UNIVERSITY OF GHANA

SCHOOL OF PHYSICAL AND MATHEMATICAL SCIENCES

DEPARTMENT OF CHEMISTRY

SYNTHESIS OF NOVEL ANTIMALARIAL AND ANTITUBERCULAR ISONICOTINOHYDRAZIDES

BY

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IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF MASTER OF PHILOSOPHY DEGREE IN CHEMISTRY

NOVEMBER 2018

DECLARATION

I, **Crystal Funmilayo Ade,** do hereby declare that this dissertation is the result of my own work and that, to the best of my knowledge, all the contents presented originated from my research with the exception of references and quotations which have been duly acknowledged, and that the same work or no part of it has been presented for the award of a degree in this university or any other institution of higher education.

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DEDICATION

This book is dedicated to all Ghanaian families who have lost loved ones either through Malaria or Tuberculosis.

ACKNOWLEDGEMENT

To the maker of life, the originator of science and the omniscient, who imparts knowledge and manipulates the human mind to hidden truths and amazing discoveries.

I am grateful to my parents, Mr. L. B Ade, the financial backbone behind my education and his beautiful wife Mrs. Vida Ade, a great source of motivation in all areas of my life, and for the special prayers showered daily upon their precious Jewel.

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LIST OF ABBREVIATIONS

ACT	Artemisinin Combination Therapie
AL	Artemether-lumefantrin
AS	Artesunate-sulfadoxine-pyrimethamine
DOTS	Directly Observed Treatment Short-course
DP	Dihydroartemisinin-piperaquine
DST	Drug Susceptibility Test
FBS	Fetal Bovine Serum
FC	Friedel-Crafts
IPTP	Intermittent Preventive Therapy in Pregnancy
MABA	Microplate Alamar Blue Assay
MDR	Multi-drug Resistance
MIC	Minimum Inhibitory Concentrations
MMV	Medicines for Malaria Venture
RBC	Red blood cells
SAR	Structure-Activity Relationship
SDG	Sustainable Development Goal
SP	Sulphadoxine-pyrimethamine

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ABSTRACT

Malaria and tuberculosis (TB) though curable and preventable, remain serious public health problems globally, with devastating consequences. Co-infection of these two deadly diseases worsens the situation and particularly makes treatment very difficult. While the current mainstay for malaria treatment, artemisinins, may gradually lose their potency due to the development of resistance by *P. falciparum, M. tuberculosis* has developed Multi-Drug Resistance (MDR) and Extensive Drug Resistance (XDR) to current anti-TB drugs, due to patient incompliance resulting from long treatment regimen. This project focused on the synthesis of isonicotinohydrazides with the concept of molecular hybridisation, by incorporating 1,2,4,5- tetraoxane and hydrazine moieties to yield a single molecule that will exhibit both antimalarial and antitubercular activites. In all, ten novel hybrid molecules were designed, synthesised and characterised using FTIR, ^{1}H and ^{13}C NMR spectroscopy. All the compounds returned acceptable physicochemical parameters as well as good antimalarial activities against 3D7 strain of *P. falciparum*. *In vitro* IC_{50} values ranging from **0.060 ± 0.033 - 0.491 ± 0.012 µM** were obtained**.** Five of the hybrid molecules tested against H37rv and *M. aurum* strains of *Mtb* using Microplate Alamar Blue Assay (MABA), gave satisfactory results while four of the compounds **101**, **102**, **103**, **104,** exhibited very good activity against H37rv with MIC values between **0.003-0.5 mg/mL**, and *M. aurum* was resistant to all five tested compounds.

CHAPTER ONE

1.0 INTRODUCTION

Tuberculosis (TB) and malaria top the list of bacterial and parasitic infections, respectively, in the world. (Blank et al., 2016). Though curable and preventable, they remain serious public health problems globally, with devastating consequences. These diseases kill approximately more than two million people annually, and are known to thrive where poverty exists, thus prevalent in poor countries (Vitoria et al., 2009). Malaria and TB have been reported to have the highest number of cases in Africa, and together with neglected tropical diseases, cause more than 32 % of diseases in the continent (Chukwuanukwu et al., 2016). This has threatened the general health of the public and substantially contributed to the high cost of health care. An important Sustainable Development Goal (SDG) by the United Nations in 2015 is to end the epidemics of both diseases alongside AIDS by the year, 2030 (Bosa et al., 2017).

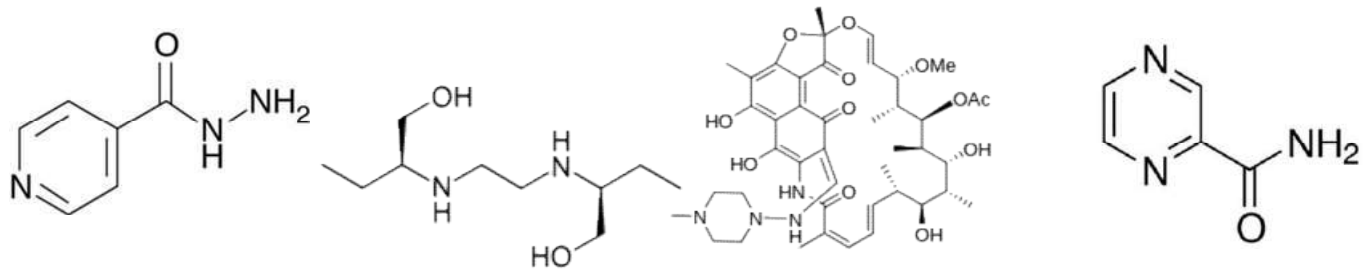
1.1 Tuberculosis

TB is an airborne disease believed to have evolved sometime between the seventh and sixth millennia BC (Hudson et al., 2013). It is also described as the most dangerous microbial disease which comes second after HIV-AIDS among the death-causing diseases in the world (Nayak et al., 2015) and the top infectious killer. The disease has been reported to be responsible for nearly 3 million deaths worldwide annually (Cinu et al., 2015), and is said to be the only disease which can be transported among humans without a vector. Ghana ranks 48 in the world and 30 in Africa with an incidence of 156 TB infections per 10,000 of her population. In the year 2016 alone, up to 14,632 TB cases were recorded in the country (GhanaWeb, 2017, WHO, 2016, WHO, 2017).

Being an airborne disease without a vaccine, TB is the single largest disease not limited to developing countries, but also afflicts developed countries (Maste et al., 2011). The causative agent of TB; *Mycobacterium tuberculosis* (*Mtb*), discovered by Robert Koch in the year 1882 is carried in droplet nuclei, generated when people infected with pulmonary or laryngeal TB shout, sing, sneeze or cough. These droplet nuclei can be suspended in the air for several hours. Transmission occurs when they are inhaled, and travel through the mouth or nasal passages, upper respiratory tract, and bronchi to the alveoli of the lungs. In more than 90 % of TB cases, the pathogen is contained as asymptomatic latent infection which decreases the risk of reinfection on repeated exposure (Zumla et al., 2013). However, the immune system is unable to completely eradicate the tubercle bacillus, thus, new active cases are constantly triggered and the disease is easily transmitted. According to WHO reports, the majority of new cases and deaths occur in Asia and Africa (WHO, 2013). Active TB on the other hand is associated with a about 5-15 % of *Mtb* infected patients (Blank et al., 2016). The risk of the active disease is approximately estimated to be 5 % in the first 18 months after infection and approximately 5 % for the remaining lifetime (Zumla et al., 2013). Symptoms of TB include long lasting (about three weeks or more) and bloody coughs, chest pain associated with coughing or breathing, loss of appetite, fever, chills and night sweats (Public Health, 2018, Zumla et al., 2013).

For about 50 years, the same drugs have been used for the treatment of TB. These drugs erased the notion that the disease could not be controlled and raised hopes of possible eradication (Hudson et al., 2013). The regimen for the administration of these drugs termed the DOTS (Directly Observed Treatment Short-course) programme, involves a six-month combination therapy of four first-line anti-TB drugs; isoniazid (INH), **1**, rifampicin (RIF), **2**, ethambutol (EMB), **3**, pyrazinamide (PZA), **4** (**Figure 1.1**). This regimen is normally reserved for new TB patients who have not been

previously treated for TB. The first two months involve the administration of all four drugs, after which INH and RIF are taken for four months (Nayak et al., 2015). Refabutin (RBT) is normally administered for drug-drug interaction cases which do not involve RIF, and together with Rifapentine (RPT) are considered to be first-line drug under certain circumstances (CDC, 2013, Arbex et al., 2010). **Table 1.1** shows a detailed description of commonly used anti-TB drugs and their uses.



1, Isoniazid (INH) **2**, Ethambutol (EMB) **3**, Rifampicin (RIF) **4**, Pyrazinamide (PZA)

Figure 1.1: First-line anti-TB drugs used in DOTS regimen.

Table 1.1: Commonly used anti-TB drugs (CDC, 2013).

Drug Classes	**Anti-TB drugs**	**Comments**
First-line drugs	INH RIF PZA EMB	Form the core of initial treatment regimen.
	RBT	Occasionally substituted for RIF for all forms of TB treatment.
	RPT	Could be used with INH for treatment of HIV negative patients with non-cavitary, drug

		susceptible pulmonary TB whose sputum smears tests negative after initial TB treatment.
Second-line drugs	Streptomycin (SM)	Initially (and currently in some cases) considered to be a first-line drug. Increased global resistance has drastically limited its overall usefulness.
	Cycloserine	Normally used for rare occasions like drug intolerance or resistance.
	Capreomycin	
	ρ-Aminosalicylic acid	
	Levofloxacin	
	Moxifloxacin	
	Gatifloxacin	
	Amikacin/Kanamycin	
	Ethionamide	

Apart from drug toxicity and intolerance, adverse drug-drug interactions with anti-HIV drugs, and patient incompliance due to lengthy treatment duration (Eldehna et al., 2015), a major limitation associated with current anti-TB drugs is the emergence of multi-drug resistant (MDR) TB and extensively drug resistant (XDR) TB (Kesicki et al., 2016). An intervention for the treatment of MDR and XDR TB is the use of second-line anti-TB drugs. These, however, require a longer

duration of treatment (up to two years) (The Union, 2018). Quite recently, a shorter regimen known as the Bangladesh regimen; a 9-12-month regimen, for the treatment of uncomplicated MDR TB was introduced. Though the regimen was comparably shorter, it did not solve all problems; especially in resisting patient incompliance (Aung et al., 2014, Sotgiu et al., 2017). However, the Bangladesh regimen proved that a shorter regimen could be as successful as a longer regimen and partially caused a change in recommendations by the WHO for MDR-TB treatment in May 2016 (WHO, 2016). Trending currently for drug resistant TB are two new drugs; bedaquiline (**6**) sold under the brand name sirturo and delamanid (**5**) sold under the brand name, deltyba (**Figure 1.2**).

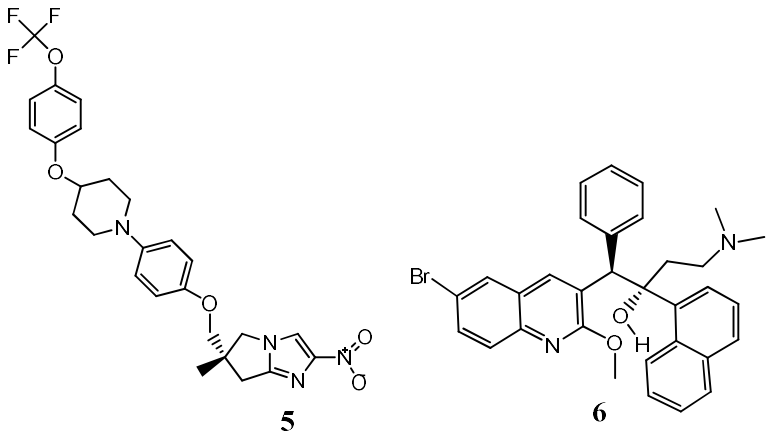
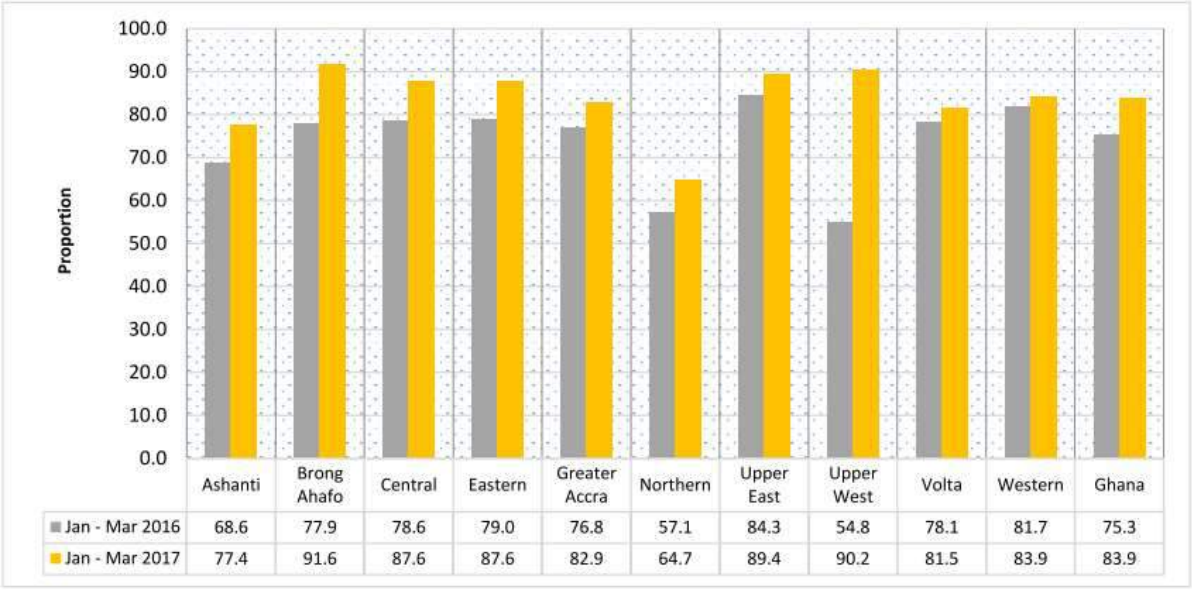


Figure 1.2: Delamanid (**5**) and Bedaquiline (**6**) current drugs for MDR-TB treatment.

These are in stage two of clinical studies in Mongolia (The Union, 2018). However, not all patients with drug resistant TB are eligible for shorter treatment regimens. Patients with RIF resistant or multi-drug resistant TB who have not received any treatment with second-line drugs, and who are resistant to the fluoroquinolone group of drugs as well as excluded from second-line injectable drugs are considered most suitable for shorter treatment regimens (tbfacts.org, 2018).

1. 2 Malaria

Malaria is a parasitic infection with high mortality rates, prevalent in the tropical and subtropical regions, which extracts huge health and economic costs (Blackie, 2014), leading to developmental and economic stagnancy or retrogression especially on a continent that already battles with the effect of other diseases like TB and HIV/AIDS (Murphy, 2015). According to WHO reports, about one million deaths, 450 million *Plasmodium falciparum (P. falciparum)* and 390 million *P. vivax* cases occur each year (Cinu et al., 2015). Those at greatest risk are children under the age of five, pregnant women and visitors from non-endemic areas (Tripathy & Roy, 2014). In Ghana, malaria tops Out Patient Department (OPD) cases with 37 %, and is responsible for the deaths of three children daily. According to the Ghana Health Service, 10.4 million cases of malaria were recorded in the year 2016 alone. **Figure 1.3** compares malaria test rates of the first quarter of 2016 to the first quarter of 2017, there was an increase in all 10 regions from 75.3 % in 2016 to 83.9 % in 2017 (Ghana Health Service, 2017).



	Ashanti	Brong Ahafo	Central	Eastern	Greater Accra	Northern	Upper East	Upper West	Volta	Western	Ghana
Jan - Mar 2016	68.6	77.9	78.6	79.0	76.8	57.1	84.3	54.8	78.1	81.7	75.3
Jan - Mar 2017	77.4	91.6	87.6	87.6	82.9	64.7	89.4	90.2	81.5	83.9	83.9

Figure 1.3: Testing rate of all health facilities in Ghana by region within the months of January - March 2016 and 2017 (Courtesy Ghana Health Service, 2017).

Malaria is caused by protozoa belonging to five species of the genus *Plasmodium; P. falciparum*, *P. vivax*, *P. ovale*, *P. knowles*, and *P. malaria.* These parasites are transmitted via bites of the female *Anopheles* mosquito. Dominant in Africa, *P. falciparum*, is the most pathogenic one and responsible for most fatalities (Staines & Krishna, 2012). The life cycle of *plasmodium* parasites (**Figure 1.4**) begins when sporozoites infect hepatocytes and proliferate into thousands of merozoites in the liver. The merozoites then rip apart from the hepatocytes, and through blood circulation, invade red blood cells (RBCs).

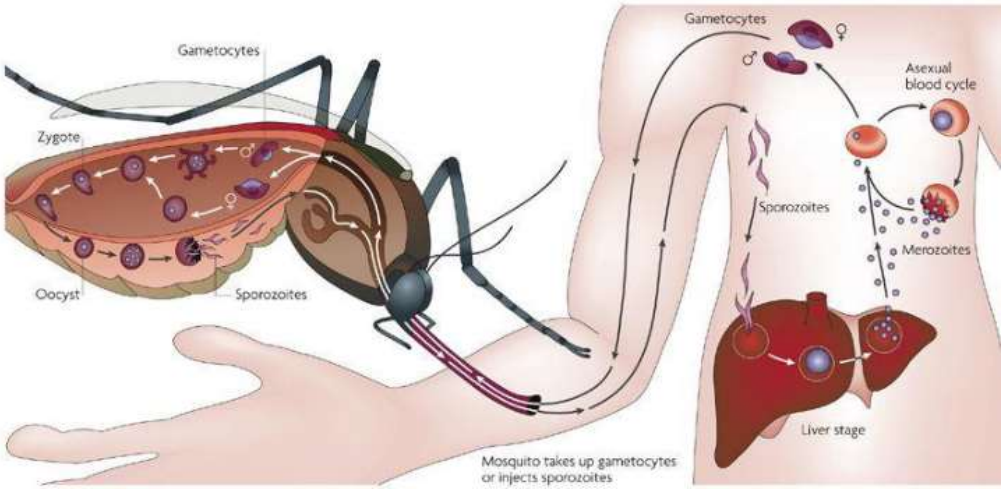


Figure 1.4: The Life cycle of *P. falciparum* (Picture courtesy, Su et al., 2007).

In the RBC, they expand first into rings and then into trophozoites and schizonts. Schizont-infected RBCs burst and liberate more merozoites, which begin the blood cycle again (Matteelli & Castelli, 2015; Tripathy & Roy, 2014). Malaria patients exhibit flu-like symptoms, which include fever, nausea and vomiting, back pain and shaking chills, usually between 10-15 days after the mosquito bite (Murphy, 2015). There has been a global awakening with regards to the fight against malaria. Attempts towards its control and eventual eradication include the use of insecticide-treated bed

nets, indoor residual spraying, use of Intermittent Preventive Therapy in Pregnancy (IPTP) and improved access to diagnosis and treatment (Marco et al., 2009). Several drugs have been lunched for the treatment of malaria. Currently, artemisinin and its derivatives (**Figure 1.5**; **7-9**) are the most effective drugs for malaria chemotherapy and are recommended by the WHO for use in combination with other antimalarials such as chloroquine, proguanil, amodiaquine, lumefantrine, mefloquine, sulfadoxime/ pyrimethamine and primaquine (Staines & Krishna, 2012, Nzila & Chibale, 2011, Tripathy & Roy, 2014). Artemisinin Combination Therapy (ACT) is applied in Ghana. Artesunate-amodiaquine is used for first-line treatment of uncomplicated malaria while artemether-lumefantrine and dihydroartemisinin-piperaquine are used alternatively for patients who cannot tolerate artesunate-amodiaquine. Oral quinine or a combination of oral quinine and clindamycin are administered to pregnant women in the first trimesters, while oral quinine or a combination of artesunate-amodiaquine or artemether-lumefantrine are used for the second and third trimesters. In the event of treatment failure of uncomplicated or severe malaria, quinine is used. Sulphadoxine-pyrimethamine (SP) is used for IPTP while doxycycline, mefloquine, proguanil and atovaquone/proguanil are used for malaria prophylaxis in non-immune visitors to the country (Ministry of Health, 2009).

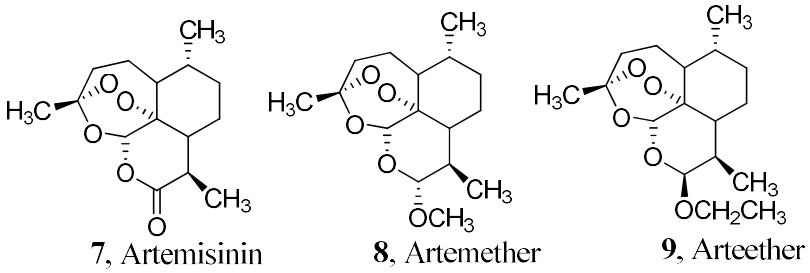


Figure 1.5: Artemisinin and its derivatives.

Reports of *P. falciparum* parasite resistance to artemisinin derivatives in South East Asia however questions the longevity of ACT strategies (Nzila & Chibale, 2011, Straimer et al., 2017). Various researchers have attempted to replace the semisynthetic components of ACTs with fully synthetic components to enhance cost of production, as well as improve overall pharmacological qualities (O'Neill et al., 2017). Outstanding amongst pharmocophoric moieties explored are 1,2,4,5-tetraoxanes; the most structurally simple class of peroxides known to be chemically stable with many methods available for synthesis. The presence of a double endoperoxide 'war-head' and IC_{50} values in the nanomolar range make these peroxides ideal for the synthesis of potential new drugs for malaria.

1.3 Co-infection

For the past seventy years, several studies have reported the co-infection of TB with parasitic diseases (Li & Zhou, 2013). A higher population of latent TB cases have been recorded to live in malaria-endemic areas (Wiwanitkit, 2006). Reports indicate that TB/malaria co-infection is prevalent in the African region, especially sub-Saharan Africa (Blank et al., 2015, Drabe et al., 2016, Chukwuanukwu et al., 2016). Regardless of HIV status, malaria has infected TB patients already afflicted by malnutrition and deprived immunity or disseminated disease (Colombatti et al., 2011). For instance, in a large hospital in Angola, 37.5 % comprising 1/3 of TB patients were found to be co-infected with malaria (Valadas et al., 2013). Apart from both diseases sharing common host defense pathways, they have been reported to generate more severe pathology in co-infections than either diseases on their own (Hawkes, 2012). In another study conducted in Hospital Raoul Follereau (HRF) in Guinea-Bissau, mortality of TB patients dropped from 26.46 % in 2005 to 18.76 % in 2007 (p-value = 0.003), due to the significant reduction of mortality in the rainy season, where malaria burden increases in many African countries, while the dry season

mortality remained constant. The study results indicate that interactions between malaria and TB produce a mutual effect of increased mortality (Colombatti et al., 2011).

Co-infection of TB and malaria, coupled with the ability of *Mtb* to lie dormant for years as a latent infection therefore makes it particularly deadly and difficult to treat, since the co-infection trigger the conversion of the latent TB into active transmissible infections (Shankar et al., 2014).

1.4 Problem statement

Apart from reports of multi-drug and strain resistance to existing drugs, as well as complexity of current treatment regimen and in some cases toxicity, which necessitates the need for new medications for both diseases, malaria/TB coinfection is the least addressed in both clinical and immunological studies, as compared to other co-infections of TB (Blank et al., 2016). Moreover, malaria parasites have been reported to decrease the hosts' effective humeral and cellular immune responses to *Mtb*, thus, making treatment complicated in cases of co-infection. Malaria-TB interactions have been proven by both *in vitro* and *in vivo* studies. These studies show that *P. falciparum* modulates *Mtb* infection and malaria exacerbates mycobacterial infection. Though reasons are not completely explored, it was inferred that parasite-parasite interaction and host-parasite interaction in malaria further depresses the immune system through a qualitative and quantitative defect (Colombatti et al., 2011). Blank et al. (2015) also found some degree of nonspecific protection against rodent *Plasmodium* parasites in the presence of mycobacterial infection.

Though tropical medicine has focused on aberration in pathogenesis of infection in an episode of malaria and tuberculosis co-occurrence for some years (Wiwanitkit, 2006), there are currently no drugs for the concurrent treatment of both diseases. Not only will new drugs for each disease offset a more effective treatment for both diseases, but a single drug that will simultaneously treat both

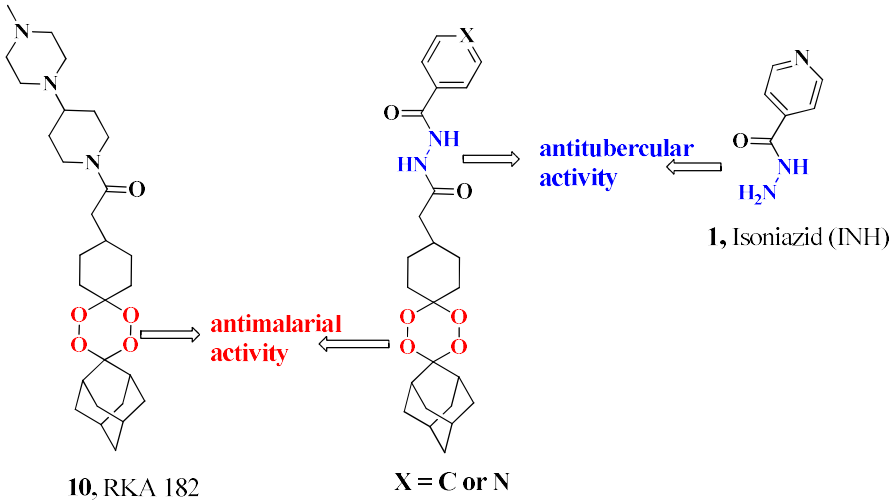
diseases could also resolve strain resistance, long treatment regimens, drug-drug interactions and possible eradication of malaria and TB.

1.5 Objective

The purpose of this study was to develop novel isonicotinohydrazides using the concept of molecular hybridization; the combination of two or more active pharmacophoric groups into a single chemical entity.

1.5.1 Specific objectives

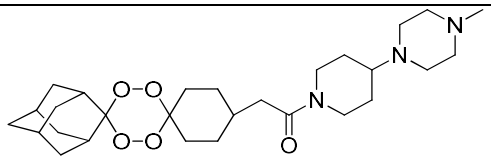
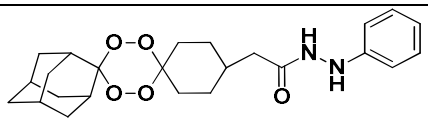
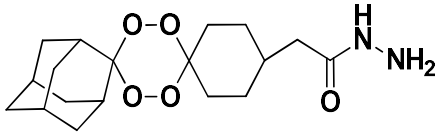
- Synthesise pure isonicotinohydrazides by the incorporation of both peroxide and hydrazine functional groups, to yield a compound that will exhibit both anti-malarial and anti-TB activities. Synthesis of the isonicotinohydrazides will be based on the **RKA 182** (**10**) scaffold as illustrated in **Scheme 1.1**. **RKA 182** is a 1,2,4,5-tetraoxane derivative reported to demonstrate superior *in vitro* and *in vivo* activity to artemether and artesunate.
- Screen synthesized compounds for both antimalarial and antitubercular activities.
- Carry out a comprehensive SAR study on synthesized compounds.



Scheme 1.1: Schematic diagram showing the pharmacophores of anti-malarial 1,2,4,5-tetraoxanes and anti-TB INH.

Preliminary anti-malarial activities of representative hybrid molecules have been determined to be similar or superior to the clinically used drugs (**Table 1.2**).

Table 1.2: *In vitro* Antimalarial Activities of 1,2,4,-tetraoxane hybrid molecules against Chloroquine-sensitive (3D7) and Resistant (K1) Strains (O'Neill et al., 2010).

Compound	**IC_{50} values of *p.falciparum* stains**	
	3D7 (nM)	**K1 (nM)**
	4.9	2.4
	0.3	4.0
	14.7	65.1
Chloroquine	12.5	250.0
Artemether	2.6	Nd

3D7 is a chloroquine sensitive strain of *P. falciparum*, K1 is a chloroquine-resistant strain of *P. falciparum*. All the compounds were tested as free bases. Nd = Not determined.

1.6 Justification

Malaria/TB co-infections appear to generate more severe pathology than either disease on its own (Hawkes, 2012). In experimental mice, malaria co-infection has exacerbated chronic TB while rendering mice vulnerable to *Plasmodium* (Mueller et al., 2013, Li et al., 2012, Hawkes, 2012). In humans, co-infection diverted immune response to an anti-inflammatory response which might breed disease progression (Chukwuanukwu et al., 2016, Li & Zhou, 2013). Respiratory distress due to metabolic acidosis and pulmonary edema and Acute Respiratory Distress Syndrome in children and adults respectively, a frequent occurrence during acute malaria aggravate the respiratory difficulties associated with TB, thus leading to increased mortality (Colombatti et al., 2011). Malaria treatment and prevention have reduced mortality in TB co-infected patients (Valadas et al., 2013) and selections of antibiotic drugs have been combined with other drugs for the treatment of malaria (Dahl & Rosenthal, 2007, Gaillard et al., 2016). Similarly, selections of anti-malaria drugs show good activity towards TB when used in combination with existing anti-TB agents (Murphy et al., 2012). A typical example is the situation of a 34-day old female newborn with co-occurrence of perinatal *falciparum* malaria and tuberculosis, who dramatically responded to combined antimalarial and antitubercular therapy (Thapa et al., 2010). Currently, there are no drugs for the simultaneous treatment of both diseases. A single drug that could have both antimalarial and anti-Tb functionalities could therefore be used for the concurrent treatment of both diseases. While the peroxide functionality of artemisinin is reported to be responsible for its potent antimalarial properties (Opsenica & Šolaja, 2009), hydrazine is also attributed to the antitubercular properties of potent front-line drug isoniazid (Souza et al., 2013, Hearn & Cynamon, 2004). A molecule with both peroxide and hydrazine functional groups could therefore exhibit both antimalarial and antitubercular activities.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1.0 Artemisinin

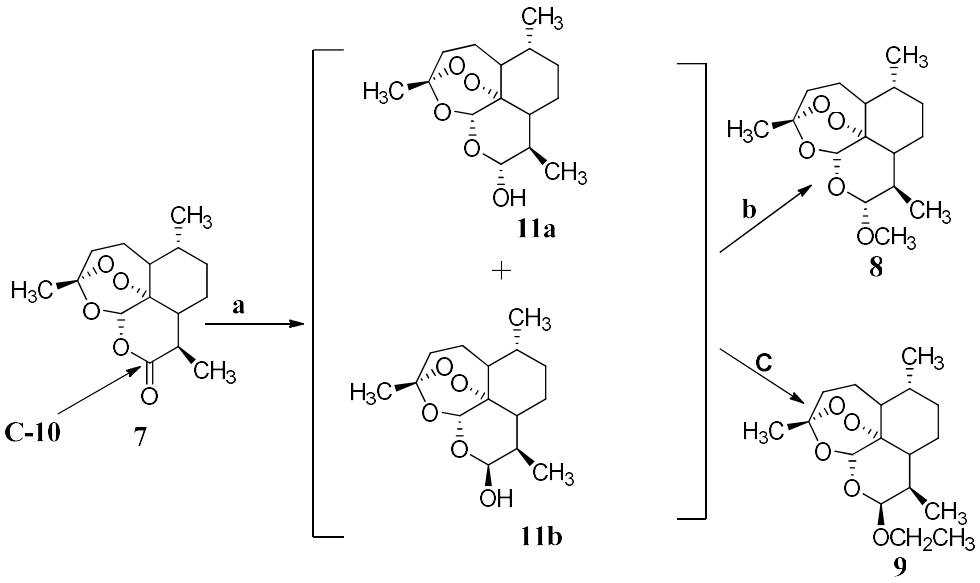
Artemisinin ($C_{15}H_{22}O_5$), is a sesquiterpene lactone, originating from *Artemisia annua*; an ancient Chinese herb, known as Quing Hao (a remedy for relapsing fever for over 2000 years) (Snow et al., 1996, Blackie, 2014, Tilley et.al, 2016). It was first isolated in the early 1970s by the Belgrade group (Staines & Krishna, 2012, Opsenica & Šolaja, 2009). However, the wrong structure was proposed. In 1979, its structure was elucidated by X-ray analysis and total synthesis. Artemisinin (**7**) is soluble in several aprotic organic solvents, but exhibits poor solubility in water and oil. It has a melting point of 156-157 ^oC, molecular mass is 282.3 Da and is unstable in the presence of both alkali and acid (Snow et al., 1996). It is described as an erythrocyte schizonticide, exhibiting rapid biomass action against all types of human and most animal malaria. It is effective against Chloroquine-sensitive (CQS) and Chloroquine-resistant (CQR) strains of *P. falciparum*. Though the precise mechanism of action of the artemisinin remains controversial (Opsenica & Solaja, 2009), it is described to cause rapid parasite biomass reduction (WHO, 2001), at all stages of the parasite life cycle, followed by the rapid elimination of the drug from the body, avoiding the lingering sub-therapeutic blood levels that create conditions conducive to selection of resistance after treatment with slower and longer-acting drugs (Plowe, 2007).

Artemisinin has a distinctive **1,2,4** triaxone system with a peroxide bridge and two adjacent oxygen atoms linked via a carbon atom to a non-peroxide oxygen atom (**Scheme 2.1 (a)**). All active compounds possess a characteristic endoperoxide moiety to which the antimalarial activity of this drug class is attributed. This was affirmed by the fact that metabolites isolated without peroxide function had no antimalarial activity (Opsenica & Šolaja, 2009). The good activity of **7** is partially

attributed to its amphiphilic structure that allows easy permeation into the cell membrane. A major failure associated with its usage is the high re-occurrence of symptoms resulting from its short half-life, low oral bioavailability and auto-induction of metabolism (Opsenica & Šolaja, 2009). Successful attempts have therefore been made to make semi-synthetic artemisinins.

2.1.1 Semi-Synthetic Artemisinin Derivatives

The first attempt was on the lactone group. The lactone carbonyl at C-10 was reduced with $NaBH_4$ in CH_3OH to yield Dihydroartemisinin (DHA) **11 (Scheme 2.1; 2)**.



Scheme 2.1: The preparation of dihydroartemisinin (**11a & 11b**) from artemisinin (**7**), with its conversion into artemether (**8**) and arteether (**9**) using (**a**) $NaBH_4$, CH_3OH, 0 °C, (**b**) CH_3OH and (**c**) CH_3CH_2OH (Vlok, 2008).

DHA was found to be six times greater in antimalarial activity than that of its parent compound, but considerably less stable *in vivo*. Lactol ethers of DHA **8** and **9** were however oil-soluble and therefore well absorbed intramuscularly. Another derivative of artemisinin; artesunate (**12**) was derived from DHA (**11**) through esterification (**Figure 2.1**). **12** is soluble in water and therefore

used intravenously for advanced malaria treatment. Arteether **8**, artemether **9** and artesunate **11** were reported to be fast-acting against uncomplicated *P. falciparum* infections and severe malaria. However, their rapid metabolism to DHA causes them to have short half-lives. Artelinic acid (**13**) (**Figure 2.1**), a water-soluble derivative of artemisinin later exhibited higher hydrolytic stability in aqueous solution in comparison with artemisinin and its derivatives, as well as better oral bioavailability and longer elimination half-life. It was however found to be three times more toxic than artesunate and thus, its use was discontinued (Vil' et al., 2017).

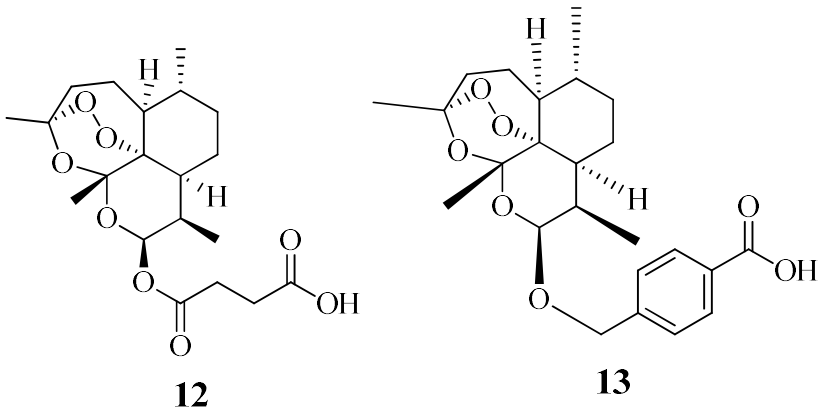


Figure 2.1: Artemisinin derivatives artesunate (**12**) and artelinic acid (**13**).

2.1.2 Artemisinin Combination Therapies (ACTs)

Synthetic variants of artemisinin are currently administered in combination with longer lasting antimalarial drugs such as primaquine **14**, amodiaquine **16,** lumefantrine **17**, Pyrimethamine **20**, , etc. (**14**-**22**; **Figure 2.2**), in Artemisinin Combination Therapies (ACTs) (WHO, 2011), due to the short half-lives and high recrudescence rates (which occurs as early as after 5 days) rendering them unsuitable as monotherapies (Fisher & Blackie, 2014, Tilley et al., 2016). The combination of artemisinin derivatives with longer lasting partner drugs enhance the sustenance of the therapeutic levels of these short-lived antimalarials after plasma concentration of the artemisinin derivative

has dropped (Fisher & Blackie, 2014). The most common combinations are artemether-lumefantrin (AL), artesunate-amodiaquine (AS+AQ), artesunate-sulfadoxine-pyrimethamine (AS+SP), artesunate-mefloquine (AS+MQ), and dihydroartemisinin-piperaquine (DP) (**Table 2.1**). Among these combinations, AL and DP have been better tolerated and exhibited excellent efficacy in most studies conducted (Price & Douglas, 2009).

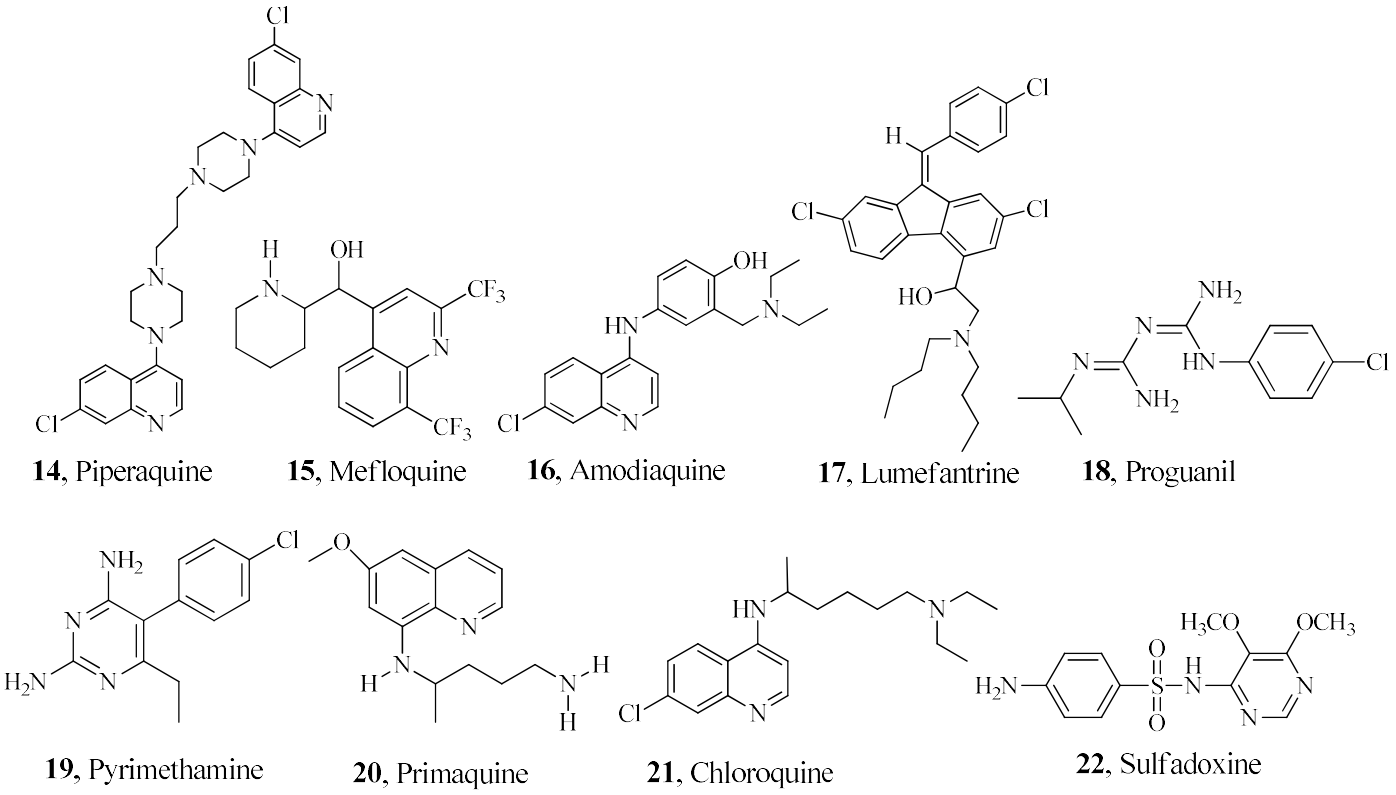


Figure 2.2: Partner drugs usually combined with artemisinin based drugs in ACTs.

