UNIVERSITY OF NAIROBI

PHYTOCHEMICAL INVESTIGATION OF TEPHROSIA RHODESICA AND TEPHROSIA POLYPHYLLA FOR ANTIPLASMODIAL PRINCIPLES

BY

MACH PAUL ACHUOTH

(I56/89425/2016)

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN CHEMISTRY AT THE UNIVERSITY OF NAIROBI

2019

DECLARATION

This thesis is composed of my original work and contains no material previously published or written by another person except where due reference has been made in the text. It has not been submitted to qualify for the award of any other degree or diploma in any university or other tertiary institution. The research was carried out in the Department of Chemistry of the University of Nairobi.

……..

Mach Paul Achuoth (BSc)

(I56/89425/2016)

This thesis has been submitted for examination with our approval as University supervisors.

Prof. Abiy Yenesew …….

Department of Chemistry

University of Nairobi

Prof. Jacques M. Kabaru ……

School of BiologiacalSciences

University of Nairobi

DEDICATION

In honor of my father Achuoth Mach Achuoth (Palek) and my late mother Apiu Jok Agwang (Ciir) for having taught me how to value the little I have. I thank my wives, Akweth Garang Golong (Koch) and Ajith Kuol Ngong (Gwala) for bearing all responsibilities of raising children in my absence. My children: Ajoh, Mabil, Thon, Apiu, Golong and Athou, you have sacrificed all the happiness and fatherly love to let me complete this project. My siblings: Lou, Kuot, Akur, Achol and Mathiang for supporting me when I was away during this study.

ACKNOWLEDGEMENTS

I am profoundly grateful to my supervisors, Prof. Abiy Yenesew and Prof. Jacques M. Kabaru for continues support during my MSc study and research. Prof. Abiy, this space is not enough to thank you. It is a long way from the day you introduced me to NMR spectroscopy, choosing the plant for phytochemistry during the initial stage of the project, guiding me through series of columns, inspiration and exposure.

My sincere thank goes to Prof. Mate Erdelyi, my host at Uppsala University, Sweden, along with his research group. The three months I spent in your lab with hands-on analytical machines and interpretation of various spectroscopic data was the propeller in this study. I can't find suitable adjectives to describe the generosity extended by Dr. Matthias Heydenreich and his lab co-workers for high resolution NMR analyses of my samples. I would like also to thank Dr. Hosea Akala of the Kenya Medical Research Institute (KEMRI) for conducting the antiplasmodial assay. Besides my supervisors, I would like to thank Dr. Albert Ndakala for teaching me the principles of organic synthesis and timely advice throughout my research, Dr. Solomon Derese for introducing me to the field of Natural Products Chemistry. I have benefited a lot from Dr. Yoseph Atilaw; you have been my "third" supervisor. I owe you a debt in ChemDraw, MestReNova and NMR data interpretation

I am grateful to the academic staff and technical staff of the Department of Chemistry, University of Nairobi for the assistance rendered to me during my study. The staff of the School of Physical Sciences and Graduate School, the University of Nairobi, you make this institution a regional university with a world class status; I have felt at home. I should not forget my colleagues in a research group, including undergraduate, MSc and PhD students' working in various laboratories in the department for stimulations, discussions and sharing of knowledge.

My study in the University of Nairobi would have been impossible without the financial support of the German Academic Exchange Service (DAAD) for my MSc scholarship which was awarded through In-country/In-region scheme. I thank Mr. Patrick Mutiso of

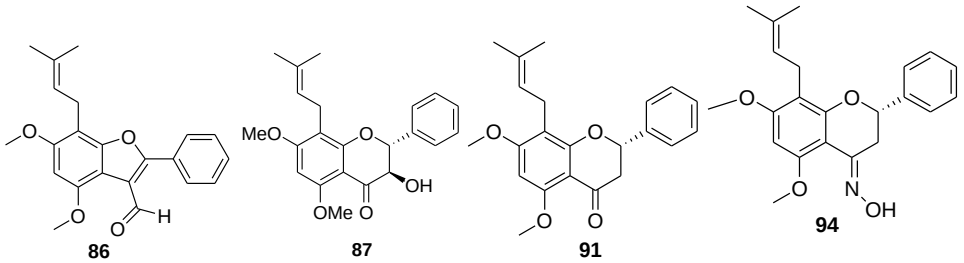
the School of Biological Sciences, University of Nairobi for helping in collection and identification of plants used in this research project.

A special group of people is not yet mentioned, you sincerely deserve some space here. My mentors: Prof. Robert M. K. Deng and Assoc. Prof. Majok Kelei Deng. You did not only inculcate the love of chemistry but also bring me closer to academia to fit your shoes, thank you for everything. The entire community of Dr. John Garang Memorial University of Science and Technology, VC, D/VCs, Deans, staff, students, cooks, cleaners, my fellow former or still teaching assistants (TAs), We are removing that TA "Jalabiya" thank you for supporting me in a way. I acknowledged, my special two friends who sacrificed their time and resources to make sure that I have an admission letter of the University of Nairobi, Mr. Mamer Thok Deng, my childhood friend and Mr. Deng Akau Wel, thank you bothers. Some brothers, you have been asking about my progress, thank you all. My other families: Clergy and congregation of St. Mark, Panapet Parish; St. Andrews Zimmerman; Board of Directors, staff and students of Alliance High School -Bor, thank you for your continued prayers. The family and friends in Diaspora, I thank relatives who hosted me at their residences under guidance of Nyandeng Awai Ajang. Palek Anyidi's crew in Nairobi: You know yourselves, your presence around me created the atmosphere of a home within Kenya.

I finish with Bor, the bottom of my energy pyramid: my family. I have an amazing family with unique structure; I love it, the way it is. You gave me invaluable support throughout my study; you gave up everything for this course to support me, thank you.

ABSTRACT

The increasing problem of drug resistance of malaria parasites, adverse side effects and the question of affordability to the inhabitants of developing countries, call for different interventions including search for new antiplasmodial lead compounds. In this regard, higher plants remain a reservoir of secondary metabolites with antiplasmodial properties. It is in this context that the present research was set up to investigate *Tephrosia rhodesica* Baker and *Tephrosia polyphylla* (Chiov.) Gillet for antiplasmodial agents against *Plasmodium falciparum* which is the major cause of malaria in humans. The roots and seed pods of *Tephrosia rhodesica* and stems of *Tephrosia polyphylla* were dried, ground and then extraction was done using dichloromethane to methanol in the ratio of 1:1 at 24 ^{o}C. The crude extracts were loaded to column and various methods of purification applied like preparative TLC, crystallization, HPLC and circular chromatography. From these two *Tephrosia* species ten compounds, including two new compounds were isolated. Six compounds from roots of *T. rhodesica* including one new compound, named rhodbenzofuran (**86**) were obtained. From the seedpods of *T. rhodesica*, a new compound, named rhodflavononol (**87**) was isolated together with a known compound and two known compounds were isolated from stems of *T. polyphylla*. A synthetic oxime, named candidone-oxime (**94**), was also prepared from candidone (**91**). Isolated compounds were characterized using various methods such as 1D-NMR (^{1}H-NMR and ^{13}C-NMR), 2D-NMR (HH COSY, NOESY,TOCSY, HMBC and HSQC), X-ray Crystallography, UV, CD and mass spectrometry. The crude extracts and compounds isolated from them were tested for antiplasmodial activities against different strains of *P. falciparum*, namely chloroquine resistant clone (W2) and chloroquine sensitive clones (3D7 and D6). The tests showed that candidone (**91**) was the most active compound against chloroquine resistance clone W2 (IC_{50} = 1.2±0.1µM) and chloroquine sensitive clone 3D7 (IC_{50} = 3.5µM). Over all, the study has showed that the two *Tephrosia* species elaborate flavonoid derivatives, some of which showing good antiplasmodial activities.



