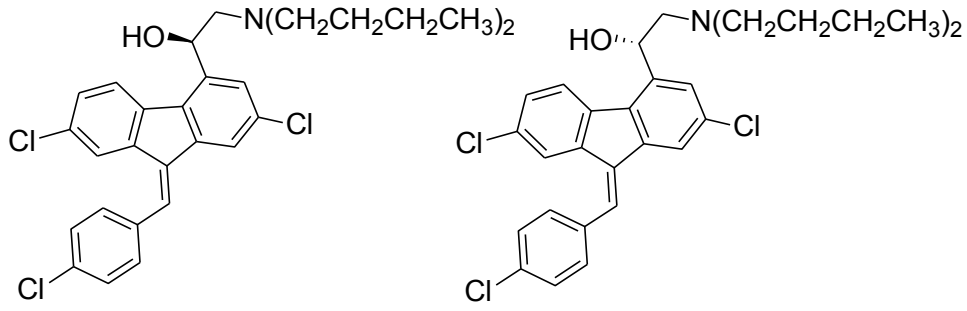
Replacement of the quinoline portion of the 4-quinolinemethanols with the phenanthrene ring system generates the class of antimalarial drugs, known as the 9-phenanthrenemethanols (Foley and Tilley, 1998). Among many others in the group, halofantrine (**12**) a blood schizonticide, was introduced as the most promising compound for the management of malaria. Its usefulness however, was later limited by reports of serious cardio-toxicity (Hombhanje et al., 1998). It exhibits low oral bioavailability and has a potential to disrupt the

heart"s electrical cycle (QT interval), causing severe ventricular arrhythmias and sudden deaths (Bouchaud et al., 2009, Leite et al., 2007).

Lumefantrine (**13**), also called benflumetol, is another arylamino alcohol, first synthesised in China in the 1970s and later registered in 1987 for the treatment of malaria (Aminake and Pradel, 2013). The drug is now widely used in ACTs being discussed below.



12



13

Figure 2.7: Chemical structures of stereoisomers of halofantrine (**12**) and lumefantrine (**13**).

a. Mechanism of action

Halofantrine and lumefantrine exert selective toxicity towards the erythrocytic stages of malaria parasites (Kakkilaya, 2004) and like the quinoline methanols, these drugs are believed to form a complex salt bridge with ferriprotoporphyrin IX (Fe(III) PPIX), a toxic by-product of haemoglobin degradation, through the π-π interactions of the aryl rings and porphyrin, and through coordination with the Fe(III) centre through the deprotonated alcohol functionality (de Villiers et al., 2008a). These altogether inhibit the conversion of the toxic Fe(III) PPIX into the non-toxic haemozoin, which effectively kills the parasite (Egan, 2006).

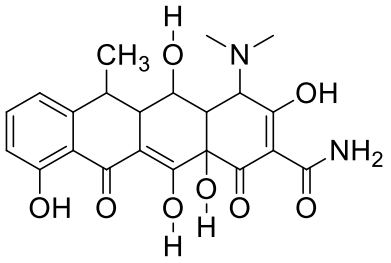
b. Resistance

Like mefloquine, resistance to halofantrine is now common in some areas of South-East Asia and has this also been reported in the Amazon region of South America and in Africa (Bloland, 2001). Mefloquine, halofantrine and lumefantrine are said to be cross resistant, a characteristic being suggested by the reduced response of malaria parasites to halofantrine and lumefantrine when used to treat mefloquine failures (Bloland, 2001, Cowman et al., 1994, Peel et al., 1994, Francois Nosten, 2012).

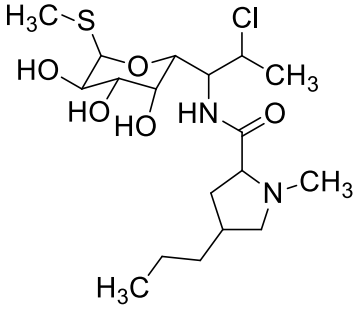
The underlying mechanism of parasite resistance to halofantrine and lumefantrine is similar to that of mefloquine (Chavchich et al., 2010), i.e. the drugs share a mechanism that is primarily mediated by the amplification of the *P. falciparum* multidrug resistant gene 1 (PfMDR1) (Woodrow and Krishna, 2006, Cowman et al., 1994).

2.8.3 Antibiotics

Tetracycline antibiotics are effective, albeit slow acting antimalarials that are used in combination with more rapidly acting drugs to treat malaria and for antimalarial chemoprophylaxis (Aminake and Pradel, 2013). Consistent with their slow clinical activity, tetracyclines exert *in vitro* antimalarial activity slowly and thus require incubation with cultured *P. falciparum* beyond a single, 48 h, asexual cycle for maximal effects (Dahl et al., 2006). Since the development of resistance by the malaria parasites to chloroquine in 1960, several studies that had been carried out in 1970, supported the recommendations of the Centre for disease control (CDC), i.e. to use doxycycline (**14**) in the chemoprophylaxis of *P. falciparum* malaria (Briolant et al., 2010a). Doxycycline, a slower acting blood schizonticide and the prototype of the group, is now recommended as a prophylactic agent and it may be used for long term chemoprophylaxis (Tan et al., 2011). When administered in conjunction with other fast acting drugs, like the artemisinins, it can be used for the treatment of malaria in non-pregnant adults and children above the age of 8 years (WHO, 2015).



14



15

Figure 2.8: Chemical structures of doxycycline (**14**) and clindamycin (**15**) antibiotics.

The distorted growth of bones in unborn babies, the altered calcification (hardening) of bones, as well as the discoloration of teeth among children have been major setbacks in the use of the tetracyclines (Draper et al., 2013). This is especially true, since this drug is contra-indicated in children and pregnant women, the two groups being at highest risk of contracting malaria and dying from it in the developing world (Tan et al., 2011).

Together with doxycycline, clindamycin (**15**) is another antibiotic that has for long been employed in the fight against malaria (Lell and Kremsner, 2002). It is a 7-chloro-lincomycin derivative that is metabolised *in vivo* into three major biologically active derivatives (Lell and Kremsner, 2002, Hall et al., 1975). Clindamycin does not possess the undesired effects of tetracyclines, and is it preferentially used for treatment of malaria in children.

a. Mechanism of action

The drugs show strong inhibitory effects on *P. falciparum*, possibly by targeting the apicoplast. The drug accumulates slowly in the parasite with the relevant inhibition being achieved through exposure of more than 3 days (Dahl et al., 2006, Lell and Kremsner, 2002). This explains the slow onset of clinical action by clindamycin and doxycycline, a character that makes these drugs undesirable in cases where fast parasite clearance is required (Lell and Kremsner, 2002). It is also believed that doxycycline and clindamycin antimalarial action may be similar to that of their bacteriostatic actions of binding to ribosomal subunits and inhibiting protein synthesis, but this has only been observed in supra-pharmacological doses (Tan et al., 2011).

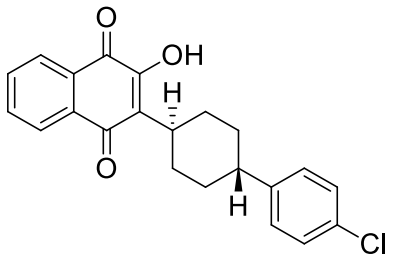
b. Resistance

Recent studies have highlighted the decreased susceptibility *in vitro* of *P. falciparum* isolates from different areas to doxycycline (Gaillard et al., 2015). Genetic markers of *P. falciparum*

metabolite drug transporter (pfmdt*)* and *P. falciparum* GTPase tetQ (pftetQ*)* genes have been identified and used in the detection and monitoring of the susceptibility of *P. falciparum* to doxycycline (Briolant et al., 2010b, Gaillard et al., 2013). However, the exact mechanism on how these markers are involved in resistance development is still not clear (Gaillard et al., 2013).

2.8.4 The naphthoquinone antimalarials

Atovaquone (**16**), a hydroxynaphthoquinone derivative, is most widely used as an antimalarial chemoprophylactic agent in combination with proguanil (Boggild et al., 2015), and in the treatment of opportunistic infections in immunosuppressed patients (Bloland, 2001). With a novel mechanism of action, atovaquone is a potent drug against the multidrug resistant malaria parasites, without any evidence of cross resistance *in vitro* (Kapadia et al., 2001).



16

Figure 2.9: Chemical structure of atovaquone (**16**).

a. Mechanisms of action

The antimalarial activity of atovaquone has been attributed to its interference with mitochondrial electron transport in the parasite (El Hage et al., 2009). It is a competitive inhibitor of the quinol oxidation site of the mitochondrial cytochrome bc1 complex and its inhibition results in the collapse of the mitochondria"s membrane potential (Fisher et al., 2012). The activity of dihydroorotate dehydrogenase, a parasitic key enzyme in the biosynthesis of pyrimidines, is also inhibited, resulting in the disruption of the plasmodial DNA synthesis and replication (El Hage et al., 2009, Fisher et al., 2012).

b. Resistance

Resistance to atovaquone, when used alone, develops rapidly and due to its high rate of treatment failures, the drug has been combined with other antimalarials, including proguanil and doxycycline (Bloland, 2001). Treatment failures by atovaquone have been associated with point mutations in the mitochondrial cytochrome b gene (Kessl et al., 2005). Molecular

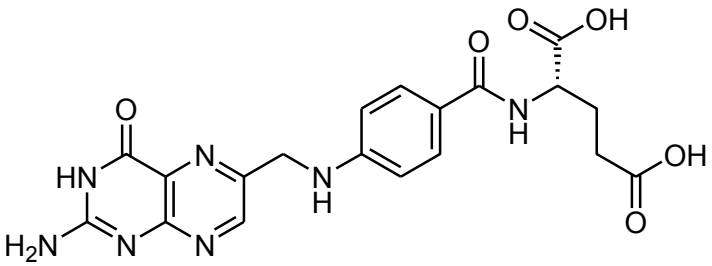
simulations have suggested that Y268S, a point mutation in cytochrome b, reduces hydrophobic interactions of cytochrome bc1 protein complex to atovaquone, resulting in activity loss (Akhoon et al., 2014).

2.8.5 The antifolates

Folate (vitamin B9) biosynthesis in the human malaria parasite *P. falciparum* is an essential activity during cell growth and replication, which is a unique metabolic pathway that occurs in the parasitic cells alone, making it a target by antimalarials (Nzila, 2006b). Antifolates are a class of compounds that interfere with folate metabolism, an important pathway of the *P. falciparum* parasites (Salcedo et al., 2001).

a. Mechanisms of action

The antifolates target two enzymes in the folate biosynthesis of the parasite,i.e. dihydropteroate synthase (DHPS) and dihydrofolate reductase (DHFR) (Figure 2.12) (Wang et al., 2010), where they inhibit the parasitic folic acid synthetic pathway.

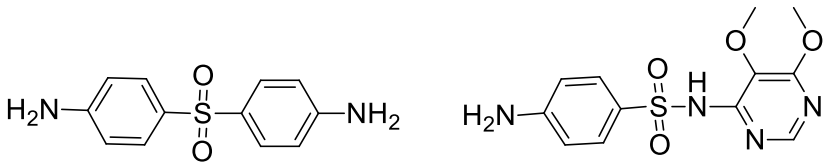


17

Figure 2.10: Chemical structure of folic acid (17).

i. Dihydropteroate synthase inhibitors: Sub-class 1

Dapsone (**18**), sulfadoxine (**19**) and other related sulfa-drugs (sulfonamides) act by interacting with the activity of the enzyme, dihydropteroate synthase (DHPS) of the bifunctional hydroxymethylpterin pyrophosphokinase-dihdropteroate synthase (HPPK)-(DHPS) protein (Nzila et al., 2000). DHPS catalyses the synthesis of 7,8-dihydropteroate (DHP) from 6-hydroxymethyl-7,8-dihydropterin pyrophosphate and *p*-aminobenzoate (*p*AB). Inhibition of the activity of this enzyme therefore renders the parasite deficient of DHP and thus blocks the entire biosynthetic pathway, resulting in its death (Wang et al., 2010).



18 **19**

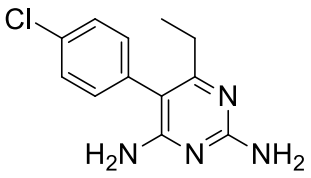
Figure 2.11: Chemical structure of dapsone (**18**) and sulfadoxine (**19**).

ii. Dihydrofolate reductase inhibitors: Sub-class 2

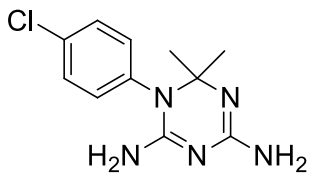
Pyrimethamine (**20**), cycloguanil (**21**), proguanil (**22**) and other related group of compounds target the dihydrofolate reductase (DHFR) activity of the parasite‟s bifunctional DHFR-thymidylate synthase (TS) protein (Nzila, 2006b). The enzyme plays the following three main roles in the folate pathway:

- It controls the *de novo* folate synthesis by mediating the synthesis of tetrahydrofolate (THF).
- It mediates the salvage of exogenous folate derivatives, DHF and the fully oxidised folate, by reducing them to THF.
- DHFR also recycles DHF from 2‟-deoxythymidine-5‟-monophosphate (dTMP) synthesis, by reducing it to THF so that it can re-enter the folate pool (Nzila, 2006a).

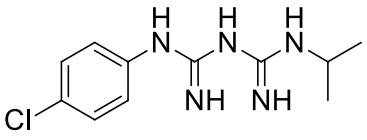
Inhibition of these enzymatic pathways therefore disrupts the constant supply of tetrahydrofolate co-factors that are required for key 1-carbon transfer reactions, including those that are critical for DNA synthesis, and therefore hinders parasite growth (Wang et al., 2010).



20



21



22

Figure 2.12: Chemical structures of pyrimethamine (**20**), cycloguanil (**21**) and proguanil (**22**).

Subsequent studies in the early 1960s revealed more rapid clinical responses and faster action when both dihydrofolate reductase and dihydropteroate synthase inhibitors were combined against *P. falciparum* and *P. ovale* malaria. Characterised by excellent compliance, good tolerance and safety, sulfadoxine-pyrimethamine (SP) became an effective and cheap antimalarial alternative to chloroquine. Currently, SP is recommended as the most effective and single-dose antimalarial prophylactic agent during pregnancy, and is it used within the approach of intermittent preventive therapy (IPT) in endemic African countries (Abdul-Ghani et al., 2013).

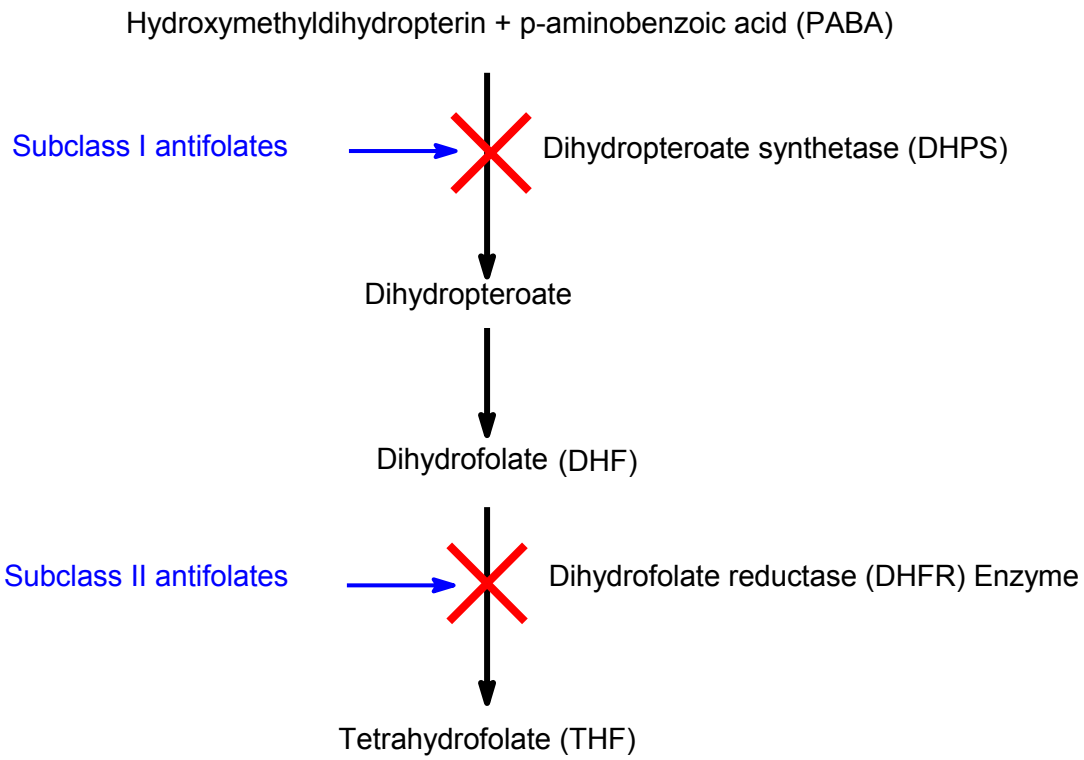


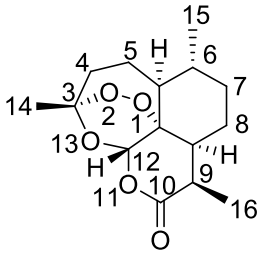
Figure 2.13: Inhibition of the parasitic folic acid biosynthetic pathway by antifolates.

b. Resistance

P. falciparum resistance against antifolates emerged rapidly (Talisuna et al., 2004). While it took about 2 decades for CQ resistant *P. falciparum* to emerge, resistance to antifolates was reported immediately after their introduction (White, 1992, Winstanley, 2000, Talisuna et al., 2004). Gene mutations (pfdhfr and pfdhps), encoding resistance to both DHPS and DHFR, the two key enzymes in the folate biosynthetic pathway targeted by antifolates, had been identified (Bloland, 2001).

2.8.6 Artemisinins

Artemisinin is a sesquiterpene lactone, containing an endoperoxide bridge, or 1,2,4-trioxane core, which is key to its antimalarial activity (Efferth and Kaina, 2010, Golenser et al., 2006, Woodrow et al., 2005). Artemisinin and its derivatives, dihydroartemisinin, artemether and artesunate (Figure 1.1) are fast acting, potent against all resistant strains of the malaria parasites, well tolerated in all phases of the disease and have the ability to reduce parasitaemia faster than any other antimalarial drug (O'Neill and Posner, 2004).



1

Figure 2.14: Chemical structure of artemisinin.

a. Mechanisms of action

The exact mechanism of action of this group of compounds has not yet been completely clarified. What is so far apparent is that the antiparasitic effects of these drugs involve the generation of reactive oxygen species (ROS), which cause oxidative stress to the malaria parasites (Aldieri et al., 2003). As to how these ROS are generated, a number of theories have been suggested. It has been postulated that the endoperoxide bond (O-1-O-2, Figure 2.14) undergoes ring opening through protonation, or formation of a complex with a metal ion (haem iron) and cleavage of the C-O bond (O-2-C-3, Figure 2.14) to generate an open hydroperoxide, or metal peroxide, which transfers oxygen to oxidisable substrates (Olliaro et al., 2001, Meshnick, 2002). It is, however, unlikely that the peroxides are able to interact with haeme to an extent that implicates the parasiticidal activity of artemisinins, as has so often been assumed, but not chemically proven (Haynes et al., 2013).

A purported structural resemblance of artemisinin with thapsigargin, a sesquiterpene lactone and an inhibitor of sarcoplasmic/endoplasmic reticulum Ca $^{2+}$-ATPase (SERCA), was used as the basis for the hypothesis that artemisinins specifically inhibit *P. falciparum* SERCA (Arnou et al., 2011). SERCA is responsible for maintaining calcium ion concentrations, which is important to the generation of calcium mediated signalling and the correct processing and post-translational folding of the parasitic proteins (Golenser et al., 2006). The inhibition of SERCA is said to occur as artemisinins and other synthetic peroxides bind into a cleft on the cytosolic side of the transporter, affecting the dynamics of Ca^{2+} homeostasis, required by the parasite for crucial intra-cellular signalling processes (Haynes et al., 2013, Golenser et al., 2006). Subsequent studies have revealed that artemisinins and fully synthetic peroxides rapidly oxidise reduced conjugates of flavin cofactors, or those of flavin cofactor precursors, such as riboflavin, which play a crucial role in maintaining intra-parasitic redox homeostasis. Inability of the parasite to control the generation and build-up of cytotoxic ROS, in turn kills it (Haynes et al., 2013).

b. Resistance

Resistance to artemisinins has been detected in five countries (Cambodia, the Lao People"s Democratic Republic, Myanmar, Thailand and Vietnam) in the great Mekong region, where artemisinin monotherapies were commonly used (WHO, 2011b). In 2011, the WHO released the Global plan for artemisinin resistance containment (GPARC) to prevent the spread of resistant parasites to other malaria endemic regions. This is especially important, because there is so far no alternative antimalarial medicine with the efficacy and tolerability than that of ACTs, as discussed below (WHO, 2012c). Despite changes in the sensitivity of parasites to artemisinins in this region, however, ACTs have remained clinically and parasitologically efficacious, provided the partner drug remains effective (WHO, 2014).

Whilst the precise mechanism of artemisinin resistance remains uncertain, mutations or amplification of gene encoding for multidrug resistance protein *Pfmdr1* (O'Brien et al., 2011), or of the encoding for *P. falciparum* sarco-endoplasmic reticulum calcium ATPase (PfSERCA) 6, have been associated with reduced susceptibility of parasites to artemisinins (Dondorp et al., 2009). Indeed, full length sequencing of the PfSERCA gene has revealed sporadic point mutations, noted as I89T, N465S, and E847K, which are proposed to confer artemisinin resistances (Dondorp et al., 2009). *Pfmdr1* amplification has been associated with increased risk of recrudescence, following artesunate-mefloquine treatment, accompanied by overall reduced susceptibility to artemisinins in South-East Asia (O'Brien et al., 2011). Additionally, a knock-out of the gene encoding for *Pfmdr* Protein increased artemisinin susceptibility, indicating the role played by this protein as an artemisinin efflux transporter (Petersen et al., 2011). More recently, artemisinin resistance has been associated with single point mutations in the propeller region of the *P. falciparum* kelch protein gene on chromosome 13 (*kelch 13*). This has led to the discovery of molecular markers, called single-nucleotide polymorphisms (SNPs) that are highly predictive of slow parasite clearance (Ashley et al., 2014, Huang et al., 2015, Wang et al., 2015).

2.8.7 Artemisinin based combination therapies

To prevent the artemisinins from meeting the same fate as other antimalarial drugs, namely the development of parasites resistance, the WHO recommended the use of artemisinins in combination, rather than in monotherapies (WHO, 2010b). This led to the adoption and introduction of artemisinin based combination therapies (ACTs) for the treatment of uncomplicated malaria in CQ resistant endemic regions. ACT combines an artemisinin derivative with a longer half-life antimalarial drug, either a quinoline or an arylmethanol. The

principle of action of ACT is that the fast acting artemisinin clears a greater portion of parasites within its short-pharmacological half-life, whilst the longer half-life partner drug then continues the clearance when the artemisinin concentration has fallen to sub-therapeutic levels. This approach aids in reducing the risk of recrudescence and drug tolerance (Woodrow et al., 2005), as well as in delaying the development of drug resistance (Dondorp and Ringwald, 2013, Klein, 2013, Bloland, 2001, WHO, 2013e, WHO, 2013a). Five ACTs, including artemether-lumefantrine, artesunate-mefloquine, artesunate-amodiaquine, artesunate-fansidar (combination of sulfadoxine and pyrimethamine), and dihydroartemisinin (DHA)-piperaquine are currently used.

In spite of this measure, safety concerns and high costs are limiting the use of ACTs in poor endemic countries. Additionally, increased tolerance to ACTs by the parasites has been noticed in South-East Asia (Dondorp et al., 2010, WHO, 2013a), which is an indication of emerging parasite resistance to the artemisinins (Meshnick, 2012).

Neurotoxicity, as indicated by a number of adverse effects, including movement disturbances, spasticity, balance deficits, brain stem tissue damage and even deaths in laboratory animals being treated with intra-muscular doses of artemether and its congener, arteether, raises concerns about their safety (Li and Hickman, 2010). By using an *in vitro* screening method on rat brain stem cultures, it has been demonstrated that artemisinins can damage the cell cytoskeleton and induce alteration in the energy state of the cell and on mitochondria through the induction of oxidative stress (Schmuck and Haynes, 2000).

This altogether emphasises the urgent need for safe, cheaper and effective new antimalarial drugs, or the enhancement of the efficacy and safety profiles of existing drugs. Progress has been made towards the development of such drugs, especially the peroxide antimalarials. Chapter 3 presents recent developments in the search for such drugs.

Bibliography

Abdul-Ghani, R., Al-Mekhlafi, A. M. & Alabsi, M. S., 2012. Microbial control of malaria: biological warfare against the parasite and its vector. *Acta Tropica*, 121, 71-84.

Abdul-Ghani, R., Farag, H. F. & Allam, A. F., 2013. Sulfadoxine-pyrimethamine resistance in *Plasmodium falciparum*: a zoomed image at the molecular level within a geographic context. *Acta Tropica*, 125, 163-90.

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. *Malaria Journal*, 10, 144.

Adams, S., Brown, H. & Turner, G., 2002. Breaking down the blood-brain barrier: signaling a path to cerebral malaria? *Trends in Parasitology*, 18, 360-6.

Adeoye, G. O. & Nga, I. C., 2007. Comparison ofquantitative buffy coat technique (QBC) with giemsa-stained thick film (GTF) for diagnosis of malaria. *International Journal for Parasitology*, 56, 308-12.

Ajwa, H., Ale, I. S., Allen, S. L., Alsop, J., Arce, G., Ashworth, D. J., Balakrishnan, S., Barnett, J. B., Barr, D. B., Barry, T., Baynes, R. E., Beauvais, S., Bentley, K. S., Bernard, C. E., Besbelli, N., Billington, R., Blacker, A. M., Blancazo, J. N., Breckenridge, C. B., Brooks, G. T., Bruckner, J., Brundage, K. M., Bui, Q., Buratti, F. M., Bus, J. S., Calvert, G. M., Carlock, L. L., Casida, J. E., Chambers, H. W., Chambers, J. E., Chan, H. P., Chester, G., Clark, J. M., Class, T., Clifton, M. S., Cochran, R., Consiglio, E. D., Dary, C. C., Dayan, F. E., De Vries, A. L., Dix, K. J., Dong, M. H., Dotson, T. A., Doull, J., Driver, J. H., Duke, S. O., Dyk, M. B., Eastmond, D. A., Eaton, D. L., Ehrich, M., Eisenbrandt, D. L., Eldridge, J. C., Evans, J. B., Farmer, D., Felsot, A. S., Fenner-Crisp, P. A., Fletcher, J. L., Flores, S., Fortmann, R., Fujita, T., Gammon, D. W., Gao, S., Garry, V. F., Gehen, S. C., Georgopoulos, P., Gollapudi, B. B., Gordon, E. B., Guerino, F., Hanley Jr, T. R., Hanson, L., Harp, P. R., Harrass, M. C., Harris, J. E., Hayes Jr, W. J., Hertner, T., Hess, F. G., Heydens, W. F., Hodgson, E., Holden, L., Hotchkiss, J. A., Hurt, S., Isukapalli, S., Iyengar, S., Iyer, P., Jensen, I. M., Jones, R. L., Jortner, B. S., Kaneko, H., Kavlock, R. J., Kelly, I. D., Kenna, M. P., Kennepohl, E., Keum, Y. S., Kim, J.-H., Kleinschmidt, L., Klonne, D. R., Knaak, J. B., Krolski, M. E., Lamb, I. C., Lampman, R. L. .2010. Hayes' handbook of pesticide toxicology. 3rd . *IN*: KRIEGER, R. (Ed.). New York, Academic Press.

Akhoon, B. A., Singh, K. P., Varshney, M., Gupta, S. K., Shukla, Y. & Gupta, S. K., 2014. Understanding the mechanism of atovaquone drug resistance in *Plasmodium*

falciparum cytochrome b mutation Y268S using computational methods. *PLoS One, 9*.

Akhwale, W. S., Lum, J. K., Kaneko, A., Eto, H., Obonyo, C., Bjorkman, A. & Kobayakawa, T., 2004. Anemia and malaria at different altitudes in the Western highlands of Kenya. *Acta Tropica*, 91, 167-75.

Aldieri, E., Atragene, D., Bergandi, L., Riganti, C., Costamagna, C., Bosia, A. & Ghigo, D., 2003. Artemisinin inhibits inducible nitric oxide synthase and nuclear factor NF-kB activation. *FEBS Letters*, 552, 141-4.

Aly, A. S., Vaughan, A. M. & Kappe, S. H., 2009. Malaria parasite development in the mosquito and infection of the mammalian host. *Annual Review of Microbiology*, 63, 195-221.

Aminake, M. N. & Pradel, G., 2013. Antimalarial drugs resistance in *Plasmodium falciparum* and the current strategies to overcome them. *Formatex Research Center*, 1, 269-282.

Aponte, J. J., Schellenberg, D., Egan, A., Breckenridge, A., Carneiro, I., Critchley, J., Danquah, I., Dodoo, A., Kobbe, R., Lell, B., May, J., Premji, Z., Sanz, S., Sevene, E., Soulaymani-Becheikh, R., Winstanley, P., Adjei, S., Anemana, S., Chandramohan, D., Issifou, S., Mockenhaupt, F., Owusu-Agyei, S., Greenwood, B., Grobusch, M. P., Kremsner, P. G., Macete, E., Mshinda, H., Newman, R. D., Slutsker, L., Tanner, M., Alonso, P. & Menendez, C., 2009. Efficacy and safety of intermittent preventive treatment with sulfadoxine-pyrimethamine for malaria in African infants: a pooled analysis of six randomised, placebo-controlled trials. *Lancet*, 374, 1533-42.

Arnou, B., Montigny, C., Morth, J. P., Nissen, P., Jaxel, C., Moller, J. V. & Maire, M., 2011. The *Plasmodium falciparum* Ca(2+)-ATPase PfATP6: insensitive to artemisinin, but a potential drug target. *Biochemical Society Transanctions,* 39, 823-31.

Asghari-Khiavi, M., Vongsvivut, J., Perepichka, I., Mechler, A., Wood, B. R., McNaughton, D. & Bohle, D. S., 2011. Interaction of quinoline antimalarial drugs with ferriprotoporphyrin IX, a solid state spectroscopy study. *Journal of Inorganic Biochemisrty*, 105, 1662-9.

Ashley, E., McGready, R., Proux, S. & Nosten, F., 2006. Malaria. *Travel Medicine and Infectious Diseases*, 4, 159-73.

Ashley, E. A., Dhorda, M., Fairhurst, R. M., Amaratunga, C., Lim, P., Suon, S., Sreng, S., Anderson, J. M., Mao, S., Sam, B., Sopha, C., Chuor, C. M., Nguon, C., Sovannaroth, S., Pukrittayakamee, S., Jittamala, P., Chotivanich, K., Chutasmit, K., Suchatsoonthorn, C., Runcharoen, R., Hien, T. T., Thuy-Nhien, N. T., Thanh, N. V., Phu, N. H., Htut, Y., Han, K. T., Aye, K. H., Mokuolu, O. A., Olaosebikan, R. R., Folaranmi, O. O., Mayxay, M., Khanthavong, M., Hongvanthong, B., Newton, P. N.,

Onyamboko, M. A., Fanello, C. I., Tshefu, A. K., Mishra, N., Valecha, N., Phyo, A. P., Nosten, F., Yi, P., Tripura, R., Borrmann, S., Bashraheil, M., Peshu, J., Faiz, M. A., Ghose, A., Hossain, M. A., Samad, R., Rahman, M. R., Hasan, M. M., Islam, A., Miotto, O., Amato, R., MacInnis, B., Stalker, J., Kwiatkowski, D. P., Bozdech, Z., Jeeyapant, A., Cheah, P. Y., Sakulthaew, T., Chalk, J., Intharabut, B., Silamut, K., Lee, S. J., Vihokhern, B., Kunasol, C., Imwong, M., Tarning, J., Taylor, W. J., Yeung, S., Woodrow, C. J., Flegg, J. A., Das, D., Smith, J., Venkatesan, M., Plowe, C. V., Stepniewska, K., Guerin, P. J., Dondorp, A. M., Day, N. P. & White, N. J., 2014. Spread of artemisinin resistance in *Plasmodium falciparum* malaria. *The New England Journal of Medicine*, 371, 411-23.

Bjorkman, A., Willcox, M., Marbiah, N. & Payne, D., 1991. Susceptibility of *Plasmodium falciparum* to different doses of quinine in vivo and to quinine and quinidine in vitro in relation to chloroquine in Liberia. *Bulletin of the World Health Organisation*, 69, 459-65.

Bloland, P. B. (2001) Drug resistance in malaria; [WEB]: http://www.who.int/csr/resources/publications/drugresist/malaria.pdf; [Date of access: 12 Feb. 2015].

Boggild, A. K., Lau, R., Reynaud, D., Kain, K. C. & Gerson, M., 2015. Failure of atovaquone-proguanil malaria chemoprophylaxis in a traveler to Ghana. *Travel Medicine and Infectious Diseases*, 13, 89-93.

Boggild, A. K. K., C.K. 2008. Protozoan Diseases: Malaria clinical features, management and prevention. *International Encyclopaedia of Public Health*, 5, 11.

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, 289.

Bousema, T. & Drakeley, C., 2011. Epidemiology and infectivity of Plasmodium falciparum and *Plasmodium vivax* gametocytes in relation to malaria control and elimination. *Clinical Microbiology Reviews*, 24, 377-410.

Bray, P. G., Martin, R. E., Tilley, L., Ward, S. A., Kirk, K. & Fidock, D. A., 2005. Defining the role of PfCRT in *Plasmodium falciparum* chloroquine resistance. *Molecular Microbiology*, 56, 323-33.

Briolant, S., Almeras, L., Belghazi, M., Boucomont-Chapeaublanc, E., Wurtz, N., Fontaine, A., Granjeaud, S., Fusai, T., Rogier, C. & Pradines, B., 2010a. *Plasmodium falciparum* proteome changes in response to doxycycline treatment. *Malaria Journal*, 9, 141.

Briolant, S., Wurtz, N., Zettor, A., Rogier, C. & Pradines, B., 2010b. Susceptibility of *Plasmodium falciparum* isolates to doxycycline is associated with pftetQ sequence

polymorphisms and pftetQ and pfmdt copy numbers. *Journal of Infectious Diseases*, 201, 153-9.

Brueckner, R. P., Lasseter, K. C., Lin, E. T. & Schuster, B. G., 1998. First-time-in-humans safety and pharmacokinetics of WR 238605, a new antimalarial. *American Journal of Tropical Medicine and Hygiene*, 58, 645-9.

Chang, K. H. & Stevenson, M. M., 2004. Malarial anaemia: mechanisms and implications of insufficient erythropoiesis during blood-stage malaria. *International Journal of Parasitology*, 34, 1501-16.

Chavchich, M., Gerena, L., Peters, J., Chen, N., Cheng, Q. & Kyle, D. E., 2010. Role of pfmdr1 amplification and expression in induction of resistance to artemisinin derivatives in *Plasmodium falciparum*. *Antimicrobial Agents and Chemotherapy* 54, 2455-64.

Cheng, G.-G., Zhang, Y., Cai, X.-H., Bao, M.-F., Gu, J., Li, Y., Liu, N., Liu, Y.-P. & Luo, X.-D., 2013. Cincholenines A and B, two unprecedented quinoline alkaloids from Cinchona ledgeriana. *Tetrahedron Letters*, 54, 4547-4550.

Chinappi, M., Via, A., Marcatili, P. & Tramontano, A., 2010. On the mechanism of chloroquine resistance in *Plasmodium falciparum*. *PLoS One*, 5.

Chua, C. L., Brown, G., Hamilton, J. A., Rogerson, S. & Boeuf, P., 2013. Monocytes and macrophages in malaria: protection or pathology? *Trends in Parasitology* 29, 26-34.

Collins, W. E. & Jeffery, G. M., 1996. Primaquine resistance in *Plasmodium vivax*. *American Journal of Tropical Medicine and Hygiene*, 55, 243-9.

Corbel, V., Akogbeto, M., Damien, G. B., Djenontin, A., Chandre, F., Rogier, C., Moiroux, N., Chabi, J., Banganna, B., Padonou, G. G. & Henry, M. C., 2012. Combination of malaria vector control interventions in pyrethroid resistance area in Benin: a cluster randomised controlled trial. *Lancet Infectious Diseases*, 12, 617-26.

Cortés-Rojas, D. F., de Souza, C. R. F. & Oliveira, W. P., 2014. Clove (Syzygium aromaticum): a precious spice. *Asian Pacific Journal of Tropical Biomedicine*, 4, 90-6.

Cowman, A. F., Berry, D. & Baum, J., 2012. The cellular and molecular basis for malaria parasite invasion of the human red blood cell. *Journal of Cellular Biology*, 198, 961-71.

Cowman, A. F. & Crabb, B. S., 2006. Invasion of red blood cells by malaria parasites. *Cell*, 124, 755-66.

Cowman, A. F., Galatis, D. & Thompson, J. K., 1994. Selection for mefloquine resistance in Plasmodium falciparum is linked to amplification of the pfmdr1 gene and cross-resistance to halofantrine and quinine. *Proceedings of the National Academy of Sciences*, 91, 1143-7.

Dahl, E. L., Shock, J. L., Shenai, B. R., Gut, J., DeRisi, J. L. & Rosenthal, P. J., 2006. Tetracyclines specifically target the apicoplast of the malaria parasite *Plasmodium falciparum*. *Antimicrobial Agents and Chemotherapy*, 50, 3124-31.

Das, B. S., 2008. Renal failure in malaria. *Journal of Vector Borne Diseases*, 45, 83-97.

de Villiers, K. A., Gildenhuys, J. & le Roex, T., 2012. Iron(III) protoporphyrin IX complexes of the antimalarial Cinchona alkaloids quinine and quinidine. *ACS Chemical Biology*, 7, 666-71.

de Villiers, K. A., Marques, H. M. & Egan, T. J., 2008a. The crystal structure of halofantrine-ferriprotoporphyrin IX and the mechanism of action of arylmethanol antimalarials. *Journal of Inorganic Biochemistry*, 102, 1660-7.

Delves, M., Plouffe, D., Scheurer, C., Meister, S., Wittlin, S., Winzeler, E. A., Sinden, R. E. & Leroy, D., 2012. The activities of current antimalarial drugs on the life cycle stages of *Plasmodium*: a comparative study with human and rodent parasites. *PLoS Medicine*, 9, e1001169.

Dembele, L., Franetich, J. F., Lorthiois, A., Gego, A., Zeeman, A. M., Kocken, C. H., Le Grand, R., Dereuddre-Bosquet, N., van Gemert, G. J., Sauerwein, R., Vaillant, J. C., Hannoun, L., Fuchter, M. J., Diagana, T. T., Malmquist, N. A., Scherf, A., Snounou, G. & Mazier, D., 2014. Persistence and activation of malaria hypnozoites in long-term primary hepatocyte cultures. *Nature Medicine*, 20, 307-12.

Desai, M., ter Kuile, F. O., Nosten, F., McGready, R., Asamoa, K., Brabin, B. & Newman, R. D., 2007. Epidemiology and burden of malaria in pregnancy. *Lancet Infectious Diseases*, 7, 93-104.

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.

Dondorp, A. M., Nosten, F., Yi, P., Das, D., Phyo, A. P., Tarning, J., Lwin, K. M., Ariey, F., Hanpithakpong, W., Lee, S. J., Ringwald, P., Silamut, K., Imwong, M., Chotivanich, K., Lim, P., Herdman, T., An, S. S., Yeung, S., Singhasivanon, P., Day, N. P., Lindegardh, N., Socheat, D. & White, N. J., 2009. Artemisinin resistance in *Plasmodium falciparum* malaria. *The New England Journal of Medicine*, 361, 455-67.

Dondorp, A. M. & Ringwald, P., 2013. Artemisinin resistance is a clear and present danger. *Trends in Parasitology* 29, 359-360.

Dondorp, A. M., Yeung, S., White, L., Nguon, C., Day, N. P., Socheat, D. & von Seidlein, L., 2010. Artemisinin resistance: current status and scenarios for containment. *Nature Reviews Microbiology*, 8, 272-80.

Doolan, D. L., Dobano, C. & Baird, J. K., 2009. Acquired immunity to malaria. *Clinical Microbiology Reviews*, 22, 13-36.

Draper, M. P., Bhatia, B., Assefa, H., Honeyman, L., Garrity-Ryan, L. K., Verma, A. K., Gut, J., Larson, K., Donatelli, J., Macone, A., Klausner, K., Leahy, R. G., Odinecs, A., Ohemeng, K., Rosenthal, P. J. & Nelson, M. L., 2013. *In vitro* and *in vivo* antimalarial efficacies of optimized tetracyclines. *Antimicrobial Agents and Chemotherapy*, 57, 3131-6.

Dutta, A. K., Avery, B. A. & Wyandt, C. M., 2006. Development and validation of a stability-indicating reversed-phase high performance liquid chromatography method for NPC 1161C, a novel 8-aminoquinoline anti-malarial drug. *Journal of Chromatography A*, 1110, 35-45.

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

Egan, T. J., 2006. Interactions of quinoline antimalarials with hematin in solution. *Journal of Inorganic Biochemistry*, 100, 916-26.

Egan, T. J., Mavuso, W. W., Ross, D. C. & Marques, H. M., 1997. Thermodynamic factors controlling the interaction of quinoline antimalarial drugs with ferriprotoporphyrin IX. *Journal of Inorganic Biochemistry*, 68, 137-45.

El Hage, S., Ane, M., Stigliani, J. L., Marjorie, M., Vial, H., Baziard-Mouysset, G. & Payard, M., 2009. Synthesis and antimalarial activity of new atovaquone derivatives. *Europian Journal of Medicinal Chemistry*, 44, 4778-82.

EWG. 2013. EWG's Guide to bug repellents: Repellent chemicals; [WEB]: http://www.ewg.org/research/ewgs-guide-bug-repellents/repellent-chemicals; [Date of access: 20 Sep. 2015].

Farooq, U. & Mahajan, R. C., 2004. Drug resistance in malaria. *Journal of Vector Borne Diseases*, 41, 45-53.

Fernando, D., Rodrigo, C. & Rajapakse, S., 2011. Primaquine in vivax malaria: an update and review on management issues. *Malaria Journal*, 10, 351.

Fisher, N., Abd Majid, R., Antoine, T., Al-Helal, M., Warman, A. J., Johnson, D. J., Lawrenson, A. S., Ranson, H., O'Neill, P. M., Ward, S. A. & Biagini, G. A., 2012. Cytochrome b mutation Y268S conferring atovaquone resistance phenotype in malaria parasite results in reduced parasite bc1 catalytic turnover and protein expression. *Journal of Biological Chemistry*, 287, 9731-41.

Florens, L., Washburn, M. P., Raine, J. D., Anthony, R. M., Grainger, M., Haynes, J. D., Moch, J. K., Muster, N., Sacci, J. B., Tabb, D. L., Witney, A. A., Wolters, D., Wu, Y., Gardner, M. J., Holder, A. A., Sinden, R. E., Yates, J. R. & Carucci, D. J., 2002. A proteomic view of the *Plasmodium falciparum* life cycle. *Nature*, 419, 520-6.

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

Franco-Paredes, C. & Santos-Preciado, J. I., 2006. Problem pathogens: prevention of malaria in travellers. *Lancet Infectious Diseases*, 6, 139-49.

Francois Nosten, P. A. P.-H., and Feiko O. ter Kuile (2012) Other 4-methanolquinolines, amyl alcohols and phentathrenes: mefloquine, lumefantrine and halofantrine. *In* HENRY M. STAINES, S. K. (Ed.) Treatment and prevention of malaria: Antimalarial Drug Chemistry, Action and Use. Switzerland, Springer Science & Business Media.

Gaillard, T., Briolant, S., Houze, S., Baragatti, M., Wurtz, N., Hubert, V., Lavina, M., Pascual, A., Travaille, C., Le Bras, J. & Pradines, B., 2013. PftetQ and pfmdt copy numbers as predictive molecular markers of decreased *ex vivo* doxycycline susceptibility in imported *Plasmodium falciparum* malaria. *Malaria Journal*, 12, 414.

Gaillard, T., Wurtz, N., Houzé, S., Sriprawat, K., Wangsing, C., Hubert, V., Lebras, J., Nosten, F., Briolant, S. & Pradines, B., 2015. Absence of association between *Plasmodium falciparum* small sub-unit ribosomal RNA gene mutations and *in vitro* decreased susceptibility to doxycycline. *Malaria Journal*, 14, 1-4.

Gautam, A., Ahmed, T., Batra, V. & Paliwal, J., 2009. Pharmacokinetics and pharmacodynamics of endoperoxide antimalarials. *Current Drug Metabolism*, 10, 289-306.

Getnet, G., Getie, S., Srivastava, M., Birhan, W., Fola, A. A. & Noedl, H., 2015. Diagnostic performance of rapid diagnostic tests for the diagnosis of malaria at public health facilities in north-west Ethiopia. *Tropical Medicine and International Health*, 20, 1564-1568.

Gildenhuys, J., Sammy, C. J., Muller, R., Streltsov, V. A., le Roex, T., Kuter, D. & de Villiers, K. A., 2015. Alkoxide coordination of iron(iii) protoporphyrin IX by antimalarial quinoline methanols: a key interaction observed in the solid-state and solution. *Dalton Transactions*, 44, 16767-77.

Gnankine, O., Bassole, I. H., Chandre, F., Glitho, I., Akogbeto, M., Dabire, R. K. & Martin, T., 2013. Insecticide resistance in bemisia tabaci gennadius (Homoptera: Aleyrodidae) and dnopheles gambiae Giles (Diptera: Culicidae) could compromise the sustainability of malaria vector control strategies in West Africa. *Acta Tropica*, 128, 7-17.

Golenser, J., Waknine, J. H., Krugliak, M., Hunt, N. H. & Grau, G. E., 2006. Current perspectives on the mechanism of action of artemisinins. *International Journal for Parasitology*, 36, 1427-1441.

Greenwood, B. M., Fidock, D. A., Kyle, D. E., Kappe, S. H., Alonso, P. L., Collins, F. H. & Duffy, P. E., 2008. Malaria: progress, perils, and prospects for eradication. *Journal of Clinical Investigations*, 118, 1266-76.

Hall, A. P., Doberstyn, E. B., Nanokorn, A. & Sonkom, P., 1975. *Falciparum* malaria semi-resistant to clindamycin. *British Medical Journal*, 2, 12-4.

Haynes, R. K., Cheu, K. W., N'Da, D., Coghi, P. & Monti, D., 2013. Considerations on the mechanism of action of artemisinin antimalarials: part 1--the 'carbon radical' and 'heme' hypotheses. *Infectious Disorders Drug Targets*, 13, 217-77.

Haynes, R. K., Fugmann, B., Stetter, J., Rieckmann, K., Heilmann, H.-D., Chan, H.-W., Cheung, M.-K., Lam, W.-L., Wong, H.-N., Croft, S. L., Vivas, L., Rattray, L., Stewart, L., Peters, W., Robinson, B. L., Edstein, M. D., Kotecka, B., Kyle, D. E., Beckermann, B., Gerisch, M., Radtke, M., Schmuck, G., Steinke, W., Wollborn, U., Schmeer, K. & Römer, A., 2006. Artemisone-a highly active antimalarial drug of the artemisinin class. *Angewandte Chemie International Edition*, 45, 1989-1989.

Hee, L., Dinudom, A., Mitchell, A. J., Grau, G. E., Cook, D. I., Hunt, N. H. & Ball, H. J., 2011. Reduced activity of the epithelial sodium channel in malaria-induced pulmonary oedema in mice. *International Journal of Parasitology*, 41, 81-8.

Hill, D. R., Baird, J. K., Parise, M. E., Lewis, L. S., Ryan, E. T. & Magill, A. J., 2006. Primaquine: report from CDC expert meeting on malaria chemoprophylaxis I. *American Journal of Tropical Medicine and Hygiene*, 75, 402-15.

Hofheinz, W. & Merkli, B. (1984) Quinine and quinine analogues. *In* PETERS, W. & RICHARDS, W. G. (Eds.) *Antimalarial Drug II.* Springer Berlin Heidelberg.

Hombhanje, F. W., Kereu, R. K., Bulungol, P. & Paika, R., 1998. Halofantrine in the treatment of uncomplicated falciparum malaria with a three-dose regimen in Papua New Guinea: a preliminary report. *Papua and New Guinea medical Journal*, 41, 23-9.

Hood, J. E., Jenkins, J. W., Milatovic, D., Rongzhu, L. & Aschner, M., 2010. Mefloquine induces oxidative stress and neurodegeneration in primary rat cortical neurons. *Neurotoxicology*, 31, 518-23.

Huang, F., Takala-Harrison, S., Jacob, C. G., Liu, H., Sun, X., Yang, H., Nyunt, M. M., Adams, M., Zhou, S., Xia, Z., Ringwald, P., Bustos, M. D., Tang, L. & Plowe, C. V., 2015. A single mutation in K13 predominates in southern China and is associated with delayed clearance of *Plasmodium falciparum* following artemisinin treatment. *Journal of Infectious Diseases*, 212, 1711-1718.

Janneck, L., Koyfman, A. & Takayesu, J. K., 2011. Clinical review of malaria for the emergency physician. *African Journal of Emergency Medicine*, 1, 126-130.

Jelinek, T., Grobusch, M. P., Schwenke, S., Steidl, S., von Sonnenburg, F., Nothdurft, H. D., Klein, E. & Loscher, T., 1999. Sensitivity and specificity of dipstick tests for rapid diagnosis of malaria in nonimmune travelers. *Journal of Clinical Microbiology*, 37, 721-3.

Kakkilaya, B. S. (2004) Life Cycle. [WEB]: http://www.malariasite.com. [Date of access 13 Jan. 2015].

Kamareddine, L., 2012. The biological control of the malaria vector. *Toxins*, 4, 748-67.

Kantele, A. & Jokiranta, T. S., 2011. Review of cases with the emerging fifth human malaria parasite, *Plasmodium knowlesi*. *Clinical Infectious Diseases*, 52, 1356-62.

Kapadia, G. J., Azuine, M. A., Balasubramanian, V. & Sridhar, R., 2001. Aminonaphthoquinones--a novel class of compounds with potent antimalarial activity against *Plasmodium falciparum*. *Pharmacological Research*, 43, 363-7.

Karle, J. M. & Bhattacharjee, A. K., 1999. Stereoelectronic features of the cinchona alkaloids determine their differential antimalarial activity. *Bioorganic & Medicinal Chemistry*, 7, 1769-74.

Katz, T. M., Miller, J. H. & Hebert, A. A., 2008. Insect repellents: historical perspectives and new developments. *Journal of the American Academy of Dermatology*, 58, 865-71.

Kessl, J. J., Ha, K. H., Merritt, A. K., Lange, B. B., Hill, P., Meunier, B., Meshnick, S. R. & Trumpower, B. L., 2005. Cytochrome b mutations that modify the ubiquinol-binding pocket of the cytochrome bc1 complex and confer anti-malarial drug resistance in Saccharomyces cerevisiae. *Journal of Biological Chemistry*, 280, 17142-8.

Khan, S. M. & Waters, A. P., 2004. Malaria parasite transmission stages: an update. *Trends in Parasitology*, 20, 575-80.

Killeen, G. F. & Smith, T. A., 2007. Exploring the contributions of bed nets, cattle, insecticides and excitorepellency to malaria control: a deterministic model of mosquito host-seeking behaviour and mortality. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 101, 867-80.

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, 311-317.

Kochar, D. K., Thanvi, I., Joshi, A., Subhakaran, Aseri, S. & Kumawat, B. L., 1998. Falciparum malaria and pregnancy. *Indian Journal of Malariology*, 35, 123-30.

Kuehn, A. & Pradel, G., 2010. The coming-out of malaria gametocytes. *Journal of Biomedical and Biotechnology*, 2010, 976827.

La-scalea, M. A., Menezes, C.M.S., Matsutami, G.C., Polli, M.C., Serrano, S.H.P. & Ferreira, E.I., 2006. Molecular modelling of the voltammetric oxidation at a glass carbon electrode of the antimalarial drug primaquine and its pro-drugs succinyl primaquine and maleylyprimaquine. *Electrochimica Acta*, 51, 8.

Lee, M. C. & Fidock, D. A., 2008. Arresting malaria parasite egress from infected red blood cells. *Nature Chemical Biology*, 4, 161-2.

Leite, E. A., Grabe-Guimaraes, A., Guimaraes, H. N., Machado-Coelho, G. L., Barratt, G. & Mosqueira, V. C., 2007. Cardiotoxicity reduction induced by halofantrine entrapped in nanocapsule devices. *Life Sciences*, 80, 1327-34.

Lell, B. & Kremsner, P. G., 2002. Clindamycin as an antimalarial drug: review of clinical trials. *Antimicrobial Agents and Chemotherapy*, 46, 2315-20.

Li, Q. & Hickman, M., 2010. Toxicokinetic and toxicodynamic (TK/TD) evaluation to determine and predict the neurotoxicity of artemisinins. *Toxicology*, 279, 1-9.

Lim, A. S., Galatis, D. & Cowman, A. F., 1996. Plasmodium falciparum: amplification and overexpression of pfmdr1 is not necessary for increased mefloquine resistance. *Experimental Parasitology*, 83, 295-303.

Lopez-Antunano, F. J., 1999. Is primaquine useful and safe as true exo-erythrocytic merontocidal, hypnozoitocidal and gametocidal antimalarial drug? *Salud Pública de México*, 41, 410-9.

Lynch, P. A., Grimm, U., Thomas, M. B. & Read, A. F., 2012. Prospective malaria control using entomopathogenic fungi: comparative evaluation of impact on transmission and selection for resistance. *Malaria Journal*, 11, 383.

Lysenko, A. J. & Beljaev, A. E., 1969. An analysis of the geographical distribution of Plasmodium ovale. *Bulletin of the World Health Organisation*, 40, 383-94.

Maia, M. F. & Moore, S. J., 2011. Plant-based insect repellents: a review of their efficacy, development and testing. *Malaria Journal*, 10, S11.

Maitland, K., Levin, M., English, M., Mithwani, S., Peshu, N., Marsh, K. & Newton, C. R., 2003. Severe *P. falciparum* malaria in Kenyan children: evidence for hypovolaemia. *Quarterly Journal of Medicine*, 96, 427-34.

Makler, M. T., Ries, L. K., Ries, J., Horton, R. J. & Hinrichs, D. J., 1991. Detection of *Plasmodium falciparum* infection with the fluorescent dye, benzothiocarboxypurine. *American Journal of Tropical Medicine and Hygiene*, 44, 11-6.

Maltha, J., Gillet, P. & Jacobs, J., 2013. Malaria rapid diagnostic tests in endemic settings. *Clinical Microbiology and Infectious Diseases*, 19, 399-407.

Marfurt, J., Chalfein, F., Prayoga, P., Wabiser, F., Wirjanata, G., Sebayang, B., Piera, K. A., Wittlin, S., Haynes, R. K., Mohrle, J. J., Anstey, N. M., Kenangalem, E. & Price, R. N., 2012. Comparative ex vivo activity of novel endoperoxides in multidrug-resistant plasmodium falciparum and P. vivax. *Antimicrobial Agents and Chemotherapy*, 56, 5258-63.

Meremo, A. J., Kilonzo, S. B., Munisi, D., Kapinga, J., Juma, M., Mwanakulya, S. & Mpondo, B., 2014. Acute renal failure in a Caucasian traveler with severe malaria: a case report. *Clinical Case Reports*, 2, 82-5.

Meshnick, S., 2012. Perspective: artemisinin-resistant malaria and the wolf. *American Journal of Tropical Medicine and Hygiene*, 87, 783-4.

Meshnick, S. R., 2002. Artemisinin: mechanisms of action, resistance and toxicity. *International Journal of Parasitology*, 32, 1655-60.

Milatovic, D., Jenkins, J. W., Hood, J. E., Yu, Y., Rongzhu, L. & Aschner, M., 2011. Mefloquine neurotoxicity is mediated by non-receptor tyrosine kinase. *Neurotoxicology*, 32, 578-85.

Mittal, P. K., 2003. Biolarvicides in vector control: challenges and prospects. *Journal of Vector Borne Diseases*, 40, 20-32.

Mohan, A., Sharma, S. K. & Bollineni, S., 2008. Acute lung injury and acute respiratory distress syndrome in malaria. *Journal of Vector Borne Diseases*, 45, 179-93.

Mohon, A. N., Elahi, R., Khan, W. A., Haque, R., Sullivan, D. J., Jr. & Alam, M. S., 2014. A new visually improved and sensitive loop mediated isothermal amplification (LAMP) for diagnosis of symptomatic falciparum malaria. *Acta Tropica*, 134, 52-7.

Moody, A., 2002. Rapid diagnostic tests for malaria parasites. *Clinical Microbiology Reviews*, 15, 66-78.

Nagelschmitz, J., Voith, B., Wensing, G., Roemer, A., Fugmann, B., Haynes, R. K., Kotecka, B. M., Rieckmann, K. H. & Edstein, M. D., 2008. First Assessment in humans of the safety, tolerability, pharmacokinetics, and ex vivo pharmacodynamic antimalarial activity of the new artemisinin derivative artemisone. *Antimicrobial Agents and Chemotherapy*, 52, 3085-3091.

Ndiaye, M., Tine, R., Faye, B., Ndiaye, J. L., Diouf, I., Lo, A. C., Sylla, K., Dieng, Y., Hallett, R., Alifrangis, M. & Gaye, O., 2013. Selection of antimalarial drug resistance after intermittent preventive treatment of infants and children (IPTi/c) in Senegal. *Comptes Rendus Biologies*, 336, 295-300.

Nosten, F. & White, N. J., 2007. Artemisinin-based combination treatment of *falciparum* malaria. *The American Journal of Tropical Medicine and Hygiene*, 77, 181-92.

Nzila, A., 2006a. Inhibitors of de novo folate enzymes in *Plasmodium falciparum*. *Drug Discovery Today*, 11, 939-44.

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

Nzila, A. M., Mberu, E. K., Sulo, J., Dayo, H., Winstanley, P. A., Sibley, C. H. & Watkins, W. M., 2000. Towards an understanding of the mechanism of pyrimethamine-sulfadoxine resistance in *Plasmodium falciparum*: genotyping of dihydrofolate reductase and dihydropteroate synthase of Kenyan parasites. *Antimicrobial Agents and Chemotherapy*, 44, 991-6.

O'Brien, C., Henrich, P. P., Passi, N. & Fidock, D. A., 2011. Recent clinical and molecular insights into emerging artemisinin resistance in *Plasmodium falciparum*. *Current Opinion in Infectious Diseasess*, 24, 570-7.