Several studies conducted in Africa and other WHO regions, have either encouraged, discouraged or caused the withdrawal of ACTs (**Table 2.1**) based on their efficacies towards malaria infections endemic in such regions (Plowe, 2007, Yeka et al., 2013, Taylor et al., 2016, Whalen, 2008, Boseley, 2008).

Table 2.1: ACTs, their efficacies and current statuses.

DRUG COMBINATION	EFFICACY AND ADVANTAGE	STATUS	References
AS-QC	Very high chloroquine failure rates (>60%) and sub-optimal efficacy of the combination (<85% cure rate)	**Not approved;** Not a viable option in areas with pre-existing moderate to high levels of *P. falciparum* resistance to Chloroquine	WHO, 2001
AS+AQ	Better efficacy than amodiaquine (cure rate >90%) Well tolerated	Approved	WHO, 2001; Nosten & White, 2007.
AS+MQ	In use for many years and the first-line treatment in several parts of Asia	Not approved; Not considered a viable option as first-line therapy in Africa	WHO, 2001; Nosten & White, 2007.
AS+SP	Well tolerated; Efficacy dependent on the level of pre-existing resistance to SP	Approved (in areas where SP efficacy is high); Resistance to SP limits the use	WHO, 2001; Nosten & White, 2007.
AL*(Coartem,™ Riamet™)*	As effective, and better tolerated, as artesunate plus mefloquine; No serious adverse reactions documented	Approved; Not recommended for use in pregnancy and lactating women	WHO, 2001; Nosten & White, 2007.
Chlorproguanil + Dapsone + AS *(Dacart™)*		Withdrawn at development stage by GSK for fear of hemolytic anemia in G6PD deficiency	WHO, 2001; Boseley, 2008.

Clinical trials of artemisinin derivatives in uncomplicated malaria have reported risk of recrudescence. Treated patients who remain in endemic areas may relapse due to liver stage hypnozoites (Price & Douglas, 2009). Though initially described as a formidable new weapon in the war against drug-resistant malaria (Plowe, 2007), recent years have witnessed the emergence

of artemisinin resistance in *P. falciparum* by K13 gene mutations. In Cambodia, malaria patients have been reported to have delayed parasitic clearance following either AS monotherapy or an ACT (Dondorp et al., 2009, Noedl et al., 2008). This may lead to a reduction in the therapeutic lifespan of artemisinins, as well as threaten the continuous use of ACTs.

Since the endoperoxide linkage in artemisinin and its derivatives has been identified as the key pharmacophore in the antimalarial activities of these molecules (Fisher & Blackie, 2014, Antoine et al., 2014), there have been attempts by research groups to replace the semi-synthetic artemisinin components of ACTs with a fully synthetic alternative due to shortcomings described above (O'Neill, et al., 2017). Success at efforts on semi-synthetic derivatives of artemisinin (artesunate and artemether) led to the creation of new synthetic peroxides as potential antimalarial agents (O'Neill et al., 2018).

2.2.0 Importance of Peroxides

Peroxides are widely applied in several areas of life. Traditional applications of peroxides include industrial radical in polymer production, chlorovinyls, styrenes, butadienes, ethylenes, acrylates, silicone rubber crosslinking, etc. (Patnaik, 2007). Though antimalarial properties are predominantly discussed in scientific literature, organic peroxides have been reported to exhibit a variety of other biological activities. These include anthelmintic, antiprotozoal, fungicidal, and antiviral (Vil' et al., 2017).

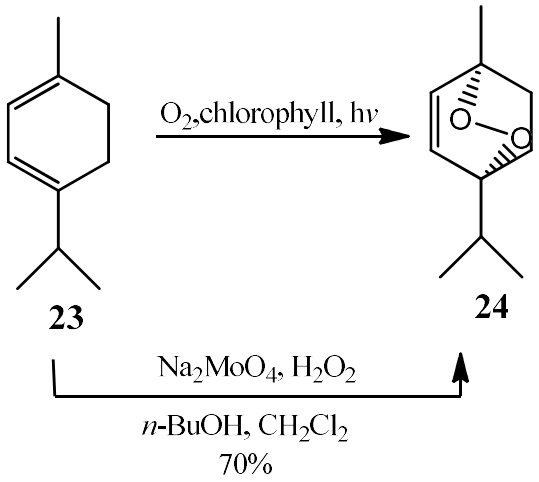
2.2.1 Naturally- Occurring Peroxides

Over a thousand peroxides including over 900 endo-peroxides from natural sources have been isolated and characterized. Sources are mainly plants, fungi, fungal endophytes, algae, invertebrates and other organisms (Dembitsky, 2015b). Extensive pharmacological screening performed on these compounds have yielded anti-tumor, antibacterial and mainly antimalarial

activities (Dembitsky, 2015a). Unfortunately, natural peroxides have short metabolic lives, hence it was a major challenge isolating and replenishing them. However, stable compounds like artemisinin were also found and have been used or modified for very important therapeutic activities over the years (Tolstikov et al., 1996).

2.2.2 Plant peroxides

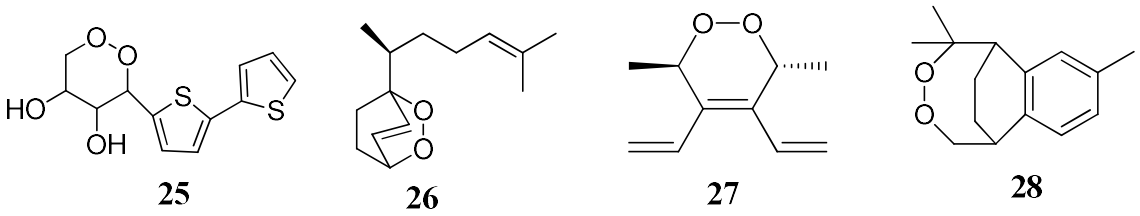
Ascaridole (1,4-epidioxy-*p*-menth-2-ene) (**24**), also known as ascarisin, described as a bicyclic monoterpene with a unique bridged peroxide (Yong-hong et al., 2012), was the first natural peroxide to be isolated in an individual state in 1895, by a German pharmacist (Yong-hong et al., 2012). It was isolated from *chenopodium*, obtained by steam distillation of the plant *Chenopodium ambrosioides L*. Ascaridole was suggested to be responsible for worm-expelling actions of the plant. The plant was also used against stomach cramps, syphilis, measles, and intestinal diseases. In the early 1900's, ascaridole was used as a major anthelmintic for the treatment of ascarids and hookworms in humans and animals. Apart from its activity against various tumor cell lines, Ascaridole is said to possess sedative and pain-relieving properties and antifungal effects as well as a potent inhibitor to *P. falciparum*, *Trypanosoma crusi* and *Leishmania amazonensis in vitro* (Dembitsky et al., 2008). The complete characterization of ascaridole was achieved in the 1950s. Its first laboratory synthesis was prepared by Schenck in 1944. It involved a photo induced addition of singlet oxygen to terpene (**23)** (**Scheme 2.2**). Due to its importance as an anthelmintic agent, ascaridole was synthesized on an industrial scale. However the drug is currently not in use, due to side effects on the gastrointestinal tract (Vil' et al., 2017).



Scheme 2.2: Synthesis of Ascaridole.

2.2.2.1 Six-membered endoperoxides.

Following the discovery of ascaridole, other six-membered endoperoxides with significant biological activity have been found (**Figure 2.3)**. Echinobithiophene (**25)**, was isolated from *Echinops ritro* and exhibited significant antimicrobial activity (Dembitsky, 2015a). 3,6-epidioxy-1,10-bisaboladiene (**26),** isolated from *Cacalia delphiniifolia,* an edible wild plant was discovered to possess cytotoxicity against leukemia K562 and prostate carcinoma LNCaP cell lines at 9.1 µM and 23.4 µM, respectively. Other peroxides, shuangkangsu (**27**) and peroxy-multiflorane triterpene ester (**28)**, isolated from the buds of *Lonicera japonica* and processed seeds of *Cucurbitaceae, Trichosanthes kirilowii,* respectively, exhibited antiviral activity by the former (Yu et al., 2008) and both antiplasmodial and cytotoxic activities by the latter (Dembitsky, 2015a).

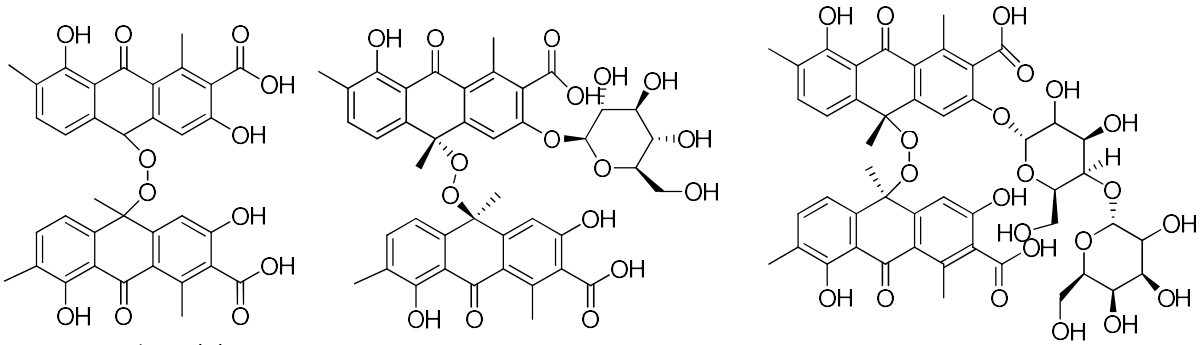


25 **26** **27** **28**

Figure 2.3: Six-membered endoperoxides of plant origin.

2.2.2.2 Rare acyclic peroxides

Oxanthromicin (**29)** (**Figure 2.4**); a natural acyclic peroxide used as an antibiotic was isolated from a fermentation broth, *Actinomadura sp*. SCC 1646 (Dembitsky, 2015a). According to Patel *et al*., who isolated the compound, oxanthromicin is a yellow amorphous compound which decomposes between 211–213 °C and was reported to exhibit good *in vitro* activity against dermatophytic fungi, as well as moderate activity against *Candida albicans* and *Staphylococcus aureus* (Patel et al., 1983). Two adxanthromycins (**30** and **31)** isolated from *Streptomyces sp.* were reported to inhibit the formation of the JY cell aggregates from 1.5 mg/mL in a dose-dependent manner. **30** and **31** were also reported to inhibit human T cell leukemia cell line SKW-3 at IC_{50} 18.8 µg/mL and 25.0 µg/mL, respectively (Nakano et al., 2000).

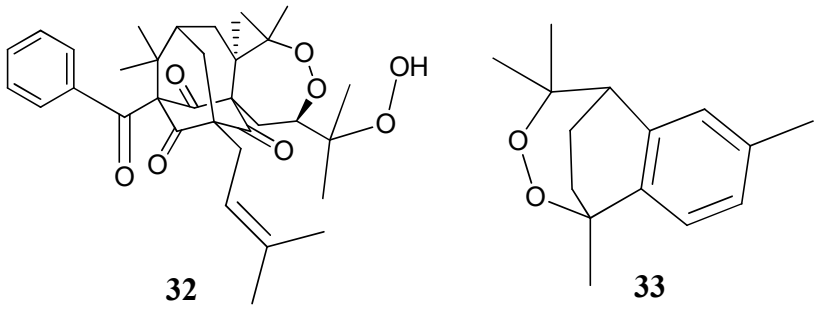


29, Oxanthromicin **30,** Adxanthromycin A **31,** Adxanthromycin B

Figure 2.4: Antifungal natural acyclic peroxide oxanthromicin and adxanthromycins A and B.

2.2.2.3 Seven-membered peroxides

Seven-membered endoperoxide Peroxysampsone **(32) (Figure 2.5)**, isolated from *Hypericum sampsonii*, a Chinese medicinal plant, displayed comparable activity with norfloxacin (Monzote et al., 2011), while 10,12-peroxycalamenene (**33),** from the *Cyperus rotundus*, showed good *in vitro* antimalarial activity (Dembitsky, 2015a).



32 **33**

Figure 2.5: Seven-membered endoperoxides of plant origin.

2.2.2.4 Four and five-membered isolated endoperoxides.

Four and five-membered isolated natural endoperoxides include (**34)** (**Figure 2.6**), from *Biden pilosa var. radiata*, a popular Taiwanese folk medicine which exhibited antitumor, antifungal, antiviral, anticancer, antimicrobial and antileishmanial activities *in vitro*, and cytotoxic sesquiterpenes, (**35-37**) from *Artemisia abrotanum* extracts. A group of guaiane 6,10-endoperoxides, isolated from the genus *Achillea* also showed cytotoxic, antibacterial and antifungal activities (Dembitsky, 2015a).

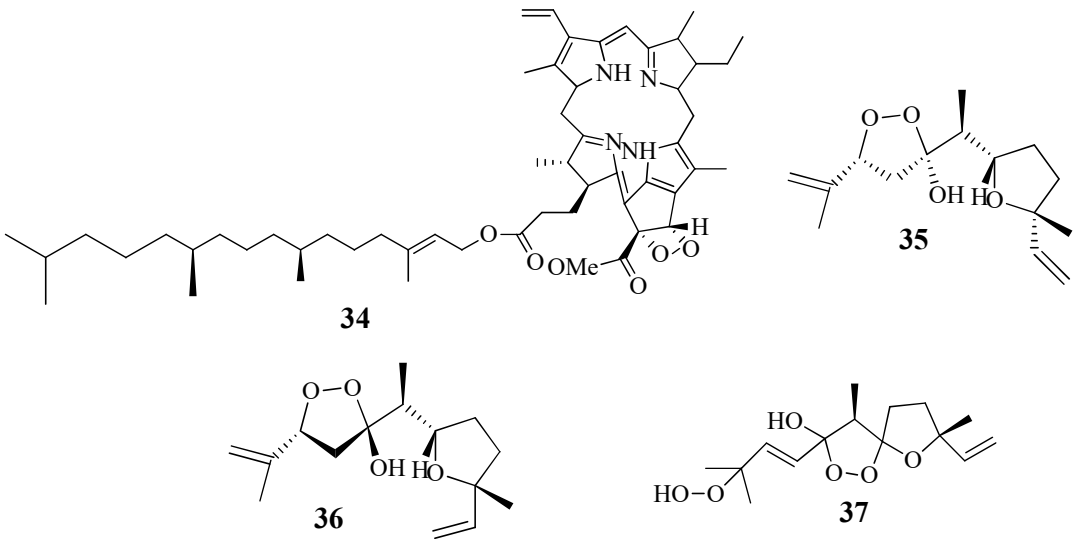


Figure 2.6: Four- and five-membered isolated natural endoperoxides from plants.

2.2.2.5 Hydroperoxides

Several natural hydroperoxides with very good biological activities have also been isolated from various plants. Compound **38** (**Figure 2.7**) was obtained from *Salvia sahendica*., known to exhibit antibacterial effects on *Klebsiella pneumonia, Staphylococcus aureus* and *Pseudomonas aeruginosa*. A *p*-menthane hydroperoxide (**39)**, from *Laurus nobilis* (Uchiyama et al., 2002), was used as a trypanocidal agent against epimastigotes of *Trypanosoma cruzi*, while (**40)** exhibited antitrypanosomal activity against *Trypanosoma brucei* (Dembitsky, 2015a). A trans-chrysanthemic monoterpenoid hydroperoxide (**41)** showed antiviral, anti-bacterial and selective cytotoxic activity when isolated from *Santolina insularis* (Cherchi et al., 2001). Hydroperoxy terpene (**42)** isolated from *Juniperus przewalskii* also exhibited effective anti-tumor activities against cervical carcinoma (HeLa) and human ovarian carcinoma (HO-8910) cell lines (Wang et al., 2002). Isolated compound (**44)** from the antimalarial plant *Liriodendron tulipifer* was found to possess antiplasmodial activity (Dembitsky, 2015a). *Inula japonica,* a flowering plant gave a hydroperoxide (**43)**, which displayed very good inhibitory activity against LPS-induced NO production in RAW264.7 macrophages (Dembitsky, 2015a).

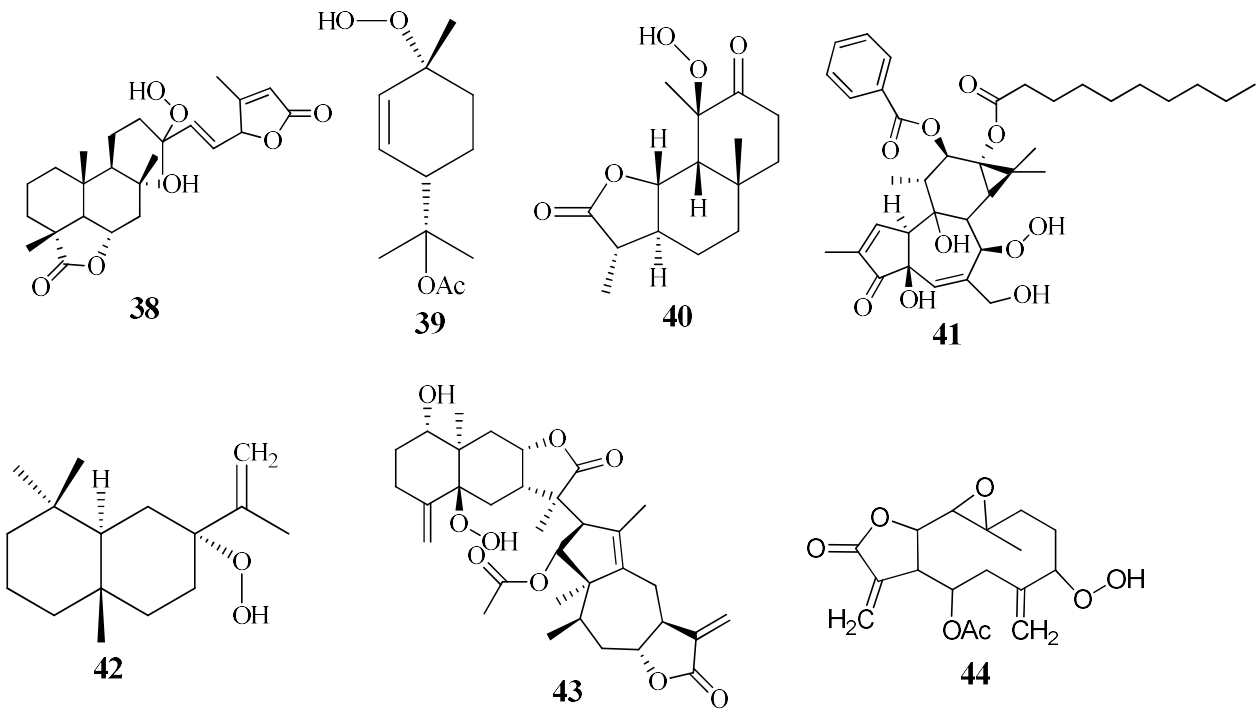


Figure 2.7: Natural hydroperoxides of plant origin.

2.2.3 Peroxides from fungi and fungal endophytes.

Steroid derivatives, ergosterol peroxides (5,8-epidioxy- 5α,8α-ergosta-6,22E-dien-3β-ol) (**45-48**; **Figure 2.8**); another class of natural peroxides have been isolated from a number of lower organisms, especially fungi and fungal endophytes (Takei et al., 2005, Dembitsky, 2015a)). These compounds demonstrated anti-inflammatory, anti-bacterial, anti-atherosclerosis and anti-proliferative properties in human T cells (Wu et al., 2012). Ergosterol peroxide from *Armillariella mellea Daedalea dickinsi, Fomitella fraxinea* and *Pleurotus cornusopiae* mushroom extracts exhibited significant inhibition of rat liver microsomes, and higher antioxidant activity when compared to well-known antioxidants, α-tocopherol and thiourea (Kim et al., 1999). *Sarcodon aspratus*, ergosterol peroxides inhibited human leukemia cells and induced apoptosis after 24-hour incubation (Takei et al., 2005). *Gomphus clavatus* ergosterol peroxide exhibited cytotoxic activity (Makropoulou et al., 2012), while ergosterol peroxide from *Ganoderma lucidum* demonstrated

(Wu et al., 2012) inhibition against breast cancer cell growth and apoptosis, thus overcoming multiple drug resistance. Edible mushroom, *Pleurotus eryngii* extracts yielded an ergosterol peroxide which showed up to 62 % inhibition rate with low cytotoxicity, even at lower concentrations (Yokoyama et al., 2012).

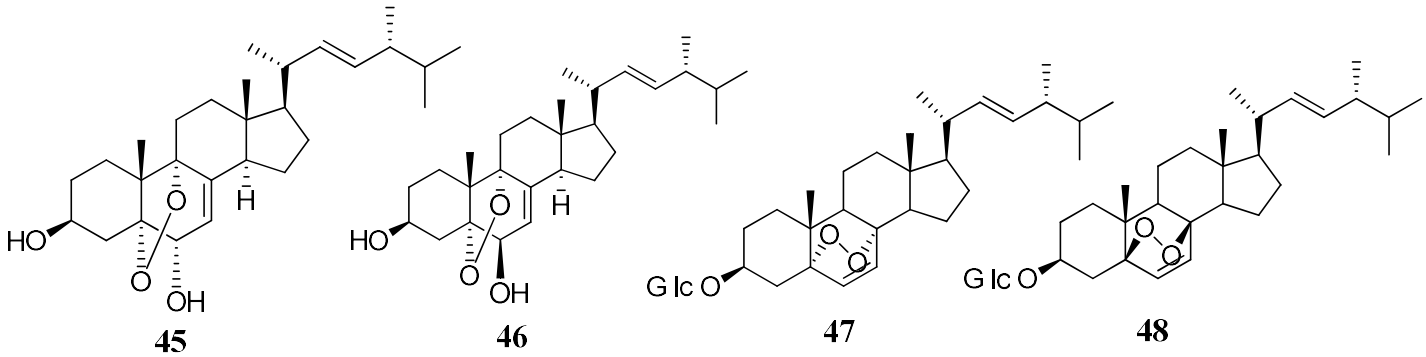


Figure 2.8: Ergosterol Peroxide and derivatives.

Isolated spiro decane sesquiterpene (**49)** (**Figure 2.9)** from cultured mycelia of parasitic fungi *Cordyceps ophioglossodes* showed cytotoxic activities (Sun et al., 2013). Eudesmene-type sesquiterpenes, HKI0595 derived from the mangrove plant *Kandelia candel* also demonstrated weak to moderate inhibitors of *B. subtilis* and *Mycobacterium vaccae* growth (Ding et al., 2012). Several nor-sesquiterpene peroxides (**50-54)**, known as talaperoxides, isolated from the culture of fungi *Talaromyces sp*. showed antineoplastic activity against mammary cancer, prostatic carcinoma, uterine cervix carcinoma or hepatic carcinoma (Li et al., 2010, Dembitsky, 2015a)). The same compounds isolated from a mangrove endophytic fungus, *Talaromyces flavus* (Li et al., 2011) and talaperoxides (**52** and **53)** showed cytotoxicity against the five human cancer cell lines with IC_{50} values between 0.70 and 2.78 µg/mL. The cultures of fungus *Cytospora sp*. yielded Cytosporolides **54-56**, caryophyllene-derived meroterpenoids with unique peroxylactone skeleton which exhibited significant antimicrobial activity against the Gram-positive bacteria *Staphylococcus aureus* and *Streptococcus pneumoniae* (Y. Li et al., 2010).

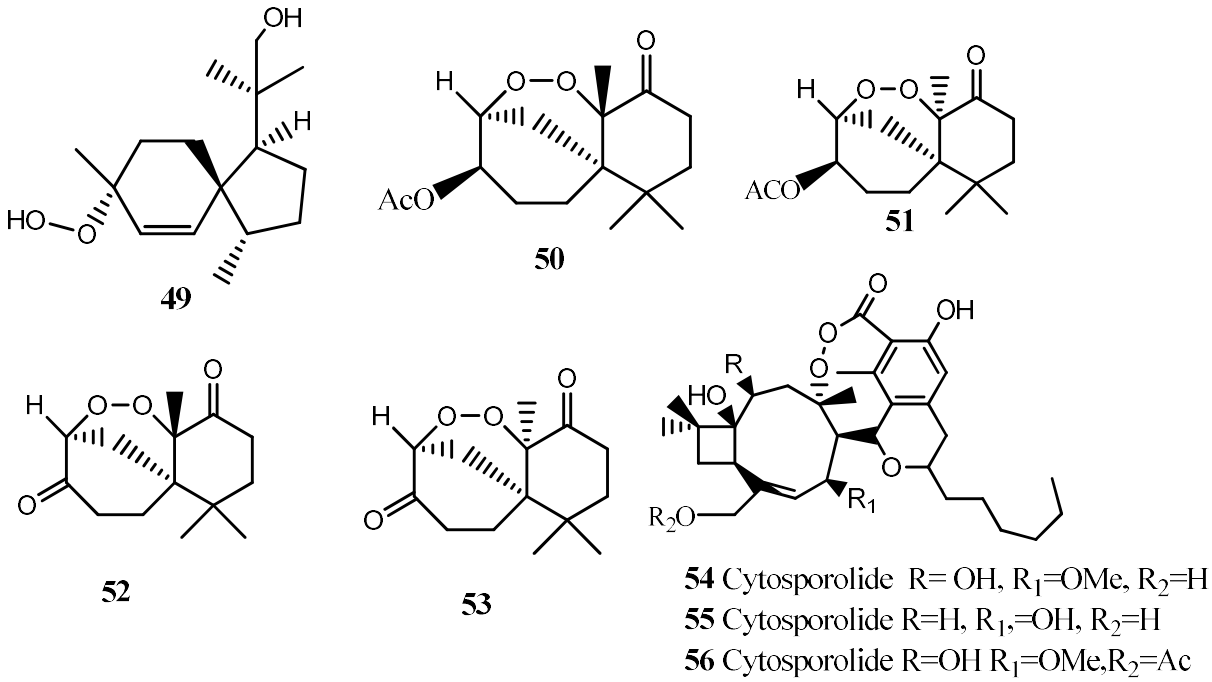
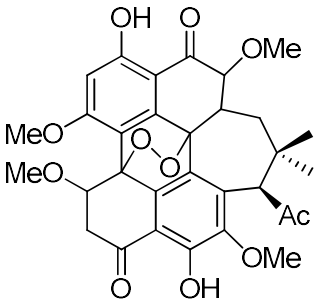
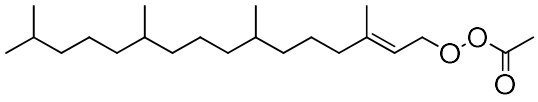


Figure 2.9: Sesquiterpene isolates from plants.

Endoperoxide verruculogen (**57**) (**Figure 2.10**) has been isolated from a number of microbiological sources including *Penicillium verruculosum* isolated from peanuts, *Aspergillus caespitosus*, *A. fumigatus*, *A. fischeri, Penicillium piscarium*, *P. paxilli*, *P. piceum*, *P. nigricans*, *P. raistrickii*, *P. estinogenum, P. simplicissmum*, *Eupenicillium sp.*, and an invasive fungal pathogen *Neosartorya fischeri*. Verruculogen is known to be a potent inhibitor of high conductance Ca activated K channel as well as exert moderate lethality on brine shrimps (Dembitsky, 2015b). Leucoperoxyterpene (**58**) with good antibacterial activity was also isolated from extracts of the aerial parts of the medicinal plant *Leucosceptrum canum* (Dembitsky, 2015a).



57



58

Figure 2.10: Acyclic peroxides of plant origin.

2.2.4 Alga Peroxides

In the early 1970s, peroxides were suspected to be present in marine organisms. Later, this assumption was proved when extracts from sea sponges were found to contain 1,2-dioxane and 1,2-dioxalane derivatives, macrocyclic peroxides and 6,9-endoperoxides of sterols. Biological assay of these peroxides (**Figure 2.11**) exhibited high antibacterial, fungicidal, cytostatic and anticarcinogenic properties (Tolstikov et al., 1996).

An endoperoxide metabolite (**59**) from *Plakortis halichondroides* was reported to inhibit protease as well as display anti-parasitic activity against *Trypanosoma brucei* at IC_{50} of 5 µM. Other endoperoxides from the plakortin family have also been reported to be active against *Plasmodia* (Oli, Abdelmohsen, Hentschel, & Schirmeister, 2014). Marine red alga *Laurencia decumbens* yielded peroxide (**60)** which showed cytotoxicity against adenocarcinomic human alveolar basal epithelial (A549) cells. Bromoditerpenes, (**61** and **62)** isolated from the marine red alga *Sphaerococcus coronopifolius* displayed cytotoxic and antibacterial activities respectively (Zhu et al., 2013). Two bioactive compounds, dictyohydroperoxide **(63)** and hydroperoxy-acetoxycrenulide (**64)**, isolated from *Dictyota dichotoma*, Showed significant cytotoxicity against human cancer cell lines (Dembitsky, 2015a).

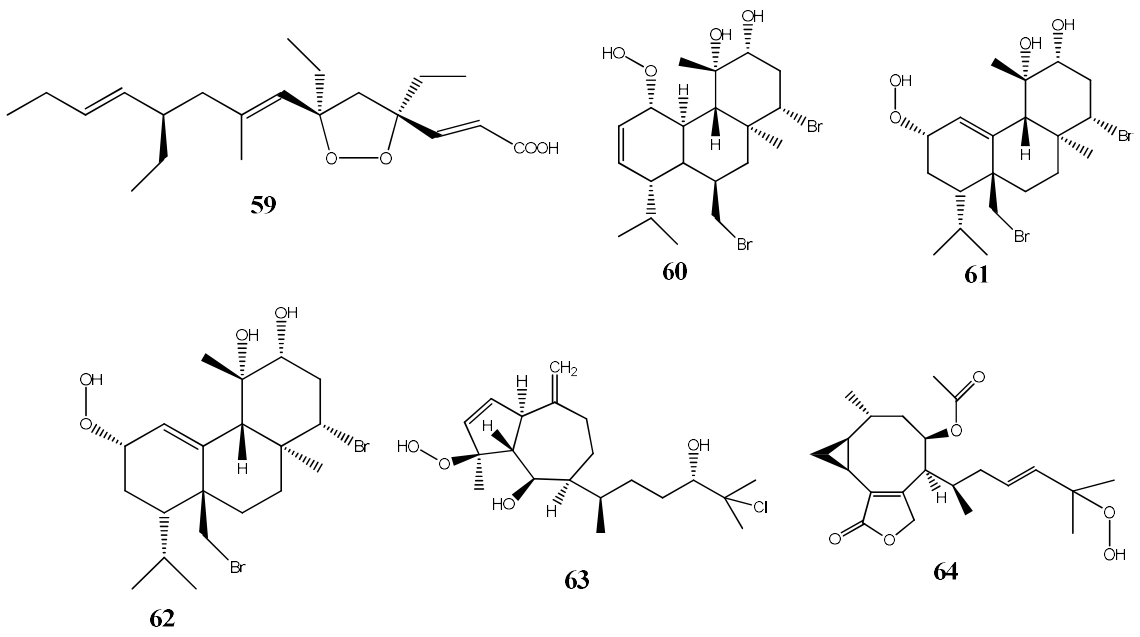
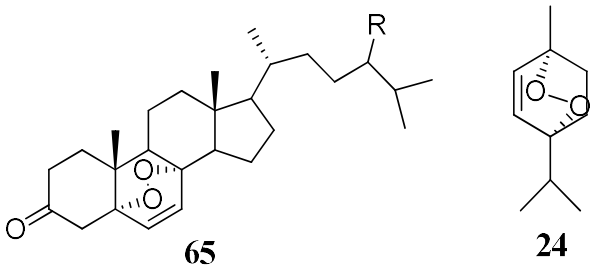


Figure 2.11: Natural peroxides from alga species.

2.3.0 Synthetic Peroxides

Since the early 1980's hundreds of semisynthetic and synthetic peroxides have been developed and reported to display antimalarial activity (Opsenica & Šolaja, 2012). The development of synthetic peroxides with superior biological activities to their natural or semi-synthetic analogues is the interest of modern synthetic chemistry. The drive for the development of synthetic peroxide is to improve pharmacological qualities (short half-lives) as well as cut down the cost involved in production (O'Neill et al., 2017). A few natural peroxides including ergosterol peroxide and ascaridole have been successful synthesised (**Figure 2.12**). Synthetic analogues of ergosterol peroxide (**65),** showed marked inhibition against the growth of Hepatitis B virus, while ascaridole (**24)** almost completely inhibited *Sclerotium rolfsii* growth (Vil' et al., 2017).



65 24

Figure 2.12: Synthetic analogues of ergosterol (**65**) peroxide and ascaridole (**24**).

Several design strategies have been initiated by MMV (Medicines for Malaria Venture) to develop more effective and cheaper synthetic peroxides to replace ART-based drugs. 1,2,4,5-tetraoxane 1,2,4-trioxane and 1,2,4-trioxolane analogues have been reported to exhibit promising antimalarial activities and therefore of much interest to synthetic chemists in search for potential antimalarial drugs (Rudrapal et al., 2017).

2.3.1 Trioxolanes

Trioxolanes are synthetic drugs with a similar peroxide pharmacophore and simpler structure to the artemisinins (Opsenica & Šolaja, 2009, Vil' et al., 2017). 1,2,4-Trioxolanes (ozonides), were initially known as intermediates during ozonolysis. It was therefore a surprising breakthrough when they were discovered to be stable compounds which possess high antimalarial activity (Opsenica & Šolaja, 2012). Endoperoxide 1,2,4-trioxane molecules have been reported to demonstrate anticancer, antimicrobial, and anti-parasitic activities (Rudrapal et al., 2017). 1,2,4-trioxolane was synthesised by Griesbaum co-ozonolysis of suitable methyl oximes and ketones. The tolerance of 1,2,4-trioxolane core to different reaction conditions has enabled the synthesis of a significant number of derivatives via the applied method (Opsenica & Šolaja, 2012).

2.3.2 Arterolane (OZ277)

The first trioxolane, arterolane (RBx11160/OZ277) (**66**) (**Figure 2.13**), to be synthesized (Dong et al., 2010), was described to be the most advanced synthetic peroxide drug (O'Neill et al., 2016),

that has proved to be well tolerated in people (Phyo et al., 2016). Weak base functional groups were substituted to give various analogues which exhibited very good schizontocidal activity against all erythrocytic stages of *P. falciparum.* In a study performed in a hospital in Gabon against *P. falciparum,* OZ277 showed excellent activity against fresh, chloroquine-resistant *P. falciparum* field isolates (Kreidenweiss et al., 2006). Phase II clinical trials of arterolane monotherapy displayed little activity while combination with piperaquine showed higher clinical activity, with no record of recrudescence in patients (Patil, Baig, Doifode, & Katare, 2014).

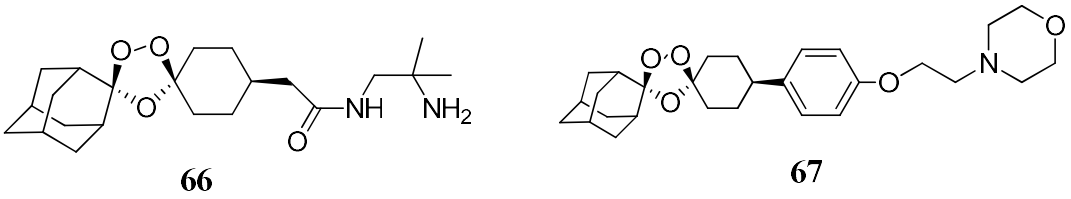


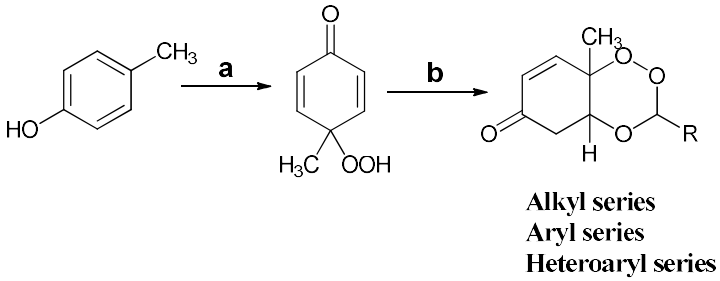
Figure 2.13: Trioxolanes OZ277 (**66**) and OZ439 (**67**).

2.3.3 Artefenomel (OZ439)

Artefenomel (OZ439) (**67**) is the second synthetic trioxolane after **OZ277** to advance to clinical candidate selection (Phyo et al., 2016). It is described as a fast-acting inhibitor of all asexual erythrocytic *P. falciparum* stages, possesses an *in vitro* potency similar to artemisinin derivatives. Its peroxide pharmacophore was optimized to eliminate slower than artemisinin derivatives and **OZ277**. **OZ439** is reported to have a longer half-life compared to other antimalarial endoperoxides, and has the potential to be used as a single-dose cure for malaria when used with a partner drug. Clinical trials are being formulated for its combination with ferroquine (SSR97193), piperaquine, or DSM265 (Phyo et al., 2016).

Quite recently, three series of trioxanes (**Scheme 2.3**) with alkyl, aryl and heteroaryl group substitutions into the 1,2,4-trioxane scaffold have been synthesised. All synthesized compounds showed good *in vitro* antimalarial activity against CQS (3D7) and CQR (RKL9) strains of *P.*

falciparum with IC_{50} values ranging from 1.24 µM to >1000 µM and 1.06 µM to >1000 µM, respectively (Rudrapal *et al*., 2017).



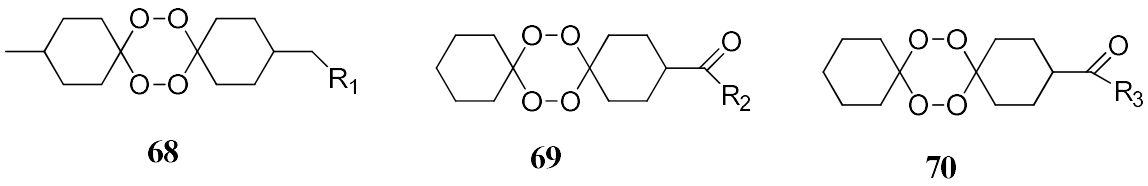
Scheme 2.3: Synthesis of target compounds. (**a**) O_3, $NaHCO_3/CH_3CN/H_2O$, rt; (**b**) RCHO, 40-50 °C, 8-12 h, CH_2Cl_2.

2.3.4 Tetraoxanes

Modern synthetic chemists are attracted to tetraoxanes mainly due to the simplicity of their synthesis. Biological properties exhibited by this class of compounds include anti-proliferative and anti-mycobacterial activities. Tetraoxanes have also been shown to work against schistosomiasis and synergistically with HIV therapies (Blackie, 2014, (Opsenica et al., 2003). However, much work has focused on the antimalarial ability of tetraoxanes. Against the usual mechanism of action proposed for endoperoxides, some groups suggest that some tetraoxane species form RO• radicals that are themselves lethal to the parasite and do not undergo rearrangement into C-centred radicals. Symmetrically substituted **3,3,6,6**-tetraalkyl-**1,2,4,5**-tetraoxane derivatives of acyclic ketones, symmetric and non-symmetric **1,2,4,5**-tetraoxane derivatives of substituted benzaldehydes have demonstrated poor antimalarial activity against CQS and CQR *P. falciparum* strains (Zmitek et al., 2006, Opsenica & Šolaja, 2009).