LIST OF ABBREVIATIONS

ACT	Artemisinin-based Combination Therapy
CD	Circular Dichroism
CI	Chemopreventive Index
COSY	Correlation spectroscopy
d	doublet
dd	doublet of doublet
D6	Chloroquine sensitive strain of *Plasmodium falciparum*
HMBC	Heteronuclear Multiple Bond Correlation
HSQC	Heteronuclear Single Quantum Coherence
Hz	Hertz
IC_{50}	50% Inhibition Concentration
MS	Mass Spectrometry
m/z	Mass to charge ratio
NMR	Nuclear Magnetic Resonance
NOESY	Nuclear Overhauster and Exchange Spectroscopy
PTLC	Preparative Thin Layer Chromatography
s	singlet
TLC	Thin Layer Chromatography
UV	Ultra Violet
WHO	World Health Organization
δ	Chemical shift

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OFSCHEMES

CHAPTER 1: INTRODUCTION

1.1 Background Information

Traditional medicine is defined, in general terms, as distinct health practice and knowledge in a particular community involving the use of plants, animals or minerals. This practice is done for the purpose of achieving wellbeing and health of the society by treatment, diagnosis or prevention (Bodeker & Ong, 2005). Statistics showed that 40% and 80% of the populations in China and Africa, repectively, use traditional medicine. The use of traditional medicine is deeply rooted in Africa where accessing modern drugs is a challenge (Chang *et al.,* 1997). The challenge come as a result of poverty and marginalization of some communities, the categories of people affected here are left with little hope on synthetic drugs but to shift to traditional medicine to satisfy their health wellbeing (Cunningham *et al.,* 2008). The rural inhabitants in Kenya are not exceptional to this, even the urban population believe in the potency of traditional medicines, especially in the condition of chronic illness such as HIV/AIDS, hypertension, cancer, peptic ulcers and haemoroids (Kigen *et al.,* 2013). Ethnobotanical studies in Kenya have revealed wide uses of plants in families such as Asteraceae, Euphobiaceae, Lamiaceae, Fabaceae, Caesalphiniaceae, Rubiaceae and Rutaceae. They are used to manage and treat a number of diseases including gastrointestinal, parasitic, metabolic, microbial, reproductive, helminthosis, snake bites, protozoa, cuts, scald, wounds and dental. Parts of the plants used include roots, leaves, flowers, seeds , pods and whole plant extracts (Gakuya *et al*., 2013).

The herbal medicine is prepared in the form of an infusion or decoctions, and administered through chewing, inhalations, smearing at the surface of swollen part.The

above families and other families have been studied and several drugs derived. Some prominent examples are quinine isolated from the bark of *Cinchona* species, a good antimalarial drug which is still in use today; artemisin isolated from aerial parts of *Artemisia annua*, a potent antimalarial drug against the parasite *Plasmodium falciparum*; convallatoxin isolated from flower and leaves of *Convallaria majal* is used as cardiotonic drug; morphine isolated from the latex of immature seedpods of *Papaver somniferum*, used as analgesic; and pyrethrin isolated from flowers of *Chrysanthenum cinnerarifolium* is a widely used insecticide (Rungsung *et al.*, 2015). Over 60 % of the drugs in the market especially the anticancer and antihypertensive were derived directly or indirectly from plants (Wheate *et al.*, 2010). It is in this line that the searches for lead compounds have been intensified including this study.

The vast phytochemical reports in literature have shown that plants act as "Biosynthetic laboratory". Several chemical compounds known as "Secondary metabolites" have been isolated from plants over the years; these compounds give plants their therapeutic properties and physiological behaviors. Secondary metabolites may not play prominent role in day to day life of a plant, instead they represent a major adaptation of plants to their environment and are part of the plant defense mechanisms against pathogens and herbivore attack (Rungsung *et al.*, 2015). They also aid in identification of plants characteristics such as odours, taste and colours, enabling pollination and dispersal (Samanta *et al.*, 2011). The interesting feature of plant secondary metabolites lies in their diverse chemical structures that falls in to three major classes of organic compounds, namely: phenolics, terpenoids and alkaloids (Veberic *et al.*, 2010). *Tephrosia* species are largely known for production of phenolic compounds, especially the flavonoids that are known to be active against a number of illnesses including malaria. A flavonoid isolated from *T. purpurea* showed a good activity when antiplasmodial biological assays was done on D6. It is in this regard that *Tephrosia rhodesica* and *Tephrosia polyphylla* were selected for investigations of active

compounds against the malaria parasite, *Plasmodium falciparum*. The impact of malaria is worrying in the world.

In the year 2015, 214 million cases of malaria were registered of which 438 000 resulted in death mostly (90 %) in Africa (World Health Organization, 2015). Malaria is a parasitic disease caused by infection of *Plasmodia* species. Among the five *Plasmodia* species which cause malaria, the most deadly is *P. falciparum* which is transmitted by the bites of female anopheles mosquito (Trampuz *et al.,* 2003)

1.2 Statement of the Problem

Malaria still kills over 438,000 people annually, most of the victims are pregnant women and children under five years old (World Health Organization, 2015). In South Sudan, complicated cases of malaria represented 62% of inpatient admission in 2018 (World Health Organisation, 2018). As a result of widespread occurrence of the malaria parasites failure to response to the available drugs as observed with the first line drugs including amodiaquine, quinine, sulphadoxine–pyrimethazine are not more effective in the treatment of malaria. (Willcox *et al.,* 2011). Artemisinin-based Combination Therapy (ACT) is the only drug recommended for use to treat uncomplicated malaria (Mutabingwa, 2005); however it is alarming to note that in Cambodia, Thailand and some parts of Africa, resistance has been observed to this combination as well (Dundorp & Nosten, 2009). Besides, the search for vaccines has not been fruitful (Bojang *et al.,* 2001). It is therefore imperative that alternative malaria drug is explored.

1.3 Objectives of the Study

1.3.1 General objective

The main objective of this research was to identify antiplasmodial principles from *Tephrosia rhodesica* and *Tephrosia polyphylla.*

1.3.2 Specific objectives

The specific objectives of this study were:

i. To establish the antiplasmodial activities of the crude extracts from *T. rhodesica* and *T. polyphylla.*
ii. To isolate and characterize secondary metabolites from roots and seedpods of *T. rhodesica* and stem of *T. polyphylla.*
iii. To establish the antiplasmodial activities of the isolated compounds from the two *Tephrosia* species.
iv. To improve the antiplasmodial activity of some of the compounds through derivatization.

1.4 Justification and Significance of the Study

Most of drugs that have dominated the market for years for treatment of malaria were obtained from plants; morphine and quinine are typical examples. Artemesinin, the lead antimalaria drug today was also obtained from a plant, *Artemesia annua*. Different classes of natural products have been tested for antiplasmodial activites. Prenylated flavonoids, including chalconoids and flavanones have shown good activities. The Genus *Tephrosia*, along with other genera found in family Fabaceae have provided a unique class of compounds, flavonoids, some of which showed antiplasmodial activites. (Atilaw *et al.*, 2017; Muiva-Mutisya *et al.*, 2014). In a preliminary assay, the crude extracts from *Tephrosia rhodesica* and *Tephrosia polyphylla* showed good to moderate antiplasmodial activities; thus in this study, phytochemical investigation of these two plants were carried out in order to identify group of compounds that lead to antiplasmodial activites of the crude extracts.

CHAPTER 2: LITERATURE REVIEW

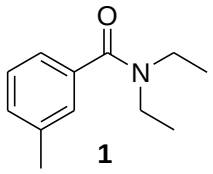
2.1 Malaria

The protozoan *Plasmodia* species that include *Plasmodium malariae, Plasmodium ovale, Plasmodium knowlessi, Plasmodium vivax* and *Plasmodium falciparum* are agents that caused malaria. Among these, the most deadly malaria is caused by *P. falciparum,* especially in Afica. The parasite is transmitted by two hosts, an infected female anopheles mosquito and human being that it bites .The severance of malaria is greater in the ageset 0-5 years and the pregnant women who have weak immunity (Duffy and Avery, 2012). Malaria trends remain high in the world, with 216 million cases of malaria registered in 2016, of which 90% of cases were in the African region. It resulted in 445,000 deaths, 91% of these were in African countries (World Health Organization, 2017).The symptoms that are common to malaria patients include high fever with chils and rigor, hyperpyrexia, acute respiratory tract infection, brain disorder, reduced cognition, gastrointestinal disorder, muscle scram, anaemia, premature delivery, still birth, miscarriages, infant low birth weight, some irreversible disabilities like blindness and may even put the patient in comma (Flannery *et al.,*2013).

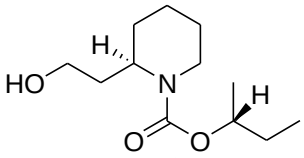
2.2 Malaria Interventions

Effective malaria management systems are necessary especially children between 0 to five years and women under antenatal care. Few precautions taken toward malaria control include: prompt treatment with effective drugs. This is done in case the prevention did not work and the patient is infected with the *plasmodium* parasite. One of these measures is through intermittent preventive treatment (IPTp) by giving antimalarial drugs to pregnant women during the monthly visits during pregnancy (Gamble *et al.,* 2006). On top of using sulphadoxine-pyrimethathine, a combination of azithromycin and chloroquine and mefloquine has been assessed for IPTp in pregnancy,

however they are poorly tolerated. The other starategy is vector control program; it works toward reducing the population of the mosquito vector, or by the use of mosquito repellents.Thus the two complimentary vector control methods are the use mosquito nets that are treated with insecticides also known as (ITNs) and spaying around the residential compounds a method called (IRS). The data from the previous studies have shown that ITNs and IRS had reduced malaria prevalence in areas where these were adequately applied. In addition, there has also been reduction in the number of low birth weight children and other inimical pregnancy abnormalities (Gamble *et al*., 2006). The other part of vector control is the use of repellents; the procedure had been used to minimize vector borne diseases due to the bites of arthropod. Some of the common repellents are: (i) DEET (N,N-Dimethyl-3-methylbenzamide) (**1**) and icaridin (**2**) (Tavares *et al*., 2018).