O'Neill, P. M. & Posner, G. H., 2004. A medicinal chemistry perspective on artemisinin and related endoperoxides. *Journal of Medicinal Chemistry,* 47, 2945-2964.

Ogbodo, S. O., Nwagha, U. I., Okaka, A. N., Ogenyi, S. C., Okoko, R. O. & Nwagha, T. U., 2009. Malaria parasitaemia among pregnant women in a rural community of eastern Nigeria; need for combined measures. *Nigerian journal of physiological sciences*, 24, 95-100.

Ogetii, G. N., Akech, S., Jemutai, J., Boga, M., Kivaya, E., Fegan, G. & Maitland, K., 2010. Hypoglycaemia in severe malaria, clinical associations and relationship to quinine dosage. *BMC Infectious Diseases*, 10, 334.

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

Osonuga, O. A., Osonuga, A. A., Osonuga, I. O., Osonuga, A. & Derkyi, K. L., 2011. Prevalence of hypoglycemia among severe malaria children in a rural African population. *Asian Pacific Journal of Tropical Disease*, 1, 192-194.

Peel, S. A., Bright, P., Yount, B., Handy, J. & Baric, R. S., 1994. A strong association between mefloquine and halofantrine resistance and amplification, overexpression, and mutation in the P-glycoprotein gene homolog (pfmdr) of Plasmodium falciparum in vitro. *American Journal of Tropical Medicine and Hygiene*, 51, 648-58.

Peter, G., Manuel, A. L. & Shetty, A., 2011. Study comparing the clinical profile of complicated cases of *Plasmodium falciparum* malaria among adults and children. *Asian Pacific Journal of Tropical Disease*, 1, 35-37.

Peters, W., 1999. The evolution of tafenoquine-antimalarial for a new millennium? *Journal of the Royal Society of Medicine*, 92, 345-52.

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

Pooley, S., Fatih, F. A., Krishna, S., Gerisch, M., Haynes, R. K., Wong, H.-N. & Staines, H. M., 2010. Artemisone uptake in *Plasmodium falciparum*-infected erythrocytes. *Antimicrobial Agents and Chemotherapy,* 55, 550-556.

Puri, S. K. & Dutta, G. P., 2003. Blood schizontocidal activity of WR 238605 (Tafenoquine) against Plasmodium cynomolgi and *Plasmodium fragile* infections in rhesus monkeys. *Acta Tropica*, 86, 35-40.

Rafiee, M. A., Hadipour, N. L. & Naderi-manesh, H., 2004. A correlation study of quinoline derivatives and their pharmaceutical behavior by ab initio calculated NQR parameters. *Journal of Computer Aided Molecular Design*, 18, 215-20.

Rajapakse, S., Rodrigo, C. & Fernando, S. D., 2015. Tafenoquine for preventing relapse in people with Plasmodium vivax malaria. *Cochrane Database of Systematic Reviews*, 4, 10458.

Rieckmann, K. H., McNamara, J. V., Frischer, H., Stockert, T. A., Carson, P. E. & Powell, R. D., 1968. Gametocytocidal and sporontocidal effects of primaquine and of sulfadiazine with pyrimethamine in a chloroquine-resistant strain of *Plasmodium falciparum*. *Bulletin of the World Health Organisation*, 38, 625-32.

Sabchareon, A., Chongsuphajaisiddhi, T., Sinhasivanon, V., Chanthavanich, P. & Attanath, P., 1988. *In vivo* and *in vitro* responses to quinine and quinidine of *Plasmodium falciparum*. *Bulletin of the World Health Organisation*, 66, 347-52.

Safdar, A., Hartman, B. J., Connor, B. A. & Murray, H. W., 1999. Pulmonary edema in malaria. *International Journal of Infectious Diseases*, 3, 217-9.

Salcedo, E., Cortese, J. F., Plowe, C. V., Sims, P. F. & Hyde, J. E., 2001. A bifunctional dihydrofolate synthetase--folylpolyglutamate synthetase in Plasmodium falciparum identified by functional complementation in yeast and bacteria. *Molecular and Biochemical Parasitology*, 112, 239-52.

Schlagenhauf, P., Adamcova, M., Regep, L., Schaerer, M. T. & Rhein, H. G., 2010. The position of mefloquine as a 21st century malaria chemoprophylaxis. *Malaria Journal*, 9, 357.

Schlagenhauf, P. & Petersen, E., 2008. Malaria chemoprophylaxis: strategies for risk groups. *Clinical Microbiology Reviews*, 21, 466-72.

Schmidt, L. H., Crosby, R., Rasco, J. & Vaughan, D., 1978. Antimalarial activities of various 4-pyridinemethanols with special attention to WR-172,435 and WR-180,409. *Antimicrobial Agents and Chemotherapy*, 14, 420-35.

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.

Shapiro, R., 2012. Prevention of vector transmitted diseases with clove oil insect repellent. *Journal of Pediatric Nursing*, 27, 346-9.

Sharew, B., Legesse, M., Animut, A., Jima, D., Medhin, G. & Erko, B., 2009. Evaluation of the performance of CareStart Malaria Pf/Pv Combo and Paracheck Pf tests for the diagnosis of malaria in Wondo Genet, southern Ethiopia. *Acta Tropica*, 111, 321-4.

Silvie, O., Mota, M. M., Matuschewski, K. & Prudencio, M., 2008. Interactions of the malaria parasite and its mammalian host. *Current Opinion in Microbiology*, 11, 352-9.

Sowunmi, A., Ogundahunsi, O. A., Falade, C. O., Gbotosho, G. O. & Oduola, A. M., 2000. Gastrointestinal manifestations of acute *falciparum* malaria in children. *Acta Tropica*, 74, 73-6.

Steinhardt, L. C., Magill, A. J. & Arguin, P. M., 2011. Review: Malaria chemoprophylaxis for travelers to Latin America. *American Journal of Tropical Medicine and Hygiene*, 85, 1015-24.

Surabattula, R., Vejandla, M. P., Mallepaddi, P. C., Faulstich, K. & Polavarapu, R., 2013. Simple, rapid, inexpensive platform for the diagnosis of malaria by loop mediated isothermal amplification (LAMP). *Experimental Parasitology*, 134, 333-40.

Takem, E. N., Achidi, E. A. & Ndumbe, P. M., 2009. Use of intermittent preventive treatment for malaria by pregnant women in Buea, Cameroon. *Acta Tropica*, 112, 54-8.

Talisuna, A. O., Bloland, P. & D"Alessandro, U., 2004. History, dynamics, and public health importance of malaria parasite resistance. *Clinical Microbiology Reviews*, 17, 235-54.

Tan, K. R., Magill, A. J., Parise, M. E. & Arguin, P. M., 2011. Doxycycline for malaria chemoprophylaxis and treatment: report from the CDC expert meeting on malaria chemoprophylaxis. *American Journal of Tropical Medicine and Hygiene*, 84, 517-31.

Taylor, W. R., Canon, V. & White, N. J., 2006. Pulmonary manifestations of malaria : recognition and management. *Treatments in Respiratory Medicine*, 5, 419-28.

Tiburcio, M., Sauerwein, R., Lavazec, C. & Alano, P., 2015. Erythrocyte remodeling by Plasmodium falciparum gametocytes in the human host interplay. *Trends in Parasitology*, 31, 270-8.

Tietje, K., Hawkins, K., Clerk, C., Ebels, K., McGray, S., Crudder, C., Okell, L. & LaBarre, P., 2014. The essential role of infection-detection technologies for malaria elimination and eradication. *Trends in Parasitology*, 30, 259-66.

Ulrich, J. N., Naranjo, D. P., Alimi, T. O., Muller, G. C. & Beier, J. C., 2013. How much vector control is needed to achieve malaria elimination? *Trends in Parasitology*, 29, 104-9.

Van den Steen, P. E., Deroost, K., Deckers, J., Van Herck, E., Struyf, S. & Opdenakker, G., 2013. Pathogenesis of malaria-associated acute respiratory distress syndrome. *Trends in Parasitology*, 29, 346-58.

van Schalkwyk, D. A. & Egan, T. J., 2006. Quinoline-resistance reversing agents for the malaria parasite Plasmodium falciparum. *Drug Resistance Updates*, 9, 211-26.

Vaughan, A. M., Aly, A. S. & Kappe, S. H., 2008. Malaria parasite pre-erythrocytic stage infection: gliding and hiding. *Cell Host Microbe*, 4, 209-18.

Wang, N., Wicht, K. J., Imai, K., Wang, M. Q., Anh Ngoc, T., Kiguchi, R., Kaiser, M., Egan, T. J. & Inokuchi, T., 2014. Synthesis, beta-haematin inhibition, and in vitro antimalarial testing of isocryptolepine analogues: SAR study of indolo[3,2-c]quinolines with various substituents at C2, C6, and N11. *Bioorganic and Medicinal Chemistry*, 22, 2629-42.

Wang, P., Wang, Q., Yang, Y., Coward, J. K., Nzila, A., Sims, P. F. & Hyde, J. E., 2010. Characterisation of the bifunctional dihydrofolate synthase-folylpolyglutamate

synthase from *Plasmodium falciparum*; a potential novel target for antimalarial antifolate inhibition. *Molecular and Biochemical Parasitology*, 172, 41-51.

Wang, Z., Wang, Y., Cabrera, M., Zhang, Y., Gupta, B., Wu, Y., Kemirembe, K., Hu, Y., Liang, X., Brashear, A., Shrestha, S., Li, X., Miao, J., Sun, X., Yang, Z. & Cui, L., 2015. Artemisinin resistance at the China-Myanmar Border and association with mutations in the K13-propeller gene. *Antimicrobial Agents and Chemotherapy*, 59, 6952-6959.

Wellems, T. E. & Plowe, C. V., 2001. Chloroquine-resistant malaria. *The Journal of Infectious Diseases*, 184, 770-6.

Wernsdorfer, W. H., 2012. Global challenges of changing epidemiological patterns of malaria. *Acta Tropica*, 121, 158-65.

White, N. J., 1992. Antimalarial drug resistance: the pace quickens. *Journal of Antimicrobial Chemotherapy*, 30, 571-85.

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

White, N. J., Warrell, D. A., Chanthavanich, P., Looareesuwan, S., Warrell, M. J., Krishna, S., Williamson, D. H. & Turner, R. C., 1983. Severe hypoglycemia and hyperinsulinemia in falciparum malaria. *The New England Journal of Medicine*, 309, 61-6.

WHO. 1990. Severe and complicated malaria; [WEB]: http://www.ncbi.nlm.nih.gov/pubmed/2219249; [Date of access : 10 Sep. 2015].

WHO 2005a. World Malaria Report; [WEB]: http://apps.who.int/iris/bitstream/10665/43213/1/9241593199_eng.pdf; [Date of access: 09 Sep. 2015].

WHO. 2009. World Health statistics report; [WEB]: http://www.who.int/gho/publications/world_health_statistics/EN_WHS09_Full.pdf; [Date of access: 15 Sep. 2015].

WHO. 2010a. Guidelines for the treatment of malaria 3rd edition; [WEB]: http://apps.who.int/iris/bitstream/10665/162441/1/9789241549127_eng.pdf; [Date of access date: 10 Sep. 2015].

WHO. 2010b. WHO Guidelines for the Treatment of Malaria 2nd Edition; [WEB]: http://www.evidence4health.org/about-ehcrc/case-studies/who-guidelines-for-the-treatment-of-malaria-2nd-edition/; [Date of access: 14 Sep. 2015].

WHO 2011a. The use of DDT in malaria vector control; [WEB]: http://apps.who.int/iris/bitstream/10665/69945/1/WHO_HTM_GMP_2011_eng.pdf; [Date of access: 31 Sep. 2015].

WHO. 2011b. World malaria report; [WEB]: http://www.who.int/malaria/publications/atoz/9789241564403/en/; [Date of access: 09 Sep. 2015].

WHO. 2012a. International travel and health; [WEB]: http://www.who.int/ith/en/: [Date of acccess: 08 Sep. 2015]

WHO 2012b. WHO Evidence Review Group:The Safety and Effectiveness of Single Dose Primaquine as a P. falciparum gametocytocide; [WEB]: http://www.who.int/malaria/mpac/sep2012/primaquine_single_dose_pf_erg_meeting_report_aug2012.pdf; [Date of access: 12 Aug. 2015].

WHO. 2012c. World malaria report; [WEB]: http://www.who.int/malaria/publications/world_malaria_report_2012/report/en/; [Date of access: 08 Sep. 2015].

WHO 2013a. Emergency response to artemisinin resistance in the Greater Mekong sub region. Regional frame work for action 2013-2015; [WEB]: http://www.who.int/malaria/publications/atoz/9789241505321/en/; [Date of access: 20 Feb. 2015].

WHO. 2013c. World malaria report; [WEB]: http://www.who.int/malaria/publications/world_malaria_report_2013/en; [Date of access: 25 Feb. 2014].

WHO. 2013d. World malaria report 2013; [WEB]: http://www.who.int/malaria/publications/world_malaria_report_2013/en/: [Date of access :13 Apr. 2014].

WHO (2013e) World malaria report; [WEB]: http://www.who.int/malaria/publications/world_malaria_report_2013/en/; [Date of access: 16 Sep. 2014].

WHO. 2014. World malaria report 2014; [WEB]: http://www.who.int/malaria/publications/world_malaria_report_2014/report/en/; [Date of access: 12 Feb. 2015].

WHO. 2015. Guidelines for the treatment of malaria 3rd edition; [WEB]: http://apps.who.int/iris/bitstream/10665/162441/1/9789241549127_eng.pdf; [Date of access: 12 Sep. 2015].

WHO 2005b. World Malaria Report; [WEB]: http://whqlibdoc.who.int/publications/2005/9241593199_eng.pdf; Access date 20 Jun. 2015].

Wikipedia (2015) Life cycle; [WEB]: https://en.wikipedia.org/wiki/Plasmodium_malariae; [Date of access : 12 Sep. 2015].

Wilairatana, P., Krudsood, S. & Tangpukdee, N., 2010. Appropriate time for primaquine treatment to reduce *Plasmodium falciparum* transmission in hypoendemic areas. *Korean Journal of Parasitology*, 48, 179-82.

Wilson, A. L., Chen-Hussey, V., Logan, J. G. & Lindsay, S. W., 2014. Are topical insect repellents effective against malaria in endemic populations? A systematic review and meta-analysis. *Malaria Journal*, 13, 446.

Winstanley, P. A., 2000. Chemotherapy for *falciparum* malaria: the armoury, the problems and the prospects. *Parasitology Today*, 16, 146-53.

Wongsrichanalai, C., Barcus, M. J., Muth, S., Sutamihardja, A. & Wernsdorfer, W. H., 2007. A review of malaria diagnostic tools: microscopy and rapid diagnostic test (RDT). *American Journal of Tropical Medicine and Hygiene*, 77, 119-27.

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

Woodrow, C. J. & Krishna, S., 2006. Antimalarial drugs: recent advances in molecular determinants of resistance and their clinical significance. *Cellular and Molecular Life Sciences,* 63, 1586-96.

Wurtz, N., Fall, B., Pascual, A., Fall, M., Baret, E., Camara, C., Nakoulima, A., Diatta, B., Fall, K. B., Mbaye, P. S., Diémé, Y., Bercion, R., Wade, B. & Pradines, B., 2014. Role of Pfmdr1 in *in vitro*. *Antimicrobial Agents and Chemotherapy*, 58, 7032-40.

Zheng, H., Tan, Z. & Xu, W., 2014. Immune evasion strategies of pre-erythrocytic malaria parasites. *Mediators of Inflammation*, 2014, 362605.

Ziegler, J., Linck, R. & Wright, D. W., 2001. Heme Aggregation inhibitors: antimalarial drugs targeting an essential biomineralization process. *Current Medicinal Chemistry Journal*, 8, 171-89.

Zollner, G. E., Ponsa, N., Garman, G. W., Poudel, S., Bell, J. A., Sattabongkot, J., Coleman, R. E. & Vaughan, J. A., 2006. Population dynamics of sporogony for Plasmodium vivax parasites from western Thailand developing within three species of colonized Anopheles mosquitoes. *Malaria Journal*, 5, 68.

CHAPTER 3

REVIEW ARTICLE

Chapter 3 comprizes of a comprehensive literature review on the topic, **Malaria drug discovery: Recent advances in antimalarial peroxides** that will in November 2015 be submitted to the ChemMedChem Journal for publication. The article presents an Introduction, as well as three sections of interest, i.e. Limitations of the current clinical artemisinins, Second generation semi-synthetic derivatives of artemisinins, and Synthetic peroxides. The article was prepared in accordance with the author"s guidelines, as outlined in the author information pack on the journal"s homepage at the following internet address:

onlinelibrary.wiley.com/journal/10.1002/(ISSN)18607187/homepage/2452_authors.html

Malaria drug discovery: Recent advances in antimalarial peroxides

Richard J. Mabilika[1], Frans J. Smit[2], Richard K. Haynes[2], David D. N'Da*[2]

[1]Pharmaceutical Chemistry, School of Pharmacy, North-West University, Potchefstroom 2520, South Africa

[2]Centre of Excellence for Pharmaceutical Sciences, North-West University, Potchefstroom 2520, South Africa

*Corresponding author. Tel.: +27 18 299 2256; Fax: +27 18 299 4243; Email: david.nda@nwu.ac.za

Abstract

Methods to prevent malaria, such as the use of insecticide treated nets, indoor residual spraying and chemotherapy, have dramatically reduced the number of fatalities from approximately 1 000 000 during the preceding decade, to 584 000 deaths in 2013. Pregnant women and children under the age of five, however, still remain most vulnerable to infection, leaving the endemic landscape unchanged. To this day, therefore, malaria remains a critical public health challenge globally, as it had been over the past decades. Present day chemotherapy of uncomplicated malaria relies upon artemisinin based combination therapies (ACTs), which comprize of combinations of artemisinins with either quinolines, or arylmethanols. The mounting development of tolerance by malaria parasites towards ACTs, and in particular to the artemisinin compounds was originally observed in South-East Asia. Such tolerance is now classified as resistance, based upon gene expression in resistant phenotypes. A worldwide spread of resistance towards artemisinins would pose a major threat to current initiatives to control and eradicate the disease, since these drugs currently are the only effective antimalarials left to try and restrict malaria infections. The threat of the increased development of resistance towards this drug necessitates the urgent development of new, clinically effective, cheaper and safer antimalarial drugs that would help curb the disease. As part of this ongoing search, advances in organic chemistry have allowed for the preparation of more potent and stable semi-synthetic and fully synthetic peroxide derivatives, with superior antimalarial activity, compared to the currently used artemisinins.

This review covers recent advances in the search for new antimalarial peroxides, with an emphasis on those listed in the 2015 Medicines for malaria venture‟s (MMV) Global portfolio of new antimalaria medicines, currently being developed and investigated, as reported in recent literature sources.

Keywords: *malaria, artemisinin based combination therapies (ACTs), artemisinin,* peroxide

1. Introduction

Malaria is a devastating disease that arizes from the invasion of red blood cells by protozoa of the genus, *Plasmodium*.[1] Five species of *Plasmodium*, i.e. *P. falciparum, P. ovale, P. vivax, P. malariae* and *P. knowlesi* cause human malaria. Of these, *P. falciparum* is responsible for the most severe form of malaria, followed by *P. vivax*.[2] These species are transmitted to humans, following the bite of an infected female *anopheles* mosquito.

The disease is endemic in 106 countries and were an estimated 3.4 billion people, representing 40% of the world"s population, infected in 2013. During 2013, roughly 198 million malaria cases, with 584 000 related deaths, were reported.[3] The incidence had been the highest in the African region, where an estimated 90% of all malaria fatalities occurred, of which children, aged under five years, accounted for 78% of all deaths.[3] In the past decade, however, significant progress in the control of malaria had also been witnessed. Between 2000 and 2013, the estimated malaria mortality rates had decreased by 47% worldwide and by 54% in the World Health Organization"s (WHO"s) African Region.[3] These successes had been attributed to a combination of factors, including the expanded use of chemotherapy, insecticide treated nets and indoor residual spraying.

The sole malaria treatment option to date comprises of chemotherapy and this is expected to continue into the foreseeable future, since no effective vaccine against the disease is yet available.

Artemisinin **1** and its derivatives **2**-**5** (Figure 1), referred to as the artemisinins, comprize the most important of the currently used classes of antimalarial drugs. In the early 1970s, the remarkable discovery of qinghaosu, subsequently called artemisinin, and isolated from the Chinese herbal plant, *Artemisia annua,*[4] resulted in the introduction of artemisinin and its derivatives for the treatment of malaria. Given the earlier demize of the 4-aminoquinoline chloroquine through the development of resistance in South-East Asia, and hence elsewhere, the introduction of these unique peroxidic compounds was particularly fortuitous. Up until very recent times, they have served as key chemotherapeutic agents for the treatment of malaria.

Artemisinin is a sesquiterpene, with a peroxide bridge that is linked through a carbon atom to a third, non-peroxidic, oxygen atom. This 1,2,4-tri-oxane core is key to the antimalarial activity of this compound.[5] The artemisinins are fast acting drugs that are potently active

and are they well tolerated during all stages of a malaria infection. Artemisinin has the ability to reduce malaria parasitaemia more rapidly than any other antimalarial drug.[6] However, because of the short half-lives of the artemisinins, protracted treatment regimens had to be introduced to avoid the phenomenon of recrudescence, i.e. the redevelopment of parasitaemia in the absence of external re-infection. Li *et. al.* were the first researchers who used artemisinin in combination with the long half-life drug, mefloquine, to suppress both recrudescence and the development of resistance.[7] The strategy of using artemisinins in combination with other longer half-life drugs, the so-called artemisinin combination therapies (ACTs), was only much later adopted by others, including the World Health Organization (WHO), for the treatment of non-severe malaria.[8-10] The principle of action of an ACT is that the fast acting artemisinin clears a larger portion of the parasites within its short pharmacological half-life, whilst the longer half-life partner drug continues the clearance, once the artemisinin concentration has fallen to sub-therapeutic levels. Until recently, this approach has successfully aided in reducing the risk of recrudescence and drug tolerance,[11] whilst also delaying the development of drug resistance.[12] Five ACTs are currently used, i.e. artemether-lumefantrine, artesunate-mefloquine, artesunate-amodiaquine, dihydro-artemisinin (DHA)-piperaquine, and the triple combination of artesunate with sulfadoxine and pyrimethamine.

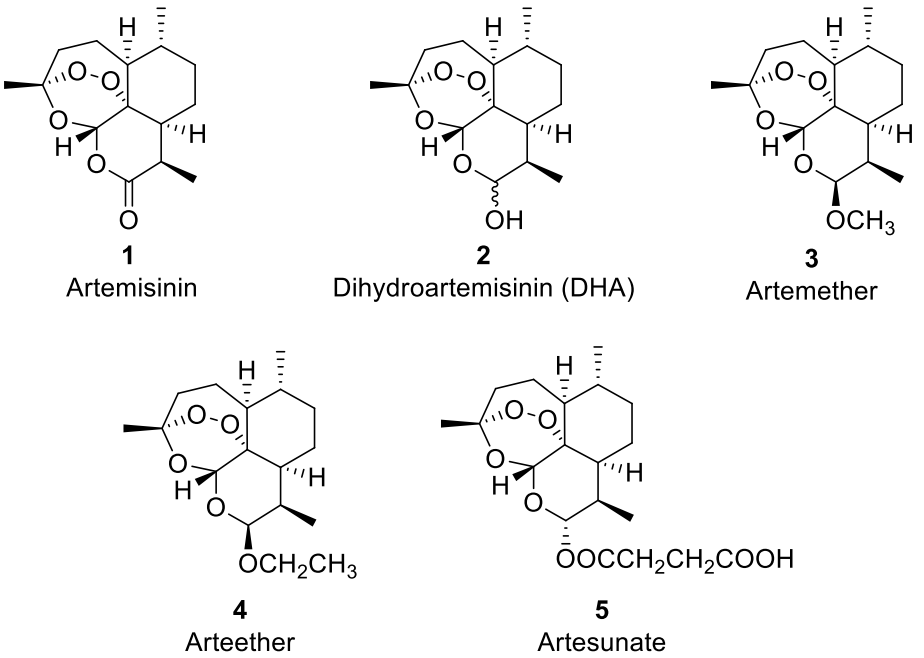


Figure 1: Chemical structures of artemisinin and its semi-synthetic derivatives.

In spite of this measure to use such combination therapies, safety concerns and the high costs have limited the use of ACTs in poor, malaria endemic countries, and even worse,

have these factors led to an undesirable and thriving fake drug market.[13-16] Furthermore, increasing tolerance to ACTs have also been confirmed, as artemisinin resistance by parasites had been observed for some time in South-East Asia.[17, 18] Various studies have investigated the mechanism of artemisinin resistance. Phosphatidylinositol-3-phospate (PI3P), a lipid product of *P. falciparum* Phosphatidylinositol-3-kinase (PfPI3K), was found to form an important channel in the transport of haemoglobin to the parasite food vacuole.[19] Dihydro-artemisinin (DHA), the active metabolite of artemisinin based drugs, has demonstrated that it binds to and inhibits the activity of PfPI3K, resulting in deficient haemoglobin levels in the food vacuole, which deprives the parasite of important amino acids.[20] Indeed, artemisinin resistant strains have abnormally high levels of PfPI3K, a characteristic that is associated with point mutations in the *P. falciparum* Kelch13 (PfKelch13) gene, now known as a primary marker of artemisinin resistance.[21] This phenomenon emphasizes the urgent need for the development of methods that would enhance the efficacy and safety profiles of existing antimalarial drugs and to identify and develop safe, cheap and effective new drugs.

Advances in the development of new antimalarials have been extensively reviewed in recent years.[22-26] This review, however, specifically focuses on semi-synthetic and fully synthetic peroxidic compounds, designed to address specific shortcomings of the currently used clinical artemisinins, including their reduced efficacy, thermal instability, short biological half-lives, safety concerns, and the limitations that exist with regards to their large scale production possibilities. The rationale for choosing these topics is that peroxides are still the most fast acting and effective drugs among all classes of drugs, currently being used to treat malaria.

2. Limitations of current clinical artemisinins

Although artemisinins are widely used in current antimalarial chemotherapeutic treatment regimens, they unfortunately have significant shortcomings, such as their low solubility, thermal instability and short pharmacological half-lives. These aspects may limit parasite clearance and hence result in recrudescence when used in monotherapy, which may contribute towards the development of parasite resistance.[27] The production of the parent artemisinin from agricultural sources largely falls short of the estimated global demand, which further contributes towards the high costs of these drugs.

2.1. Solubility

For a drug to pass through a biomembrane, it must possess limited aqueous solubility and it must be neither too lipophilic, nor too hydrophilic. Lipophilic drugs show poor aqueous solubility and tend to be absorbed by fatty globules in the intestine. Once they reach the blood stream, they may be adsorbed into tissues and released slowly into the circulation. This results into prolonged cellular exposure which exacerbates toxicity, such as neurotoxicity. Contrary, hydrophilic, water soluble drugs may be excreted directly through the kidneys, but if they succeed to penetrate a cell membrane, they become entrapped in intracellular aqueous media. An ideal drug must therefore possess balanced lipophilic and hydrophilic properties in order to permeate biological membranes and enter to the systemic circulation. The *n*-octanol/water partition coefficient (Log P) offers a good measure of this balance and targets values between 1-5, whilst values ranging between ~1-3 are ideal.[28]

The benefits of artemisinin are offset by its poor solubility in both oil and water. This has led to the structural modifications that had been made to this sesquiterpene to form its first generation of semi-synthetic derivatives, *viz.* dihydro-artemisinin (DHA) **2**, the oil soluble artemether **3** and arte-ether **4**, and the water soluble artesunate **5**. The measured Log P values of the two clinically used drugs, artesunate (2.77) and DHA (~2.6), are well within the ideal range that would enable their optimal absorption through biological membranes, while artemether with a Log P value above the ideal range (3.98) is expected to be absorbed to a lesser extent.

2.2. Chemical stability and pharmacological half-lives

Since all current clinically used artemisinins are structurally either alkyl- or ester acetals, they are chemically and/or metabolically unstable. In addition, O-alkyl ethers generally are subject to facile metabolic de-alkylation by the P-450 super-family.[29-31] It is therefore not surprising that the first generation artemisinins have short pharmacological half-lives when administered orally, or in the case of artesunate, through intramuscular injection. The lability of a drug is reflected by its rapid metabolism, or in the case of artesunate, by its extent of hydrolysis into DHA, the principal metabolite of artemisinin. Artemether has a pharmacological half-life of 2-3 h[32] and artesunate, 20-45 min, following oral administration.[33] DHA itself has a relatively short half-life of ~45 min, indicative of its deep seated decomposition pathways,[34-37] and of its conversion into the water soluble glucuronide.[38] It is therefore noteworthy that, since the derivatives, artemether **3** and artesunate **5** rapidly decompose into DHA **2**, and because DHA itself is used as an artemisinin component in ACTs, malaria parasites are *in vivo* predominantly exposed to DHA. In hindsight, it is hence no surprise that resistance towards the artemisinins has also

developed, an event that could have been avoided by a more flexible adoption of other peroxidic antimalarials, incapable of forming DHA *in vivo*.[39]

2.3. Thermal stability

Poor thermal stability is another disadvantage of the artemisinins.[40] The clinically used DHA is thermally the least stable of all the artemisinins. At 40°C and a relative humidity (RH) of 70%, solid DHA undergoes 2% of decomposition after 1 month, and 2.9% after 3 months.[34] As a result, the poor thermal stabilities of these drugs and of DHA in particular, raise concerns regarding the risk of decomposition of these formulated materials in malaria endemic countries that are known for their hot and humid weather. In general, the International Conference of Harmonization (ICH) and the WHO have guidelines that prescribe accelerated thermal stress testing of antimalarial drugs over a period of 6 months, by exposing these formulated drugs to 40 ±2°C and a relative humidity of 75 ±5%.[41] The threshold of unknown decomposition products, based upon a daily dose of 100 mg, should not exceed 0.2%, whilst less than 1.5% of decomposition is allowed for known degradants for which their toxicity and efficacy profiles have been quantified, as for the parent drug. The application of these guidelines to the development of artemisinin drugs is therefore quite challenging, and, because malaria is endemic in tropical areas, thermal stability especially is an essential consideration in the design of new antimalarials.

2.4. Safety and toxicity

DHA, the principal metabolite of current artemisinins, is neurotoxic.[42] The derivatives of artemether and arte-ether, however, also possess intrinsic neurotoxicity that is accompanied by several adverse effects, including movement disturbances, spasticity, balance deficits, brain stem tissue damage, and even the death of laboratory animals that have been treated with intramuscular doses of both derivatives.[43] Most reports on artemisinins induced neurotoxicity relate to studies carried out on laboratory animals.[5, 44] Although few clinical studies unambiguously support these findings in humans,[44] a safety concern does exist with regards to the use of more extended and higher doses of artemisinins in humans, such as those that are being employed in patients,[45] who do not respond to initial treatment with ACTs in areas, such as Cambodia, where resistance has become a serious threat.

Cytotoxicity and embryotoxicity are other safety concerns being associated with the use of artemisinins. In a report regarding the safety of artemisinin compounds during pregnancy, the WHO underlined an important knowledge gap between the cytotoxic and embryotoxic properties of peroxidic compounds.[46] Currently, the regulations with regards to ACT therapy

and pregnant women state that artemisinin drugs can only be safely used in the second and third trimesters of pregnancy, and should they only be administered in the first trimester, if no other suitable antimalarial treatment is available and only if the treatment is considered life-saving.[46]

2.5. Large scale production

Artemisinin **1**, the parent compound of the current clinically used derivatives, is extracted from *Artemisia annua* and purified at an overall cost of $350-400/kg.[47] The total synthesis of artemisinin, owing to the molecular complexity of its structure, is not commercially viable, despite several routes having been reported.[48-51] The duration of these chemical processes, the reagents and conditions involved, and the low overall yields all indicate that total synthesis is not a feasible source of artemisinin.

An alternative production method exists that involves a yeast fermentation process to deliver the biosynthetic precursor, artemisinic acid[52] (Figure 2). Early work by Roth and Acton[53, 54] and by Haynes *et. al.*[55-58] mapped a (bio) synthetically feasible pathway from dihydro-artemisinic acid into artemisinin. These researchers showed that photo-oxygenation of dihydro-artemisinic acid **7** yields hydroperoxide **8** that can be converted by protic acids,[53] or catalytic amounts of copper (II) trifluoromethanesulfonate[58] to yield artemisinin (39%). Haynes and Vonwiller[55] demonstrated that the treatment of hydroperoxide **8** occurred through Hock cleavage to generate enol **9**, which subsequently underwent a completely stereo-selective thermal reaction with ground state (triplet) oxygen, to give the opened ring hydroperoxy carboxylic acid **10**. This ring then closed smoothly to generate artemisinin **1**. The process was later adopted in its entirety by Seeberger and Lévesque into a continuous flow reactor, better suited for conducting the photochemical step on an industrial scale to produce parent artemisinin **1** in 40% yields from dihydro-artemisinic acid (Figure 2) on a 3 g scale.[59] Chen *et. al.* reported a higher yield (41%) of artemisinin on the same scale, from compound **7**, featuring molybdate induced disproportionation of hydrogen peroxide to thermally generate the singlet oxygen, required during the *ene* reaction step of the process leading to hydroperoxide **8**, without recourse to the continuous-flow technique.[60] More recently, artemisinic acid was converted into artemisinin **1** in a 55% yield by employing a photochemical oxidation process that had been implemented by Sanofi,[61] that apparently is capable of producing 50-60 tonnes of semi-synthetic artemisinin per annum[52]. This is estimated to represent about one-third of the global need. Kopetzki *et. al.* reported a 65% yield of artemisinin from dihydro-artemisinic acid, by employing the photochemical process.[62] It is noteworthy that in this latter example, artemisinic acid was extracted as a waste product from *Artemisia annua*, while extracting artemisinin. However, the adjustment

to the extraction process of *A. annua* to provide both artemisinin and artemisinic acid, was first developed more recently and aimed at extracting artemisinin directly, as well as to enable the semi-synthesis of artemisinin and its derivatives from artemisinic acid.[56, 63] The routes for the conversion of dihydro-artemisinic acid into artemisinin are illustrated in Figure 2.

These semi-synthetic routes to produce artemisinin from a relatively readily available precursor, bypass the problem of the complexity of total synthesis. However, the semi-synthetic flow operations are themselves also complex. The specialized equipment that is required for the photochemical reaction represents a substantial capital investment and operational costs, and is this alternative not likely to significantly reduce the costs of supplying ACTs to malaria endemic regions. These routes would, however, help to ensure a secure supply of artemisinin. Alternative strategies include the development of newer artemisinin derivatives with significantly enhanced efficacies, such that less material may be used in chemotherapeutic regimens so as to reduce the demand for the parent artemisinin **1**, and to develop totally synthetic routes for delivering peroxides that exhibit potencies that are comparable to that of current artemisinins. This would have the advantage of providing supplies of antimalarial drugs that are independent of the limitations of an agricultural source.[64]

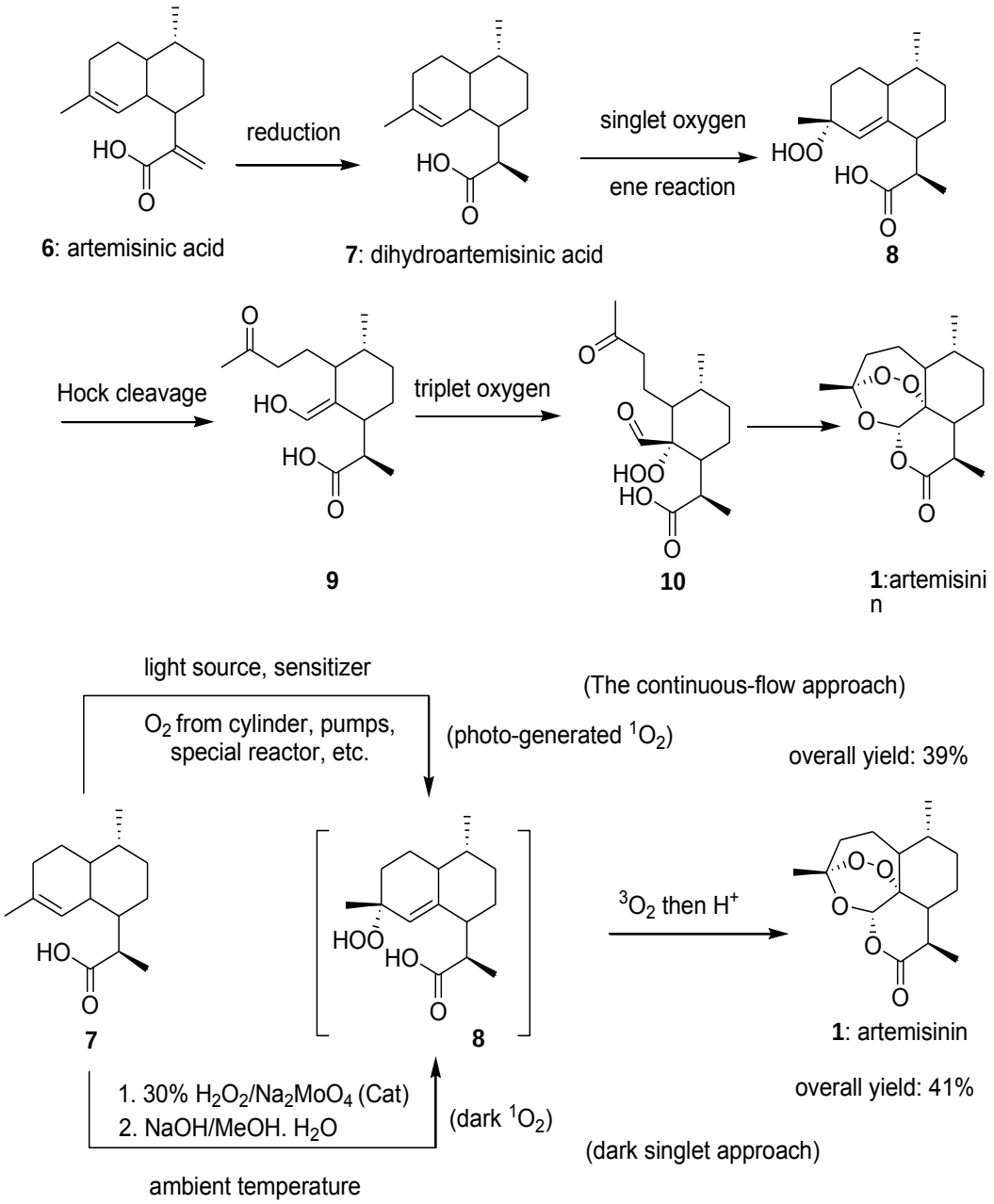


Figure 2: Reaction sequences in the synthesis of artemisinin (1) from artemisinic acid, as developed by Roth and Acton[53, 54] and by Haynes and Vonwiller,[55], (2) through continuous-flow alterations[59] and (3) the thermal singlet oxygen method[60] of conversion of dihydro-artemisinic acid into artemisinin.

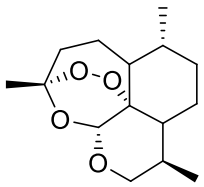
3. Second generation, semi-synthetic artemisinin derivatives

As noted, artemisinin derivatives need to have significantly enhanced efficacies, compared to current derivatives, and should they ideally possess more favorable toxicity profiles than the current artemisinins, as well as metabolic and pharmacokinetic properties that won't offset the drugs' efficiencies.[65]

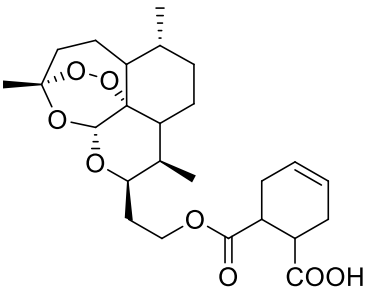
3.1. Deoxo-artemisinins

The readily accessible, semi-synthetic derivative, (+)-deoxo-artemisinin **11**, is devoid of an oxygen bearing functionality at C-10 and does it thereby have the properties of a cyclic ether. It is likely to be more stable than the alkyl lactol ethers, which has been confirmed in at least a simulated stomach acid study.[66] (+)-Deoxo-artemisinin is easily prepared from DHA **2** through the reduction with Lewis acidic reducing agents that do not affect this biologically important peroxide.[67, 68] It was found to be eight-fold more active *in vitro* than artemisinin **1**, and two-fold more active than artemether **3**, and it was clearly more effective in curing *P. berghei* in a murine model than artemisinin. Unfortunately, the pharmacokinetic and toxicity profiles of 10-deoxo-artemisinin is unknown. It may be a useful compound for evaluation as possible future antimalarial, given that it cannot be converted into DHA, and it is likely to have a longer half-life than artemisinin itself.

Other C-10 deoxy derivatives have an alkyl group attached to C-10. Like 10-deoxo-artemisnin, these are simple ethers with longer half-lives than artemisinin itself.[66] The derivative **12** (Figure 3) was the most potent against both CQR and CQS strains of *P. falciparum*, and up to twenty times more active than artemisinin **1**.[69] No further investigations have been carried out on these derivatives.



11
IC_{50}: W-2 0.15, D-6 2.33 ng/ml
[artemisinin **1**: W-2 1.21; D-6 2.33 ng/ml]

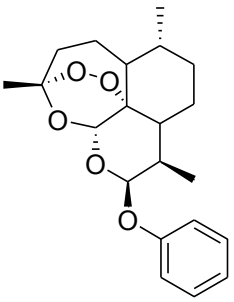


12
IC_{50}: GHA 0.4, W-2 0.5 nM
[artemisinin **1**: GHA 10, W-2 9 nM]

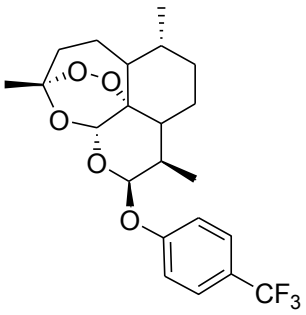
Figure 3: In vitro antimalarial activity of deoxo-artemisinin **11** against CQS D-6 and CQR W-2[67] and of analogue **12** against CQS GHA (Ghana) and CQR W-2 strains of *P. falciparum*.[69]

3.2. C-10, O-phenyl substituted derivatives

The replacement of an *O*-alkyl group with an *O*-aryl group in the first generation artemisinin ethers, i.e. artemether **3** and arte-ether **4**, will prevent oxidative de-alkylation by CYP-450 enzymes, characteristic of the decomposition of current alkyl ethers into DHA. The *p*-(trifluoromethyl)phenyl compound **14** was as active as artemether and had activity superior to that of artesunate **5** against *P. berghei* and *P. yoelii in vivo*.[70] Compound **14** was found to be metabolically stable, compared to compound **13**, indicating that the *p*-trifluoromethyl group blocked P-450 mediated aromatic hydroxylation and its subsequent metabolism (Figure 4). However, toxicity profiles of the aryl derivatives were not recorded, and no further development was pursued.



13
IC_{50}: HB3 3.42; K1 3.66 nM
[artemether **3**: HB3 3.42; K1 4.55 nM]



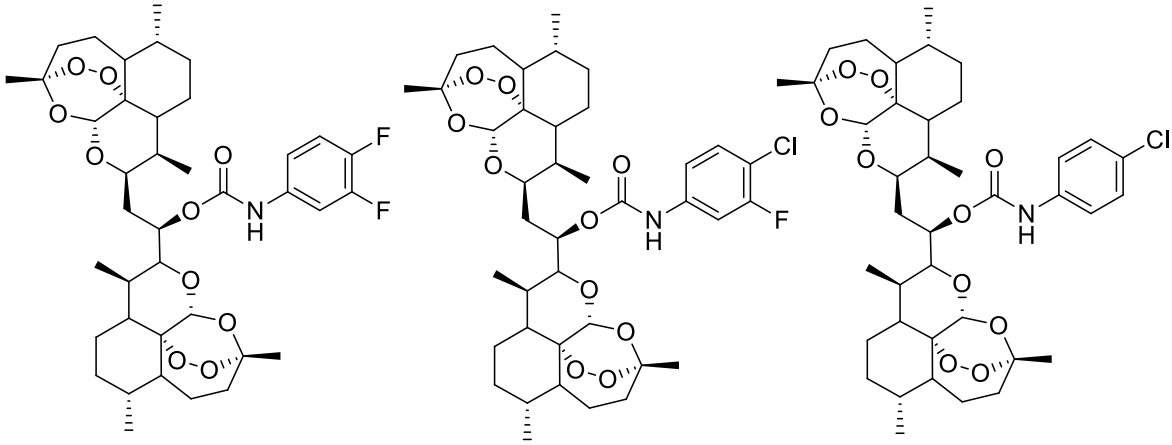
14
IC_{50}: HB3 3.42, K1 4.62 nM
[artemether **3**: HB3 3.42, K1 4.55 nM]
P. berghei (po): ED_{50} 2.7, ED_{90} 5.4 mg/kg
P. yoelii (sc): ED_{50} 2.2, ED_{90} 3.1 mg/kg
[artesunate **5**: P. berghei: ED_{50} 4; ED_{90} 13 mg/kg;
P. yoelii: ED_{50} 3.6; ED_{90} 13.5 mg/kg]

Figure 4: C-10 O-aryl substituted derivatives of artemisinin: comparison of activities in vitro of **14** with artemether **3** against HB3 and K1 strains of *P. falciparum*, and activities in vivo of **14** with artesunate **5** vs. P. berghei and P. yoelli ssp. NS.[71]

3.3. Artemisinin dimers

A number of C-10 linked artemisinin dimers (Figure 5) were found to be active against both CQR and CQS strains of *P. falciparum*, some at nano-molar concentrations.[72] Recently, prolonged survival times of malaria infected mice were noted, when treated with two-carbon linked dimeric carbamates **15**, **16**, and **17** and administered in a single dose (5 mg/kg), in combination with mefloquine hydrochloride (15 mg/kg) to *P. berghei* infected mice. These survival times were longer than that of artemether **3** (Figure 5).[73]

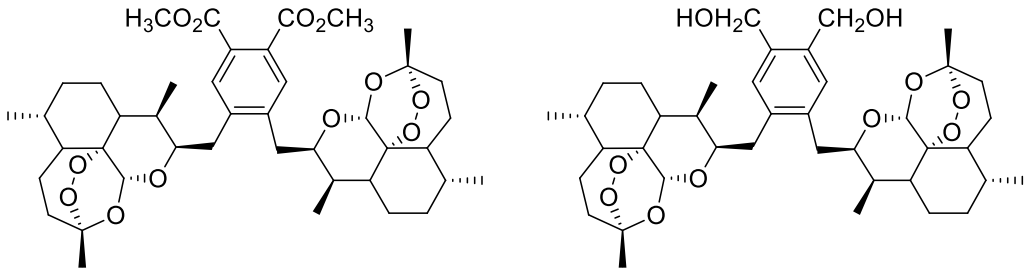
Aryl-linked artemisinin dimers, including the phthalate and the bis-benzyl alcohol dimers **18** and the diol **19** (Figure 5), also displayed enhanced activity against malaria parasites, as well as against cancer cells. These had lower oral ED_{50} of 2.6 mg/kg, compared to the 4 mg/kg of artesunate **5**, when administered under similar test conditions.[74] Despite their excellent activities, it may not be cost effective to produce these dimers, due to the complexity of their synthesis processes.



15
P. berghei, survival time 25.5 days
[artemether **3**: 20.3 days]

16
P. berghei,
survival time 24.8 days

17
P. berghei,
survival time 26.8 days



18
IC_{50}: NF54 1.6 nM
[artemisinin **1**: 6.6 nM]
P. berghei: ED_{50} 0.71 mg/kg (sc)]

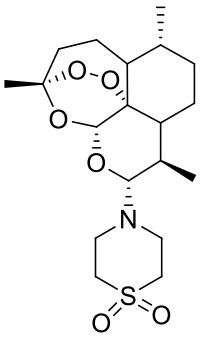
19
IC_{50}: NF54 0.78 nM [artemisinin **1**: 6.6 nM]
P. berghei: ED_{50} 0.06 mg/kg (sc);
ED_{50} 2.6 mg/kg (po); [artesunate **5**:
P. berghei: ED_{50} 2.2 mg/kg (sc);
ED_{50} 4.0 mg/kg (po)]

Figure 5: In vivo antimalarial efficacies measured when using a single oral dose of trioxane dimers **15-17** (5 mg/kg) in combination with mefloquine hydrochloride (15 mg/kg) in *P. berghei* infected mice.[73] *In vitro* antimalarial activity of artemisinin dimers **18** and **19** against the CQS NF54 strain of *P. falciparum*, and *in vivo* antimalarial activity in *P. berghei* infected (ANKA strain) mice, after employing a single oral dose.[74]

3.4. Artemisone

To avoid the potential side effects of neurotoxicity of the current artemisinins, the Medicines for malaria venture (MMV) in the late 1990s prioritized the development of new derivatives that would not metabolize into DHA, both *in vitro* and *in vivo*. This led to the preparation of several 10-amino derivatives,[75] the most important of which was artemisone **20** (Figure 6).

Artemisone is therefore a second generation semi-synthetic derivative of artemisinin. It did not demonstrate any measurable neurotoxicity in both *in vitro* and *in vivo* assays.[76-79] Artemisone also showed superior activity against both CQR and CQS *P. falciparum* strains, and proved substantially more potent *in vivo* in rodent *P. berghei* and in primate *P. falciparum* infected models,[80] than artesunate.



20: **Artemisone**
IC_{50}: W2 0.6, D10 0.72 , 3D7 1.0, K1 1.0 nM
[artesunate **5**: W2 1.3, 3D7 3.9, K1 5.7 nM]

Figure 6: Artemisone and its antimalarial activity.[80]

Further *in vivo* data on the antimalarial efficacy of artemisone is summarized in Table 1.[81] The data also indicates the superior efficacy of artemisone against a *P. yoelii* artemisinin resistant line, underlining its potential to successfully destroy artemisinin resistant *P. falciparum*. Artemisone has a Log P value of 2.49 and more importantly, an aqueous solubility of 89 mg/l, which exceeds the limiting value that is required for a drug to permeate biological membranes.[80] It also possesses a pharmacological half-life of 3.1 h, longer than that of artemether (1.5 h), but still shorter than the 3 days being suggested by the MMW for the next generation of drugs that are urgently required for malaria eradication.[82] Indeed, according to MMV, a suitable drug candidate for malaria extermination should be able to kill gametocytes, hypnozoites and other liver stages, thereby inhibiting transmission, relapse, as well as providing prophylaxis against the disease. Ideally, such a candidate should also have a minimum half-life of 3 days, although in practice such properties may be difficult to achieve.

Table 1: Summary of *in vivo* activity of artemisone **20** and artesunate **5** against drug susceptible and drug resistant rodent malaria lines in a 4-day Peters" test[81]

Plasmodium	Administration route	Artemisone **20**			Artesunate **5**		
		ED_{50} mg/kg	ED_{90} mg/kg	I_{90}	ED_{50} mg/kg	ED_{90} mg/kg	I_{90}
P. berghei NY	Sc	1.24	9.62	1.0	7.8	41.21	1.0
	Po	2.12	11.67	1.0	12.66	111.94	
P. berghei P	Sc	0.63	1.92	0.2	21.62	18.2	0.4
P. berghei KFY	Sc	0.22	0.83	0.1	0.7	5.38	0.1
P. yoelii NS	Sc	1.28	11.0	1.0	2.21	49.54	1.0
	Po	2.34	27.99	1.0	12.97	179.47	1.0
P. yoelii ART	Sc	1.14	12.13	1.1	2.14	87.5	1.8
P. chabaudi AS	Sc	0.42	1.38	1.0	0.69	19.68	1.0

sc, subcutaneous route; po, oral route. *P. berghei* NY, drug susceptible; *P. berghei* P, primaquine resistant; *P. berghei* KFY, sulfadoxine/pyrimethamine resistant; *P. yoelii* NS, CQR; *P. yoelii* ART, artemisinin resistant; *P. chabaudi* AS, drug susceptible. I_{90}, ED_{90} resistant line/ED_{90} parent line.

Artemisone is the only second generation, semi-synthetic artemisinin derivative that has ever been subjected to clinical trials. During the Phase IIA clinical trials in Thailand, it effected complete cure within a period of two days, when administered at one third the dose of its comparative drug, artesunate, followed by a final dose of mefloquine on day 3. Examination of the drug and its metabolites in the plasma of patients that had been treated with artemisone, indicated that DHA was not among the metabolites, which was hence in fulfilment of one of the criteria for the development of artemisone. Unfortunately, a planned Phase II clinical trial, during which artemisinin resistant *falciparum* malaria would have been treated in Western Cambodia, was not carried out due to operational reasons.[83] Overall, further investigations on artemisone was not continued by the MMV, as it was considered to not have demonstrated overwhelming benefits, compared to existing clinical artemisinins.[84] It can, however, be argued that artemisone is a better candidate drug than artesunate for the treatment of cerebral malaria, for various reasons, such as that of artemisone having equal thermal stability to artesunate,[40] and like artesunate, it is hydrolytically unstable at low pH, unlike artesunate, artemisone is stable at pH 7.4,[76, 85] and of particular interest is the fact that artemisone has low hemolytic potential. Developmental and reproductive toxicity studies that had been conducted on animals, however, revealed that artemisone elicited both

embryotoxicity and fetoxicity, and did it also induce cardiac ventricular septal defects and retarded ossification at dosages that resulted in total litter loss and abortions.[86] Unfortunately, the study was not carried out in parallel with one or more of the current clinical artemisinins that are likely to be problematic, and could the effects not be compared. Even so, the precautionary measures by the WHO to recommend that current clinically used artemisinins for malaria treatment be only administered during the second and third trimester of pregnancy,[46] based upon various developmental and reproductive toxicity studies in rats and rabbits, are likely to apply to artemisone also.

3.5. 11-Aza-artemisinin and its derivatives

Amides generally are thermally, hydrolytically and metabolically more robust than esters. This also applies to cyclic amides (lactams) that tend to have such enhanced properties with regards to cyclic esters (lactones). As a result, one possible route of stabilizing artemisinin is to convert its lactone into a lactam, by replacing O-11 with a nitrogen (N) atom. The method of converting artemisinin **1** into 11-aza-artemisinin **21** through the formal replacement of 11-O with N (Figure 7), was developed by Ziffer,[87] and the method has been used by other groups[40, 88] to deliver substituted 11-aza-artemisinins. The attachment of electron withdrawing groups to the N atom of aza-artemisinin itself, bestows its enhanced thermal stability. *N*-sulfonyl-11-aza-artemisinins hence display better thermal stability than artemisinin, the clinical artemisinins and aza-artemisinin **21**. The *N*-methanesulfonyl derivative **22** has a melting point of 245 °C, which appears to be the highest for any artemisinin.[40] Most of these derivatives showed no detectable signs of decomposition over periods of three years, or more.[40]

The N-sulfonyl derivatives **23**-**25** were approximately equipotent to artesunate *in vitro* [40] against CQS 3D7 and CQR K1 strains of *P. falciparum*. Derivative **22** was also equipotent to artesunate against the 3D7 strain, whilst it possessed a significantly higher efficacy against the K1 strain. The aza-artemisinin derivatives **27-30** (Figure 7) also possessed higher *in vivo* activity than arte-ether **4**.[88] However, no toxicity data for any of these derivatives are available in current literature. Table 2 summarizes the efficacies of promising aza-artemisinin derivatives.

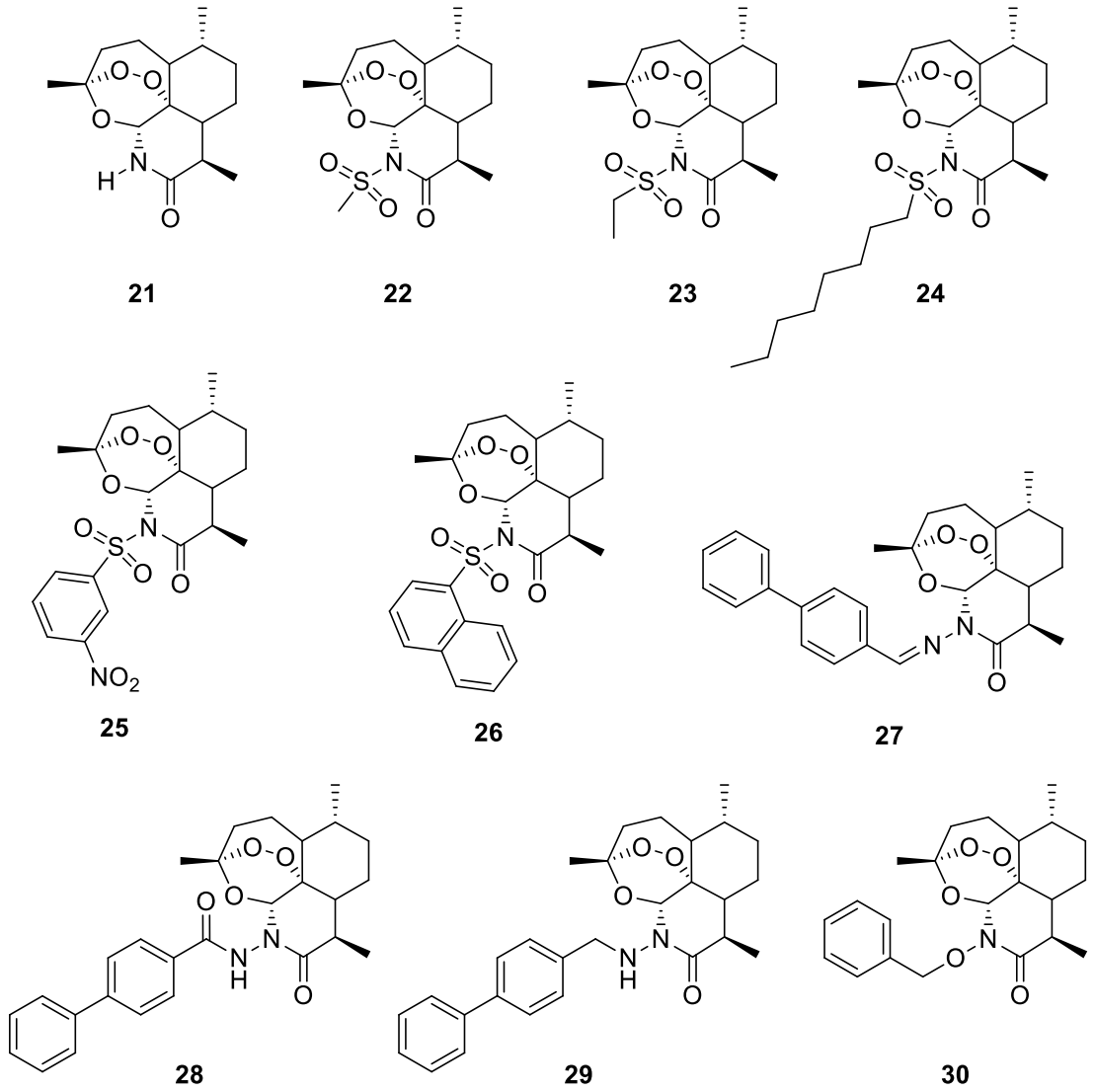


Figure 7: Chemical structures of promising antimalarial 11-aza-artemisinins.