2.3.5 1,2,4,5-Tetraoxanes

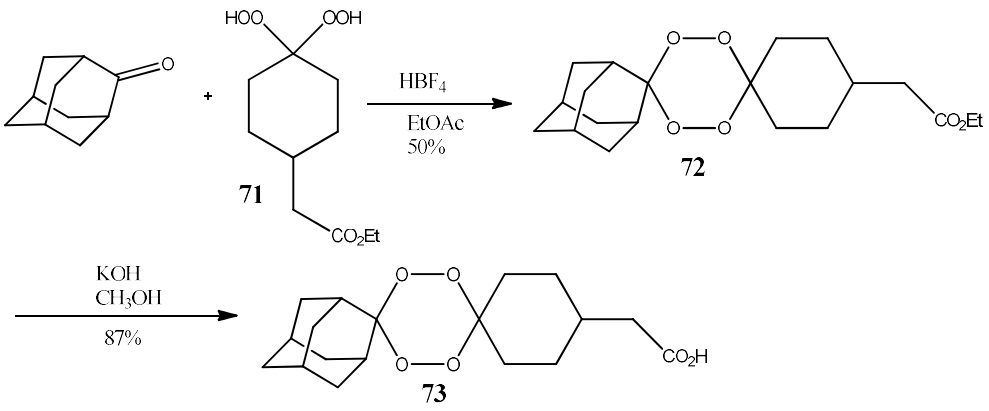
1,2,4,5-Tetraoxanes are peroxides which have demonstrated excellent antimalarial activity against both CQS and CQR strains of *P. falciparum* (Marti, et al., 2011). They are considered as the most structurally simple class of endoperoxides and they can be synthesised from widely available cyclic ketones (Blackie, 2014). **3,6**-Substituted derivatives of **1,2,4,5**-tetraoxacyclohexane were initially used for other purposes, and their discovery has demonstrated pronounced antimalarial activity and opened new possibilities in the treatment of malaria. Since then, efforts have been geared towards better synthetic processes and new derivatives as key precursors, with improved activities (Opsenica & Solaja, 2009). The initial appearance of tetraoxanes in literature gave their description as mixed tetraoxanes (Dong et al., 2007). Later, dicyclohexylidene tetraoxanes (**Figure 2.14**) were synthesized with the aim of obtaining the simplest amphiphilic structures, to minimize the influence of steric effects (Opsenica & Solaja, 2009).



68 **69** **70**

Figure 2.14: Dicyclohexylidene tetraoxanes.

These compounds, **68-70,** showed significant stability under basic and acidic conditions and under oxidative, reductive and reductive amination conditions (Opsenica & Solaja, 2009). Synthesised 1,2,4,5-tetraoxane **73**, via condensation of bis-hydroperoxide (**71)** with adamantanone followed by hydrolysis of the ester (**72**; **Scheme 2.4**), showed high *in vivo* activity against helminths (Wang et al., 2011, Vil' et al., 2017).



Scheme 2.4: Synthesis of 1,2,4,5-tetraoxane (**73)** with high anthelmintic activity.

Monospiro derivatives of these compounds were reported to have a much lower antimalarial potency, confirming the superiority of **3,6**-dispiro-**1,2,4,5**-tetraoxane structural motif, while adamantyl derivatives showed 100 % inhibition at 30 mg kg^{-1} doses. Amphiphilic adamantyl-based mixed tetraoxanes were additionally synthesized, and these showed that the adamantyl substituents could stabilize the structure and improve antimalarial activity (Opsenica & Šolaja, 2009). Bridged 1,2,4,5-tetraoxanes exhibited high *in vitro* and *in vivo* activity against trematodes *Schistosoma mansoni*. **74** (**Figure 2.15**) caused 75 % worm burden reductions in *S. mansoni* (Ingram et al., 2012, Vil' et al., 2017).

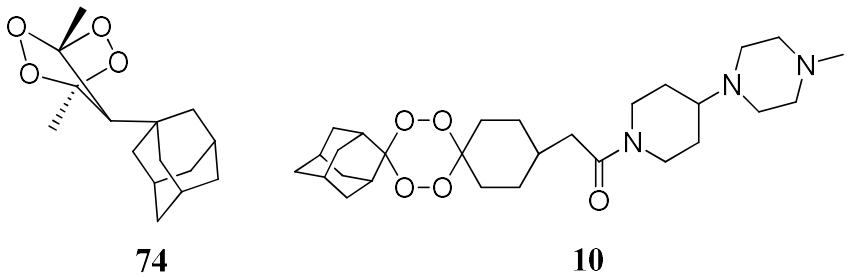
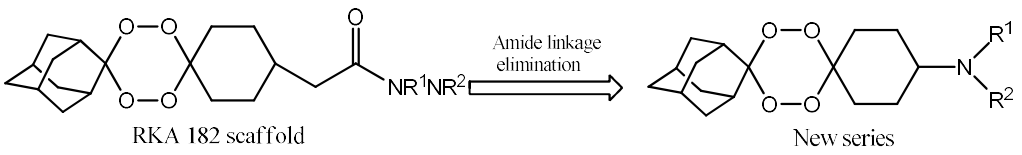


Figure 2.15: Synthetic 1,2,4,5-tetraoxanes.

2.3.6 RKA 182

RKA 182 (**10**), is a 1,2,4,5-tetraoxane derivative reported to demonstrate superior *in vitro* and *in vivo* activity to artemether and artesunate. It was also reported to have good oral bioavailability in rodent models and more stable than arterolane in malaria infected human RBCs (O'Neill et al., 2010).

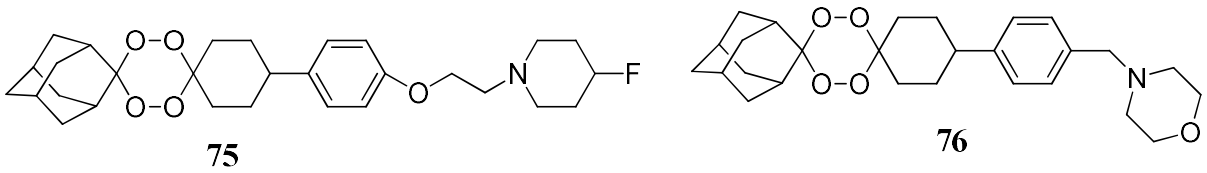
Following these impressive results, a new series of compounds (**Scheme 2.5**) were later designed to improve the metabolic stability of **RKA 182**. These compounds exhibited excellent *in vitro* activity in the low nanomolar range with promising oral activity in the *P. berghei* ANKA mouse model of malaria (Marti, et al., 2011). Although the PK profile for **RKA 182** was compatible with that of a 3-day dosing regimen, the need for drugs for single dose cure led to the synthesis of **E209** and **N205** (O'Neill et al., 2018).



Scheme 2.5: Elimination of amide linkage in **RKA 182**.

2.3.7 E209

E209 (**75**) **(Figure 2.16**) is described to have nanomolar efficacy against several strains of *P. falciparum* and *P. vivax* as well as pharmacodynamic and pharmacokinetic properties compatible with a single-dose cure even against PfK13-C580Y dependent artemisinin resistance. **E209** provides a better solution since it minimizes ring-stage resistance. It is reported to possess an elimination half-life of 24–30 hours compared to 1-2-hour half-life of artemisinins. Hence, plasma levels remain above the IC_{50} level for at least 4 days, and also retain the killing potential throughout each stage of the parasite's intra-erythrocytic cycle (O'Neill et al., 2017).



75 **76**

Figure 2.16: Synthetic antimalarial 1,2,4,5-tetraoxanes E209 (**75**) and N205 (**76**).

2.3.8 N205

N205 (**76**), a derivative of **RKA182** with improved lipophilicity (by inclusion of an aromatic ring in the side-chain) and enhanced blood stability (rodent and human) in addition to enhancing PK/PD properties in appropriate animal models showed outstanding *in vivo* antimalarial activity within the same region as **OZ439** and **E209** (O'Neill et al., 2018).

2.4.0 Molecular Hybridization

Molecular hybridization is a novel concept of drug design and development which involves the combination of pharmocophoric moieties of different bioactive substances to yield a new hybrid compound with improved affinity and efficacy when compared with parent drugs (Viegas-junior et al., 2007). Therefore, the concept, also known as "**covalent biotherapy**" or "**double drugs**," can be regarded as an extension of the concept of a fixed-dose combination of two or more drugs in a single tablet. Molecular hybridization can be classified into three basic categories: (a) mode of interaction of the individual pharmacophores with target (b) nature or form of presentation and (c) nature of the linker unit employed. **Figure 2.17** illustrates a detailed classification of hybrids based on the three categories with an example of a hybrid compound for each category (Agarwal et al., 2017).

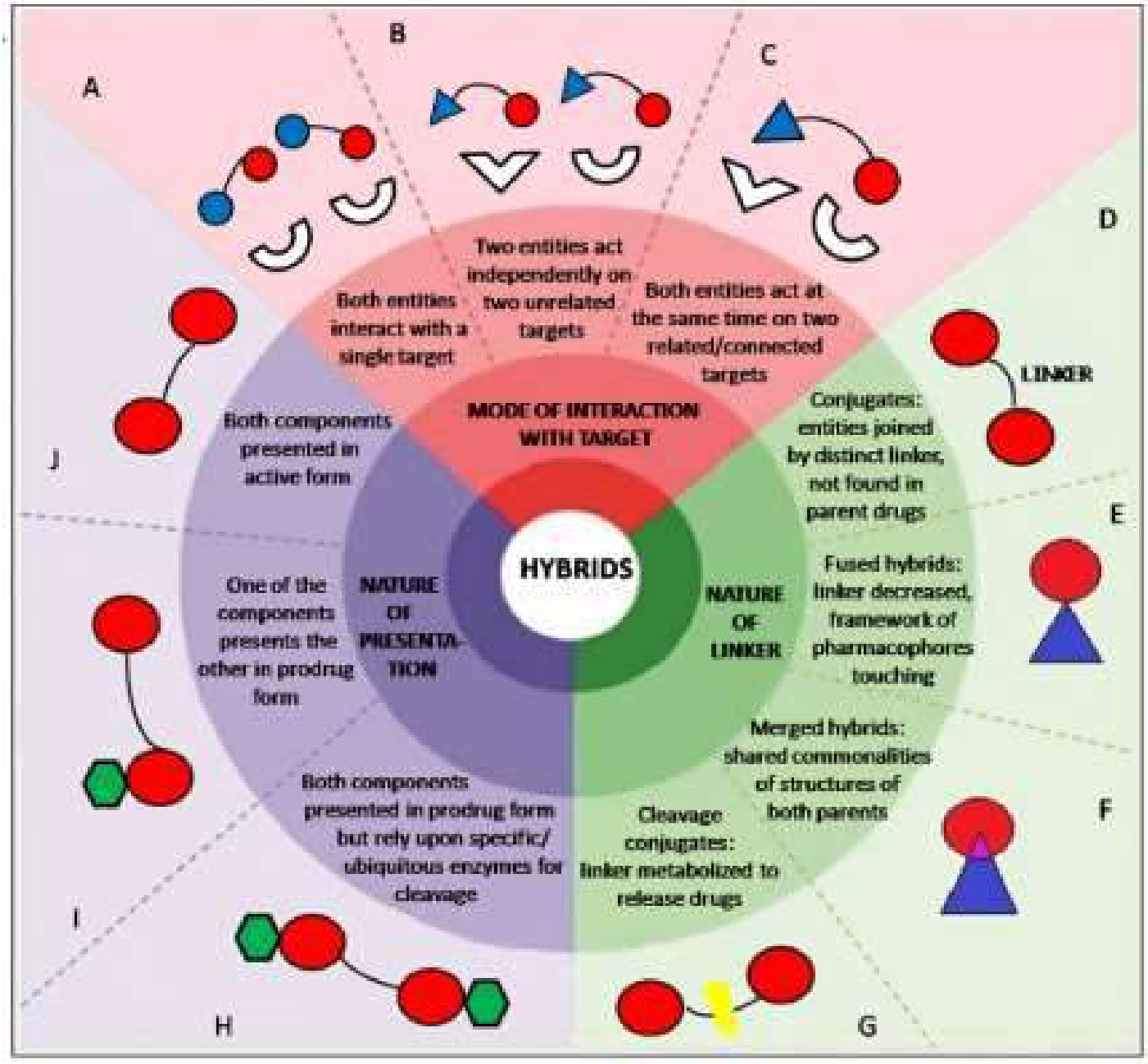


Figure 2.17: Detailed classification of hybrids (Agarwal et al., 2017).

Various molecular hybrids of natural and synthetic origins have been developed as anti-fungal, anti-malarial, anti-tuberculosis, anti-inflammatory and anti-cancer agents with significant improvements in their biological activities. Most hybrid compounds show lower toxicities and synergism. Comparison of some hybrid molecules with the individual component drugs clearly

indicates that the hybrid molecules display superior activity. (Mishra & Singh, 2016). Keri *et al.* (2013), designed and synthesized tacrine–benzothiazole hybrids (**77) (Figure 2.18**) as novel multi-potent anti-Alzheimer drug candidates. Results indicated that the multi-functional effects of the new hybrids qualified them as potential anti-AD drugs.

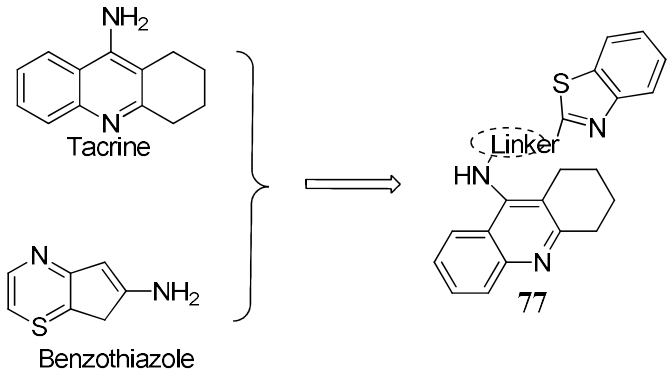


Figure 2.18: Design strategy for tacrine-benzothiazole hybrids.

Similarly, benzoate-tacrine hybrids synthesized by Zhang *et al.*, also exhibited IC_{50} values of biological activity in the nanomolar range with compound (**78)** (**Figure 2.19**) displaying the highest inhibition against Acetylcholinesterase at 5.63 nM (Zhang et al., 2016).

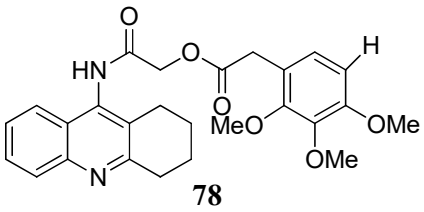


Figure 2.19: Benzoate-tacrine hybrid synthesized by Zhang *et al.*, (2016).

2.4.1 Anti-TB Hybrids

A quinoline-triazole hybrid (**79**) **(Figure 2.20**) synthesized via click chemistry exhibited 76.41 % reduction of growth at dose 5 mg/mL against *Mtb* H37Rv (Kumar et al., 2011) while **80** showed

potent activity in comparison with isoniazid against *Mtb*, H37Rv, *M. smegmatis* and *M. fortuitum* (Thomas *et al*., 2011).

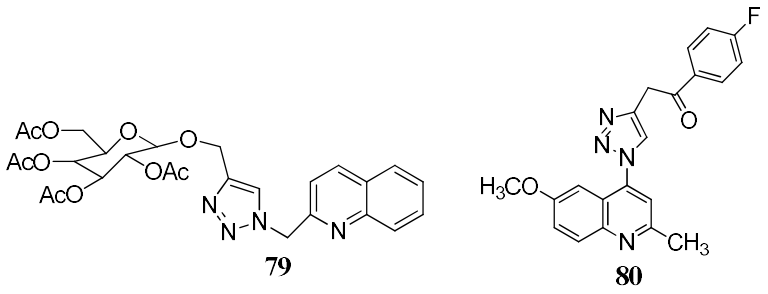


Figure 2.20: Quinoline-triazole hybrids.

1,2,3-triazole based hybrids (**Figure 2.21**), displayed potent *in vitro* anti-mycobacterial activity against *Mtb* H37Rv (ATCC 27294 strain) with very low toxicity. The most active compound (**81**) exhibited anti- mycobacterial activity with an MIC for 100 % inhibition at 1.89 µM, 26 times more active than pyrazinamide (50.08 µM), and 4 times more active than ethambutol (7.6 µM). It was also reported to have a low toxicity profile (Mishra & Singh, 2016).

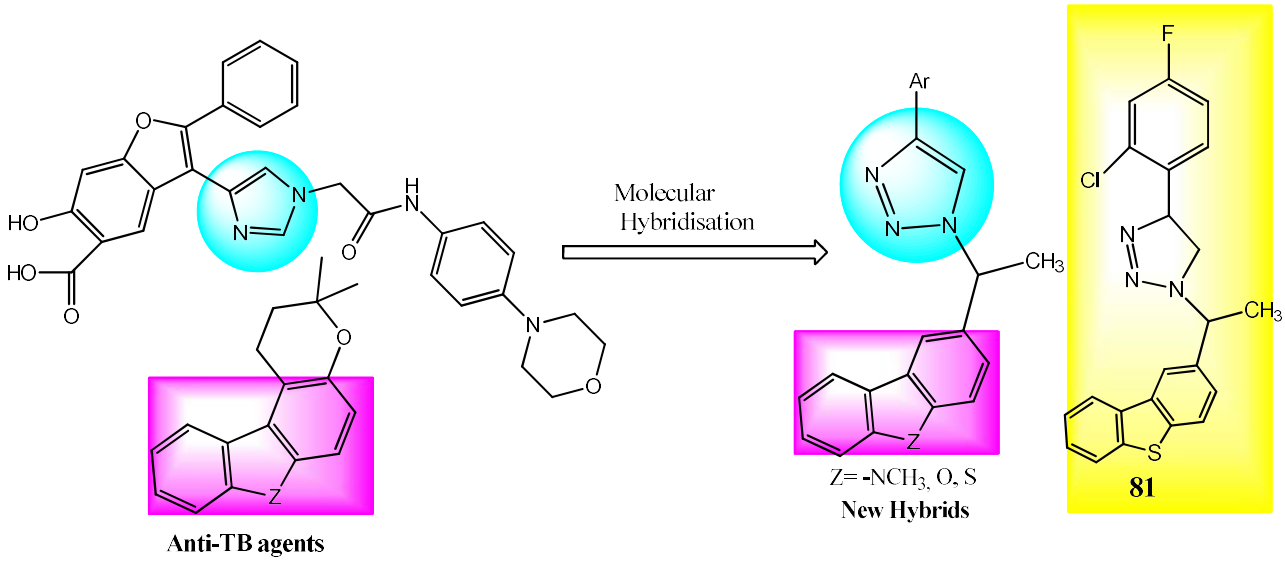


Figure 2.21: Triazole based hybrid molecules.

Gemma *et al*., (2009) synthesized 4-quinolyhydrazones, (**82-86) (Figure 2.22**) by combining anti-malarial agents; quinolines and anti-TB agents; hydrazones. At a concentration, 6.25 µg/mL, most of the synthesized quinolyhydrazones displayed 100 % inhibitory activity against *Mtb* in cellular assays. Further screening of these compounds allowed the identification of very potent anti-TB agents. Carvalho *et al*., (2008) focused on combining trans cinnamic acid; known to possess antimalarial activity, with isoniazide to yield hybrid molecules (**87-89**) which also exhibited excellent anti-TB activities.

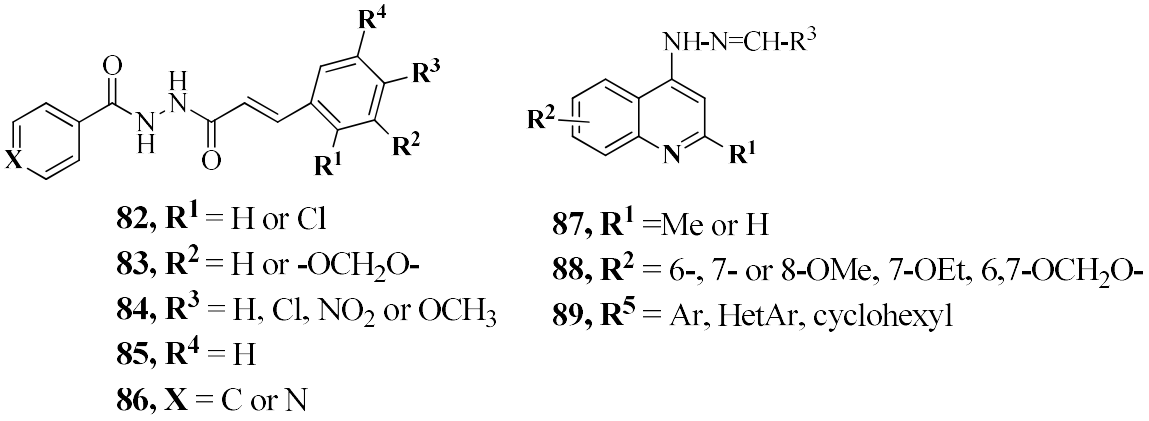


Figure 2.22: Hybrid molecules synthesized by Calvalheiro and Gemma *et al*.

Benzimidazole-oxazole/pyrimidine/pyrazole hybrids were also synthesized to exhibit both antimicrobial and antitubercular activities. While a number of the compounds showed better antimicrobial activity almost equal to reference standard Ciprofloxacin & Ketoconazole, compounds **90** and **91** (**Figure 2.23**) showed excellent antibacterial activity against some pathogenic strains of bacteria and fungi (Chikkula et al., 2018).

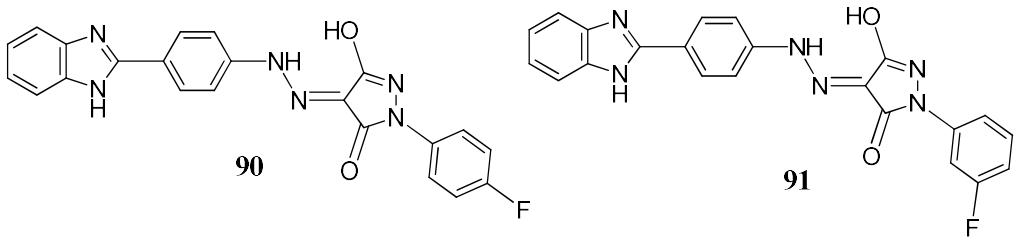


Figure 2.23: Hybrid compounds with excellent antibacterial activity.

2.4.2 Antimalarial Hybrids

Recently, Mishra and Singh have reviewed several successful antimalarial hybrids. These include **92** and **93** (**Figure 2.24**); chloroquine-ferrocene which showed superior anti-malarial activity and cytotoxicity than standard clinically used chloroquine when tested against *P. falciparum* strains (Mishra and Singh, 2016).

Ferrocenyl-pyrimidine conjugates (**94)** demonstrated antimalarial activity against NF54 *P. falciparum* strain, at 7.68 ± 0.50 µM. Furoxan-amodiaquin based hybrid molecule (**95)** demonstrated activity against several parasites including *P. falciparum, S. mansoni*, and *Ancylostoma ceylanicum*. Hybrids of cinnamic acid and chalcones (**96**) have also shown promising anti-malarial activity against both CQR (W2) and CQS (3D7) strain of *P. falciparum* (Mishra & Singh, 2016).

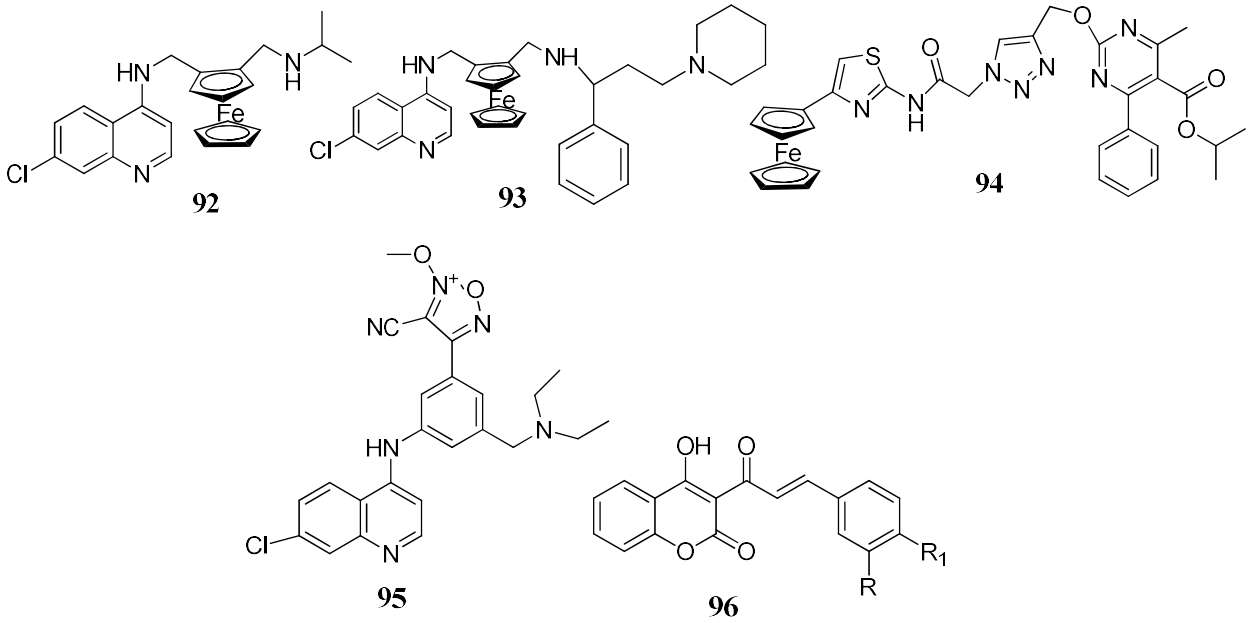


Figure 2.24: Antimalarial hybrids.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Reagents

With the exception of those stated, all reagents were obtained from commercial suppliers. Sure seal dichloromethane, triethylamine and THF were used throughout the experiments.

3.2 Chromatography

Analytical thin layer chromatography was performed on pre-coated silica gel (0.25 mm layer of silica gel F254) aluminium sheets. UV light (254 nm) was used for all visualizations and flash column chromatography was performed using Merck 938S Kieselgel 60 Silica gel.

3.3 FTIR

FTIR spectra were run using a Perkin-Elmer FTIR spectrometer spectrum 2. Solid samples were applied neat on to sodium chloride discs.

3.4 NMR

^{1}HNMR spectra were recorded using a Bruker 500 MHz NMR spectrophotometer situated at the Department of Chemistry, UG, Legon. Spectra were referenced to the residual solvent peak and chemical shifts expressed in ppm from TMS as the internal reference peak. All NMR experiments were performed at room temperature. The following annotations are used to describe multiplicity; s, singlet, bs, broad-singlet, d, doublet, t, triplet, q, quartet, m, multiplet and coupling constants are expressed in Hertz.

3.5 LC-MS

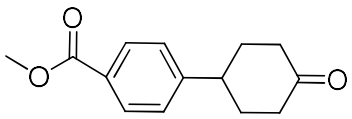
Mass spectra were recorded between 20-70 eV using a VG7070E and/or Micromass LCT mass spectrometers. The molecular ion M^+ with intensities in parenthesis is given, followed by peaks corresponding to major fragment losses.

3.6 Melting Points

Melting points are expressed in degree Celsius (ºC) and performed using the Gallenkamp melting point apparatus and capillary tubes.

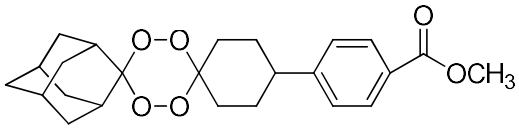
3.7 Experimental

3.7.1 Preparation of methyl 4-(4-oxocyclohexyl)benzoate (97)



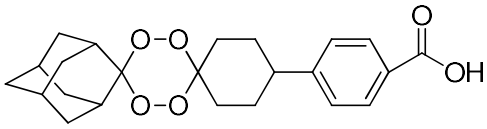
A solution of oxalyl chloride 4.51 mL (53.3 mmol) in DCM (50 mL) was added to a suspension of 4-phenylcyclohexanone (7 g, 40 mmol) and $AlCl_3$ (16.07 g, 120 mmol) in DCM (150 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 1 h then at room temperature for 2 h. A mixture of methanol (10 mL) and pyridine (8.1 mL) was added drop wise to the reaction mixture and left to stand overnight. The reaction mixture was then washed with water, 3N HCl, $NaHCO_3$, dried over $NaSO_4$, filtered and concentrated. Purification by flash column chromatography gave methyl 4-(4-oxocyclohexyl)benzoate in 65 % yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.00 (d, 2H, J = 8.3 Hz, Ar), 7.32 (d, 2H, J = 8.3 Hz, Ar), 3.92 (s, 3H, OCH_3), 3.10 (tt, 1H, J = 11.4 Hz, 3.0 Hz, CH), 2.53 (dd, 4H, J = 9.6 Hz, 5.0Hz, CH_2), 2.28-2.20 (m, 2H, CH_2), 2.03-1.90 (m, 2H, CH_2); ^{13}C NMR(100 MHz, $CDCl_3$-d_6) δ_C 211.0, 167.3, 150.5, 130.4, 129.0, 127.2, 52.5, 43.2, 41.6, 34.2; MS (ES+), $[M + Na]^+$ (100) 255.1 HRMS calculated for 255.0997 $C_{14}H_{16}O_3Na$, found 255.0989.

3.7.2 Preparation of compound 98



To a solution of methyl 4-(4-oxocyclohexyl)benzoate (4 g, 17 mmol) in acetonitrile (75 mL) at 0 °C was added formic acid (8 mL) and 30% H_2O_2 (16 mL). The resulting reaction mixture was stirred for 30 min at 0 °C, warmed to room temperature and diluted with water (30 mL). The resulting mixture was extracted with DCM (3 x 50 mL), dried over $MgSO_4$ and concentrated to give a crude *gem*-bishydroperoxide which was used without further purification. The *gem*-bishydroperoxide was dissolved in CH_2Cl_2 (50 mL) and added to a stirring solution of the required adamantanone (1.5 eq) and rhenium (VII) oxide (0.02 eq) in CH_2Cl_2 (50 mL) at room temperature. The reaction mixture was stirred for 1 hour, filtered through a plug of silica and concentrated. Purification by flash column chromatography gave the compound in 48 % as a white powder. ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.97 (d, J = 7.6 Hz, 2H), 7.31 (d, J = 7.6 Hz, 2H), 3.91 (s, 3H), 2.69 (t, J = 10.6 Hz, 1H), 2.56 (s, 2H), 2.16 – 1.54 (m, 20H); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 167.2, 151.1, 129.7, 128.1, 126.7, 110.6, 107.2, 51.9, 47.1, 43.6, 39.3, 37.0, 36.3, 33.2, 27.5, 27.1

3.7.3 Preparation of compound 99



A solution of the tetraoxane ester (**98**) (3.86 mmol) in 10 % w/v potassium hydroxide/methanol (12.6 mL) was stirred at reflux for 90 min. The solution was allowed to cool to room temperature and concentrated under reduced pressure. The resulting residue was taken up in water (15 mL) and washed with diethyl ether (3 × 12 mL). The aqueous layer was acidified with concentrated

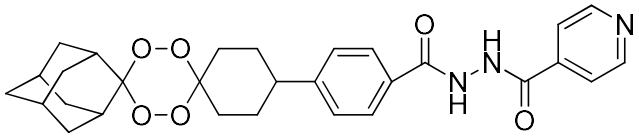
hydrochloric acid and a white precipitate formed. Diethyl ether (18 mL) was added to dissolve the precipitate and the aqueous phase extracted with diethyl ether (2 × 12 mL). The combined organic phases were washed with brine (10 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a white solid. Recrystallization from ethanol gave the carboxylic acid as a white solid in 91 % yield.

^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.97 (d, J = 8.0 Hz, 2H), 7.26 (d, J = 8.0 Hz, 2H), 2.62 (dd, J = 15.6, 7.7 Hz, 1H), 2.06 – 1.42 (m, 22H); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 171.3, 152.2, 130.8, 127.3, 110.6, 106.8, 46.6, 43.34, 38.8, 37.2, 32.8, 29.6, 27.2. MS (ES-); $[M - H]^-$ (100) 399.2 HRMS calculated for 399.1808 $C_{23}H_{27}O_6$, found 399.1808.

3.7.4 General procedure for the amide formation

To a solution of the acid (2.33 mmol) in dry DCM (30 mL) was added triethylamine (3.50 mmol, 1.5 eq) and ethylchloroformate (2.33 mmol, 1.0 eq). The reaction was stirred for 60 min at 0 °C. (2.33 mmol, 1.0 eq) of the required amide was added, and after stirring for 30 min, the reaction mixture was warmed to room temperature and stirred for a further 90 min. The reaction mixture was then diluted with water and extracted with DCM (3 x 30 mL). The combined organic extracts were washed with brine, dried over anhydrous Na_2SO_4 and concentrated. Purification by flash column chromatography afforded the required amide.

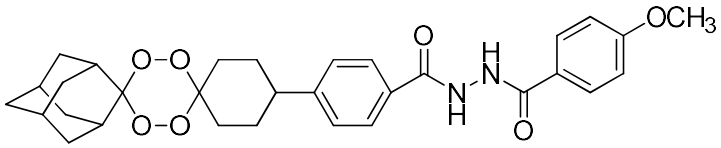
3.7.4.1 Preparation of compound 100



This product was prepared according to the general procedure for preparing amides in 56 % as a white solid. V_{max}(neat)/cm^{-1} 3286.6, 2913.1, 2857.6, 1652.6, 1530.0, 1325.0, 1062.2, 997.1. Mpt:

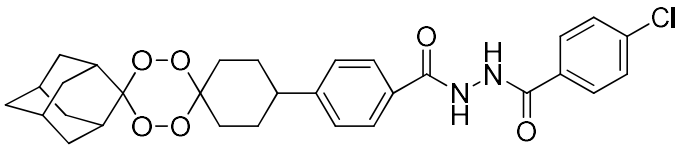
148-150 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.70 (d, J = 5.4 Hz, 2H), 8.00 (s, 1H), 7.98 (s, 1H), 7.77 (d, J = 8.1 Hz, 2H), 7.68 (d, J = 2.0 Hz, 2H), 7.29 (d, J = 8.1 Hz, 2H), 2.71 – 2.61 (m, 1H), 2.10 – 1.60 (m, 22H); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 165.1, 162.75, 151.1, 150.5, 143.8, 138.4, 128.8, 127.6, 127.4, 120.9, 110.6, 107.3, 43.5, 39.0, 34.0, 37.0, 36.0, 33.3, 27.7, 27.0.

3.7.4.2 Preparation of compound 101



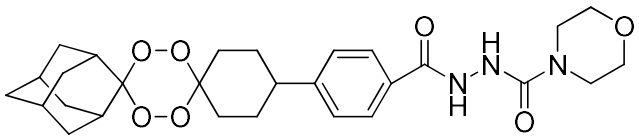
This product was prepared according to the general procedure for preparing amides in 64 % as a white solid. V_{max} (neat)/cm^{-1} 3256.6, 2932.7, 2862.4, 1605.2, 1460.0, 1252.2, 1060.9, 842.0 Mpt: 179-181 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 9.40 (s, 1H), 9.36 (s, 1H), 7.77 (d, J = 8.8 Hz, 2H), 7.72 (d, J = 8.2 Hz, 2H), 7.23 (d, J = 8.2 Hz, 2H), 6.86 (d, J = 8.8 Hz, 2H), 3.78 (s, 3H), 2.66 – 2.53 (m, 1H), 2.05 – 1.49 (m, 22H); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 164.14, 162.93, 150.69, 129.20, 127.49, 127.27, 123.59, 114.01, 110.59, 107.32, 77.27, 76.76, 55.47, 45.87, 43.61, 36.97, 33.18, 29.70, 27.08.

3.7.4.3 Preparation of compound 102



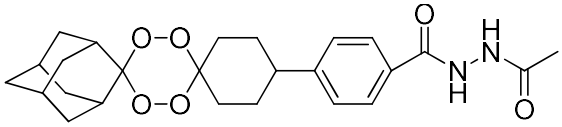
This product was prepared according to the general procedure for preparing amides in 64 % as a white solid. V_{max} (neat)/cm^{-1} 3216.7, 2915.5, 2855.2, 1626.1, 1486.3, 1264.6, 1060.0; Mpt:148-150 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.01 (s, 1H), 8.00 (s, 1H), 7.83 (d, J = 8.5 Hz, 2H), 7.75 (d, J = 8.3 Hz, 2H), 7.38 (d, J = 8.5 Hz, 2H), 7.32 (d, J = 8.3 Hz, 2H), 2.74 – 2.48 (m, 1H), 2.11 – 1.57 (m, 22H); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 170.09, 166.06, 152.00, 138.79, 130.41, 129.00, 128.78, 127.02, 110.60, 107.33, 77.27, 76.77, 43.76, 36.97, 33.17, 29.70, 27.08, 14.37.

3.7.4.4 Preparation of compound 103



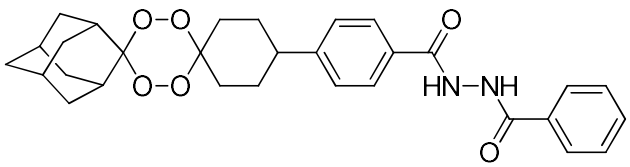
This product was prepared according to the general procedure for preparing amides in 50 % as a white solid. V_{max} (neat)/cm^{-1} 3277.0, 2919.0, 2857.6, 1645.0, 1537.0, 1450.2, 1257.4, 1115.17, 1060, 997.1 Mpt: 136-138 $^{\circ}$C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 9.21 (s, 1H), 7.92 (s, 1H), 7.75 (d, J = 8.1 Hz, 2H), 7.21 (d, J = 8.0 Hz, 2H), 3.58–3.54 (m, 4H), 3.40–3.35 (m, 4H), 2.62–2.53 (m, 1H), 2.05-1.51 (m, 22H); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 166.2, 157.0, 150.3, 129.5, 127.6, 127.1, 110.3, 107.1, 66.3, 47.0, 43.9, 43.6, 39.2, 37.0, 36.3, 33.2, 27.4, 26.9.

3.7.4.5 Preparation of compound 104



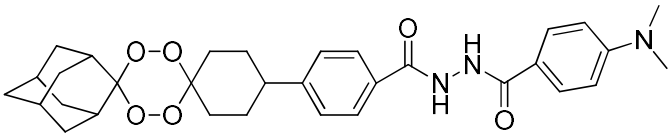
This product was prepared according to the general procedure for preparing amides in 60 % as a white solid. V_{max} (neat)/cm^{-1} 3248.1, 2932.3, 2860.1, 1652.0, 1447.8, 1252.2,1060.0, 997.1. Mpt: 138-140 $^{\circ}$C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 9.61 (d, J = 3.9 Hz, 1H), 9.47 (d, J = 3.8 Hz, 1H), 7.76 (d, J = 7.9 Hz, 2H), 7.27 (d, J = 9.0 Hz, 2H), 2.67 (t, J = 11.6 Hz, 1H), 2.11 (s, 3H), 2.06 – 1.57 (m, 22H); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 167.6, 164.3, 150.6, 141.1, 129.0, 127.6, 127.2, 110.3, 107.7, 43.5, 39.3, 37.0, 33.2, 27.1, 20.8.

3.7.4.6 Preparation of compound 105



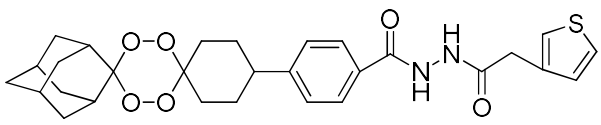
This product was prepared according to the general procedure for preparing amides in 70 % as a white solid. V_{max} (neat)/cm^{-1} 3223.8, 2919.1, 2857.2, 1676.7, 1628.28, 1446.1, 1293.6, 1056.5, 997.7. Mpt: 158-160 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 9.59 (s, 1H), 9.54 (s, 1H), 8.01 (d, J = 7.5 Hz, 2H), 7.90 (d, J = 6.7 Hz, 2H), 7.82 (s, 1H), 7.46 (d, J = 7.2 Hz, 2H), 7.32 (d, J = 7.5 Hz, 2H), 2.76–2.53 (m, 1H), 2.19–1.60 (m, 22H); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 170.30, 152.07, 150.88, 130.44, 128.79, 127.57, 127.33, 127.03, 110.60, 107.32, 77.27, 76.76, 46.98, 43.77, 43.63, 39.28, 36.97, 33.17, 27.08.