1

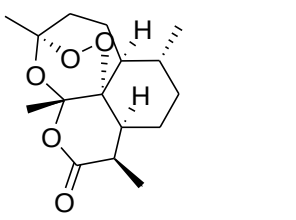


2

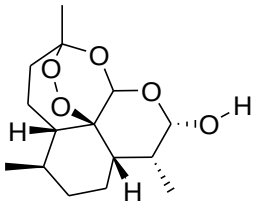
2.3 Antimalarial Drugs

In the treatment of malaria infections, information about the area in which malaria was contracted, patient's medical history, whether this malaria is severe or not, national guidelines and availability of antimalarial drugs are important factors to consider. A combination of artimisinin (**3**) derivatives with long acting antimalarial drugs such as dihydroartemisinin (**4a**) plus piperaquine phosphate under trade name P-ALAXIN reduces the time of treatment to only three days. Furthermore, early detection and timely treatment of malaria is necessary inorder to avoid the progress of *Plasmodium falciparum* infection that may cause death. The leading Artemisinin-based combination therapies (ACTs) are artemether (**4b**) plus lumefantrine, Artesunate (**5a**) plus mefloquine

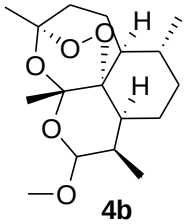
or amodiaquine (**5b**). For severe malaria, intramuscular administration of quinine (**6**) is still recommended. These drugs are safe and well tolerated, particularly in children (Stauffer and Fischer, 2003).



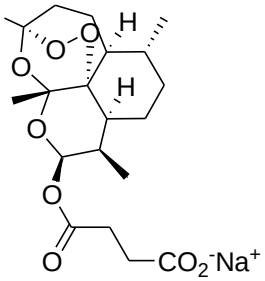
3



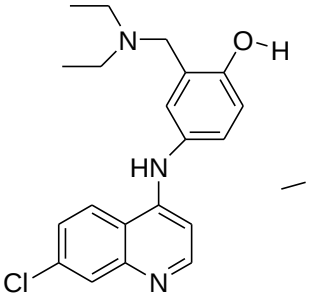
4a



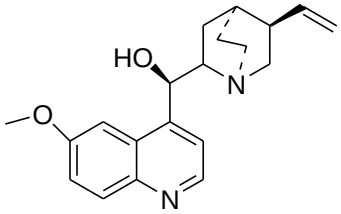
4b



5a



5b



6

2.4 Antimalarial Drug Resistance

The efforts to reduce incidences of malaria have been confronted by the parasites resistance to available drugs. The mosquito vector has also developed resistance to insecticides in use (Fong, 2013). Way back in 1910, it was noticed that quinine (**6**) was becoming ineffective to *plasmodium* parasite in areas of Southern America and some parts of Asia (Farooq & Mahajan, 2004). Ever since, resistance has been reported for all antiplasmodial drugs; the reliable drug artemisinin (**3**) and its derivatives artemether (**4b**) and artesunate (**5a**) are not exception to resistance of *P. falciparum*. There is a failure of parasite to response to these drugs in, Thailand, Cambodia, Vietnam, Lao people's Democratic Republic and Myanmar (Dundorp and Nosten, 2009). In related development, the common type of *Plasmodium* parasite in Africa, *P. falciparum* has it

artemisinin–resistance strain reported in Equatorial Guinea. It is therefore suggested that Equatorial Guinea and other counties in Africa with comparable malaria prevalence should remain vigilant (Lu *et al*., 2017).

2.5 Phytochemicals with Antiplasmodial Activities

A number of new anti-plasmodial natural products have been reported. They belong to the major classes of flavonoids, alkaloids, terpenoids, anthraquinones and quinones. The flavone numbered as compound (**7)** isolated from *Tephrosia* species was strongly active against chloroquine sensitive strain of *Plasmodium falciparum* (D6) with IC_{50} value of 1.7 ±0.1µM. Refer to the Table 2.1 in the text.

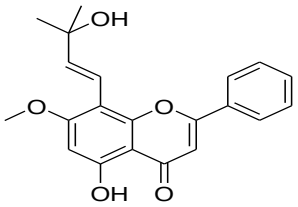
Table 2.1: Antiplasmodial flavonoids reported from *Tephrosia* species and related taxa

Compound	Antiplasmodial activity IC_{50} (µM)	Plant	Reference
(Trans)-5-Hydroxy-8-(3-hydroxy-3-methylbut-1-en-1-yl)-7-methoxy-2-phenyl-4H-chromen-4-one (**7**)	D6=1.7 ±0.1	*Tephrosia purpurea* subsp. *Leptostachya* (ST)	Atilaw *et al*., 2017
8-(5,5-Dimethyl-4-oxotetrahydrofuran-3-yl)-5-methoxy-4-oxo-2-phenyl-4H-chromen-7-yl acetate (**8**)	D6=14.8 ±3.2	*Tephrosia purpurea* subsp. *Leptostachya* (ST)	Atilaw *et al*., 2017

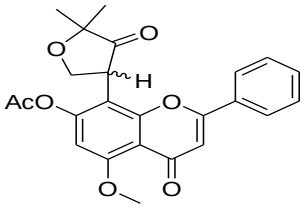
Terpurinflavone (**9**)	D6=3.1±0.3; W2=6.3±2.7	*Tephrosia purpurea* (ST)	Juma *et al*., 2011; Muiva-Mutisya *et al*., 2014.
Lanceolatin A (**10**)	D6=11.4±2.9; W2=14.9±3.1	*Tephrosia purpurea* (ST)	Juma *et al*., 2011; Muiva-Mutisya *et al*., 2014.
Semiglabrin (**11**)	D6=25.77±6.08; W2=35.58±5.41,	*Tephrosia purpurea* (ST)	Juma *et al*., 2011; Muiva-Mutisya *et al*., 2014.
Lanceolatin B (**12**)	D6=27.02±2.65; W2=35.99±4.24	*Tephrosia purpurea* (ST)	Juma et al., 2011; Muiva-Mutisya *et al*., 2014.
Abyssinone (**13**)	D6=4.9±0.8 W2=6.1±1.3	*Erythrina abyssinica* (RT)	Yenesew *et al*., 2003
Sigmoidin A (**14**)	D6=5.8 ±0.6 W2=5.9±1.1	*Erythrina abyssinica* (RT)	Yenesew *et al*., 2003
Abyssinin III (**15**)	D6=5.8 ±1.1 W2=5.2±1.7	*Erythrina abyssinica* (RT)	Yenesew *et al*., 2003
Abyssinone IV (**16**)	D6=5.4 ±1.5 W2=5.9±1.8	*Erythrina abyssinica* (RT)	Yenesew *et al*., 2003
Erythrabssin-II (**17**)	D6=8.1±1.4; W2=6.5±0.6	*Erythrina abyssinica* (RT)	Yenesew *et al*., 2003

ST= Stems; RT= Roots

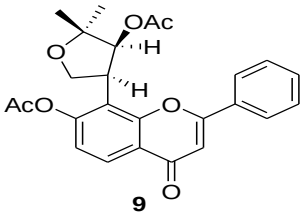
The alkaloids protopine (**18**) and chelanthifoline (**19**) isolated from *Corydalis calliantha* showed good antiplasmodial activities with IC_{50} values of 4.25 ± 0.69 µM for compound (**18**) and 2.78 ± 0.39 µM for compound (**19**) and 4.21 ± 1.24 µM for compound (**18**) and 3.76 ± 1.00 µM for comound(**19**) against the TM4 and K1 strains of *P. falciparum* parasite respectively.