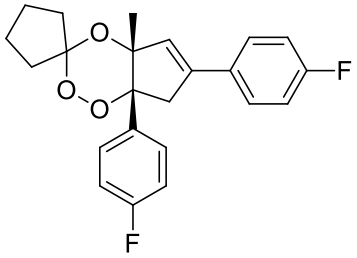
Table 2: In vitro antimalarial activity of aza-artemisinin derivatives **23-26**[40] and *in vivo* activity of derivatives **27**-**30** against multi-drug resistant *P. yoelii* in Swiss mice following oral administration[88]

Compound	*In vitro* IC_{50} ng/ml (nM)		*In vivo vs. P. yoelii*			
	3D7	K1		Dose (mg/kg)	% Parasitaemia suppression	Survival rate
Artesunate **5**	1.5 (3.9)	2.2 (5.7)	**27**	12	100	5/5
22	4.1 (11.4)	3.2 (8.9)	**28**	12	100	5/5
23	1.7 (4.6)	3.7 (9.9)	**29**	12	100	5/5
24	1.1 (2.4)	2.1 (4.6)	**30**	12	100	4/5
25	2.1 (4.5)	2.2 (4.7)	Arte-ether **4**	48	100	5/5
26	2.8 (5.9)	0.2 (0.4)				

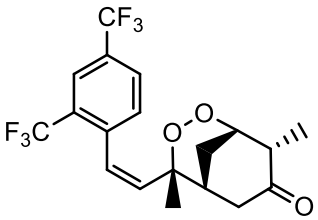
4. Synthetic peroxides

The peroxide group within the artemisinin compounds is essential to their activity. From a synthetic chemistry viewpoint, it is relatively easy to prepare compounds so that they contain a peroxide group. According to the literature, many synthetic peroxides have been prepared, of which many have displayed antimalarial activities. The most important compounds are reviewed in this section.

The first fully synthetic peroxide drug candidates that had been developed were Fenozan B07[89] and Arteflene (Figure 8).[90] Arteflene was selected as antimalarial drug candidate, primarily because of its evidently relatively stable peroxide that is incorporated into a 1,2-dioxane unit, the lower associated rate of malaria recrudescence and its longer plasma half-life than both artemether **3** and artesunate **5**. After having progressed to a phase II clinical trial, inconsistent results were obtained during the trials and further development was discontinued.[91] Other detracting factors that prevented the further development of arteflene included difficulties with scaling up its rather long synthesis process and its poor oral bio-availability.[92]



31: **Fenozan B07**
IC_{50}: W2 14.17, D6 14.17 nM
ED_{50} 2.5 mg/kg; ED_{90} 1.8 mg/kg



32: Arteflene
IC_{50}: K1 75.9 , W2 51.7 nM
[artemisinin **1** K1 12.4, W2 9.6 nM]

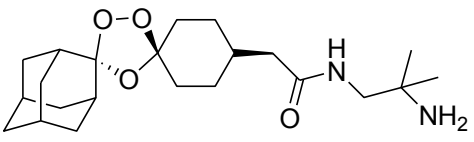
Figure 8: Early promising fully synthetic antimalarial peroxides.

4.1. 1,2,4-Tri-oxolanes

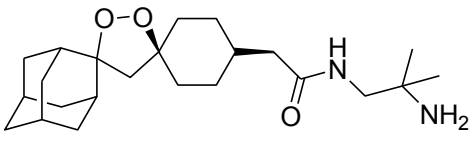
The antimalarial activity of 1,2,4-tri-oxolanes, or ozonides, was first reported by Vennerstrom *et. al.,* whilst examining 1,2,4,5-tetra-oxanes, which eventually led to the development of two candidate tri-oxolanes, i.e. OZ277and OZ439.[93]

4.1.1. OZ277

A series of 1,2,4-tri-oxolanes were synthesized through the Griesbaum co-ozonolysis[94] of the corresponding oximes with ketones.[95, 96, 93] Most compounds were active against the NF54 (multi-drug sensitive) and K1 (CQ and pyrimethamine resistant) strains of *P. falciparum*. However, tri-oxolane **33** (Figure 9), the tosylate salt, was the most potent against both strains[97] (Table 3) and appeared to be relatively stable towards metabolism by CYP-450 enzymes.[98] Intravenous administration of OZ277 to rats showed measurable plasma concentrations for up to 6 h, with a significantly longer terminal elimination half-life than that of DHA. OZ277 was selected as a pre-clinical candidate, based upon its improved toxicological profile and the reduced concentrations in brain tissues after oral dosing, compared to those of the other derivatives.[99]



33: **(OZ277)**



34: 1,2-dioxolane (inactive)

Figure 9: OZ277 and inactive 1,2-dioxolane.

Table 3: In vitro antimalarial activity of semi-synthetic artemisinins and synthetic ozonide OZ277 against NF54 and K1 strains of *P. falciparum*, and pharmacokinetic (PK) parameters after intravenous and oral administration to rats

Compound	*In vitro* inhibitory concentration (ng/ml)				*In vivo* PK		
	NF54		K1				
	IC_{50}	IC_{90}	IC_{50}	IC_{90}	$t_{1/2}$ (min)	(%) Oral Bioavailability	Ref.
Artemether **3**	0.49	0.98	0.45	0.80	52	1.4	[97, 100]
Artesunate **5**	0.98	1.9	0.6	1.8			[97]
DHA **2**					26		[100]
OZ277 **33**	0.82	1.6	0.17	0.42	76	26	[97] [101]

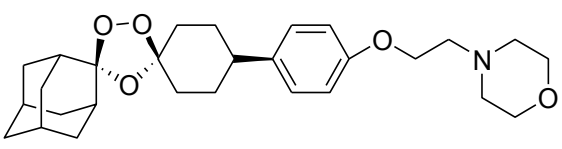
Examination of structure-activity relationships (SAR) of 1,2,4-tri-oxolanes, relative to OZ277, indicated that optimal antimalarial activity within this class was dependent upon (i) the spiro-adamantane ring system, (ii) the 1,2,4-tri-oxolane unit, (iii) increased lipophilicity and (iv) the presence of neutral and/or basic functional groups.[101, 102] An apparent proof of the important biological role of 1,2,4-tri-oxolane was presumed as being evident from the inactivity of 1,2-dioxolane **34**, an analogue of **33** that has a carbon atom in the place of the the non-peroxidic oxygen atom of the tri-oxolane,[103] although this was a surprising and counter-intuitive observation.[104]

Although OZ277 had a promising start as an orally available and highly potent antimalarial candidate, its development was discontinued, before completion of phase III clinical trials, due to disappointing clinical outcomes. It was found to be clinically less active than artemisinin, and it showed inadequate clinical and parasitological responses at day 28 (60-70%), after seven days of treatment, compared to artesunate (95%). Plasma exposure was found to be non-linear above 100 mg doses, and this compound was unstable in infected blood.[25] Despite these limitations, Ranbaxy Laboratories in India completed a phase III trial of OZ277 as a maleate salt (arterolane maleate; Rbx 11160), in combination with piperaquine phosphate. In 2012, the product was approved for the Indian market for malaria treatment. The lower efficacy of this combination, however, compared to that of artesunate-piperaquine, is expected to accelerate the emergence of resistance against piperaquine,

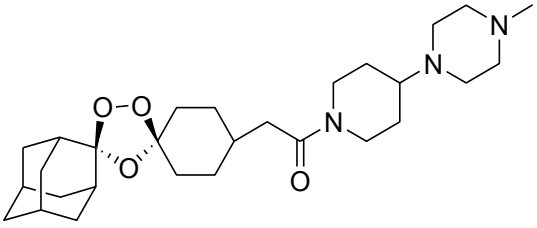
which has already been reported in Cambodia.[103] Such pharmaceutical practices pose serious questions about non-compliance by countries to international norms and recommendations regarding the requirements for a drug candidate to become a clinical/therapeutic agent, as recommended by the MMV and WHO.

4.1.2. OZ439

Further structural optimization to identify new synthetic ozonides, during which the limitations of OZ277 had been purportedly addressed, led to the synthesis of OZ439 (Figure 10). Nonetheless, the seeming strategy being used by the group to develop OZ439 was to design a compound that would be 'more stable towards reactions with iron(II) than OZ277, whilst also showing sufficient reactivity with iron(II) to still exert antimalarial activity'.[104] The mesylate salt of OZ439 **35** illustrated its potential as an antimalarial agent during both *in vitro* (*P. falciparum*) and *in vivo* (rodent, *P. berghei*) evaluations, even though this drug was less active than OZ277 during the *in vitro* screenings. In murine malaria models, however, OZ439 exhibited a prolonged exposure profile, resulting in an improved efficacy, relative to OZ277, artesunate **5** and other antimalarial drugs.[105] *In vitro*, OZ439 acted rapidly against all asexual erythrocytic *P. falciparum* stages, with inhibitory concentration (IC_{50}) values comparable to clinical artemisinins. Unlike other synthetic and semi-synthetic peroxidic antimalarials, however, OZ439 completely cured *P. berghei* infected mice with a single oral dose of 20 mg/kg, whilst exhibiting prophylactic activity, superior to currently used antimalarials, including mefloquine (Table 4). OZ439 also demonstrated substantially increased pharmacokinetic half-lives and blood concentrations *versus* time profiles, in three pre-clinical animal species.[106] Another evident difference to the artemisinins was that OZ439 displayed a strong inhibition of gametocyte maturation/gamete formation, whilst it also impacted on the sporogonic phase of the malaria parasite.[107] With such notable pharmacological and pharmacokinetic features, OZ439 was selected as a potentially credible alternative to the current artemisinins. OZ439 therefore is a second generation 1,2,4-tri-oxolane that entered clinical trials.



35: OZ439 (artefenomel)



36

Figure 10: Chemical structures of OZ439 **35** and related active tri-oxolane **36.**

Table 4: In vivo activity of a single dose of comparative antimalarial drugs, OZ277 and OZ439 against murine P. berghei[106]

Compound	*In vivo* efficacy 1 × 30 mg/kg (po)			*In vivo* prophylaxis 1 × 30 mg/kg (po) given 24 h before infection		
	Activity (%)	Survival (days)	Mouse cures	Activity (%)	Survival (days)	Protection (%)
OZ277 **33**	99.8	11	0/5	0/	7	0
OZ439 **35**	>99.9	>30	5/5	98.8	>30	100
Artesunate **5**	92	9	0/5	21	7	0
Mefloquine	99.6	22	0/5	99.9	27	60

In vivo efficacy of single dose comparative antimalarial drugs, OZ277 and OZ439 against murine *P. berghei* during treatment and during prophylaxis. The percentage activity of the compounds were assessed on day 3 post-infection. Percentage cure means no detectable parasites at day 30 post-infection.

In a first-in-man, double blind study, designed to assess the safety and pharmacokinetic profiles of OZ439, single and multiple doses were administered to healthy volunteers. OZ439 displayed impressive results after single oral doses of 800 mg, including a mean plasma concentration above 10 ng/ml and a notably long terminal half-life of about 30 h, which was about fifteen-fold higher than that of artesunate, or DHA. The clinical trials also revealed OZ439 as a safe and well tolerated compound at dose levels that offered prolonged plasma exposure.[105]

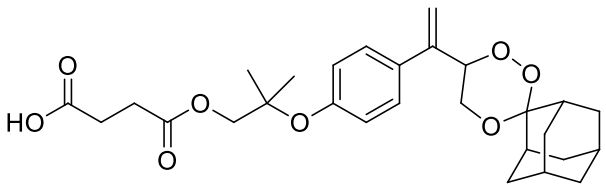
In a phase IIa trial to assess the efficacy of OZ439 in clearing parasitaemia, as a measure of antimalarial activity, an estimated parasite clearance half-life, in the range of 4.1-5.6 h for both the artemisinin sensitive and resistant *P. falciparum*, was noted. This was slower than that of artesunate (2-3 h) against the sensitive parasites, but faster than artesunate (6 h) against the resistant parasites. Following OZ439 administration, parasites were undetectable in blood smears at 30-36 h in *P. falciparum* patients and at 18-24 h in *P. vivax* patients. The clearance time was also not substantially affected in patients with *P. falciparum* artemisinin resistant parasites, associated with mutations in the kelch 13 propeller region (*P441I, N458Y, F446I, P527H, G538V, C580Y, A675A/V*, or *P667T*), in which the clearance time was 5.5 h, compared to 4.4 h in patients with *P. falciparum* without mutations in the propeller region.[108] After successfully completing phase IIa trials,[106] in July 2014, OZ439 was

submitted to a phase IIb trial, involving a randomized, double-blind study to establish the efficacy, safety, tolerability and pharmacokinetics of a single dose regimen of OZ439, in loose combination with piperaquine phosphate in adults and children, diagnosed with uncomplicated *P. falciparum* malaria.[109] The outcomes of the 2014 IIb trial however, are yet to be reported.

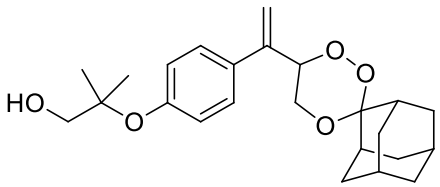
To date, little is known about the neurotoxicity of OZ439 in humans at repetitive and high doses, in contrast with the many reports in this regard on current clinical artemisinins. However, the embryotoxic and cytotoxic effects of tri-oxolane FBEG100 **36**, an analogue of OZ439, in comparison with artesunate **5** in rat embryos and in some human cell lines, have been examined.[110] Like artesunate, the tri-oxolane is embryotoxic and depletes primitive erythroblasts in a rodent model. Additionally, cytotoxicity towards a panel of established and primary human cell lines, with caspase dependent apoptosis and caspase independent necrosis underlying the induction of cell death, was also observed. As a result, while OZ439 seems like a promising next generation antimalarial, based upon its pharmacological and pharmacokinetic features, the data for tri-oxolane FBEG100 indicates that those adverse effects that are associated with the artemisinins, including embryotoxicity, cannot be ruled out for OZ439.

4.2. 1,2,4-Tri-oxane (CDRI 97/78)

Compound **37** (CDRI 97/78, Figure 11) is a fully synthetic 1,2,4-tri-oxane being developed by the Indian Central Drug Research Institute (CDRI).[111]



37: CDRI 97/78



38: CDRI 97/63 (active metabolite of **36**)

Figure 11: Fully synthetic 1,2,4-tri-oxanes.

CDRI 97/78 completed pre-clinical efficacy and regulatory tests, during which both CDRI 97/78 and its metabolite 97/63 have shown significant *in vivo* antimalarial activities against the multi-drug resistant strain of *P. yoelii Nigeriensis* in Swiss mice (Table 5) and *P. cynomolgi* in the Rhesus monkey model (Table 6).[112, 113] In a phase I clinical trial study, with a single ascending dose in the 80-700 mg range, CDRI 97/78 was found to be well tolerated, with only a few self-limiting, grade 2 severity adverse events observed.[114]

Table 5: *In vivo* antimalarial activity of tri-oxane CDRI 97/78 **37** and its metabolite **38** against multi-drug resistance strain of *P. yoelii* Nigeriensis in Swiss mice

Component	Administration route	Dose mg/kg	Mouse number	% Protection on day 4	Cured/ treated
CDRI 97/78 **37**	*im	64	6	100	6/6
		72	6	100	6/6
		48	6	100	5/6
		24	6	100	3/6
	*po	96	6	100	6/6
		48	6	100	2/6
CDRI 97/63 **38**	*im	96	6	100	6/6
		72	6	100	6/6
		48	6	100	5/6
	*po	96	6	100	6/6
		72	6	100	6/6
		48	6	100	1/6

*im (intramuscular), *po (oral)

Table 6: *In vivo* antimalarial activity of tri-oxane CDRI 97/78 **37** and its metabolite **38** against *P. cynomolgi* in the Rhesus monkey model

Compound	Administration route	Dose mg/kg per days	Regimen	Parasitaernia clearance time (h)	Cure Rate
CDRI 97/78 **37**	iv	10 x4	Divided	48	2/2
	im	10 x4	Divided	48	2/2
		10 x4	Single	48-72	0/2
	po	20 x 5	Divided	48-72	2/2
		10 x5	Divided	96	0/2
		20 x 1 + 10 x 4	Divided	48-72	5/5
CDRI 97/63 **38**	im	15 x4	Divided	48	2/2
		10 x4	Divided	72	2/2
	po	20 x 5	Divided	48	2/2
		20 x5	Divided	72	2/2

		15 x 5	Divided	72	2/2

Following oral administration, the compound is rapidly converted into its primary active metabolite, CDRI 97/63, which has a mean half-life of about 12 h.[114] CDRI 97/78 may thus very well be a potential candidate for future malaria therapy, if the single dose results can be replicated in multiple dose studies and its benefit be proven during confirmatory trials. The clinical development of both antimalarial drug candidates is being pursued under licensing agreements with IPCA Pharmaceuticals Ltd. in Mumbai.[115]

4.3. Tetra-oxanes

1,2,4,5-Tetra-oxanes comprize another class of synthetic peroxides that have been investigated in the quest for new antimalarial drugs. Structurally, they are distinct from the artemisinins and are they readily prepared from substituted cyclohexanones.[116] The compound, WR149888 **39** (Figure 12), was the first tetra-oxane, reported to possess antimalarial activity. It unfortunately displayed poor *in vivo* activity, due to its poor bio-availability, indicative of its high first pass metabolism.[117] In search for more active, stable and orally bio-available tetra-oxanes, a series of analogues was synthesized and their biological activities evaluated. Replacement of methyl with ethyl groups in positions 1 and 10 of the cyclohexyl rings resulted in derivative **40**, which possessed a slightly higher potency than WR149888. Further lengthening of the alkyl chain beyond ethyl resulted in derivatives **41** and **42**, which were less active than WR149888. This suggested that two-carbon chains comprized of the optimum alkyl substituent length for achieving maximum activity for the di-substituted tetra-oxanes. Tetramethyl substituted tetra-oxane isomers **43**-**45** were found to be four- to five-fold more active than WR149888, but still less potent than artemisinin **1** and arte-ether **4**.[118]

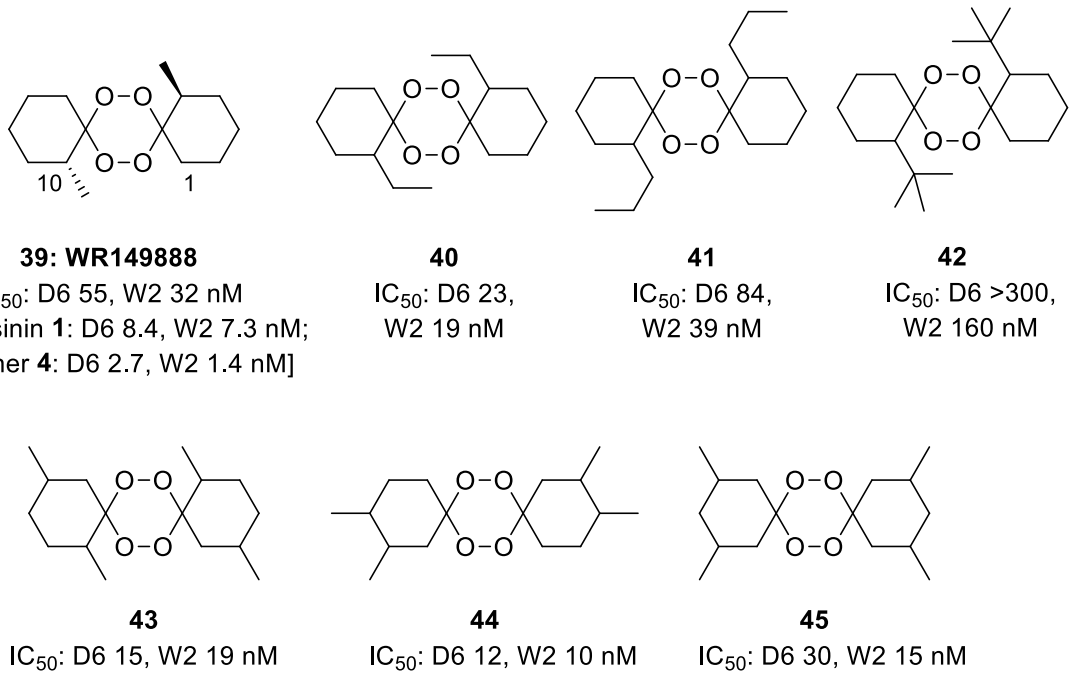
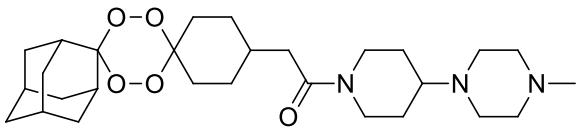
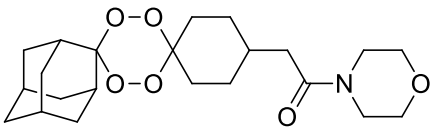


Figure 12: WR14988 and other 1,2,4,5-tetra-oxanes.[118]

Based upon previous observations that dispiro-1,2,4-tri-oxolanes were substantially less active than dispiro-1,2,4,5-tetra-oxanes[119] and that, in order to achieve higher antimalarial activity in 1,2,4-tri-oxolanes, fusion of the 1,2,4-tri-oxolane ring system to an adamantane core was required,[98] the O'Neill group synthesized new dispiro-1,2,4,5 tetra-oxanes, bearing an adamantyl end group and a variety of polar side chains to counterbalance the lipophilicity of the adamantane group[119, 120, 121, 122] (Figure 13). The *in vitro* and *in vivo* activities, as well as the pharmacokinetic parameters of these compounds were evaluated. The piperidinyl piperazine derivative **46** as a tosylate salt (RKA182) **47** and the morpholinyl ozonide **48** (RKA216) emerged as the most active of all out of some two-hundred compounds. The tosylate salt of **47** had excellent *in vitro* (IC_{50}<1 nM) and *in vivo* activities (ED_{50} 1.33 and ED_{90} 4.18 mg/kg) (Table 7). The efficacy of this tetra-oxane was found superior to those of artemether and artesunate. It also possessed good oral bio-availability in rodent models and showed better stability than OZ277 in infected human red blood cells.[106] RKA182 showed higher *in vitro* activity (IC_{50}<5 nM) than mefloquine, artesunate and artemisinin against South-East Asian isolates of parasites, taken from patients who had not responded well to treatment with ACTs.[123] The relatively straight forward, industrial scale synthesis process that produces 70% of material at a projected low cost,[122] is an additional advantage of RKA182 over the artemisinins and may this qualify it even further as a credible alternative. RKA182 represents the first generation of synthetic 1,2,4,5-tetra-oxane antimalarials being selected for pre-clinical development.



46 (tosylate salt **47: RKA182**)



48: **RKA216**

Figure 13: Chemical structures of potent antimalarial dispiro adamantyl tetra-oxanes.

Table 7: *In vitro* and *in vivo* activity data and pharmacokinetic parameters of tetra-oxanes

Compound	*In vitro*		*In vivo*				
	IC_{50} (nM)		ED_{50} (mg/kg)	ED_{90} (mg/kg)	$t_{1/2}$(h) (po)	(%) Bio-availability	Ref
	3D7	K1					
1	9.2		8.4				[119]
3	7.8	3.2	3.8	12.2		1.4[98]	[122]
5	1.8	1.6	4.0	11.7			[122]
46	4.9	1.9	1.8	8.4	3.5	24	[122]
47 RKA182	0.8	1.1	1.3	4.2	2.4	38	[122]
48 RKA216	5.2	0.8	3.2	3.9	5.9	11	[122]

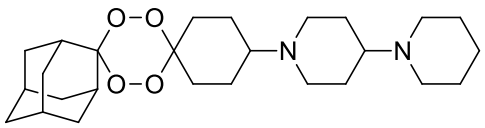
Investigation of the embryotoxic and cytotoxic effects of tetra-oxane RKA182, relative to those of artesunate **5** in rat embryos and in some human cell lines, was conducted. This study identified levels of embryotoxicity in the early stages of development in the rat embryos, which was attributed to anaemia and had been caused by the depletion of primitive erythroblasts. Although this was only observed in rodents, these findings clearly supported current guidelines with regards to the use of artemisinins in pregnant women.[110] Increased cytotoxicity in human cells was also identified in the presence of high levels of haem, as is observed during malaria infection.[110] The therapeutic index, however, favors the 1,2,4,5-tetra-oxane, when comparing the pharmacological efficacy and toxicity levels of RKA182 and artesunate.

In summary, RKA182 presented improved pharmacokinetics, relative to the semi-synthetic artemisinins. However, its half-life had been found too short for use in combination chemotherapies, as required by the MMV and, while these drugs showed superior efficacies, compared to the currently used artemisinins, some of the adverse effects that are associated

with the artemisinins, including embryotoxicity, were also evident among this class of compounds.

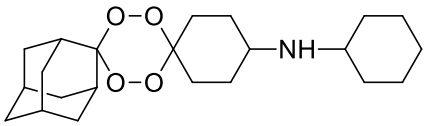
In an attempt to further stabilize the amide link, featured in RKA182, which may potentially result from its metabolic instability, a second generation of RKA182 analogues were designed and synthesized (Figure 14).[124] All of these derivatives showed notable *in vitro* activities, with IC_{50} values in the low nano-molar range (0.2-3.7 nM). The analogues **49** and **50** in particular displayed an impressive twenty-five-fold higher potency, than artesunate against the K1 strain.

In vivo, promising oral activity against the *P. berghei* ANKA strain in mice was also observed after oral administration of a 30 mg/kg dose of selected compounds. The free base derivatives **51** and **53** and their salts, **52** (hydrochloride) and **54** (ditosylate), respectively, were the most active, with the latter two salts being found more potent than artesunate, based upon comparison of their ED_{90} values. Assessment of metabolic stabilities indicated that compound **53** was slightly more stable than RKA182, with 57% of this compound remaining, following 1 h of incubation with human liver microsomes, compared to the 40% of RKA182.[124] This new series of 1,2,4,5-tetra-oxanes, with their enhanced metabolic stabilities, coupled with their excellent activity profiles, are potentially suitable antimalarial candidates.



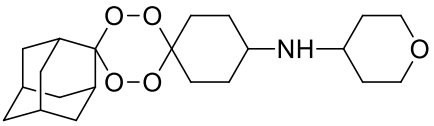
49

IC_{50}: 3D7 2.4, K1 0.2 nM
[artesunate **5** K1 4.9 nM;
ED_{50} 2.8, ED_{90} 18 mg/kg;
RKA182 3D7 0.8, K1 1.1 nM]



50

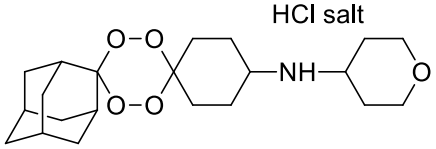
IC_{50}: 3D7 0.4, K1 0.2 nM



51

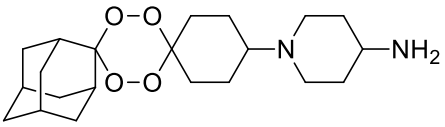
IC_{50}: 3D7 0.7, K1 0.4 nM
ED_{50} 2.8, ED_{90} 18 mg/kg

HCl salt



52

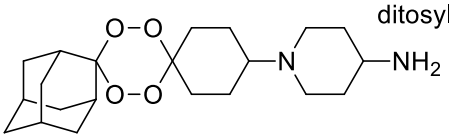
IC_{50}: 3D7 1 nM
ED_{50} 2.3, ED_{90} 11 mg/kg



53

IC_{50}: 3D7 1.2, K1 1.2 nM
ED_{50} 9.4, ED_{90} 34 mg/kg

ditosylate salt



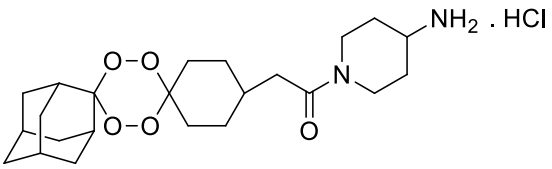
54

IC_{50}: 3D7 2.5 nM
ED_{50} 3.7, ED_{90} 10 mg/kg

Figure 14: Analogues of RKA182, devoid of the amide link and *in vitro* activity against 3D7 and K1 strains of *P. falciparum* and *in vivo* activity against the *P. berghei* ANKA strain in mice**.**

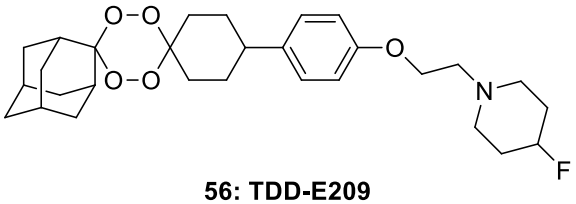
Truncation of the side chain of RKA182 led to compound **55** (J3-39) (Figure 15), which maintained the antimalarial activity of RKA, whilst additionally displaying increased metabolic stability, enhanced drug exposure, with 76% bio-availability and with less toxicity in repeated toxicity studies in pre-clinical animal studies.[123]

Replacement of the tri-oxolane core in OZ439 with the 1,2,4,5-tetra-oxane, provided, among others, compounds **56** (TDD-E209) and **57** (TDD-N205) that were identified as lead compounds.[123] *In vitro* efficacy (Table 8) of TDD-E209 against several sensitive and resistant strains of the malarial parasite was less than that of artesunate. The efficacy of both tetra-oxanes were also lower than that of OZ439 (Table 9), which is considered as the most potent, fully synthetic antimalarial peroxide being synthesized to date. The tetra-oxanes **55-57** are currently undergoing research led optimization, with the support of the MMV.



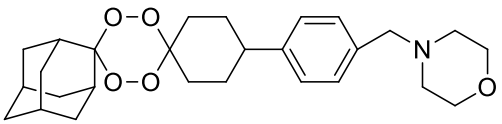
55: JC3-39

IC_{50}: 3D7 1.4 nM; ED_{50} 0.5, ED_{90} 3.5 mg/kg;
Mouse survival (3 x 10 mg/kg p.o. dosing): 17 days (free base) vs. 8 days [artesunate **5**];
PK in rat: 2 mg/kg (i.v.), 20 mg/kg (p.o.);
$t_{1/2}$: 2h (i.v.), 7 h (p.o.);
Solubility >50 mg/ml in H_2O (22°C)



56: TDD-E209

IC_{50}: 3D7 5.1 *vs* 8 nM [OZ439 **35**]
oral bioavailability in rat: 60-100%; $t_{1/2}$: 21 h (i.v.);
single dose ED_{90} Pfscid: 11 mg/kg,
P. berghei MSD: 25 days (2/3 cures)



57: TDD-N205

IC_{50}: 3D7 1.3 nM vs. 8 nM [OZ439 **35**]
oral bioavailability in rat: 60-100%;
$t_{1/2}$: 5.7 h (i.v.); single dose ED_{90} Pfscid: 8.6 mg/kg,
P. berghei MSD: 25 days (2/3 cures)

Figure 15: Third generation tetra-oxane antimalarials [123].

Table 8: Comparative *in vitro* activity of TDD-E209 **56**, artesunate and chloroquine against CQS and CQR strains of the malarial parasite[123]

Parasite strain	*In vitro* activity, IC_{50} (nM)		
	TDD-E209 **56**	Artesunate **5**	CQ
NF54	5.2	4.7	5.9
K1	4.2	2.8	173
HB3	4.2	2.8	9
7G8	2.9	1.8	55
TM90C2B	`7.3	3.8	121
D6	8.5	5.8	8.6
V1/S	4.2	2.7	193.5
Dd2	7.7	5.4	150.5
FCB	14.0	5.8	66

NF54, HB3, D6 (CQS strains); K1, 7G8, TM90C2B, V1/S, Dd2 and FCB (CQR strains)

Table 9: *In vivo* activity and average mouse survival rates of third generation tetra-oxanes versus OZ439[123]

Compound	% Activity	Average mouse survival following administration of 30 mg/kg doses
OZ439 **35**	99.4	30 (30, 30, 30)
TDD-E209 **56** citrate	99.65	25 (15, 30, 30)
TDD-N205 **57** citrate	99.42	26 (16, 30, 30)
TDD-E209 **56** mesylate	99.42	14 (13, 14, 14)
TDD-N205 **57** mesylate	99.30	25 (13, 30, 30)

Conclusion

Artemisinins in ACTs to date remain the drugs of choice for the treatment of uncomplicated malaria. However, the unfavorable physicochemical and pharmacological features of these drugs and the increased tolerance of malaria parasites to these drugs in South-East Asia, are threatening their continued use and are thus jeopardizing current strategies to fight malaria. It is therefore imperative to search for and identify new antimalarial drugs, such as new artemisinins that do not degrade into DHA upon hydrolysis or metabolism *in vivo*, or fully synthetic peroxides.

The past decade has seen substantial efforts and resources being invested in the discovery and development of novel semi- and fully synthetic peroxidic antimalarials, with higher demonstrated *in vitro* and *in vivo* efficacies than current, clinically used artemisinins. Limited successes have been achieved to date though. Indeed, only one of the semi-synthetic artemisinins, i.e. artemisone, that has been prepared in recent times, has advanced to clinical trials, although its development was then discontinued by the MMV, for the reasons as outlined above. New data has, however, emerged with regards to this compound that is indicative thereof that its gametocytocidal activity is more potent than any other antimalarial drug to date, with potentially promising activity against a mosquito stage of the malaria parasite, namely oocysts. It is also potently active towards parasite isolates, obtained from patients who had failed to respond to ACT therapy. Three completely new synthetic peroxides, i.e. RKA182 (pre-clinical), CDRI 97/78 (phase I) and OZ439 (phase IIb) have been approved by the MMV. It would appear that the last compound, with its remarkably long pharmacological half-life, may be the most promising for use in a single dose combination for the possible future treatment of malaria. Overall, the expectation currently is that much research is still required for identifying new semi-synthetic artemisinins and fully

synthetic peroxide drugs for future use as credible alternatives to the current clinically used artemisinins. Artemisinin, irrespective of its mode of preparation, whether from *Artemisia annua*, or through semi-synthetic processes involving photochemistry, currently remains an important molecule for the treatment of malaria.

Acknowledgements

The authors wish to thank the North-West University for financial support.

This research project was funded by the South African Medical Research Council (MRC) with funds from National Treasury under its Economic Competitiveness and Support Package.

References

[1] K. Na-Bangchang, J. Karbwang, *Fundam Clin Pharmacol* **2009**, *23*(4), 387-409.

[2] C. A. Guerra, P. W. Gikandi, A. J. Tatem, A. M. Noor, D. L. Smith, S. I. Hay, R. W. Snow, *PLoS Med* **2008**, *5*(2), e38.

[3] WHO, *WHO* **2014**.

[4] Z. Jianfang, *Yangcheng Evening News Publishing Company, Yang Cheng, China* **2006**.

[5] T. Efferth, B. Kaina, *Crit Rev Toxicol* **2010**, *40*(5), 405-421.

[6] P. M. O'Neill, G. H. Posner, *J Med Chem* **2004**, *47*(12), 2945-2964.

[7] G. Li, X. Guo, K. Arnold, H. Jian, L. Fu, *Lancet* **1984**, *324*(8416), 1360-1361.

[8] F. Nosten, N. J. White, *Am J Trop Med Hyg* **2007**, *77*(6 Suppl), 181-192.

[9] F. T. Aweeka, P. German, *Clin Pharmacokinet* **2008**, *47*(2), 91-102.

[10] R. T. Eastman, D. A. Fidock, *Nat Rev Microbiol* **2009**, *7*(12), 864-874.

[11] C. J. Woodrow, R. K. Haynes, S. Krishna, *Postgrad Med J* **2005**, *81*(952), 71-78.

[12] A. M. Dondorp, P. Ringwald, *Trends Parasitol* **2013**, *29*(8), 359-360.

[13] P. N. Newton, R. McGready, F. Fernandez, M. D. Green, M. Sunjio, C. Bruneton, S. Phanouvong, P. Millet, C. J. M. Whitty, A. O. Talisuna, S. Proux, E. M. Christophel, G. Malenga, P. Singhasivanon, K. Bojang, H. Kaur, K. Palmer, N. P. J. Day, B. M. Greenwood, F. Nosten, N. J. White, *PLoS Med* **2006**, *3*(6), e197.

[14] P. N. Newton, M. D. Green, F. Fernandez, *PLoS Med* **2007**, *4*(3), e139.

[15] R. Bate, P. Coticelli, R. Tren, A. Attaran, *PLoS ONE* **2008**, *3*(5), e2132.

[16] P. N. Newton, M. D. Green, F. M. Fernandez, N. P. J. Day, N. J. White, *Lancet Inf Dis* **2006**, *6*(9), 602-613.

[17] WHO, *WHO* **2013**.

[18] S. Meshnick, *Am J Trop Med Hyg* **2012**, *87*(5), 783-784.

[19] A. Vaid, R. Ranjan, W. A. Smythe, H. C. Hoppe, P. Sharma, *Blood* **2010**, *115*(12), 2500-2507.

[20] A. M. Van Hook, *Science Signaling* **2015**, *8*(375), ec118.

[21] 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* **2015**, *520*(7549), 683-687.

[22] J. Held, S. Jeyaraj, A. Kreidenweiss, *Expert Opin Invest Drugs* **2015**, *24*(3), 363-382.

[23] E. L. Flannery, A. K. Chatterjee, E. A. Winzeler, *Nat Rev Microbiol* **2013**, *11*(12), 849-862.

[24] M. A. Biamonte, J. Wanner, K. G. Le Roch, *Bioorg Med Chem Lett* **2013**, *23*(10), 2829-2843.

[25] M. Anthony, J. Burrows, S. Duparc, J. J. Moehrle, T. Wells, *Malar J* **2012**, *11*(1), 316.

[26] D. S. Barnett, R. K. Guy, *Chem Rev* **2014**, *114*(22), 11221-11241.

[27] WHO, *WHO* **2013**.

[28] C. A. Lipinski, F. Lombardo, B. W. Dominy, P. J. Feeney, *Adv Drug Deliv Rev* **2001**, *46*, 3-26.

[29] J. M. Grace, A. J. Aguilar, K. M. Trotman, T. G. Brewer, *Drug Metab Dispos* **1998**, *26*(4), 313-317.

[30] V. Navaratnam, S. Mahsufi Mansor, N. W. Sit, J. Grace, Q. Li, P. Olliaro, *Clin Pharmacokinet* **2000**, *39*(4), 255-270.

[31] F. Aweeka, P. German, *Clin Pharmacokinet* **2008**, *47*(2), 91-102.

[32] G. Lefevre, M. S. Thomsen, *Clin Drug Invest* **1999**, *18*(6), 467-480.

[33] C. Morris, S. Duparc, I. Borghini-Fuhrer, D. Jung, C. S. Shin, L. Fleckenstein, *Malar J* **2011**, *10*(1), 263.

[34] 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* **2007b**, *2*(10), 1448-1463.

[35] H. F. Jansen, S. A. Soomro, *Curr Med Chem* **2007**, *14*(30), 3243-3259.

[36] WHO, *WHO* **2013**.

[37] F. Jansen, *Malar J* **2010**, *9*(1), e212.

[38] S. Pooley, F. A. Fatih, S. Krishna, M. Gerisch, R. K. Haynes, H.-N. Wong, H. M. Staines, *Antimicrob Agents Chemother* **2010**, *55*(2), 550-556.

[39] R. Harmse, H. N. Wong, F. J. Smit, R. K. Haynes, D. D. N'Da, *Curr Med Chem* **2015**, *in press*.

[40] R. K. Haynes, H.-N. Wong, K.-W. Lee, C.-M. Lung, L. Y. Shek, I. D. Williams, S. L. Croft, L. Vivas, L. Rattray, L. Stewart, V. K. W. Wong, B. C. B. Ko, *ChemMedChem* **2007a**, *2*(10), 1464-1479.

[41] S. Kopp, *RAJ Pharma* **2006**, *5*(291-294).

[42] G. Schmuck, R. Haynes, *Neurotox Res* **2000**, *2*(1), 37-49.

[43] Q. Li, M. Hickman, *Toxicology* **2010**, *279*, 1-9.

[44] T. Gordi, E.-I. Lepist, *Toxicol Lett* **2004**, *147*(2), 99-107.

[45] 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, *New Eng J Med* **2014**, *371*(5), 411-423.

[46] WHO, **2006**.

[47] M. Peplow, *Chemistryworld online (RSC); http://www.rsc.org/chemistryworld/2013/04/sanofi-launches-malaria-drug-production; Access date: May 29, 2015* **2013**.

[48] L. Hsing-Jang, Y. Wen-Lung, Y. C. Sew, *Tetrahedron Lett* **1993**, *34*(28), 4435-4438.

[49] G. Schmid, W. Hofheinz, *J Am Chem Soc* **1983**, *105*(3), 624-625.

[50] J. S. Yadav, B. Thirupathaiah, P. Srihari, *Tetrahedron* **2010**, *66*(11), 2005-2009.

[51] C. Zhu, S. P. Cook, *J Am Chem Soc* **2012**, *134*(33), 13577-13579.

[52] C. J. Paddon, P. J. Westfall, D. J. Pitera, K. Benjamin, K. Fisher, D. McPhee, M. D. Leavell, A. Tai, A. Main, D. Eng, D. R. Polichuk, K. H. Teoh, D. W. Reed, T. Treynor, J. Lenihan, H. Jiang, M. Fleck, S. Bajad, G. Dang, D. Dengrove, D. Diola, G. Dorin, K. W. Ellens, S. Fickes, J. Galazzo, S. P. Gaucher, T. Geistlinger, R. Henry, M. Hepp, T. Horning, T. Iqbal, L. Kizer, B. Lieu, D. Melis, N. Moss, R. Regentin, S. Secrest, H. Tsuruta, R. Vazquez, L. F. Westblade, L. Xu, M. Yu, Y. Zhang, L. Zhao, J. Lievense, P. S. Covello, J. D. Keasling, K. K. Reiling, N. S. Renninger, J. D. Newman, *Nature* **2013**, *496*(7446), 528-532.

[53] R. J. Roth, N. Acton, *Planta Med* **1987**, *53*(06), 576-576.

[54] R. J. Roth, N. Acton, *J Chem Educ* **1989**, *66*(4), 349-350.

[55] R. K. Haynes, S. C. Vonwiller, *J Chem Soc Chem Commun* **1990**(6), 451-453.

[56] R. K. Haynes, S. C. Vonwiller, *Trans R Soc Trop Med Hyg* **1994**, *88, Supplement 1*(0), 23-26.

[57] R. K. Haynes, S. C. Vonwiller, *Acc Chem Res* **1997**, *30*(2), 73-79.

[58] S. C. Vonwiller, J. A. Warner, S. T. Mann, R. K. Haynes, *J Am Chem Soc* **1995**, *117*(45), 11098-11105.

[59] F. Lévesque, P. H. Seeberger, *Angew Chem Int Ed* **2012**, *51*(7), 1706-1709.

[60] H.-J. Chen, W.-B. Han, H.-D. Hao, Y. Wu, *Tetrahedron* **2013**, *69*(3), 1112-1114.

[61] J. Dhainaut, A. Dlubala, R. Guevel, A. Medard, G. Oddon, N. Raymond, J. Turconi, Photochemical process for producing artemisinin, US 8507697 B2, 2013; http://www.google.com/patents/US8507697, Google Patents, **2013**.

[62] D. Kopetzki, F. Lévesque, P. H. Seeberger, *Chem Eur J* **2013**, *19*(17), 5450-5456.

[63] S. C. Vonwiller, R. K. Haynes, G. King, H.-J. Wang, *Planta Med* **1993**, *59*(06), 562-563.

[64] A.-C. Uhlemann, S. Wittlin, H. Matile, L. Y. Bustamante, S. Krishna, *Antimicrob Agents Chemother* **2007**, *51*(2), 667-672.

[65] P. Ploypradith, *Acta Tropica* **2004**, *89*(3), 329-342.

[66] M. Jung, S. Lee, *Bioorg Med Chem Lett* **1998**, *8*(9), 1003-1006.

[67] M. Jung, X. Li, D. A. Bustos, H. N. ElSohly, J. D. McChesney, W. K. Milhous, *J Med Chem* **1990**, *33*(5), 1516-1518.

[68] B. Ye, Y. Wu, G. Li, X. Jiao, *Acta Pharm Sin* **1991**, *26*(3), 228-230.

[69] V. T. Khac, T. N. Van, S. T. Van, *Bioorg Med Chem Lett* **2005**, *15*(10), 2629-2631.

[70] I. Opsenica, D. Opsenica, K. S. Smith, W. K. Milhous, B. A. Å olaja, *J Med Chem* **2008**, *51*(7), 2261-2266.

[71] P. M. O'Neill, A. Miller, L. P. D. Bishop, S. Hindley, J. L. Maggs, S. A. Ward, S. M. Roberts, F. Scheinmann, A. V. Stachulski, G. H. Posner, B. K. Park, *J Med Chem* **2001**, *44*(1), 58-68.

[72] B. T. Mott, A. Tripathi, M. A. Siegler, C. D. Moore, D. J. Sullivan, G. H. Posner, *J Med Chem* **2013**, *56*(6), 2630-2641.

[73] R. C. Conyers, J. R. Mazzone, M. A. Siegler, A. K. Tripathi, D. J. Sullivan, B. T. Mott, G. H. Posner, *Bioorg Med Chem Lett* **2014**, *24*(5), 1285-1289.

[74] I.-H. Paik, S. Xie, T. A. Shapiro, T. Labonte, A. A. Narducci Sarjeant, A. C. Baege, G. H. Posner, *J Med Chem* **2006**, *49*(9), 2731-2734.

[75] R. K. Haynes, W.-Y. Ho, H.-W. Chan, B. Fugmann, J. Stetter, S. L. Croft, L. Vivas, W. Peters, B. L. Robinson, *Angew Chem Int Ed* **2004**, *43*(11), 1381-1385.

[76] W. A. Guiguemde, N. H. Hunt, J. Guo, A. Marciano, R. K. Haynes, J. Clark, R. K. Guy, J. Golenser, *Antimicrob Agents Chemother* **2014**, *58*(8), 4745-4754.

[77] R. K. Haynes, B. Fugmann, J. Stetter, K. Rieckmann, H. D. Heilmann, H. W. Chan, M. K. Cheung, W. L. Lam, H. N. Wong, S. L. Croft, L. Vivas, L. Rattray, L. Stewart, W. Peters, B. L. Robinson, M. D. Edstein, B. Kotecka, D. E. Kyle, B. Beckermann, M. Gerisch, M. Radtke, G. Schmuck, W. Steinke, U. Wollborn, K. Schmeer, A. Romer, *Angew Chem Int Ed Eng* **2006**, *45*, 2082 - 2088.

[78] J. Nagelschmitz, B. Voith, G. Wensing, A. Roemer, B. Fugmann, R. K. Haynes, B. M. Kotecka, K. H. Rieckmann, M. D. Edstein, *Antimicrob Agents Chemother* **2008**, *52*(9), 3085-3091.

[79] M. Ramharter, D. Burkhardt, J. Nemeth, A. A. Adegnika, P. G. Kremsner, *Am J Trop Med Hyg* **2006**, *75*(4), 637-639.

[80] R. K. Haynes, B. Fugmann, J. Stetter, K. Rieckmann, H.-D. Heilmann, H.-W. Chan, M.-K. Cheung, W.-L. Lam, H.-N. Wong, S. L. Croft, L. Vivas, L. Rattray, L. Stewart, W. Peters, B. L. Robinson, M. D. Edstein, B. Kotecka, D. E. Kyle, B. Beckermann, M. Gerisch, M. Radtke, G. Schmuck, W. Steinke, U. Wollborn, K. Schmeer, A. Römer, *Angew Chem Int Ed* **2006**, *45*(13), 1989-1989.

[81] L. Vivas, L. Rattray, L. B. Stewart, B. L. Robinson, B. Fugmann, R. K. Haynes, W. Peters, S. L. Croft, *J Antimicrob Chemother* **2007**, *59*(4), 658-665.

[82] J. Burrows, R. Hooft van Huijsduijnen, J. Mohrle, C. Oeuvray, T. Wells, *Malar J* **2013**, *12*(1), 187.

[83] www.clinicaltrials.gov;, **2010**.

[84] M. Enserink, *Science* **2010**, *328*(5980), 846.

[85] J. Waknine-Grinberg, N. Hunt, A. Bentura-Marciano, J. McQuillan, H.-W. Chan, W.-C. Chan, Y. Barenholz, R. Haynes, J. Golenser, *Malar J* **2010**, *9*(1), 227.

[86] G. Schmuck, A. M. Klaus, F. Krötlinger, F. W. Langewische, *Birth Defects Res Part B: Develop Reprod Toxicol* **2009**, *86*(2), 131-143.

[87] D. S. Torok, H. Ziffer, S. R. Meshnick, X.-Q. Pan, A. Ager, *J Med Chem* **1995**, *38*(26), 5045-5050.

[88] C. Singh, V. P. Verma, M. Hassam, A. S. Singh, N. K. Naikade, S. K. Puri, *J Med Chem* **2014**, *57*(6), 2489-2497.

[89] C. W. Jefford, S. Kohmoto, D. Jaggi, G. Timári, J.-C. Rossier, M. Rudaz, O. Barbuzzi, D. Gérard, U. Burger, P. Kamalaprija, J. Mareda, G. Bernardinelli, I. Manzanares, C. J. Canfield, S. L. Feck, B. L. Robinson, W. Peters, *Helv Chim Acta* **1995**, *78*(3), 647-662.

[90] S. H. Jaquet C, Chollet J, Peters W *Trop Med Parasitol* **1994**, *45*(3), 266−271.

[91] G. R. Salako LA, Mittelholzer M-L, Haller L, Sorenson F, Sturchler D, *Trop Med Parasitol* **1994**, *45*(3), 284-287.

[92] J. R. Girometta MA, Ponelle C, Guenzi A, Wiegand-Chou RC, *Trop Med Parasitol* **1994**, *45*(3), 272–277.

[93] J. L. Vennerstrom, Y. Dong, J. Chollet, H. Matile, Spiro and dispiro 1,2,4-trioxolane antimalarials, Google Patents, **2002**.

[94] K. Griesbaum, X. Liu, A. Kassiaris, M. Scherer, *Liebigs Annalen: org bioorg chem* **1997**(7), 1381.

[95] Y. Tang, Y. Dong, J. M. Karle, C. A. DiTusa, J. L. Vennerstrom, *J Org Chem* **2004a**, *69*(19), 6470-6473.

[96] J. L. Vennerstrom, S. Arbe-Barnes, R. Brun, S. A. Charman, F. C. K. Chiu, J. Chollet, Y. Dong, A. Dorn, D. Hunziker, H. Matile, K. McIntosh, M. Padmanilayam, J. Santo

Tomas, C. Scheurer, B. Scorneaux, Y. Tang, H. Urwyler, S. Wittlin, W. N. Charman, *Nature* **2004**, *430*(7002), 900-904.

[97] O. Abiodun, R. Brun, S. Wittlin, *Malar J* **2013**, *12*(1), 43.

[98] J. L. Vennerstrom, Y. Dong, J. Chollet, H. Matile, M. Padmanilayam, Y. Tang, W. N. Charman, Spiro and dispiro 1,2,4-trixolane antimalarials, Google Patents, **2004**.

[99] Y. Tang, Y. Dong, J. L. Vennerstrom, *Med Res Rev* **2004b**, *24*(4), 425-448.

[100] Y. Dong, J. Chollet, H. Matile, S. A. Charman, F. C. K. Chiu, W. N. Charman, B. Scorneaux, H. Urwyler, J. Santo Tomas, C. Scheurer, C. Snyder, A. Dorn, X. Wang, J. M. Karle, Y. Tang, S. Wittlin, R. Brun, J. L. Vennerstrom, *J Med Chem* **2005**, *48*(15), 4953-4961.

[101] Y. Dong, S. Wittlin, K. Sriraghavan, J. Chollet, S. A. Charman, W. N. Charman, C. Scheurer, H. Urwyler, J. Santo Tomas, C. Snyder, D. J. Creek, J. Morizzi, M. Koltun, H. Matile, X. Wang, M. Padmanilayam, Y. Tang, A. Dorn, R. Brun, J. L. Vennerstrom, *J Med Chem* **2010**, *53*(1), 481-491.

[102] Y. Dong, Y. Tang, J. Chollet, H. Matile, S. Wittlin, S. A. Charman, W. N. Charman, J. S. Tomas, C. Scheurer, C. Snyder, B. Scorneaux, S. Bajpai, S. A. Alexander, X. Wang, M. Padmanilayam, S. R. Cheruku, R. Brun, J. L. Vennerstrom, *Bioorg Med Chem* **2006**, *14*(18), 6368-6382.

[103] X. Wang, Y. Dong, S. Wittlin, S. A. Charman, F. C. K. Chiu, J. Chollet, K. Katneni, J. Mannila, J. Morizzi, E. Ryan, C. Scheurer, J. Steuten, J. Santo Tomas, C. Snyder, J. L. Vennerstrom, *J Med Chem* **2013**, *56*(6), 2547-2555.

[104] R. K. Haynes, K. W. Cheu, D. N'Da, P. Coghi, D. Monti, *Infect Disord Drug Targets* **2013**, *13*(4), 217-277.

[105] J. J. Moehrle, S. Duparc, C. Siethoff, P. L. M. Giersbergen, J. C. Craft, S. Arbe-Barnes, S. A. Charman, M. Gutierrez, S. Wittlin, J. L. Vennerstrom, *Br J Clin Pharmacol* **2013**, *75*(2), 524-537.

[106] S. A. Charman, S. Arbe-Barnes, I. C. Bathurst, R. Brun, M. Campbell, W. N. Charman, F. C. K. Chiu, J. Chollet, J. C. Craft, D. J. Creek, Y. Dong, H. Matile, M. Maurer, J. Morizzi, T. Nguyen, P. Papastogiannidis, C. Scheurer, D. M. Shackleford, K. Sriraghavan, L. Stingelin, Y. Tang, H. Urwyler, X. Wang, K. L. White, S. Wittlin, L. Zhou, J. L. Vennerstrom, *Proc Natl Acad Sci* **2011**, *108*(11), 4400-4405.

[107] M. Delves, D. Plouffe, C. Scheurer, S. Meister, S. Wittlin, E. A. Winzeler, R. E. Sinden, D. Leroy, *PLoS Med* **2012**, *9*(2), e1001169.

[108] A. P. Phyo, P. Jittamala, F. H. Nosten, S. Pukrittayakamee, M. Imwong, N. J. White, S. Duparc, F. Macintyre, M. Baker, J. J. Mohrle, *Lancet Infect Dis* **2015**.

[109] NIH, *US Natl Inst Health* **2015**.

[110] I. M. Copple, A. E. Mercer, J. Firman, G. Donegan, B. Herpers, M. H. Wong, J. Chadwick, A. D. Bringela, M. L. S. Cristiano, B. van de Water, S. A. Ward, P. M. O'Neill, B. K. Park, *Mol Med* **2012**, *18*, 1045-1055.

[111] C. Singh, N. Gupta, S. K. Puri, *Bioorg Med Chem Lett* **2003**, *13*(20), 3447-3450.

[112] C. Singh, S. K. Puri, Substituted 1,2,4-trioxanes as antimalarial agents and a process of producing the substituted 1,2,4-trioxanes, Google Patents, **2001**.

[113] P. Gupta, P. Srivastava, *Pharmacol Clin Toxicol* **2014**, *2*(1).

[114] N. Shafiq, S. Rajagopalan, H. N. Kushwaha, N. Mittal, N. Chandurkar, A. Bhalla, S. Kaur, P. Pandhi, G. D. Puri, S. Achuthan, A. Pareek, S. K. Singh, J. S. Srivastava, S. P. S. Gaur, S. Malhotra, *Malar Res Treat* **2014**, *2014*, 10.

[115] CSIR, **2015**.

[116] J. L. Vennerstrom, H. N. Fu, W. Y. Ellis, A. L. Ager, J. K. Wood, S. L. Andersen, L. Gerena, W. K. Milhous, *J Med Chem* **1992**, *35*(16), 3023-3027.

[117] J. L. Vennerstrom, A. L. Ager, S. L. Andersen, J. M. Grace, V. Wongpanich, C. K. Angerhofer, J. K. Hu, D. L. Wesche, *Am J Trop Med Hyg* **2000a**, *62*(5), 573-578.

[118] J. L. Vennerstrom, Y. Dong, S. L. Andersen, A. L. Ager, H.-n. Fu, R. E. Miller, D. L. Wesche, D. E. Kyle, L. Gerena, S. M. Walters, J. K. Wood, G. Edwards, A. D. Holme, W. G. McLean, W. K. Milhous, *J Med Chem* **2000b**, *43*(14), 2753-2758.

[119] G. L. Ellis, R. Amewu, S. Sabbani, P. A. Stocks, A. Shone, D. Stanford, P. Gibbons, J. Davies, L. Vivas, S. Charnaud, E. Bongard, C. Hall, K. Rimmer, S. Lozanom, M. Jesus, D. Gargallo, S. A. Ward, P. M. O'Neill, *J Med Chem* **2008**, *51*(7), 2170-2177.

[120] R. Amewu, A. V. Stachulski, S. A. Ward, N. G. Berry, P. G. Bray, J. Davies, G. Labat, L. Vivas, P. M. O'Neill, *Org Biomol Chem* **2006**, *4*(24), 4431-4436.

[121] F. Bousejra-El Garah, M. H.-L. Wong, R. K. Amewu, S. Muangnoicharoen, J. L. Maggs, J.-L. Stigliani, B. K. Park, J. Chadwick, S. A. Ward, P. M. O'Neill, *J Med Chem* **2011**, *54*(19), 6443-6455.

[122] P. M. O'Neill, R. K. Amewu, G. L. Nixon, F. Bousejra ElGarah, M. Mungthin, J. Chadwick, A. E. Shone, L. Vivas, H. Lander, V. Barton, S. Muangnoicharoen, P. G. Bray, J. Davies, B. K. Park, S. Wittlin, R. Brun, M. Preschel, K. Zhang, S. A. Ward, *Angew Chem Int Ed* **2010**, *49*(33), 5693-5697.

[123] S. A. Ward, *Joint Int Trop Med Meeting JITMM* **2013**, *on "Towards global health: an Asian Paradigm of Tropical Medicine", Dec. 11-13, 2013, Centera Grand & Bangkok Convention Centre, Cenentral World in Bangkok, Thailand; http://www.jitmm.com/uploadDoc/Archive/AbstractBook_2013.pdf; Access date: May 15, 2015*.