3.7.4.7 Preparation of compound 106



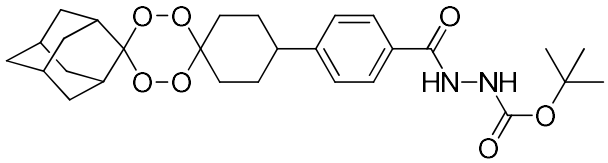
This product was prepared according to the general procedure for preparing amides in 60 % as a white solid. V_{max} (neat)/cm^{-1} 3212.7, 2918.8, 2856.7, 1767.1, 1607.0, 1521.6, 1282.5, 1059.9, 924.8. Mpt: 165-167 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 9.42 (s, 1H), 9.20 (s, 1H), 7.78 (d, J = 10.5 Hz, 2H), 7.68 (d, J = 6.7 Hz, 2H), 7.26 (d, J = 3.6 Hz, 2H), 6.72 (d, J = 8.9 Hz, 2H), 3.05 (s, 6H), 2.72–2.52 (m, 1H), 2.09–1.59 (m, 22H); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 152.97, 128.70, 127.29, 127.19, 127.09, 111.43, 111.01, 110.48, 107.24, 77.16, 76.91, 76.65, 43.51, 39.96, 36.87, 33.07, 29.59, 26.98, 14.00.

3.7.4.8 Preparation of compound 107



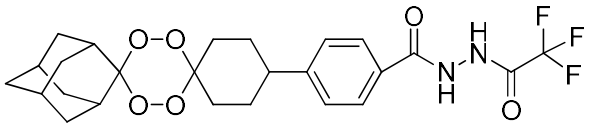
This product was prepared according to the general procedure for preparing amides in 89 % as a white solid. V_{max} (neat)/cm^{-1} 3259.9, 2918.6, 2858.0, 1679.0, 1426.0, 1298.9, 1244.1, 1058.7, 997.9. Mpt: 150-152 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.01 (d, J = 8.0 Hz, 2H), 7.30 (dd, J = 8.2, 6.1 Hz, 3H), 7.26 (s, 1H), 7.19 (d, J = 3.0 Hz, 2H), 7.04 (d, J = 4.9 Hz, 1H), 3.63 (s, 2H), 2.69 (tt, J = 11.9, 4.0 Hz, 1H), 2.06–1.19 (m, 22H); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 170.73, 170.49, 151.91, 130.40, 128.37, 126.99, 126.53, 123.55, 110.59, 107.34, 77.31, 76.80, 62.43, 43.74, 36.97, 35.80, 33.17, 29.70, 27.08, 14.35.

3.7.4.9 Preparation of compound 108



This product was prepared according to the general procedure for preparing amides in 45 % as a white solid. V_{max} (neat)/cm^{-1} 2920.4, 2851.5, 1681.2, 1609.3, 1427.4, 1299.9, 1059.1, 998.2. Mpt: 155-157 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.04 (s, 1H), 8.02 (s, 1H), 7.33 (d, J = 8.1 Hz, 2H), 7.26 (d, J = 8.1 Hz 2H), 2.70 (tt, J = 11.8, 4.0 Hz, 1H), 2.06–1.61 (m, 22H), 1.26 (s, 9H); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 171.25, 171.05, 152.03, 130.36, 126.92, 110.48, 107.21, 77.16, 76.65, 68.07, 43.66, 36.86, 33.06, 29.59, 26.97, 22.58.

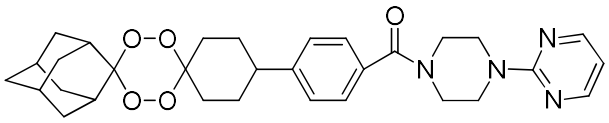
3.4.10 Preparation of compound 109



This product was prepared according to the general procedure for preparing amides in 62 % as a white solid. V_{max} (neat)/cm^{-1} 2918.0, 2852.2, 1720.0, 1679.6, 1609.0, 1451.5, 1300.0, 1058.3, 997.7. Mpt: 153-156 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.04 (s, 1H), 8.03 (s, 1H), 7.33 (d, J = 7.9

Hz, 2H), 7.26 (d, J = 7.9 Hz, 2H), 2.70 (tt, J = 11.8, 4.0 Hz, 1H), 2.13–1.59 (m, 22H); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 171.19, 152.16, 130.47, 127.04, 110.59, 107.32, 77.28, 76.77, 46.98, 43.78, 39.28, 33.17, 29.70, 27.46, 27.09.

3.21 Preparation of compound 110



This product was prepared according to the general procedure for preparing amides, however, an amine (2-(piperazin-1-yl)pyrimidine) instead of a hydrazine was coupled in a mixed anhydride to yield 57 % white solid. V_{max} (neat)/cm^{-1} 2936.3, 2904.8, 2860.12, 1720.5, 1618.5, 1584.8, 1547.9, 1471.1, 1437.3, 1354.2, 1280.7, 1251.0, 1062.5, 802.1. Mpt: 163-166 $^{\circ}$C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_C 8.32 (d, J = 4.8 Hz, 2H), 8.00 (d, J = 8.0 Hz, 2H), 7.37 (d, J = 8.0 Hz, 2H), 7.32–7.25 (m, 1H), 3.87 (t, J = 12.7 Hz, 4H), 3.55 (t, J = 12.7 Hz, 4H), 2.71–2.62 (m, 1H), 2.09–1.57 (m, 22H); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 170.61, 161.42, 157.68, 151.74, 147.83, 133.37, 130.25, 127.30, 126.94, 110.43, 107.25, 77.16, 76.65, 43.62, 43.40, 36.84, 33.04, 26.96.

3.5 Physicochemical properties

Lipophilicity (Log P), CLogP and topological polar surface area (tPSA) were calculated using ChemDraw Ultra 12.0.

3.6 *P. falciparum* (3D7) *in vitro* assay (Drug Discovery Unit, University of Dundee).

Chloroquine-sensitive *P. falciparum strain* 3D7 cultures were maintained in a 5 % suspension of human red blood cells cultured in RPMI 1640 medium (pH 7.3). These were supplemented with 0.5 % Albumax II (Gibco Life Technologies, San Diego, CA), 12 mM sodium bicarbonate, 0.2

mM hypoxanthine, and 20 mg/L gentamicin at 37 ºC, in 1 % O_2, 3 % CO_2 atmosphere and a nitrogen balance (Trager & Jensen, 1988). Fluorescence assay was used to quantify growth inhibition, utilising the binding of SYBR Green (Bennett et al., 2004) to double stranded DNA which after excitation at 485 nm emitted a fluorescent signal at 528 nm. Mefloquine was the control used for assay quality monitoring. Compound bioactivity was expressed as EC_{50}. The most potent hits from each series identified by the primary screening were reconfirmed by testing in a [^{3}H]-Hypoxanthine incorporation assay.

3.6.1 *In vitro* Cell Assay Data Analysis

All data were processed using IDBS Activity Base. Raw data was converted into per cent inhibition through linear regression by setting the high inhibition control as 100 % and the no inhibition control as 0 %. Quality control criteria for passing plates were as follows: z'> 0.5, S:B> 3, %. $CV_{(no\ inhibition\ control)}$ < 15. Z' was calculated with;

$$1 - \frac{3 \text{ x } (StDev\_high + StDev\_low)}{ABS\ (Mean\_high - Mean\_low)}$$

Curve fitting was calculated with the formula;

$$y = A + \frac{B - A}{1 + (C/x)^D}$$

Where A = % inhibition at bottom, B = % inhibition at the top, C = EC_{50}, D = slope, x = inhibitor concentration and y = % inhibition. B was fixed to 100 if curve definition was poor (Baragaña et al., 2015).

3.7 Alamar Blue Assay for Anti-TB activity (Bacteriology Department, NMIMR).

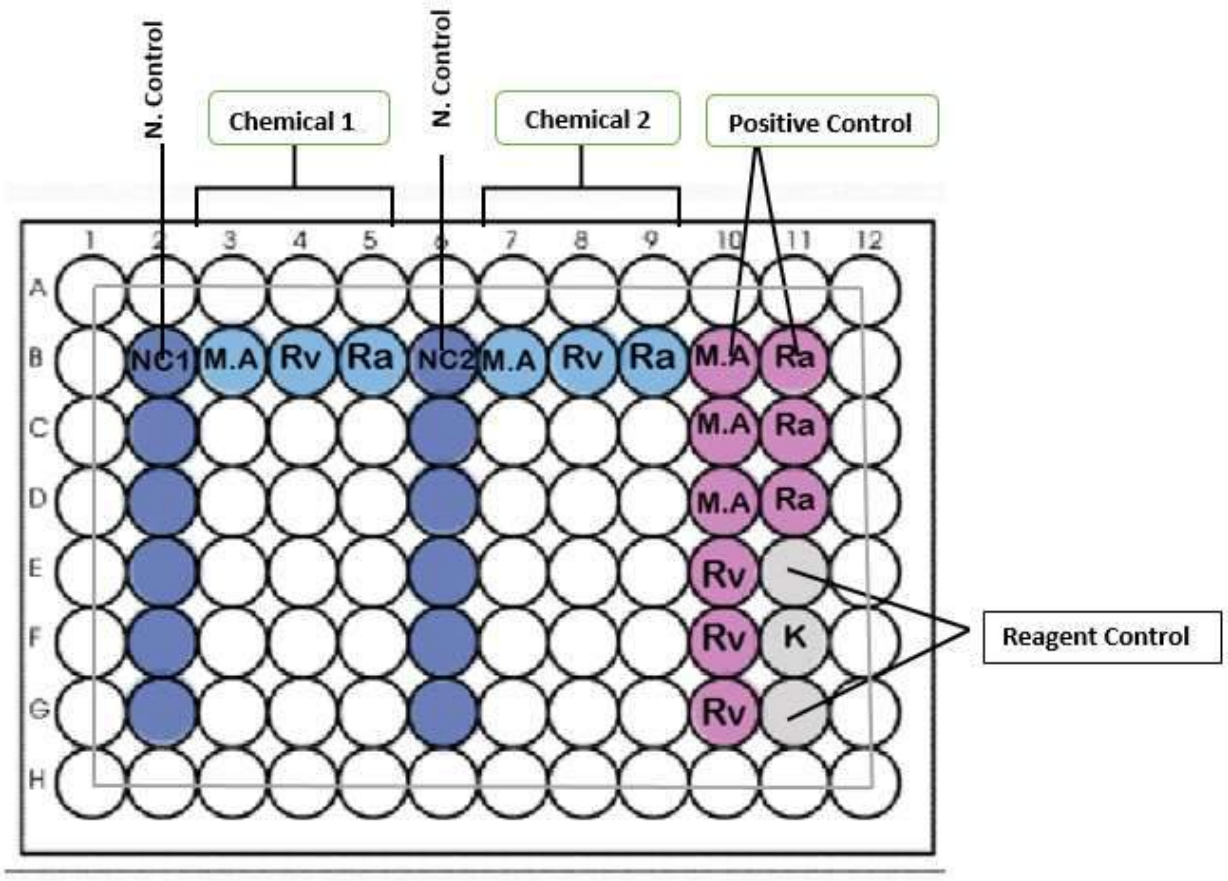
3.7.1 Stock Preparation of Test Compounds

Ten (10) mg of powdered compound was dissolved in 125 µL of dimethyl sulfoxide (DMSO) to give a concentration of 80 mg/mL. A 1 in 10 dilutions was done for the stock by adding 675 µL of DMSO to 75 µL of stock solution resulting in a 8 mg/mL working concentration. The concentration of test compound in each well down the column after a two-fold serial dilution from Column B2 to G2 on a 96 well plate is as shown below;

8 mg/mL ⟶ 4 mg/mL ⟶ 2 mg/mL ⟶ 1 mg/mL ⟶ 0.5 mg/mL ⟶ 0.250 mg/mL

3.10.2 Preparation of 96 well plates

Plate Map for Assay



3.10.3 Presentation of 7H9 culture media

For mycobacteria species; H37*rv* and *M. aurum* Middlebrook 7H9 medium (Becton Dickson company Sparks MD) supplemented with 0.2 % glycerol, 1.0 g casitone per litre, 10 % heat inactivated Fetal Bovine Serum (FBS), and 0.05 % tween 80 (sterile- filtered through 0.2 µL Millipore filters). For every 500 mL of medium solution, 2.35 g of middlebrook 7H9 is added to 450 mL of distilled water and supplemented with 0.5 g casitone and 2 mL glycerol. The 7H9 broth with glycerol and casitone (7H9-GC) was autoclaved at 121 ºC for 10 minutes, allowed to cool and stored at 4 °C. The growth media (7H9-GC supplemented with FBS and filter-sterile Tween 80 (7H9-GCFT)) were constituted fresh just before use. Five (5) mL FBS and 250 µL 10 % tween were added to 45 mL of 7H9GC medium, to give 7H9 GCFT medium (medium for the assay).

3.10.4 Inoculation and Incubation

Suspension of mycobacterial isolates (H37*rv* and *M. aurum*) were prepared by adding a loopful of mycobacteria colonies in 500 µL of 7H9GC medium and homogenized by votexing. The turbidity of the suspension was adjusted to 1 McFarland. A 1 in 20 dilutions was made by adding 200 µL of the suspension to 3800 of 7H9GC medium. Plates were inoculated by adding 10 µL of the suspension to test wells and positive control wells with exception to the negative control wells and incubated at 37 ºC for 7 days.

3.10.5 Reading of DST Plates

The plates were checked for mycobacterial growth after 7 days. Alamar blue reagent was added to the first negative and positive control wells (i.e. B_2/B_6 and $B_{10}/B_{11}/E_{10}$) and incubated for 24 hours and observed for color change in the positive control well. Alamar blue reagent was added to the remaining wells and incubated for 24 hours to observe for change in color. Wells with no

growth/no color change remained blue whereas wells with mycobacteria growth turned pink. Picture of plates are shown in appendix.

The Minimum Inhibition Concentration (MIC) is the lowest concentration of drug that prevents a visible growth of bacteria for which there is a color change from blue to pink (inhibits more than 99 % of growth of bacterial culture). For therapy guidance, isolates with MIC of 0.2 or 0.5 µg/mL for INH and 0.4 µg/mL for rifampicin are reported as immediate resistant.

3.10.6 Quality control

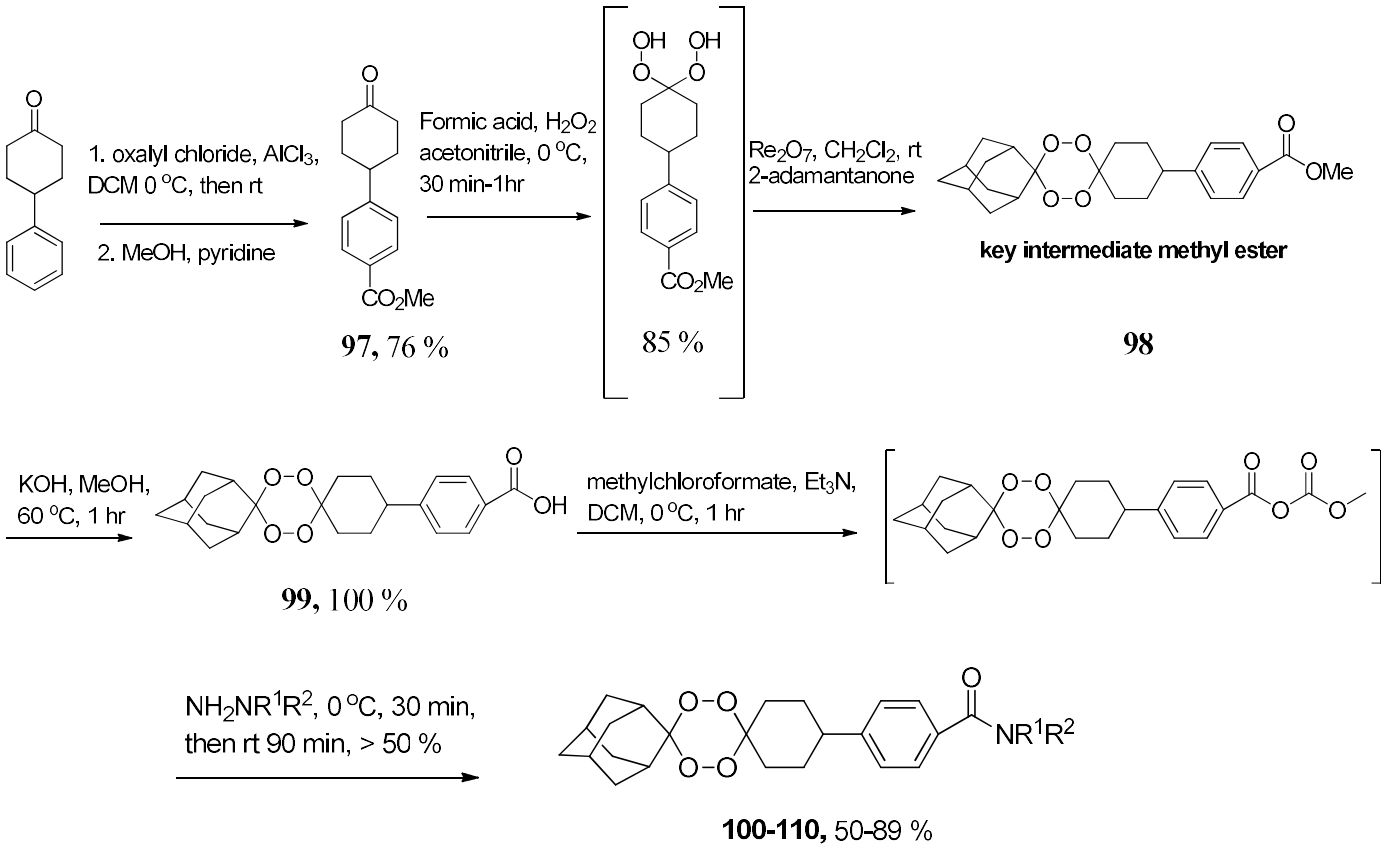
Quality control during DST assay was ensured at different levels. The outer wells were filled with sterile distilled water to minimize errors by evaporation. Each 96 well plate contains 9 positive control wells and two columns negative control, as well as 3 wells for media control to check the purity and identification of each of the isolates and test drugs as well as the medium used.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

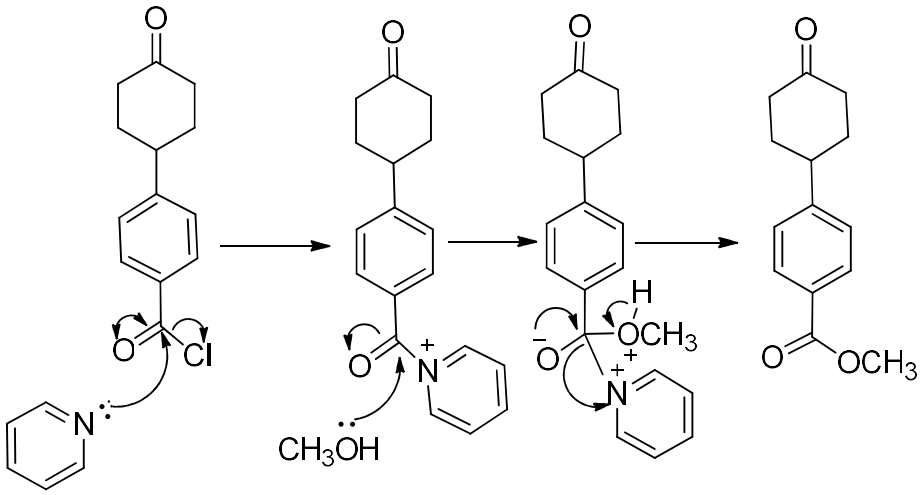
4.1 Chemistry

The isonicotinohydrazides were synthesized in six (**6**) reaction steps in moderate to high yields involving an already established synthetic route developed by O'Neill *et al*. (O'Neill et al., 2010). The key intermediate methyl ester (**98**), was synthesized via a modified Friedel-Crafts (FC) reaction with 4-phenylcyclohexanone to produce methyl 4-(4-oxocyclohexyl)benzoate (**97**) (**Scheme 4.1**).



Scheme 4.1: Synthetic route for the preparation of the isonicotinohydrazides.

FC is one of the most versatile methods used for synthesizing substituted aromatic compounds (Ghodke & Chudasama, 2015). 4-phenylcyclohexanone was treated with oxalyl chloride in the presence of aluminum chloride as a catalyst to yield the acyl chloride which was further treated with pyridine and quenched *in situ* by methanol to give the methyl ester **97**. The mechanism for the FC modification is shown in **Scheme 4.2**.



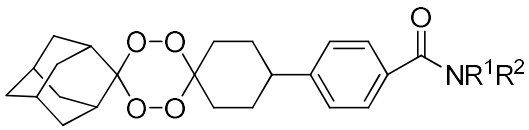
Scheme 4.2: The mechanism of methyl ester formation.

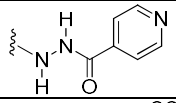
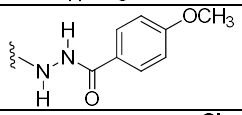
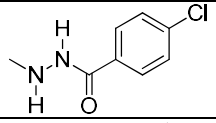
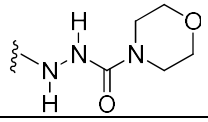
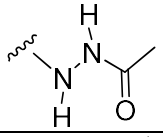
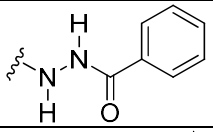
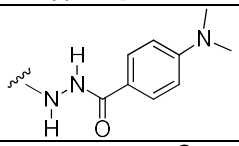
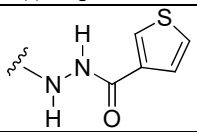
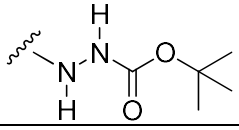
The methyl 4-(4-oxocyclohexyl)benzoate, **97** was then treated with 30 % hydrogen peroxide in acetonitrile with catalytic amount of formic acid to give a bishydroperoxide intermediate which was then condensed with 2-adamantanone in the presence of 2 % rhenium oxide catalyst to yield a methyl ester **98**. The use of Re_2O_7 catalyst for the tetraoxane formation was developed by Ghorai and Dussault (Ghorai & Dussault, 2009).

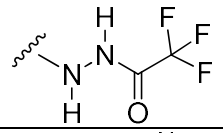
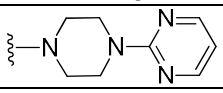
The application of an adamantanone in drug design has been extensively used by several scientists (Opsenica & Šolaja, 2009). For example, Amewu and co-workers observed in SAR studies when adamantanone fused to the tetraoxane core led to increased potency and metabolic stability (Amewu et al., 2013).

Next, the methyl ester **98** was hydrolyzed under basic conditions to the carboxylic acid followed by an amide bond formation to give the isonicotinohydrazides. All the analogues prepared and their respective yields are represented in **Table 4.1** below.

Table 4.1: Compounds Synthesised and their Percentage Yields.



COMPOUND NUMBER	CODE	-NR¹R²	Yield (%)
100	**CFA/003**		56
101	**CFA/004**		64
102	**CFA/005**		64
103	**CFA/006**		50
104	**CFA/007**		60
105	**CFA/027**		70
106	**CFA/029a**		60
107	**CFA/029b**		89
108	**CFA/030**		45

109	**CFA/032**		62
110	**CFA/025**		57

All the compounds synthesized were fully characterized using 1D and 2D NMR as well as FTIR

The ^{1}HNMR of compound **101** (**Figure 4.1**) gave two singlets at chemical shifts of 9.40 and 9.36 ppm respectively corresponding to hydrazine NH (**H4** and **H5**). The protons at chemical shifts 7.77, and 6.86 ppm with coupling constant of **8.8 Hz** correspond to **H6** and **H7** respectively while the protons at chemical shifts of 7.72 ppm, 7.23 ppm with coupling constant of **8.2 Hz** corresponds to **H3** and **H2** respectively. The singlet at the chemical shift of 3.78 ppm correspond to the methoxy peak **H1** and the multiplet between 2.64-2.56 ppm correspond to the **H8** whereas the multiplet between 2.64-1.50 ppm correspond to the proton on the adamantyl and cyclohexyl rings **H9-H15**.

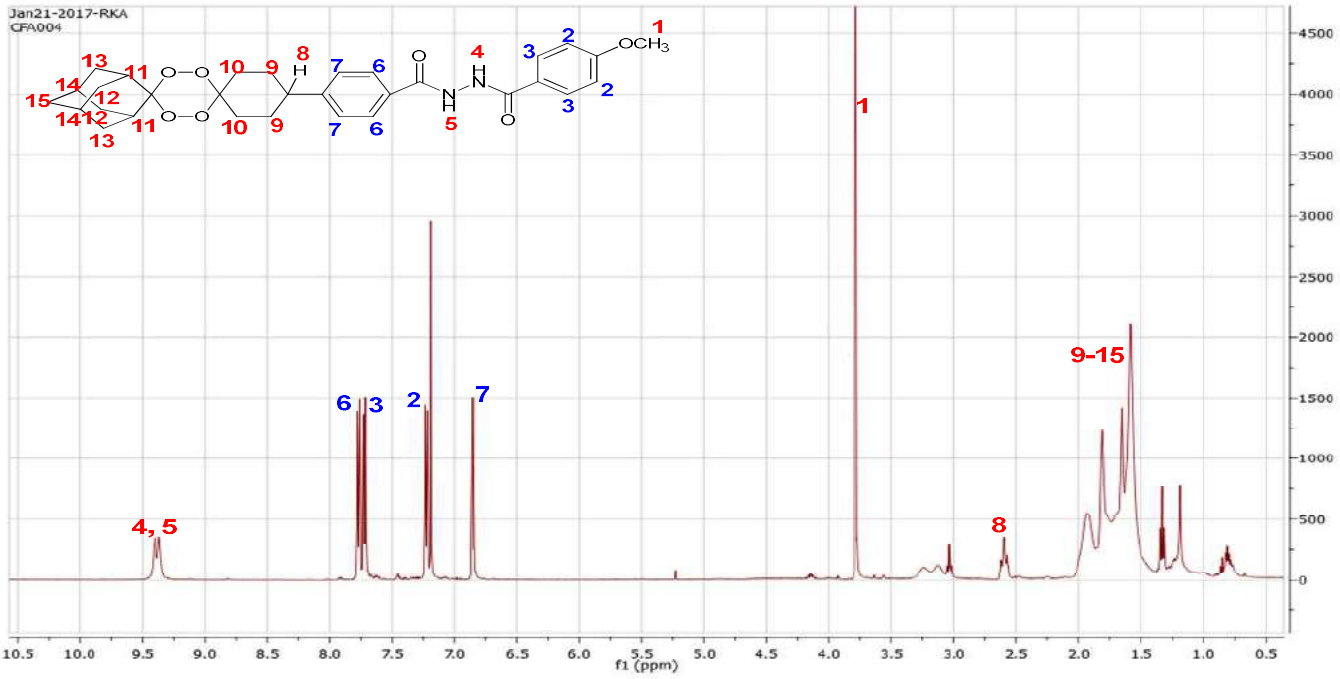


Figure 4.1: 1H NMR spectra for compound 101 **(CFA/004).**

The ^{13}C NMR (**Figure 4.2**) revealed 19 distinct carbons with a number of them equivalent. The peaks at 164.2 ppm correspond to the carbonyl carbons **C6** while the peak at 164.0 ppm correspond to the carbon bearing the methoxy group **C2**. The peaks between 162.9-114.0 ppm correspond to the aromatic carbons while the peaks at 110.6 and 107.3 ppm correspond to the quaternary carbons **C14** and **C15** respectively. The remaining peaks are the cyclohexyl and adamantyl carbons (**C12-C14**, **C17-C19**).

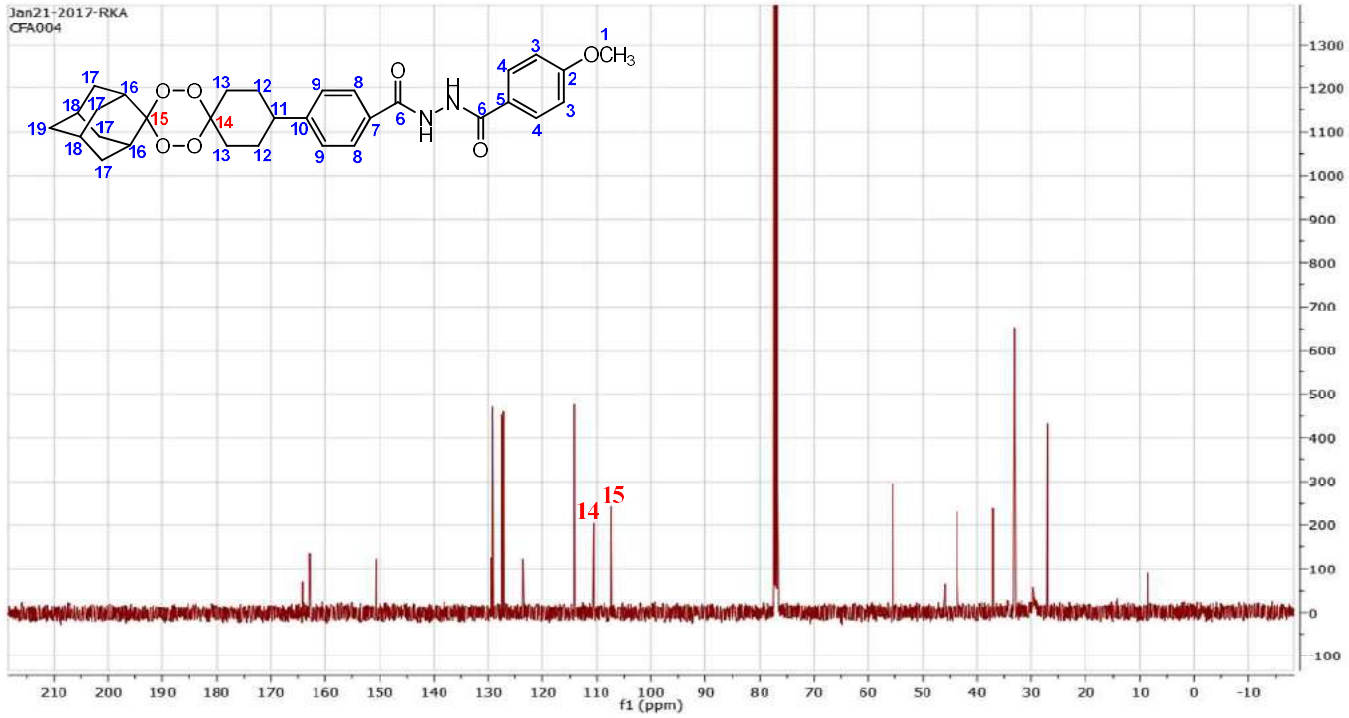


Figure 4.2: ^{13}C NMR spectra for compound **101** (**CFA/004**).

Infrared spectrum of **CFA/004** (**Figure 4.3**) confirmed the presence of aromatic **C=C** stretch at 1604.22 cm^{-3}. Absorption frequencies at 3251.98 cm^{-3} and 1456.78 cm^{-3} correspond to amide **N-H stretch,** and **C-O** respectively, while frequencies at 942.12 cm^{-3} and 2927.34 cm^{-3} correspond to aromatic **C-H** bend, **C-H** stretch respectively.

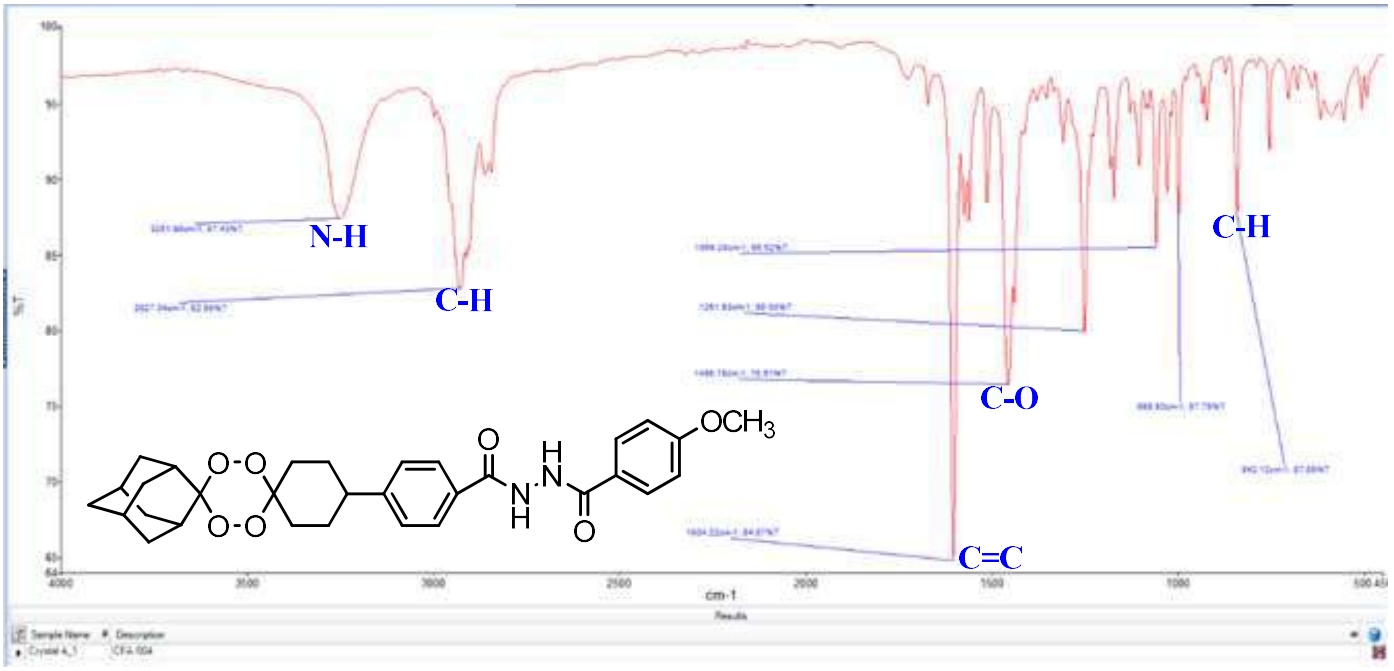
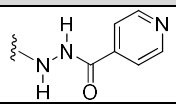


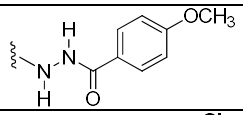
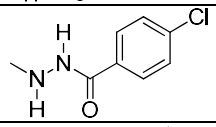
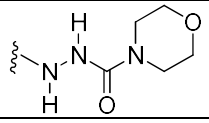
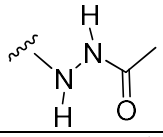
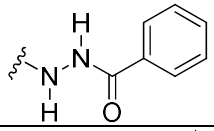
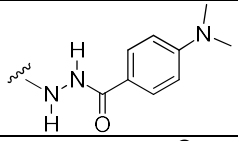
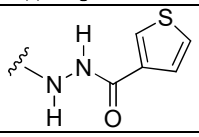
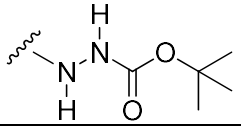
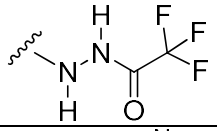
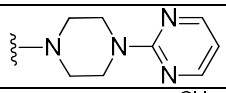
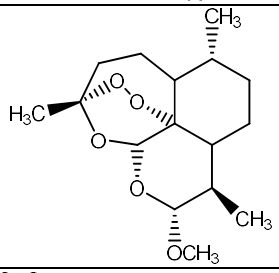
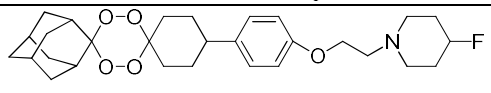
Figure 4.3: IR spectrum for **CFA/004.**

4.2 Physicochemical properties

The physicochemical parameters of the synthesized compounds (**Table 4.2**) were calculated to establish if there were any correlations between any of the parameters and observed activity. All the compounds were lipophilic with LogP values between **4.54 -7.25** while the tPSA ranged from **95.12-108.8.** Calculated tPSA values for all analogues were below 140, suggesting good cell membrane permeability should any pass for clinical studies. While most compounds displayed the same value at **95.12**, compound **110** had the lowest tPSA value of **85.19** while **100** displayed the highest value of **107.8**.

Table 4.2: Calculated physicochemical properties of synthesized compounds.

COMPOUND NUNMBER	CODE	-NR^1R^2	Log P	CLogP	tPSA
100	**CFA/003**		5.36	4.98	107.80

101	**CFA/004**		6.57	5.93	104.35
102	**CFA/005**		7.25	6.65	95.12
103	**CFA/006**		4.54	4.502	107.59
104	**CFA/007**		4.80	3.82	95.12
105	**CFA/027**		6.69	5.73	95.12
106	**CFA/029a**		5.89	4.40	121.4
107	**CFA/029b**		6.56	5.24	95.12
108	**CFA/030**		6.26	5.30	104.35
109	**CFA/032**		5.94	4.93	95.12
110	**CFA/025**		6.21	4.56	85.19
Artemether			3.51	3.06	46.15
E209			6.44	6.47	49.39

***Calculated using ChemDraw Ultra 12**

4.3 Antimalarial activity

All compounds synthesized were tested *in vitro* against the 3D7 strain of *P. falciparum* and they displayed activities in nanomolar range. All tetraoxane analogues displayed activities between **0.06-0.269 µM** with compound **101** being the most potent possessing IC_{50} value **0.060 ± 0.033 µM (Table 4.3),** with Artemether as the control. The IC_{50} curve of compound **CFA 004**, **101** is shown in **Figure 4.4** below and the rest of the curves are shown in **Appendix 1**.

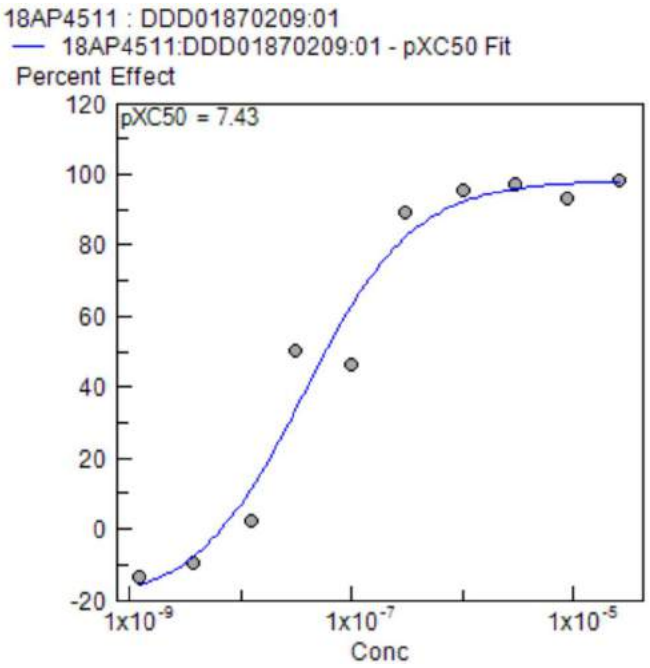


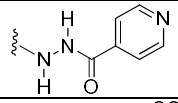
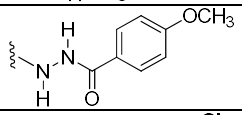
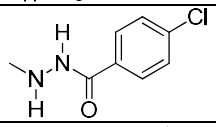
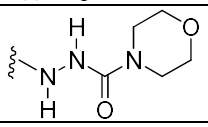
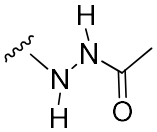
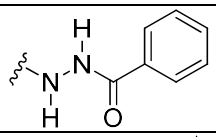
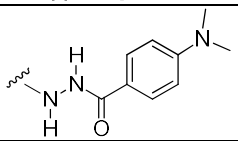
Figure 4.4: IC_{50} curve for compound **101** (**CFA004**).