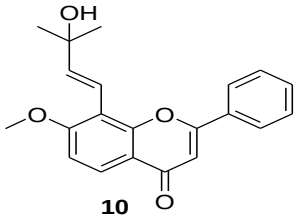
7



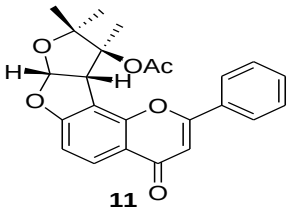
8



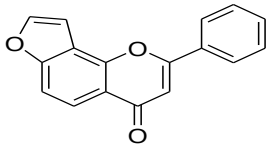
9



10

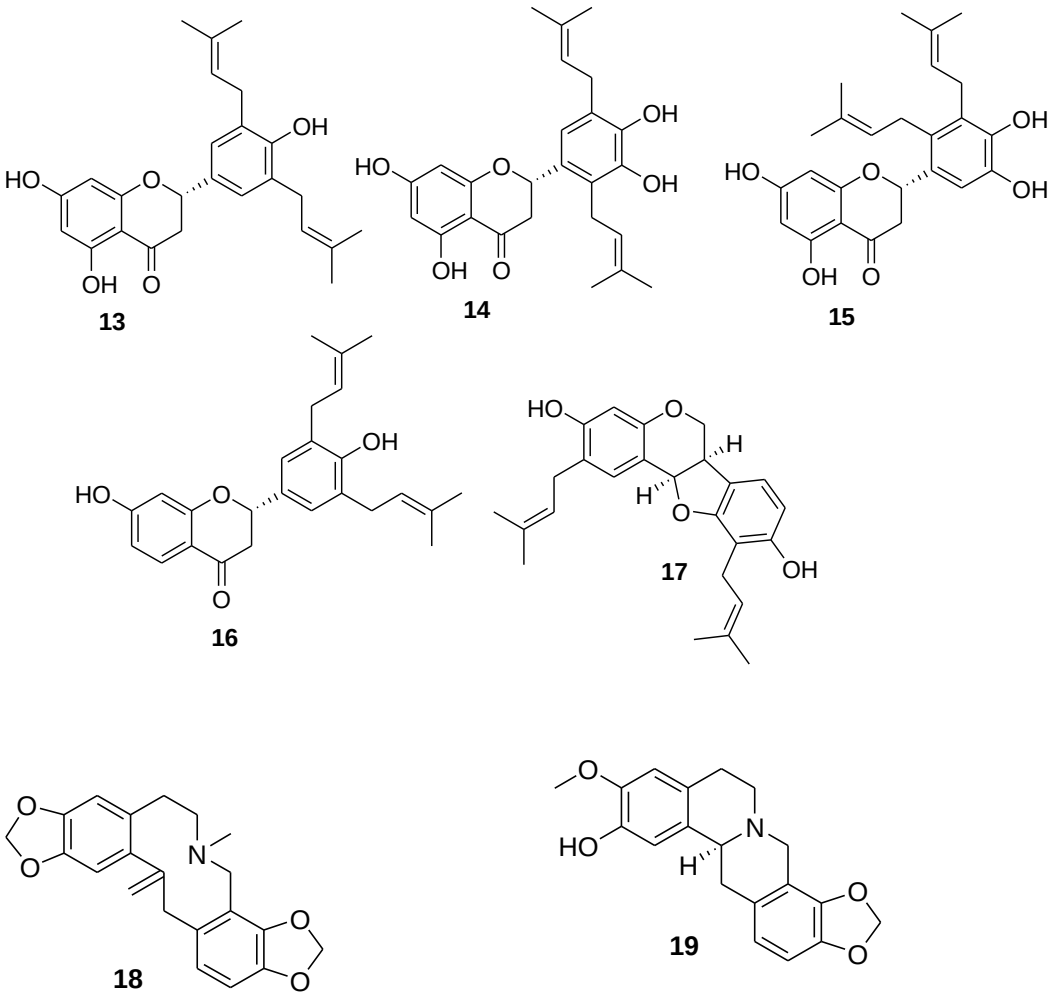


11



12

.



2.6 The Family Fabaceae

The genus *Tephrosia* is a member of the family Fabaceae. This family is having other name as Leguminosae, it has over 650 genera and 20,000 species. This family of shrubs, herbs and trees is distributed in tropics and subtropical region of the world (Tarus *et al*., 2002). The family is characterized by producing seed pods and isoflavonoids. For centuries, the family has provided man with timbers, food, and fodder for animals, medicine and fragrances. The family is subdivided into four subfamilies, namely:

Papilionoideae, Caesalpinioideae, Mimosoideae and Dialioideae. The genus *Tephrosia* is found within the subfamily Papilionoideae, uniquely identify by papilionoid flowers (Polhill, 1981).

2.6.1 The Genus *Tephrosia*

The genus *Tephrosia* consists of over 350 species of soft and hard wood shrubs that are widely distributed in the temperate region of the world (Zhi & Pedley, 2010). Up to 30 *Tephrosia* species are known to occur in Kenya (Tarus *et al*., 2002). The genus is characterized by odd-pinnate leaves with no stipples, a white flower with flattened pod and fruit of 10 to 15 by 1.6 cm (Sikolia *et al*., 1994). Among them are *T. aequilata, T. villosa, T. elata, T. hildebrandtii, T. purpurea, T. pumila, T. rhodesica, T. polypphylla, T. holstii, T. interupta, T.linearis, and T. pentaphylla*.

2.6.1.1 *Tephrosia rhodesica*

The description of morphology: Much-branched small shrub which grow upto 2 m tall. A short-leaved annual plant; leaflets 11-19, 25×9 mm; dense pink flowers that are on the upper axils; standard long hairy, about 11 mm long; a pale hairy pod with pale margins parts (Beentje *et al*., 1994)

Figure 2.1: *Tephrosia rhodesica*

Distribution: Kitui district Kalunka; Kisumu; Nguruman hills in Kenya, Somalia, Ethiopia, South Sudan, Sudan and Zimbabwe.

2.6.1.2 *Tephrosia Polyphylla*

The description of morphology: Annual villose herb up to 1 m tall. Leaf-rachis of upto 5-11 mm long, leaflets 7-17 mm and not more than 20 mm, long by 7 mm wide. Purple flowers located at upper axils only. Calyx densely is villose with lobes of 9 mm long. It has Standard villose with short hairs that are brown in colour and 10–13 mm long. Pods ovate, sessile or almost so, measuring 11 x 6 mm (Beentje *et al*., 1994).

Figure 2.2: *Tephrosia polyphylla.*

Distribution: Turkana province; central province and coastal province of Kenya, Somalia, Ethiopia, Northern Uganda, Northern Tanzania.

2.7 Ethnobotanical Information of the Genus *Tephrosia*

The genus *Tephrosia* is traditionally used by different communities to depend on when affected by diseases like respiratory disorders, inflammation, pain, syphilis, diarrhea, diuretic, stomachache (Dzenda *et al*; 2007). *Tephrosia purpurea*, one of the most widely used species in the genus *Tephrosia*, is used as laxative, antivenom, medicine against gastric disorders and ulcer, a tonic, antidiarrheal and also in leprosy (Sharma *et al*., 2003). The Table 2.2 below summarizes the traditional uses of some *Tephrosia* species.

Table 2.2: Some traditional uses of selected *Tephrosia* species

Tephrosia species	Plant part	Ethnomedical use(s)	Reference
T. aequilata	Roots	Used to treat venereal diseases when chewed in combination with salt	Kokwaro, 2009; Tarus *et al*., 2002
T.apollinea	Aerial part	Used to treat cough, headache, nasal and bronchitis congestion, wounds and bone fractures	Ammar *et al*., 2013
T. elata	Roots	Chewed as to treat stomach pain, fever and body weakness	Muiva *et al*., 2009
T.calophylla	Roots	Used for treatment of diabetes and the leaf extracts used for treating ulcers.	Parine *et al*., 2015
T.holstii	Roots	Used to cure Stomach pain and weakness.	Beentje *et al*., 1994

T.linearis	Leaves	Used to treat babies' cough	Kokwaro, 2009
T.interupta	Roots	Used to treat cough	Kokwaro, 2009
T.noctiflora	Roots	Used to treat cough	Kokwaro, 2009
T.pauncijuga	Roots and leaves	Used to treat wounds	Kokwaro, 2009
T. purpurea	Roots, stems and leaves	Used to cure gastroduodenal disorders, haemoroids, anaemia, stomach pains, and skin diseases	Kokwaro, 2009
T.pentaphylla	Roots	Used to cure chest and throat pain	Kokwaro, 2009
T.pumila	Roots	Used to cure chest pain and common cold	Kokwaro, 2009
T.obovata	Seeds	Used to catch fish	Chen *et al*., 1978
T.uniflora		Poisonous bites remedy	Abreu & Luis, 1996
T.vilosa	Roots	Used to treat Liver malfunctions and respiratory problems	Kokwaro, 2009
T. vogelii	Leaves	Used to treat scabies and eradication of ticks and fleas in cattle and poultry respectively.	Kokwaro, 2009

2.8 Biological Activities of the Genus *Tephrosia*

A number of *Tephrosia* species have been investigated for activities against different biological conditions (Table 2.3). The seedpods of *T. elata* on testing were found to posses some antiplasmodial activities (Muiva *et al*., 2009). Different parts of *T. deflexa, T. linearis* and *T. purpurea* showed antibacterial activities (Hussain *et al*., 2012; Kare *et al., 2006;* Ratsimamanga-Urverg *et al.,1994).* The roots of *T. vogelii* and *T. aequilata* were examined and showed antimicrobial activity (Tarus *et al*., 2002; Wanga *et al*., 2006). Other crucial biological activities that include: antioxidant, antimicrobial, antibacterial, antileishmanial, anti-inflamatory, anticancer, antidiabetic and Hepatoprotective were able to be detected from the roots of mostly used species of *Tephrosia*, which is *Tephrosia purpurea*. (Choudhary, 2007; Gupta *et al*.,2008; Hussain *et al*., 2012; Pavana *et al* ., 2007; Shah *et al* .,2011; Sharma *et al*., 2003); The roots and leaves of *Tephrosia. Vogelii* was found to be antimicrobial against a number of negative and positive gram bacteria.