[124] F. Marti, J. Chadwick, R. K. Amewu, H. Burrell-Saward, A. Srivastava, S. A. Ward, R. Sharma, N. Berry, P. M. O'Neill, *MedChemComm* **2011**, *2*(7), 661-665.

CHAPTER 4

ARTICLE FOR SUBMISSION

Chapter 4 comprises the manuscript of an article to be submitted to the European Journal of Medicinal Chemistry for publication. The article presents the Introduction, Results, Discussion, Conclusion, Materials and methods of the compounds prepared in this study. This article is prepared according to the author‟s guidelines, available in the author information pack on the journal‟s home page:

https://www.elsevier.com/journals/european-journal-of-medicinal-chemistry/0223-5234?generatepdf=true

Synthesis and biological evaluation of novel artemisone derivatives

Richard J. Mabilika[a], Frans J. Smit[b], Lyn-Marie Birkholtz[c], Dina Coertzen[c], Richard K. Haynes*[b],David D. N"Da*[b]

[a] Pharmaceutical Chemistry, School of Pharmacy, North-West University, Potchefstroom 2520, South Africa

[b] Centre of Excellence for Pharmaceutical Sciences, North-West University, Potchefstroom 2520, South Africa

[c] Department of Biochemistry, Centre for Sustainable Malaria Control, University of Pretoria, Pretoria 0002, South Africa

* Corresponding authors.

E-mail: Richard.haynes@nwu.ac.za, Tel.: +27 (0)18 299 4466; E-mail: david.nda@nwu.ac.za, Tel.: +27 18 299 2256; Fax: +27 18 299 4243

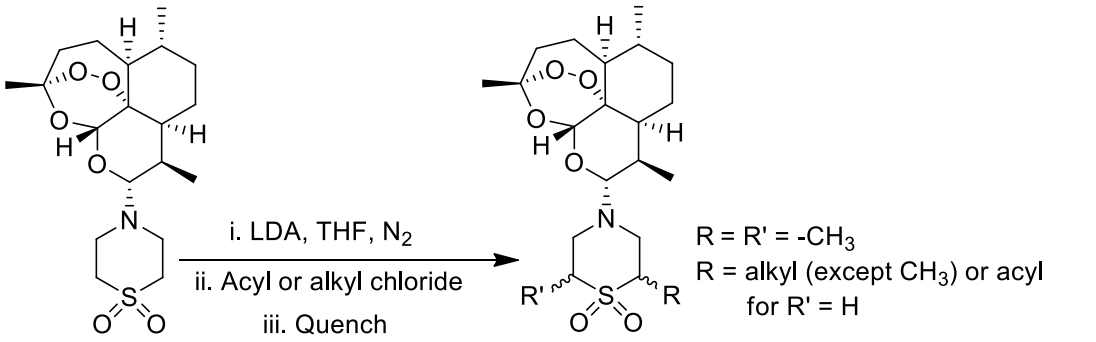
Abstract

By exploiting the acidity of the methylene protons adjacent to the sulfone group in the thiomorpholine-*S*,*S*-dioxide ring in artemisone, the latter could be converted in straightforward fashion by treatment with lithium *N*,*N*-diisopropylamide and then with alkyl and acyl halides into alkylated and acylated products in low to moderate yields. Evaluation of *in vitro* antimalarial activities of the products against chloroquine (CQ) resistant K1 and W2 strains and the CQ sensitive NF54 strains of intraerythrocytic *Plasmodium falciparum* parasites indicated potent antimalarial activities, with IC_{50} values ranging from 0.4 – 22, 0.5 – 34 and 1 – 32 nM against the NF54, K1 and W2 strains respectively. Artemisone derivatives bearing short chain α-alkyl or acyl groups proved to be the most potent in the series. They were almost equipotent with artemisone, 2 - 4 times more active than artesunate and 3- to 6-fold more active than artemether against both NF54 and K1 strains. Additionally, cytotoxicity of the compounds was evaluated against the WI-38 normal human fetal lung fibroblast cell line. All compounds displayed highly selective toxicity towards the malaria parasite, most with selectivity indices greater than 70,000 indicating their antiparasitic effects result from intrinsic activity and not from general cytotoxicity. Owing to their impressive resistance index across all the parasite lines and their high selective toxicity towards the malaria parasites, compounds **6** and **10** were identified as the best candidates for further investigation as potential drugs in the search for new, effective and safe antimalarial drugs.

Keywords: malaria, ACTs, artemisone, artemisinin, artesunate, antimalarial

Graphical abstract

A series of substituted artemisone derivatives were prepared by deprotonation of artemisone and reaction with alkyl and acyl chloride. Their *in vitro* antiplasmodial activity against NF54, K1 and W2 strains as well as early stages *P. falciparum* gametocytocidal (EG) activity determined. Additionally, their cytotoxicity against WI-38 cell line was assessed.



Artemisone

Artemisone derivatives
6 - 22

Antimalarial activity, IC_{50}
- **NF54**: 0.4 - 22 nM
- **K1**: 0.5 - 34 nM
- **W2**: 1 - 32 nM
- **EG**: 10 - 26 nM

Cytotoxicity, HFLF WI-38, IC_{50}: >100 µM

1. Introduction

Malaria is a massive global public health problem in more than 100 countries inhabited by roughly 3.4 billion people, accounting for nearly 40% of the world"s population [1]. Five species of the genus *Plasmodium* namely; *P. falciparum, P. vivax, P. ovale, P. malariae* and *P. knowlesi* cause malaria infections in humans [2]. *P. falciparum* is the most lethal specie and, together with *P. vivax* are responsible for most cases and deaths reported worldwide [3]. The female *Anopheline* mosquito remains the only known vector of the disease [4].

There was an estimated 198 million malaria cases and 584,000 deaths globally in 2013 [1]. The burden is heaviest in the African Region, where an estimated 90% of all malaria deaths occurred in 2013, with children aged under 5 years accounting for 78% of all deaths [1]. However, significant progress has been made in the fight against malaria in the past decade; between 2000 and 2013, as malaria mortality rates decreased by 47% worldwide and by 54% in the WHO African Region. This success has been accomplished as a result of the combinations of several factors including the expanded use of chemotherapy, and the use of insecticide treated nets (ITNs) and in-door residual sprays (IRS) [1].

The treatment of malaria continues to rely on antimalarial chemotherapeutic agents, this is especially so as an effective vaccine against the disease is not yet available. A number of vaccines are in clinical trials, with the most advanced candidate, *i.e.* RTS, S/AS01 having completed Phase III. A reduction in severe malaria of 46% among children (2-12 years) and 27% among infants (<24 months) who received all the planned doses of RTS/AS01 as compared to their control group counterparts has been reported [1].

Several drugs have been used throughout the past century for malaria treatment. Following its introduction, chloroquine (CQ); an inexpensive fully synthetic quinoline antimalarial, proved to be one of the most successful and important drugs ever deployed against an infectious disease in the 20th century [5]. Unfortunately, the drug was unwisely used, which resulted in the rapid emergence and spread of CQ-resistant (CQR) strains of *P. falciparum* parasites which has drastically proscribed its use [6]. The effectiveness of CQ against *P. vivax* parasites has also progressively declined across *P. vivax* malaria endemic areas. [7].

Artemisinin (**1**) a structurally unique sesquiterpene peroxide obtained from the indigenous herb *Artemisia annua* was introduced for treatment of malarial by the Chinese in the 1970s [8, 9]. The isolation of artemisinin, its characterization as a peroxide and preparation of its

derivatives such as dihydroartemisinin (**2**), artemether (**3**) and artesunate (**4**) (**2**-**4**, Figure 1), by Chinese scientists, collectively represents one of the great events in medicine of the latter half of the 20th century [8]; the timing of its introduction associated with the demise of chloroquine was particularly fortuitous. Artemisinins are highly potent, well tolerated in all phases of the disease and act more rapidly than any other antimalarial drug [10]. However, they suffer from drawbacks such as low solubility, thermal instability, and short pharmacological half-lives. These properties result in incomplete parasite clearance leading to recrudescence, that may contribute to the development of parasite resistance [11, 12].

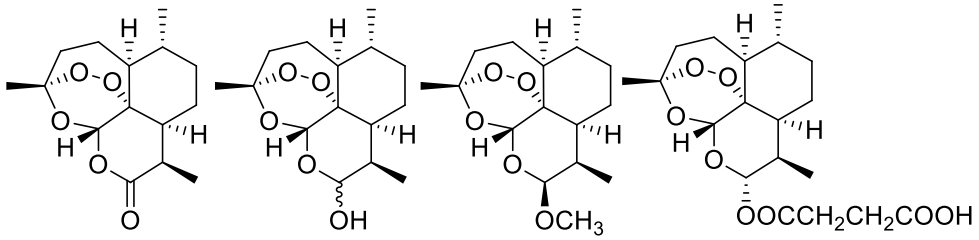
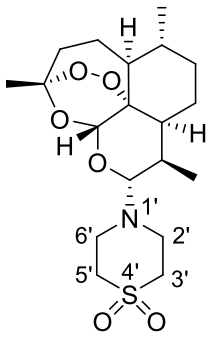


Figure 1: Artemisinin (**1**), dihydroartemisinin (**2**), artemether (**3**) and artesunate (**4**)

To protect artemisinins from resistance, the WHO recommends the use of these drugs in combination with a longer half-life drug [13, 14]. Thus, artemisinin-based combination therapy (ACT) for the treatment of uncomplicated malaria were introduced and adopted in almost all CQ-resistant endemic regions [3]. ACT combines an artemisinin derivative with a longer half-life antimalarial, either a quinoline or an arylmethanol [9]. The rationale behind ACT use is that the short-acting but highly potent artemisinin derivative induces a rapid reduction in parasite biomass [15] and the slower acting and more slowly eliminated drug partner continues the clearance when the artemisinin concentration has fallen to sub-therapeutic levels [16, 17].

Because of the poor solubility of artemisinin in both oil and water, this was structurally modified to give the the oil soluble derivative artemether **3**, and the water soluble artesunate **4** [9]. Thus, all artemisinin derivatives in clinical use are structurally either alkyl acetals or an ester acetal [18]. Solubility aside, another limitation with these compounds, however, is their short pharmacological half-lives [19], a reflection of their acid lability and facile metabolism to dihydroartemisinin (DHA), their active metabolite that is associated with neurotoxicity in laboratory animals [20]. Additionally, increased tolerance to ACTs by the parasites leading to resistance has now been confirmed in South East Asia [3, 18, 21].

Initiatives to counter artemisinin induced neurotoxicity by Medicines for Malaria Venture (MMV) resulted in the development of artemisone **5** (Figure 2), a second generation semi-synthetic derivative that has no measurable neurotoxicity both *in vitro* and *in vivo* [22]. It has a longer half-life (3.1 h) than the other derivatives, artesunate (~50 min), artemether (1.3 h) and DHA (~ 45 min) [22]. The measured Log *P* value of 2.49 of artemisone is also lower than those of artesunate (2.77), artemether (3.98), and DHA (~2.6) [23]. Furthermore, artemisone shows superior activity to artesunate against both CQ-resistant and -sensitive *P. falciparum* strains. It has substantially greater *in vivo* activity in rodent *P. berghei* and *P. falciparum* in primate model [24] making it a drug-like compound. It has an aqueous solubility of 89 mg/L which exceeds the limiting requirement for a drug to permeate across biological membranes [23].



5

Figure 2: Artemisone (**5**): the 4'-sulfone group renders the methylene protons at C3' and C5' acidic (pKa ~ 29-32).

Structural modifications by substituting selected lipophilic groups on the 10-alkylamino side chain are likely to enhance artemisone"s lipophilicity and therefore its permeation through biological membranes. However, it is at the same time important to establish if the enhancement of lipophilicity will not also render the drug more neurotoxic and cytotoxic.

As part of our program focusing on the discovery of novel antimalarial compounds, and in light of the above considerations lipophilic derivatives of artemisone were prepared from artemisone and selected acyl or alkyl halides. The *in vitro* antimalarial activity against NF54, K1 and W2 and cytotoxicity against the WI-38 cell line of normal human fetal lung fibroblast of all synthesized compounds were determined.

2. Results

2.1. Chemistry

Artemisone (Figure. 2) contains a S,S-dioxothiomorpholino group attached to C-10 of the artemisinin nucleus. As such, the protons at C3' and C5' flanking the sulfone group are acidic, and should have an approximate pKa in the range of that of aliphatic sulfones, namely ~29-32 [25]. Thus, artemisone should be amenable to deprotonation with lithium *N*,*N*-diisopropyamide (LDA) (pKa of *N*,*N*-diisopropyamine 35.7). In the event, LDA was generated *in situ* in anhydrous tetrahydrofuran at -78 °C from *N*,*N*-diisopropyamine by treatment with n-butyllithium in the presence of the deprotonation endpoint indicator 2,2'-bipyridyl according to a standard technique [26]. Addition of artemisone was then carried out to generate the α-sulfonyl carbanion. The resulting solution was treated with the alkyl or acyl halide and after quenching isolated in the usual manner. The compounds **6**-**22** (Scheme 1) were thereby obtained in low to moderate yields (11 - 38%). The compounds were usually obtained as diastereomeric mixtures; attempts to separate the diastereomers by silica gel flash chromatography and recrystallisation were not successful, and the mixtures were characterized by spectroscopic methods. In general, two overlapping singlets due to H-12 appear in the ^{1}H spectra of the majority of compounds at 5.6-5.2 ppm, which is indicative of the presence of the two epimers designated A and B. The ratio of the integrals of these peaks corresponds to the ratio of each isomer in the mixture. Arbitrarily assigning isomer A to the highest integral and isomer B to lowest integral revealed all derivatives to contain predominantly isomer A in the 60-95% range (Supplementary information, Table 2). Compound **7** was the only pure derivative evidently consisting of a single compound. However, crystals were not suitable for X-Ray structural determination. Compound **6** is the dimethylated compound; with stereocentres at C-3" and C-5', one would expect four diastereoisomers. However, the ^{1}H NMR spectrum based on the signal due to H-12 revealed the presence of only two isomers.

2.2. In vitro antimalarial activity and cytotoxicity

The compounds were screened *in vitro*, alongside artemisone **5**, artemether **3**, and artesunate **4** against NF54, K1 and W2 asexual stages as well as early stage gametocytes of *P. falciparum* parasites. The IC_{50} values (concentrations resulting in 50% inhibition of parasite proliferation as measured fluorimetrically over 96 h) are as shown in Table 1.

With the exception of **13** and **14**, all the compounds were highly active, with IC_{50} values ranging from 0.42 - 5.9, 0.50 - 3.26 and 1 - 6.7 nM against NF54, K1 and W2 strains, respectively.

Compounds **6** - **11**, **17**, **19** and **21** were the most active against NF54, while compounds **6**, **7**, **9** and **20** were the most active against K1, in both cases with IC_{50} values of less than 1 nM. Compounds **7** - **9** were equipotent to artemisone, 2 - 4 times more active than artesunate against NF54 while being 3 to 6-fold more potent than artemether againt K1 parasites. Compounds **13** with IC_{50} values of 22, 34.54 and 32.1 nM against NF54, K1 and W2, respectively, was the second least active derivative while **14** was found inactive with IC_{50} higher than 500 nM. The exact IC_{50} of compound **14** against asexual parasites was not determined as it did not show complete inhibition of asexual NF54 *P. falciparum* parasites at 500 nM.

A similar observation was made with respect to the antiplasmodial activities of these compounds against the CQ-resistant W2 *P. falciparum* parasites. The most active compounds against NF54 (Excepted compounds **11**, **17** and **19**), conserved their activity against W2 with IC_{50} values of less than 1.8 nM. They were equipotent to artemether but displayed 4 – 6 times more activity than artesunate against W2.

Majority of the compounds were also very active against the early stage gametocytes. Compounds, **6** - **12**, **17** - **19**, **21** and **22** were 2-fold times more active than artesunate while possessing 5 - 6 times more activity than artemether. Screens at 1 μM concentration revealed the compounds to possess improved activity against early stage gametocytes as compared to the parent drug, artemisone.

The majority of the synthesized compounds had resistance indices values in the range of 0.4 – 1.5, implying that the derivatives were almost equally active against both CQR and CQS parasite strains.

None of the compounds was significantly cytotoxic as their experimental IC_{50} values against normal human fetal lung fibroblast (HFLF), WI-38 cell line were substantially higher (>100 μM) than that of the reference drug emetine (0.05 μM). Most of the compounds displayed selectivity towards intraerythrocytic *P. falciparum* parasites as compared to mammalian cells with selectivity indices greater than ~17000. Compounds **13** and **14** regarded as the least active in the series were also non-cytotoxic as can be seen from their high selectivity indices; 4500 and 200, respectively.

Table 1. IC_{50} values of compounds tested in vitro for antiplasmodial activity against asexual blood stages of NF54, K1 and W2 strains of Plasmodium falciparum and against sexual blood stages-EG (early gametoctyes) of NF54, cytotoxicity their cytotoxicity against WI-38 HFLF cells. Data are averaged from n=3 independent biological repeats, performed in triplicate ± SEM, Cells were incubated with compounds at various concentrations for 96 h; antimalarial activities and cytotoxicities were determined using SYBR Green I and SRB assays, respectively.

		Antiplasmodial activity, IC_{50} (nM)±SE				**Resistance Index**		**Cytotoxicity** IC_{50} (µM)	**Selectivity Index**
Cpd	**cLog P**[a]	**NF54**	**K1**	**W2**	**EG of NF54**	**RI**[b]	**RI**[c]	**WI-38 HFLF**[d]	**SI**[e]
Artemisone 5	2.94± 0.74	0.57±0.01	0.80±0.50	0.37±0.11	nd	1.4	0.6	nd	nd
6	4.35±0.90	0.98±0.24	0.59±0.05	1.20±0.33	16.6±0.90	0.6	1.2	>100	>102040
7	4.92±0.90	0.49±0.11	0.94±0.13	1.30±0.40	11.47±2.65	1.9	2.7	>100	>204080
8	3.56±0.93	0.46±0.10	1.04±0.11	1.00±0.27	13.62±2.6	2.2	2.2	>100	>217390
9	4.09±0.93	0.42±0.11	0.98±0.10	1.10±0.26	19.31±1.23	2.3	2.6	>100	>238090
10	4.62±0.93	1.03±0.12	1.04±0.06	1.80±0.40	10.59±2.97	1.0	1.7	>100	>97080
11	5.68±0.93	0.83±0.07	2.30±0.70	2.90±0.50	0.1195*	2.8	3.5	>100	>120480
12	6.75±0.93	1.42±0.22	2.10±0.50	3.70±1.00	18.4±4.80	1.5	2.6	>100	>70420
13	8.87±0.93	22.00±3.06	34.54±3.1	32.10±6.7	105*	1.6	1.5	>100	>4500
14	11.00±0.93	>500	>500	>500	2.959*	nd	nd	>100	>200
15	6.44±0.95	5.90±2.00	7.3±1	20.67±4.0	nd	1.2	3.5	>100	>16950
16	3.39±1.08	2.53±0.29	1.11±0.14	4.70±10	nd	0.4	1.9	>100	>39520
17	4.82±0.98	0.72±0.13	1.16±0.11	2.71±0.14	17.01±2.64	1.6	3.8	>100	>136980
18	4.08±0.98	1.00±0.4	1.21±0.10	2.10±0.50	12.4±3.90	1.2	2.1	>100	>100000
19	5.28±0.98	0.73±0.06	1.47±0.55	2.33±0.31	13.89±2.47	2.0	3.2	>100	>136980
20	5.23±0.94	1.10±0.05	0.5±0.04	2.00±0.40	26.6±3.40	0.5	1.8	>100	>90910
21	5.30±0.94	0.83±0.18	1.10±0.10	1.69±0.26	11.83±5.65	1.3	2.0	>100	>120480
22	5.31±0.97	1.30±0.22	1.76±0.30	2.56±0.25	16.1±4.70	1.4	2	>100	>76920
Artemether 3	3.07± 0.68	3.00±0.29	3.26±0.20	2.40±0.20	37.74±2.08	1.1	0.8	nd	nd
Artesunate 4	3.37±0.90	1.86±0.17	2.64±0.18	6.70±0.60	62.83±3.41	1.4	3.6	>100	>12690
Emetine								0.05	

* data represents a single biological repeat (n=1); nd (not determined); [a]calculated using ACD/Chemsketch v 14.02; [b]Resistance Index (RI) = IC_{50}K1/IC_{50}NF54; [c]Resistance Index (RI) = IC_{50}W2/IC_{50}NF54; [d]WI-38 cell line of normal human fetal lung fibroblast; [e]Selectivity Index (SI) = IC_{50}WI-38/IC_{50}NF54.; Cpd (Compound) .

3. Discussion

3.1 *In vitro* antimalarial activities and cytotoxicities

The *P. falciparum* NF54 strain is susceptible to all known antimalarials; with the highest gametocytemia obtained in cell cultures, NF54 has often been used to evaluate *in vitro* gametocytocidal activity of potential antimalarial compounds [27]. *P. falciparum* K1 strain on the other hand, is a resistant strain from Thailand; it has shown resistance against the antimalarial drugs chloroquine and pyrimethamine [28]. However, the Indochina/Laos *P. falciparum* W2 strain is a multi-drug resistant strain known to be the most resistant of all *P. falciparum* strains and has shown resistance against most antimalarial drugs including chloroquine, cycloguanil, pyrimethamine and sulfadoxine with the exception of artemisinins [29, 30]. The three strains were used in the determination of *in vitro* antiplasmodial activities of the target compounds.

Artemisone, a potently active semisynthetic 10-alkylaminoartemisinin has an aqueous solubility of 89 mg/L and is a relatively polar compound (measured Log P value of 2.49), which compromises its ability to cross biological membranes and hence its clinical utility [23]. The target compounds were designed to be more lipophilic than the parent compound but yet expected to retain good activity.

With the exception of the highly lipophilic compounds **13** (cLog *P* 8.87) and **14** (cLog *P* 11), all the compounds in the series were active at low nanomolar concentrations, namely, IC_{50}<10 nM and IC_{50}<30 nM against asexual parasites and early stage gametocytes, respectively. The compounds with the exception of **13**-**15** were even more active against both NF54 (sensitive) and K1 (resistant) *P. falciparum* strains than the clinically used drugs artesunate and artemether.

None of the target compounds outperformed the parent drug artemisone against the W2 strain. However, derivatives, **7**-**9** were more active against NF54 while **6** and **20** outperformed against the K1 strain.

Compound **14**, which happened to be the most lipophilic derivative with a cLog *P* of 11.0, did not show any inhibition of parasite growth even at doses as high as 500 nM, indicative of complete loss of activity of the artemisone moiety in this derivative. Compounds, **15** (cLog *P*

6.44) bearing the lipophilic adamantane ring and **13** (cLog *P* 8.87) were also among the least active compounds in the series. However, the short chain acyl and alkyl mono and di-substituted derivatives **6** - **10** with cLog *P* < 5 and classified as less lipophilic, displayed overall higher potencies (IC_{50}<1.8 nM) than the more lipophilic compounds with longer chains, **12** - **14**. Thus, in this sub-series of acyl/alkyl derivatives, the antimalarial activity decreases as the chain length increases reaching the minimum measurable activities with derivative **13** (14 carbons chain) then inactivity with **14** (16 carbon chain).

All the aromatic/heterocyclic substituted derivatives, **17** - **22** were active with IC_{50} values of less than 2.7 nM regardless of the strains. In particular, compounds **17** and **19** bearing the thiophene ring and **21** featuring with the 4-methoxy- aryl substituent were the most active (IC_{50}<0.9 nM). No clear structure-activity relationship could be drawn from this sub-series.

Surprisingly, however, compound **12** with a cLog *P* value of 6.75 was more active than its less lipophilic counterpart namely **15** (cLog *P* 6.44) irrespective of the strain considered, although still less active than those lesser lipophilic (cLog *P* <5.5) in the series. Compound **16**, on the other hand, the most hydrophilic (lowest cLog *P* 3.4) of the series was also surprisingly less potent with IC_{50} values in the 2.5 -4.7 nM range against all the strains. This suggests that the antiplasmodial activities in the series were better with compounds displaying a balance between lipophilic and hydrophilic properties possessing cLog P values in the 3.6 – 5.3 range.

The compounds also showed impressive gametocytocidal activities. Compounds **6** - **10**, **12** and **17** - **22** with cLog *P* values in the range of 3.56 – 6.75, were approximately 2 - 4 and 3 - 6 times more active than the clinically used drugs artemether and artesunate against the early stage gametocytes, respectively. The general trend in the series however, was that the activity of the compounds decreased from NF54 to K1 to W2, which in accordance the latter as the most antimalarial drugs resistant among all *P. falciparum* strains currently available.

The resistance index (RI) of the compounds between the sensitive strain NF54 and the resistant K1 and W2 strains varied significantly with most of them having impressive RI (IC_{50} K1/IC_{50} NF54) of less than 1.5, making them equally active between the CQ sensitive and resistant parasites. In particular, artemisone derivatives, **6** and **16** had RI (IC_{50} K1/IC_{50} NF54) < 1, suggesting that these compounds were more active against K1 than NF54. Compound **6** was the only one with RI (IC_{50} W2/IC_{50} NF54) of less than 1.5 and therefore the only W2 non-cross resistant compound in the series. Overall, compound **6** was the most active in the series with impressive RIs against all three strains.

Furthermore, cytotoxicity screens using the WI-38 cell line revealed all derivatives regardless of their antimalarial efficacy to be non-cytotoxic. Analysis of the selectivity index (SI), which is indicative of a drug‟s ability to clear only parasitic cells and sparing healthy host cells, showed all the target compounds to be highly selective in their antiplasmodial effects, which is indicative of their high safety profiles. Subsequently, the observed antiplasmodial activity of each compound was therefore intrinsic.

Overall, on the basis of safety profile and efficacy against all the strains and more importantly against the early stage gametocytes, compounds **6** and **10** could be identified as the best candidates for further investigation in the search for new, effective and safe antimalarial drugs.

4. Conclusion

A series of novel lipophilic derivatives of artemisone was prepared in a single step involving the reaction of the α-sulfonyl carbanion of artemisone with acyl and alkyl chlorides. Isolated as epimeric mixtures, the derivatives were screened as such for antimalarial activities together with artemisone, artesunate, and artemether against the NF54, K1 and W2 strains of *P. falciparum* . The cytotoxicity was assessed using the HFLF WI-38 cell line. The majority of derivatives were active, non-cytotoxic and conserved the high antiplasmodial activity of the parent, artemisone against all strains despite the increased lipophilicity. In general, none of these compounds, however, was able to outperform artemisone. In comparison with the current clinically used drugs, artesunate and artemether, however, a different picture was seen. Not only were the most promising derivatives, **6** featuring 3‟‟,5‟‟-dimethyl substituent and **10** containing 3‟‟-(butan-1-one) chain, more active than both clinically drugs but also possessed outstanding early gametocytocidal activity than these drugs. These two derivatives may qualify as potential candidates for further investigation in the search for new and effective antimalarials. A prospective study may entail separation of the diastereomers by chiral HPLC chromatography and their full characterization/identification, and more importantly, assessment of their transmission blocking ability through determination of activities against late stage gametocytes, as well as liver stages.

5. Materials and methods

5.1. Materials

The reagents; *n*-butyllithium, diisopropylamine, 2,2"-bipyridyl, butoyl chloride, benzoyl chloride, propionyl chloride, acetyl chloride, 4-morpholinecabonyl chloride, 3-methylthiophene-2-carbonyl chloride, methoxylbenzoyl chloride, 2-thiophenecarbonyl chloride, adamantanecarbonyl chloride, 2- furoyl chloride, biphenyl-4-carbonyl chloride, benzofuran-2- carbonyl chloride, octanoyl chloride palmitoyl chloride, lauroyl chloride, octanoyl chloride, hexanoyl chloride and iodopropane were bought from Sigma-Aldrich and used without further purifications. Other reagents including; methyl iodide, acetyl chloride, and propionyl chloride were obtained from Unilab, Acros-Organics and Fluka Chemica, respectively, and were used without further purification. Artemisone (batch no: VRD-753-02-49) generously donated by Cipla (India) was recrystallized from ethyl acetate/hexane mixture prior to use. Analytical grade solvents, THF and DCM purified by distillation according to standard procedures were used.

5.2. General procedures

All ^{1}H, and ^{13}C NMR spectra of synthesized compounds were obtained on a 600 MHz Bruker Avance™ III spectrometers as solutions in deuterated solvents ($CDCl_3$). All chemical shifts (δ) are reported in parts per million (ppm) values. ^{1}H chemical shifts are reported downfield of tetramethylsilane (TMS), and were internally referenced to the residual proton in $CDCl_3$ (7.26 ppm), or DMSO-d_6 (2.50 ppm). ^{13}C chemical shifts were internally referenced to resonances in $CDCl_3$ (δ 77.16 ppm). Peak multiplicities are abbreviated as follows: s (singlet), d (doublet), dd (doublet of doublet), t (triplet), q (quartet), p (pentet), and m (multiplet). Coupling constant (*J*) are reported in Hz. NMR data were analysed using MestReNova Software, version 5.3.2-4936. Exact mass measurements were recorded on Bruker MicroTOFQII mass spectrometer with an APCI and ESI source set at 300 °C and 180 °C respectively, using Bruker Compass data analysis 4.0 software. A full scan, ranging between 50–3000 *m/z*, was generated at a capillary voltage of 4500 V, an end plate offset voltage of -500 V and a collision cell RF voltage of 100 Vpp for APCI and 150.0 Vpp for ESI.

Infrared (IR) spectra were recorded on a Bruker Alpha-P FTIR instrument and, melting points (mp) were determined with a BÜCHI melting point B-545 instrument and were uncorrected.

Thin layer chromatography (TLC) was performed using silica gel plates (60F254), acquired from Merck, and components were visualized by ultraviolet light (254 nm). Silica gel 230-400 (particle size 40-63µm) mesh was used for all flash column chromatography.

5.3. Biological evaluation

5.3.1. *In vitro* antimalarial assay

Malaria parasite proliferation can be directly monitored in their intra-erythrocytic environment through detecting and monitoring DNA replication (without background forthcoming from erythrocytes, which lack DNA) [31]. SYBR Green I is a fluorescent dye that interacts with DNA, therefore a correlation between DNA content (SYBR Green I signal) and parasitaemia can be used to monitor decrease in parasitaemia as a measurement of inhibition of parasite proliferation [31, 32].

5.3.1.1. Anti-plasmodial assay against erythrocyte stages of *P. falciparum* parasites (asexual parasites)

P. falciparum parasites were maintained at 37°C in human erythrocytes (O+) suspended in complete culture medium [RPMI 1640 medium (Sigma-Aldrich) supplemented with 25 mM HEPES (Sigma-Aldrich), 20 mM D-glucose (Sigma-Aldrich), 200 µM hypoxanthine (Sigma-Aldrich), 0.2% sodium bicarbonate, 24 µg/ml Gentamycin (Sigma-Aldrich) and 0.5% AlbuMAX II] in a gaseous environment of 90% N_2, 5% O_2, and 5% CO_2 as described by Verlinden et al.[33] *In vitro* ring-stage intra-erythrocytic *P. falciparum* parasite cultures (genotyped drug sensitive/resistant strains; K1 (chloroquine, pyrimethamine, mefloquine and cycloguanil resistant) and NF54 (drug sensitive)) (200 µl at 1% haematocrit, 1% parasitaemia) were treated with the compounds.

The controls for this assay included chloroquine disulphate (1 µM, as positive control) and complete RPMI media (as negative control) and grown for 96 h at 37°C under the 90% N_2, 5% O_2, and 5% CO_2 gas mixture in 96-well plates. At the conclusion of the 96 h growth period, equal volumes (100 µl each) of the *P. falciparum* parasite cultures were combined with SYBR Green I lysis buffer (0.2 µl/ml 10 000xSYBR Green I, Invitrogen; 20 mM Tris, pH 7.5; 5 mM EDTA; 0.008% (w/v) saponin; 0.08% (v/v) Triton X-100). The samples were incubated at 37°C for 1 h after which the fluorescence was measured using a Fluoroskan

Ascent FL microplate fluorometer (Thermo Scientific, excitation at 485 nm and emission at 538 nm).

The „background" fluorescence (i.e. that measured in the samples derived from chloroquine-treated iRBC samples in which parasite proliferation was completely inhibited) was subtracted from the total fluorescence measured for each sample to provide a measure of parasite proliferation. Data obtained were analysed in Excel 2007 and sigmoidal dose-response curves were plotted using GraphPad 5.0. Experiments were performed in triplicate, and repeated mainly n=3 times.

The compounds were screened at 5 μM and 1 μM against the NF54 strain of asexual *P. falciparum* parasites to determine their % inhibition. Compounds showing near complete inhibition at these concentrations were further screened at 500 and 100 nM against NF54 parasites to determine the most active compounds in the series.

Compounds were weighed (1 mg) and dissolved in 100% DMSO and made up to a 10 mM stock and stored at 4°C; which were then incubated at room temperature (~1 hrs) prior to an assay to prepare working solutions. Compounds **12**, **14**, **15**, **16** and **22** precipitated out of solution after being stored at 4°C, and were incubated at 37°C for ~1 h prior to the assay.

The final starting concentrations (5, 1 μM and 500, 100 nM) were prepared in complete RPMI-1640 media and aliquoted in triplicate into a 96-well plate. The %DMSO in the starting concentration was 0.05% for the 5 μM, 0.000125% for the 1 μM, 0.05% for the 500 nM and 0.00000125% for the 100 nM drug concentrations, below the parasite inhibition threshold as previously determined in the laboratory. Compounds in solution were only used for up to 3 weeks, as a marked decrease in compound activity was observed following longer periods of storage.

Following dual point assays, compounds were subjected to *in vitro* IC_{50} determinations against asexual *P. falciparum* NF54, K1 and W2 parasites. All compounds in the series showed activity >100 nM and therefore, the respective IC_{50} values for the whole series was determined. The same compound dissolving and storage conditions were applied as described for the dual point assays.

All assays were performed in triplicate for at least three independent biological repeats.

5.3.1.2. Antiplasmodial activity determination against early stage *P. falciparum* gametocytes

The compounds were also screened for *in vitro* gametocytocidal activity against early stage *P. falciparum* gametocytes using dual point assays and dose-response curves for IC_{50} determination. The compounds were weighed, prepared and screened at three concentrations; 5 µM, 1 µM, 100 nM and 10 nM to determine their % inhibition as a decrease in *P. falciparum* gametocyte viability. Based on the % inhibition activities derived from dual point assay the starting concentrations for each independent compound was calculated to produce a 9-point dose-response curve in order to obtain the IC_{50} for each compound. Gametocytes were produced as per Reader *et al*.[34] Inhibition of gametocyte cell viability was assayed with a Luciferase reporter gene assay, using the luciferase reporter cell lines, early [NF54-PfS-GFP-Luc (EG16)] stage promoter, which accurately quantifies and distinguishes the early (Stage I/II) gametocidal activity of a compound. The working concentrations of the compounds were prepared in complete RPMI-1640 medium and transferred into a 96-well plate. Early stage (>85% stage I and II distribution, no stages later than III was detected) gametocytes at 2% parasitaemia and 2% haematocrit were transferred into a 96-well plate. An untreated gametocyte control was included and a 5 µM Methylene Blue positive control was included. Plates were incubated for 48 h in a gaseous environment of 90% N_2, 5% O_2, and 5% CO_2 at 37ºC. Following incubation cells were lysed at room temperature using 3x luciferin lysis buffer (15 M DTT, 0.6 mM CoA, 0.45 mM ATP, 0.42 mg/ml luciferin, 10 mM $MgCl_2$, 10 mM Tris Base, 10 mM Tris-HCl and 0.03% Triton X-100). Luciferase activity was measured at 560 nm in a Glomax luminescence plate reader (Promega). Following subtraction of the background readings luciferase counts for the untreated controls were normalised against the average of the triplicate values to indicate % cell viability. Data obtained were analysed in Excel 2007 and sigmoidal dose-response curves were plotted using GraphPad 5.0. Experiments were performed in triplicate, and repeated for three independent gametocyte populations n=3 times.

5.3.2. *In vitro* cytotoxicity assay

The cytotoxic effects of the compounds were tested by sulforhodamine B (SRB) assay on the WI-38 cell line. 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] The SRB Assay was performed at CSIR in accordance with the protocol of the Drug Evaluation Branch and NCI, the assay was adopted for this screen.

The WI-38 cell line - normal human fetal lung fibroblast from ECACC was routinely maintained as a monolayer cell culture at 37ºC, 5% CO_2, 95% air and 100% relative humidity in minimum essential medium (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 additions served as control. The blank contained 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 10mM 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.

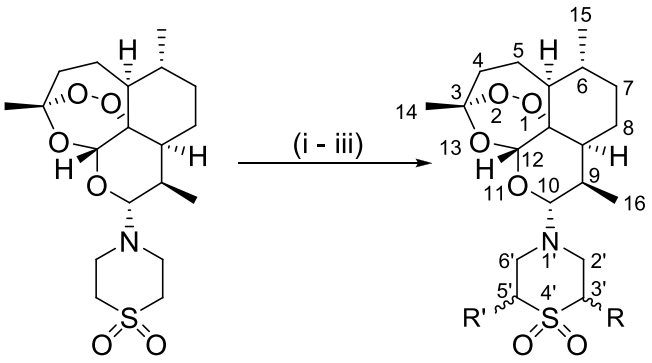
5.4. Synthesis

5.4.1. General procedure

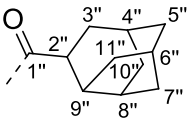
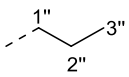
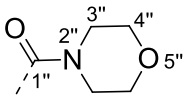
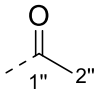
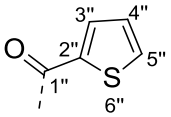
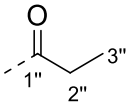
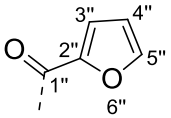
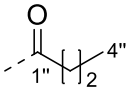
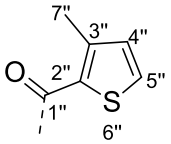
In nitrogen supplied reaction flask containing 10 ml of THF, was successively added diisopropylamine (701 µl, 5 mmol, 2 eq.), n-butyllithium (2.5 ml, 5 mmol, 2 eq.) and 2,2" bipyridyl (4 mg, 0.025 mmol, 0.01eq.) and the resulting mixture stirred at -78 ºC for 30 min. Artemisone (1 g, 2.5 mmol, 1 eq.) was dissolved in 5 ml of THF and introduced in the reaction flask. , The reaction mixture allowed to stir at -78ºC for another 90 min or more until the mixture turned red (indicative of total deprotonation of artemisone).

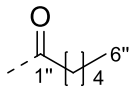
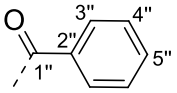
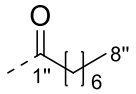
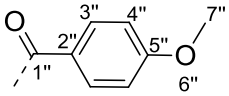
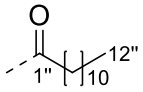
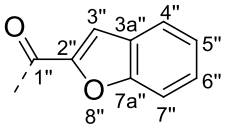
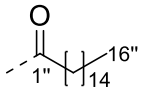
The acyl or alkyl chloride (1.5 eq.) was added into the above reaction mixture then stirring was continued for another for 20 h whilst allowing the temperature to rise from -78 °C to room temperature. The reaction mixture was then quenched with a saturated solution of

ammonium chloride (40 mL), washed twice with saturated $NaHCO_3$ solution (40 mL) and water (40 mL). The combined extracts were dried over $MgSO_4$, filtered and the filtrate evaporated under reduced pressure to give a crude pale yellow oil, which was then purified by column chromatography with ethyl acetate:hexane (3:7, v/v) as eluent.



Scheme 1: Multi-stage single step synthesis of compounds **6 - 22.**
Reagents and conditions: (i). *n*-Bu-Li, diisopropylamine, 2,2"-bipyridyl, dry THF, -78 °C, 30 min, (ii) artemisone in THF, -78 °C, 90 min, (iii) Acyl or alkyl chloride, -78 °C - rt, 20 h.

Comp	R	R'	%Yield	Comp	R	R'	%Yield
6	CH_3	CH_3	11	**15**		H	18
7		H	18	**16**		H	11
8		H	25	**17**		H	17
9		H	23	**18**		H	16
10		H	19	**19**		H	15

11		H	35	**20**		H	16
12		H	38	**21**		H	18
13		H	38	**22**		H	11
14		H	24				

5.4.1. 3',5'-dimethyl-4-((3R,5aS,6R,9R,10R,12R,12aR)-3,6,9-trimethyldecahydro-3H-3,12-epoxy[1,2]dioxepino[4,3-i]isochromen-10-yl)thiomorpholine 1,1-dioxide, **6.** Yield: 12%; mp: 165.1°C; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 5.26 (s, 1H, H-12), 4.14 (d, *J* = 10.2 Hz, 1H, H-10), 3.54 (m, 2H, H-3", H-5"), 3.19 – 3.03 (m, 4H, H-2", H-6"), 2.59 – 2.48 (m, 1H, H-9), 2.38 – 2.26 (m, 1H, H-5a), 1.98 (ddd, *J* = 14.6, 5.0, 3.0 Hz, 1H, H-8a), 1.69 (dddd, *J* = 14.4, 9.9, 7.8, 4.1 Hz, 2H, H-4), 1.54 (m, 6H, H-5, H-7, H-8), 1.49 – 1.16 (m, 10H, H-1'", H-2", H-14, H-6), 1.08 (d, *J* =11.5 3H, H-16), 0.74 (m,3H, H-15); ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 104.19 (C-3), 91.82 (C-10), 91.07 (C-12), 80.12 (C-12a), 76.99 (C-5a), 76.78 (C-2"), 55.81 (C-3"), 51.42 (C-5"), 45.61 (C-6"), 37.42 (C-8a), 36.15 (C-4), 34.17 (C-9), 31.55 (C-7), 28.95 (C-6), 25.89 (C-14), 24.76 (C-8), 21.57 (C-15), 20.21 (C-5), 13.49 (C-16), 7.86 (C-2'"), 7.68 (C-1'"); IR (ATR) ν_{max}/cm^{-1}: 2928, 1452, 1376, 1308, 1256, 1127, 1036, 976; HRMS- APCI *m/z* $[M+H]^+$430.2300 (calcd for $C_{21}H_{35}NO_6S$: 430.2385).

5.4.2. 3'-propyl-4-((3R,5aS,6R,9R,10R,12R,12aR)-3,6,9-trimethyldecahydro-3H-3,12-epoxy[1,2]dioxepino[4,3-i]isochromen-10-yl)thiomorpholine 1,1-dioxide, **7.** Yield: 20%; mp: 139.4°C; ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 5.26 (s, 1H, H-12), 4.17 (d, *J* = 10.3 Hz, 1H, H-10), 3.46 – 3.28 (m, 6H, H-2", H-5", H-6"), 2.91 – 2.85 (m, 1H, H-3"), 2.54 (dqd, *J* = 14.4, 7.1, 4.4 Hz, 1H, H-9), 2.38 – 2.28 (m, 1H, H-8a), 2.04 – 1.93 (m, 2H, H-4), 1.85 (ddt, *J* = 13.8, 6.8, 3.6 Hz, 1H, H-5a), 1.70 (ddq, *J* = 13.0, 9.8, 3.5 Hz, 2H, H-5), 1.61 – 1.15 (m, 12H, H-6, H-7, H-8, H-14, H-1'", H-2'"), 1.06 – 0.82 (m, 6H, H-15, and H-3"), 0.77 (d, *J* = 7.1 Hz, 3H, H-16); ^{13}C NMR (151 MHz, $CDCl_3$) δ ppm 105 (C-3), 91.91 (C-12), 91.03 (C-10), 80.09 (C-12a), 76.78 (C-3"), 60.40 (C-5a), 51.71 (C-5"), 51.44 (C-2"), 45.62 (C-8a), 37.43 (C-6"), 36.15 (C-4) , 34.17 (C-9), 29.02 (C-7), 25.88 (C-1"), 25.38 (C-6), 24.78 (C-14), 21.59 (C-8), 20.16 (C-15), 17.96 (C-2"), 15.71 (C-5), 14.00 (C-16), 13.45 (C-3"); IR (ATR) ν_{max}/cm^{-1}: 2929,

1450, 1390, 1376, 1308, 1250, 1207, 1187, 1011, 976; HRMS-ESI *m/z* $[M+H]^+$444.2404 (calcd for $C_{22}H_{37}NO_6S$: 444.2398).

5.4.3. 1-(1,1-dioxido-4-((3R,5aS,6R,9R,10R,12R,12aR)-3,6,9-trimethyldecahydro-3H-3,12-epoxy[1,2]dioxepino[4,3-i]isochromen-10-yl)thiomorpholin-3'-yl)ethanone, **8.** Yield: 28.5%; mp 146.8-174.5°C; ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 5.28 (s, 1H, H-12), 4.97 (d, *J* = 10.7 Hz, 1H, H-10), 4.25 (t, *J* = 10.4 Hz, 1H, H-3''), 3.78 – 3.65 (m, 2H, H-2''), 3.63 – 3.50 (m, 2H, H-5''), 3.41 – 3.32 (m, 2H, H-6''), 2.57 – 2.44 (m, 1H, H-9), 2.43 (s, 3H, H-2'''), 2.37 – 2.29 (m, 1H, H-5a), 2.00 (ddd, *J* = 14.5, 5.0, 2.8 Hz, 1H,H-8a), 1.86 (ddt, *J* = 13.8, 6.9, 3.6 Hz, 1H, H-8), 1.70 (tt, *J* = 9.0, 3.6 Hz, 2H, H-4), 1.49 – 1.38 (m, 2H, H-7), 1.30 (s, 3H, H-14), 1.25 – 1.0 (m, 4H, H-5, H-6), 0.91 (d, 3H, H-15) 0.76 (d, *J* = 7.1 Hz, 3H, H-16); ^{13}C NMR (151 MHz, $CDCl_3$) δ ppm 198.17 (C-1''), 104.37 (C-3), 92.16 (C-10), 90.66 (C-12), 80.26 (C-12a), 77.00 (C-3''), 76.78 (C-5a), 69.91 (C-5''), 52.80 (C-2''), 51.19 (C-8a), 45.45 (C-6''), 37.48 (C-4), 36.06 (C-9), 34.11 (C-7), 32.99 (C-2'''), 28.81 (C-6), 25.85 (C-14), 24.81 (C-8), 21.52 (C-15), 20.17 (C-5), 13.24 (C-16); IR (ATR) ν_{max}/cm^{-1}: 2926, 1712, 1360, 1313, 1126, 1036, 979, 934,875. HRMS-ESI *m/z* $[M+H]^+$ 444. 2020 (calcd for $C_{21}H_{33}NO_7S$: 444.1977).

5.4.4. 1-(1,1-dioxido-4-((3R,5aS,6R,9R,10R,12R,12aR)-3,6,9-trimethyldecahydro-3H-3,12-epoxy[1,2]dioxepino[4,3-i]isochromen-10-yl)thiomorpholin-3'-yl)propan-1-one, **9.** Yield: 26%; mp: 144.2-168.1°C; ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 5.26 (s, 1H, H-12), 4.95 (d, *J* = *12.1,* 1H, H-10), 4.22 (dd, *J* = 25.2, 10.4 Hz, 1H, H-3''), 3.81 – 3.73 (m, 2H, H-2''), 3.58 (dddd, *J* = 19.1, 15.6, 9.2, 6.4 Hz, 4H, H-5'',H-6''), 3.45 – 3.32 (m, 4H, H-2'', H-9, H-5a), 2.97 – 2.80 (m, 5H, H-4,H-5,H-8a), 2.69 (ddq, *J* = 53.2, 19.1, 7.1 Hz, 2H, H-8), 2.59 – 2.46 (m, 2H, H-7), 2.23 (s, 3H, H-14), 2.03 – 1.95 (m, 4H, H-6, H-3'''), 1.86 (d, *J* = 13.7 Hz, 3H, H-16), 1.12 (d, 3H, H-15); ^{13}C NMR (151 MHz, $CDCl_3$) δ ppm 200.82 (C-1''), 104.36 (C-3), 92.17 (C-10), 90.67 (C-12), 80.28 (C-12a), 76.78 (C-3''), 69.07 (C-5a), 52.66 (C-5''), 51.25 (C-2''), 45.49 (C-8a), 39.34 (C-6''), 37.49 (C-4), 36.07 (C-9), 34.11 (C-7), 28.92 (C-2'''), 25.89 (C-6), 24.81 (C-14), 21.54 (C-8), 20.16 (C-15), 13.27 (C-5), 7.21 (C-16), 4.9 (C-3''); IR (ATR) ν_{max}/cm^{-1}: 2934, 1716, 1444, 1356, 1308, 1272, 1212, 1126, 1021, 956; HRMS-ESI *m/z* $[M+H]^+$ 458.2192 (calcd for $C_{22}H_{35}NO_7S$: 458.2134).

5.4.5. 1-(1,1-dioxido-4-((3R,5aS,6R,9R,10R,12R,12aR)-3,6,9-trimethyldecahydro-3H-3,12-epoxy[1,2]dioxepino[4,3-i]isochromen-10-yl)thiomorpholin-3'-yl)butan-1-one, **10.** Yield: 22%; mp: 132.3-147.6°C; ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 5.28 (s, 1H, H-12), 4.24 (d, *J* = 10.4 Hz, 1H, H-10), 3.76 (d, *J* = 13.9 Hz, 1H,H-3''), 3.55 (ddd, *J* = 15.5, 12.4, 2.9 Hz, 2H, H-2''), 3.45 – 3.32 (m, 2H, H-5''), 2.92 – 2.78 (m, 2H, H-6''), 2.64 (ddd, *J* = 18.1, 8.1, 6.2 Hz, 1H, H-

9), 2.51 (dqd, *J* = 14.4, 7.1, 4.4 Hz, 1H, H-6), 2.34 (ddd, *J* = 14.7, 13.5, 4.0 Hz, 1H, H-8a), 2.00 (ddd, *J* = 14.6, 4.9, 2.7 Hz, 1H, H-5a), 1.75 (s, 3H, H-14), 1.50 – 1.36 (m, 4H, H-4,H-5), 1.36 – 1.18 (m, 4H,H-2‴,H-3‴), 1.04 – 0.98 (m, 7H, H-7,H-8,H-4‴), 0.85 (d, *J* =10.5 3H, H-15), 0.76 (d, *J* = 7.1 Hz, 3H, H-16); ^{13}C NMR (151 MHz, $CDCl_3$) δ ppm 200.43 (C-1‴),104.38 (C-3), 92.16 (C-10), 90.74 (C-12), 80.27 (C-12a), 76.78 (C-3′), 69.23 (C-5a), 52.69 (C-5′), 51.24 (C-2′), 47.81 (C-8a), 45.49 (C-6′), 37.50 (C-2‴), 36.09 (C-4), 34.12 (C-9), 28.88 (C-7), 25.91 (C-6), 24.82 (C-14), 21.56 (C-8), 20.18 (C-5), 16.60 (C-15), 13.46 (C-3‴), 13.30 (C-4‴), 13.26 (C-16); IR (ATR) ν_{max}/cm^{-1}: 2934, 1713, 1444, 1307, 1269, 1207, 1042, 1022, 981; HRMS- APCI *m/z* $[M+H]^+$ 472.2275 (calcd for $C_{23}H_{37}NO_7S$: 472.2290).

5.4.6. 1-(1,1-dioxido-4-((3R,5aS,6R,9R,10R,12R,12aR)-3,6,9-trimethyldecahydro-3H-3,12-epoxy[1,2]dioxepino[4,3-i]isochromen-10-yl)thiomorpholin-3'-yl)hexan-1-one, **11.** Yield: 44%; mp:117°C; ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 5.28 (s, 1H, H-12), 4.24 (d, *J* = 10.4 Hz, 1H, H-10), 3.75 (m, 1H, H-3′), 3.44 – 3.31 (m, 6H, H-2′, H-5′, H-6′), 2.91 – 2.78 (m, 2H, H-2‴), 2.38 – 2.29 (m, 1H, H-5a), 2.04 – 1.96 (m, 12H, H-4, H-5, H-6, H-7, H-8, H-9, H-3‴), 1.70 (s, 3H, H-14), 1.64 – 1.50 (m, 4H, H-4‴, H-5‴), 1.02 – 0.95 (m, 1H, H-8a), 0.93 (d, *J* = 6.3 Hz, 3H, H-16), 0.85 (t, *J* = 6.9 Hz, 3H, H-6‴), 0.76 (d, *J* = 7.1 Hz, 3H, H-15); ^{13}C NMR (151 MHz, $CDCl_3$) δ ppm 104.38 (C-3), 92.19 (C-10), 90.72 (C-12), 80.26 (C-12a), 76.78 (C-3′), 69.24 (C-5a), 52.72 (C-5′), 51.24 (C-2′), 46.03 (C-8a), 45.49 (C-6′), 37.50 (C-2‴), 36.09 (C-4), 34.13 (C-9), 33.87 (C-7), 31.88 (C-6), 29.50 (C-5‴), 25.93 (C-3‴), 24.83 (C-14), 23.07 (C-8), 22.66 (C-5), 21.56 (C-15), 20.18 (C-4‴), 14.10 (C-16), 13.26 (C-6‴); IR (ATR) ν_{max}/cm^{-1}: 2928, 1713, 1451, 1375, 1308, 1131, 1041,978, 940, 876; HRMS-ESI *m/z* $[M+H]^+$ 500.2661 (calcd for $C_{25}H_{42}NO_7S$ 500.2635).

5.4.7. 1-(1,1-dioxido-4-((3R,5aS,6R,9R,10R,12R,12aR)-3,6,9-trimethyldecahydro-3H-3,12-epoxy[1,2]dioxepino[4,3-i]isochromen-10-yl)thiomorpholin-3'-yl)octan-1-one, **12.** Yield: 50%; mp: 129.1°C; ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 5.28 (s, 1H, H-12), 4.24 (d, *J* = 10.4 Hz, 1H, H-10), 3.75 (m, 1H, H-3′), 3.44 – 3.31 (m, 6H, H-2, H-5, H-6), 2.91 – 2.78 (m, 2H, H-2‴), 2.38 – 2.29 (m, 1H, H-5a), 2.04 – 1.96 (m, 15H, H-4, H-5, H-6, H-7, H-8, H-9, H-3‴), 1.70 (s, 1H, H-14), 1.64 – 1.50 (m, 8H, H-4‴, H-7‴, H-5‴, H-6‴), 1.02 – 0.95 (m, 1H, H-8a), 0.93 (d, *J* = 6.3 Hz, 3H, H-16), 0.85 (t, *J* = 6.9 Hz, 3H, H-8‴), 0.76 (d, *J* = 7.1 Hz, 3H, H-15); ^{13}C NMR (151 MHz, $CDCl_3$) δ ppm 104.38 (C-3), 92.17 (C-10), 90.72 (C-12), 80.26 (C-12a), 76.78 (C-3′), 69.24 (C-5a), 52.72 (C-5′), 51.24 (C-2′), 46.03 (C-8a), 45.49 (C-6′), 37.50 (C-2‴), 36.09 (C-4), 34.13 (C-9), 33.87 (C-7), 31.88 (C-6), 29.50 (C-5‴), 29.41 (C-6‴), 29.10 (C-7‴), 25.93 (C-3‴), 24.83 (C-14), 23.07 (C-8), 22.66 (C-5), 21.56 (C-15), 20.18 (C-4‴), 14.10 (C-16),

13.26 (C-8‴); IR (ATR) ν_{max}/cm^{-1}: 2927, 1715, 1447, 1377, 1309, 1132, 1039,938, 874; HRMS-ESI *m/z* $[M+H]^+$ 528.2999 (calcd for $C_{27}H_{45}NO_7S$: 528. 2987).

5.4.8. 1-(1,1-dioxido-4-((3R,5aS,6R,9R,10R,12R,12aR)-3,6,9-trimethyldecahydro-3H-3,12-epoxy[1,2]dioxepino[4,3-i]isochromen-10-yl)thiomorpholin-3'-yl)dodecan-1-one, **13.** Yield: 55%; mp: 122.8°C; ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 5.28 (s, 1H, H-12), 4.24 (d, *J* = 10.4 Hz, 1H, H-10), 3.75 (m, 1H, H-3‴), 3.44 – 3.31 (m, 6H, H-2‴, H-5‴, H-6‴), 2.91 – 2.78 (m, 2H, H-2'''), 2.38 – 2.29 (m, 1H, H-5a), 2.04 – 1.96 (m, 12H, H-4, H-5, H-6, H-7, H-8, H-9, H-3'''), 1.70 (s, 3H, H-14), 1.64 – 1.50 (m, 16H, H-10‴, H-4‴, H-11‴, H-8‴, H-7‴, H-5‴, H-6‴, H-9‴), 1.02 – 0.95 (m, 1H, H-8a), 0.93 (d, *J* = 6.3 Hz, 3H, H-16), 0.85 (t, *J* = 6.9 Hz, 3H, H-12‴), 0.76 (d, *J* = 7.1 Hz, 3H, H-15); ^{13}C NMR (151 MHz, $CDCl_3$) δ ppm 200.08 (C-1‴), 104.38 (C-3), 92.17 (C-10), 90.72 (C-12), 80.26 (C-12a), 76.78 (C-3‴), 69.24 (C-5a), 52.72 (C-5‴), 51.24 (C-2‴), 46.03 (C-8a), 45.49 (C-6‴), 37.50 (C-2'''), 36.09 (C-4), 34.13 (C-9), 33.87 (C-7), 31.88 (C-6), 29.58 (C-10'''), 29.50 (C-5‴), 29.41 (C-6‴), 29.15 (C-9'''), 29.10 (C-7'''), 28.81 (C-8‴), 25.93 (C-3‴), 24.83 (C-14), 24.67 (C-11‴), 23.07 (C-8), 22.66 (C-5), 21.56 (C-15), 20.18 (C-4‴), 14.10 (C-16), 13.26 (C-12‴); IR (ATR) ν_{max}/cm^{-1}: 2918, 2850, 1713, 1446, 1375, 1306, 1132, 1040, 938, 874; HRMS-ESI *m/z* $[M+H]^+$ 584.3610 (calcd for $C_{31}H_{53}NO_7S$ 584.3601).