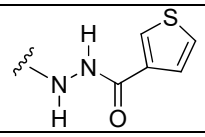
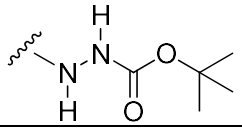
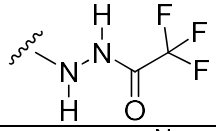
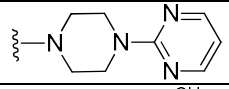
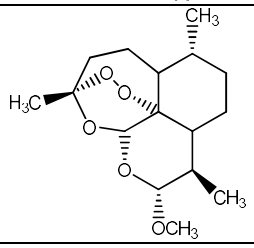
Other compounds including **100**, **104**, **105** and **106** retained antimalarial potency below 100 nM while the remaining compounds **102**, **103**, **107**, **108**, **109** and **110** gave activities below 500 nM. It was observed that apart from the analogue prepared from isoniazid, other isonicotinohydrazides with or without substitution on the aromatic ring were potent suggesting that the incorporation of the phenyl ring might have impacted positively on the antimalarial activity. Whereas the compounds with electron donating groups as substituents on the aromatic ring **101** and **106** were highly potent, the only analogue with an electron withdrawing group **102**, which incidentally was

the most lipophilic (**LogP = 7.24**), was the least active (**IC_{50} = 0.491 ± 0.012 µM**) suggesting that the nature of the substituent is critical for antimalarial activity. Of the analogues without the incorporation of an aromatic group, compound **104** was the most active, followed by the least lipophilic analogue containing the morpholine substituent with LogP value of 4.54, compound **103.** Compounds **108**, **107** and **109** also followed the order of decreasing activity respectively.

An analogue with 2-(piperazin-1-yl) pyrimidine, without a hydrazine functionality (**110**) was found to be less potent compared with **101** but more active than **102**. The activity of **0.211 ± 0.071 µM** observed was lower than previously reported for tetraoxanes.

Table 4.3: *In vitro* antimalarial activity profiles of synthesized compounds.

COMPOUND	CODE	-NR^1R^2	IC_{50} (µM)
100	CFA/003		0.072 ± 0.039
101	CFA/004		0.060 ± 0.033
102	CFA/005		0.491 ± 0.012
103	CFA/006		0.113 ± 0.029
104	CFA/007		0.061 ± 0.003
105	CFA/027		0.082 ± 0.009
106	CFA/029a		0.099 ± 0.019

107	CFA/029b		0.261 ± 0.030
108	CFA/030		0.140 ± 0.004
109	CFA/032		0.269 ± 0.008
110	CFA/025		0.211 ± 0.071
8 (Artemether)			0.0078 ± 0.009 (O'Neill et al., 2010)

4.4 Antitubercular activity

Five (**5**) synthesized compounds **100**, **101**, **102**, **103**, **104** were subjected to drug susceptibility tests against standard pathogenic *M. tuberculosis* strain; H37rv (Rv) and fast-growing mycobacteria surrogate for Anti-TB drug test; *M. aurum* (M.A) using Microplate Alamar Blue Assay (MABA). Different concentrations ranging from 0.25-8 mg/mL were used to investigate whether the compounds will exhibit activity towards the selected strains. Apart from compound **101** which showed activity from 8 mg/L, compounds **100**, **102**, **103** and **104** gave impressive activity against H37*rv* from 0.25 mg/mL (**Table 4.4**). On the other hand, only compound **103** was active at 4 mg/mL while the rest were active from 8 mg/mL with **103** and **104** inactive against *M. aurum*.

Table 4.4: Activity of compounds at different concentrations against H37rv and *M. aurum* (Courtesy Bacteriology Department NMIMR).

Concentration of compound down the column (mg/mL)	**100 (CFA 003)**		**101 (CFA 004)**		**102 (CFA 005)**		**103 (CFA 006)**		**104 (CFA 007)**		**75 E209**	
	M.A	Rv	M.A	Rv	M.A	Rv	M.A	Rv	M.A	Rv	M.A	Rv
8	+	+	-	+	+	+	+	+	-	+	-	+
4	-	+	-	-	-	+	+	+	-	+	-	+
2	-	+	-	-	-	+	-	+	-	+	-	+
1	-	+	-	-	-	+	-	+	-	+	-	+
0.5	-	+	-	-	-	+	-	+	-	+	-	-
0.25	-	+	-	-	-	-	-	+	-	+	-	-

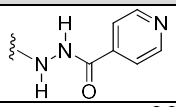
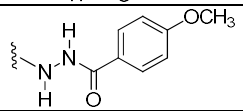
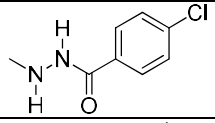
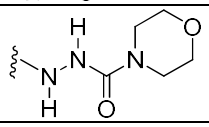
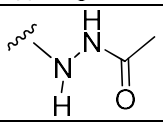
+ represents Activity; - represents No Activity

4.4.1 Minimum Inhibitory Concentrations (MIC).

Following the impressive results obtained in the *in vitro* screening, the compounds were subjected to MIC measurements for both strains within the concentration range shown below.

0.125 → 0.0625 → 0.0312 → 0.01562 → 0.007 → 0.0039 → 0.0015 → 0.00075 → 0.00037 → 0.00018 ↓ 0.00009 → 0.000045

Table 4.5: MIC's of test compounds with *M. aurum* and H37rv.

COMPOUND	CODE	-NR^{1}R^2	MIC for *M. aurum* (mg/mL)	MIC for H37rv (mg/mL)
100	CFA/003		8.000	0.003
101	CFA/004		Above 8.000	8.000
102	CFA/005		8.000	0.500
103	CFA/006		0.400	0.125
104	CFA/007		8.000	0.125
75 (E209)	-		Above 8	1

Apart from **103** which displayed the least MIC value (0.4 mg/mL) against *M. aurum*, the remaining compounds tested had MIC of 8 mg/mL (**Table 4.5)**. Interestingly, while compound **101** possessed the most active antimalarial activity with IC_{50} of 60 nM, it gave the least anti-TB activity with MIC of 8 mg/mL. A similar trend was observed for the MIC determination with respect to the two strains investigated. All the compounds tested showed activity against H37rv with compound **100** the most active with MIC of 0.003 mg/mL followed by **103**, **104** and **102** with MIC's of 0.125, 0.125, 0.500 and 8.000 mg/mL respectively. **Figure 4.5** represents the plate showing MIC's of the compounds against H37rv strain.

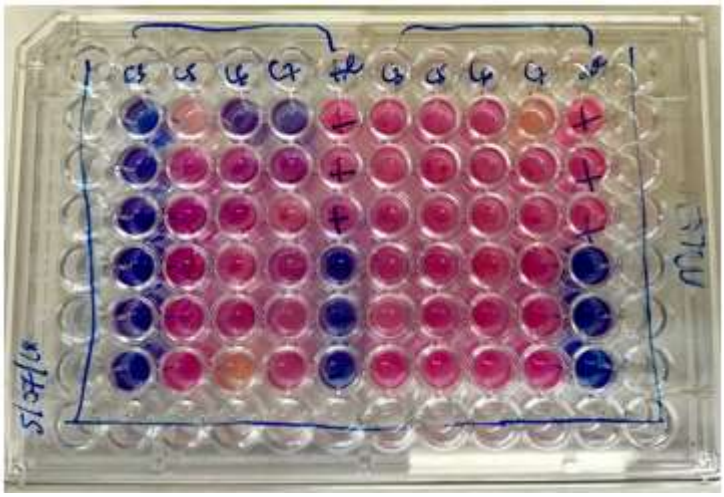


Figure 4.5: Plate showing MICs of the 4 active compounds using H37rv.

Even though the non-hydrazide variant **75** was found to be more potent than the least active hydrazide, with MIC of 1 mg/mL, it was observed that the incorporation of the hydrazides motif was essential for antitubercular activity. The electronic effect of the substituent on the phenyl ring was also observed to influence antitubercular activity. While the activity of hydrazide with an electron donating substituent on the phenyl ring **101** was low, the analogue with an electron withdrawing group **102** gave a superior activity. Though not overly surprising, it was observed that the hybrid with isonizid incorporated gave the most active antitubercular result suggesting that the pyridyl nitrogen might be critical for activity. The least lipophilic compounds; **100**, **103**, **104** were observed to be the most active anti-TB agents.

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1.0 Conclusion

Ten isonicotinohydrazides were designed, synthesized and characterized with ^{1}H-NMR, ^{13}C-NMR and FTIR. All synthesized compounds exhibited antimalarial activity in nanomolar range with compound **101** being the most active, possessing an IC_{50} value of **0.060 ± 0.033 µM,** against the 3D7 strain of *P. falciparum***.** Five of the hybrid molecules were further tested for their antitubercular activity, first by *in vitro* test using varying concentrations, followed by an MIC measurement using *M. aurum* and H37rv strains of *M. tuberculosis*. Of the five hybrid molecules tested, compound **100** was the most potent with an MIC of 0.003 mg/mL. Four of the analogues, **100**, **102**, **103** and **104**, showed both antimalarial and anti-TB activity with very good physicochemical parameters. Thus, the hybrid molecules has the potential of being developed further as potential antimalarial and antitubercular molecules Meanwhile, **E209** a very potent antimalarial drug candidate devoid of a hydrazide functional group, showed poor antitubercular activity. Hence, the hydrazide functionality is critical for antitubercular activity.

5.2.0 Recommendations

- All synthesized analogues should be tested against *M. aurum* and H37rv strains of *M. tuberculosis*.
- All analogues should be tested against resistant clinical isolates and wild-type *M. tuberculosis* strains.
- Additional analogues should be synthesized to ensure a comprehensive Structure-Activity Relationship (SAR) study.

REFERENCES

Agarwal, D., Gupta, R. D., & Awasthi, K. (2017). Are Antimalarial Hybrid Molecules a Close Reality or a Distant Dream? *Antimicrobial Agents and Chemotherapy*, *61*(5), 1–12.

Amewu, R. K., Chadwick, J., Hussain, A., Panda, S., Rinki, R., Janneh, O., … O'Neill, P. M. (2013). Synthesis and evaluation of the antimalarial, anticancer, and caspase 3 activities of tetraoxane dimers. *Bioorganic & Medicinal Chemistry*, *21*(23), 7392–7397.

Antoine, T., Fisher, N., Amewu, R., O'Neill, P. M., Ward, S. A., & Biagini, G. A. (2014). Rapid kill of malaria parasites by artemisinin and semi-synthetic endoperoxides involves ROS-dependent depolarization of the membrane potential. *Journal of Antimicrobial Chemotherapy*, *69*(4), 1005–1016.

Arbex, M. A., & Marília de Castro Lima Varella, Hélio Ribeiro de Siqueira, F. A. F. de M. (2010). Antituberculosis drugs: Drug interactions, adverse effects, and use in special situations. *Jornal Brasileiro de Pneumologia*, *36*(April), 626–640.

Aung, K. J. M., Van Deun, A., Declercq, E., Sarker, M. R., Das, P. K., Hossain, M. A., & Rieder, H. L. (2014). Successful "9-month Bangladesh regimen" for multi-drug resistant tuberculosis among over 500 consecutive patients. *International Journal of Tuberculosis and Lung Disease*, *18*(10), 1180–1187.

Baragaña, B., Hallyburton, I., Lee, M. C. S., Norcross, N. R., Grimaldi, R., Otto, T. D., … Gilbert, I. H. (2015). A novel multiple-stage antimalarial agent that inhibits protein synthesis. *Nature*, *522*(7556), 315–320.

Bennett, T. N., Paguio, M., Gligorijevic, B., Seudieu, C., Kosar, A. D., Davidson, E., & Roepe, P. D. (2004). Novel , Rapid , and Inexpensive Cell-Based Quantification of Antimalarial Drug Efficacy. *Antimicrobial Agents and Chemotherapy*, *48*(5), 1807–1810.

Blackie, M. (2014). Tetraoxanes as Antimalarials : Harnessing the Endoperoxide. *Mini Reviews in Medicinal Chemistry*, *14*(2), 123–135.

Blank, J., Behrends, J., Jacobs, T., & Schneider, B. E. (2015). Mycobacterium tuberculosis co-infection has no impact on Plasmodium berghei ANKA induced experimental cerebral

malaria in C57BL/6 mice. *Infection and Immunity*, *84*(2), 502–510.

Blank, J., Eggers, L., Behrends, J., Jacobs, T., & Schneider, B. E. (2016). One episode of self-resolving Plasmodium yoelii infection transiently exacerbates chronic Mycobacterium tuberculosis infection. *Frontiers in Microbiology*, *7*(152).

Bosa, L., L, D. S., Mendes, D. V, Sifna, A., M, S. M., Riccardi, F., & Colombatti, R. (2017). Feasibility and Effectiveness of Tuberculosis Active Case-Finding among Children Living with Tuberculosis Relatives : a Cross-Sectional Study in Guinea-Bissau. *Mediterranean Journal of Hematology and Infectious Diseases*, *9*(1), 5–11.

Boseley, S. (2008, March 1). Malaria drug off market over side-effect fears _ World news _. *The Guardian*. Retrieved from https://www.theguardian.com/world/2008/mar/01/kenya.glaxosmithklinebusiness

Carvalho, S. A., da Silva, E. F., de Souza, M. V. N., Lourenço, M. C. S., & Vicente, F. R. (2008). Synthesis and antimycobacterial evaluation of new trans-cinnamic acid hydrazide derivatives. *Bioorganic and Medicinal Chemistry Letters*, *18*(2), 538–541.

CDC. (2013). Transmission and Pathogenesis of Tuberculosis (pp. 21–43).

Cherchi, G., Deidda, D., Gioannis, B. De, Marongiu, B., Pompei, R., Porcedda, S., … Sestu, S. P. (2001). Extraction of Santolina insularis essential oil by supercritical carbon dioxide : influence of some process parameters and biological activity. *Flavour and Fragrance Journal*, *16*(1), 35–43.

Chikkula, K. V., Raja, S., & Raja, S. (2018). Anti-tubercular and antimicrobial activities of novel heterocyclic substituted benzimidole derivatives. *Der Pharmacia Lettre*, *10*(2), 60–72.

Chukwuanukwu, R. C., Onyenekwe, C. C., Agbakoba, N. R., & Okoye, J. O. (2016). Modulation of the immune response to Mycobacterium tuberculosis during malaria / M . tuberculosis co-infection. *Clinical and Experimental Immunology*, *187*(2), 259–268.

Chukwuanukwu, R. C., Onyenekwe, C. C., Martinez-Pomares, L., Flynn, R., Singh, S., Amilo, G. I., … Okoye, J. O. (2016). Modulation of the immune response to Mycobacterium tuberculosis during malaria/M. tuberculosis co-infection. *Clinical and Experimental*

Immunology, *187*(2), 259–268.

Cinu, T. A., Sidhartha, S. K., Indira, B., Varadaraj, B. G., Vishnu, P., Shenoy, G., & Gautham, S. (2015). Synthesis and Evaluation of Antitubercular Activity of Novel Diphenyl Ether Derivatives. *Indo Global Journal of Pharmaceutical Sciences*, *5*(1), 19–25.

Colombatti, R., Penazzato, M., Bassani, F., Vieira, C. S., Lourenço, A. A., Vieira, F., … Riccardi, F. (2011). Malaria prevention reduces in-hospital mortality among severely ill tuberculosis patients : a three- step intervention in Bissau , Guinea-Bissau. *BMC Infectious Diseases*, *11*(1), 57.

Dahl, E. L., & Rosenthal, P. J. (2007). Multiple Antibiotics Exert Delayed Effects against the Plasmodium falciparum Apicoplast. *Antimicrobial Agents and Chemotherapy*, *51*(10), 3485–3490.

Dembitsky, V. M. (2015a). Astonishing Diversity of Natural Peroxides as Potential Therapeutic Agents. *Journal of Molecular and Genetic Medicine*, *9*(1), 1–18.

Dembitsky, V. M. (2015b). Bioactive Fungal Endoperoxides. *IMedPub Journals*, *1*(15), 1–7.

Dembitsky, V., Shkrob, I., & Ondrej, L. (2008). Ascaridole and related peroxides from the genus chenopodium. *Biomedical Papers of the Medical Faculty of the University Palacky Olomouc Czechoslovakia*, *152*(2), 209–215.

Ding, L., Maier, A., Fiebig, H.-H., Lin, W.-H., Peschel, G., & Hertweck, C. (2012). Kandenols A–E, Eudesmenes from an Endophytic Streptomyces sp. of the Mangrove Tree Kandelia candel. *Journal of Natural Products*, *75*(12), 2223–2227.

Dondorp, A. M., Nosten, F., Poravuth, Y., Das, D., Phyo, A. P., Tarning, J., … White, N. J. (2009). Artemisinin Resistance in Plasmodium falciparum Malaria. *New England Journal of Medicine*, *361*(5), 455–467.

Dong, Y., Creek, D., Chollet, J., Matile, H., Charman, S. A., Wittlin, S., … Vennerstrom, J. L. (2007). Comparative antimalarial activities of six pairs of 1,2,4,5-tetraoxanes (peroxide dimers) and 1,2,4,5,7,8-hexaoxonanes (peroxide trimers). *Antimicrobial Agents and Chemotherapy*, *51*(8), 3033–3035.

Dong, Y., Wittlin, S., Sriraghavan, K., Chollet, J., Charman, S. A., Charman, W. N., … Vennerstrom, J. L. (2010). The Structure−Activity Relationship of the Antimalarial Ozonide Arterolane (OZ277). *Journal of Medicinal Chemistry*, *53*(1), 481–491.

Drabe, C. H., Vestergaard, L. S., Helleberg, M., Nyagonde, N., Rose, M. V., Francis, F., … Ravn, P. (2016). Performance of interferon-gamma and IP-10 release assays for diagnosing latent tuberculosis infections in patients with concurrent malaria in Tanzania. *American Journal of Tropical Medicine and Hygiene*, *94*(4), 728–735.

Eldehna, W. M., Mohamed, F., Marwa, M., Hatem, A., & Abdel-Aziz. (2015). Design, Synthesis and Antitubercular Activity of Certain Nicotinic Acid Hydrazides. *Molecules*, *20*, 8800–8815.

Fisher, L. C., & Blackie, M. A. (2014). Tetraoxanes as antimalarials: harnessing the endoperoxide. *Mini Reviews in Medicinal Chemistry*, *14*(2), 123–135.

Gaillard, T., Madamet, M., Tsombeng, F. F., Dormoi, J., & Pradines, B. (2016). Antibiotics in malaria therapy: which antibiotics except tetracyclines and macrolides may be used against malaria? *Malaria Journal*, *15*(1), 556.

Gemma, S., Savini, L., Altarelli, M., Tripaldi, P., Chiasserini, L., Coccone, S. S., … Butini, S. (2009). Development of antitubercular compounds based on a 4-quinolylhydrazone scaffold. Further structure-activity relationship studies. *Bioorganic and Medicinal Chemistry*, *17*(16), 6063–6072.

Ghana Health Service. (2017). Roll Back malaria, (9), 1–12.

GhanaWeb. (2017, March 17). Tuberculosis on the upsurge; 14,632 cases in 2016 alone _ General News 2017-03-17. Retrieved from https://www.ghanaweb.com/GhanaHomePage/NewsArchive/Tuberculosis-on-the-upsurge-14-632-cases-in-2016-alone-519782

Ghodke, S. V, & Chudasama, U. V. (2015). Friedel-Crafts alkylation and acylation of aromatic compounds under solvent-free conditions using solid acid catalysts. *International Journal of Chemical Studies*, *2*(5), 27–34.

Ghorai, P., & Dussault, P. H. (2009). Broadly Applicable Synthesis of 1,2,4,5-Tetraoxanes - Organic Letters. *Organic Letters*, *11*(1), 213–216.

Hawkes, M. (2012). *Host-pathogen interactions in malaria and tuberculosis: Experimental models and translation to novel adjunctive therapies*. *ProQuest Dissertations and Theses*. University of Toronto. Retrieved from http://search.proquest.com/docview/1351351028?accountid=37552

Hearn, M. J., & Cynamon, M. H. (2004). Design and synthesis of antituberculars: Preparation and evaluation against Mycobacterium tuberculosis of an isoniazid Schiff base. *Journal of Antimicrobial Chemotherapy*, *53*, 185–191.

Hudson, A., Imamura, T., Gutteridge, W., Kanyok, T., & Nunn, P. (2013). *The current anti-TB drug research and development pipeline*.

Ingram, K., Yaremenko, I. A., Krylov, I. B., Hofer, L., Terentev, A. O., & Keiser, J. (2012). Identification of antischistosomal leads by evaluating bridged 1,2,4,5-tetraoxanes, alphaperoxides, and tricyclic monoperoxides. *Journal of Medicinal Chemistry*, *55*(20), 8700–8711.

Karthik Kumar, K., Prabu Seenivasan, S., Kumar, V., & Mohan Das, T. (2011). Synthesis of quinoline coupled [1,2,3]-triazoles as a promising class of anti-tuberculosis agents. *Carbohydrate Research*, *346*(14), 2084–2090.

Keri, R. S., Quintanova, C., Marques, S. M., Esteves, A. R., Cardoso, S. M., & Santos, M. A. (2013). Design, synthesis and neuroprotective evaluation of novel tacrine-benzothiazole hybrids as multi-targeted compounds against Alzheimer's disease. *Bioorganic and Medicinal Chemistry*, *21*(15), 4559–4569.

Kesicki, E. A., Bailey, M. A., Ovechkina, Y., Early, J. V., Alling, T., Bowman, J., … Parish, T. (2016). Synthesis and evaluation of the 2-aminothiazoles as anti-tubercular agents. *PLoS ONE*, *11*(5).

Kim, S., Park, S., Min, T., & Yu, K. (1999). Antioxidant Activity of Ergosterol Peroxide (5,8-Epidioxy-5 ,8 -ergosta-6,22E-dien-3 -ol) in *Armillariella mellea*. *Bulletin of the Korean Chemical Society*, *20*(7), 819–823.

Kreidenweiss, A., Mordmu, B., Krishna, S., & Kremsner, P. G. (2006). Antimalarial Activity of a Synthetic Endoperoxide (RBx-11160 / OZ277) against Plasmodium falciparum Isolates from Gabon. *Antimicrobial Agents and Chemotherapy*, *50*(4), 1535–1537.

Li H, Huang H, Shao C, Huang H, Jiang J, Zhu X, Liu Y, Liu L, Lu Y, Li M, Lin Y, S. Z. (2011). Cytotoxic norsesquiterpene peroxides from the endophytic fungus Talaromyces flavus isolated from the mangrove plant Sonneratia apetala. *J Nat Prod*, *74*(5), 1230–1235.

Li, X. X., & Zhou, X. N. (2013). Co-infection of tuberculosis and parasitic diseases in humans: A systematic review. *Parasites and Vectors*, *6*(79).

Li, Y., Niu, S., Sun, B., Liu, S., Liu, X., & Yongsheng Che. (2010). Cytosporolides A−C, Antimicrobial Meroterpenoids with a Unique Peroxylactone Skeleton from Cytospora sp. *Org. Lett.*, *12*(14), 3144–3147.

Makropoulou, M., Aligiannis, N., Gonou-Zagou, Z., Pratsinis, H., Skaltsounis, A.-L., & Fokialakis, N. (2012). Antioxidant and Cytotoxic Activity of the Wild Edible Mushroom Gomphus clavatus. *Journal of Medicinal Food*, *15*(2), 216–221.

Marti, F., Chadwick, J., Amewu, R. K., Burrell-Saward, H., Srivastava, A., Ward, S. A., … O'Neill, P. M. (2011). Second generation analogues of RKA182: synthetic tetraoxanes with outstanding in vitro and in vivo antimalarial activities. *MedChemComm*, *2*(7), 661–665.

Maste, M. M., Jeyarani, P., Kalekar, M. C., & Bhat, A. R. (2011). Synthesis and Evaluation of Benzimidazole Derivatives for Anti-tubercular and Antimicrobial Activities. *Asian Journal of Research in Chemistry*, *4*(7), 1055–1058.

Matteelli, A., & Castelli, F. (2015). *Life cycle of malaria parasites*.

Ministry of Health. (2009). *Drug policy for ghana*. Retrieved from https://www.who.int/countries/gha/news/2006/anti.malaria.drug.policy/en/

Mishra, S., & Singh, P. (2016). Chemistry Hybrid molecules : The privileged scaffolds for various pharmaceuticals. *European Journal of Medicinal Chemistry*, *124*, 500–536.

Monzote, L., Rubio, O. C., Matheeussen, A., Assche, T. Van, Maes, L., & Cos, P. (2011). Antimicrobial Evaluation of the Polyisoprenylated Benzophenones Nemorosone and

Guttiferone A. *Phytotherapy Research*, *25*(3), 458–462.

Murphy, K. V. (2015). *Design and Synthesis of Novel Chloroquine-based Antimalarials by*. Portland State University. Retrieved from https://pdxscholar.library.pdx.edu/open_access_etds/2623

Murphy, M. E., Singh, K. P., Laurenzi, M., Brown, M., & Gillespie, S. H. (2012). Managing malaria in tuberculosis patients on fluoroquinolone-containing regimens : assessing the risk of QT prolongation. *International Journal of Tuberculosis and Lung Disease*, *16*(2), 144–149.

Nakano T., Koiwa T., Takahashi S., N. A. (2000). Adxanthromycins A and B, new inhibitors of ICAM-1_LFA-1 mediated cell adhesion molecule from Streptomyces sp. *Journal of Antibiotics*, *53*(1), 12–18.

Nayak, N., & Jurupula, Ramprasad Udayakumar, D. (2015). New INH-pyrazole analogs: Design, synthesis and evaluation of antitubercular and antibacterial activity. *Bioorganic and Medicinal Chemistry Letters*, *25*(23), 5540–5545.

Nayak, N., Ramprasad, J., & Dalimba, U. (2015). New INH-pyrazole analogs: Design, synthesis and evaluation of antitubercular and antibacterial activity. *Bioorganic and Medicinal Chemistry Letters*, *25*(23), 5540–5545.

Noedl, H., Youry, S., Kurt, S., Bryan, L. S., Duong, S., & Mark, M. F. (2008). Evidence of Artemisinin-Resistant Malaria in Western Cambodia. *New England Journal of Medicine*, *359*(24), 2619–2620.

Nosten, F., & White, N. J. (2007). Artemisinin-based combination treatment of falciparum malaria. *The American Journal of Tropical Medicine and Hygiene*, *77*(6 Suppl), 181–192.

Nzila, A., & Chibale, K. (2011). Drug repositioning in the treatment of malaria and TB. *Future Medicinal Chemistry*, *3*(11), 1413–1426.

O'Neill, P., Amewu, R., Nixon, G., Elgarah, F., Mungthin, M., Chadwick, J., … Ward, S. (2010). Identification of a 1,2,4,5-Tetraoxane Antimalarial Drug-Development Candidate (RKA 182) with Superior Properties to the Semisynthetic Artemisinins. *Angewandte*

Chemie International Edition, *49*(33), 5693–5697.

O'Neill, P. M., Amewu, R. K., Charman, S. A., Sabbani, S., Gna, N. F., Fidock, D. A., … Rochford, R. (2017). A tetraoxane-based antimalarial drug candidate that overcomes PfK13-C580Y dependent artemisinin resistance. *Nature Communications*, *8*, 1–10.

O'Neill, P. M., Sabbani, S., Nixon, G. L., Schnaderbeck, M., Roberts, N. L., Shore, E. R., … Amewu, R. K. (2016). Optimisation of the synthesis of second generation 1, 2, 4, 5 - tetraoxane antimalarials. *Tetrahedron*, *72*(40), 6118–6126.

O'Neill, P. M., Stocks, P. A., Sabbani, S., Roberts, N. L., Amewu, R. K., Shore, E. R., … Ward, S. A. (2018). Bioorganic & Medicinal Chemistry N205 : Towards tetraoxane scaffolds with potential for single dose cure of malaria. *Bioorganic & Medicinal Chemistry*, *26*(11), 2996–3005.

Oli, S., Abdelmohsen, U. R., Hentschel, U., & Schirmeister, T. (2014). Identification of plakortide e from the Caribbean sponge Plakortis halichondroides as a trypanocidal protease inhibitor using bioactivity-guided fractionation. *Marine Drugs*, *12*(5), 2614–2622.

Opsenica, D., Kyle, D. E., Milhous, W. K., & Olaja, B. A. (2003). Antimalarial, antimycobacterial and antiproliferative activity of phenyl substituted mixed tetraoxanes. *Journal of the Serbian Chemical Society*, *68*(4–5), 291–302.

Opsenica, D. M., & Solaja, B. A. (2009). Antimalarial peroxides. *Journal of the Serbian Chemical Society*, *74*(11), 1155–1193.

Opsenica, D. M., & Šolaja, B. A. (2009). Antimalarial peroxides. *J. Serb. Chem. Soc*, *74*(11), 1155–1193.

Opsenica, D. M., & Šolaja, B. A. (2012). Artemisinins and synthetic peroxides as highly efficient antimalarials. *Macedonian Journal of Chemistry and Chemical Engineering*, *31*(2), 137–182.

Patel, M., Horan, A. C., Gullo, V. P., Loebennerg, D., Marquez, J. A., Miller, G. H., & Waltz, J. A. (1983). Oxanthromicin, a novel antibiotic from actinomadura. *The Journal of Antibiotics*, *XXXVII*(4), 413–415.

Patil, C., Baig, M., Doifode, S., & Katare, S. (2014). Fixed dose combination of arterolane and piperaquine: A newer prospect in antimalarial therapy. *Annals of Medical and Health Sciences Research*.

Patnaik, P. (2007). *A Comprehensive Guide to the Hazardous Properties of Chemical Substances* (3rd ed.). Hoboken: John Wiley & Sons, Inc.

Phyo, A. P., Jittamala, P., Nosten, F. H., Pukrittayakamee, S., Imwong, M., White, N. J., … Möhrle, J. J. (2016). Antimalarial activity of artefenomel (OZ439), a novel synthetic antimalarial endoperoxide, in patients with Plasmodium falciparum and Plasmodium vivax malaria: an open-label phase 2 trial. *The Lancet Infectious Diseases*, *16*(1), 61–69.

Plowe, C. V. (2007). Combination Therapy for Malaria: Mission Accomplished? *Clinical Infectious Diseases*, *44*(8), 1075–1077.

Price, R. N., & Douglas, N. M. (2009). Artemisinin Combination Therapy for Malaria : Beyond Good Efficacy. *Clinical Infectious Diseases*, *49*(11), 1638–1640.

Public Health, E. (2018). Tuberculosis – the disease, its treatment and prevention. *Department of Health*. Retrieved from https://www.gov.uk/government/publications/tuberculosis-the-disease-its-treatment-and-prevention

Rudrapal, M., Chetia, D., & Singh, V. (2017). Novel series of 1,2,4-trioxane derivatives as antimalarial agents. *Journal of Enzyme Inhibition and Medicinal Chemistry*, *32*(1), 1159–1173.

Shankar, E. M., Vignesh, R., Elleg, R., Barathan, M., Chong, Y. K., & Kahar, M. (2014). Mycobacterium tuberculosis co-infection : a ‘ danger-couple model ’ of disease pathogenesis. *Pathogens and Disease*, *70*, 110–118.

Snow, R., Molyneux, C., Warn, P., Omumbo, J., Nevill, C., Gupta, S., & Marsh, K. (1996). Infant parasite rates and immunoglobulin M seroprevalence as a measure of exposure to Plasmodium falciparum during a randomized controlled trial of insecticide-treated bed nets on the Kenyan coast. *Am J Trop Med Hyg.*, *55*(2), 144–149.

Sotgiu, G., Tiberi, S., Centis, R., D’Ambrosio, L., Fuentes, Z., Zumla, A., & Migliori, G. B.

(2017). Applicability of the shorter 'Bangladesh regimen' in high multidrug-resistant tuberculosis settings. *International Journal of Infectious Diseases*, *56*, 190–193.

Souza, G. D. De, Rodrigues, A., Silva, P., & Cristina, E. (2013). A New Complex of Palladium (II) With 2-Furoic Hydrazide: Synthesis, Characterization, Theoretical Calculations and Biological Studies. *Croatica Chemica Acta*, *86*(2), 201–206.

Staines, H. M., & Krishna, S. (2012). *Treatment and Prevention of Malaria*. (H. M. Staines & S. Krishna, Eds.) (illustrate). Springer Science & Business Media.

Su, X., Hayton, K., Wellems, T. E., & Walliker, D. (2007). Genetic linkage and association analyses for trait mapping in. *Nature Reviews Genetics*, *8*(July), 497–506.

Sun, Y., Zhao, Z., Feng, Q., Xu, Q., Lü, L., Liu, J. K., … Li, Y. Q. (2013). Unusual spirodecane sesquiterpenes and a fumagillol analogue from cordyceps ophioglossoides. *Helvetica Chimica Acta*, *96*(1), 76–84.

Takei, T., Yoshida, M., Ohnishi-Kameyama, M., & Kobori, M. (2005). Ergosterol peroxide, an apoptosis-inducing component isolated from Sarcodon aspratus (Berk.) S. Ito. *Bioscience, Biotechnology, and Biochemistry*, *69*(1), 212–215.

Taylor, A. R., Flegg, J. A., Holmes, C. C., Guérin, P. J., Sibley, C. H., Conrad, M. D., … Rosenthal, P. J. (2016). Artemether-lumefantrine and dihydroartemisinin-piperaquine exert inverse selective pressure on Plasmodium falciparum drug sensitivity associated haplotypes in Uganda. *Open Forum Infectious Diseases*, 1–5.

TBFACTS.ORG. (2018). Treatment of Drug Resistant TB _ Shorter regimens. TBFACTS.ORG. Retrieved from https://www.tbfacts.org/treatment-of-drug-resistant-tb/

Thapa, R., Mallick, D., & Biswas, B. (2010). Perinatal malaria and tuberculosis co-infection : A case report. *International Journal of Infectious Diseases*, *14*(3), 254–256.

The Union. (2018). STREAM stage 2 clinical study enrols patients in first trial to include bedaquiline to test shortened MDR-TB treatment regimens _ The Union. Retrieved from https://www.theunion.org/news-centre/features/stream-video

Thomas, K. D., Adhikari, A. V., Chowdhury, I. H., Sandeep, T., Mahmood, R., Bhattacharya, B.,

& Sumesh, E. (2011). Design, synthesis and docking studies of quinoline-oxazolidinone hybrid molecules and their antitubercular properties. *European Journal of Medicinal Chemistry*, *46*(10), 4834–4845.

Tilley, L., Straimer, J., Gnädig, N. F., Ralph, S. A., & Fidock, D. A. (2016). Artemisinin Action and Resistance in Plasmodium falciparum. *Trends in Parasitology*, *32*(9), 682–696.

Tolstikov, G. A., Tolstikov, A. G., & Tolstikova, O. V. (1996). Natural peroxides . Chemistry and biological activity Natural peroxides . Chemistry and biological activity. *Russian Chemical Reviews*, *65*(9), 769–783.

Trager, W., & Jensen, J. B. (1988). Human malaria parasites in Continuous culture. *Science*, *193*, 673–675.

Tripathy, S., & Roy, S. (2014). A review of age-old antimalarial drug to combat malaria : efficacy up- gradation by nanotechnology based drug delivery. *Asian Pacific Journal of Tropical Medicine*, *7*(9), 673–679.

Uchiyama, N., Matsunaga, K., Kiuchi, F., Honda, G., Tsubouchi, A., Nakajima-Shimada, J., & Aoki, T. (2002). Trypanocidal terpenoids from Laurus nobilis L. *Chemical & Pharmaceutical Bulletin*, *50*(11), 1514–1516.

Valadas, E., Gomes, A., Sutre, A., Brilha, S., Wete, A., Hänscheid, T., & Antunes, F. (2013). Tuberculosis with malaria or HIV co-infection in a large hospital in Luanda, Angola. *Journal of Infection in Developing Countries*, *7*(3), 269–272.

Viegas-junior, C., Danuello, A., Bolzani, S., Barreiro, E. J., Alberto, C., & Fraga, M. (2007). Molecular Hybridization : A Useful Tool in the Design of New Drug Prototypes. *Current Medicinal Chemistry*, *14*, 1829–1852.

Vil', V. A., Yaremenko, I. A., Ilovaisky, A. I., & Terent'ev, A. O. (2017). Peroxides with Anthelmintic, Antiprotozoal, Fungicidal and Antiviral Bioactivity: Properties, Synthesis and Reactions. *Molecules*, *22*(1881).

Vitoria, M., Granich, R., Gilks, C. F., Gunneberg, C., Hosseini, M., Were, W., … Cock, K. M. De. (2009). The Global Fight Against HIV / AIDS , Tuberculosis , and Malaria Current

Status and Future Perspectives, (May), 844–848.

Vlok, M. C. (2008). *Literature Review: Artemisinin*.

Wang, W. S., Li, W. E., & Jia, Z. J. (2002). Terpenes from Juniperus przewalskii and their antitumor activities. *Pharmazie*, *57*(5), 343–345.

Whalen, J. (2008, March 3). Glaxo Halts Malaria Drugs. *The Wall Street Journal*. Retrieved from https://www.wsj.com/articles/SB120430791681703371?mod=googlenews_wsj

WHO. (2001). *Antimalarial Drug Combination Therapy; Report of a WHO Technical Consultation*. *World Health Organization, Geneva*. Retrieved from http://www.rbm.who.int/

WHO. (2011). World Malaria Report 2011, (December), 1–3.

WHO. (2016). *WHO treatment guidelines for drug- resistant tuberculosis 2016*. Retrieved from http://www.who.int/tb/areas-of-work/drug-resistant-tb/treatment/resources/en/

WHO. (2017). *Global Tuberculosis Report 2017*. Retrieved from http://www.who.int/tb/publications/C2_2017GLOBAL_FACTSHEET.pdf?ua=1

Wiwanitkit, V. (2006). Co-infection between tuberculosis and malaria: A consideration on interaction of molecules and pathogenesis. *Journal of Vector Borne Diseases*, *43*(4), 195–197.

Wu, Q. P., Xie, Y. Z., Deng, Z., Li, X. M., Yang, W., Jiao, C. W., … Yang, B. B. (2012). Ergosterol Peroxide Isolated from Ganoderma lucidum Abolishes MicroRNA miR-378-Mediated Tumor Cells on Chemoresistance. *PLoS ONE*, *7*(8), 44579.

Xiaofang Wang, Qingjie Zhao, Mireille Vargas, Yuxiang Dong, Kamaraj Sriraghavan, Jennifer Keiser, J. L. V. (2011). The Activity of Dispiro Peroxides Against Fasciola hepatica. *Bioorg Med Chem Lett.*, *21*(18), 5320–5323.

Yeka, A., Tibenderana, J., Achan, J., D'Alessandro, U., & Talisuna, A. O. (2013). Efficacy of Quinine, Artemether-Lumefantrine and Dihydroartemisinin-Piperaquine as Rescue Treatment for Uncomplicated Malaria in Ugandan Children. *PLoS ONE*, *8*(1).

Yokoyama, S., Bang, T. H., Shimizu, K., & Kondo, R. (2012). Osteoclastogenesis inhibitory effect of ergosterol peroxide isolated from Pleurotus eryngii. *Natural Product Communications*, *7*, 1163–1164.

Yong-hong, Z. H. U., Xin-xin, L. I., Hong-mei, M. O., Li-hua, Z., Lan-lan, Z., & Shui-ping, Z. (2012). Gastroprotective Effects of Ascaridole on Gastric Ulcer in Rats. *Chinese Herbal Medicines*, *4*(1), 58–62.

Yu, D., Chen, R., Huang, L., Xie, F., Zhou, K., Li, H., & Tong, K. (2008). The structure and absolute configuration of Shuangkangsu: a novel natural cyclic peroxide from Lonicera japonica. *Journal of Asian Natural Products Research*, *10*(9), 851–856.

Zhang, C., Du, Q. Y., Chen, L. Di, Wu, W. H., Liao, S. Y., Yu, L. H., & Liang, X. T. (2016). Design, synthesis and evaluation of novel tacrine-multialkoxybenzene hybrids as multi-targeted compounds against Alzheimer's disease. *European Journal of Medicinal Chemistry*, *116*, 200–209.