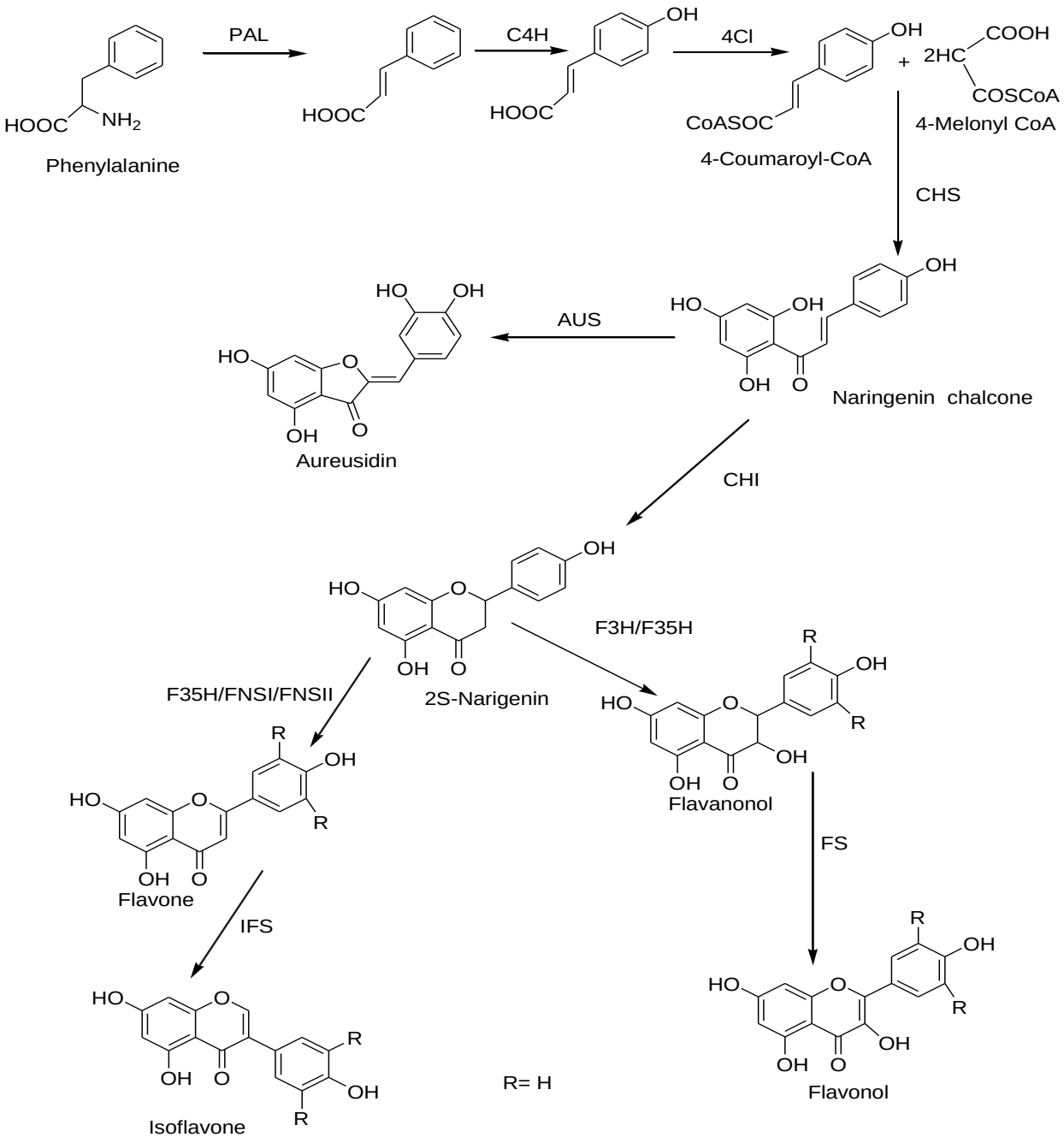
Table 2.3: Some *Tephrosia* species and their biological activities

Tephrosia Species	Plant Part	Biological activity	Reference
T. aequilata	Roots	Antimicrobial Parasitic	Tarus *et al*., 2002
T. calophylla	Roots	Hepatoprotective Antihyperlipidemic	Adinarayana *et al*., 2009; Mohan, 2011
T. deflexa	Seeds	Antibacterial	Kare *et al*., 2006
T. elata	Seedpods	Antiplasmodial	Muiva *et al*., 2009
T. hidebrandtii	Roots	Antifeedant	Lwande *et al*.,1986
T. linearis	Roots	Antibacterial	Ratsimamanga-Urverg *et al*., 1994
T. pumila	Roots	Antiprotozoal	Ganapaty *et al*., 2008
T. purpureaa	Roots	Antioxidant Antimicrobial Antibacterial Antileishmanial Anti-inflamatory Anticancer Antidiabetic Hepatoprotective	Choudhary, 2007; Gupta *et al*.,2008; Hussain *et al*., 2012; Pavana *et al* ., 2007; Shah *et al* .,2011; Sharma *et al*., 2003
T. sinapou	Roots	Anti-inflamatory	Martinez *et al*., 2012
T.spinosa	Aerial parts	Anti-inflamatory	Chakradhar *et al*., 2005
T. toxicaria	Roots	Chemopreventive	Jang *et al*., 2003
T. villosa		Hyperglycemc	Balakrishnan *et al*., 2007
T. vogelii	Roots and leaves	Antimicrobial	Wanga *et al*., 2006

2.9 Biosynthesis of Flavonoids

Two metabolic pathways are basically involved in the biosynthesis of flavanoids, the phenylpropanoids and shikimate pathways. At the first step, through phenylpropanoids pathway part of the basic skeleton of flavonoid is formed; here the aromatic amino acid phenylalanine is converted into 4-coumaroyl-CoA as shown in (scheme 2.1). Then, a tetraketide linked to the 4- coumaroyl-CoA intermediate is formed through incorporation of three malonyl CoA units (Dewick, 2002; Winkel-Shirley, 2001). Hence, the synthesis of narigenin chalcone is enabled by the enzyme chalcone synthase (CHS) from p-hydroxycoumaroyl CoA with three molecules of malonyl CoA to form an intermediate of tetraketide that cyclized into hydroxylated aromatic ring system to form a scaffold of a chalcone. (Dewick, 2002).

Different subclasses of flavonoids are then formed from a chalcone skeleton. The enzyme chalcone isomerase (CHI) converts narigenin chalcone to naringenin, the same enzyme also converts isoliquiritigenin chalcone to liquiritigenin. Another enzyme, Aurone synthase (AUS), converts narigenin chalcone to Aureusidin. The other enzyme involved in this pathway is Isoflavone synthase (IFS) that converts flavones to isoflavones. Narigenin is converted to the corresponding flavanonol by F3H (flavanone-3-hydroxylase), followed by conversion of the favanonol to flavonol by FS (flavonoid synthase). The introduction of alkyl group at position 3 and 5 is facilitated by F3′5′H (flavonoid-3′,5-′hydroxylase). Scheme 2.1 shows how the different subclasses of flavanoids are biosynthesized (Dewick, 2002; Winkel-Shirley, 2001).



Scheme 2.1: Biosynthetic pathways of flavonoids (Dewick, 2002; Winkel-Shirley, 2001)

2.10 Phytochemistry of the Genus *Tephrosia*

The literature has shown that the Genus Tephrosia produced a vast number of phytochemcials. Most of them are flavonoids isolated from various *Tephrosia* species. The compounds so far reported from this genus belonging to different subclasses of flavonoids are reviewed in the following sections.