5.4.9. 1-(1,1-dioxido-4-((3R,5aS,6R,9R,10R,12R,12aR)-3,6,9-trimethyldecahydro-3H-3,12-epoxy[1,2]dioxepino[4,3-i]isochromen-10-yl)thiomorpholin-3'-yl)hexadecan-1-one, **14.** Yield: 40%; mp: 119.7°C; ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 5.28 (s, 1H, H-12), 4.24 (d, *J* = 10.4 Hz, 1H, H-10), 3.75 (m, 1H, H-3‴), 3.44 – 3.31 (m, 6H, H-2, H-5‴, H-6‴), 2.91 – 2.78 (m, 2H, H-2‴), 2.38 – 2.29 (m, 1H, H-5a), 2.04 – 1.96 (m, 15H, H-4, H-5, H-6, H-7, H-8, H-9, H-3‴), 1.70 (s, 1H, H-14), 1.64 – 1.50 (m, 24H, H-10‴, H-4‴, H-11‴, H-8‴, H-7‴, H-5‴, H-6‴, H-9‴,H-12‴, H-13‴, H-14‴, H-15‴), 1.02 – 0.95 (m, 1H, H-8a), 0.93 (d, *J* = 6.3 Hz, 3H, H-16), 0.85 (d, *J* = 6.9 Hz, 3H, H-16‴), 0.76 (d, *J* = 7.1 Hz, 3H, H-15); ^{13}C NMR (151 MHz, $CDCl_3$) δ ppm 178.68 (C-1‴), 104.38 (C-3), 92.17 (C-10), 90.72 (C-12), 80.26 (C-12a), 76.78 (C-3‴), 69.24 (C-5a), 52.72 (C-5‴), 51.24 (C-2‴), 46.03 (C-8a), 45.49 (C-6‴), 37.50 (C-2‴), 36.09 (C-4), 34.13 (C-9), 33.87 (C-7), 31.88 (C-6), 29.58 (C-4‴), 29.50 (C-5‴), 29.41 (C-6‴), 29.15 (C-9‴), 29.10 (C-7‴), 28.81 (C-8‴), 25.93 (C-3‴), 24.83 (C-14), 24.67 (C-11‴), 23.07 (C-8), 22.66 (C-5), 21.56 (C-15), 21.32 (C-12‴), 21.22 (C-13‴), 20.90 (C-14‴), 20.19 (C-15‴), 20.18 (C-10‴), 14.10 (C-16), 13.26 (C-16‴); IR (ATR) ν_{max}/cm^{-1}: 2917, 2849, 1713, 1469, 1307, 1271, 1132, 1039, 939, 875,845; HRMS-ESI *m/z* $[M+H]^+$ 650.4219 (calcd for $C3_5H_{61}NO_7S$ 650.4215).

5.4.10. (1s,3S)-adamantan-1-yl(1,1-dioxido-4-((3R,5aS,6R,9R,10R,12R,12aR)-3,6,9-trimethyldecahydro-3H-3,12-epoxy[1,2]dioxepino[4,3-i]isochromen-10-yl)thiomorpholin-3'-

yl)methanone, **15.** Yield: 26%; mp: 165.6°C; ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 5.66 (s, 1H, H-12), 5.12 (d, *J* = 11.6 Hz, 1H, H-10), 4.27 (m, 1H, H-3‴), 3.93 (dd, *J* = 14.8, 11.1 Hz, 2H, H-2‴), 3.40 (dt, *J* = 15.6, 2.9 Hz, 2H, H-5‴), 3.13 (dt, *J* = 14.8, 2.9 Hz, 2H, H-7‴), 2.22 – 2.16 (m, 1H, H-5a), 2.54 – 2.40 (m, 2H, H-3‴), 2.13 – 2.05 (m, 7H, H-4, H-10‴, H-5, H-9‴), 2.04 – 1.82 (m, 13H, H-8, H-8a, H-8‴, H-7, H-11‴, H-6‴, H-4‴, H-5‴, H-9), 1.81 (s, 3H, H-14), 1.46 (m, 1H, H-6), 0.99 (d, 3H, H-16), 0.79 (m, 3H, H-15); ^{13}C NMR (151 MHz, $CDCl_3$) δ ppm 204.30 (C-1‴), 104.42 (C-3), 91.94 (C-10), 91.38 (C-12), 77.00 (C-12a), 80.52 (C-3‴), 76.78 (C-2‴), 63.44 (C-5a), 63.15 (C-5‴), 56.52 (C-2‴), 51.32 (C-8a), 48.20 (C-6‴), 48.18 (C-11‴), 47.76 (C-9‴), 45.56 (C-4), 37.46 (C-6‴), 37.00 (C-8‴), 36.54 (C-9), 36.27 (C-7), 36.34 (C-7‴), 36.17 (C-10‴), 36.02 (C-6), 34.09 (C-5‴), 29.60 (C-4‴), 27.69 (C-3‴), 26.17 (C-14), 24.82 (C-8), 21.89 (C-5), 20.22 (C-15), 13.02 (C-16); IR (ATR) ν_{max}/cm^{-1}: 2907, 1700, 1450, 1331, 1243, 1135, 1039, 972, 931, 874; HRMS-ESI *m/z* $[M+H]^+$ 564.3147 (calcd $C_{30}H_{45}NO_7S$: 564.2917).

5.4.11. (1,1-dioxido-4-((3R,5aS,6R,9R,10R,12R,12aR)-3,6,9-trimethyldecahydro-3H-3,12-epoxy[1,2]dioxepino[4,3-i]isochromen-10-yl)thiomorpholin-3'-yl)(morpholino)methanone, **16.** Yield: 14.5%; mp: 157.2-169.1°C; ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 5.27 (s, 1H, H-12), 4.93 (dd, *J* = 10.4, 3.7 Hz, 1H, H-3‴), 4.27 (d, *J* = 10.2 Hz, 1H, H-10), 3.92 – 3.76 (m, 4H,, H-3‴, H-7‴), 3.75 – 3.32 (m, 10H, H-2‴, H-5‴, H-6‴, H-4‴, H-6‴), 2.90 (dt, *J* = 13.4, 2.6 Hz, 1H, H-5a), 2.54 – 2.45 (m, 1H, H-9), 2.34 (td, *J* = 14.0, 4.0 Hz, 1H, H-6), 2.03 – 1.95 (m, 1H, H-8a), 1.87 (ddt, *J* = 13.7, 6.9, 3.6 Hz, 2H, H-8), 1.71 (dddd, *J* = 22.7, 13.4, 7.1, 3.5 Hz, 2H, H-4), 1.51 – 1.38 (m, 4H, H-5, H-7), 1.27(s, 3H, H-14), 0.96 (d, 3H, H-15), 0.75 (d, 3H, H-16); ^{13}C NMR (151 MHz, $CDCl_3$) δ ppm 161.09 (C-1‴), 104.35 (C-3), 91.88 (C-10), 91.12 (C-12), 80.77 (C-12a), 76.79 (C-3‴), 66.82 (C-4‴), 66.56 (C-6‴), 59.51 (C-5a), 52.62 (C-5‴), 51.35 (C-2‴), 47.05 (C-6‴), 45.46 (C-8a), 42.81 (C-4), 37.53 (C-3‴), 37.63 (C-7‴), 36.13 (C-9), 34.05 (C-7), 29.41 (C-6), 26.13 (C-14), 24.76 (C-8), 21.85 (C-5), 20.23 (C-15), 13.16 (C-16); IR (ATR) ν_{max}/cm^{-1}: 2927,1654 , 1436, 1374, 1307, 1225, 1114, 1042, 941; HRMS-ESI *m/z* $[M+H]^+$ 515.2373 (calcd for $C_{24}H_{38}N_2O_8S$: 515.2348).

5.4.12. (1,1-dioxido-4-((3R,5aS,6R,9R,10R,12R,12aR)-3,6,9-trimethyldecahydro-3H-3,12-epoxy[1,2]dioxepino[4,3-i]isochromen-10-yl)thiomorpholin-2-yl)(thiophen-3'-yl)methanone, **17.** Yield: 21%; mp: 137.9°C; ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 7.99 (d, *J* = 4.1 Hz, 1H, H-5‴), 7.75 (d, *J* = 4.6, 1H, H-3‴), 7.14 – 7.06 (m, 1H, H-4‴), 5.32 (s, 1H, H-12), 5.29 (m, 1H, H-10), 4.29 (m, 1H, H-3‴), 3.84 (dtd, *J* = 31.5, 13.5, 13.0, 3.8 Hz, 2H, H-2‴), 3.76 – 3.59 (m, 2H, H-5‴), 3.52 – 3.31 (m, 2H, H-6‴), 3.22 – 3.14 (m, 1H, H-5a), 3.01 (dt, *J* = 13.5, 3.0 Hz, 2H, H-4), 2.65 – 2.43 (m, 1H, H-9), 2.42 – 2.22 (m, 2H, H-5), 2.14 (s, 3H, H-14), 2.07 – 1.94 (m,

1H, H-8a), 1.87 (qdq, *J* = 10.2, 6.9, 3.6 Hz, 2H, H-7), 1.70 (ddq, *J* = 20.0, 13.1, 3.5 Hz, 1H, H-6), 1.31 (m, 2H, H-8), 0.97 (m, 3H, H-15), 0.80 (m, 3H, H-16); ^{13}C NMR (151 MHz, $CDCl_3$) δ ppm 182 (C-1‴), 136.00 (C-2‴), 135.73 (C-3‴), 128.29 (C-5‴), 104.40 (C-4‴), 91.89 (C-3), 91.22 (C-10), 77.00 (C-12), 76.78 (C-12a), 66.23 (C-3″), 52.51 (C-5a), 51.79 (C-5″), 51.39 (C-2″), 45.52 (C-8a), 37.56 (C-6″), 36.13 (C-4), 34.09 (C-9), 29.34 (C-7), 26.27 (C-6), 24.76 (C-14), 21.76 (C-8), 21.58 (C-5), 20.20 (C-15), 13.17 (C-16); IR (ATR) ν_{max}/cm^{-1}: 2926, 1644, 1410, 1311, 1125, 1025, 1041, 978, 940, 843, 826, 726; HRMS-ESI *m/z* $[M+H]^+$ 512.1771 (calcd for $C_{24}H_{33}NO_7S_2$: 512.1698).

5.4.13. (1,1-dioxido-4-((3R,5aS,6R,9R,10R,12R,12aR)-3,6,9-trimethyldecahydro-3H-3,12-epoxy[1,2]dioxepino[4,3-i]isochromen-10-yl)thiomorpholin-3'-yl)(furan-2-yl)methanone, **18.** Yield: 20%; mp: 137.6°C; ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 7.65 (d, *J* = 1.9 Hz, 1H, H-5‴), 7.48 (d, *J* = 3.7 Hz, 1H, H-3‴), 7.29 (m, 1H, H-4‴), 6.63 – 6.57 (s, 1H, H-12), 5.38 (m, 1H, H-10), 4.31 – 4.20 (m, 1H, H-3″), 3.78 (ddd, *J* = 25.8, 15.6, 11.5 Hz, 6H, H-2″, H-5″, H-6″), 3.65 (m, 10H, H-5a, H-4, H-5, H-7, H-8, H-9), 3.55 – 3.38 (m, 1H, H-6), 3.07 (s, 3H, H-14), 2.52 (m, 1H, H-8a), 1.08 (d, *J* = 13.7, 3H, H-16), 0.76 (d, *J* = 10.3 Hz, 3H, H-15); ^{13}C NMR (151 MHz, $CDCl_3$) δ ppm 177.81 (C-1‴), 152.37 (C-2‴), 147.86 (C-5‴), 120.94 (C-3‴), 112.81 (C-4‴), 104.35 (C-3), 91.90 (C-10), 91.15 (C-12), 80.39 (C-12a), 77.00 (C-3″), 76.78 (C-5a), 65.68 (C-5″), 52.38 (C-2″), 51.38 (C-8a), 45.52 (C-6″), 37.46 (C-4), 36.12 (C-9), 34.09 (C-7), 29.21 (C-6), 26.05 (C-14), 24.76 (C-8), 21.69 (C-5), 20.21 (C-15), 13.15 (C-16);IR (ATR) ν_{max}/cm^{-1}: 2929, 1662, 1462, 1392, 1310, 1127, 1041, 940, 872, 844, 770; HRMS-ESI *m/z* $[M+H]^+$ 496. 59 (calcd for $C_{24}H_{33}NO_8S$: 496.59).

5.4.14. (1,1-dioxido-4-((3R,5aS,6R,9R,10R,12R,12aR)-3,6,9-trimethyldecahydro-3H-3,12-epoxy[1,2]dioxepino[4,3-i]isochromen-10-yl)thiomorpholin-2-yl)(3-methylthiophen-3'-yl)methanone, **19.** Yield: 20%; mp: 141.5-162.3°C; ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 7.50 (d, *J* = 9.5, Hz, 1H, H-5‴), 6.94 (d, *J* = 4.9, Hz, 1H, H-4‴), 5.54 (s, 1H, H-12), 5.28 (d, *J* = 24.1 Hz, 1H, H-10), 4.22 (dd, *J* = 29.0, 10.3 Hz, 1H, H-3″), 3.94 (dd, *J* = 14.8, 10.4 Hz, 2H, H-2″), 3.81 – 3.70 (m, 2H, H-5″), 3.67 – 3.51 (m, 2H, H-6″), 3.33 (s, 3H, H-7‴), 2.96 – 2.90 (m, 2H, H-4), 2.58 (m, *J* = 22.5 Hz, 8H, H-5, H-7, H-8, H-9, H-6,), 2.49 – 2.06 (m, 1H, H-5a), 1.82 (s, 3H, H-14), 1.79 – 1.58 (m, 1H, H-8a), 0.99 (d, *J* = 13.2 Hz, 3H, H-15), 0.93 (d, *J* = 12.3 Hz, 3H, H-16); ^{13}C NMR (151 MHz, $CDCl_3$) δ ppm 182.61 (C-1‴), 147.62 (C-2‴), 133.08 (C-3‴), 132.40 (C-4‴), 104.41 (C-5‴), 92.23 (C-3), 91.41 (C-10), 90.90 (C-12), 80.20 (C-12a), 77.00 (C-3″), 76.78 (C-5a), 68.46 (C-5″), 51.79 (C-2″), 45.56 (C-8a), 51.28 (C-6″), 37.48 (C-4), 36.17 (C-9), 34.12 (C-7), 29.08 (C-6), 25.97 (C-14), 24.82 (C-8), 21.62 (C-15), 20.21 (C-5),

13.27 (C-7''), 13.09 (C-16); IR (ATR) ν_{max}/cm^{-1}: 2966, 1616, 1445, 1369, 1326, 1208, 1153, 1095, 1028, 837 HRMS-ESI *m/z* $[M+H]^+$ 526.1867 (calcd for $C_{25}H_{35}NO_7S_2$: 526.1854).

5.4.15. (1,1-dioxido-4-((3R,5aS,6R,9R,10R,12R,12aR)-3,6,9-trimethyldecahydro-3H-3,12-epoxy[1,2]dioxepino[4,3-i]isochromen-10-yl)thiomorpholin-3'-yl)(phenyl)methanone, **20.** Yield: 20%; mp: 153.1-156.1°C; ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 8.13 – 8.02 (m, 2H, H-3'',H-7''), 7.61 – 7.52 (m, 1H, H-5''), 7.52 – 7.38 (m, 2H, H-4'', H-6''), 5.64 – 5.58 (s, 1H, H-12), 5.30 (d, 1H, H-10), 4.30 – 4.16 (m, 1H, H-3'), 3.82 (td, *J* = 12.8, 3.9 Hz, 2H, H-2'), 3.76 – 3.60 (m, 2H, H-6'), 3.44 (dq, *J* = 14.8, 3.3 Hz, 2H, H-5'), 2.98 (dqd, *J* = 14.5, 7.1, 4.3 Hz, 1H, H-5a), 2.35 (td, *J* = 14.0, 4.0 Hz, 1H, H-9), 2.07 – 1.97 (m, 2H, H-4), 1.87 (m, 2H, H-5), 1.69 (tp, *J* = 14.1, 3.8 Hz, 2H, H-7), 1.57 – 1.40 (m, 2H, H-8), 1.37 (s, 3H, H-14), 1.28 (m, 2H, H-6, H-8a), 0.90 (d, 3H, H-16), 0.72 (d, 3H, H-15); ^{13}C NMR (151 MHz, $CDCl_3$) δ ppm 190.67 (C-1''), 136.73 (C-2''), 134.03 (C-5''), 129.53 (C-3''), 128.50 (C-7''), 104.41 (C-4''), 92.02 (C-6''), 91.31 (C-3), 80.47 (C-10), 76.78 (C-12), 65.17 (C-12a), 60.35 (C-3'), 52.52 (C-5a), 51.80 (C-5'),51.43 (C-2'), 45.56 (C-8a), 45.50 (C-6'), 37.45 (C-4), 36.17 (C-9), 34.07 (C-7), 29.38 (C-6), 26.15 (C-14), 24.75 (C-8), 21.77 (C-5), 20.22 (C-15), 13.20 (C-16); IR (ATR) ν_{max}/cm^{-1}: 2935, 1676, 1448, 1317, 1279, 1193, 1132, 1039, 973; HRMS-ESI *m/z* $[M+H]^+$ 506.2185 (calcd for $C_{26}H_{35}NO_7S$: 506.2134).

5.4.16. (1,1-dioxido-4-((3R,5aS,6R,9R,10R,12R,12aR)-3,6,9-trimethyldecahydro-3H-3,12-epoxy[1,2]dioxepino[4,3-i]isochromen-10-yl)thiomorpholin-3'-yl)(4-methoxyphenyl)methanone, **21.** Yield: 24%; mp: 160.1°C; ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 8.11 – 8.03 (m, 2H, H-3'', H-7''), 6.91 – 6.84 (m, 2H, H-4'', H-6''), 5.56 (s, 1H, H-12), 5.30 (d, *J* = 10.2 1H, H-10), 4.27 (m, 1H, H-3'), 4.09 (q, *J* = 7.2 Hz, 2H, H-2'), 3.84 (s, 3H, H-8''), 3.76 – 3.58 (m, 6H, H-6', H-5', H-4), 3.43 (dq, *J* = 14.6, 3.4 Hz, 3H, H-5, H-5a), 3.35 (m, *J* = 14.4 Hz, 1H, H-9), 2.97 (dt, *J* = 13.3, 3.0 Hz, 2H, H-8), 2.48 (dqd, *J* = 14.3, 7.1, 4.4 Hz, 2H, H-7), 2.40 – 2.29 (m, 1H, H-6), 2.07 – 1.97 (m, 4H, H-14, H-8a), 1.18 (d, *J* = 8.0 Hz 3H, H-16), 0.76 (d, *J* = 7.1 Hz, 3H, H-15); ^{13}C NMR (151 MHz, $CDCl_3$) δ ppm 188.61 (C-1''), 164.32 (C-5''), 132.06 (C-3''), 129.93 (C-2''), 113.70 (C-7''), 104.37 (C-4''), 91.99 (C-6''), 91.30 (C-3), 80.49 (C-10), 76.78 (C-12), 64.80 (C-12a), 60.35 (C-3'), 55.50 (C-8''), 52.52 (C-5a), 51.43 (C-5'), 45.50 (C-2'), 37.45 (C-8a), 36.14 (C-6'), 34.07 (C-4), 29.37 (C-9), 26.17 (C-7), 24.75 (C-6), 21.77 (C-14), 20.22 (C-8), 14.16 (C-15), 13.41 (C-5), 13.21 (C-16); IR (ATR) ν_{max}/cm^{-1}: 2950, 1667, 1597, 1515, 1315, 1270, 1172, 1129, 1036, 969; HRMS-ESI *m/z* $[M+H]^+$ 536.2267 (calcd for $C_{27}H_{37}NO_8S$: 536.2239).

5.4.17. benzofuran-2-yl(1,1-dioxido-4-((3R,5aS,6R,9R,10R,12R,12aR)-3,6,9-trimethyldecahydro-3H-3,12-epoxy[1,2]dioxepino[4,3-i]isochromen-10-yl)thiomorpholin-3'-yl)methanone, **22.** Yield: 15%; mp: 158.8°C; ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 7.82 (s, 1H, H-3‴), 7.67 (dd, *J* = 7.8, 0.9 Hz, 1H, H-5‴), 7.63 – 7.53 (m, 1H, H-6‴), 7.47 (ddd, *J* = 8.5, 7.1, 1.4 Hz, 1H, H-4‴), 7.31 – 7.23 (d, *J* = 9.3, 1H, H-7‴), 5.31 (s, 1H, H-12), 4.27 (d, *J* = 10.2 Hz, 1H, H-10), 4.19 - 4.17 (m, 1H, H-3″), 3.83 – 3.72 (m, 4H, H-5″, H-2″), 3.72 – 3.60 (m, 2H, H-6″), 3.47 (dq, *J* = 14.7, 3.5 Hz, 1H, 5a), 3.36 (dt, *J* =14.2, 5.5 Hz, 2H, H-4), 3.06 (dt, *J* = 13.0, 3.2 Hz, 1H, H-9), 2.34 (dtd, *J* = 18.8, 14.0, 4.0 Hz, 1H, H-6), 2.15 (s, 3H, H-14), 2.08 – 1.93 (m, 2H, H-5), 1.87 (dddt, *J* = 14.1, 10.5, 6.5, 3.4 Hz, 1H, H-8a), 1.70 (tdd, *J* = 13.5, 7.0, 3.6 Hz, 2H, H-8), 1.38 – 1.18 (m, 2H, H-7), 0.90 (d, *J* = 10.1, 3H, H-16), 0.76 (dd, *J* = 7.1 Hz, 3H, H-15); ^{13}C NMR (151 MHz, $CDCl_3$) δ ppm 171.15 (C-1‴), 156.25 (C-2‴), 152.13 (C-7a‴), 129.12 (C-3a‴), 126.97 (C-6‴), 124.02 (C-5‴), 123.72 (C-4‴), 117.40 (C-3‴), 112.66 (C-7‴), 104.40 (C-3), 91.97 (C-10), 91.25 (C-12), 80.46 (C-12a), 76.78 (C-3″), 66.17 (C-5a), 60.37 (C-5″), 52.47 (C-2″), 51.40 (C-8a), 45.49 (C-6″), 37.48 (C-4), 36.16 (C-9), 34.09 (C-7), 29.27 (C-6), 26.32 (C-14), 21.74 (C-8), 20.22 (C-5), 14.17 (C-15), 13.19 (C-16); IR (ATR) ν_{max}/cm^{-1}: 2935, 1675, 1549, 1319, 1129, 1037, 972, 938, 876, 840; HRMS-ESI *m/z* $[M+H]^+$ 546.2162 (calcd for $C_{28}H_{35}NO_8S$: 546.2083).

Acknowledgements:

This work is as a result of research supported by the Medical Research Council of South Africa (MRC). The authors thank the MRC and the North-West University, Potchefstroom Campus for the financial support, Dr. J Jordaan for MS analysis, Mr. A Jourbert for NMR analysis and the University of Pretoria for conducting biological screening.

References

[1] WHO, [WEB]: http://www.who.int/malaria/publications/world_malaria_report_2014/report/en/. [Accessed: 12/02/ 2015, (2014).

[2] A. Kantele, T.S. Jokiranta, Clin Infect Dis 52 (2011) 1356-1362.

[3] N.J. White, S. Pukrittayakamee, T.T. Hien, M.A. Faiz, O.A. Mokuolu, A.M. Dondorp, Lancet, 383 (2014) 723-735.

[4] WHO, [WEB]: http://apps.who.int/iris/bitstream/10665/43213/1/9241593199_eng.pdf. [Accessed: 09/09/2015], in, 2005.

[5] T.E. Wellems, C.V. Plowe, J Infect Dis, 184 (2001) 770-776.

[6] M. Foley, L. Tilley, Pharmacol Ther, 79 (1998) 55-87.

[7] J. Marfurt, F. Chalfein, P. Prayoga, F. Wabiser, G. Wirjanata, B. Sebayang, K.A. Piera, S. Wittlin, R.K. Haynes, J.J. Mohrle, N.M. Anstey, E. Kenangalem, R.N. Price, Antimicrob Agents Chemother, 56 (2012) 5258-5263.

[8] R. Haynes, K. Cheu, D. N'Da, P. Coghi, D. Monti, Infect Disord Drug Targets, 13 (2013) 217 - 277.

[9] L. Cui, X. Su, Expert Rev Anti Infect Ther, 7 (2009) 999-1013.

[10] S. Krishna, A.C. Uhlemann, R.K. Haynes, Drug Resist Updates, 7 (2004) 233-244.

[11] A. Gautam, T. Ahmed, V. Batra, J. Paliwal, Curr Drug Metab, 10 (2009) 289-306.

[12] WHO, [WEB]: http://www.who.int/malaria/publications/atoz/9789241505321/en/. [Accessed: 20/02/2015], (2013).

[13] WHO, [WEB]:http://apps.who.int/iris/bitstream/10665/162441/1/9789241549127_eng.pdf. [Accessed: 10/09/2015], (2010).

[14] WHO, [WEB]: http://www.who.int/malaria/publications/atoz/afr_mal_04_01/en/. [Accessed:09/09/2015], (2004).

[15] R.N. Price, N.M. Douglas, Clin Infect Dis 49 (2009) 1638-1640.

[16] F. Nosten, N.J. White, Am J Trop Med Hyg, 77 (2007) 181-192.

[17] E.Y. Klein, Int J Antimicrob Agents, 41 (2013) 311-317.

[18] A.M. Dondorp, S. Yeung, L. White, C. Nguon, N.P. Day, D. Socheat, L. von Seidlein, Nature Rev Microbiol, 8 (2010) 272-280.

[19] T. Gordi, D.X. Huong, T.N. Hai, N.T. Nieu, M. Ashton, Antimicrob Agents Chemother 46 (2002) 1026-1031.

[20] G. Schmuck, R.K. Haynes, Neurotox Res, 2 (2000) 37-49.

[21] WHO, [WEB]: http://www.who.int/malaria/publications/world_malaria_report_2013/en/. [Accessed: 25/02/2014], (2013).

[22] S. Pooley, F.A. Fatih, S. Krishna, M. Gerisch, R.K. Haynes, H.-N. Wong, H.M. Staines, Antimicrob Agents Chemother 55 (2010) 550-556.
[23] R.K. Haynes, B. Fugmann, J. Stetter, K. Rieckmann, H.-D. Heilmann, H.-W. Chan, M.-K. Cheung, W.-L. Lam, H.-N. Wong, S.L. Croft, L. Vivas, L. Rattray, L. Stewart, W. Peters, B.L. Robinson, M.D. Edstein, B. Kotecka, D.E. Kyle, B. Beckermann, M. Gerisch, M. Radtke, G. Schmuck, W. Steinke, U. Wollborn, K. Schmeer, A. Römer, Angewandte Chemie, 45 (2006) 1989-1989.
[24] J. Nagelschmitz, B. Voith, G. Wensing, A. Roemer, B. Fugmann, R.K. Haynes, B.M. Kotecka, K.H. Rieckmann, M.D. Edstein, Antimicrob Agents Chemother, 52 (2008) 3085-3091.
[25] R.J.S. Francis A. Carey, in, Springer, Verlag US, 2007, pp. 592-593
[26] M. Gall, H.O. House, in, John Wiley & Sons, Inc., 2003.
[27] M. Roncalés, J. Vidal-Mas, D. Leroy, E. Herreros, Int J Parasitol Res, 2012 (2012) 7.
[28] R.G. Ridley, W. Hofheinz, H. Matile, C. Jaquet, A. Dorn, R. Masciadri, S. Jolidon, W.F. Richter, A. Guenzi, M.A. Girometta, H. Urwyler, W. Huber, S. Thaithong, W. Peters, Antimicrob Agents Chemother, 40 (1996) 1846-1854.
[29] F.J. Smit, R.A. van Biljon, L.M. Birkholtz, D.D. N'Da, Eur J Med Chem, 90 (2015) 33-44.
[30] S. Kaneko, http://www.m.ehime-u.ac.jp/school/parasitology/eng/Strain1.htm; Access date 09/10/2015., in, 1999.
[31] M. Smilkstein, N. Sriwilaijaroen, J.X. Kelly, P. Wilairat, M. Riscoe, Antimicrob Agents Chemother 48 (2004) 1803-1806.
[32] J.D. Johnson, R.A. Dennull, L. Gerena, M. Lopez-Sanchez, N.E. Roncal, N.C. Waters, Antimicrob Agents Chemother 51 (2007) 1926-1933.
[33] B.K. Verlinden, J. Niemand, J. Snyman, S.K. Sharma, R.J. Beattie, P.M. Woster, L.M. Birkholtz, J Med Chem, 54 (2011) 6624-6633.
[34] J. Reader, M. Botha, A. Theron, S.B. Lauterbach, C. Rossouw, D. Engelbrecht, M. Wepener, A. Smit, D. Leroy, D. Mancama, T.L. Coetzer, L.M. Birkholtz, Malar J, 14 (2015) 213.
[35] W. Voigt, Methods Mol. Med, 110 (2005) 39-48.

Supplementary information

Synthesis and biological evaluation of novel artemisone derivatives

Richard J. Mabilika[a], Frans J. Smit[b], Lyn-Marie Birkholtz[c], Dina Coertzen[c], Richard K. Haynes*[b],David D. N'Da*[b]

[a] Pharmaceutical Chemistry, School of Pharmacy, North-West University, Potchefstroom 2520, South Africa

[b] Centre of Excellence for Pharmaceutical Sciences, North-West University, Potchefstroom 2520, South Africa

[c] Department of Biochemistry, Centre for Sustainable Malaria Control, University of Pretoria, Pretoria 0002, South Africa

* Corresponding authors.

E-mail: Richard.haynes@nwu.ac.za, Tel.: +27 (0)18 299 4466; E-mail: david.nda@nwu.ac.za, Tel.: +27 18 299 2256; Fax: +27 18 299 4243

Table 2: Percentage abundance of isomers A and B, calculated from the ratio of H-12 integral heights of each isomer.

Compound	H-12 integral heights (cm)		Diastereomic ratio: A^a/B^b	A%	B%
	A^a	B^b			
6	4.8	0.3	16	94.1	5.9
7	4.8	0	-	100	0
8	4.3	0.3	14.3	93.5	6.5
9	4.9	1.1	4.5	81.8	18.2
10	5	0.3	16.7	94.3	5.7
11	5	2.6	1.9	66.2	33.8
12	5	2.4	2.1	67.7	32.3
13	3	0.3	10	90.9	9.1
14	1.3	0.1	13	93.1	6.9
15	5.1	0.1	51	98.1	1.9
16	5.6	1	5.6	84.8	15.2
17	4.8	1.1	4.4	81.5	18.5
18	5.8	0.3	19	95	5
19	4	1.9	2.1	67.7	32.3
20	5.6	0.4	14	93.3	6.7
21	3.7	0.4	9.3	90.3	9.7
22	4.8	0.7	6.9	87.3	12.7

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

CHAPTER 5

SUMMARY AND CONCLUSION

In 2013 alone, a total of 198 million malaria cases and over 580 000 deaths were reported worldwide, with 82% of the cases and 90% of the deaths occurring in Africa (WHO, 2014). The disease remains endemic in most sub-Saharan African countries, the Middle East, Latin America and in South-East Asia, where communities are exposed to the highest risks associated with malaria and yet, have the least access to effective services for prevention and treatment of the disease (WHO, 2013).

With no licenced vaccine in the armoury against malaria to date, the treatment of this disease has remained dependent on antimalarial chemotherapeutic agents (WHO, 2014). Since their introduction in the early 1970s, artemisinins have continued to be the most active antimalarial agents and have thus remained to be the basis for the current malaria treatment regimens (Woodrow et al., 2005, WHO, 2013). They are highly potent, well tolerated in all phases of the disease and reduce parasitaemia more rapidly than any other antimalarial drug (Gautam et al., 2009, O'Neill and Posner, 2004). However, they suffer from drawbacks such as low solubility, thermal instability, short pharmacological half-lives, and neurotoxicity a character associated with their principal metabolite, dihydroartemisinin (DHA) (Woodrow et al., 2005, WHO, 2013).

In the late 1990s, the Medicines for Malaria Venture (MMV) placed priority on the development of new derivatives that do not metabolize to DHA and therefore are non-neurotoxic in screens and *in vivo*. Artemisone, a second generation semi-synthetic derivative of artemisinin, was thereby prepared, and did not show any measurable neurotoxicity in both *in vitro* and *in vivo* assays (Haynes et al., 2006). The drug has substantially greater *in vivo* activity in rodent *P. berghei* and *P. falciparum* in primate model (Nagelschmitz et al., 2008), making it a drug-like compound. Although artemisone has a good aqueous solubility suitable for permeation across a biological membrane, its measured Log P value of 2.49 marks artemisone as a polar compound. Thus it is of considerable interest to prepare more lipophilic derivatives and to evaluate their efficacies with respect to artemisone itself (Haynes et al., 2006).

The aim of this study was to prepare a series of substituted artemisone derivatives with enhanced lipid solubility profiles and metabolic stability as compared to artemisone, as well as to assess their *in vitro* antimalarial activity against both CQS and CQR strains of *P. falciparum* and cytotoxicity profiles against mammalian cells. The target compounds were designed to be less polar than artemisone in order to facilitate permeation across biological membranes

To achieve the stated aim of the study, the following objectives were set:

- To prepare and characterize a series of lipophilic derivatives of artemisone
- To assess the *in vitro* antimalarial activity of the compounds against both CQR and CQS strains of *P. falciparum.*
- To assess the cytotoxicity profiles of the compounds on *ex vivo* animal and human cells

The compounds **6**-**22** were prepared in in low to moderate yields (12-38%) *via* nucleophilic acylation or alkylation of the α-sulfonyl carbanion derived by deprotonation of artemisone with lithium *N*,*N*-diisopropylamine in anhydrous THF under nitrogen. All compounds were characterized ^{1}H and ^{13}C NMR and IR spectroscopies and mass spectrometry. The cLog *P* values (Chemsketch computer software) of the compounds were in the range of 3.39–11, above that of artemisone with cLog P of 3.37.

The compounds **6**-**22** were screened together with artesunate, artemether, and artemisone against the asexual erythrocytic stages of NF54, K1 and W2 strains of *P. falciparum* parasites, and against early gametocyte stages of the NF54 strain. Cytotoxicities of the target compounds were evaluated against the normal human fetal lung fibroblast WI-38 cell line.

With the exception of the highly lipophilic compounds, *viz.*, **13** (cLog P = 8.87) and **14** (cLog P = 11), all the remaining compounds in the series were active at low nanomolar concentrations namely, IC_{50}<10 nM and IC_{50}<30 nM against the asexual parasites and early stage gametocytes, respectively. All the target compounds except **13**, **14** and **15** were even more potent against both NF54 (sensitive) and K1 (resistant) *P. falciparum* strains than the clinically used drugs artesunate and artemether.

Compounds **6**-**10**, **12** and **17**-**22** with cLog *P* values in the range of 3.56–6.75, were approximately 2-4 and 3-6 times more potent than the clinically used drugs artemether and

artesunate against early stage gametocytes, respectively. At 1 μM concentration, the compounds showed improved potency against gametocytes and asexual parasites in comparison to the parent artemisone.

The compounds had resistance indices (RIs) (IC_{50} K1 or W2/ IC_{50} NF54) in the range of 0.4–3.8 across the three parasite strains. Most compounds, however, had impressive RIs (IC_{50} K1/IC_{50} NF54) of less than 1.5, making them almost equally active between the CQ sensitive and resistant parasites. In particular, compounds **6** and **16** had RIs (IC_{50} K1/ IC_{50} NF54) of less than 1 suggesting that they were more active against the resistant K1 than the sensitive NF54 strains. Compound **6** was the only artemisone derivative with RI (IC_{50} W2/IC_{50} NF54) of less than 1.5 and therefore the only compound that showed no cross resistance between W2 and NF54 in the series.

Short chain acyl and alkyl mono-substituted derivatives **7**, **8**, and **9** displayed overall higher activities (IC_{50}<0.5 nM) than compounds with longer chains while all the aromatic substituted derivatives **17** - **22** were active with IC_{50} values of less than 2.7 nM. In particular, compounds **17** and **19** bearing the thienylcarbonyl substituent and compound **21** with the methoxybenzoyl substituent were the most potent (IC_{50}<0.9 nM) among the aromatic substituted compounds. Generally, none of the compounds was found to be cytotoxic meaning they are likely to display selective activity against parasites cells in the presence of mammalian cells. It comes as a surprise that compound **15** bearing the highly lipid soluble adamantane ring, famous for enhancing activity of synthetic trioxolanes and tetraoxanes which incorporate that group, was among the least active compounds in the series (IC_{50}>5 nM). This however could be explained by the general trend in the series that a marked increase in lipophilicity tends to translate into lower activity.

Overall compounds **6** and **10** with impressive resistance index across all the parasite lines and high selective toxicity towards the malaria parasites were identified as the best candidates for further investigation as potential antimalarial drugs.

Bibliography

Gautam, A., Ahmed, T., Batra, V. & Paliwal, J., 2009. Pharmacokinetics and pharmacodynamics of endoperoxide antimalarials. *Current Drug Metabolism*, 10, 289-306.

Haynes, R. K., Fugmann, B., Stetter, J., Rieckmann, K., Heilmann, H.-D., Chan, H.-W., Cheung, M.-K., Lam, W.-L., Wong, H.-N., Croft, S. L., Vivas, L., Rattray, L., Stewart, L., Peters, W., Robinson, B. L., Edstein, M. D., Kotecka, B., Kyle, D. E., Beckermann, B., Gerisch, M., Radtke, M., Schmuck, G., Steinke, W., Wollborn, U., Schmeer, K. & Römer, A., 2006. Artemisone-a highly active antimalarial drug of the artemisinin class. *Angewandte Chemie International Edition,* 45, 1989-1989.

Nagelschmitz, J., Voith, B., Wensing, G., Roemer, A., Fugmann, B., Haynes, R. K., Kotecka, B. M., Rieckmann, K. H. & Edstein, M. D., 2008. First assessment in humans of the safety, tolerability, pharmacokinetics, and *ex vivo* pharmacodynamic antimalarial activity of the new artemisinin derivative artemisone. *Antimicrobial Agents and Chemotherapy,* 52, 3085-3091.

O'Neill, P. M. & Posner, G. H., 2004. A Medicinal chemistry perspective on artemisinin and related endoperoxides. *Journal of Medicinal Chemistry,* 47, 2945-2964.

WHO. 2013. World malaria report; [WEB]: http://www.who.int/malaria/publications/world_malaria_report_2013/en/: [Date of access : 25 Feb. 2014].

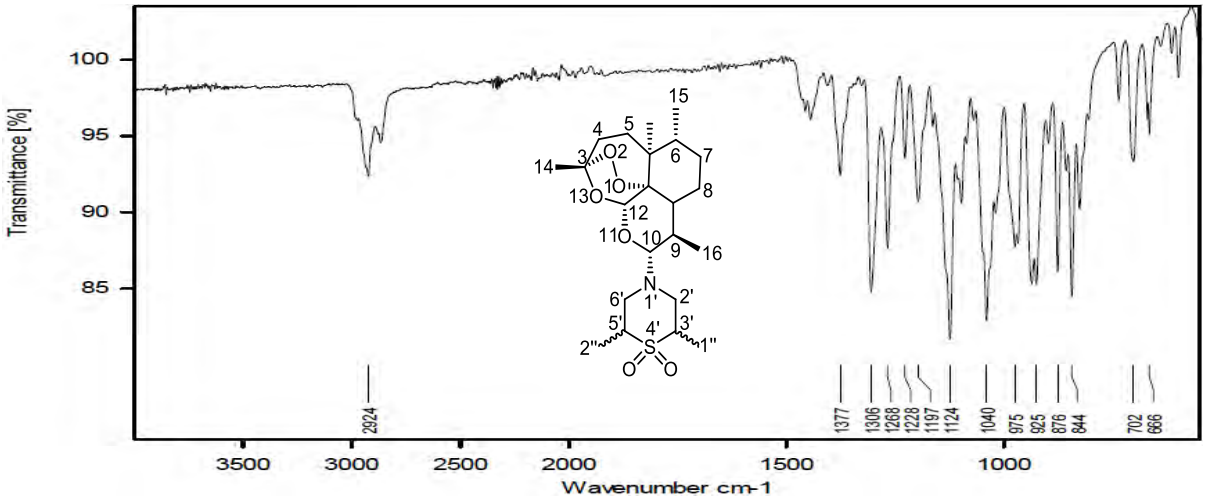
WHO. 2014. World malaria report; [WEB]: http://www.who.int/malaria/publications/world_malaria_report_2014/report/en/; [Date of access: 12 Feb. 2015].

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

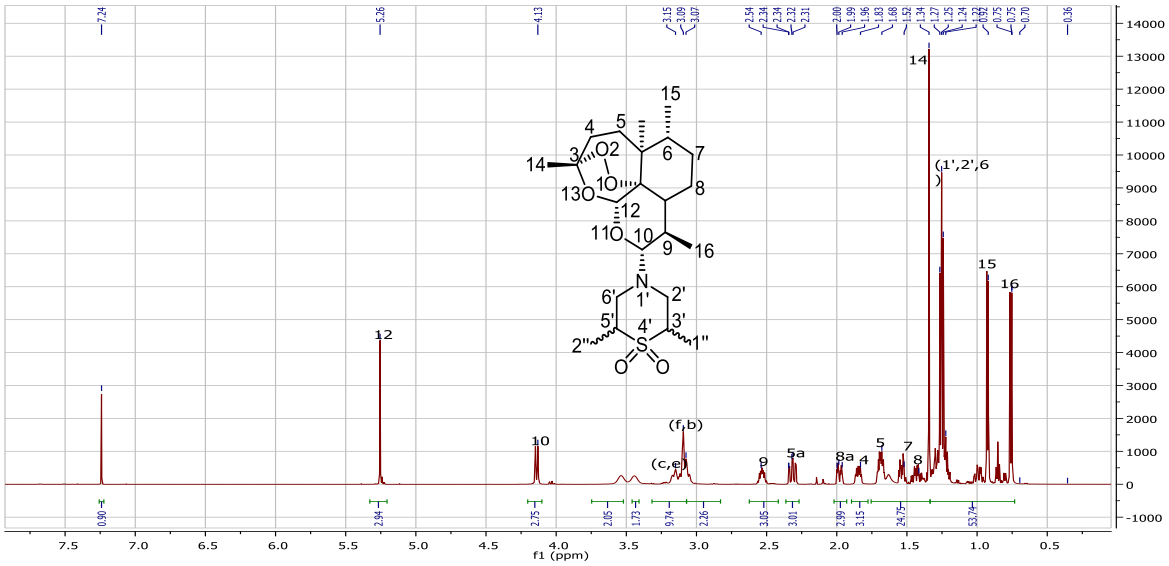
APPENDIX A: SPECTRA

Compound 6:

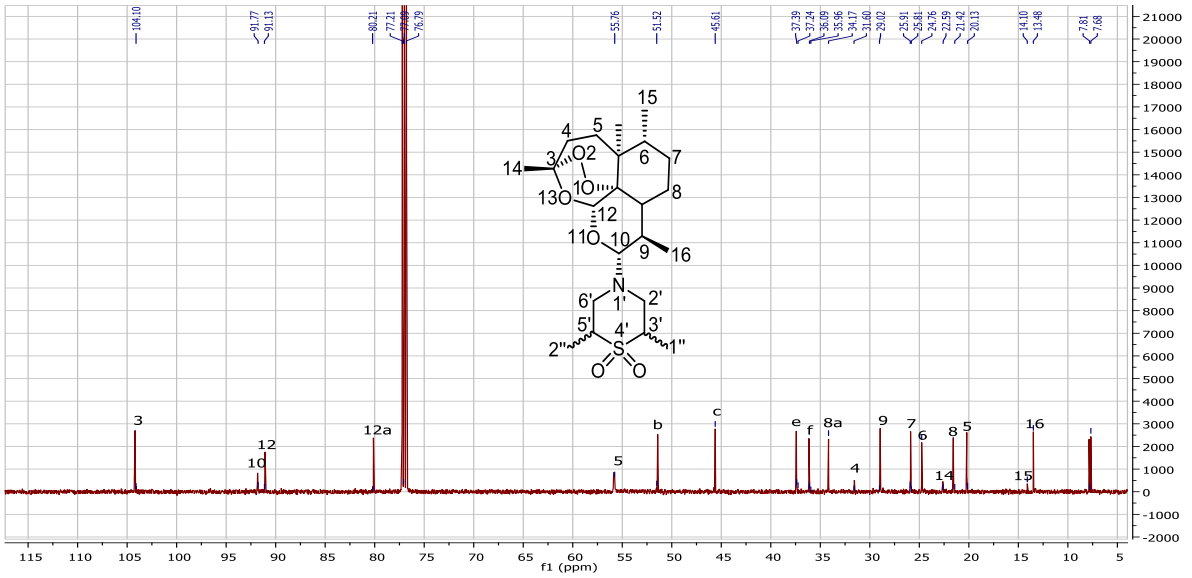
IR



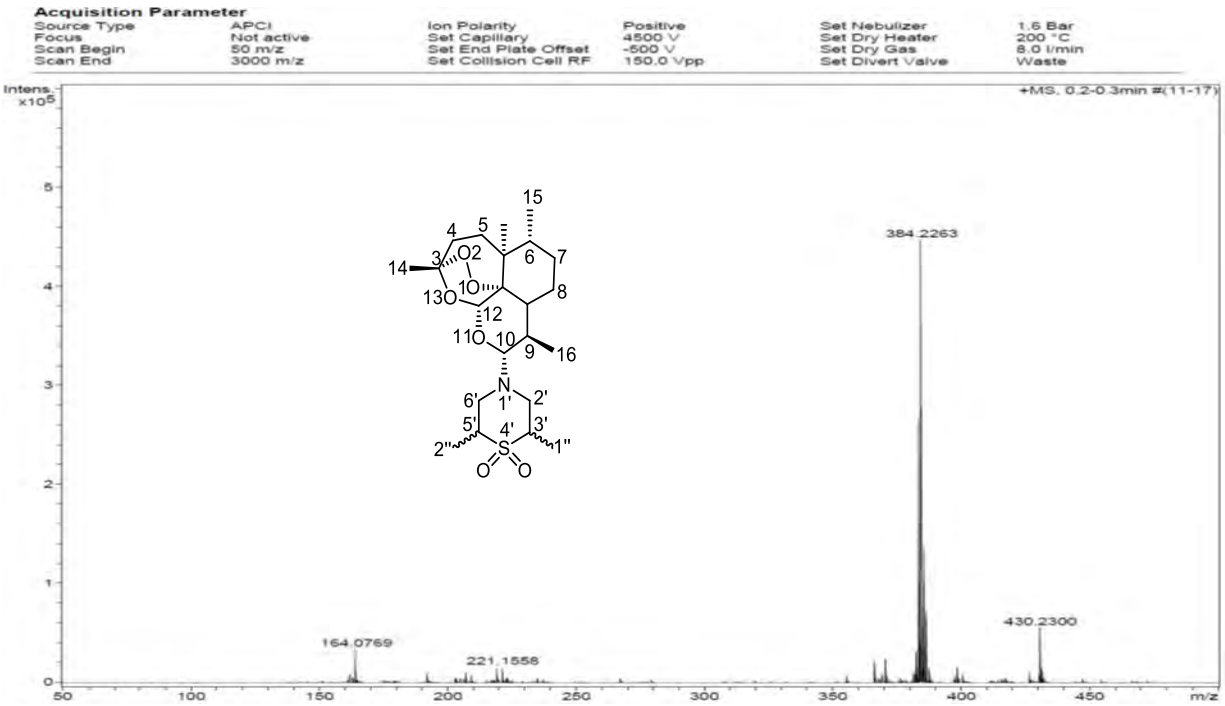
^{1}H NMR



^{13}C NMR

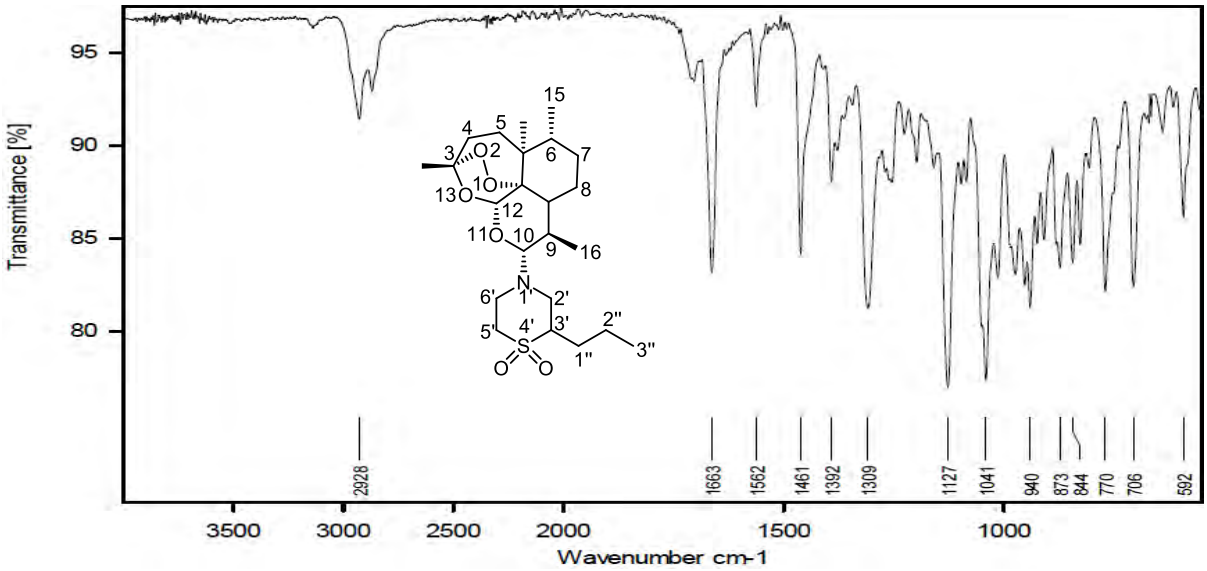

f1 (ppm)

HRMS

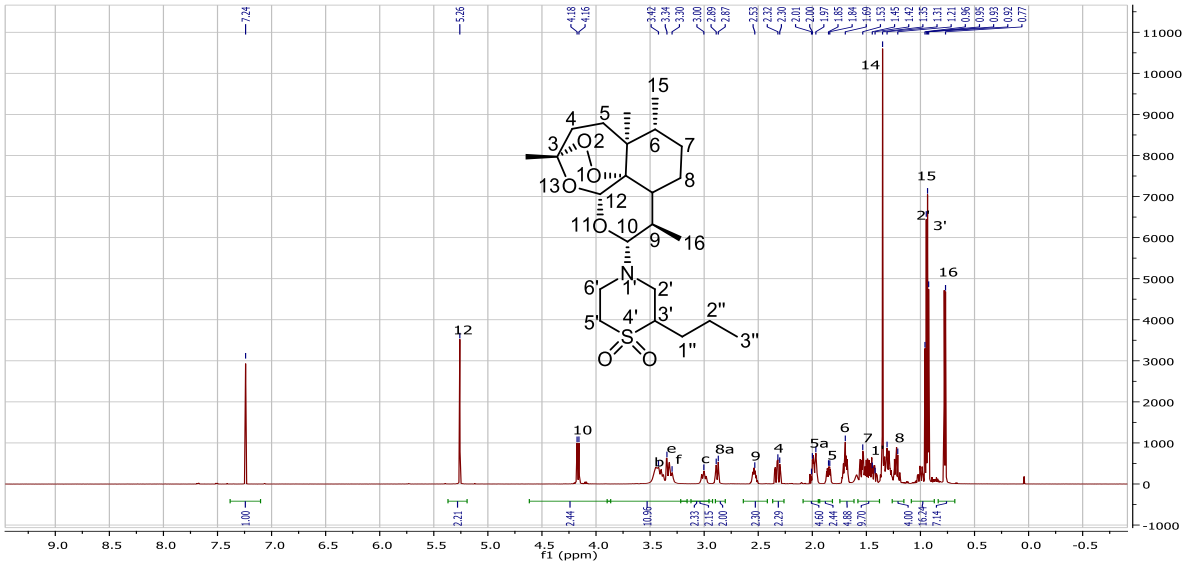

Acquisition Parameter
Source Type APCI
Ion Polarity Positive
Set Nebulizer 1.6 Bar
Focus Not active
Set Capillary 4500 V
Set Dry Heater 200 °C
Scan Begin 50 m/z
Set End Plate Offset -500 V
Set Dry Gas 8.0 l/min
Scan End 3000 m/z
Set Collision Cell RF 150.0 Vpp
Set Divert Valve Waste
+MS, 0.2-0.3min #(11-17)
384.2263
430.2300
164.0769
221.1556
m/z

Compound 7:

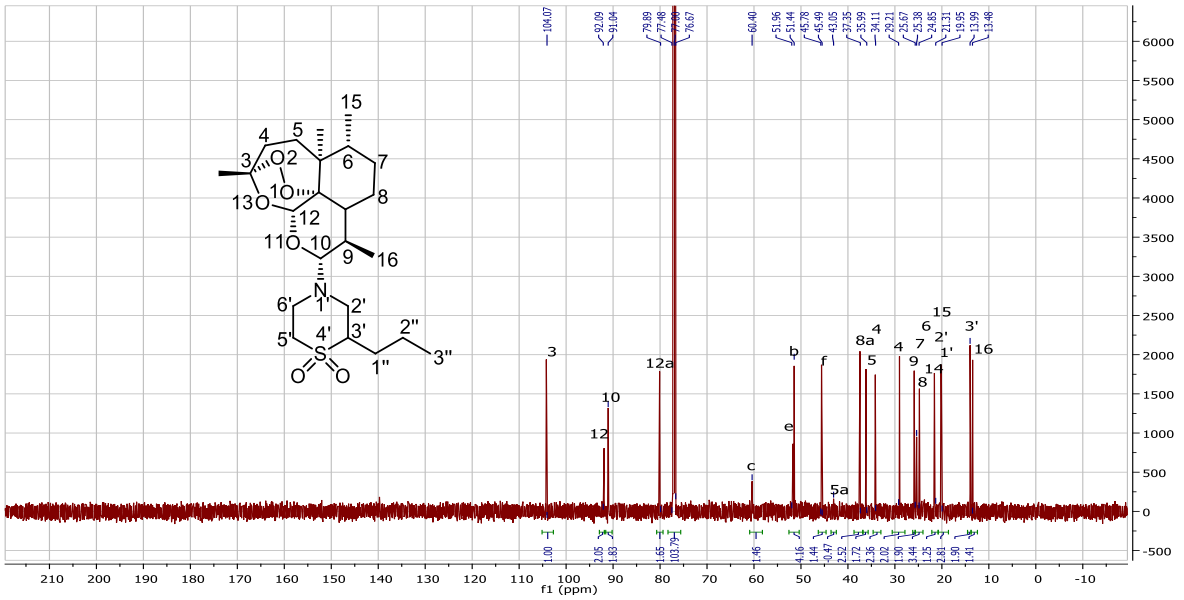
IR



^{1}H NMR



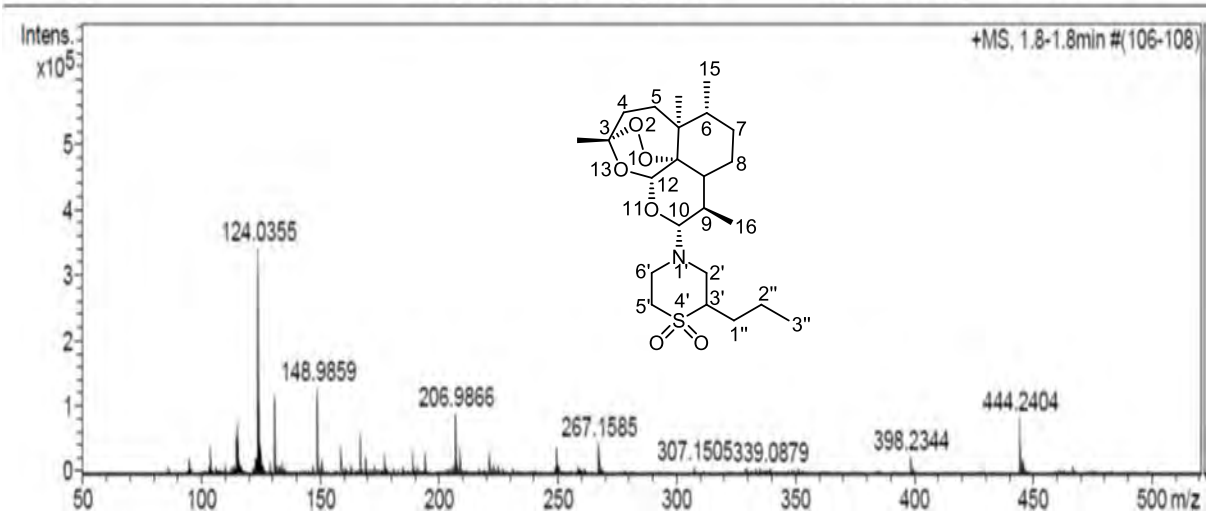
^{13}C NMR



HRMS

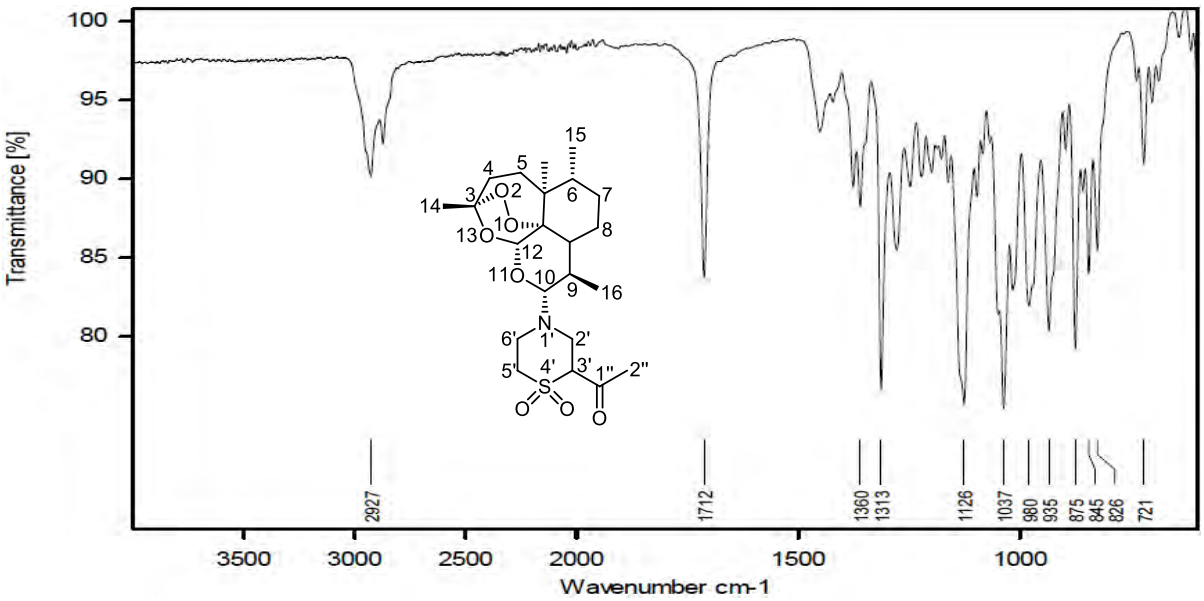
Acquisition Parameter

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	3000 m/z	Set Collision Cell RF	150.0 Vpp	Set Divert Valve	Waste