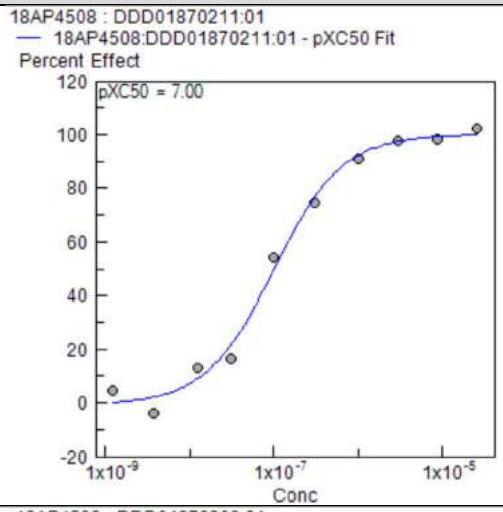
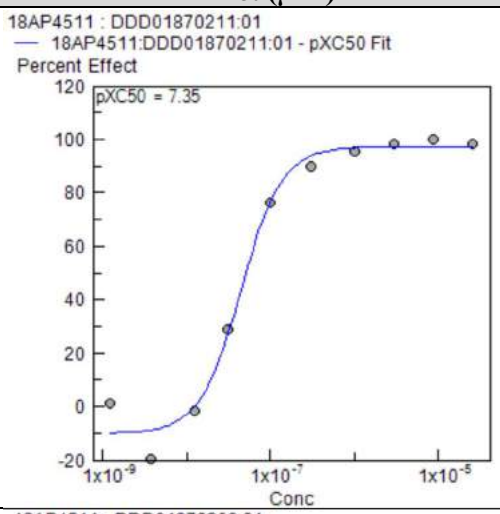
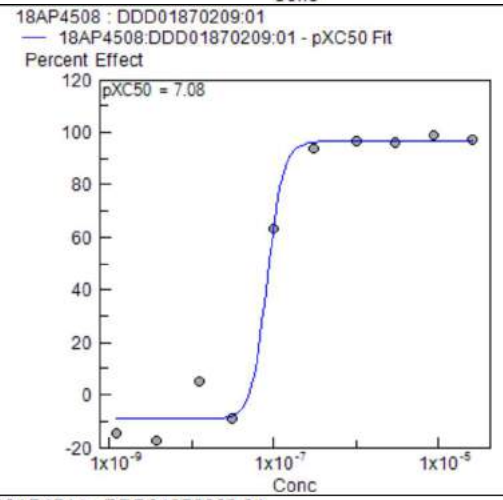
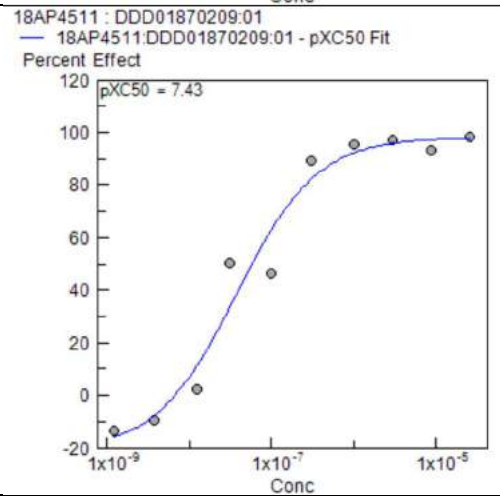
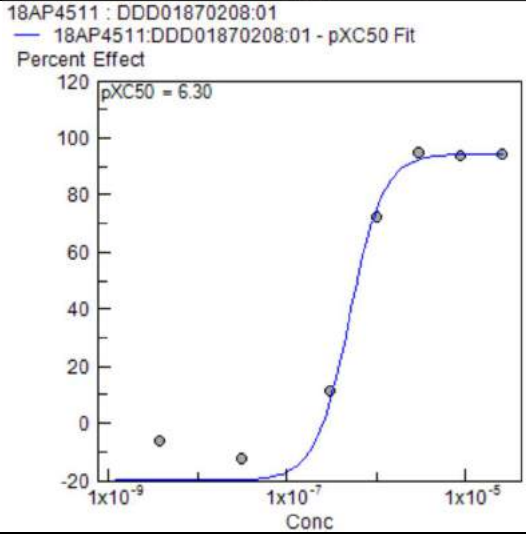
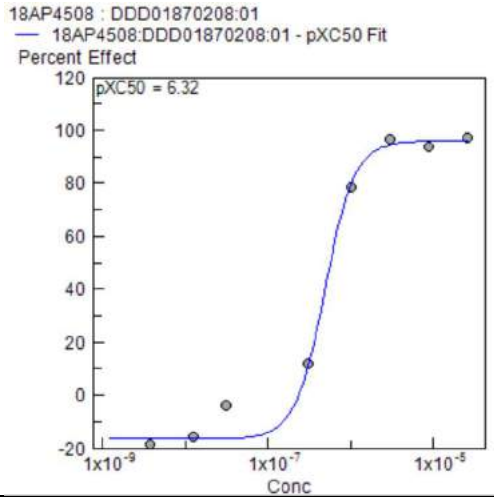
Zhu, J. X., Qin, J. J., Jin, H. Z., & Zhang, W. D. (2013). Japonicones Q-T, four new dimeric sesquiterpene lactones from Inula japonica Thunb. *Fitoterapia*, *84*(1), 40–46.

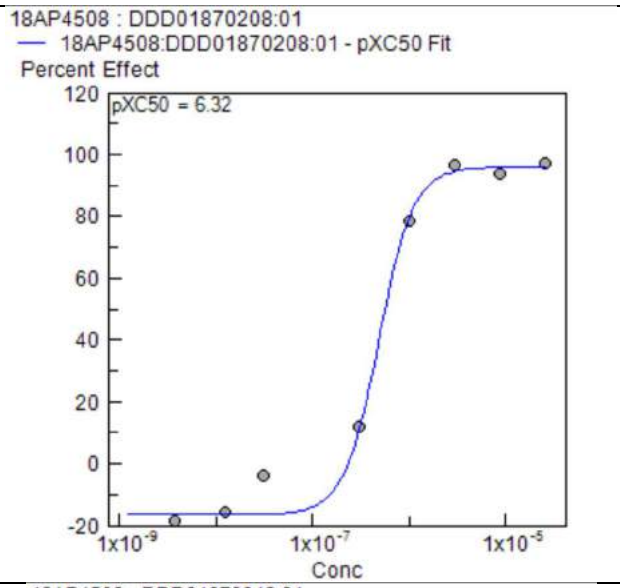
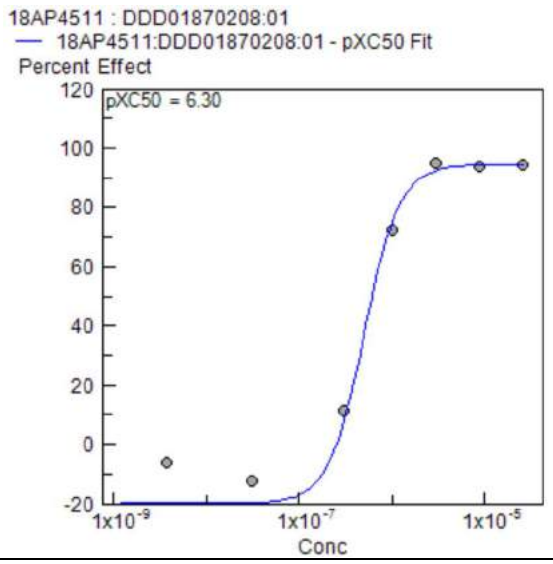
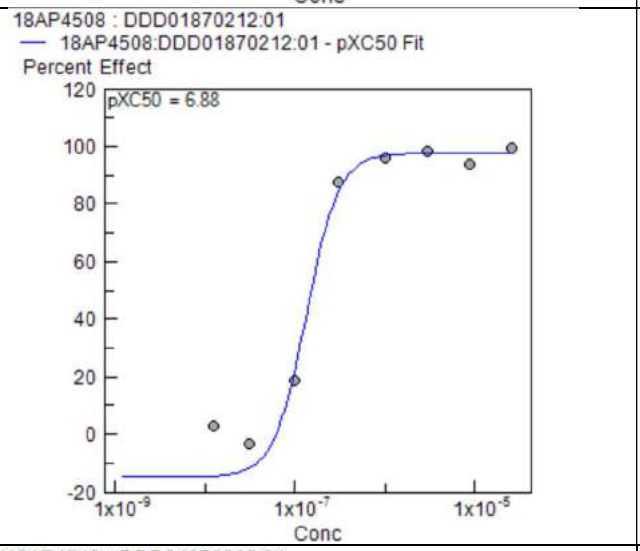
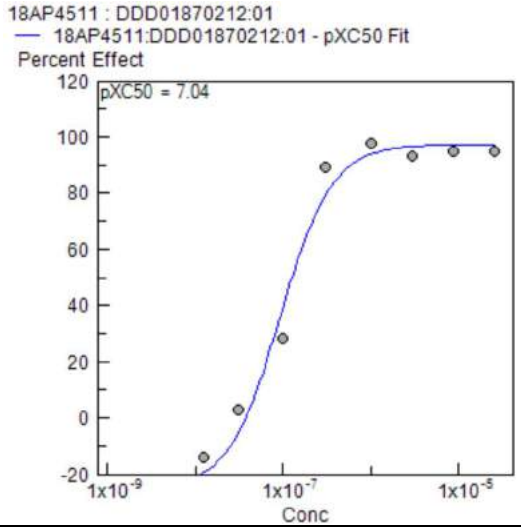
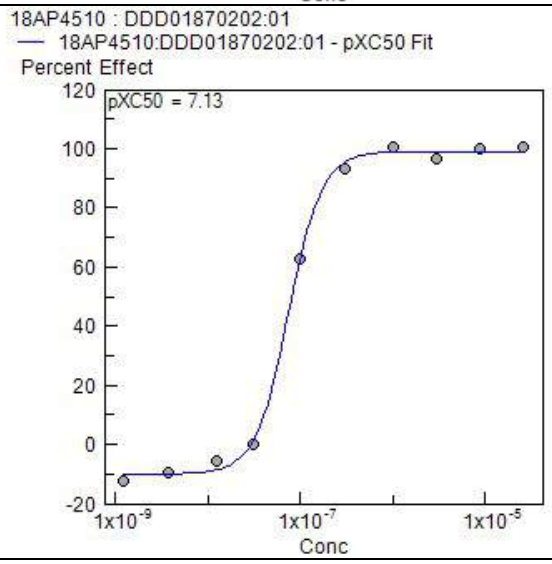
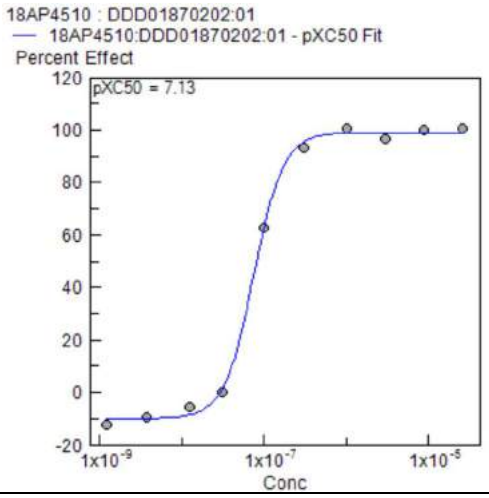
Zmitek, K., Stavber, S., Zupan, M., Bonnet-delpon, D., Charneau, S., Grellier, P., & Iskra, J. (2006). Synthesis and antimalarial activities of novel 3,3,6,6-tetraalkyl-1,2,4,5-tetraoxanes. *Bioorganic & Medicinal Chemistry*, *14*, 7790–7795.

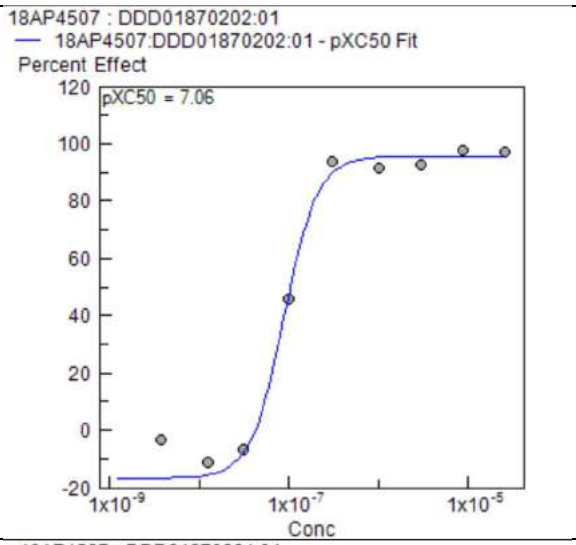
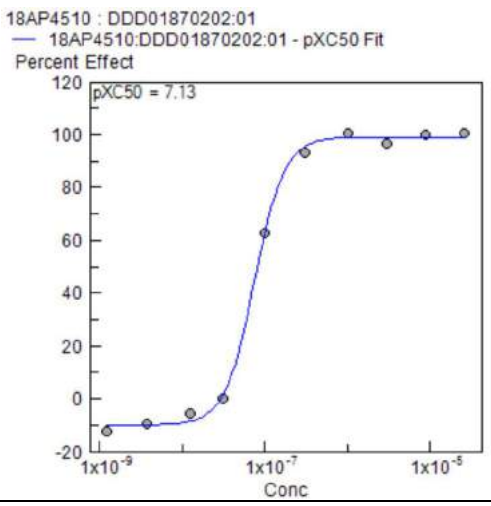
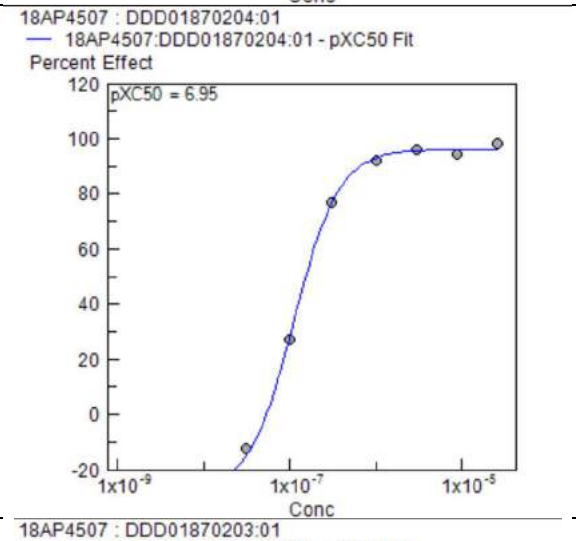
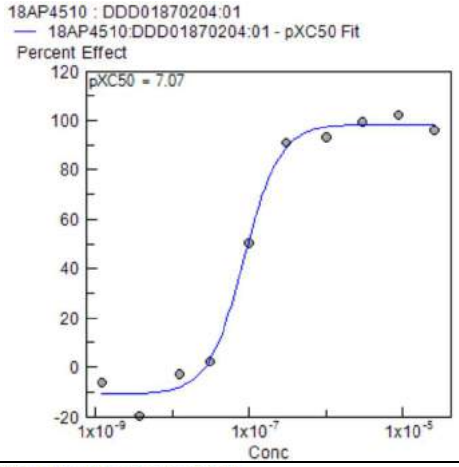
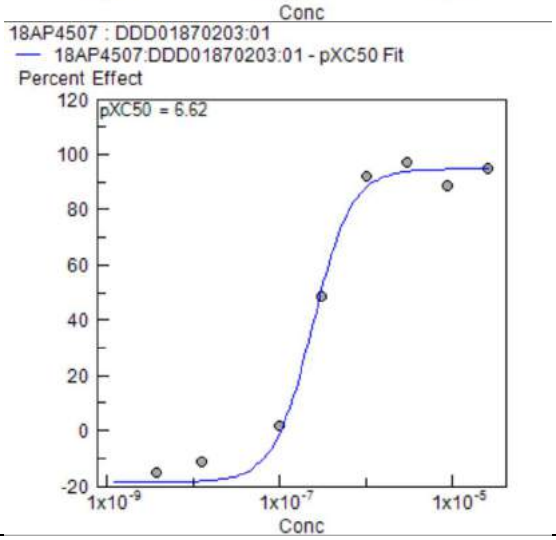
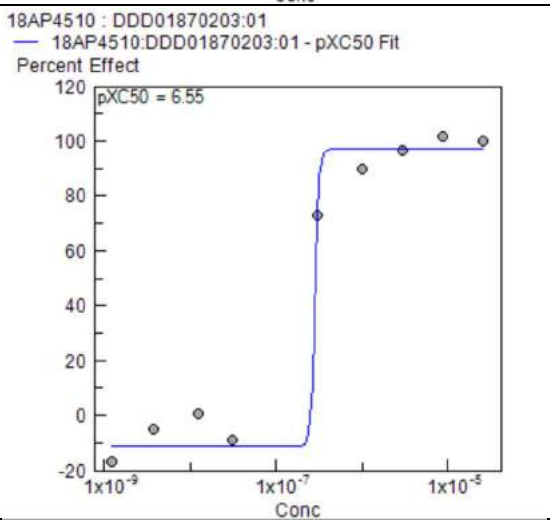
Zumla, A., Raviglione, M., Hafner, R., & Reyn., C. F. von. (2013). Tuberculosis. *New England Journal of Medicine*, *368*(8), 745–755.

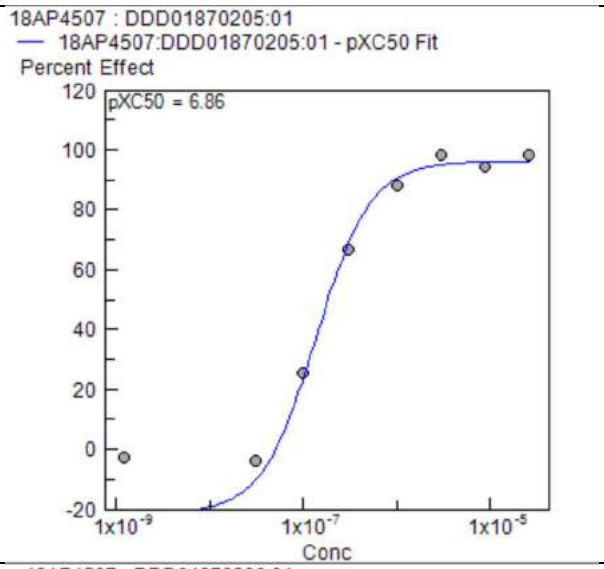
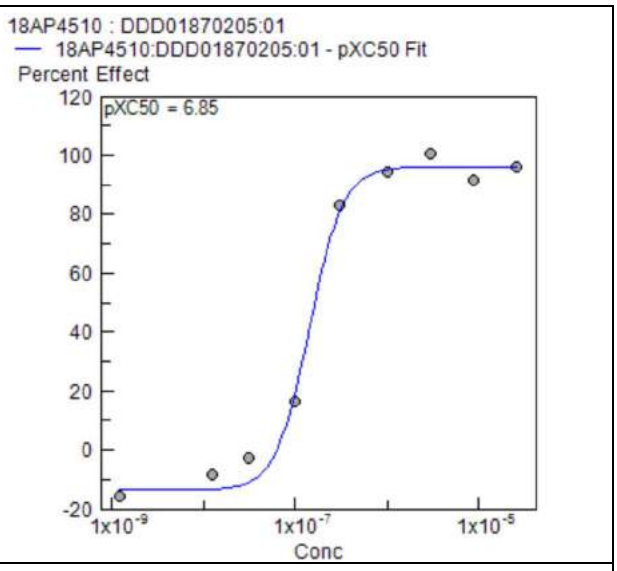
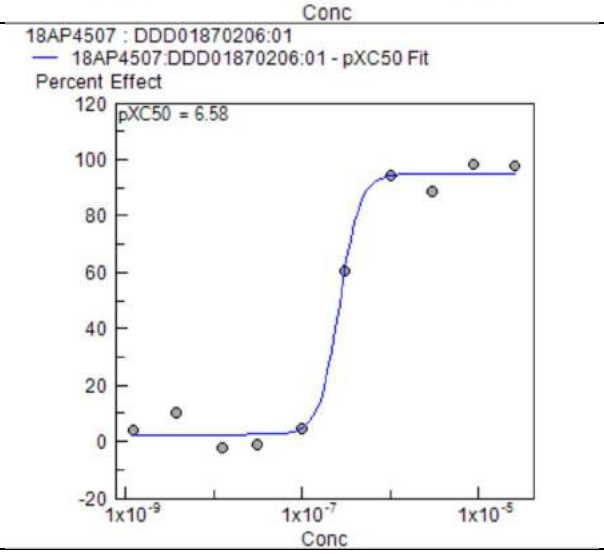
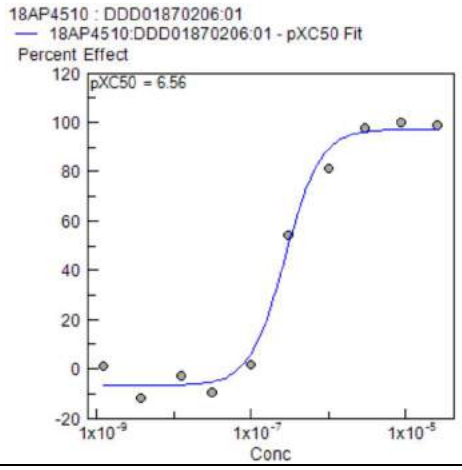
APPENDICES

Appendix 1: IC_{50} curves of synthesized analogues.

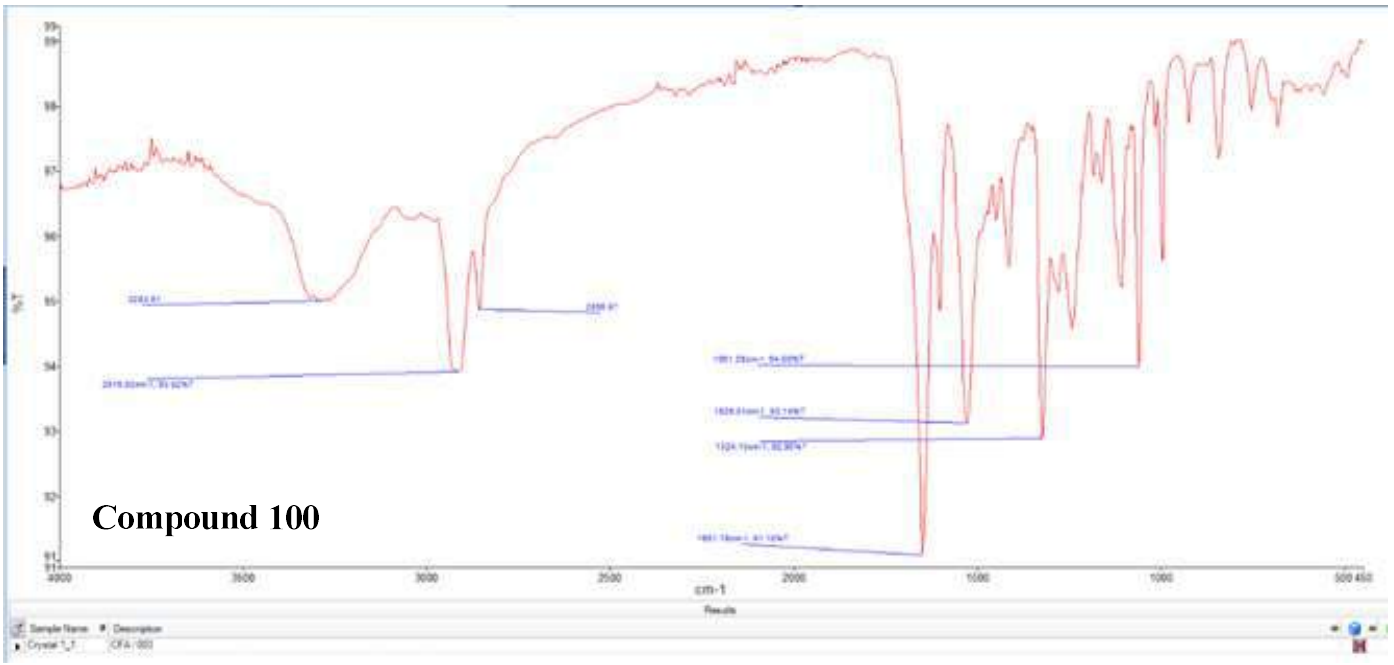
COMPOUND	CODE	IC_{50} (µM)
100		
101		
102		