2.10.1 Flavonoids

Flavonoid is the term used to describe the broad spectrum of natural products that have a C6-C3-C6 carbon skeleton. They made up one of the largest groups of naturally occurring phenols (Butler, 2004) which are found in the advanced algae and terrestrial plants (Schijlen *et al*., 2004). Flavonoids are the major metabolites produced by the genus *Tephrosia*. These flavonoids are divided depending on the arrangement of the basic C6-C3-C6 skeletal structure (Chen, *et al*, 2014). Each of the C-6 at the periphery represents an aromatic ring linked by a three carbon unit that forms a mid heterocycle ring containing atleast one oxygen atom. The aromatic rings are labeled as C at the middle but oxygenated, B at the periphery, mostly unsubstituted and A some time oxygenated or prenylated. This group of compounds categorically fall under three sub-classes depending on the location of the aromatic ring to the benzopyrano moiety, these classes are: Flavonoids (2-phenyl benzopyrans) latter subdivided into flavanone, flavones, flavanonol and flavonol. Isoflavonoids (3-benzopyrans) and neoflavonoids (4-benzopyrans) having a chalcone as a precursor. Therefore all the classes of flavonoids are structurally and biogenetically similar. The biogenesis from chalcone can also produced rotenoids and pterocarpans as summarized in the following sections.

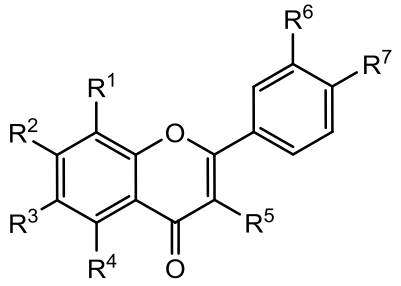
2.10.1.1 Flavonols of the Genus *Tephrosia*

One of the flavonols, 6-hydroxykaempferol 4'-methyl ether (**20**) isolated from *T. candida* contains a glycosyl and rhamnosyl substituent at C-2 and C-5. The other flavonols are all aglycones. Interestingly, 7-ethoxy-3,3',4'-trihydroxyflavone (**23**)

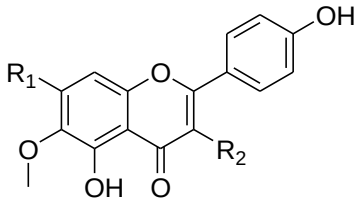
isolated from *T. procumbens*, contains the unusual ethoxy group at C-7. The Table 2.4 below shows some flavonols isolated from the genus *Tephrosia*.

Table 2.4: Flavonol of the Genus *Tephrosia*

Flavonol	Plant source	Reference
6-Hydroxykaempferol-4'-methyl ether (**20**)	*T. candida* (WP)	Sarin, *et al*., 1976
Candidol (**21**)	*T. candida* (SD)	Dutt & Chibber, 1983
Candidrone (**22**)	*T. candida* (SD)	Parmar *et al*.,1987, Horie *et al*.,1994
7-Ethoxy-3,3',4'-trihydroxyflavone (**23**)	*T.procumbens* (RT)	Venkataratnam *et al*., 1987
6-Hydroxykaempferol-6-methyl ether3-O– α–Rhamnopyranosyl(1→6)-β-galactopyranoside-7-O-α-rhamnopyranoside (**24**)	*T.vogeli* (LF)	Belmain *et al* .,2012
6-Hydroxykaempferol-6-methyl ether3-O– α–Rhamnopyranosyl(1→2)[α-rhamnopyranosyl(1→6)]-β-galactopyranoside (**25**)	*T. vogeli* (LF)	Belmain *et al*., 2012
Compound (**26**)	*T. vogeli* (LF)	Belmain *et al*., 2012
6-Hydroxykaempferol6-methyl ether3-O-a-rhamnopyranosyl (1→2)[(3-O-E-feruloyl)-α-rhamnopyranosyl(1→6)]-β-galactopyranosides (**27**)	*T. vogeli* (LF)	Belmain *et al*., 2012



	R^1	R^2	R^3	R^4	R^5	R^6	R^7
20	H	ORha	OH	OH	Orha	OMe	H
21	H	OMe	OMe	OMe	OH	OH	OH
22	OMe	H	OMe	OH	OMe	OH	OH
23	H	OEt	H	H	OH	OH	OH



24 R^1**=** O-(α-Rha)**,** R^2**=** O-(α-Rha-(1-2)-[O-(α-Rha-(1-6)]-β-Gal

25 R^1=O-(α-Rha)**,** R^2 =O-(α-Rha-(1-6)-β-Gal

26 R^1=OH**,** R^2=O-(α-Rha-(1-2)-[O-(α-Rha-(1-6)]-β-Gal-7-O-

27 R^1=OH, R^2=O-(α-Rha-(1-2)-[(3-O-E-Feruloyl)-α-Rha-(1-6)]-β-Gal

2.10.1.2 Flavanonols of the Genus *Tephrosia*

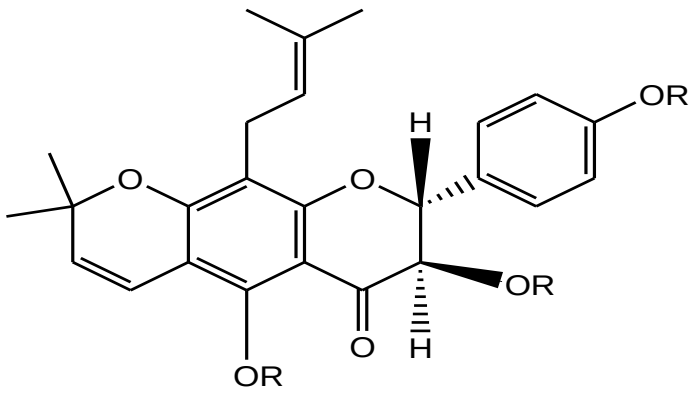
Very few flavanonols have been isolated from the genus *Tephrosia*, these include lupinifolinol (**28**) and lupinifolinol triacetate (**29**) from the stem of *T.Lupinifolia* (Smalberger*et al*., 1974).

The Table 2.5 below shows the list of some flavanonols isolated from Genus *Tephrosia*.

Table 2.5: Flavanonols of the Genus *Tephrosia*

Flavanonol	Plant source	Reference
Lupinifolinol (**28**)	*T. lupinifolia* (ST)	Smalberger et al., 1974
Lupinifolinol triacetate (**29**)	*T. lupinifolia* (ST)	Smalberger et al., 1974

Key: ST-Stem



	R
28	H
29	Ac

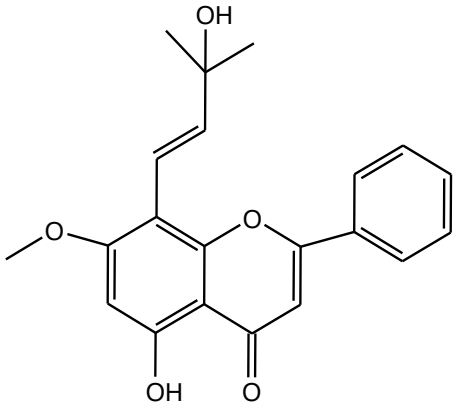
2.10.1.3 Flavones of the Genus *Tephrosia*

Flavones are 2,3-dehydroderivative of flavanones, with 2,3-olefinic bond (Agrawal, 2013). A number of flavones have been isolated from *Tephrosia* species, and some of these are listed in (Table 2.6).

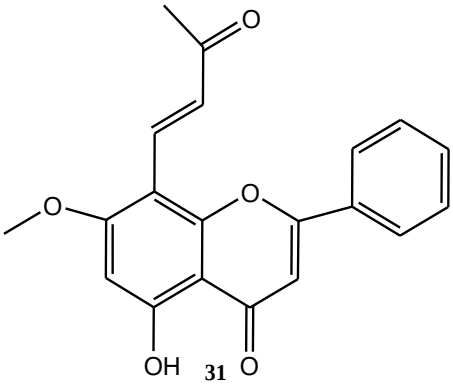
Table 2.6: Flavones of the Genus *Tephrosia*

Flavone	Plant source	Reference
(E)-5-Hydroxytephrostachin (**30**)	*T. purpurea* (ST)	Atilaw *et al*., 2017
Purleptone (**31**)	*T. purpurea* (ST)	Atilaw *et al*., 2017
(E)-5-Hydroxyan Hydrotephrostachin (**32**)	*T. purpurea* (ST)	Atilaw *et al*., 2017
Tephpurlepflavone (**33**)	*T. purpurea* (ST)	Atilaw *et al*., 2017
Terpurinflavone (**34**)	*T. purpurea* (ST)	Juma *et al*., 2011
Lanceolatin A (**35**)	*T. purpurea* (ST)	Juma *et al*., 2011
Semiglabrin (**36**)	*T. purpurea* (ST)	Juma *et al*., 2011
Lanceolatin B (**37**)	*T. purpurea* (ST)	Juma *et al*., 2011