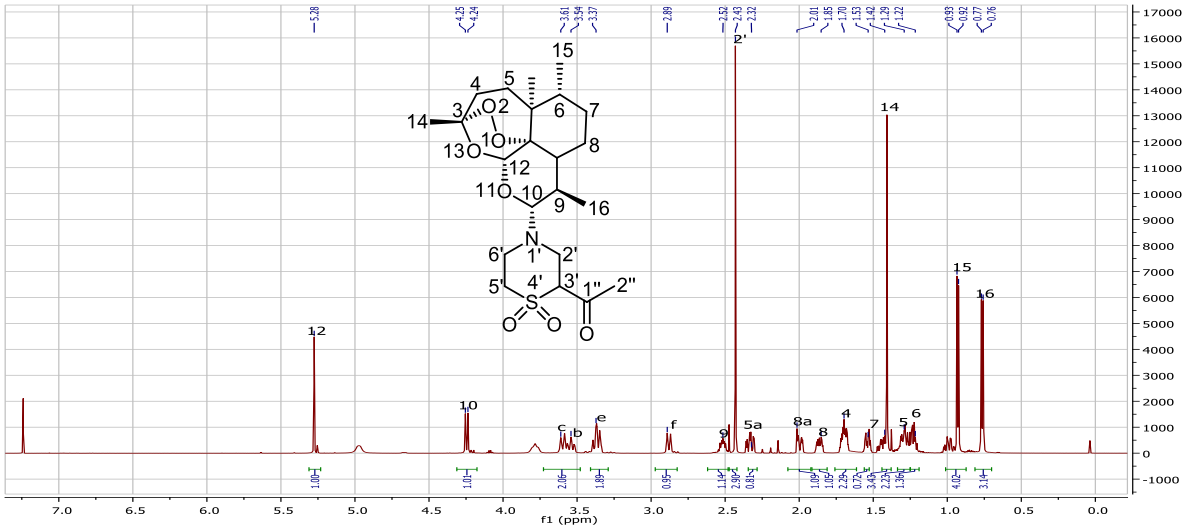


Compound 8:

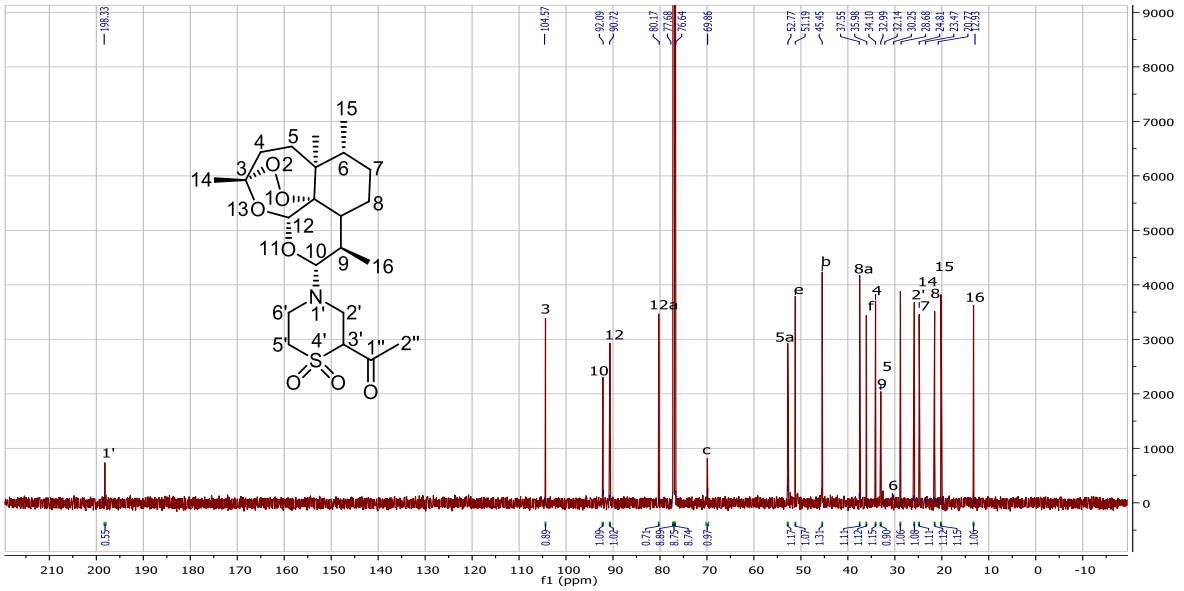
IR



^{1}H NMR



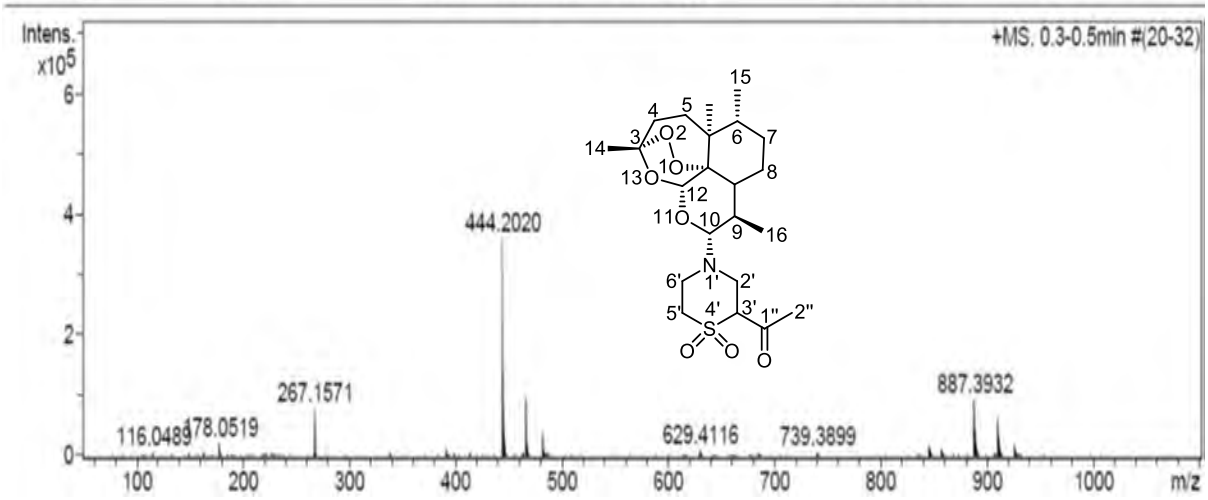
^{13}C NMR



HRMS

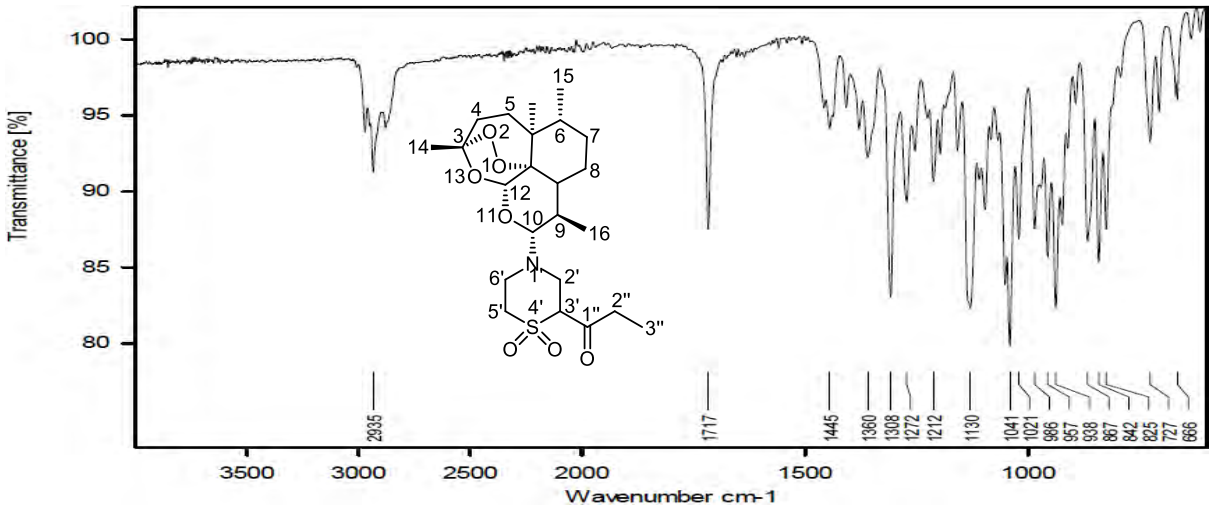
Acquisition Parameter

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	3000 m/z	Set Collision Cell RF	150.0 Vpp	Set Divert Valve	Waste

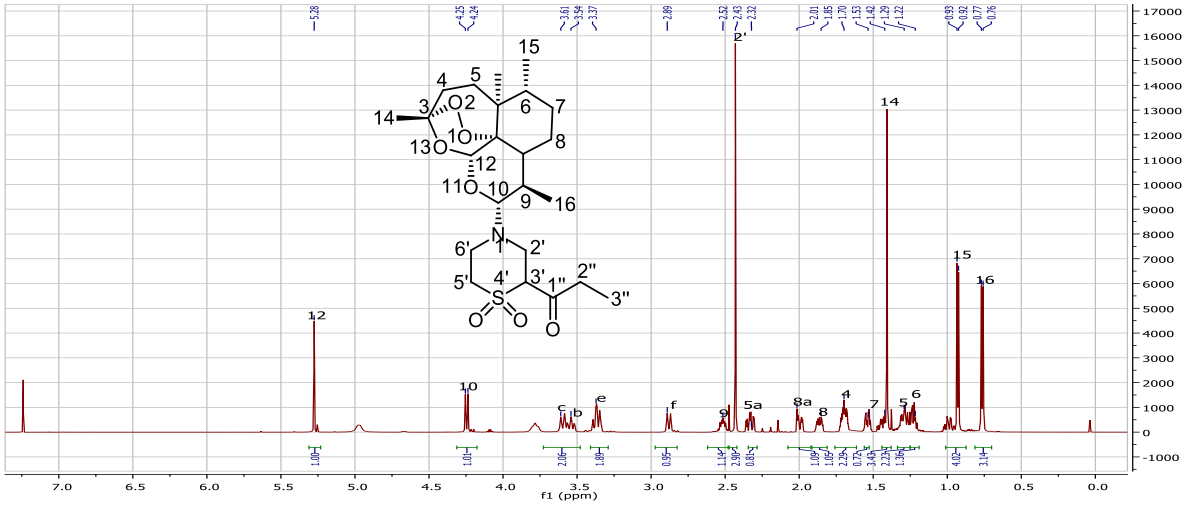


Compound 9:

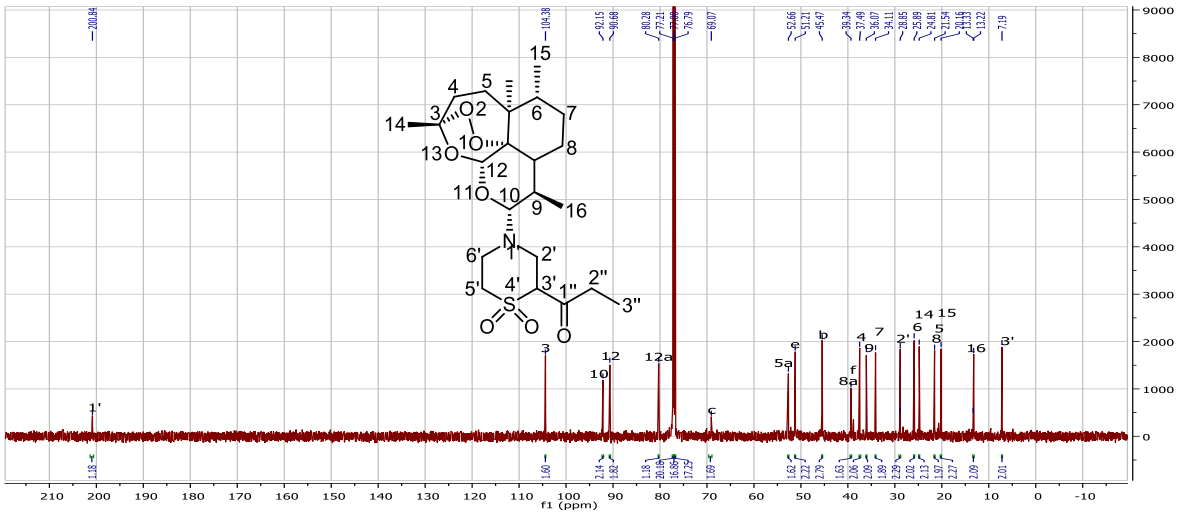
IR



^{1}H NMR



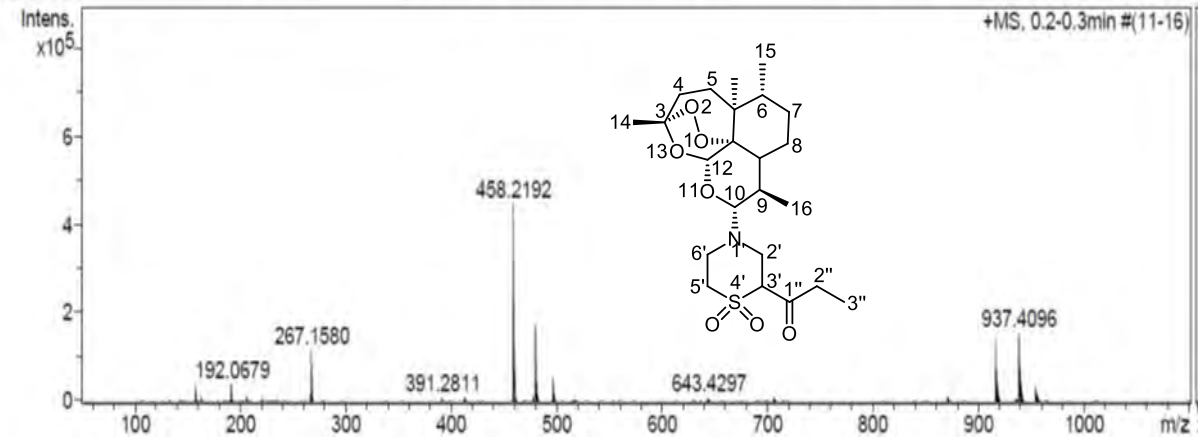
^{13}C NMR



HRMS

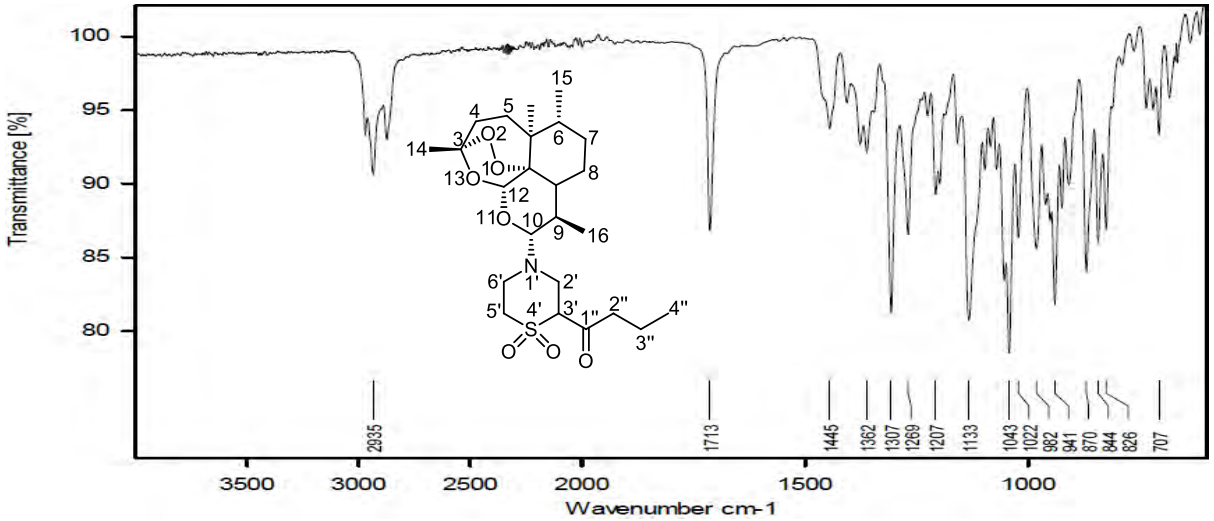
Acquisition Parameter

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	3000 m/z	Set Collision Cell RF	150.0 Vpp	Set Divert Valve	Waste

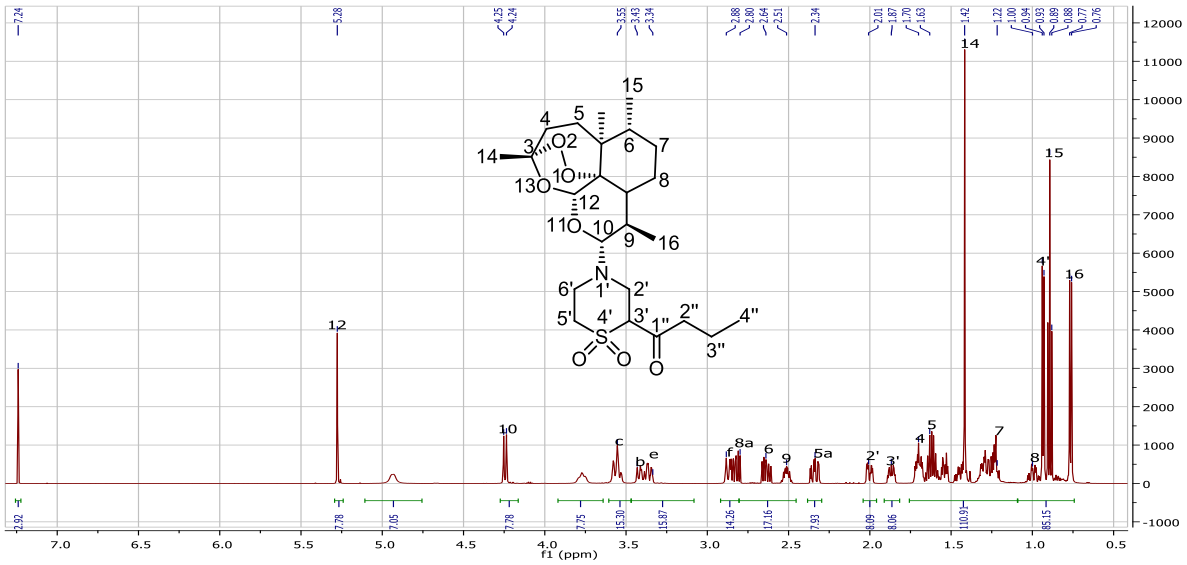


Compound 10:

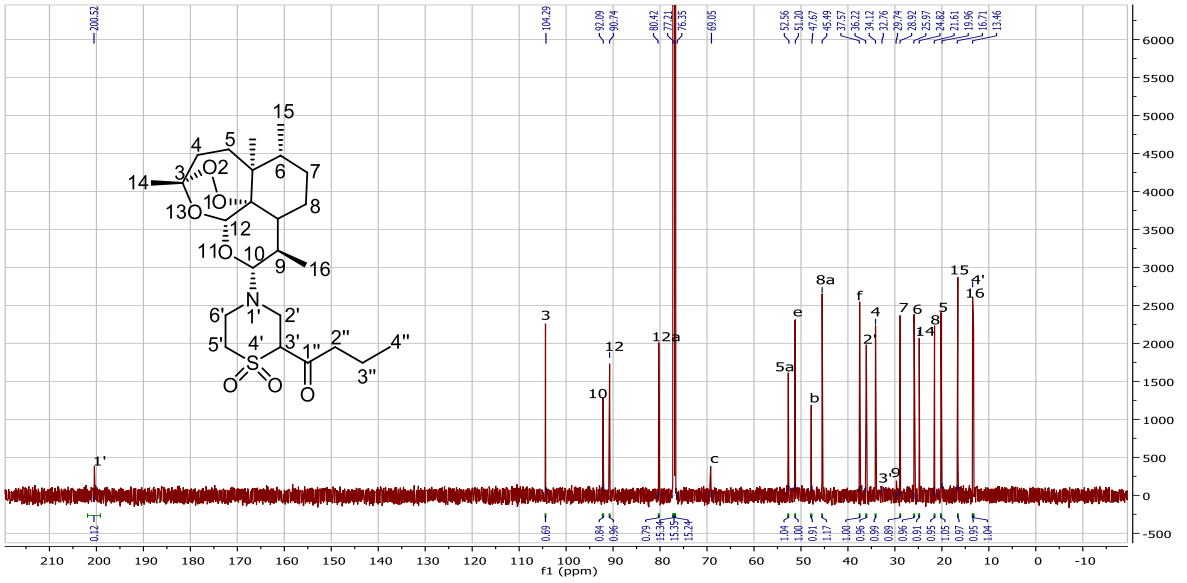
IR



^{1}H NMR



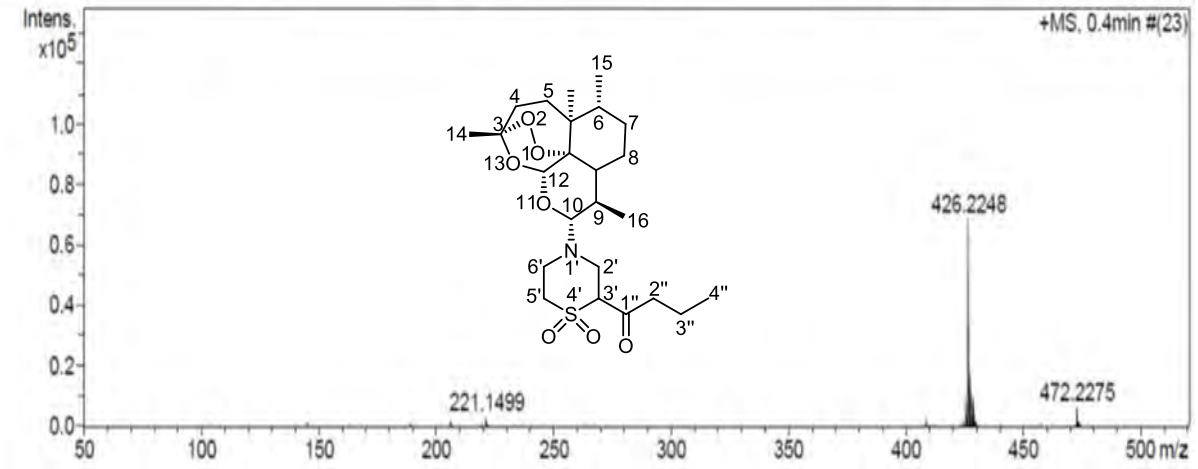
^{13}C NMR



HRMS

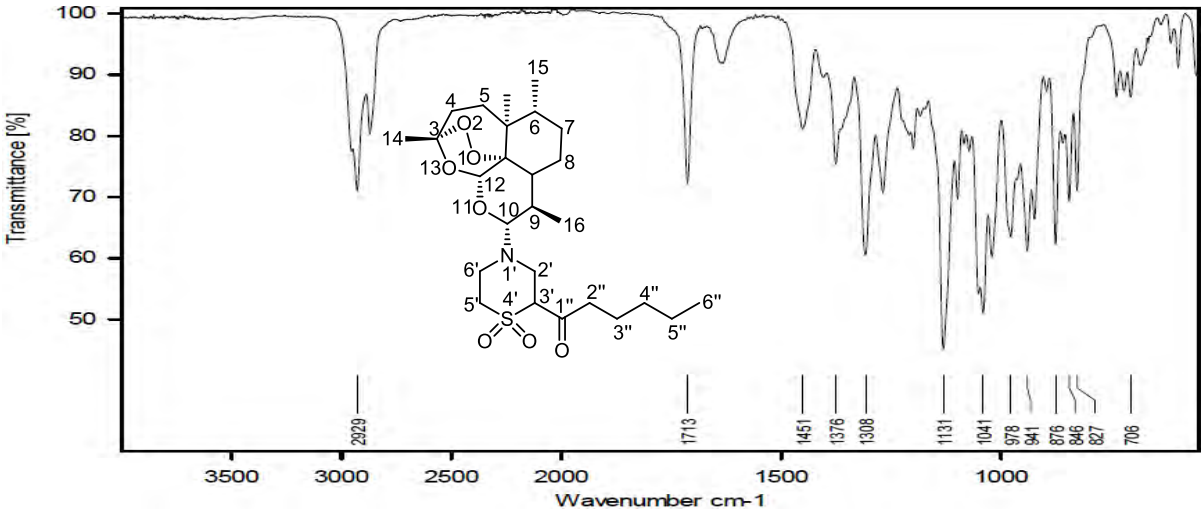
Acquisition Parameter

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	3000 m/z	Set Collision Cell RF	150.0 Vpp	Set Divert Valve	Waste

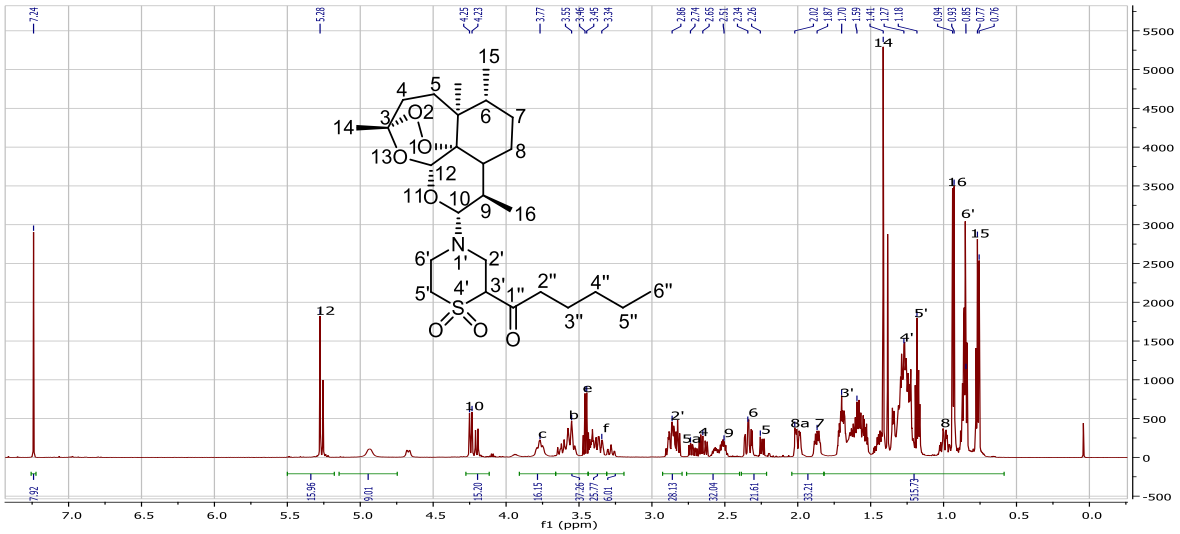


Compound 11:

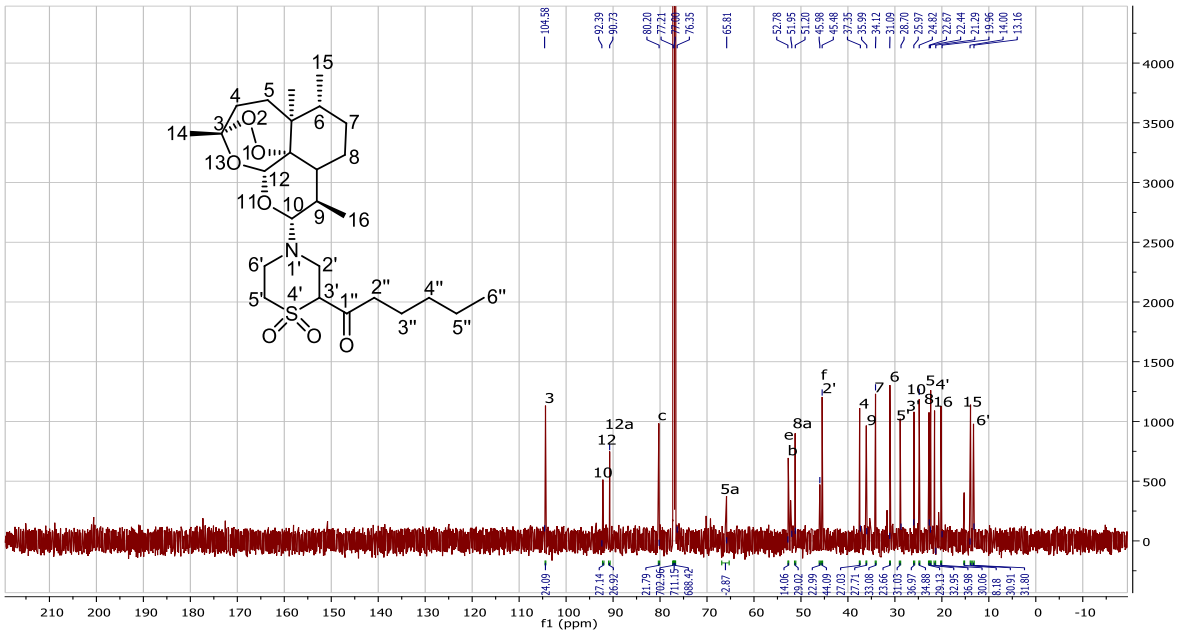
IR



^{1}H NMR



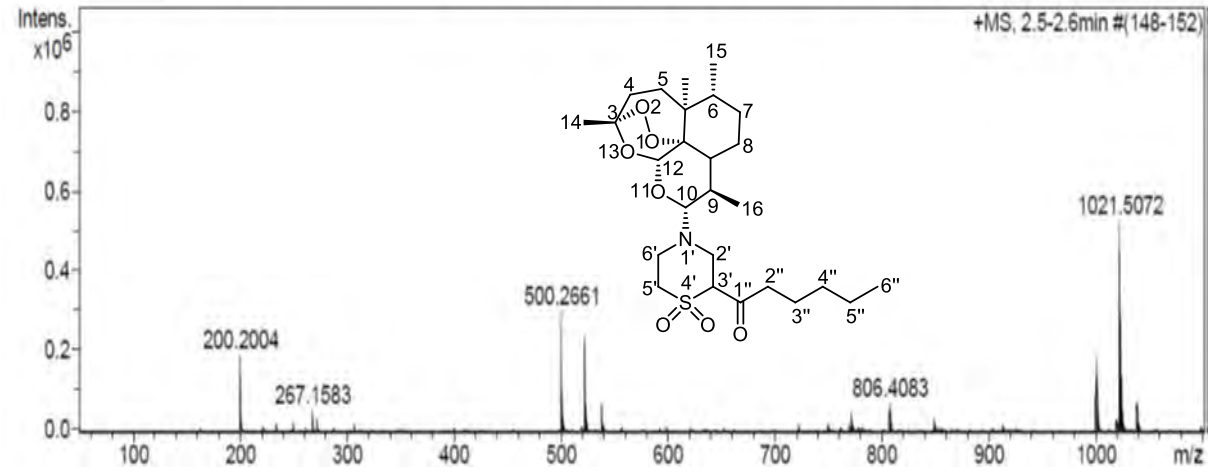
^{13}C NMR



HRMS

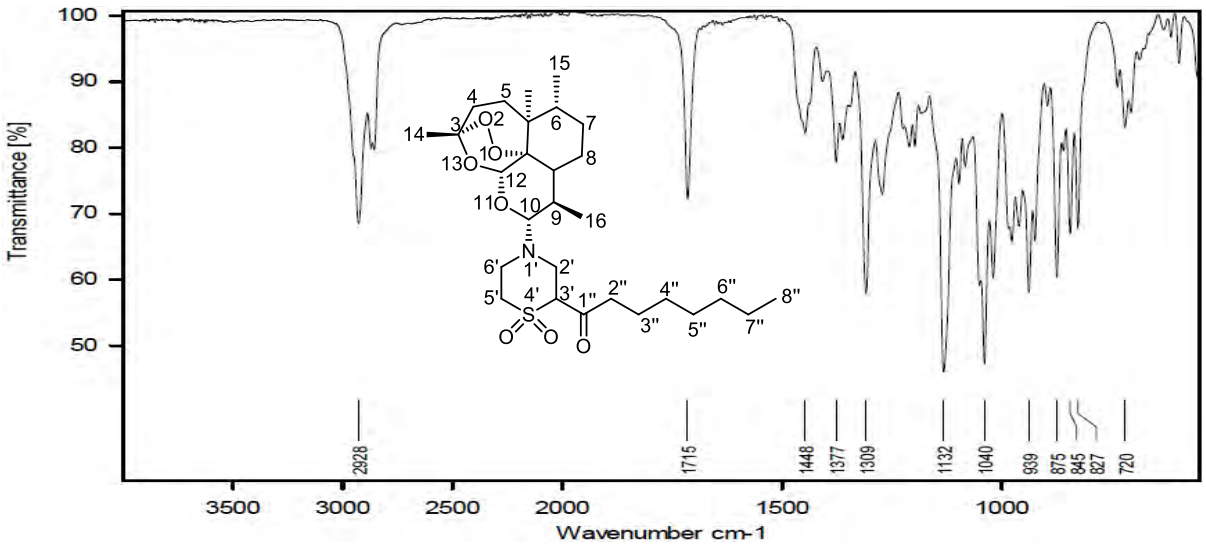
Acquisition Parameter

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	3000 m/z	Set Collision Cell RF	150.0 Vpp	Set Divert Valve	Waste

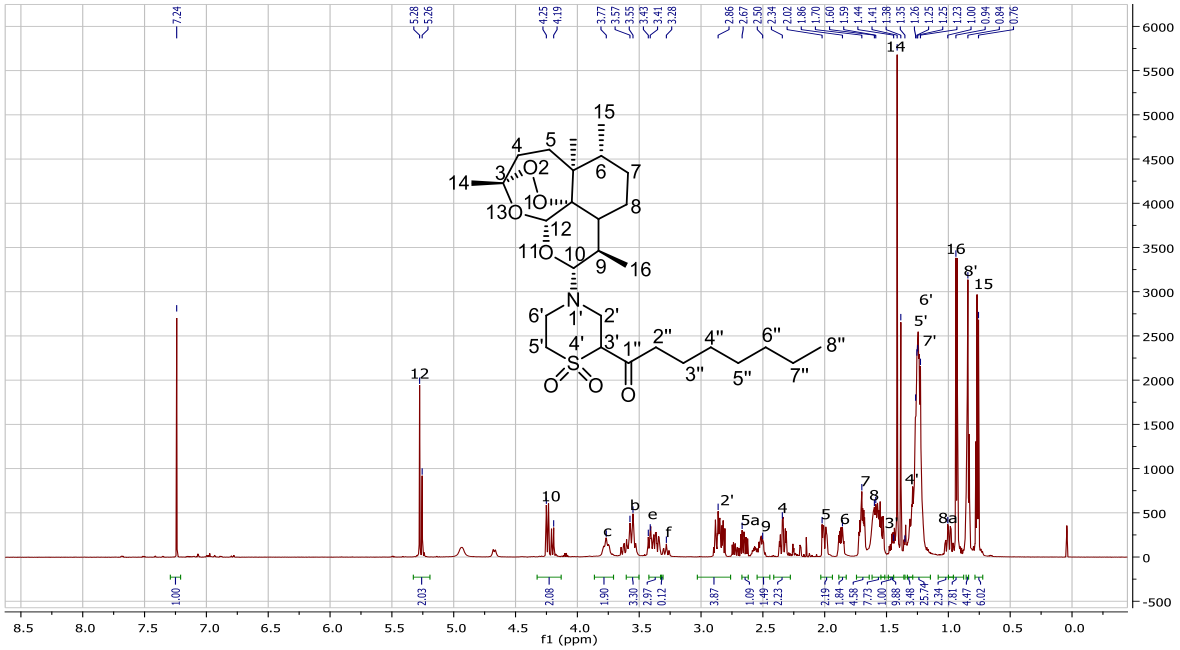


Compound 12:

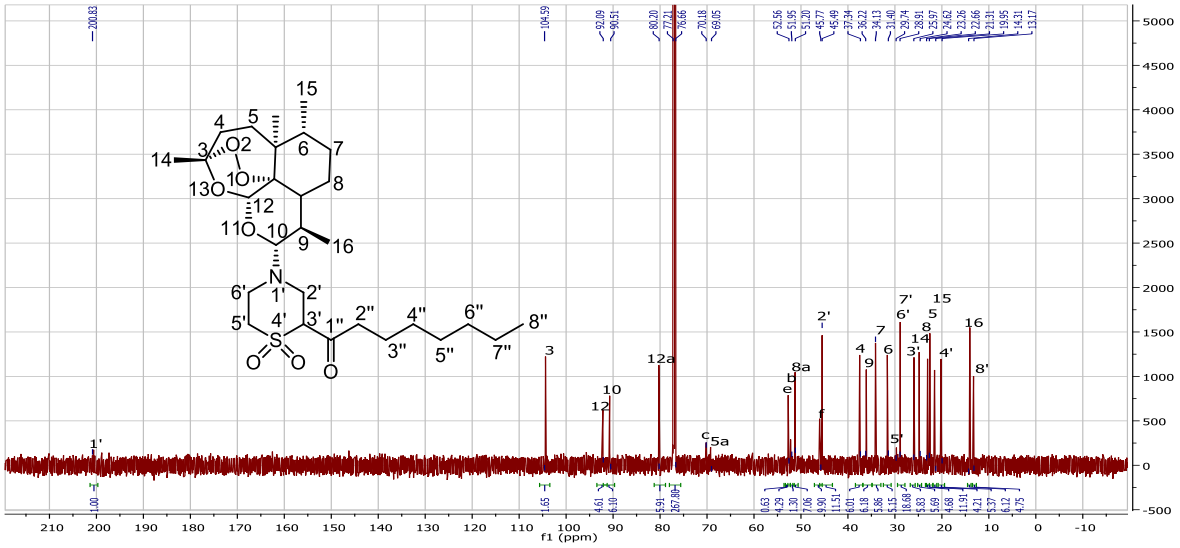
IR



^{1}H NMR



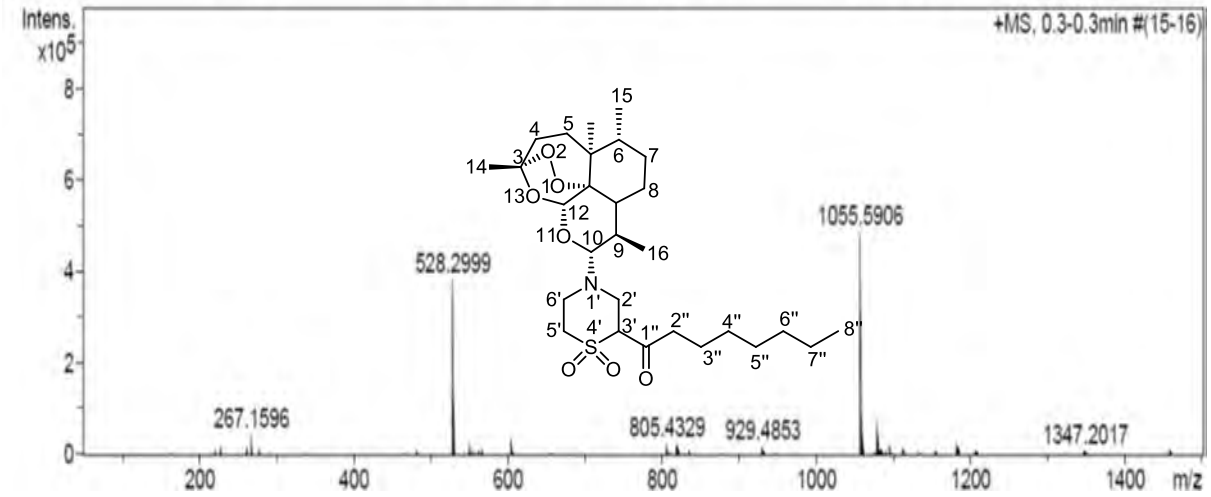
^{13}C NMR



HRMS

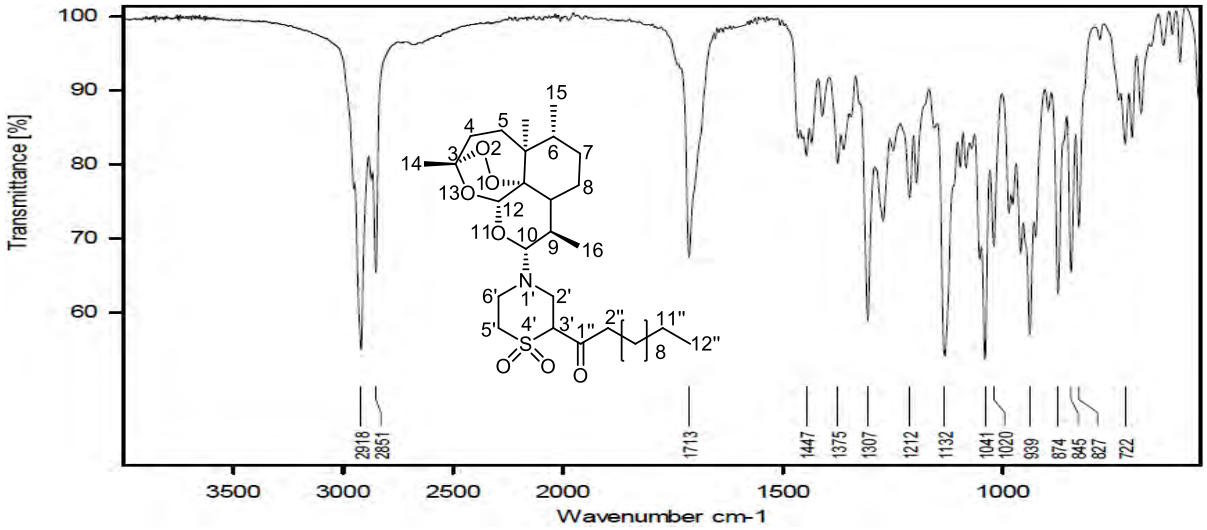
Acquisition Parameter

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	3000 m/z	Set Collision Cell RF	150.0 Vpp	Set Divert Valve	Waste

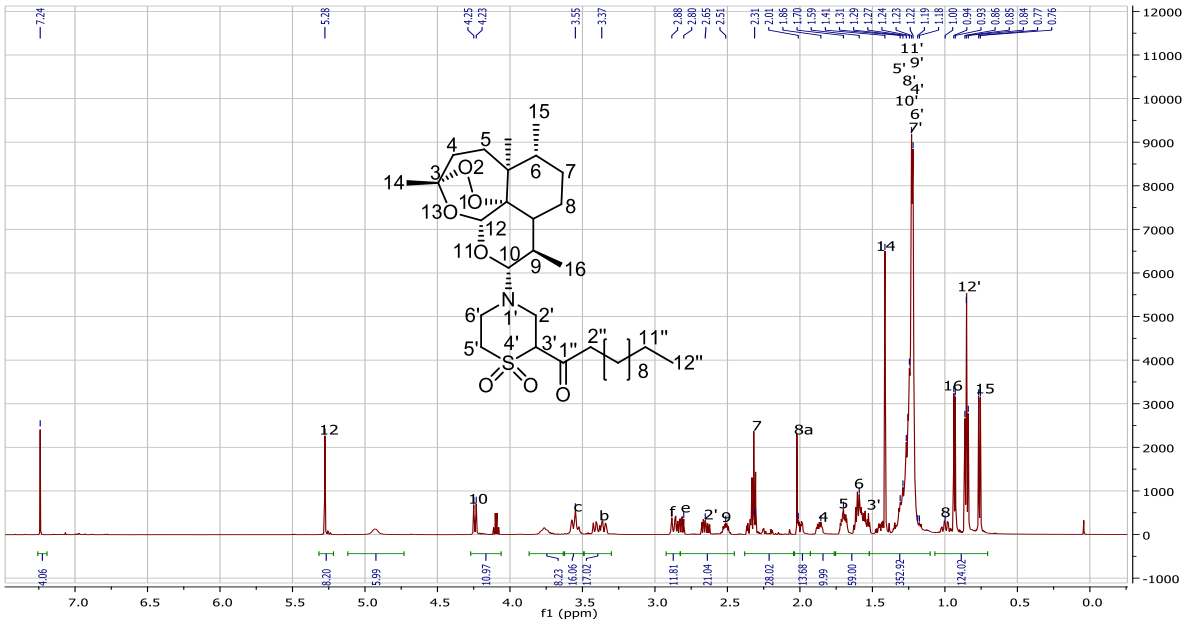


Compound 13:

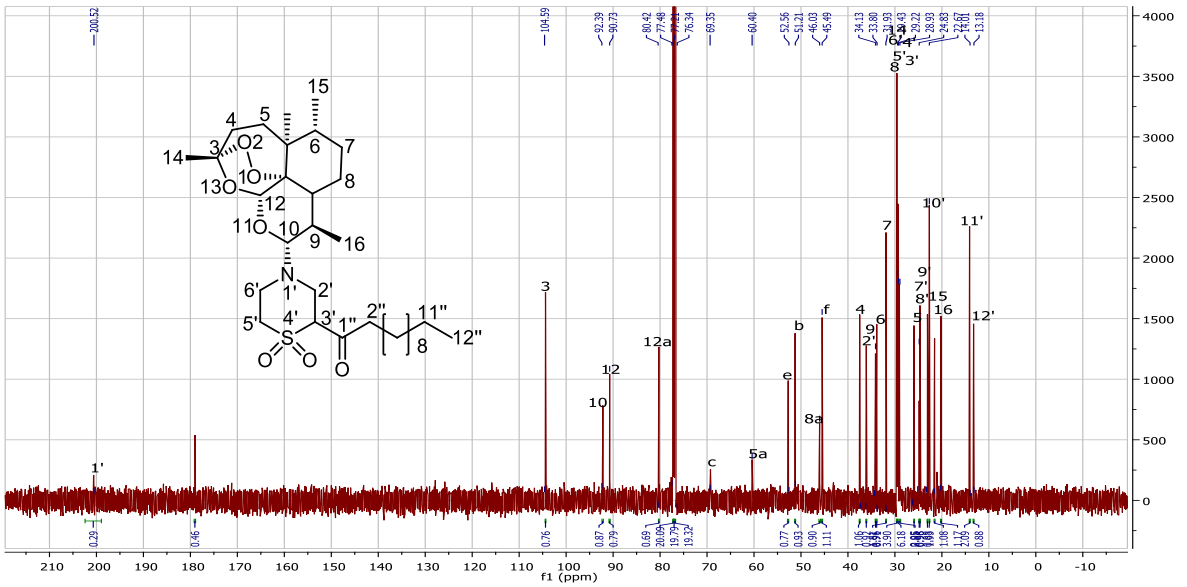
IR



^{1}H NMR



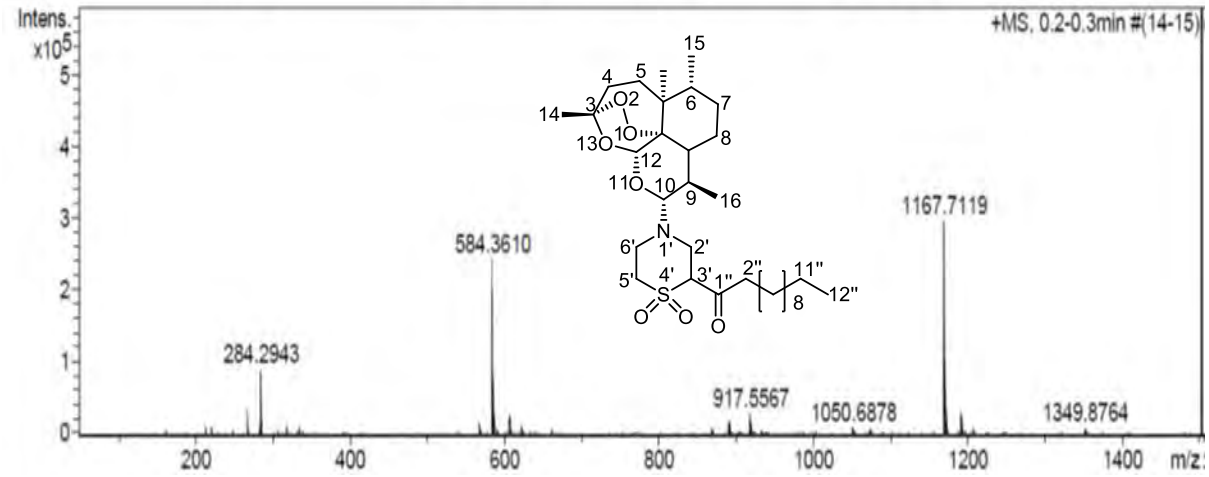
^{13}C NMR



HRMS

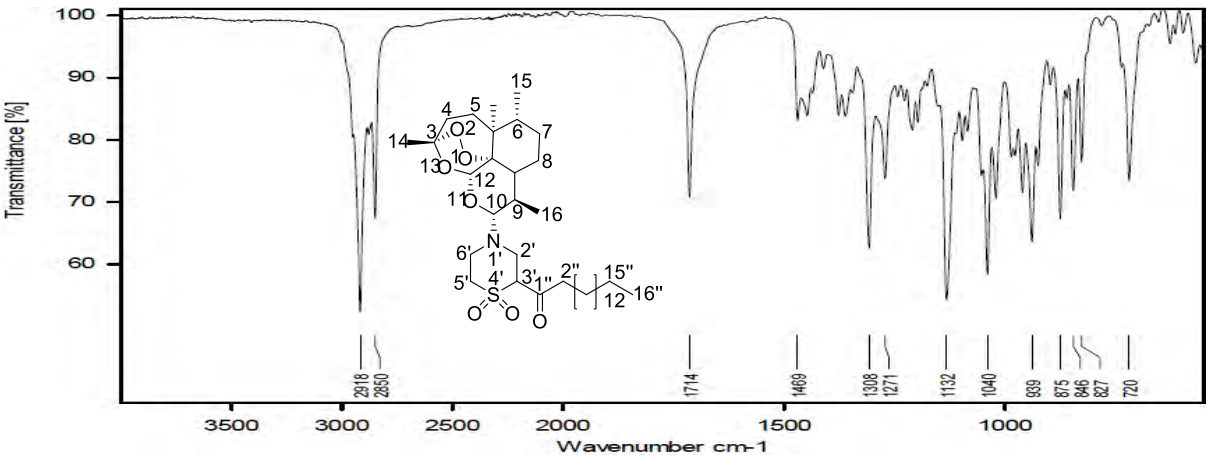
Acquisition Parameter

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	3000 m/z	Set Collision Cell RF	150.0 Vpp	Set Divert Valve	Waste

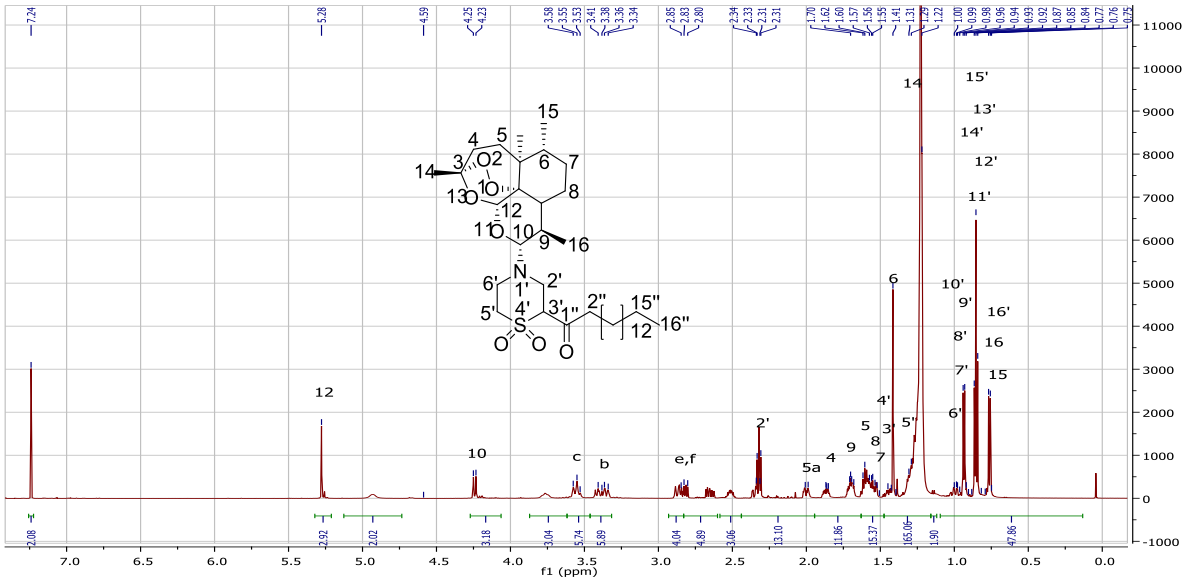


Compound 14:

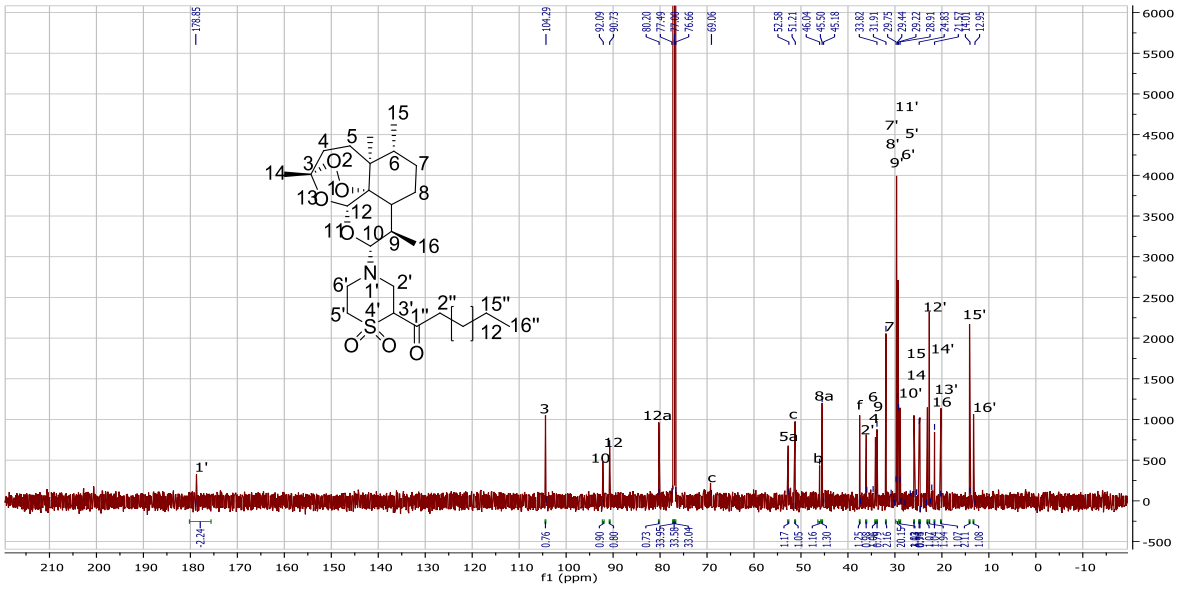
IR



^{1}H NMR



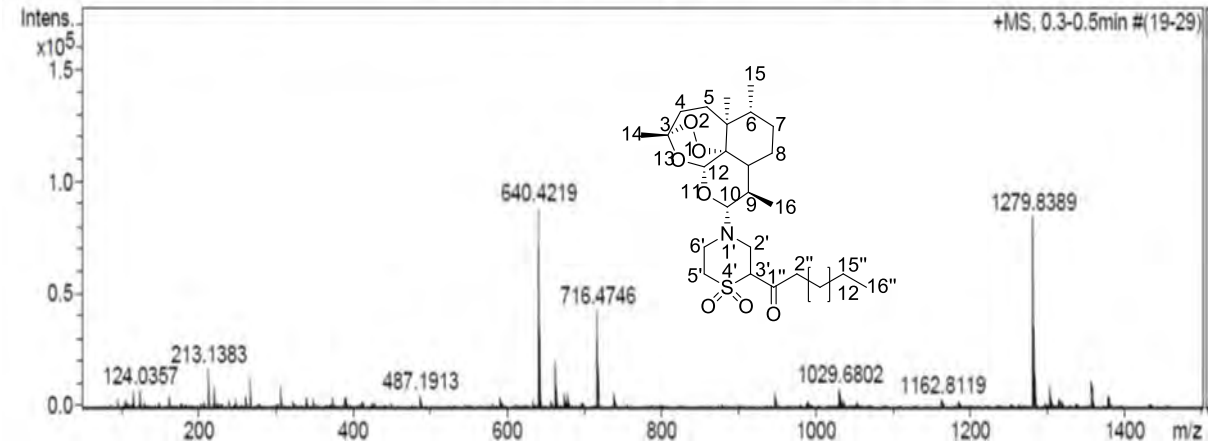
^{13}C NMR



HRMS

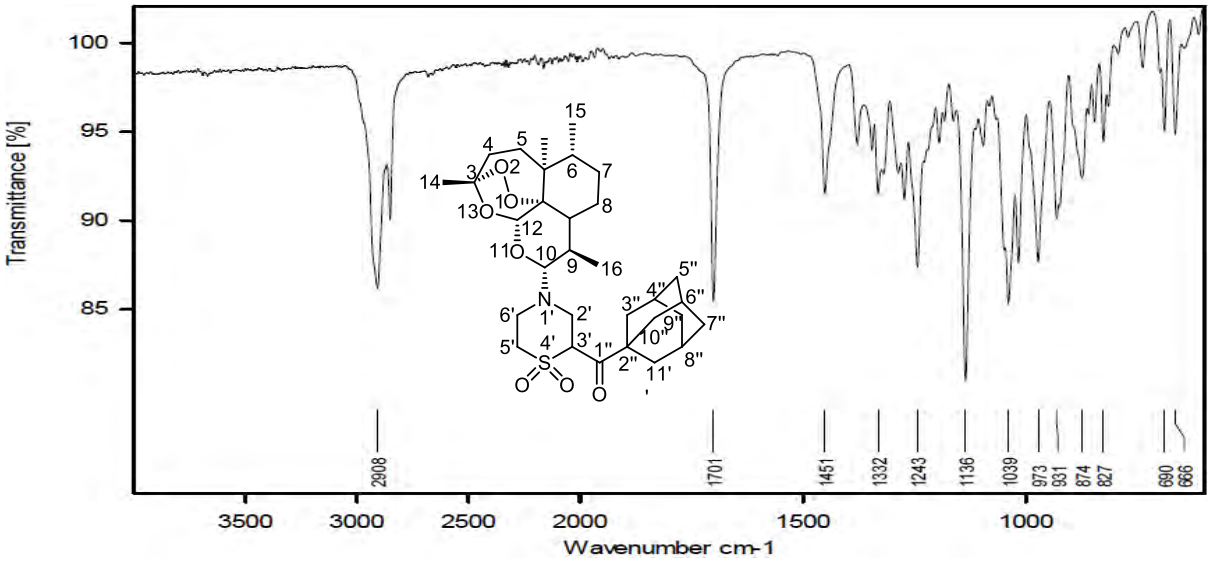
Acquisition Parameter

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	3000 m/z	Set Collision Cell RF	150.0 Vpp	Set Divert Valve	Waste