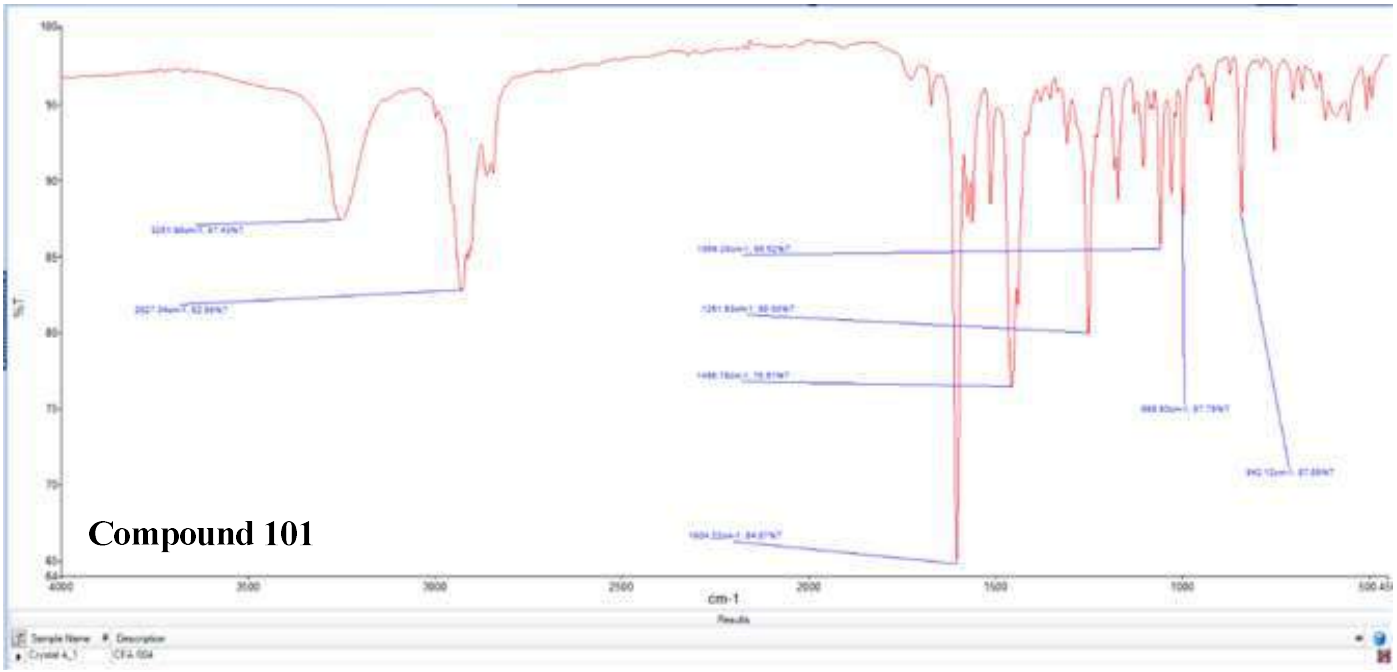
103		
104		
105		

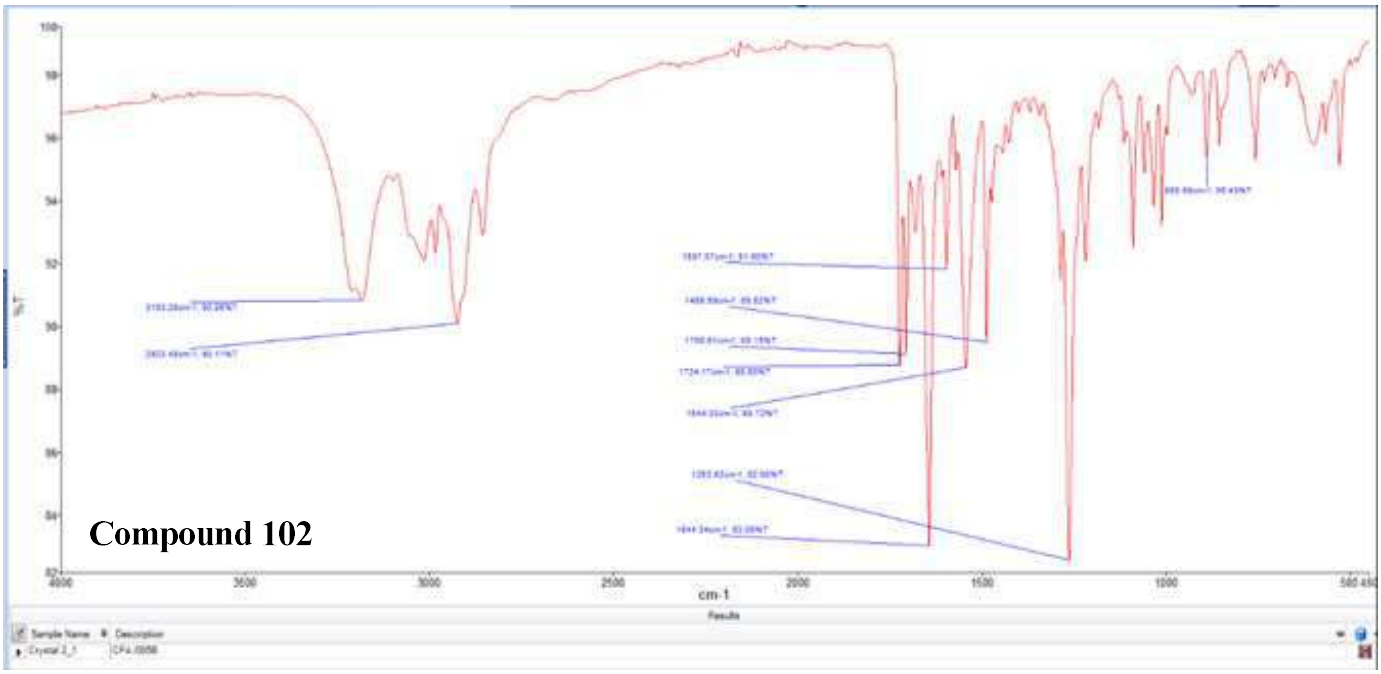
106		
107		
108		

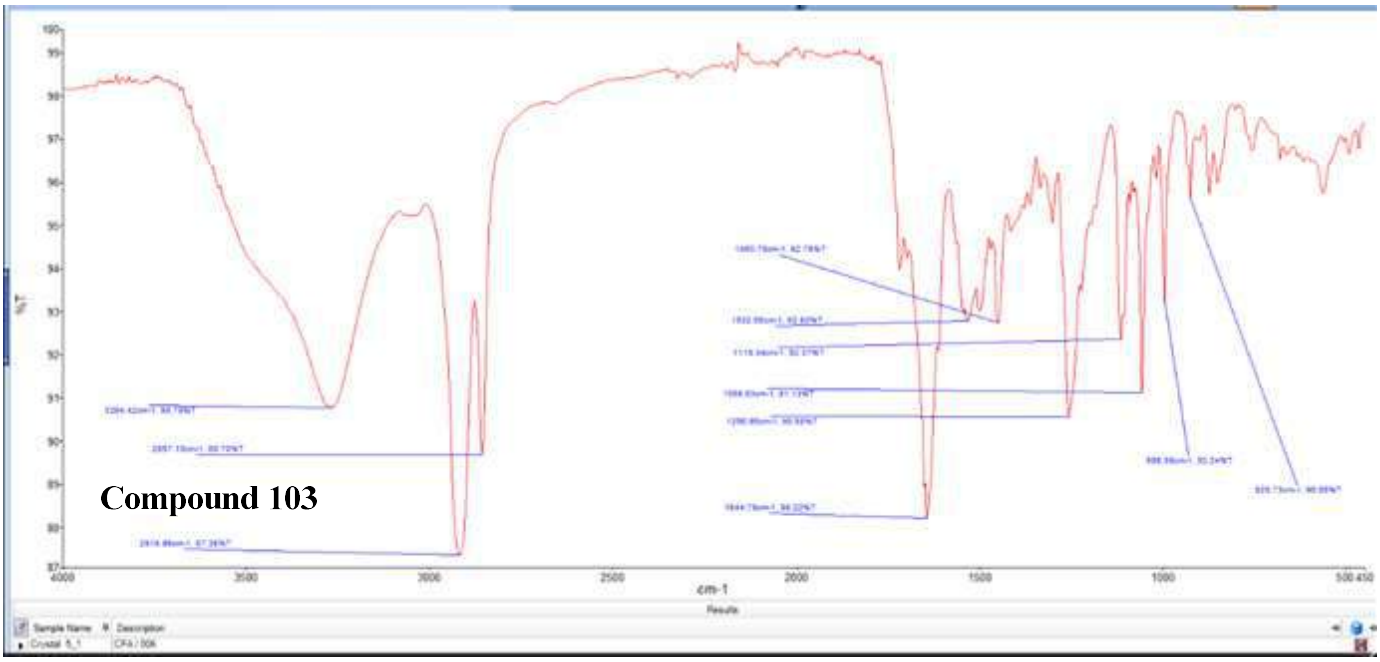
109		
110		

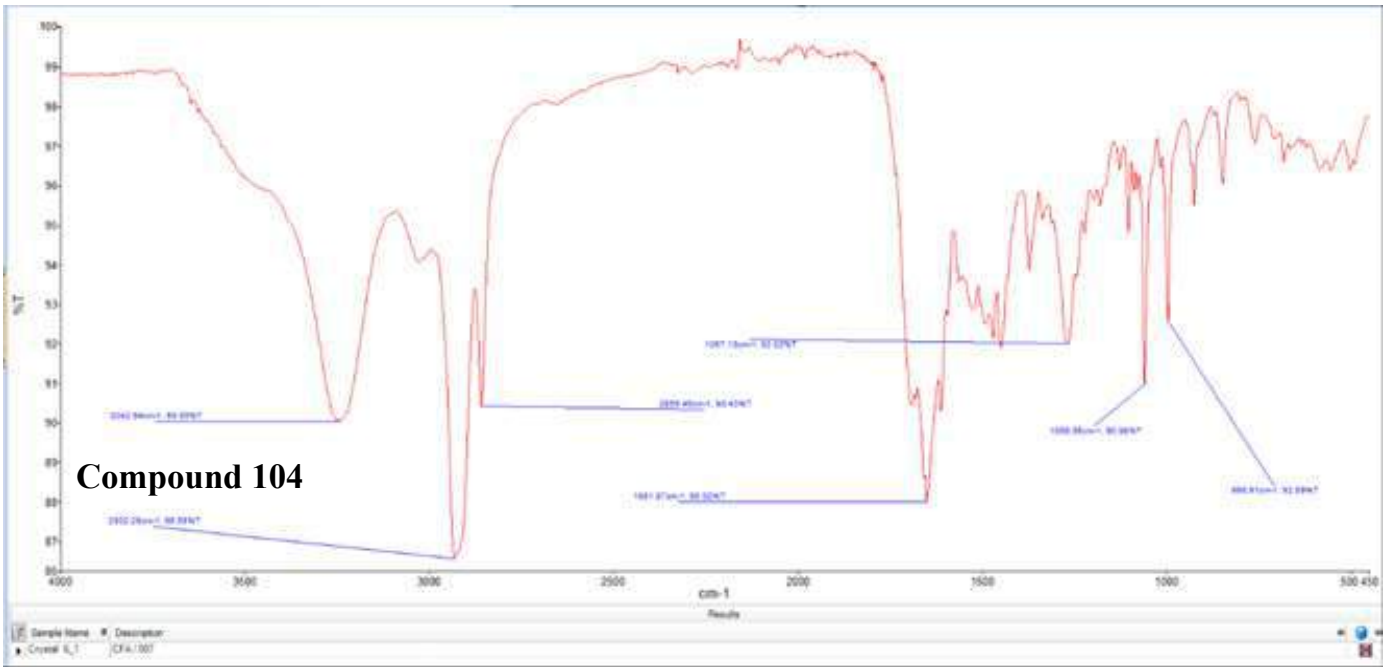
Appendix 2: IR Spectra

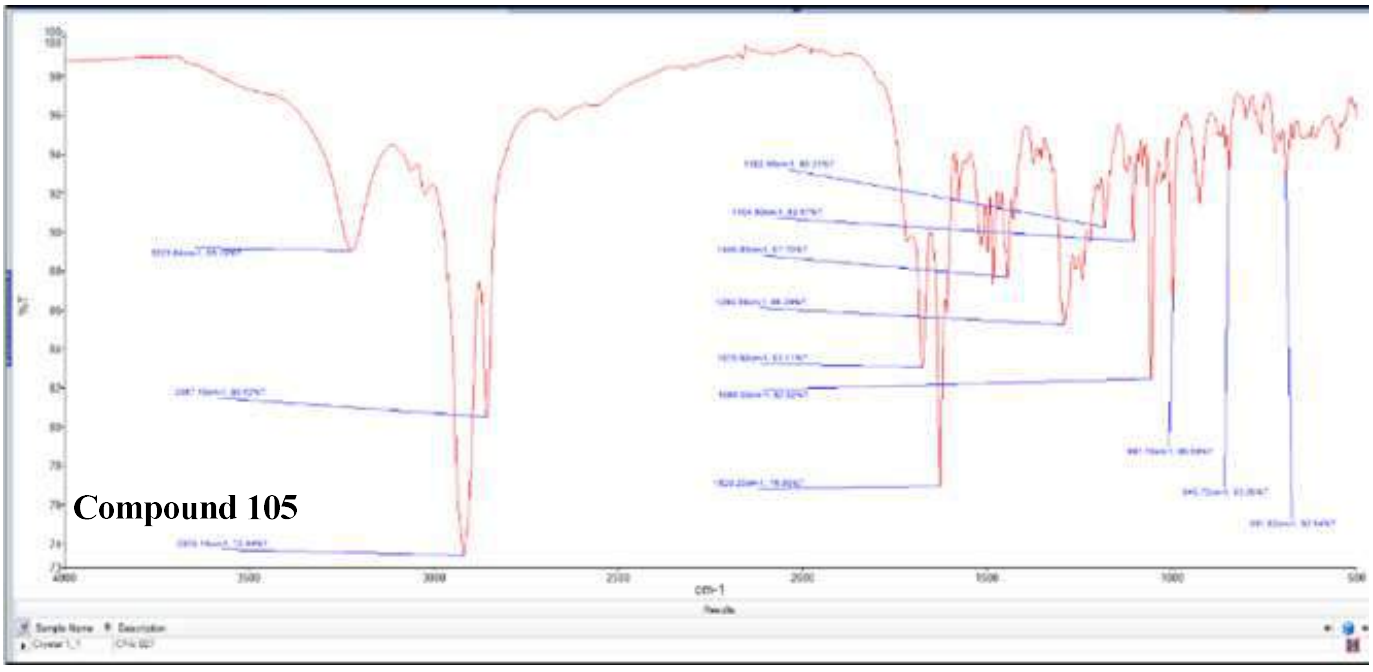


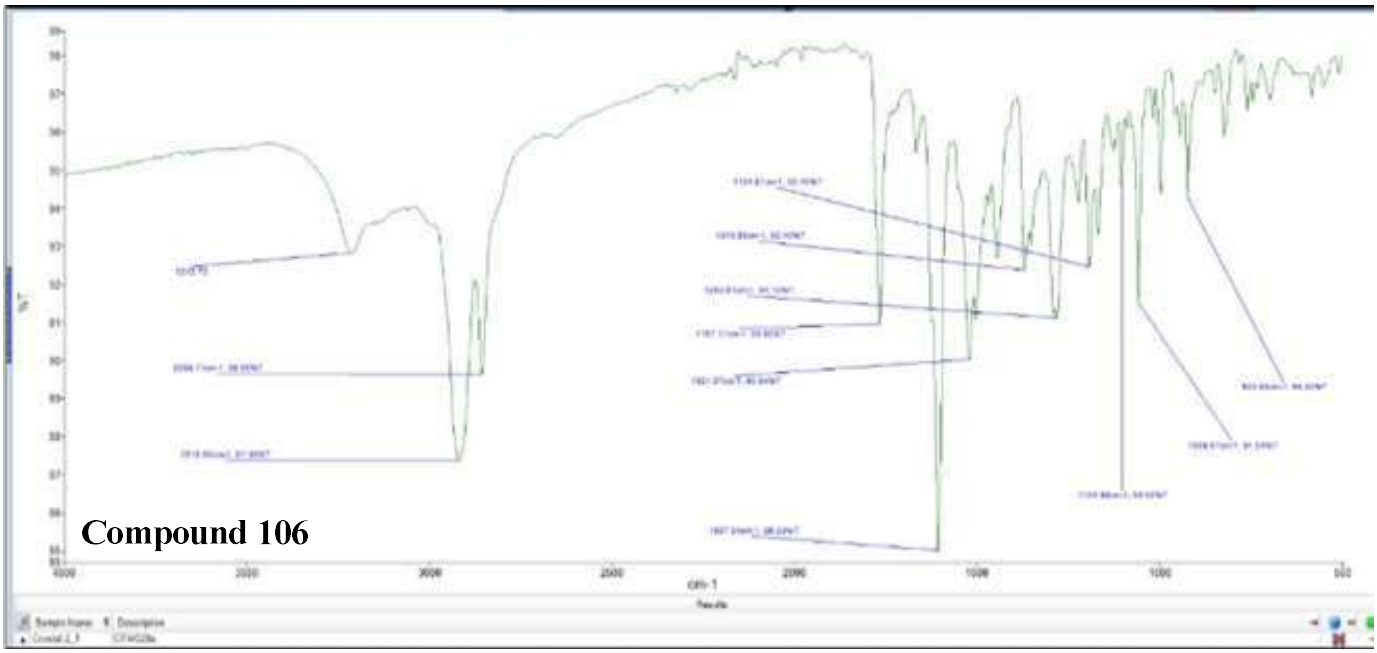


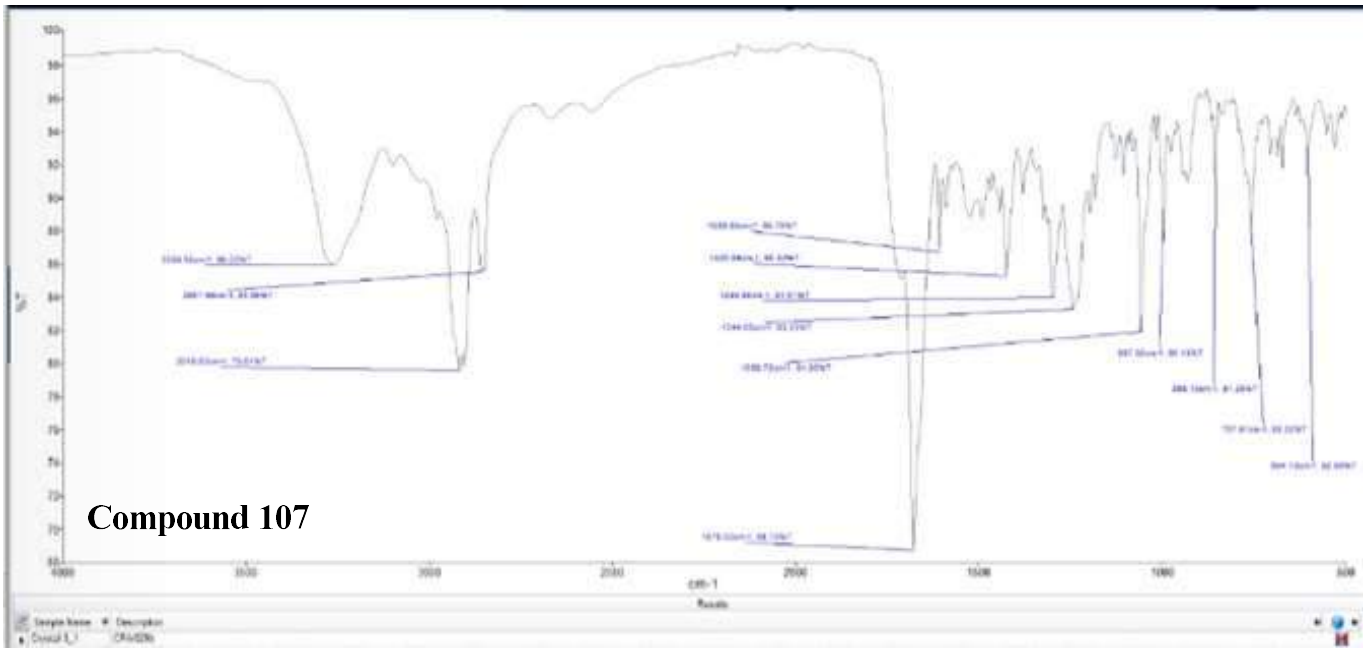


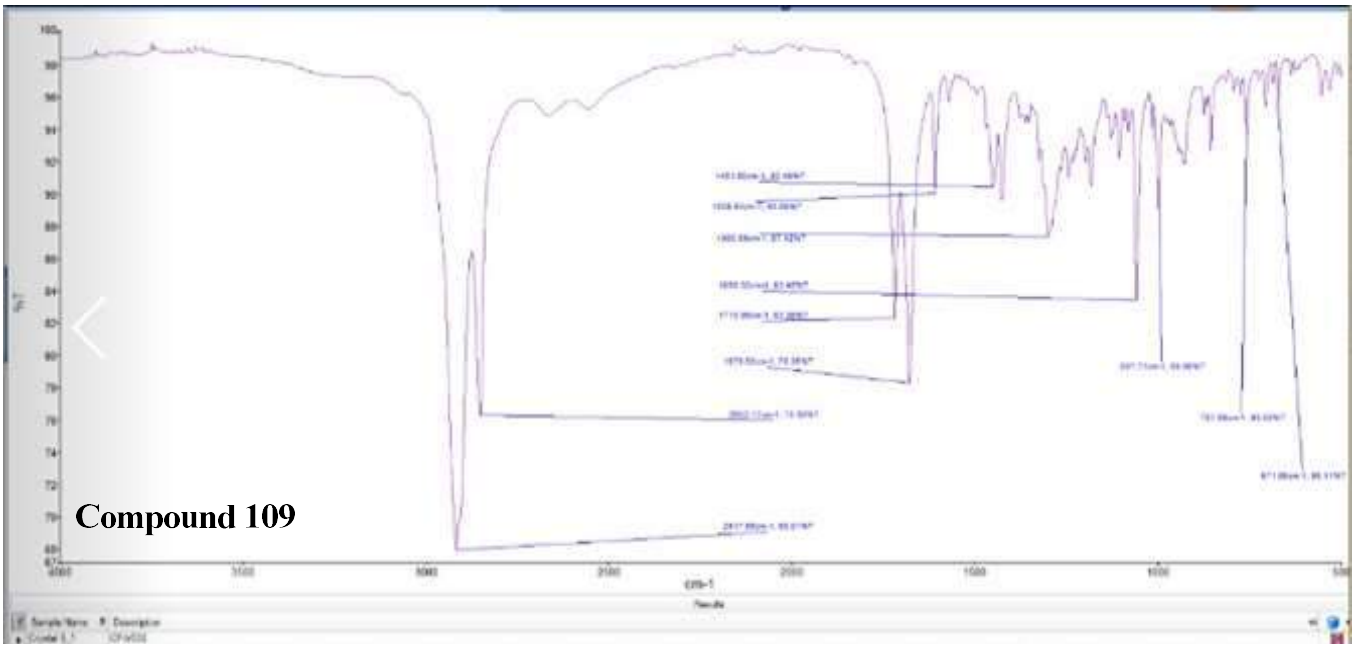


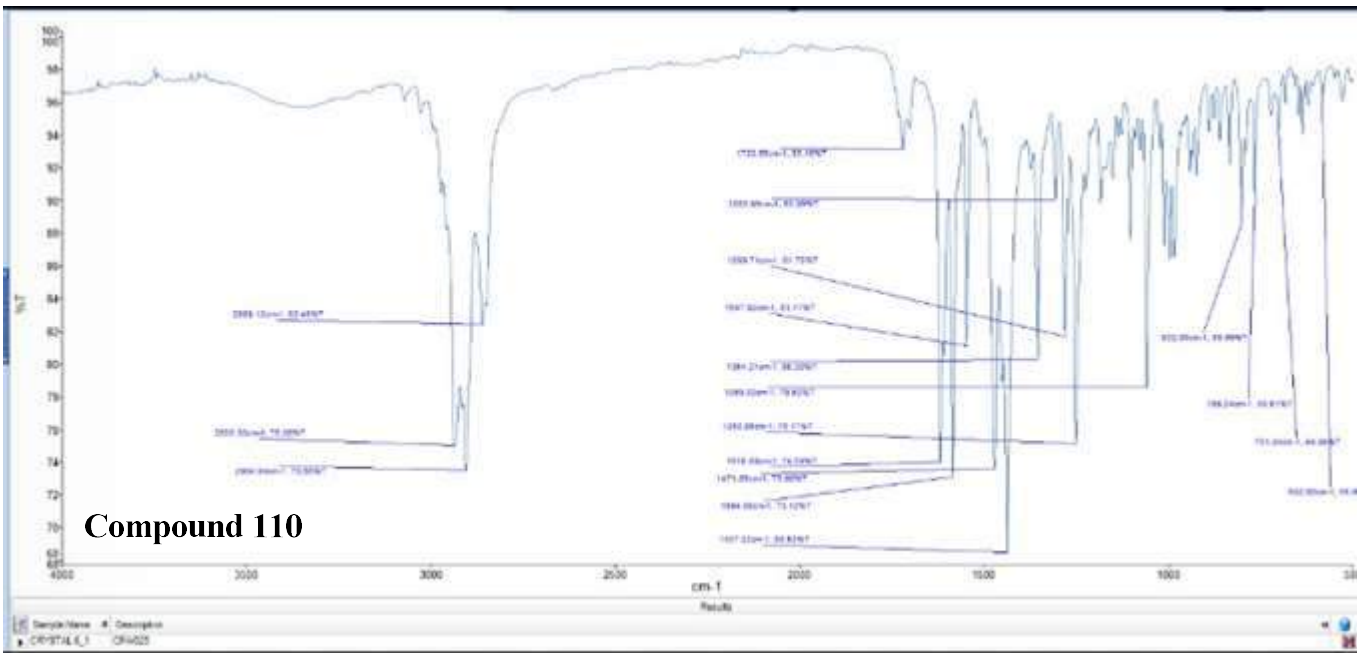




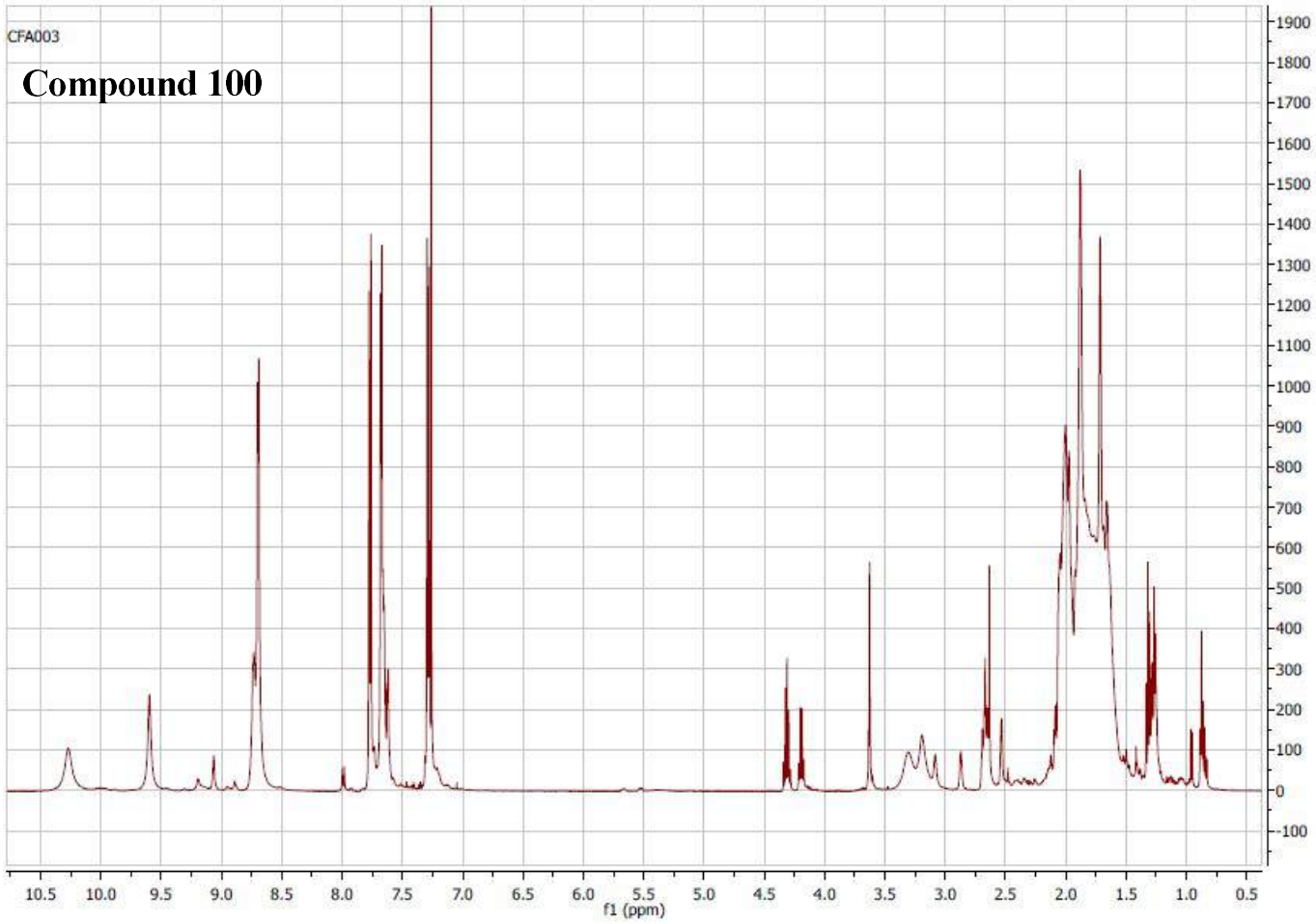


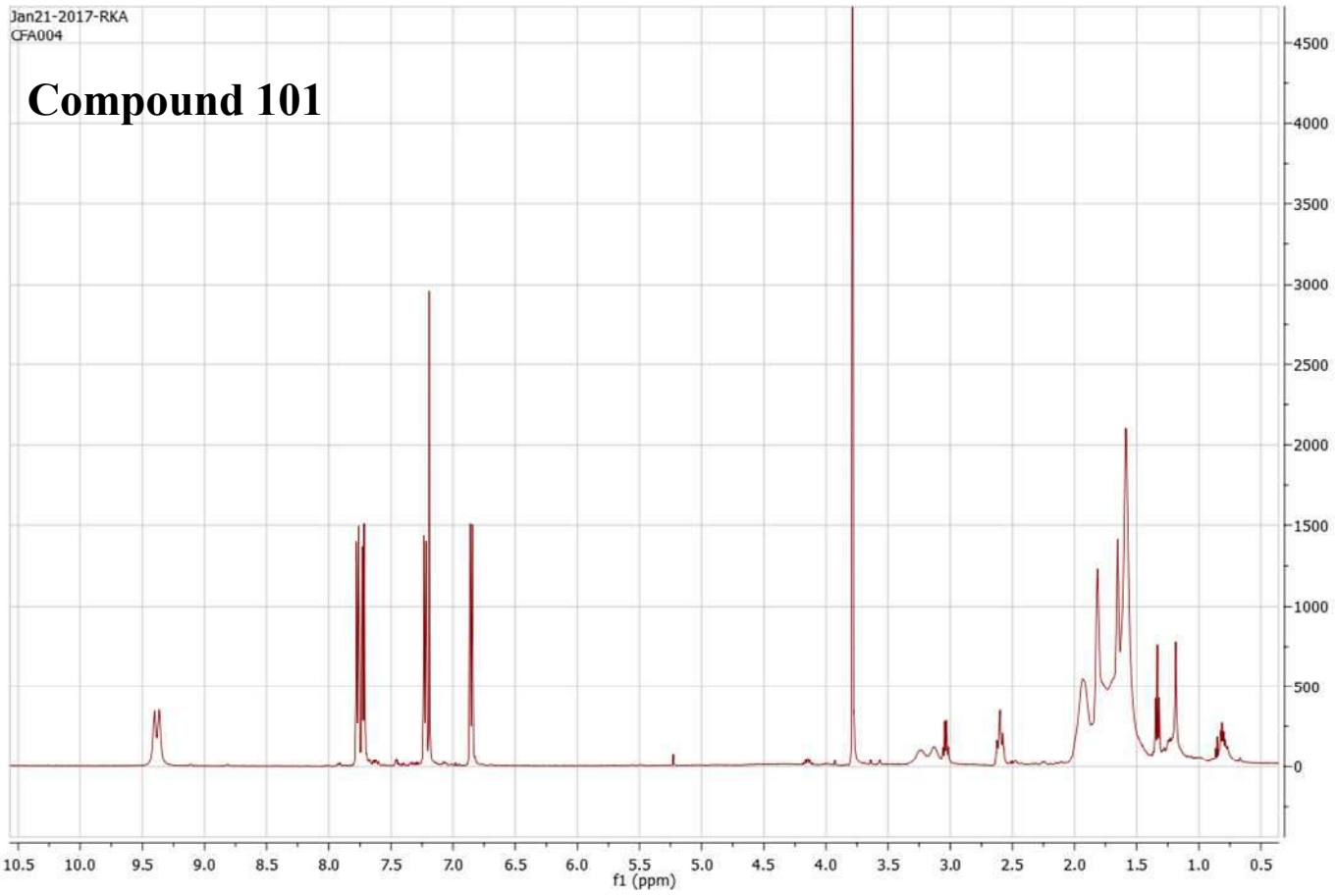


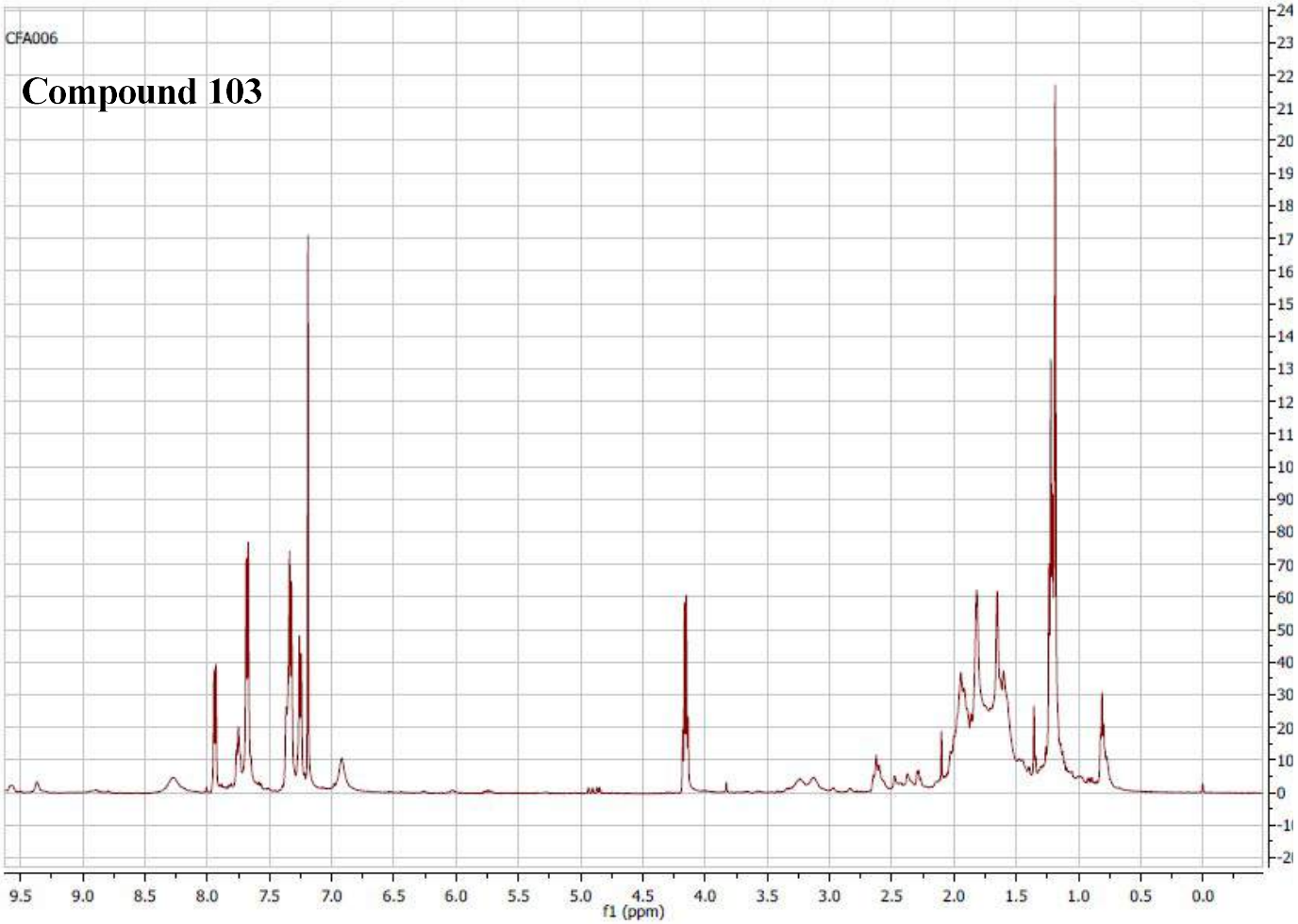


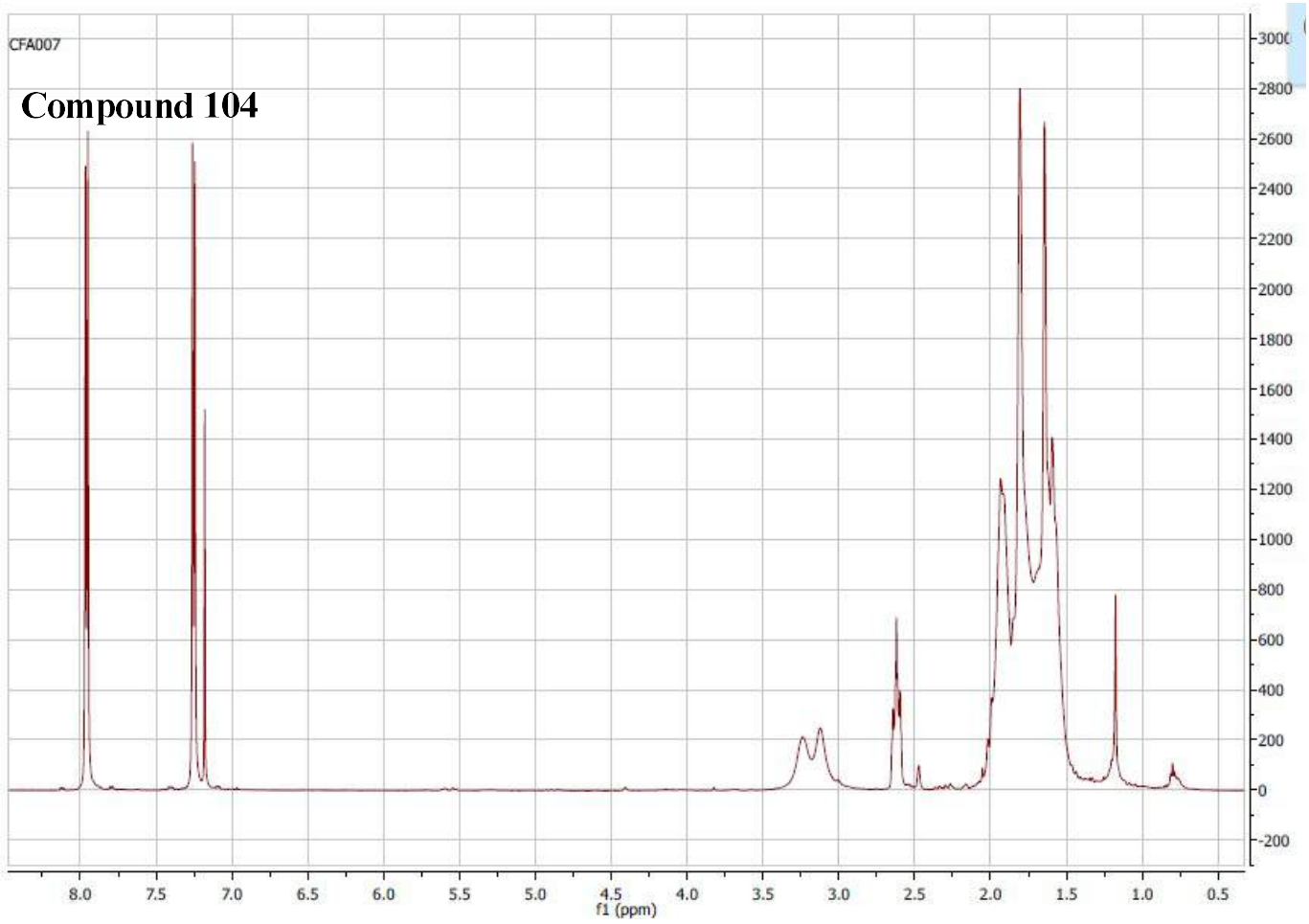


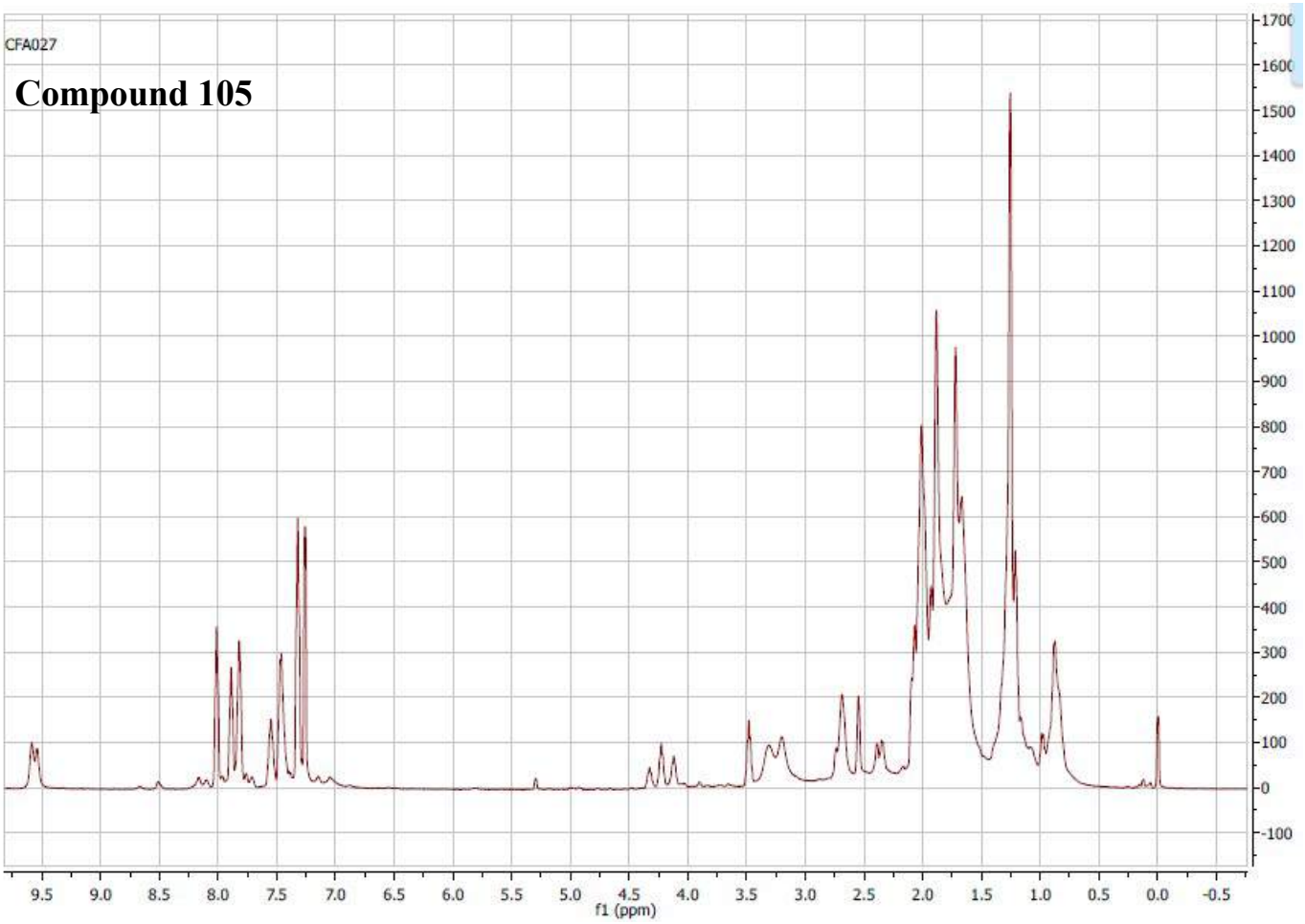
Appendix 3: ^{1}H NMR Spectra