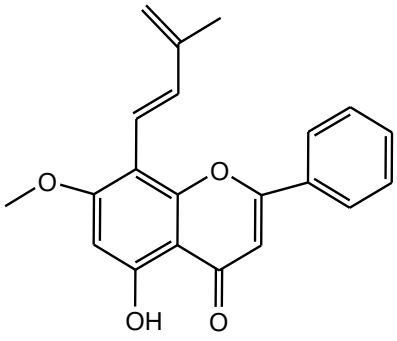
Key: ST- stem



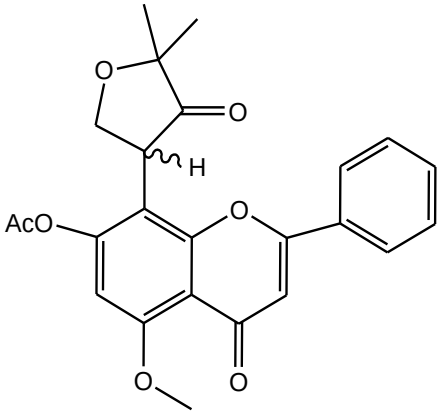
30



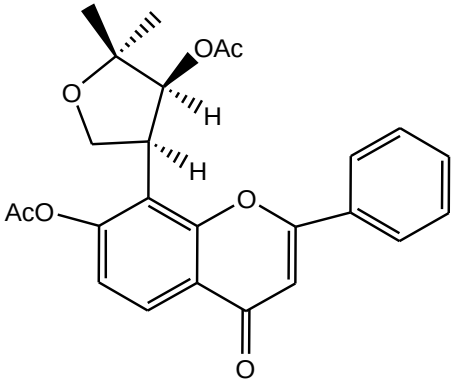
31



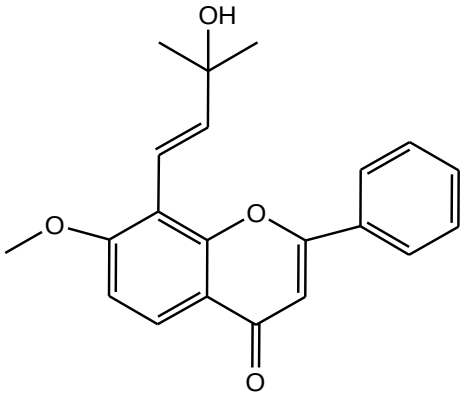
32



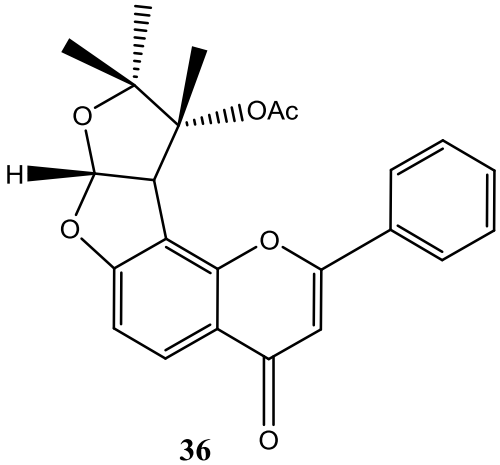
33



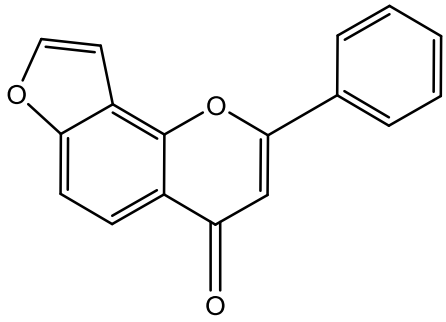
34



35



36



37

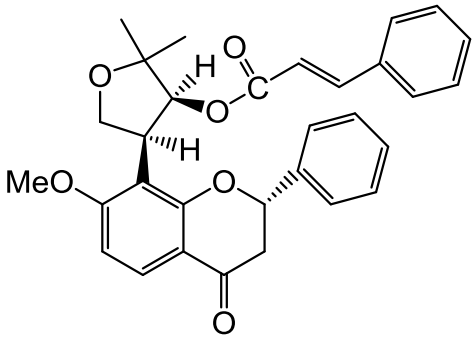
2.10.1.4 Flavanones of the Genus *Tephrosia*

The parent skeleton of flavanones posses 2-phenylchromanone units. Most of the naturally occurring flavanones of this genus have unsubstituted ring B (Agrawal, 2013). The Table 2.7 below shows the list of some flavanones isolated from the genus *Tephrosia*.

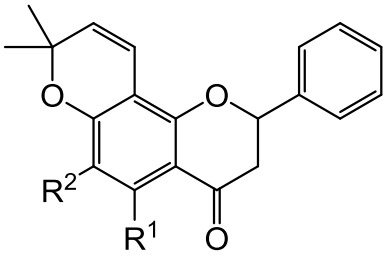
Table 2.7: Flavanones of the Genus *Tephrosia*

Flavanone	Plant source	Reference
Tephrorin B (**38**)	*T. purpurea* (AP)	Chang *et al*., 2000
Fulvinervin A (**39**)	*T. fulvinevis* (SD)	Rao *et al*.,1985
Maximaflavone A (**40**)	*T. maxima* (RT)	Rao *et al*., 1994
Isolonchocarpin (**41**)	*T.purpurea* (RT)	Waterman & Khalid, 1980
Spinoflavanone A (**42**)	*T. spinosa* (RT)	Rao & Prasad, 1992
Teproleocarpin B (**43**)	*T.leiorcarpa* (RT)	Go *et al*., 1991
Dehydroisoderricin (**44**)	*T. purpurea (*RT)	Rao & Raju, 1984

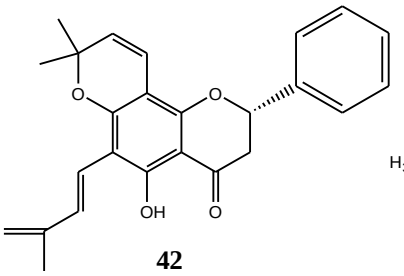
Key:RT-roots, SD-seeds, AP- Aerialparts



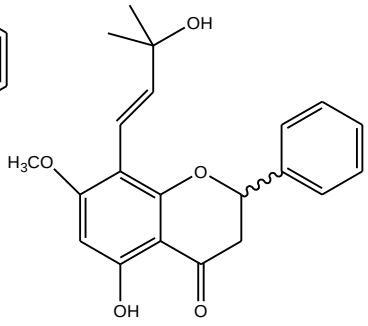
38



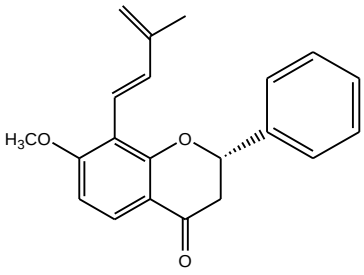
	R^1	R^2
39	OH	Prenyl
40	H	Prenyl
41	H	H



42



43



44

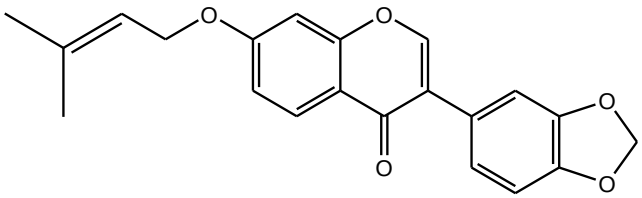
2.10.1.5 Isoflavones of the Genus *Tephrosia*

Isoflavonoids are a diverse class of flavonoids found to have a C-15 unit. This unit is generated from a flavones molecule which is known to originate from phenylpropanoid precursors and melonyl CoA (Phillips & Kapulnik, 1995). Most of them have oxygenation at C-4' which as required on the basis of their biogenesis. Examples include pumilaisoflavone A (**45**) and pumilaisoflavone D (**46**) isolated from *T. pumila* (Yenesew *et al.*,1989). Table 2.8 shows the list of some isoflavones isolated from the genus *Tephrosia*.