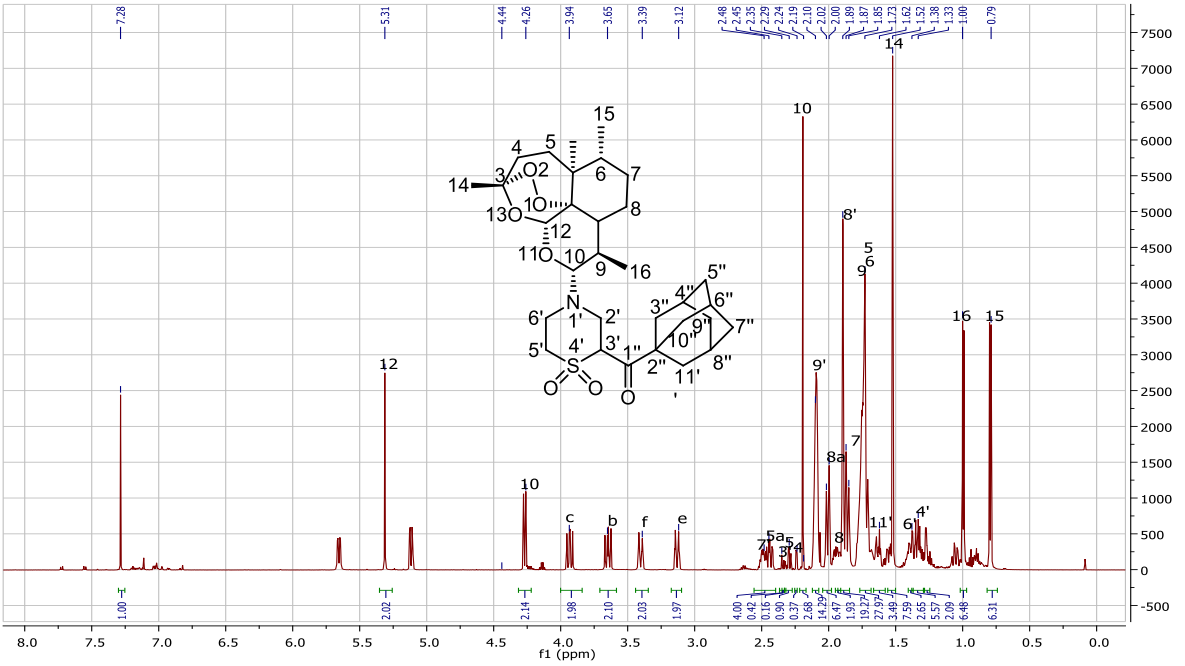


Compound 15:

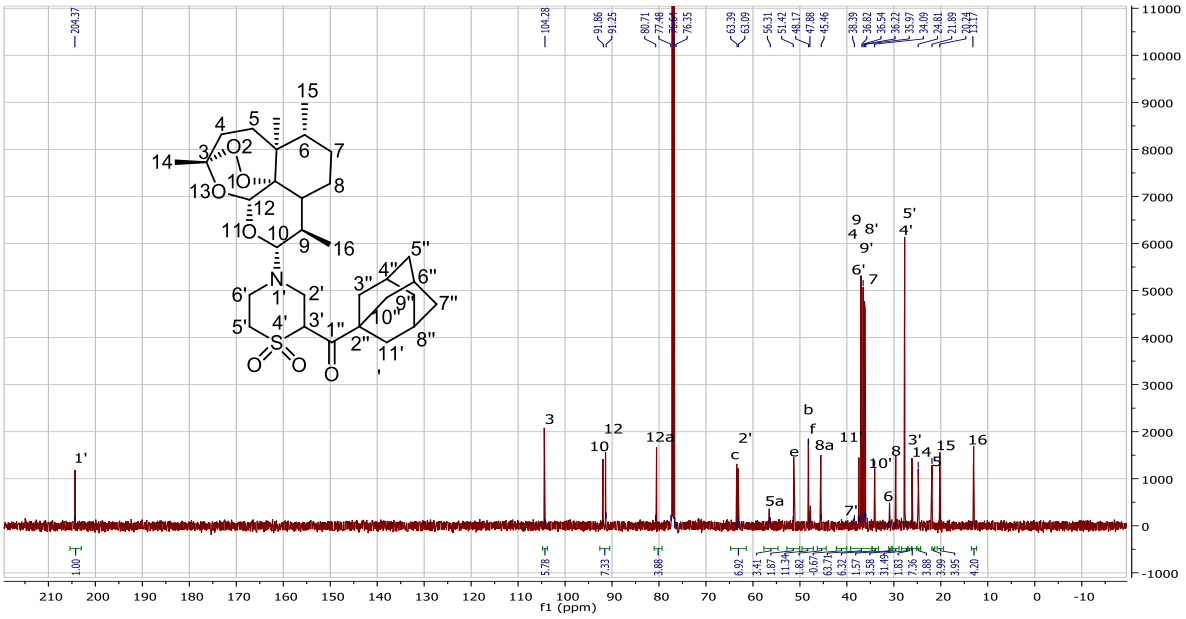
IR



^{1}H NMR



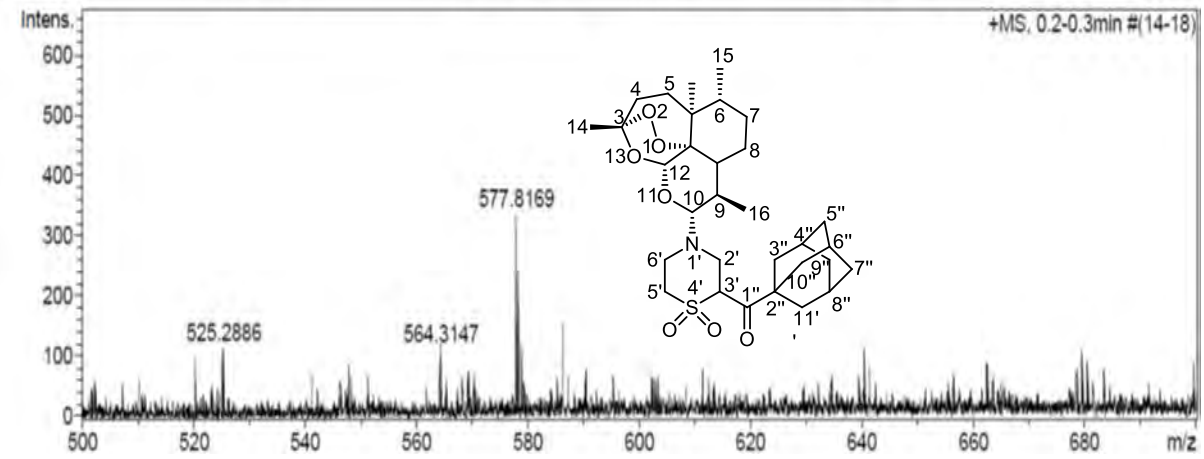
^{13}C NMR



HRMS

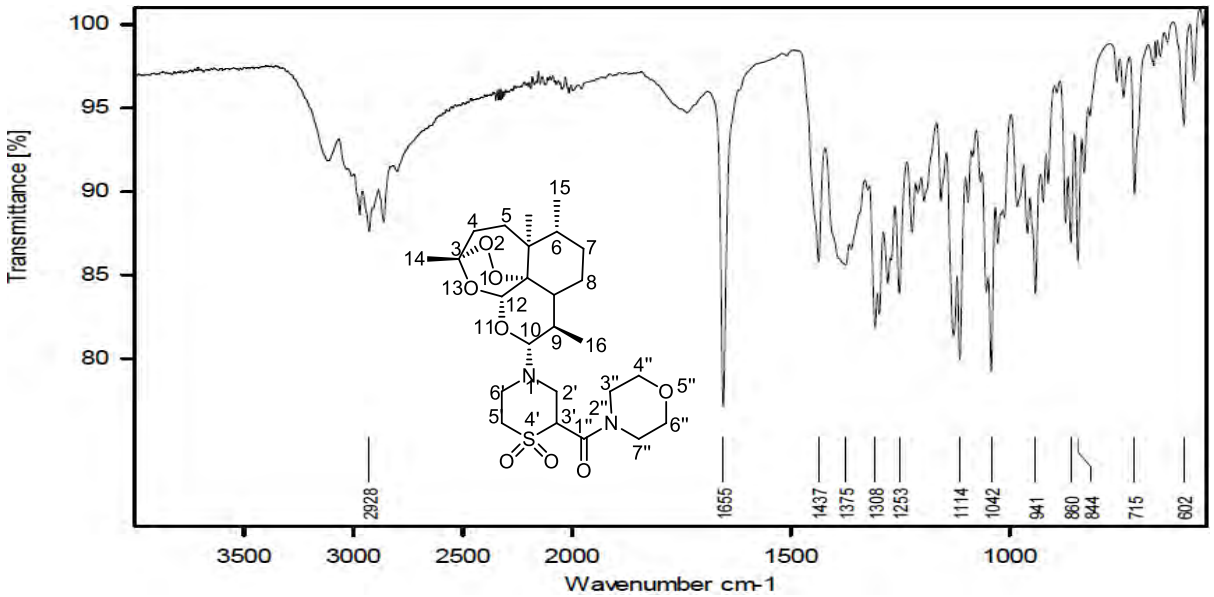
Acquisition Parameter

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	3000 m/z	Set Collision Cell RF	150.0 Vpp	Set Divert Valve	Waste

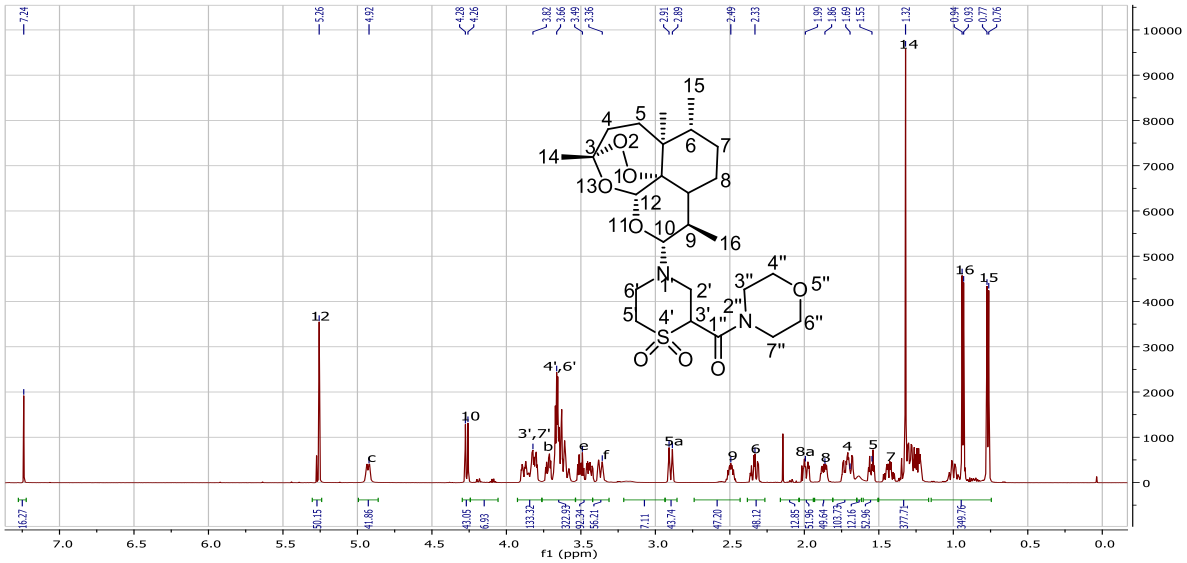


Compound 16:

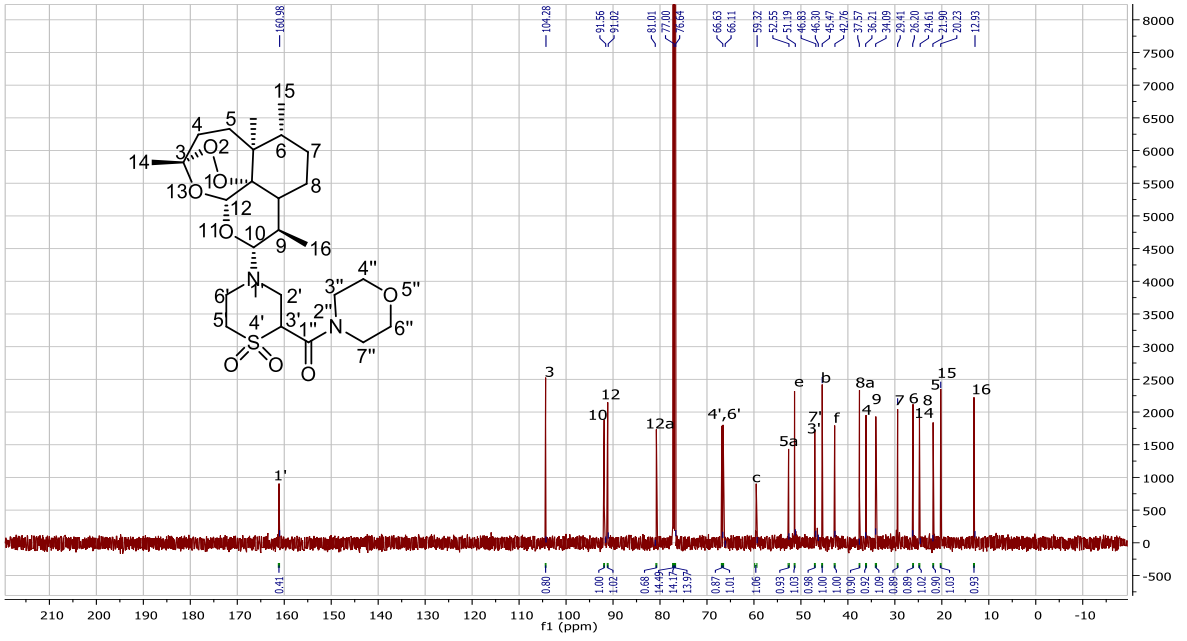
IR



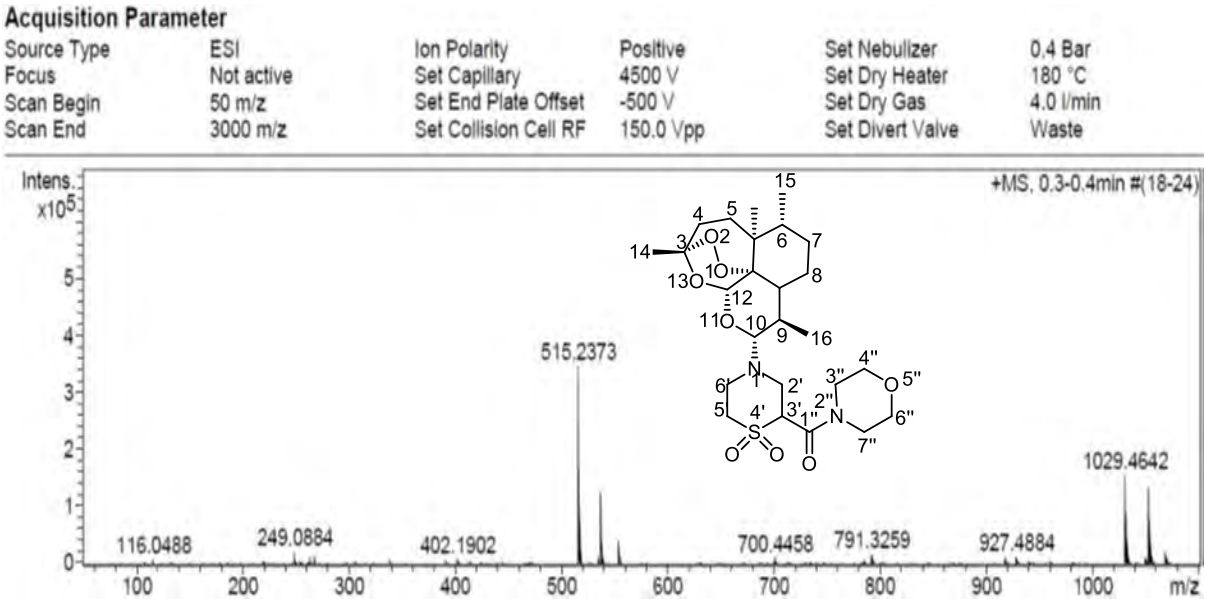
^{1}H NMR



^{13}C NMR

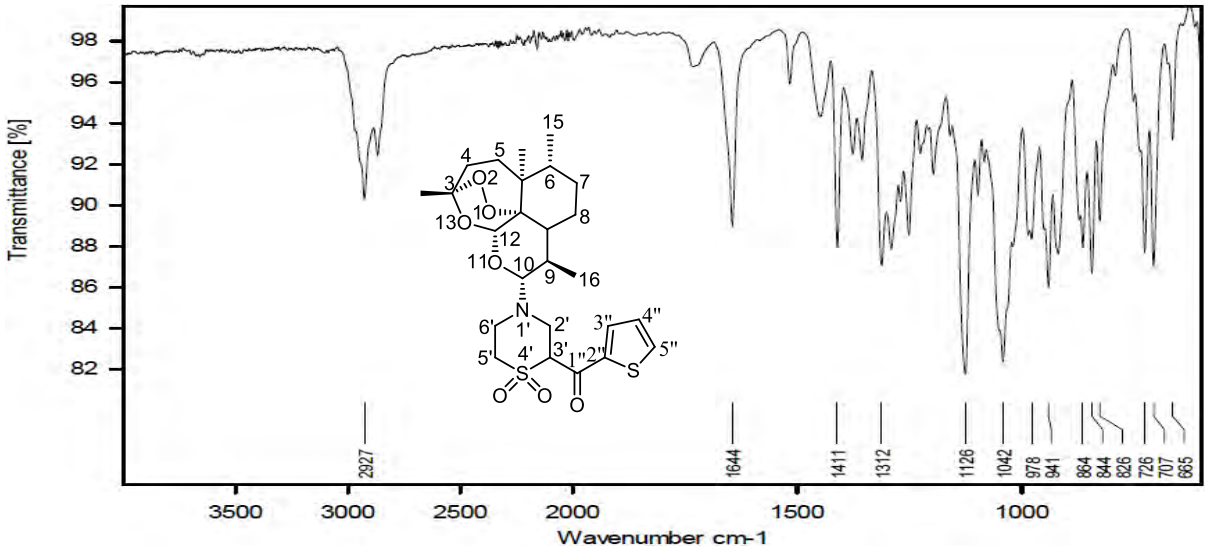


HRMS

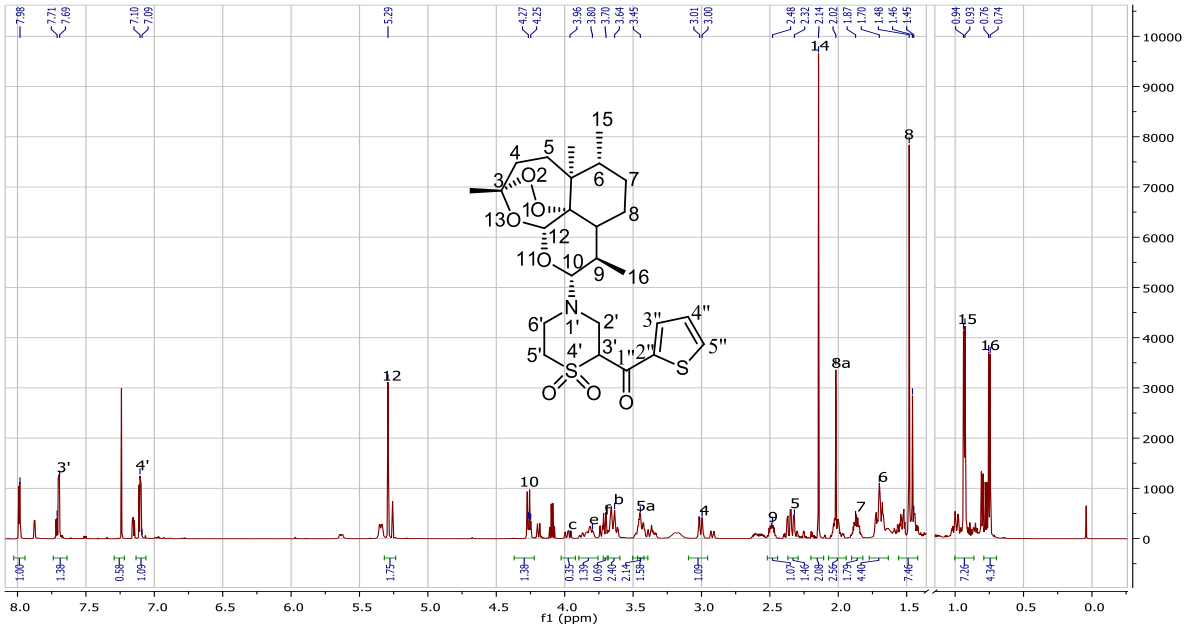


Compound 17:

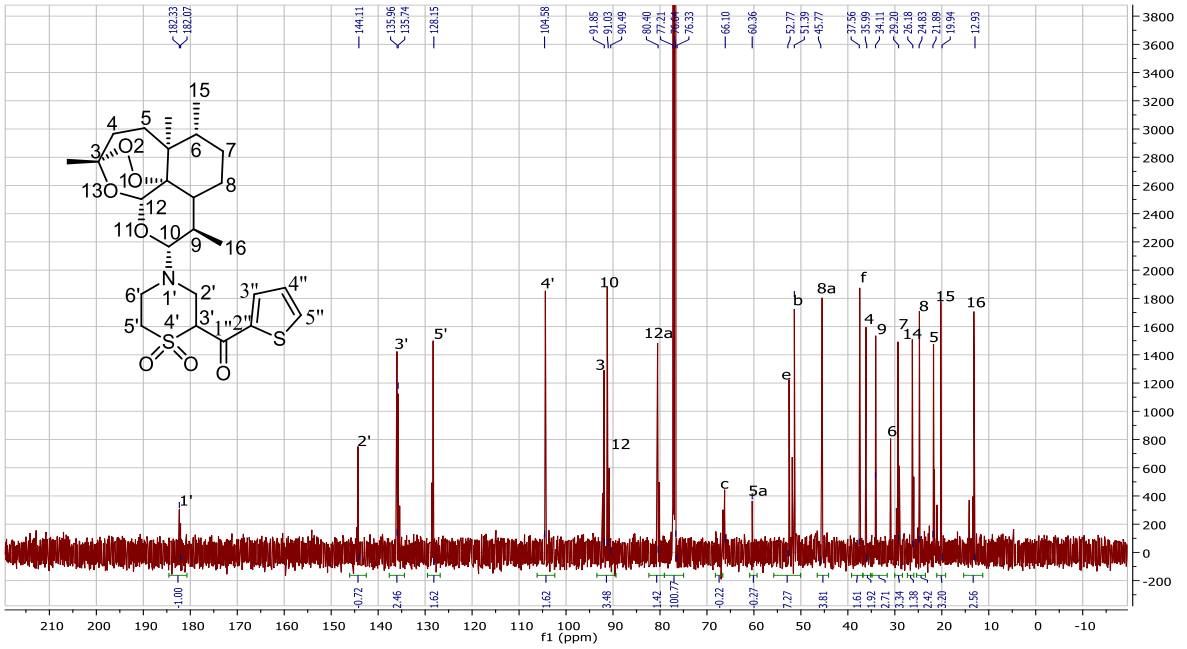
IR



^{1}H NMR



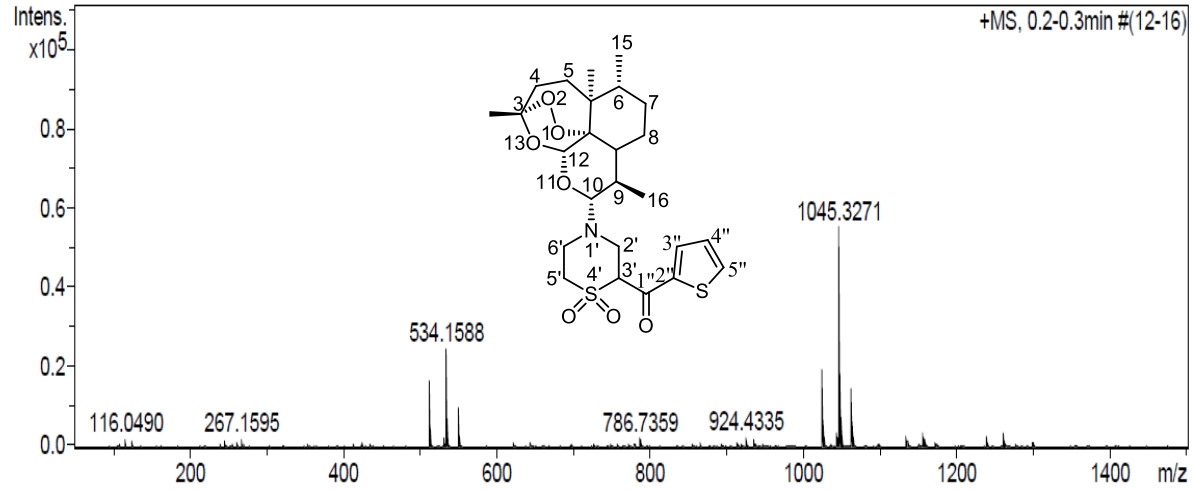
^{13}C NMR



HRMS

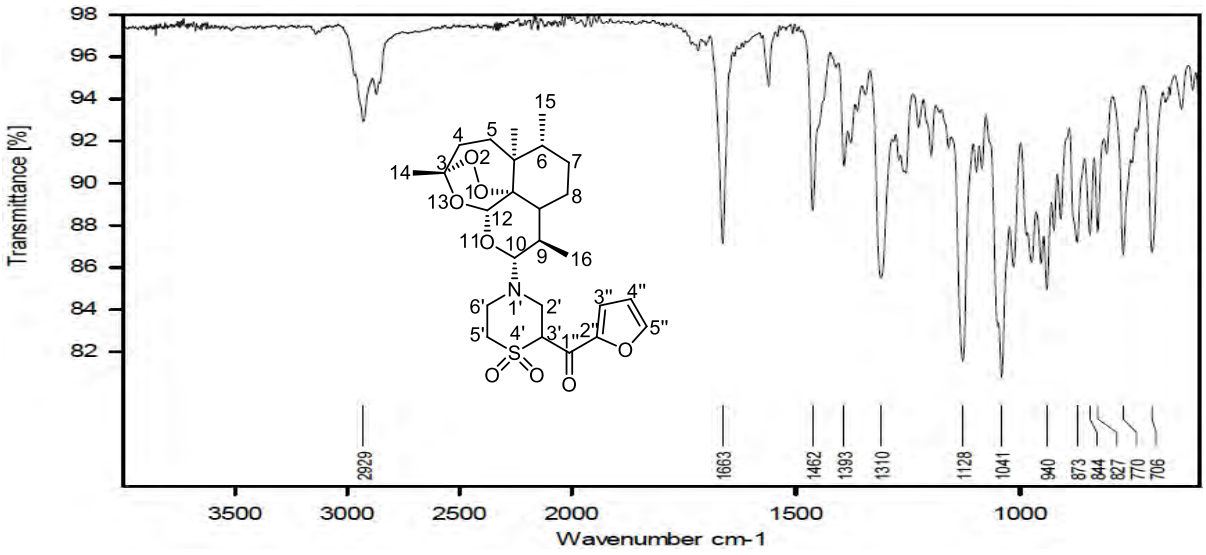
Acquisition Parameter

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	3000 m/z	Set Collision Cell RF	150.0 Vpp	Set Divert Valve	Waste

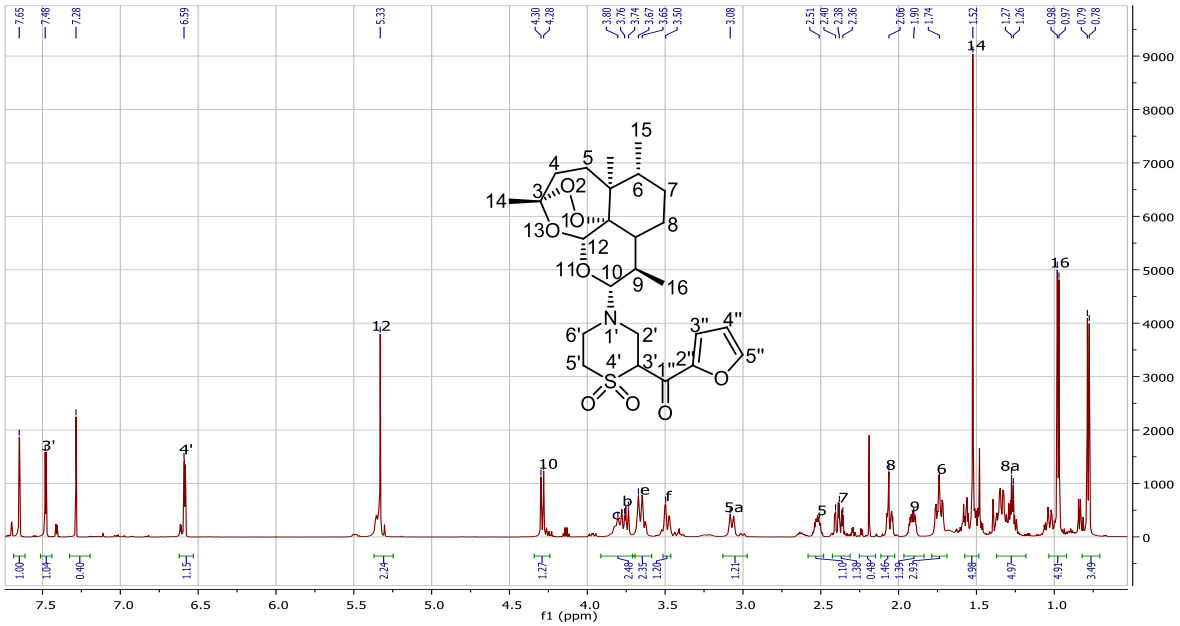


Compound 18:

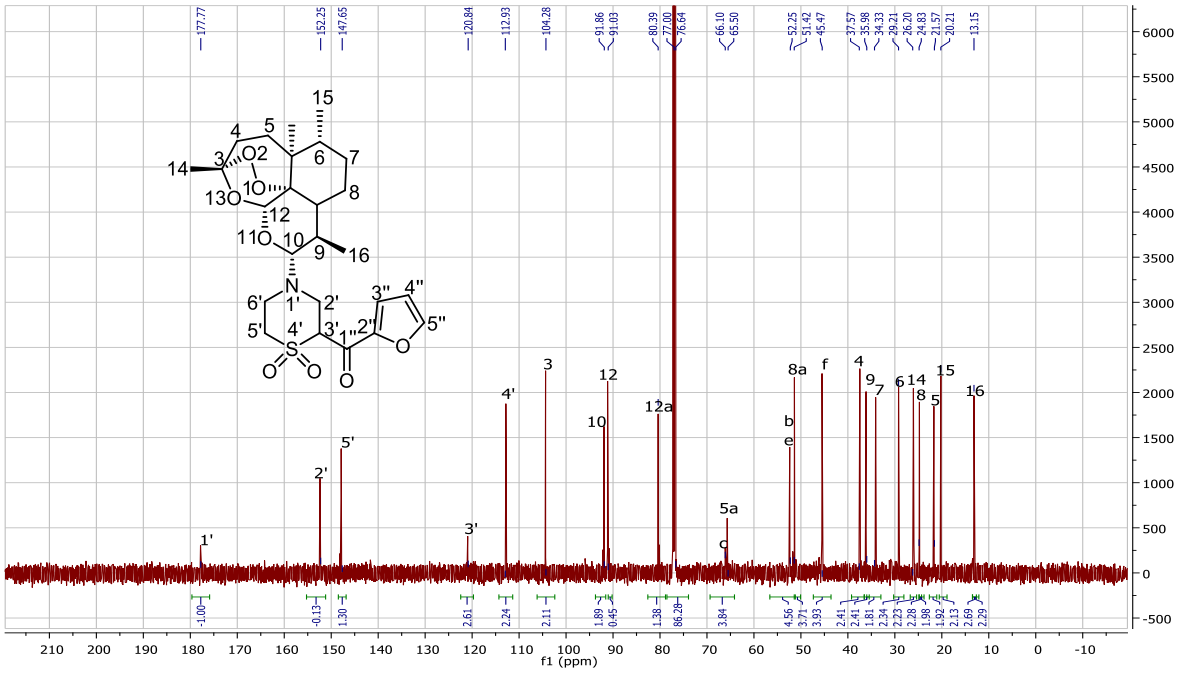
IR



^{1}H NMR



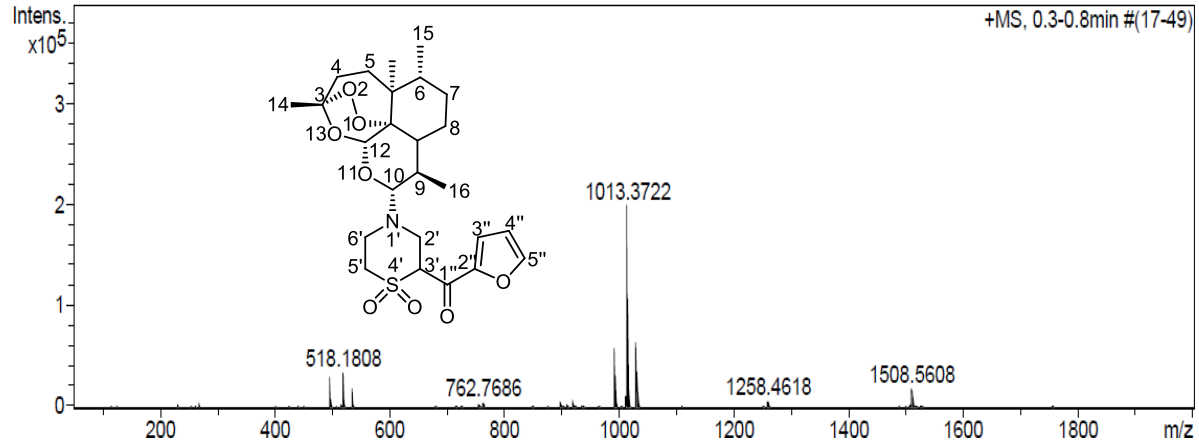
^{13}C NMR



HRMS

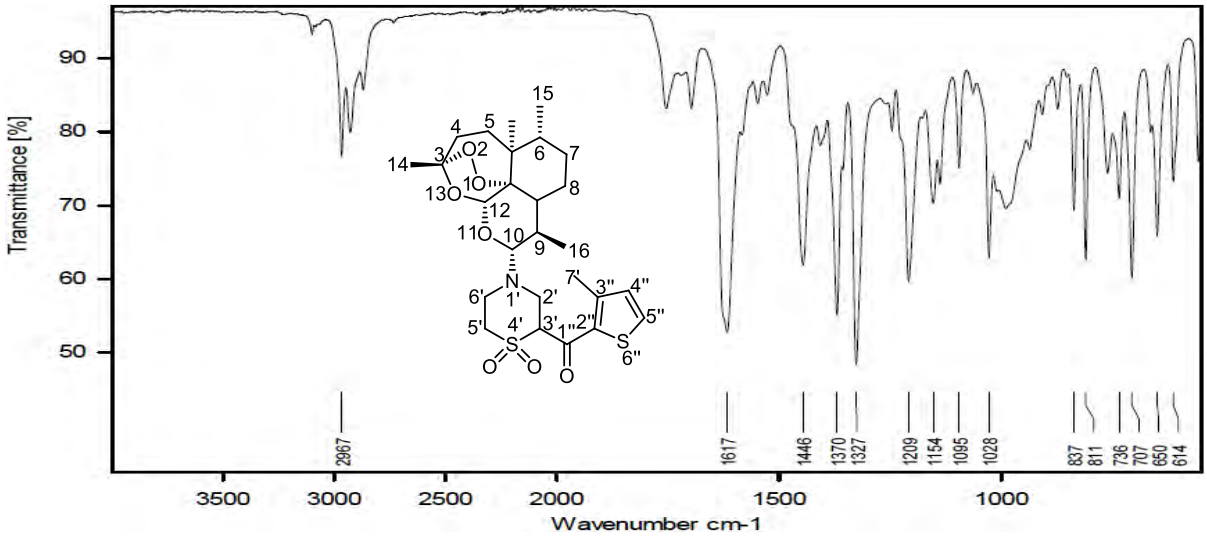
Acquisition Parameter

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	3000 m/z	Set Collision Cell RF	150.0 Vpp	Set Divert Valve	Waste

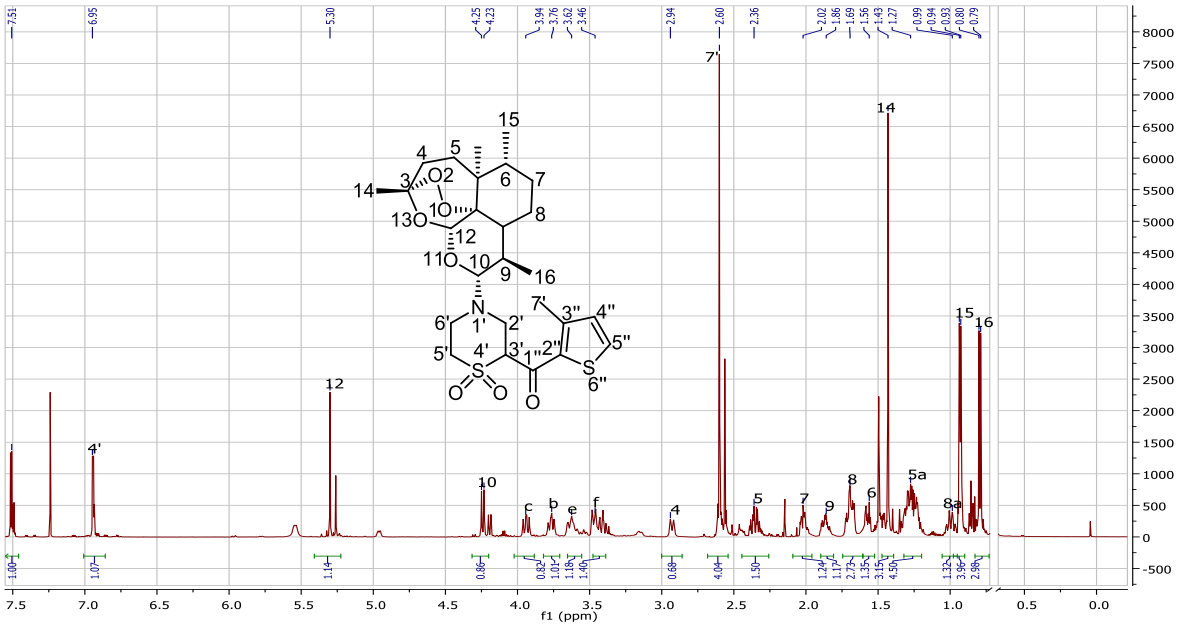


Compound 19:

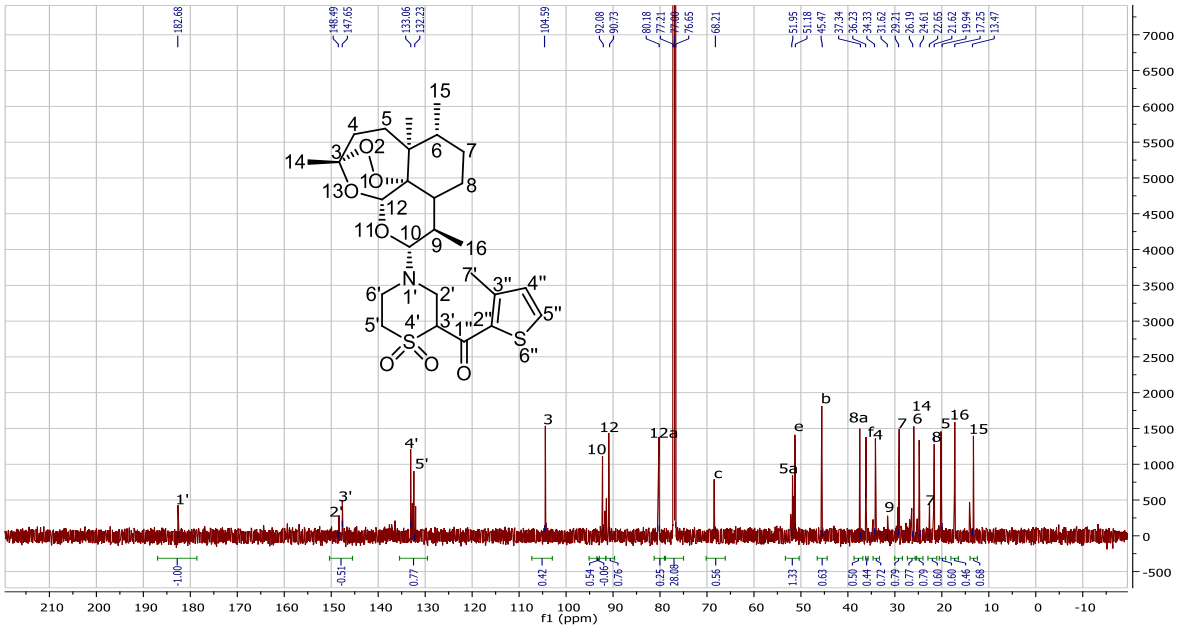
IR



^{1}H NMR



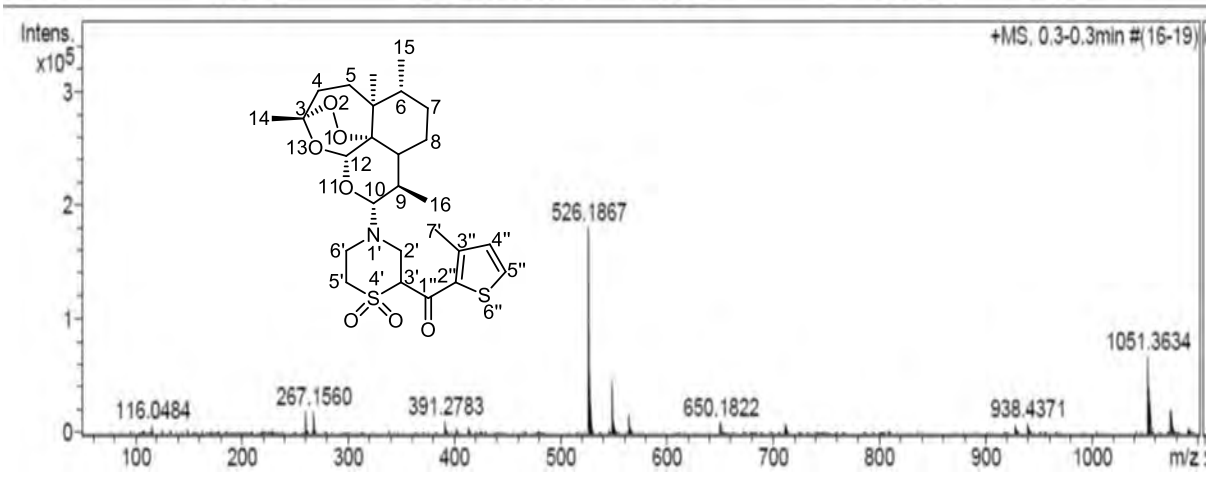
^{13}C NMR



HRMS

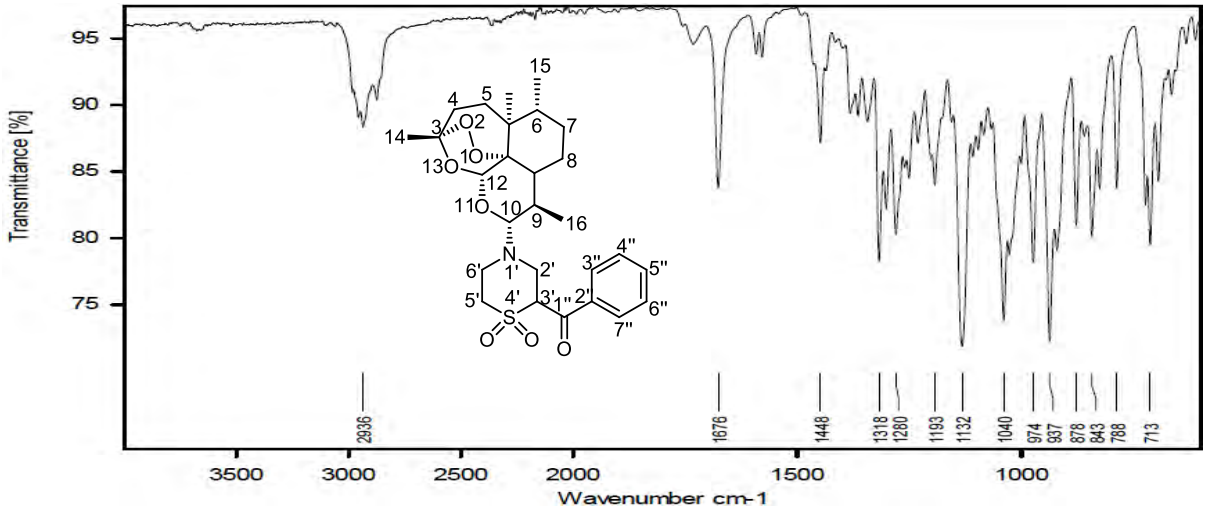
Acquisition Parameter

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	3000 m/z	Set Collision Cell RF	150.0 Vpp	Set Divert Valve	Waste

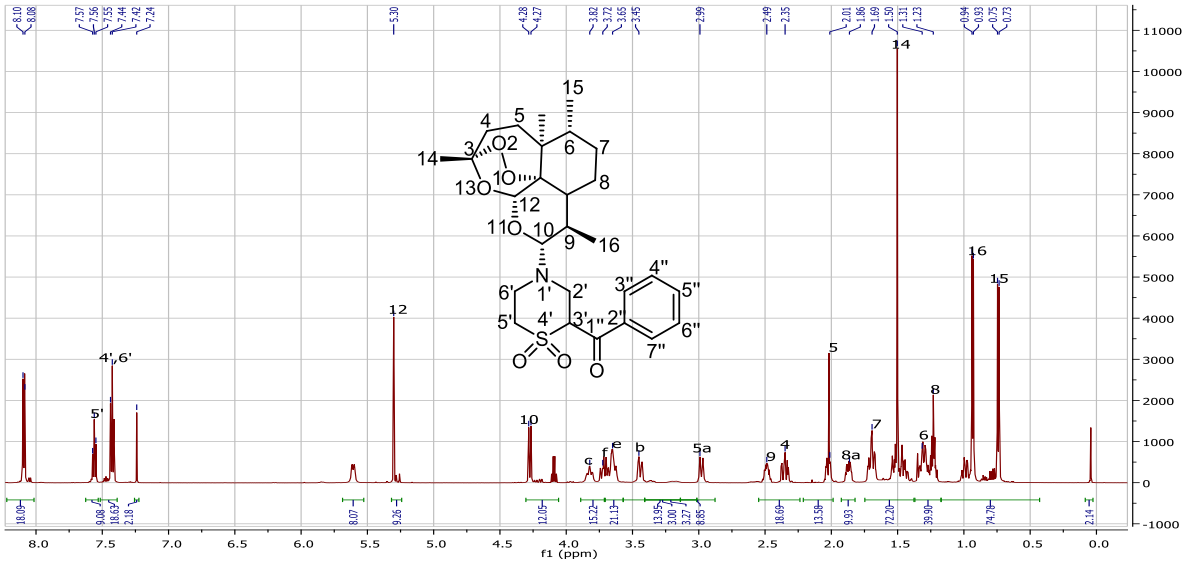


Compound 20:

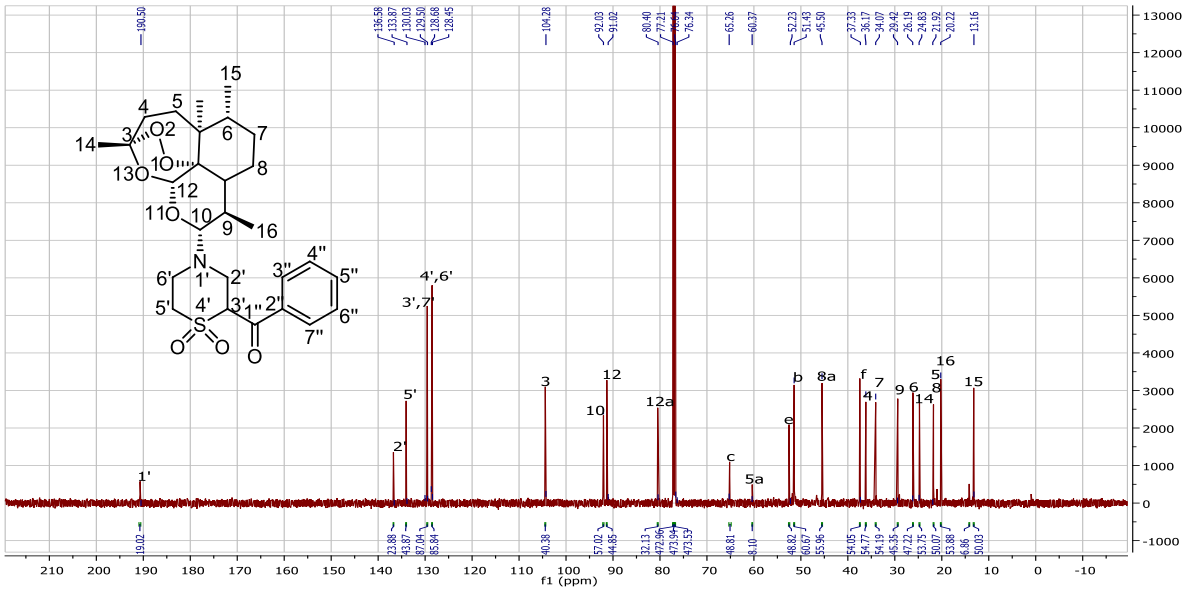
IR



^{1}H NMR



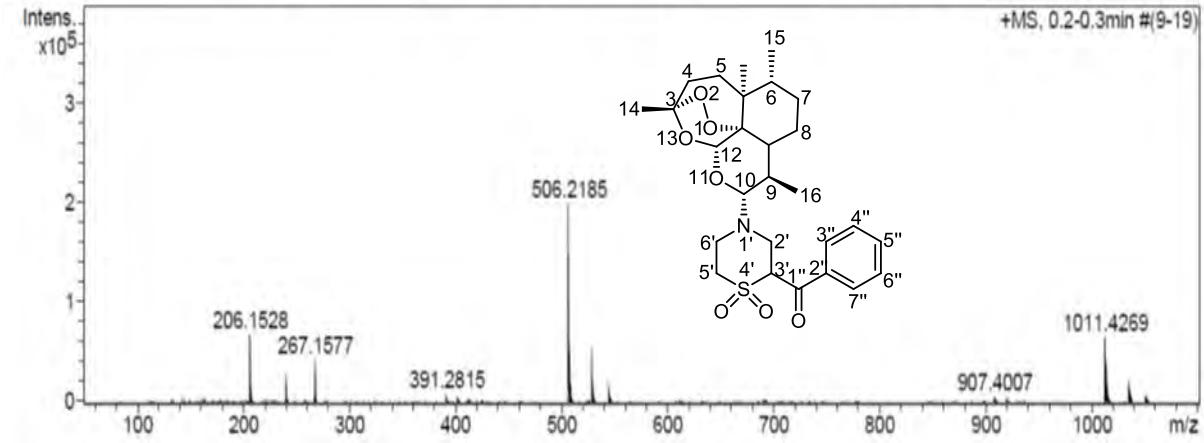
^{13}C NMR



HRMS

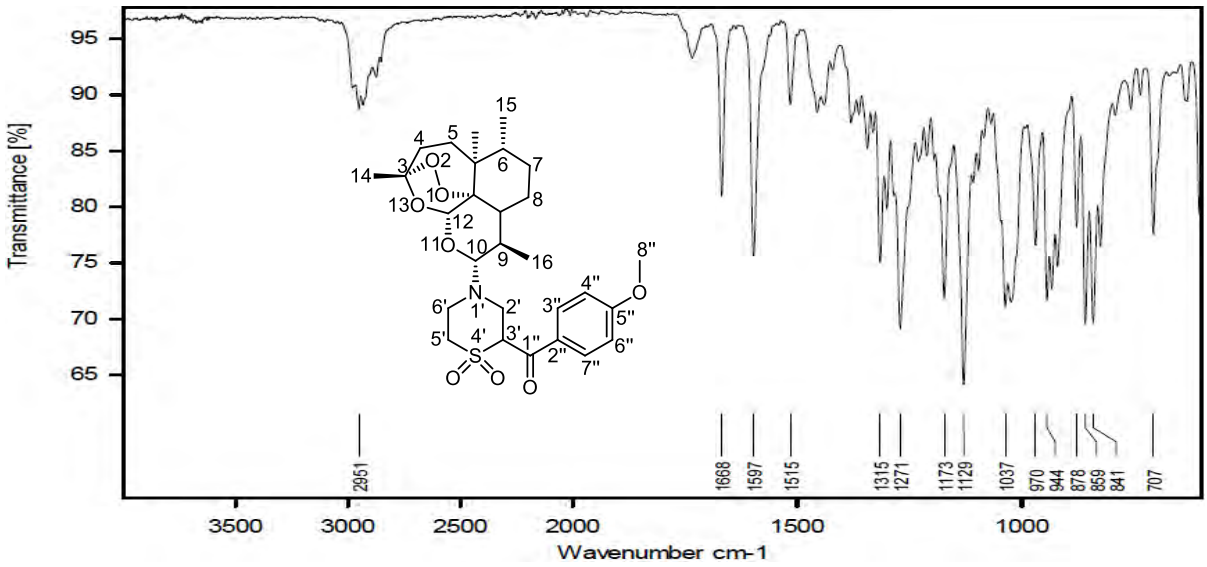
Acquisition Parameter

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	3000 m/z	Set Collision Cell RF	150.0 Vpp	Set Divert Valve	Waste

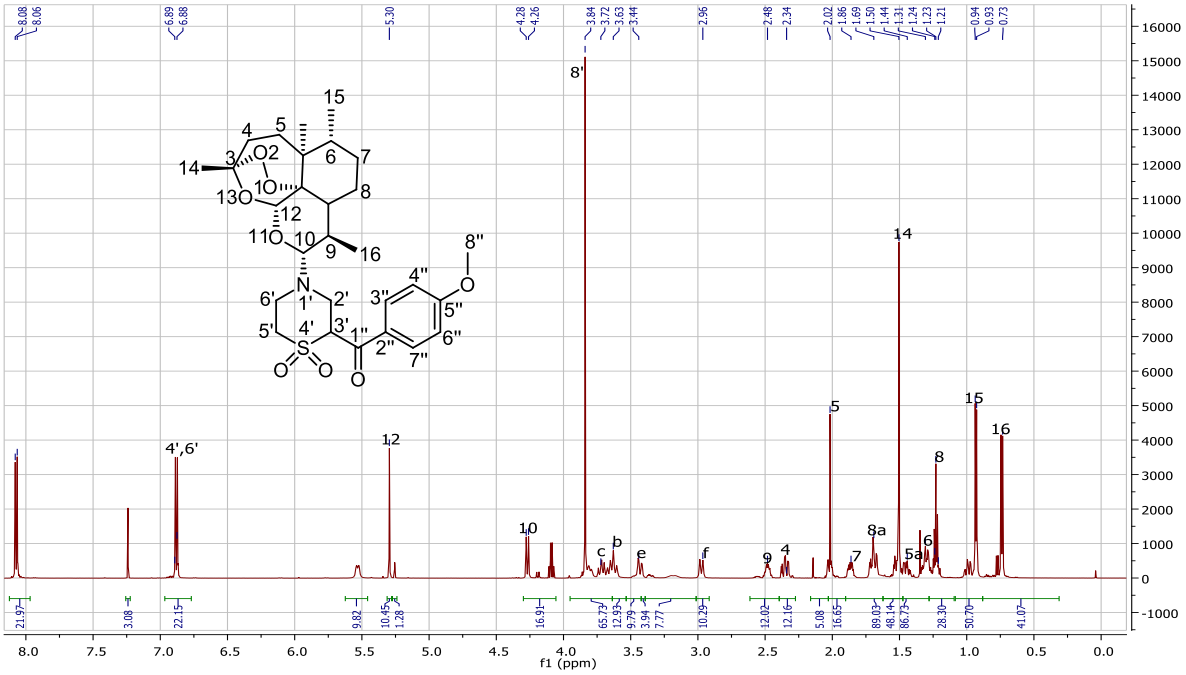


Compound 21:

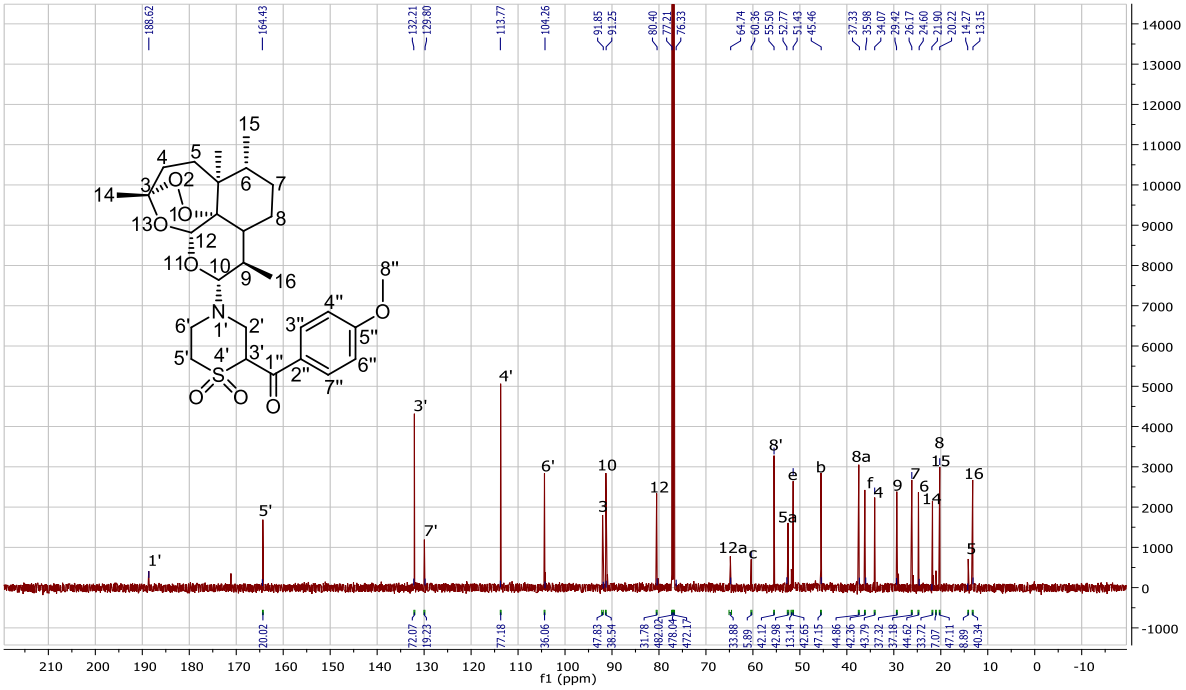
IR



^{1}H NMR



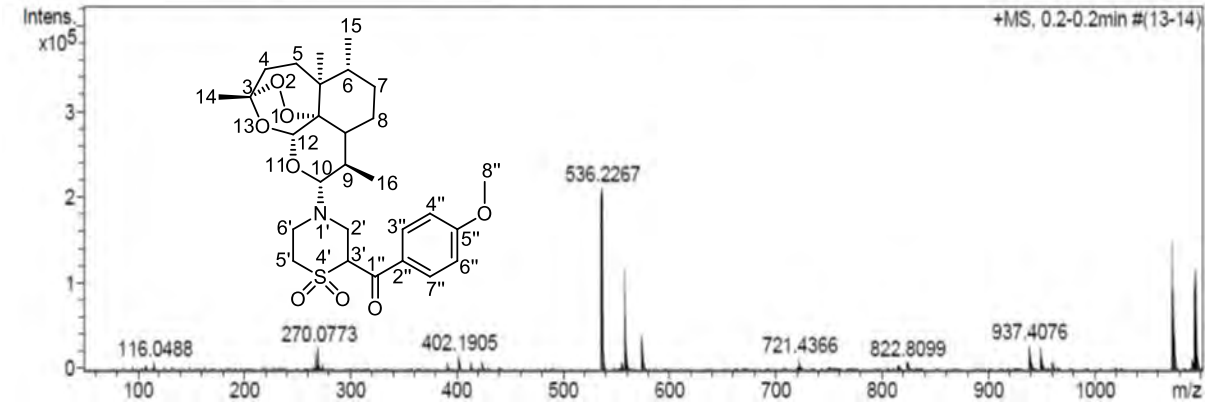
^{13}C NMR



HRMS

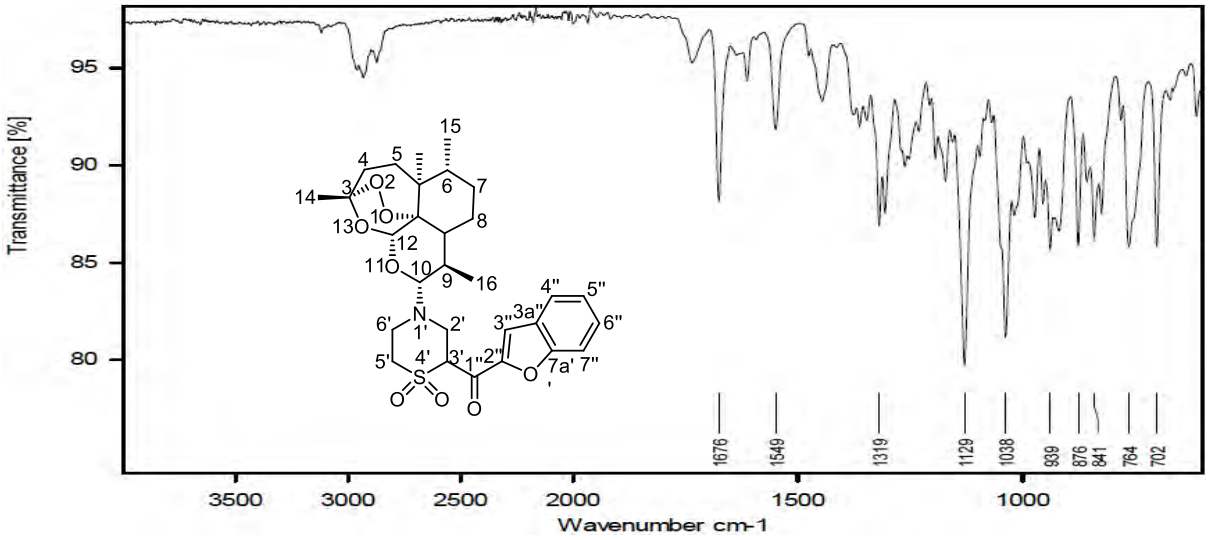
Acquisition Parameter

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	3000 m/z	Set Collision Cell RF	150.0 Vpp	Set Divert Valve	Waste

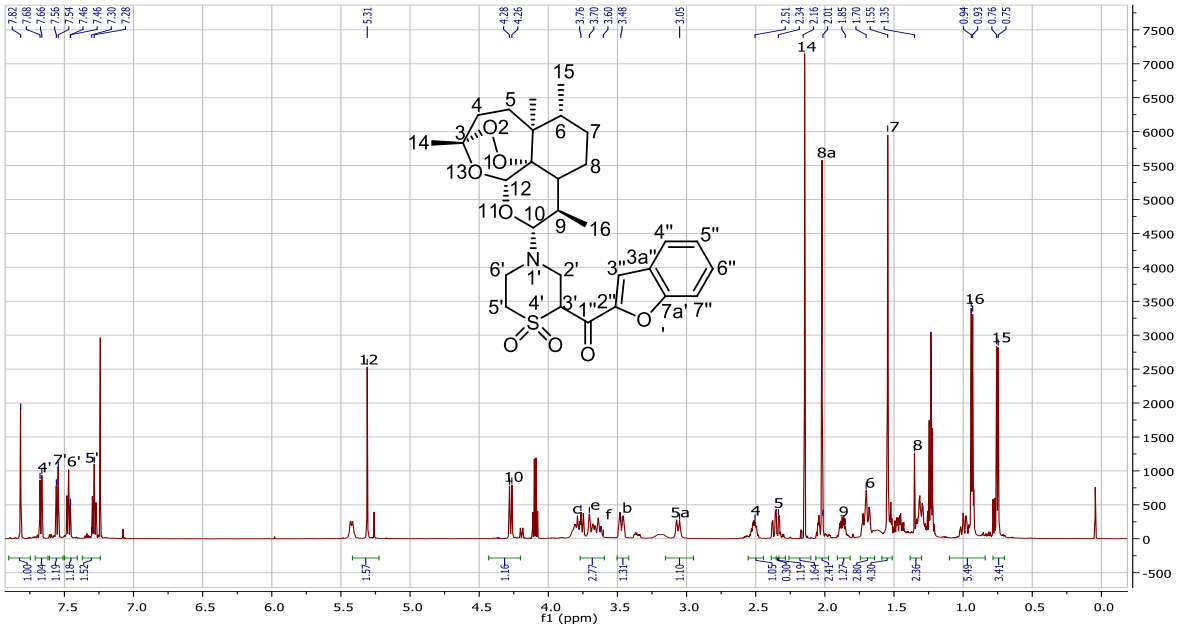


Compound 22:

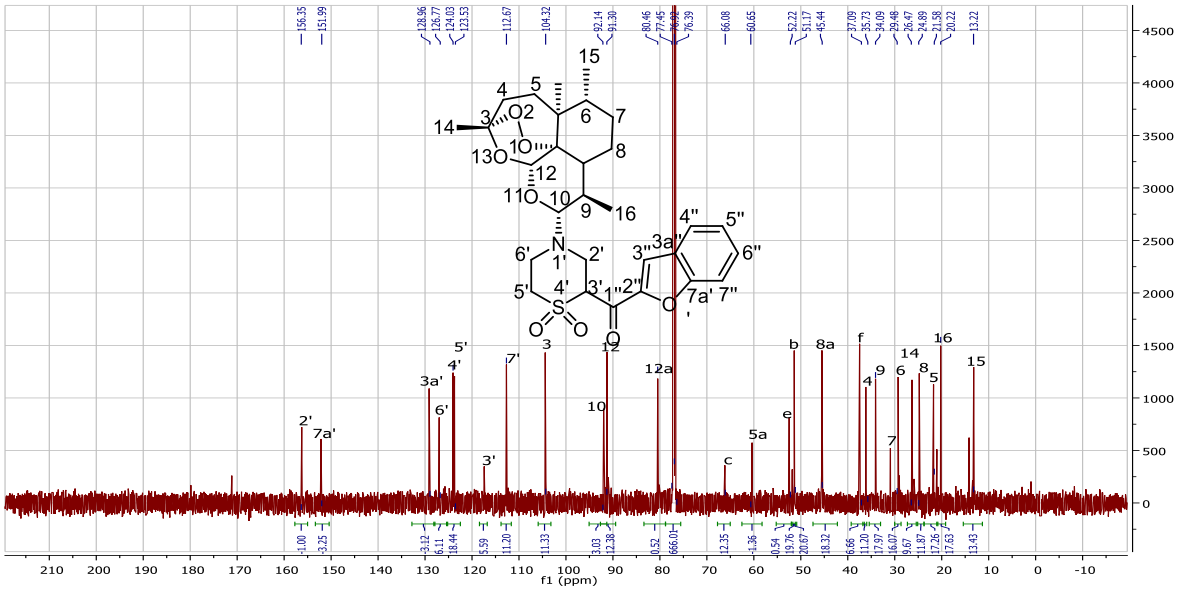
IR



^{1}H NMR



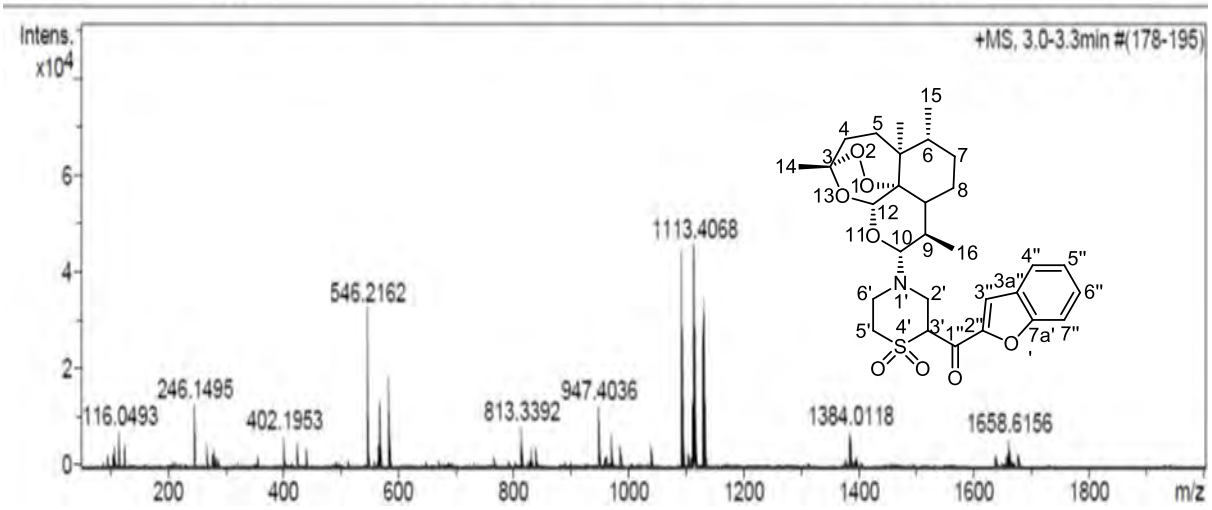
^{13}C NMR



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	3000 m/z	Set Collision Cell RF	150.0 Vpp	Set Divert Valve	Waste



APPENDIX B: GUIDE FOR AUTHORS

CHEMMEDCHEM Chemistry & Drug Discovery

In preparing a manuscript for submission, we ask authors to note the following formal manuscript guidelines. Detailed information can be found on our homepage: http://www.chemmedchem.org or can be obtained from the editorial staff at (+49) 6201-606-142 or chemmedchem@wiley-vch.de.

General

You should submit you **manuscript** using http://www.manuscriptxpress.com/osm.

Templates for each type of contribution can be found on our home page http://www.chemmedchem.org.

Graphical material

To assist referees in the peer-review process, graphics should be integrated into the document, appearing as they are mentioned.

Graphics are:

Equations (without legend)
Formulas (without legend but with Arabic numeral(s) in boldface, e.g. **1a, 28**)
Schemes (with legend)
Figures (with legend);
please note, we do not use 'Charts'.

Preferred graphics programs: ChemDraw, FreeHand, CorelDraw 7, Photoshop.

Acceptable formats: TIFF (preferred), EPS, WMF, BMP, CDX (preferred), CDR, PPT.

Unusable graphic programs: C-Design, Origin, Claris-Draw, ChemIntosh, MacDraw Pro.

Restricted use: ChemWindow, Illustrator, Quark-XPress, PowerPoint, PageMaker, ISIS-Draw.

Final format for **vector graphics** (stick diagrams, etc.): Encapsulated Postscript (EPS) with bound fonts, and the characters must be converted into outlines, not Postscript (PS). The "bounding boxes" must be of an appropriate size.

The resolution for **raster figures** (e.g., ORTEP representations) and for color figures must be at least 300 dpi. The resolution for stick diagrams in a bitmap format (*.bmp files) must be at least 1000 dpi.

Manuscript Organization

- **Reviews**: order of the manuscript sections: title—author(s)—[*] footnote—lead-in—main text—acknowledgements—keywords—references—suggestion for the table of contents.
- **Highlights**, **Minireviews**, and **Communications**: order of the manuscript sections: title—author(s)—dedication—[*] footnote—[**] footnote—main text—experimental section—acknowledgements—keywords—references—suggestion for the table of contents.
- **Full paper**: order of the manuscript sections: title—author(s)—dedication—[*] footnote—[**] footnote—abstract—main text—experimental section—acknowledgements—keywords—references—suggestion for the table of contents.

Contribution Titles and Author Affiliations

- **Title** line: The title should be as succinct as possible and without a reference. Please also try to avoid the use of words such as 'new', 'novel', and 'first' in the title.
- **Author** line: Academic title, first name, other initials, and surname of each author are listed, and an asterisk indicates each correspondence author. Other symbols to indicate different companies or academic institutions are not necessary.
- **Addresses** ([*] footnote): The names of all authors (with academic title and all first names as initials) and the address, fax number, and e-mail address(es) of the correspondence author(s) are listed. If the authors belong to different research groups, the names belonging to each group, and the university or company only of non-correspondence authors, should be indicated after the address of the correspondence author.

Additional Materials Required

- A **short text and color image for the table of contents** should be supplied using the template provided. The text should be about five lines (300 characters) and refer to the figure supplied. The picture should contain only the most essential information. Please restrict any text in the picture to a minimum and avoid large schemes.
- A maximum of five **keywords** should be given in alphabetical order, at least two of which must come from the core keyword list given on our homepage at http://www.chemmedchem.org under the link sequence: For Authors / Basic Keyword List.
- **Supporting Information**: Detailed facts of importance to specialist readers can be submitted as Supporting Information and will be made accessible online, should the article be accepted. Color and animated multimedia applications are welcome. In particular, superfluous physical and experimental data should be included here.

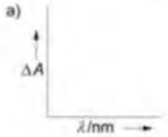
General Remarks

- **Spelling** may be British or American but consistency should be maintained within a manuscript. Authors are asked to make their manuscripts suitable for a heterogeneous readership of biologists and chemists and to be considerate to our many readers for whom English is a foreign language—please use a simple, clear style and avoid jargon.
- Names of organisms should comply with genetic conventions, with genus and species names written in italics and spelled out in full on first appearance. Abbreviations for genes should be written in lower-case letters and italicized, those of the corresponding protein products should start with a capital letter and should not be italicized (e.g., *hsp70* and Hsp70, respectively). Enzyme names should be accompanied by the respective Enzyme Commission (EC) numbers.

Tables, Equations, Figures and Schemes

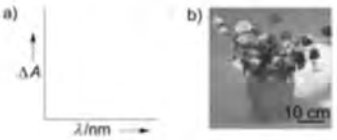
- All **tables, equations, figures,** and **schemes** should be mentioned in the text in numerical order, starting with number 1, e.g. Table 1; [Eq. (1)] or, in text, Equation (1); (Figure 1); (Scheme 1). Please start these words with a capital letter, write them in full, and use Arabic (not Roman) numerals.
- Please transfer explanatory text in the diagram (such as the legends for symbols and lines, e.g. circles=compound 1, dotted line=compound 2) and comprehensive experimental details and other information (e.g. bond lengths and angles) to figure legends.
- **Axis labels**: If possible, the ordinate is labeled **perpendicular** to the axis. The axis labels contain a **standard abbreviation** for the physical quantity (e.g. A for absorption, ν for frequency, I_{rel} for relative intensity, E_i for ionization energy) in italic font. If special abbreviations are used, please explain them in the legend. Units are in normal lower-case letters and separated by a slash (e.g. δ/ppm, λ/nm, t/min, V_{abs}/mL g^{-1}).

Example:



- Please use a), b) to indicate partitions of the figure. Please place them in the upper left corner of the respective figure partition.

Example:



- Please use Roman lower-case letters or Arabic numerals with a bracket to indicate reaction sequences. Do not use i), ii). Example: a,b) → a)↓ or a,b → a↓

- Common abbreviations such as Me, *i*Pr, *s*Bu, *t*Bu, and Ph should be used; general substituents should be indicated by R^1, R^2 (not by R_1, R_2).
- Please use only one font style. The journal style requires a sans serif font (Arial, Helvetica).
- Other insertions in diagrams should be of the same font size as that in the main diagram.
- Please remove the circles surrounding "+" or "–" charges in chemical structure drawings.
- Minus signs (–) should be longer than a hyphen (-).
- For structural formulae the line width should be at least 0.2 pt or 0.1 mm.
- Ideal size of symbols for elements, numbers, **3 mm** (writing above a reaction arrow may be a little smaller); formula numbers (always boldface), **3.5 mm**; interatomic bond lengths, **6 mm**; total maximum width, **14 cm** (or 29.5 cm); writing above a reaction arrow may be a little smaller. Other sizes, but following the proportions 3:3.5:6, are also acceptable. Please refer to a ChemDraw template provided on the *ChemMedChem* homepage at http://www.chemmedchem.org under the For Authors link.
- Further guidelines on the graphical representation standards for chemical structure diagrams can be found at http://old.iupac.org/publications/pac/80/2/0277/
- **Computer-aided image enhancement** is often unavoidable. However, such manipulation cannot result in data that are less relevant or unrepresentative being shown and/or genuine and significant signals being lost. A clear relationship must remain between the original data and the electronic images that result from those data. If an image has been electronically modified, the form of the modification must be given in the Figure caption. If computer-aided processing or modification of an image is a fundamental part of the experimental work, then the form that this processing takes must be clearly described in the Experimental Section.

Format

- Formula numbers should be in **boldface** (Arabic numerals and, if necessary, Roman lowercase letters). Please avoid Roman numerals.
- **Abbreviations** and **acronyms** should be used sparingly and consistently. Where they first appear in the text, the complete term—apart from the most common acronyms such as NMR, IR, and *t*Bu—should also be given.
- ***Italics***: Please italicize letters that symbolize physical quantities such as *T* for temperature, *E* for energy, *n* for an unspecific number (other examples: K_i, log *P*, R_f, t_R, etc.). Latin phrases such as "in vitro" "et al." and "in vacuo" are not italicized. Stereochemical information (*cis*, *Z*, *R*), locants in a compound name (*N*-acetylimidazole), symmetry and space groups (C_{2v}), and prefixes in formulas or compound names such as *tert*-butyl or *p*-aminosalicylic acid should also be italicized.
- Common abbreviations for formulas can be used such as Me, Pr, Bu, and Ph. Only in this case are prefixes such as *iso*-, *n*-, *tert*- written as a single italic letter without a dash: *i*Pr, *n*Bu, *t*Bu. Examples: *n*BuLi, but *n*-butyllithium; *n*Oct, but *n*-C_8H_{17}.
- Please indicate general substituents by R^1, R^2, not by R_1, R_2.
- D- and L- (stereochemical assignments), molar (M), and normal (N), should be in SMALL CAPITALS.
- The oxidation state used with names of elements should be in capital Roman numerals within parentheses (e.g. iron(II)); with element symbols it should be superscripted: Fe^{II}; not Ru(III) but Ru^{III}.
- Please enclose formulas for **coordination compounds** in square brackets (IUPAC recommendation).

References

- Please type references to literature or footnotes in the text in **square brackets** as superscripts **after** any punctuation.
- Please type [1,2] rather than [1],[2] and [1-5] rather than [1,2,3,4,5]
- Please mention all authors names in the citation itself (not "et al.") if the number of authors is fewer than 20.
- Please cite the author names as follows: author initials then surnames, comma, next author. The penultimate and the last name should **not** be separated by "and", but just by a comma.
- Please do not use a comma between the *Journal name* and the **year**.
- Please separate composite references by a)...; b)...; c)... . etc. (not (a), (b), (c)). Please separate them with semicolons.
- Please do not use commas within a journal name.
- At the end of the main text, the references are listed with the numbers **in square brackets** (but not as superscripts).
- Please cite the references as follows: Citation number in square brackets, author initials then surnames, comma, Journal name abbreviated according to the "Chemical Abstracts Service Source Index" (*italicized*), year of publication (**boldface**), comma, volume number (*italicized*), comma, first page or page range, full stop (or semicolon within a composite reference). Examples:

  Journals:
  [1] a) H. J. Ache, *Angew. Chem.* **1989**, *101*, 1–21; *Angew. Chem. Int. Ed. Engl.* **1989**, *28*, 1–20; b) H. Frey, *Angew. Chem.* **1998**, *110*, 2313–2318; *Angew. Chem. Int. Ed.* **1998**, *37*, 2193–2197; c) G. M. Sheldrick, SHELXS-96, Program for the Solution of Crystal Structures, University of Göttingen, Göttingen (Germany), **1996**.
  [2] a) B. Wrackmeyer, *J. Chem. Soc. Chem. Commun.* **1995**, 1624–1626; b) A. Kraft, *Chem. Commun.* **1996**, 77–79, and references therein; c) S. C. Stinson, *Chem. Eng. News* **1998**, *76*(28), 57–73; d) B. Krebs, H. U. Hürter, *Acta Crystallogr. Sect. A* **1981**, *37*, 163; e) "Synthesis in Biochemistry": R. Robinson, *J. Chem. Soc* **1936**, 1079. f) G. Eulenberger, *Z. Naturforsch. B* **1981**, *36*, 521; g) D. Bruss, *Appl. Phys. B*, DOI 10-1007/s003409900185.

  Books:
  [1] E. Wingender, *Gene Regulation in Eukaryotes*, VCH, Weinheim, **1993**, p. 215.
  [2] a) T. D. Tullius in *Comprehensive Supramolecular Chemistry, Vol. 5* (Eds.: J. L. Atwood, J. E. D. Davies, D. D. MacNicol, F. Vögtle, K. S. Suslick), Pergamon, Oxford, **1996**, pp. 317–343; b) G. Maas, *Methoden Org. Chem. (Houben-Weyl) 4th ed. 1952–*, Vol. E21/1, **1983**, pp. 379–397.

  Others:
  [1] a) C. R. A. Botta (Bayer AG), DE-B 2235093, **1973** [*Chem. Abstr.* **1974**, *80*, 55356c]; b) A. Student, PhD thesis, University of Newcastle (UK), **1991**.
  [2] a) W.-D. Becker, *Abstr. Pap. 11th Conf. Int. Sci. Technol.* (San Diego, CA) **1996**, p. 156; b) A. Kleemann, K. Drauz, J. Engel, B. Kautscher, E. Wünsch, *Proc. 4th Akabori Conf.* (Shizuoka, Japan) **1991**, pp. 96–101; c) S. Novick, "Biography of Rotational Spectra for Weakly Bound Complexes" to be found under http://www-wesleyan.edu/chem.bios/vdw.html, **1999**.
- When citing publications from *Angewandte Chemie*, please quote both German and International Editions. The page numbers of the German version can be found at the end of the article itself and in the annual index of the International Editions. Example: [1] a) H. J. Ache, *Angew. Chem.* **1989**, *101*, 1–21; *Angew. Chem. Int. Ed. Engl.* **1989**, *28*, 1–20; b) H. Frey, *Angew. Chem.* **1998**, *110*, 2313–2318; *Angew. Chem. Int. Ed.* **1998**, *37*, 2193–2197.

Experimental Section

- **Minimum spectral requirements** for **Communications and Full Papers**: ^{1}H NMR, ^{13}C NMR and MS data are required for intermediate compounds, and ^{1}H NMR, ^{13}C NMR, IR and HRMS data for all final compounds and those used in biological testing. $[\alpha]_D^{20}$ should be given for all optically active compounds described. The following data are desirable for all compounds: melting point ranges for solids, R_f with solvent details.

- The **Experimental Section** in **Full Papers** is not limited in length, however, this section in **Communications** should only contain the most pertinent information; additional physical data and protocol descriptions should be submitted as Supporting Information. Spectral requirements and formatting are detailed in full below.

- Equipment (including make, model, and software version used) and conditions used for the measurement of physical data as well as any organisms, proteins, or nucleic acids used should be described at the beginning of the Experimental Section. Sources of less-common starting materials must be given and solvent details should also be described.

- Procedures should be given in sufficient detail to enable others to repeat your work.

- In so far as is practical, authors should use a systematic name for each title compound (as suggested by the International Union of Pure and Applied Chemistry (IUPAC), the International Union of Biochemistry and Molecular Biology (IUBMB), or Chemical Abstracts) followed by the compound number in parentheses (e.g. Ethyl 4-cyanobenzoate (**7**)). For the sake of clarity general descriptors such as compound **1**, dendrimer **2**, or alcohol **3** should be used.

- Quantities of reactants, solvents, etc. should be included in parentheses rather than in the running text (e.g., triphenylstannyl chloride (0.964 g, 2.5 mmol) in toluene (20 mL)).

- Products should be described and yields should be given as both a quantity (mol or g) and % yield (e.g. ... compound **7** as a white powder (34 mg, 89%)).

- Data should be quoted with decimal points, not commas, and negative exponents (e.g. 25.8 J K^{-1} mol^{-1}).

- When describing acidity, either pH 2 or pH>2/pH<2 is preferred but **not** pH=2.

- Example: .....gave compound **7** as a white powder (34 mg, 89%): R_f =0.38 ($CHCl_3$/MeOH, 9:1); mp: 70-71°C; $[\alpha]_D^{20}$ = –13.5 (*c* = 0.2 in acetone); ^{1}H NMR (400 MHz, $CDCl_3$): δ=1.35 (q, *J* =8.1 Hz, 2H), 0.97 ppm (t, *J* =8.2 Hz, 3H); ^{13}C NMR (75 MHz, [D_6]DMSO): δ= 8.9, 27.3, 56.8, 64.2, 170.3 ppm; IR (KBr): $\tilde{\nu}$ = 3248, 3056, 1790, 1780, 1506, 1493 cm^{-1}; UV/Vis (CH_2Cl_2): λ_{max} (ε)=320 (5000), 270 nm (12000); MS (EI, 70 eV): *m/z* (%): 173 (32), 171 (100) [*M*+H]$^+$; HRMS-FAB: *m/z* [*M*+H]$^+$ calcd for $C_8H_7ClO_2$: 171.0135, found: 171.0142; Anal. calcd for $C_8H_7ClO_2$: C 56.32, H 4.14, O 18.76, found: C 56.35, H 4.11, O 18.79. ***Please give data in this order.***

Compound Purity

- Those compounds used in biological testing should possess purity of no less than 98% as determined by elemental analysis (to an accuracy of within ±0.4%), HRMS or HPLC analysis. Data should be included in the Experimental Section or Supporting Information.

We understand the associated limitations involved in chemical synthesis, these requirements are flexible within reason; please contact the ChemMedChem *editorial office with any questions.*

QSAR/QSPR Manuscripts

- Topics in quantitative structure-activity relationships appear frequently in *ChemMedChem*, and in light of the recent broadening of this field, it is important that prospective authors are aware of our editorial policy toward QSAR/QSPR manuscripts. First, the novelty of the QSAR/QSPR study should be clearly stated, preferably in the article's Abstract and Introduction. Second, if a new method or theory is reported, it should be validated against at least one other published dataset using at least one other commonly used method or theory; all QSAR/QSPR models must be validated using external data, and not data that were used for the development of the model. Finally, all data used in performing the QSAR study should be reported in the manuscript itself, provided in the Supporting Information, or otherwise readily available without restriction.

Crystallographic data:

- Crystallographic data for **organic** and **organometallic** compounds: Before submission of the manuscript, send your data to CCDC. The data will be assigned a registry number, which should be included with the following standard text in the manuscript: CCDC-## contain(s) the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk.

- Crystallographic data for **inorganic** compounds: Before submitting your manuscript, send the data directly to FIZ. For details, please visit http://www.fiz-karlsruhe.de. The following standard text should be included in the manuscript: Further details of the crystal structure investigation(s) may be obtained from the Fachinformationszentrum Karlsruhe, 76344 Eggenstein-Leopoldshafen, Germany (fax: (+49) 7247-808-666; e-mail: crysdata@fiz-karlsruhe.de) on quoting the depository number CSD-... (numbers CSD-... , -... , -... , and -...).

- Crystallographic data for **proteins** and **nucleic acids**: Before submitting your manuscript, send the data directly to the Protein Data Bank (PDB). For details, please visit http://www.rcsb.org/pdb.

- Sequence data for **nucleic acids**: Before submitting your manuscript, send the data directly to GenBank (http://www.ncbi.nlm.nih.gov/) or EMBL (http://www.ebi.ac.uk/embl/index.html).

- Sequence data for **proteins**: Before submitting your manuscript, send the data directly to PIR (http://pir.georgetown.edu/) or SwissProt (http://www.expasy.ch/sprot/sprot-top.html).

Notice for experiments involving live subjects (animal/human) or human tissue:

- Manuscripts containing **animal experiments** must include a statement that permission was obtained from the relevant national or local authorities. The institutional committees that have approved the experiments must be identified and the accreditation number of the laboratory or of the investigator given where applicable. If no such rules or permissions are in place in the country where the experiments were performed, then this must also be clearly stated.

- Manuscripts with experiments with **human subjects** or **tissue samples** from human subjects must contain a disclaimer in the Experimental Section to state that informed, signed consent was obtained from either the patient or next of kin.

APPENDIX C: GUIDE FOR AUTHORS

EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY

Published under the auspices of the French Société de Chimie Thérapeutique (SCT)

AUTHOR INFORMATION PACK

TABLE OF CONTENTS

ISSN: 0223-5234

DESCRIPTION

The *European Journal of Medicinal Chemistry* is a global journal that publishes studies on all aspects of **medicinal chemistry**: organic synthesis; biological behavior; pharmacological activity; drug design; QSAR; molecular modeling; drug-receptor interactions; molecular aspects of drug metabolism; prodrug synthesis and drug targeting. It provides a medium for publication of original papers, laboratory notes, short or preliminary communications, and invited reviews.

Book Proposals: Elsevier also publishes books in this area. If you have a suggestion for a book topic or would like to submit a book proposal, please contact us at: chemistry@elsevier.com

Benefits to authors
We also provide many author benefits, such as free PDFs, a liberal copyright policy, special discounts on Elsevier publications and much more. Please click here for more information on our author services.

Please see our Guide for Authors for information on article submission. If you require any further information or help, please visit our support pages: http://support.elsevier.comhttp://about.elsevier.com/images/EJMECH2012.gif

AUDIENCE

Medicinal Chemists, Pharmacologists, Pharmacists, Biochemists and Physicians.

IMPACT FACTOR

2014: 3.447 © Thomson Reuters Journal Citation Reports 2015

ABSTRACTING AND INDEXING

BIOSIS
Elsevier BIOBASE
CNRS/Pascal
Chemical Abstracts
Current Contents/Life Sciences
MEDLINE®
EMBASE
Science Citation Index
Current Awareness in Biological Sciences
Scopus

EDITORIAL BOARD

GUIDE FOR AUTHORS

Your Paper Your Way

We now differentiate between the requirements for new and revised submissions. You may choose to submit your manuscript as a single Word or PDF file to be used in the refereeing process. Only when your paper is at the revision stage, will you be requested to put your paper in to a 'correct format' for acceptance and provide the items required for the publication of your article.
To find out more, please visit the Preparation section below.

INTRODUCTION

The *European Journal of Medicinal Chemistry* (EJMECH) publishes studies on all aspects of medicinal chemistry, including organic synthesis; biological behavior; pharmacological activity; drug design; QSAR; molecular modelling; drug-receptor interactions; molecular aspects of drug metabolism; pro-drug synthesis and drug targeting. The journal accepts papers from any country in this world.

Types of Contributions

The journal provides a medium for publication of the following articles:

Original papers are full papers describing original research of high scientific level and timeliness. They are definitive reports on a full study.

Short communications are shorter pieces of work and report limited studies of the same level as described in original papers.

Preliminary communications are also limited studies but describe particularly novel and significant new findings which shall be developed further in the future.

Laboratory notes are focused on practical aspects. They may deal with an original synthesis of a series of compounds or with new pharmacological methods.

Review articles are specially commissioned by the Editors and may embrace the results of various workers in a given area or may feature recent developments from the author's own laboratory. A review that is distinguished by a novel interpretation or representation of earlier findings is especially suitable. The Editors welcome inquiries concerning the suitability of a particular topic for a review article. In addition, a book review section appears in the journal from time to time.

Contact Details for Submission

The European Journal of Medicinal Chemistry uses a web-based online manuscript submission and review system. Authors must submit their manuscript via the online submission page http://ees.elsevier.com/ejmech

BEFORE YOU BEGIN

Ethics in publishing

For information on Ethics in publishing and Ethical guidelines for journal publication see http://www.elsevier.com/publishingethics and http://www.elsevier.com/journal-authors/ethics.

Human and animal rights

If the work involves the use of human subjects, the author should ensure that the work described has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans, http://www.wma.net/en/30publications/10policies/b3/index.html; Uniform Requirements for manuscripts submitted to Biomedical journals, http://www.icmje.org. Authors should include a statement in the manuscript that informed consent was obtained for experimentation with human subjects. The privacy rights of human subjects must always be observed.

All animal experiments should be carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, the European Communities Council Directive of 24 November 1986 (86/609/EEC) or the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978) and the authors should

clearly indicate in the manuscript that such guidelines have been followed. **All animal studies need to ensure they comply with the ARRIVE guidelines.** More information can be found at http://www.nc3rs.org.uk/page.asp?id=1357.

In addition to the contents of the Directive 2010/63/EU Authors of papers submitted to EJMECH are encouraged to reduce as much as possible animal testing. Indeed, animal testing should be limited to the study of compounds that have already demonstrated enough interest in *in vitro* experiments. It should not be used as a general screening method.

Conflict of interest

All authors are requested to disclose any actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations within three years of beginning the submitted work that could inappropriately influence, or be perceived to influence, their work. See also http://www.elsevier.com/conflictsofinterest. Further information and an example of a Conflict of Interest form can be found at: http://service.elsevier.com/app/answers/detail/a_id/286/supporthub/publishing.

Submission declaration and verification

Submission of an article implies that the work described has not been published previously (except in the form of an abstract or as part of a published lecture or academic thesis or as an electronic preprint, see http://www.elsevier.com/sharingpolicy), that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright-holder. To verify originality, your article may be checked by the originality detection service CrossCheck http://www.elsevier.com/editors/plagdetect.

Contributors

Each author is required to declare his or her individual contribution to the article: all authors must have materially participated in the research and/or article preparation, so roles for all authors should be described. The statement that all authors have approved the final article should be true and included in the disclosure.

Changes to authorship

Authors are expected to consider carefully the list and order of authors **before** submitting their manuscript and provide the definitive list of authors at the time of the original submission. Any addition, deletion or rearrangement of author names in the authorship list should be made only **before** the manuscript has been accepted and only if approved by the journal Editor. To request such a change, the Editor must receive the following from the **corresponding author**: (a) the reason for the change in author list and (b) written confirmation (e-mail, letter) from all authors that they agree with the addition, removal or rearrangement. In the case of addition or removal of authors, this includes confirmation from the author being added or removed.

Only in exceptional circumstances will the Editor consider the addition, deletion or rearrangement of authors **after** the manuscript has been accepted. While the Editor considers the request, publication of the manuscript will be suspended. If the manuscript has already been published in an online issue, any requests approved by the Editor will result in a corrigendum.

Article transfer service

This journal is part of our Article Transfer Service. This means that if the Editor feels your article is more suitable in one of our other participating journals, then you may be asked to consider transferring the article to one of those. If you agree, your article will be transferred automatically on your behalf with no need to reformat. Please note that your article will be reviewed again by the new journal. More information about this can be found here: http://www.elsevier.com/authors/article-transfer-service.

Copyright

Upon acceptance of an article, authors will be asked to complete a 'Journal Publishing Agreement' (for more information on this and copyright, see http://www.elsevier.com/copyright). An e-mail will be sent to the corresponding author confirming receipt of the manuscript together with a 'Journal Publishing Agreement' form or a link to the online version of this agreement.

Subscribers may reproduce tables of contents or prepare lists of articles including abstracts for internal circulation within their institutions. Permission of the Publisher is required for resale or distribution outside the institution and for all other derivative works, including compilations and translations

(please consult http://www.elsevier.com/permissions). If excerpts from other copyrighted works are included, the author(s) must obtain written permission from the copyright owners and credit the source(s) in the article. Elsevier has preprinted forms for use by authors in these cases: please consult http://www.elsevier.com/permissions.

For open access articles: Upon acceptance of an article, authors will be asked to complete an 'Exclusive License Agreement' (for more information see http://www.elsevier.com/OAauthoragreement). Permitted third party reuse of open access articles is determined by the author's choice of user license (see http://www.elsevier.com/openaccesslicenses).

Author rights
As an author you (or your employer or institution) have certain rights to reuse your work. For more information see http://www.elsevier.com/copyright.

Role of the funding source
You are requested to identify who provided financial support for the conduct of the research and/or preparation of the article and to briefly describe the role of the sponsor(s), if any, in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication. If the funding source(s) had no such involvement then this should be stated.

Funding body agreements and policies
Elsevier has established a number of agreements with funding bodies which allow authors to comply with their funder's open access policies. Some authors may also be reimbursed for associated publication fees. To learn more about existing agreements please visit http://www.elsevier.com/fundingbodies.

Open access
This journal offers authors a choice in publishing their research:

Open access
• Articles are freely available to both subscribers and the wider public with permitted reuse
• An open access publication fee is payable by authors or on their behalf e.g. by their research funder or institution
Subscription
• Articles are made available to subscribers as well as developing countries and patient groups through our universal access programs (http://www.elsevier.com/access).
• No open access publication fee payable by authors.

Regardless of how you choose to publish your article, the journal will apply the same peer review criteria and acceptance standards.

For open access articles, permitted third party (re)use is defined by the following Creative Commons user licenses:

Creative Commons Attribution (CC BY)
Lets others distribute and copy the article, create extracts, abstracts, and other revised versions, adaptations or derivative works of or from an article (such as a translation), include in a collective work (such as an anthology), text or data mine the article, even for commercial purposes, as long as they credit the author(s), do not represent the author as endorsing their adaptation of the article, and do not modify the article in such a way as to damage the author's honor or reputation.

Creative Commons Attribution-NonCommercial-NoDerivs (CC BY-NC-ND)
For non-commercial purposes, lets others distribute and copy the article, and to include in a collective work (such as an anthology), as long as they credit the author(s) and provided they do not alter or modify the article.

The open access publication fee for this journal is **USD 2850**, excluding taxes. Learn more about Elsevier's pricing policy: http://www.elsevier.com/openaccesspricing.

Green open access
Authors can share their research in a variety of different ways and Elsevier has a number of green open access options available. We recommend authors see our green open access page for further information (http://elsevier.com/greenopenaccess). Authors can also self-archive their manuscripts

immediately and enable public access from their institution's repository after an embargo period. This is the version that has been accepted for publication and which typically includes author-incorporated changes suggested during submission, peer review and in editor-author communications. Embargo period: For subscription articles, an appropriate amount of time is needed for journals to deliver value to subscribing customers before an article becomes freely available to the public. This is the embargo period and it begins from the date the article is formally published online in its final and fully citable form.

This journal has an embargo period of 24 months.

Language (usage and editing services)

Please write your text in good English (American or British usage is accepted, but not a mixture of these). Authors who feel their English language manuscript may require editing to eliminate possible grammatical or spelling errors and to conform to correct scientific English may wish to use the English Language Editing service available from Elsevier's WebShop (http://webshop.elsevier.com/languageediting/) or visit our customer support site (http://support.elsevier.com) for more information.

Submission

Our online submission system guides you stepwise through the process of entering your article details and uploading your files. The system converts your article files to a single PDF file used in the peer-review process. Editable files (e.g., Word, LaTeX) are required to typeset your article for final publication. All correspondence, including notification of the Editor's decision and requests for revision, is sent by e-mail.

Referees

Please submit the names and institutional e-mail addresses of several potential referees. For more details, visit our Support site. Note that the editor retains the sole right to decide whether or not the suggested reviewers are used.

PREPARATION

NEW SUBMISSIONS

Submission to this journal proceeds totally online and you will be guided stepwise through the creation and uploading of your files. The system automatically converts your files to a single PDF file, which is used in the peer-review process.
As part of the Your Paper Your Way service, you may choose to submit your manuscript as a single file to be used in the refereeing process. This can be a PDF file or a Word document, in any format or lay-out that can be used by referees to evaluate your manuscript. It should contain high enough quality figures for refereeing. If you prefer to do so, you may still provide all or some of the source files at the initial submission. Please note that individual figure files larger than 10 MB must be uploaded separately.

References

There are no strict requirements on reference formatting at submission. References can be in any style or format as long as the style is consistent. Where applicable, author(s) name(s), journal title/book title, chapter title/article title, year of publication, volume number/book chapter and the pagination must be present. Use of DOI is highly encouraged. The reference style used by the journal will be applied to the accepted article by Elsevier at the proof stage. Note that missing data will be highlighted at proof stage for the author to correct.

Formatting requirements

There are no strict formatting requirements but all manuscripts must contain the essential elements needed to convey your manuscript, for example Abstract, Keywords, Introduction, Materials and Methods, Results, Conclusions, Artwork and Tables with Captions.
If your article includes any Videos and/or other Supplementary material, this should be included in your initial submission for peer review purposes.
Divide the article into clearly defined sections.

Figures and tables embedded in text

Please ensure the figures and the tables included in the single file are placed next to the relevant text in the manuscript, rather than at the bottom or the top of the file.

REVISED SUBMISSIONS

Use of word processing software
Regardless of the file format of the original submission, at revision you must provide us with an editable file of the entire article. Keep the layout of the text as simple as possible. Most formatting codes will be removed and replaced on processing the article. The electronic text should be prepared in a way very similar to that of conventional manuscripts (see also the Guide to Publishing with Elsevier: http://www.elsevier.com/guidepublication). See also the section on Electronic artwork.
To avoid unnecessary errors you are strongly advised to use the 'spell-check' and 'grammar-check' functions of your word processor.

Essential title page information

• ***Title.*** Concise and informative. Titles are often used in information-retrieval systems. Avoid abbreviations and formulae where possible.
• ***Author names and affiliations.*** Please clearly indicate the given name(s) and family name(s) of each author and check that all names are accurately spelled. Present the authors' affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with a lower-case superscript letter immediately after the author's name and in front of the appropriate address. Provide the full postal address of each affiliation, including the country name and, if available, the e-mail address of each author.
• ***Corresponding author.*** Clearly indicate who will handle correspondence at all stages of refereeing and publication, also post-publication. **Ensure that the e-mail address is given and that contact details are kept up to date by the corresponding author.**
• ***Present/permanent address.*** If an author has moved since the work described in the article was done, or was visiting at the time, a 'Present address' (or 'Permanent address') may be indicated as a footnote to that author's name. The address at which the author actually did the work must be retained as the main, affiliation address. Superscript Arabic numerals are used for such footnotes.

Graphical abstract

A Graphical abstract is mandatory for this journal. It should summarize the contents of the article in a concise, pictorial form designed to capture the attention of a wide readership online. Authors must provide images that clearly represent the work described in the article. Graphical abstracts should be submitted as a separate file in the online submission system. Image size: please provide an image with a minimum of 531 × 1328 pixels (h × w) or proportionally more. The image should be readable at a size of 5 × 13 cm using a regular screen resolution of 96 dpi. Preferred file types: TIFF, EPS, PDF or MS Office files. See http://www.elsevier.com/graphicalabstracts for examples.
Authors can make use of Elsevier's Illustration and Enhancement service to ensure the best presentation of their images also in accordance with all technical requirements: Illustration Service.

Highlights

Highlights are mandatory for this journal. They consist of a short collection of bullet points that convey the core findings of the article and should be submitted in a separate editable file in the online submission system. Please use 'Highlights' in the file name and include 3 to 5 bullet points (maximum 85 characters, including spaces, per bullet point). See http://www.elsevier.com/highlights for examples.

Keywords

Immediately after the abstract, provide a maximum of 6 keywords, using American spelling and avoiding general and plural terms and multiple concepts (avoid, for example, 'and', 'of'). Be sparing with abbreviations: only abbreviations firmly established in the field may be eligible. These keywords will be used for indexing purposes.

Abbreviations

Define abbreviations that are not standard in this field in a footnote to be placed on the first page of the article. Such abbreviations that are unavoidable in the abstract must be defined at their first mention there, as well as in the footnote. Ensure consistency of abbreviations throughout the article.

Experimental

Analytical data should be included for examination by the editor and referees. However, these data will not be printed if they agree within within ± 0.4 % with calculated values, but may be noted as follows: 'Anal. C14H15NO3 (C, H, N, O)', with the mentioning under Experimental protocols: 'Analyses indicated by the symbols of the elements or functions were within ± 0.4 % of the theoretical values'.

Characterization of prepared compounds

Organic compounds:

Sufficient experimental information should be given by the authors. In particular "supplementary material for publication online" should be constructed for all papers which should at least include representative 1H and 13C NMR spectra. Metal complexes:

X-Ray crystal structural determinations are required for metal complexes.

Acknowledgements

Collate acknowledgements in a separate section at the end of the article before the references and do not, therefore, include them on the title page, as a footnote to the title or otherwise. List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.).

Nomenclature

The author is responsible for providing the correct nomenclature which must be consistent and unambiguous. The use of chemical names for drugs is preferred.

Math formulae

Please submit math equations as editable text and not as images. Present simple formulae in line with normal text where possible and use the solidus (/) instead of a horizontal line for small fractional terms, e.g., X/Y. In principle, variables are to be presented in italics. Powers of e are often more conveniently denoted by exp. Number consecutively any equations that have to be displayed separately from the text (if referred to explicitly in the text).

Footnotes

Footnotes should be used sparingly. Number them consecutively throughout the article. Many word processors build footnotes into the text, and this feature may be used. Should this not be the case, indicate the position of footnotes in the text and present the footnotes themselves separately at the end of the article.

Artwork

Electronic artwork

General points

- Make sure you use uniform lettering and sizing of your original artwork.
- Preferred fonts: Arial (or Helvetica), Times New Roman (or Times), Symbol, Courier.
- Number the illustrations according to their sequence in the text.
- Use a logical naming convention for your artwork files.
- Indicate per figure if it is a single, 1.5 or 2-column fitting image.
- For Word submissions only, you may still provide figures and their captions, and tables within a single file at the revision stage.
- Please note that individual figure files larger than 10 MB must be provided in separate source files.

A detailed guide on electronic artwork is available on our website:
http://www.elsevier.com/artworkinstructions.

You are urged to visit this site; some excerpts from the detailed information are given here.

Formats

Regardless of the application used, when your electronic artwork is finalized, please 'save as' or convert the images to one of the following formats (note the resolution requirements for line drawings, halftones, and line/halftone combinations given below):

EPS (or PDF): Vector drawings. Embed the font or save the text as 'graphics'.

TIFF (or JPG): Color or grayscale photographs (halftones): always use a minimum of 300 dpi.

TIFF (or JPG): Bitmapped line drawings: use a minimum of 1000 dpi.

TIFF (or JPG): Combinations bitmapped line/half-tone (color or grayscale): a minimum of 500 dpi is required.

Please do not:

- Supply files that are optimized for screen use (e.g., GIF, BMP, PICT, WPG); the resolution is too low.
- Supply files that are too low in resolution.
- Submit graphics that are disproportionately large for the content.

Color artwork

Please make sure that artwork files are in an acceptable format (TIFF (or JPEG), EPS (or PDF), or MS Office files) and with the correct resolution. If, together with your accepted article, you submit usable color figures then Elsevier will ensure, at no additional charge, that these figures will appear in color online (e.g., ScienceDirect and other sites) regardless of whether or not these illustrations

are reproduced in color in the printed version. **For color reproduction in print, you will receive information regarding the costs from Elsevier after receipt of your accepted article**. Please indicate your preference for color: in print or online only. For further information on the preparation of electronic artwork, please see http://www.elsevier.com/artworkinstructions.

Figure captions
Ensure that each illustration has a caption. A caption should comprise a brief title (**not** on the figure itself) and a description of the illustration. Keep text in the illustrations themselves to a minimum but explain all symbols and abbreviations used.

Text graphics
Text graphics may be embedded in the text at the appropriate position. If you are working with LaTeX and have such features embedded in the text, these can be left. See further under Electronic artwork.

Tables

Please submit tables as editable text and not as images. Tables can be placed either next to the relevant text in the article, or on separate page(s) at the end. Number tables consecutively in accordance with their appearance in the text and place any table notes below the table body. Be sparing in the use of tables and ensure that the data presented in them do not duplicate results described elsewhere in the article. Please avoid using vertical rules.

References

Citation in text
Please ensure that every reference cited in the text is also present in the reference list (and vice versa). Any references cited in the abstract must be given in full. Unpublished results and personal communications are not recommended in the reference list, but may be mentioned in the text. If these references are included in the reference list they should follow the standard reference style of the journal and should include a substitution of the publication date with either 'Unpublished results' or 'Personal communication'. Citation of a reference as 'in press' implies that the item has been accepted for publication.

Web references
As a minimum, the full URL should be given and the date when the reference was last accessed. Any further information, if known (DOI, author names, dates, reference to a source publication, etc.), should also be given. Web references can be listed separately (e.g., after the reference list) under a different heading if desired, or can be included in the reference list.

References in a special issue
Please ensure that the words 'this issue' are added to any references in the list (and any citations in the text) to other articles in the same Special Issue.

Reference management software
Most Elsevier journals have their reference template available in many of the most popular reference management software products. These include all products that support Citation Style Language styles (http://citationstyles.org), such as Mendeley (http://www.mendeley.com/features/reference-manager) and Zotero (https://www.zotero.org/), as well as EndNote (http://endnote.com/downloads/styles). Using the word processor plug-ins from these products, authors only need to select the appropriate journal template when preparing their article, after which citations and bibliographies will be automatically formatted in the journal's style. If no template is yet available for this journal, please follow the format of the sample references and citations as shown in this Guide.

Users of Mendeley Desktop can easily install the reference style for this journal by clicking the following link:
http://open.mendeley.com/use-citation-style/european-journal-of-medicinal-chemistry
When preparing your manuscript, you will then be able to select this style using the Mendeley plug-ins for Microsoft Word or LibreOffice.

Reference formatting
There are no strict requirements on reference formatting at submission. References can be in any style or format as long as the style is consistent. Where applicable, author(s) name(s), journal title/book title, chapter title/article title, year of publication, volume number/book chapter and the pagination must be present. Use of DOI is highly encouraged. The reference style used by the journal will be

applied to the accepted article by Elsevier at the proof stage. Note that missing data will be highlighted at proof stage for the author to correct. If you do wish to format the references yourself they should be arranged according to the following examples:

Reference style
Text: Indicate references by number(s) in square brackets in line with the text. The actual authors can be referred to, but the reference number(s) must always be given.
Example: '..... as demonstrated [3,6]. Barnaby and Jones [8] obtained a different result ....'
List: Number the references (numbers in square brackets) in the list in the order in which they appear in the text.
Examples:
Reference to a journal publication:
[1] J. van der Geer, J.A.J. Hanraads, R.A. Lupton, The art of writing a scientific article, J. Sci. Commun. 163 (2010) 51–59.
Reference to a book:
[2] W. Strunk Jr., E.B. White, The Elements of Style, fourth ed., Longman, New York, 2000.
Reference to a chapter in an edited book:
[3] G.R. Mettam, L.B. Adams, How to prepare an electronic version of your article, in: B.S. Jones, R.Z. Smith (Eds.), Introduction to the Electronic Age, E-Publishing Inc., New York, 2009, pp. 281–304.

Video data

Elsevier accepts video material and animation sequences to support and enhance your scientific research. Authors who have video or animation files that they wish to submit with their article are strongly encouraged to include links to these within the body of the article. This can be done in the same way as a figure or table by referring to the video or animation content and noting in the body text where it should be placed. All submitted files should be properly labeled so that they directly relate to the video file's content. In order to ensure that your video or animation material is directly usable, please provide the files in one of our recommended file formats with a preferred maximum size of 150 MB. Video and animation files supplied will be published online in the electronic version of your article in Elsevier Web products, including ScienceDirect: http://www.sciencedirect.com. Please supply 'stills' with your files: you can choose any frame from the video or animation or make a separate image. These will be used instead of standard icons and will personalize the link to your video data. For more detailed instructions please visit our video instruction pages at http://www.elsevier.com/artworkinstructions. Note: since video and animation cannot be embedded in the print version of the journal, please provide text for both the electronic and the print version for the portions of the article that refer to this content.

AudioSlides

The journal encourages authors to create an AudioSlides presentation with their published article. AudioSlides are brief, webinar-style presentations that are shown next to the online article on ScienceDirect. This gives authors the opportunity to summarize their research in their own words and to help readers understand what the paper is about. More information and examples are available at http://www.elsevier.com/audioslides. Authors of this journal will automatically receive an invitation e-mail to create an AudioSlides presentation after acceptance of their paper.

Supplementary material

Supplementary material can support and enhance your scientific research. Supplementary files offer the author additional possibilities to publish supporting applications, high-resolution images, background datasets, sound clips and more. Please note that such items are published online exactly as they are submitted; there is no typesetting involved (supplementary data supplied as an Excel file or as a PowerPoint slide will appear as such online). Please submit the material together with the article and supply a concise and descriptive caption for each file. If you wish to make any changes to supplementary data during any stage of the process, then please make sure to provide an updated file, and do not annotate any corrections on a previous version. Please also make sure to switch off the 'Track Changes' option in any Microsoft Office files as these will appear in the published supplementary file(s). For more detailed instructions please visit our artwork instruction pages at http://www.elsevier.com/artworkinstructions.

Open data

This journal supports Open Data, enabling authors to submit any raw (unprocessed) research data with their article for open access publication on ScienceDirect under the CC BY license. For more information, please visit http://www.elsevier.com/about/research-data/open-data.

Database linking
Elsevier encourages authors to connect articles with external databases, giving readers access to relevant databases that help to build a better understanding of the described research. Please refer to relevant database identifiers using the following format in your article: Database: xxxx (e.g., TAIR: AT1G01020; CCDC: 734053; PDB: 1XFN). See http://www.elsevier.com/databaselinking for more information and a full list of supported databases.

Mol files
Mol files (optional): Elsevier would like to enrich your online article by visualizing and providing details of chemical structures you define as the main chemical compounds described. For this purpose, corresponding mol files can be uploaded via the online submission system. Each compound needs to be submitted as a separate mol file. Please use your preferred drawing tool to export chemical structures as mol files and ensure that they are unique, complete and do not contain any R-groups or other variables so that a correct InChI key can be generated. For more information see http://www.elsevier.com/mol.

Interactive plots
This journal enables you to show an Interactive Plot with your article by simply submitting a data file. For instructions please go to http://www.elsevier.com/interactiveplots.

Submission checklist
The following list will be useful during the final checking of an article prior to sending it to the journal for review. Please consult this Guide for Authors for further details of any item.

Ensure that the following items are present:

One author has been designated as the corresponding author with contact details:
- E-mail address
- Full postal address

All necessary files have been uploaded, and contain:
- Keywords
- All figure captions
- All tables (including title, description, footnotes)

Further considerations
- Manuscript has been 'spell-checked' and 'grammar-checked'
- All references mentioned in the Reference list are cited in the text, and vice versa
- Permission has been obtained for use of copyrighted material from other sources (including the Internet)

Printed version of figures (if applicable) in color or black-and-white
- Indicate clearly whether or not color or black-and-white in print is required.

For any further information please visit our customer support site at http://support.elsevier.com.

AFTER ACCEPTANCE

Use of the Digital Object Identifier
The Digital Object Identifier (DOI) may be used to cite and link to electronic documents. The DOI consists of a unique alpha-numeric character string which is assigned to a document by the publisher upon the initial electronic publication. The assigned DOI never changes. Therefore, it is an ideal medium for citing a document, particularly 'Articles in press' because they have not yet received their full bibliographic information. Example of a correctly given DOI (in URL format; here an article in the journal *Physics Letters B*):
http://dx.doi.org/10.1016/j.physletb.2010.09.059
When you use a DOI to create links to documents on the web, the DOIs are guaranteed never to change.

Online proof correction
Corresponding authors will receive an e-mail with a link to our online proofing system, allowing annotation and correction of proofs online. The environment is similar to MS Word: in addition to editing text, you can also comment on figures/tables and answer questions from the Copy Editor. Web-based proofing provides a faster and less error-prone process by allowing you to directly type your corrections, eliminating the potential introduction of errors.
If preferred, you can still choose to annotate and upload your edits on the PDF version. All instructions for proofing will be given in the e-mail we send to authors, including alternative methods to the online version and PDF.

We will do everything possible to get your article published quickly and accurately. Please use this proof only for checking the typesetting, editing, completeness and correctness of the text, tables and figures. Significant changes to the article as accepted for publication will only be considered at this stage with permission from the Editor. It is important to ensure that all corrections are sent back to us in one communication. Please check carefully before replying, as inclusion of any subsequent corrections cannot be guaranteed. Proofreading is solely your responsibility.

Offprints

The corresponding author, at no cost, will be provided with a personalized link providing 50 days free access to the final published version of the article on ScienceDirect. This link can also be used for sharing via email and social networks. For an extra charge, paper offprints can be ordered via the offprint order form which is sent once the article is accepted for publication. Both corresponding and co-authors may order offprints at any time via Elsevier's WebShop (http://webshop.elsevier.com/myarticleservices/offprints). Authors requiring printed copies of multiple articles may use Elsevier WebShop's 'Create Your Own Book' service to collate multiple articles within a single cover (http://webshop.elsevier.com/myarticleservices/booklets).

AUTHOR INQUIRIES

You can track your submitted article at http://www.elsevier.com/track-submission. You can track your accepted article at http://www.elsevier.com/trackarticle. You are also welcome to contact Customer Support via http://support.elsevier.com.