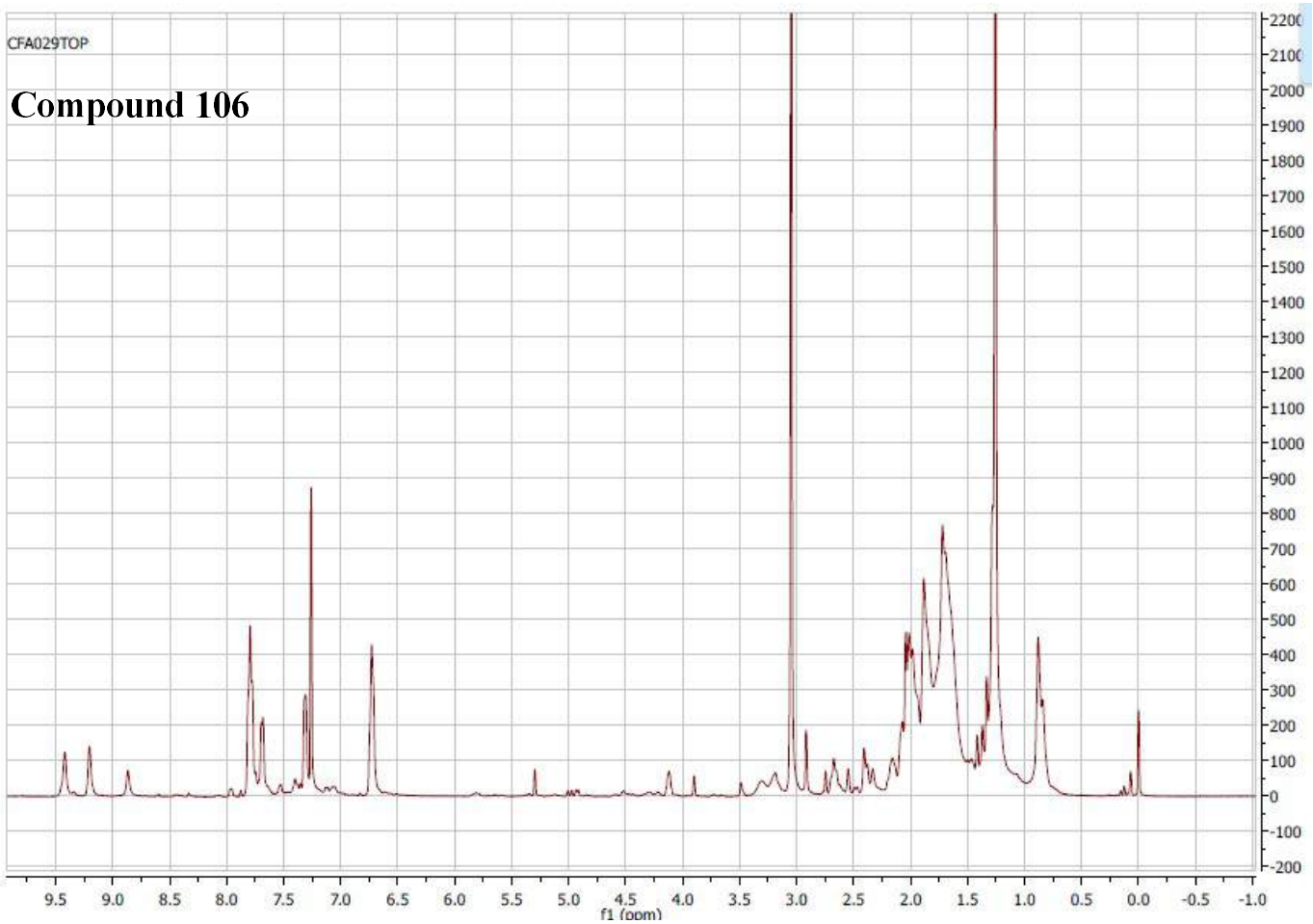


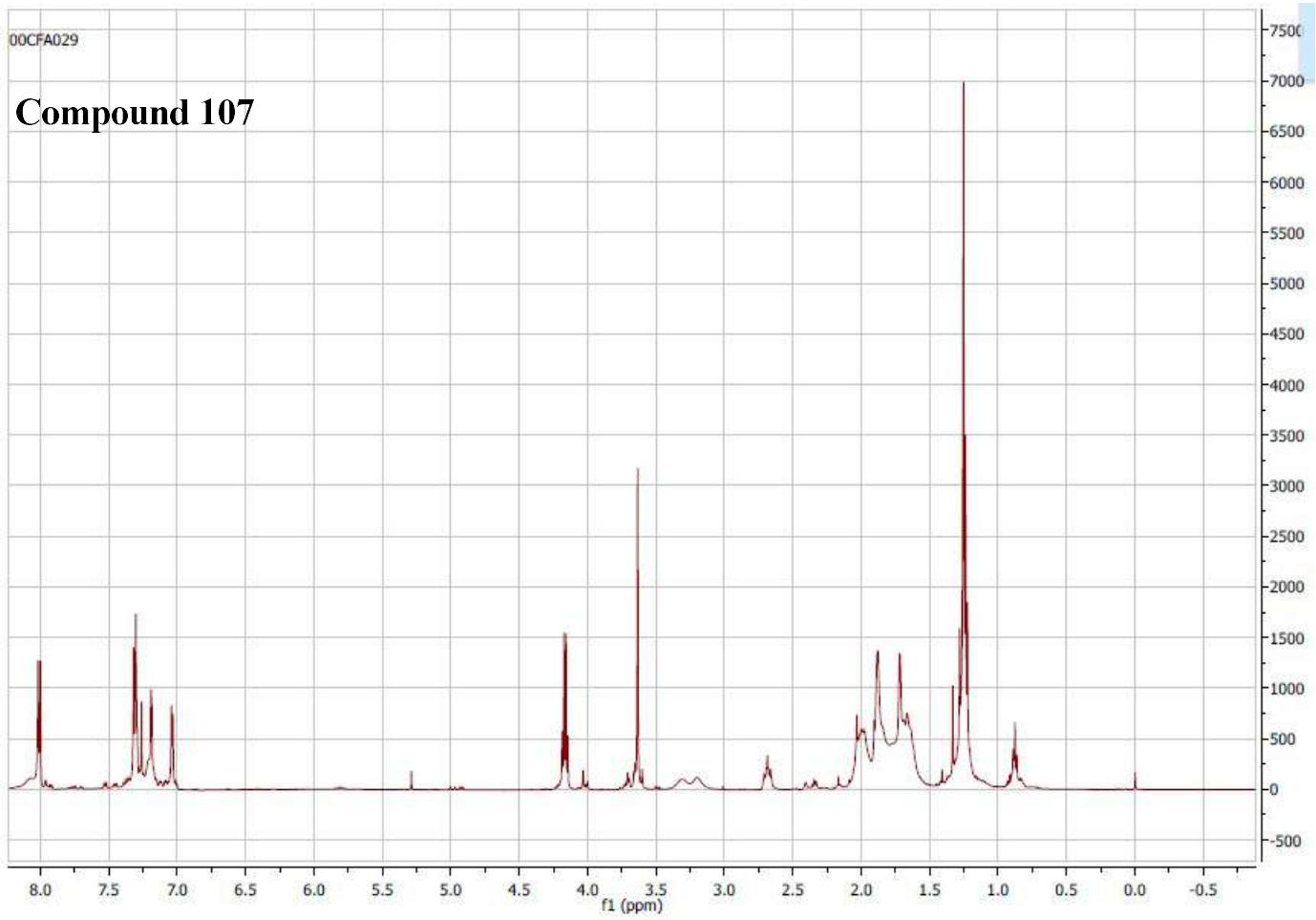


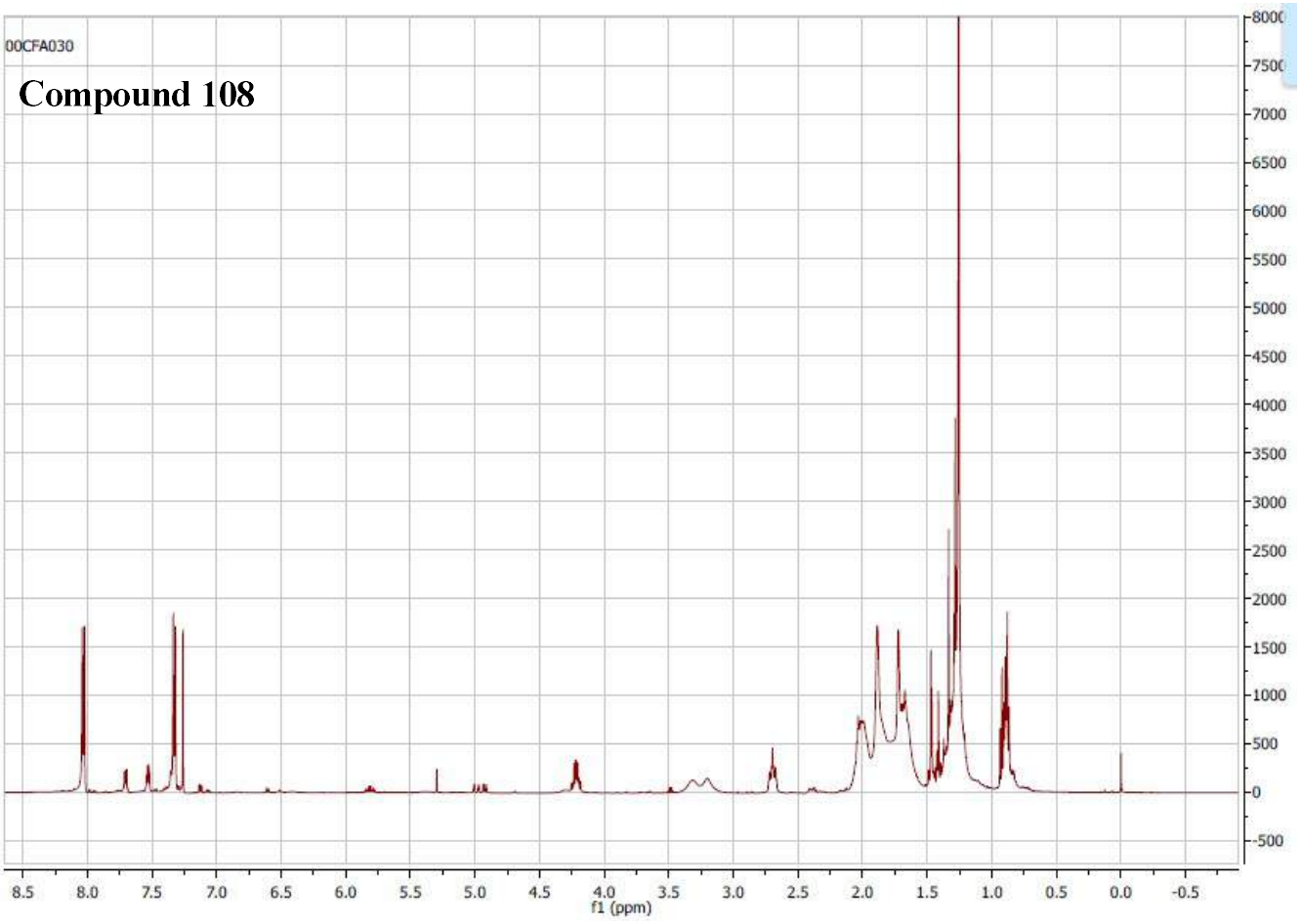


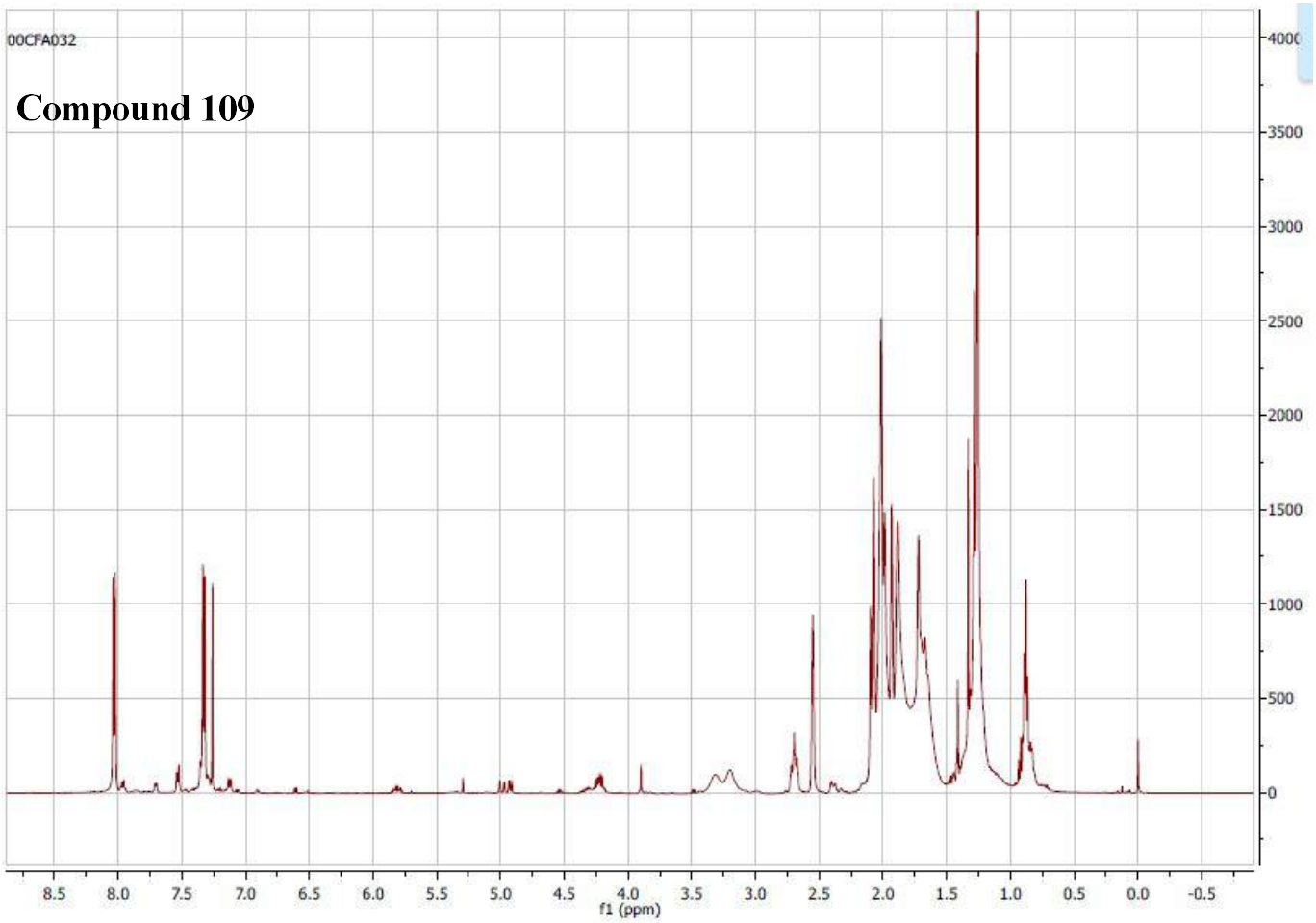


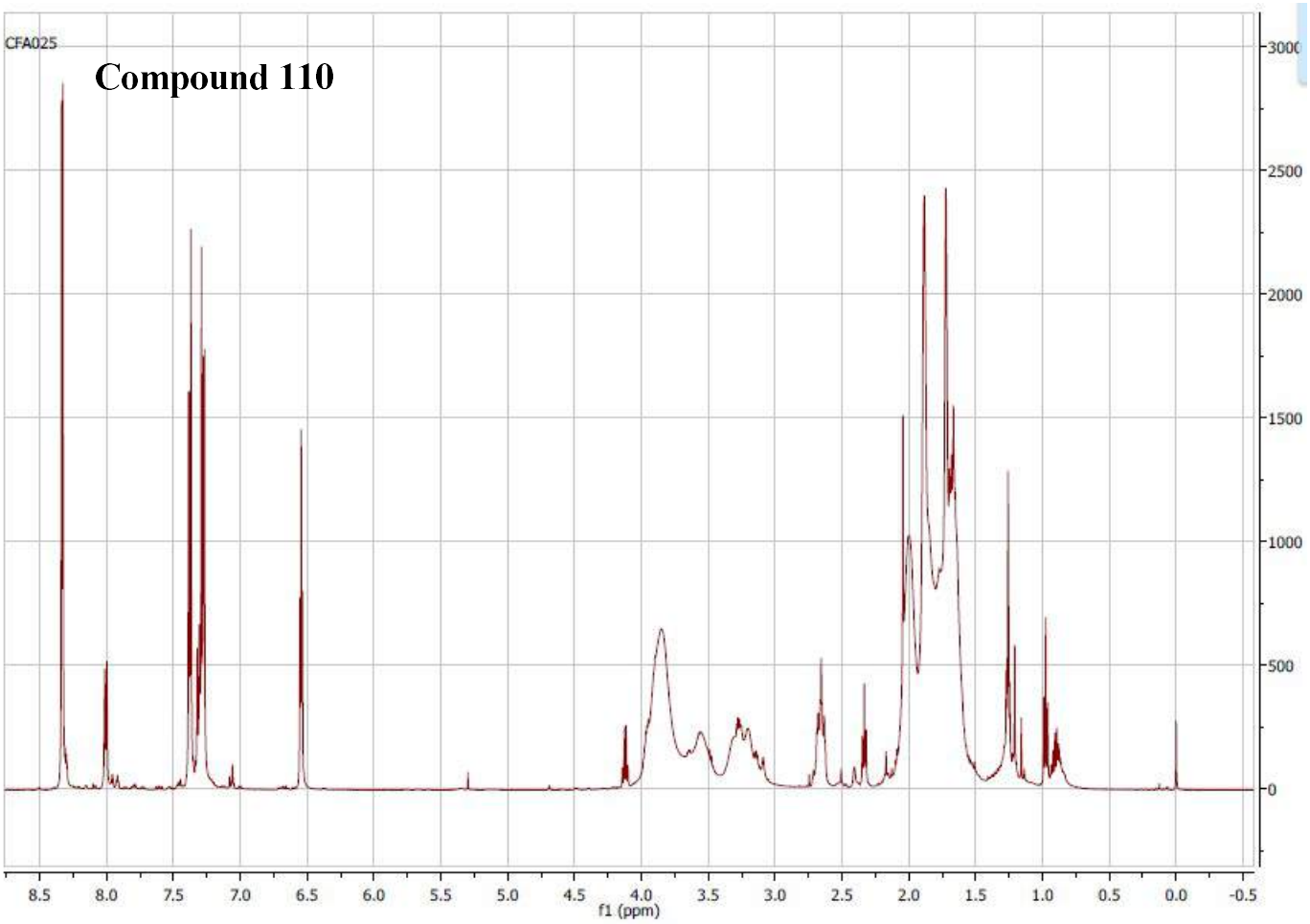




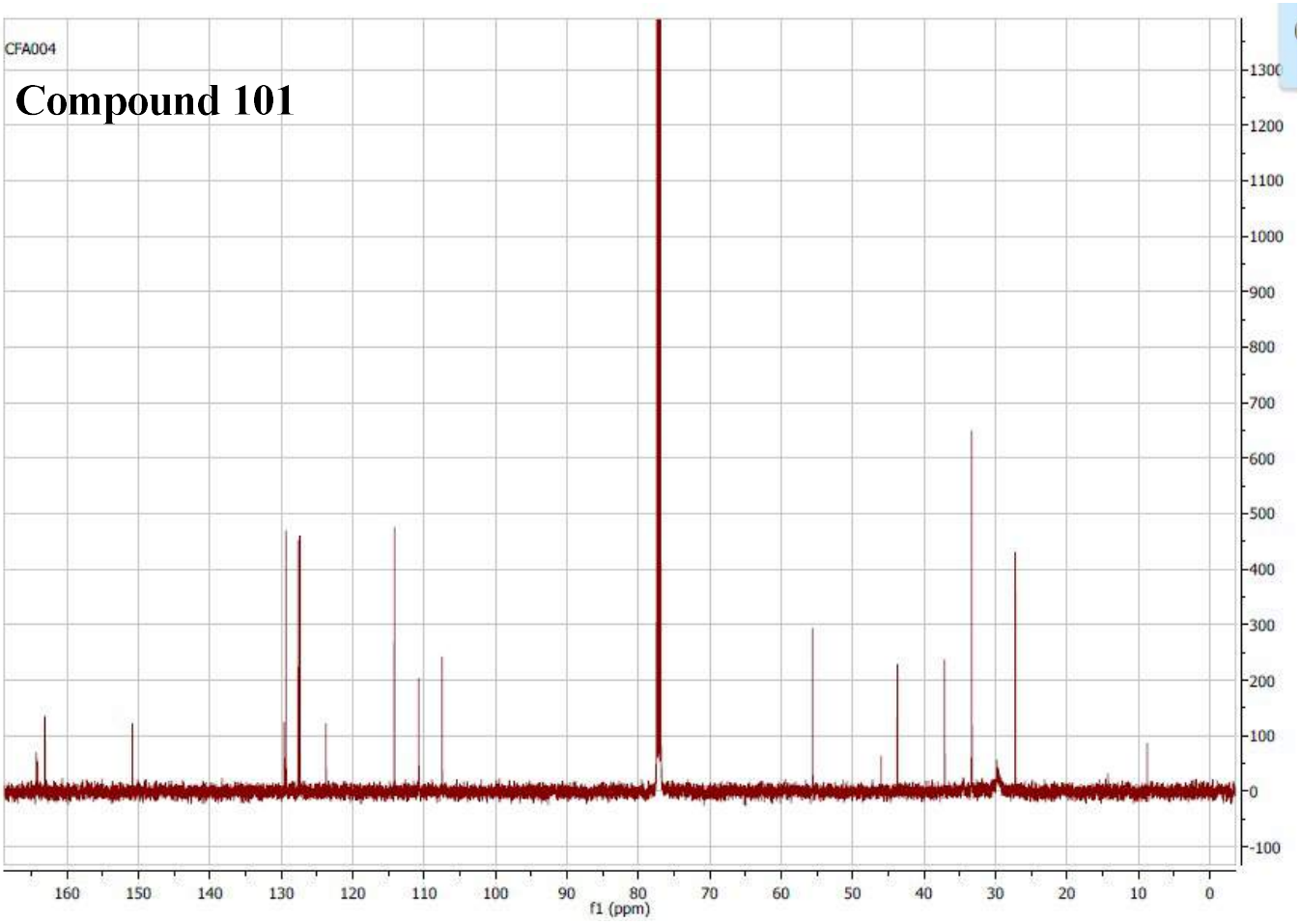


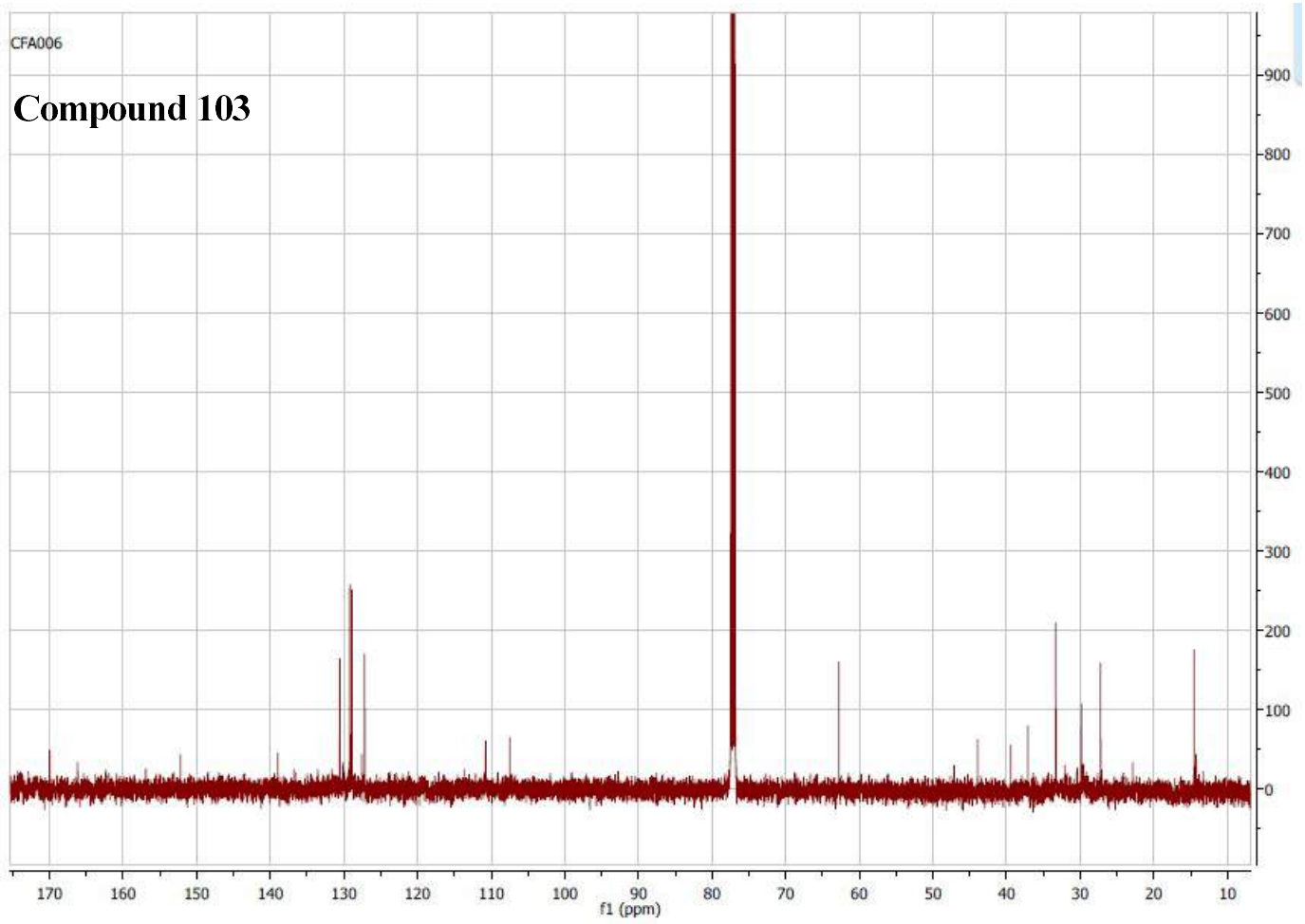


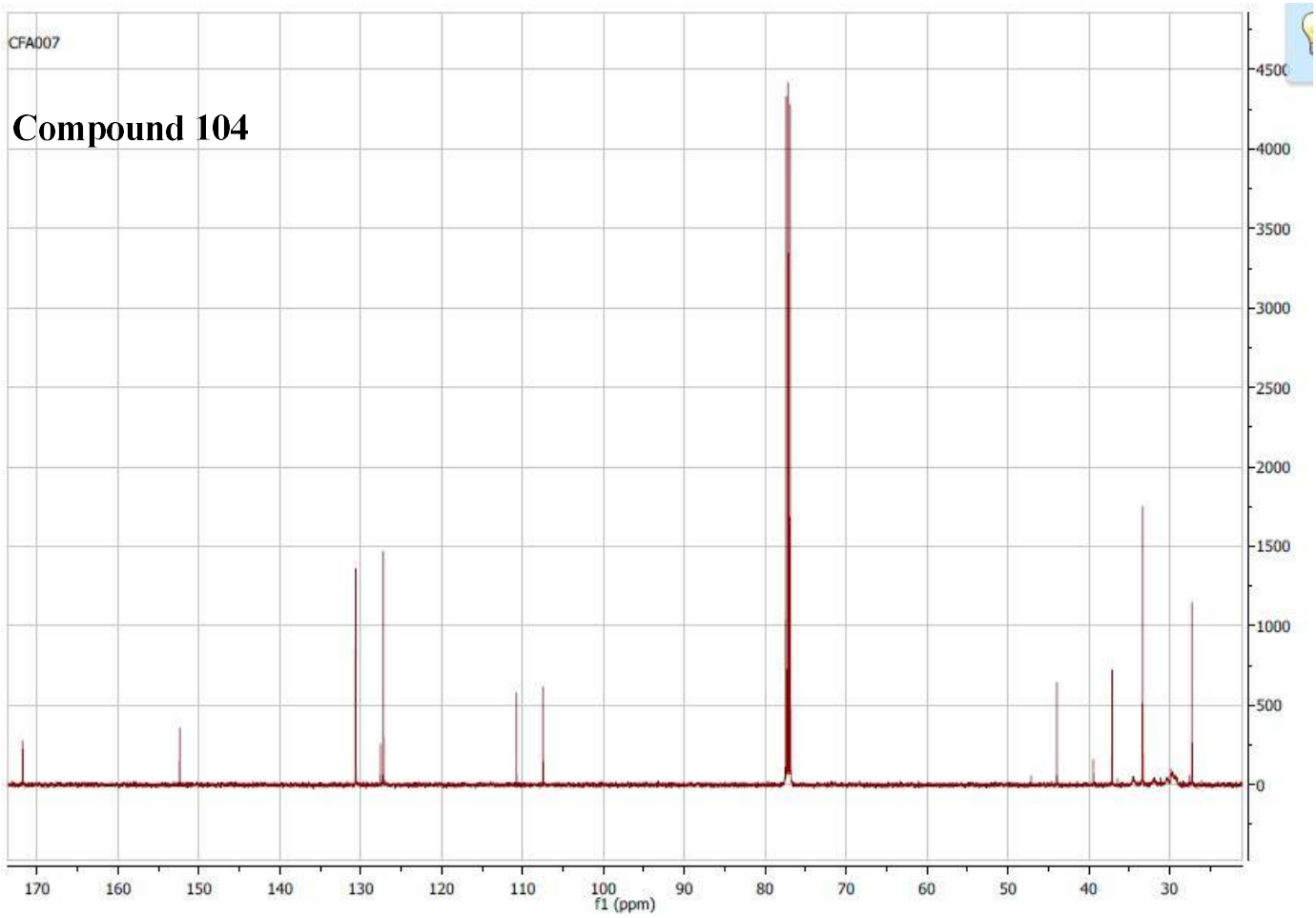


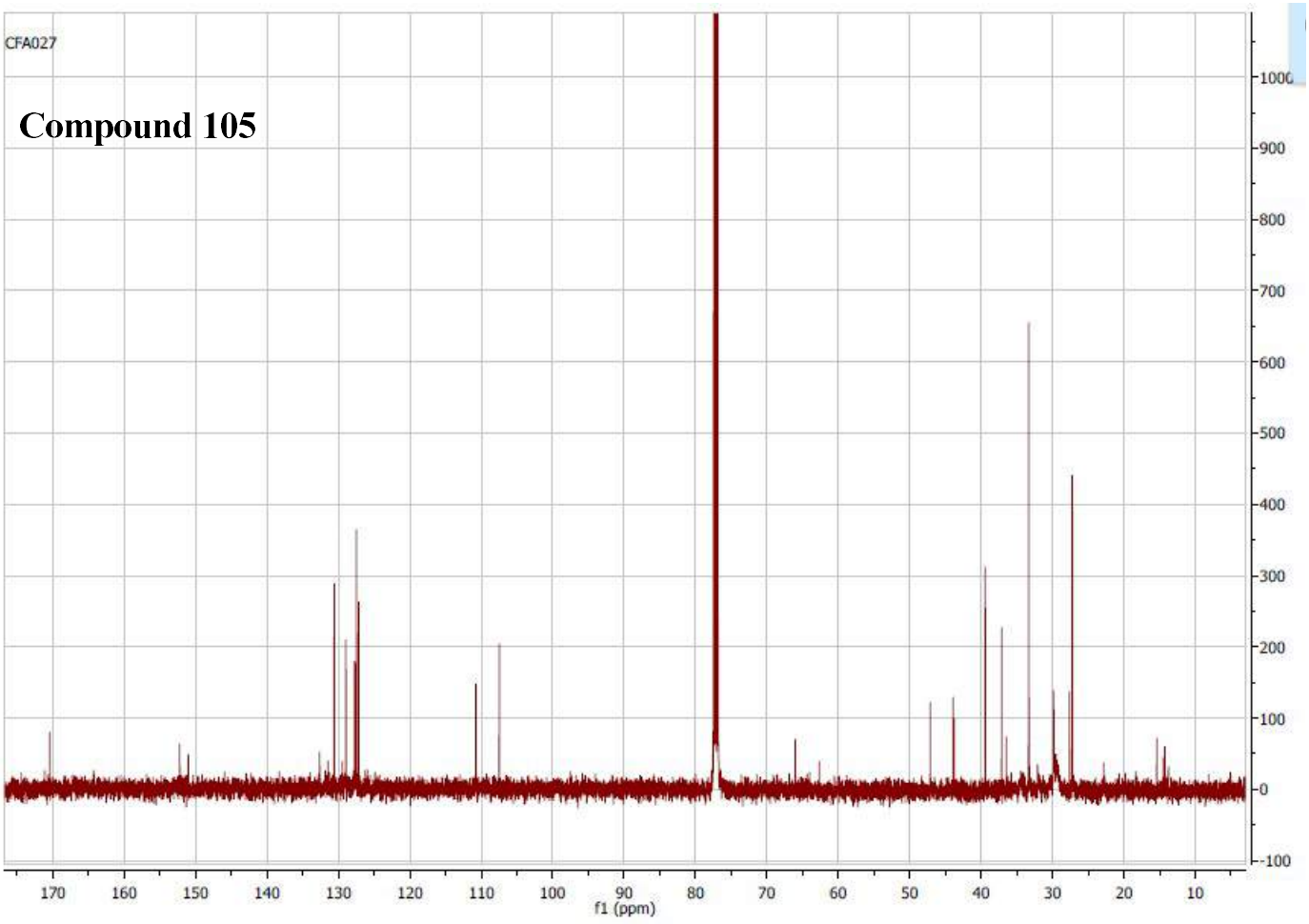


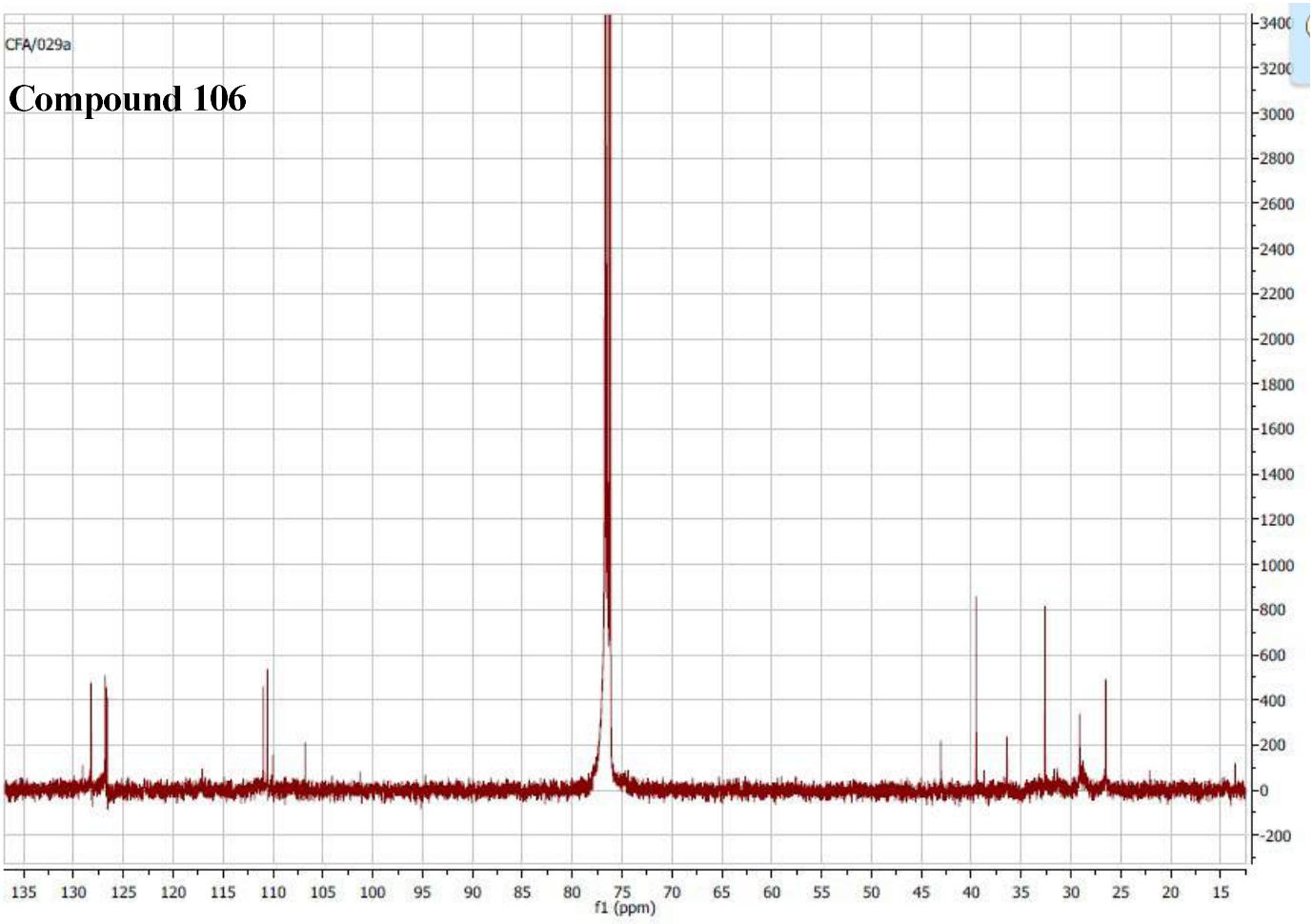
Appendix 4: ^{13}C NMR Spectra

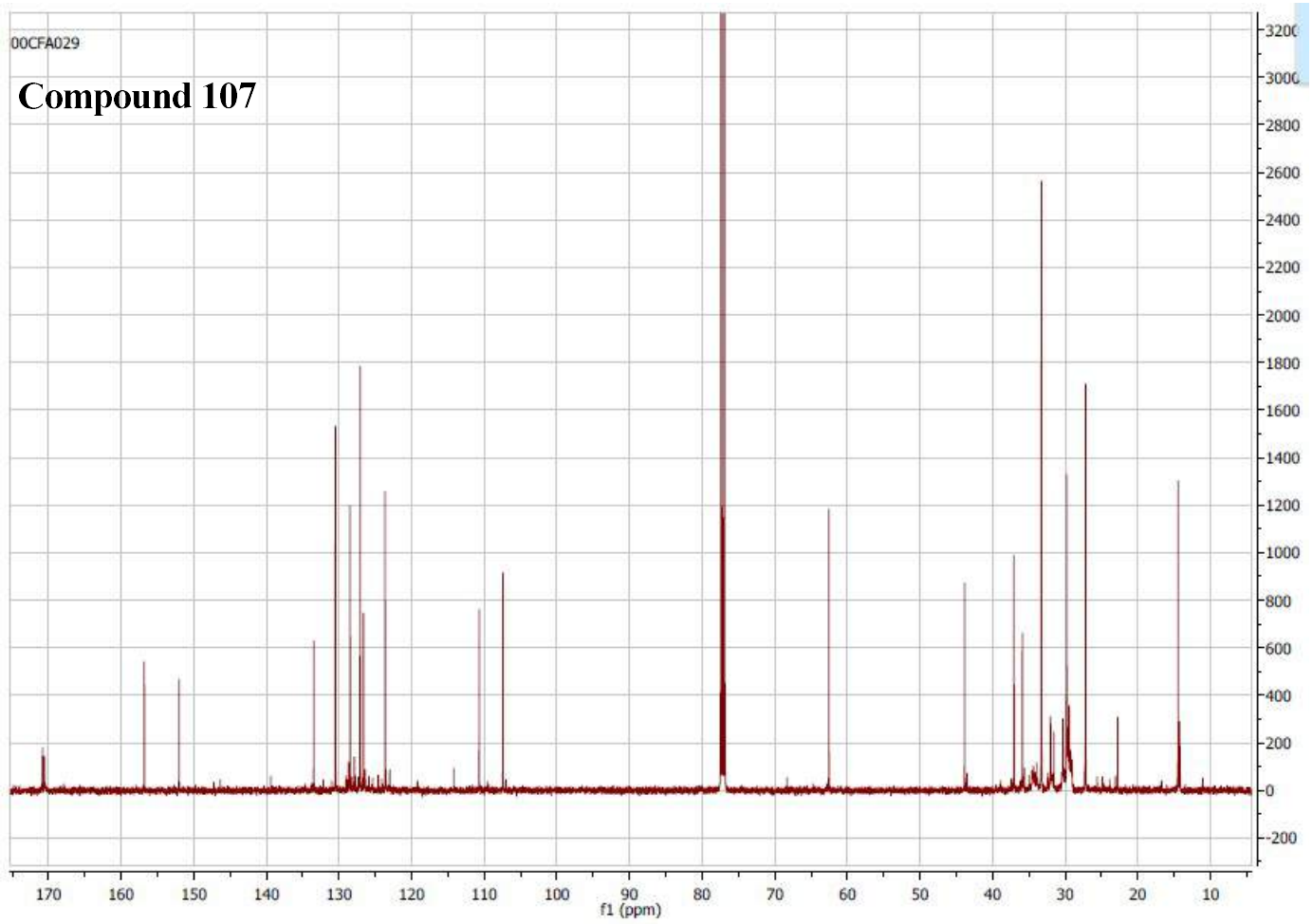


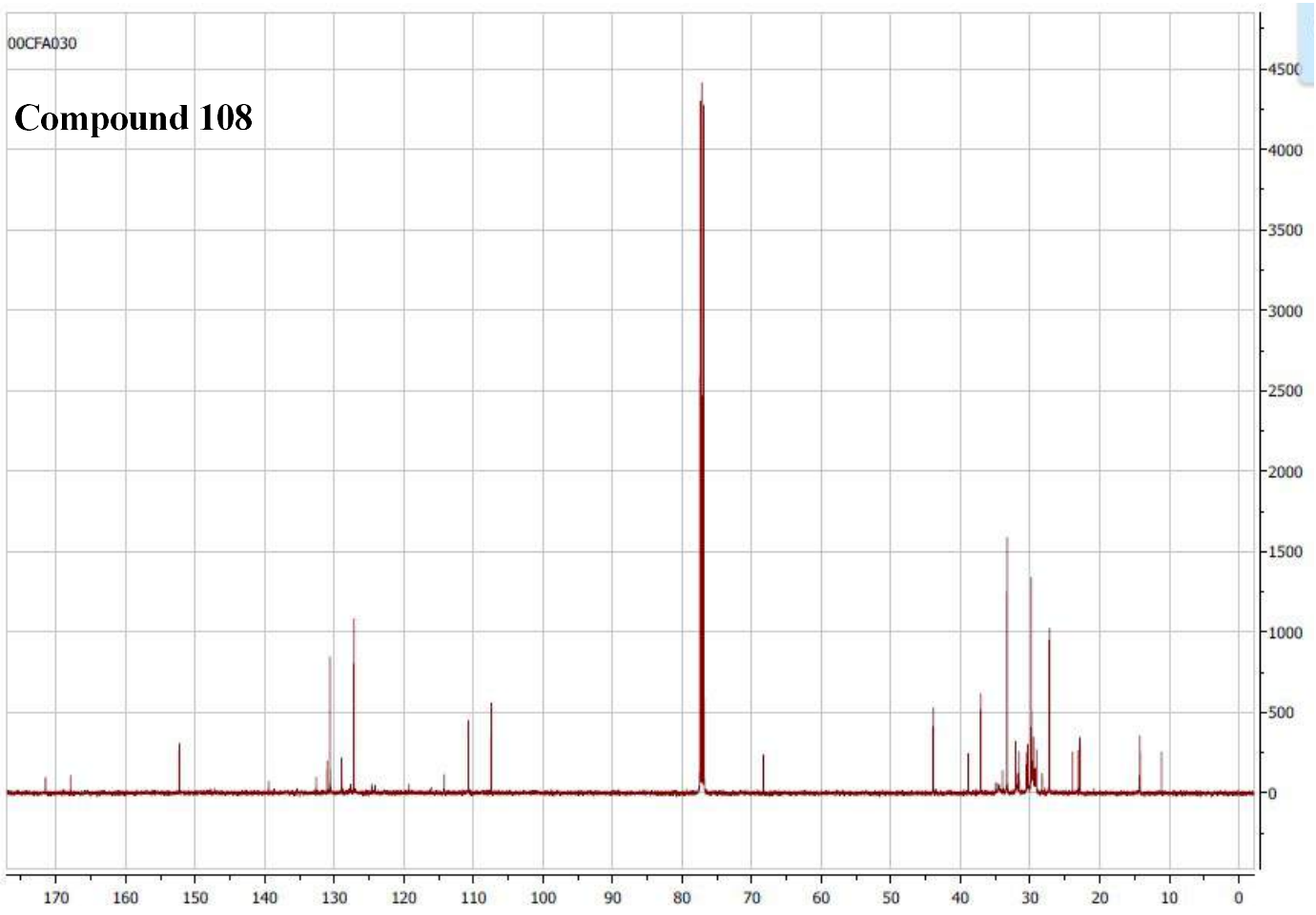


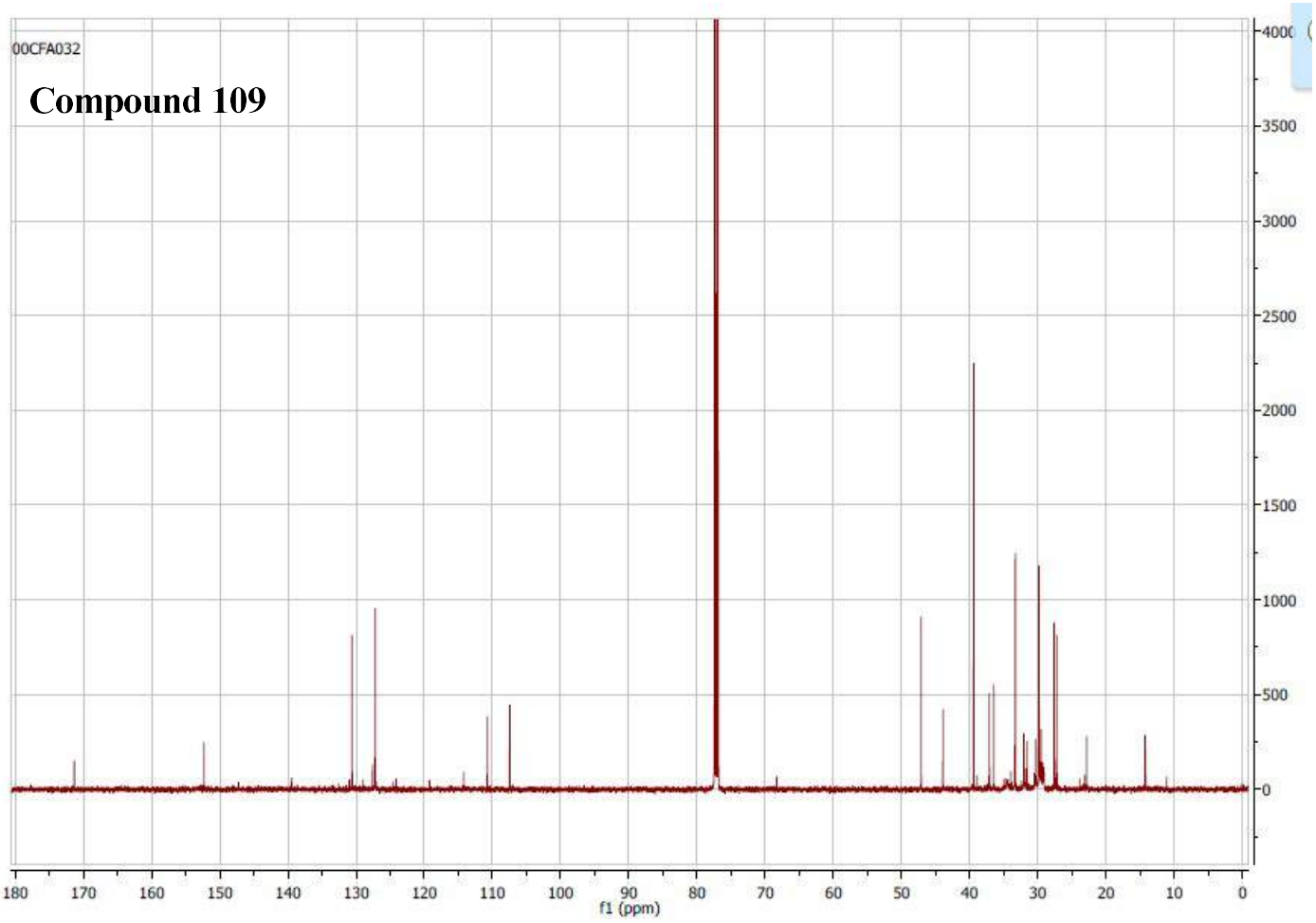


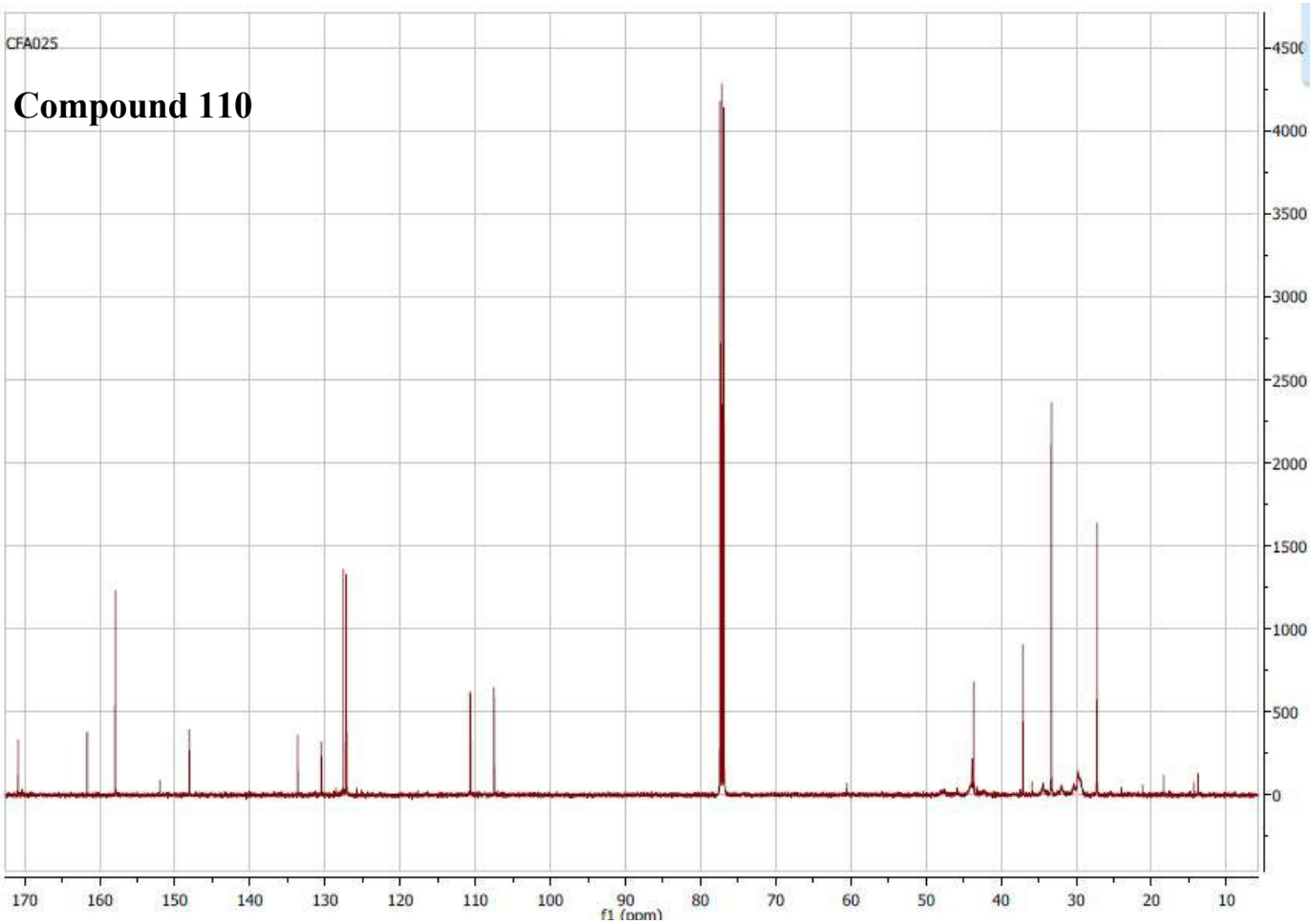












Appendix 5: Drug Susceptibility Testing (DST) plates

(Courtesy Bacteriology Department NMIMR)