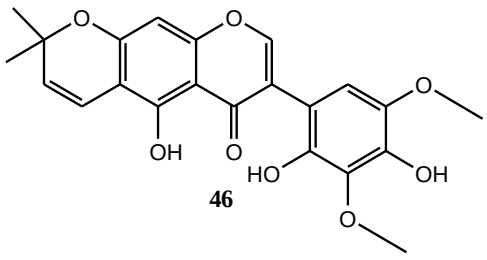
Table 2.8: Isoflavones of the Genus *Tephrosia*

Isoflavone	Plant source	Reference
PumilaisoflavoneA(**45**)	*T. pumila* (WP)	Yenesew *et al*., 1989
PumilaisoflavoneD (**46**)	*T. pumila* (WP)	Yenesew *et al*., 1989
PumilaisoflavoneB(**47**)	*T. pumila* (WP)	Dagne *et al*., 1988
Dimethoxyisoflavone (**48**)	*T. purpurea (*AP)	Shawl *et al*.,1984
4'-Demethyltoxicarol isoflavone (**49**)	*T. polyphylla* (RT)	Dagne *et al*., 1992
2',4',5,5'-Tetramethoxy-2",2"-dimethylpyrano-[6',5"-h]isoflavone (**50**).	*T. polyphylla* (RT)	Dagne *et al*., 1992
Calopogoniumisoflavone B (**51)**	*T. elata* (RT)	Lwande *et al* .,1985
Maximaisoflavone J (**52**)	*T. maxima* (RT)	Murthy & Rao, 1985
Maximaflavone B (**53**)	*T. maxima* (RT)	Murthy & Rao, 1985

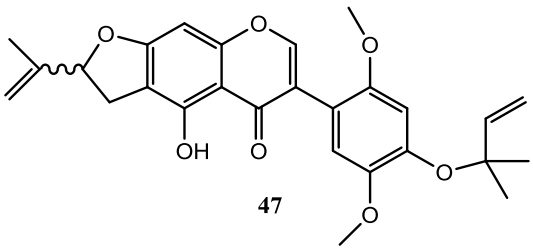
Key: RT- roots, AP- aerial parts, WP- whole plant



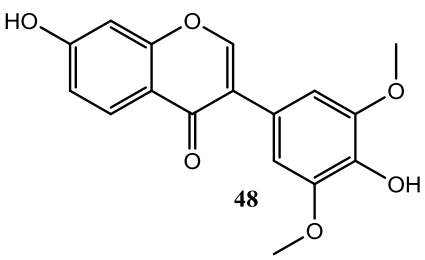
45



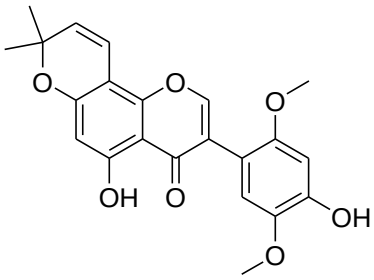
46



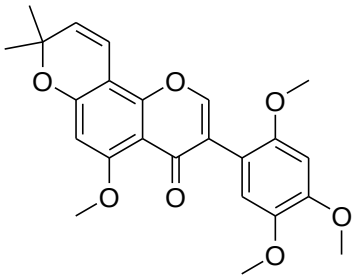
47



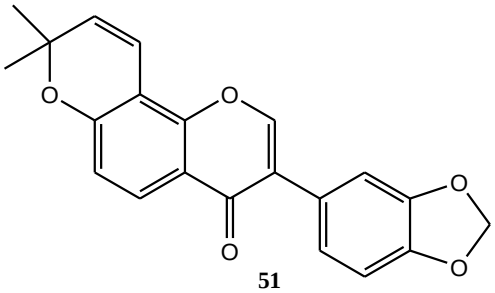
48



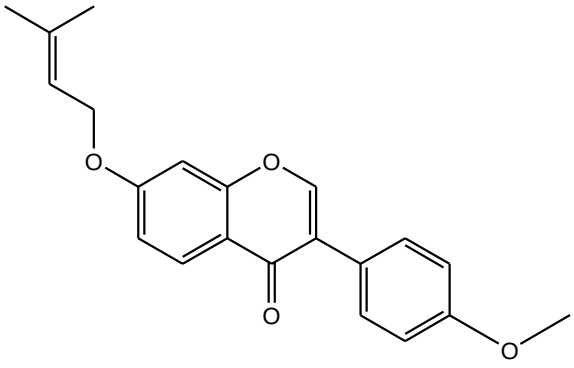
49



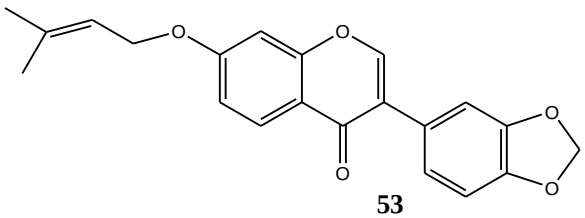
50



51



52



53

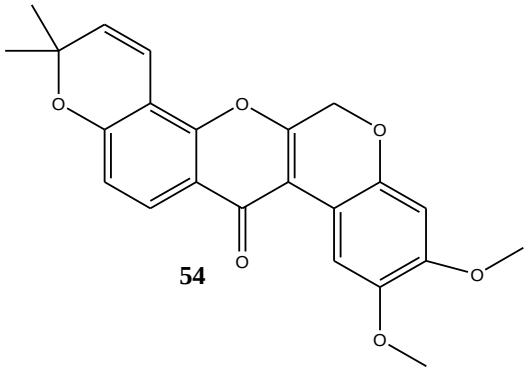
2.10.1.6 Rotenoids of the Genus *Tephrosia*

In *Tephrosia* species, some rotenoids are oxygenated at C-6. Examples of dehydrorotenoids such as dehydrodeguelin (**54**) isolated from *T. candida* (Crombie *et al*.,1998) and 6-*O*-acetyldihydrostemonal (**56**) isolated from *T. pentaphylla* (Dagne *et al*.,1989) showed the said observation. The Table .2.9 shows a list of some rotenoids isolated from the genus *Tephrosia.*

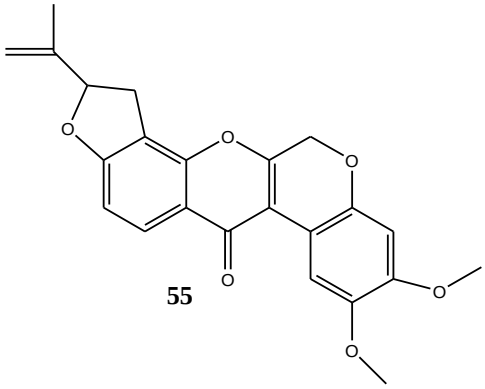
Table 2.9: Rotenoids of the genus *Tephrosia*

Rotenoids	Plant source	Reference
Dehydrodeguelin (**54**)	*T. candida* (RT)	Crombie *et al*., 1998
Dehydrorotenone (**55**)	*T. candida* (RT)	Crombie *et al*., 1998
6-*O*-Acetyldihydrostemonal (**56**)	*T. pentaphylla* (RT)	Dagne *et al*., 1989
9-Dimethyldihydrostemonal (**57**)	*T. pentaphylla* (RT)	Dagne *et al*., 1989
6-Hydroxyrotenone (**58**)	*T. pentaphylla* (RT)	Dagne *et al*., 1989
Villosol (**59**)	*T. villosa* (PD)	Prashant & Krupadanam, 1993
Villinol (**60**)	*T. villosa* (PD)	Prashant & Krupadanam, 1993
Villosone (**61**)	*T. villosa* (PD)	Prashant & Krupadanam, 1993

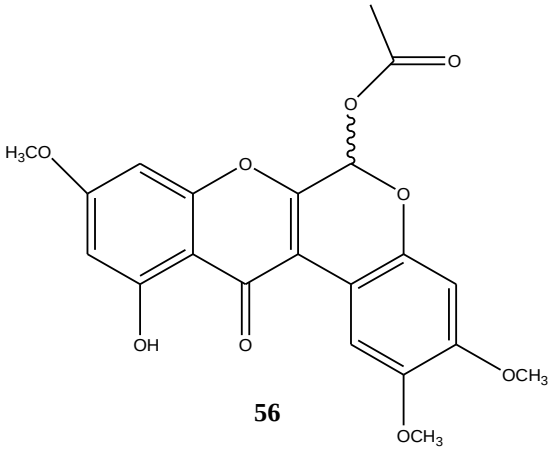
Key: RT- roots, PD- pods



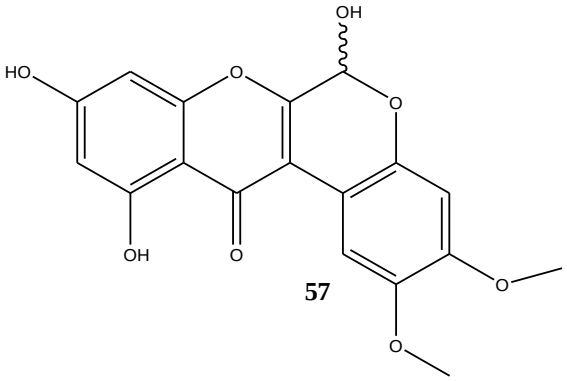
54



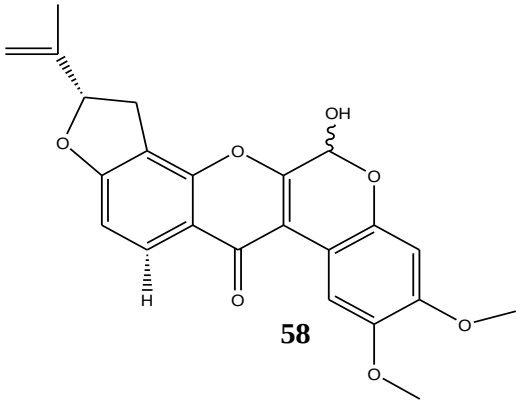
55



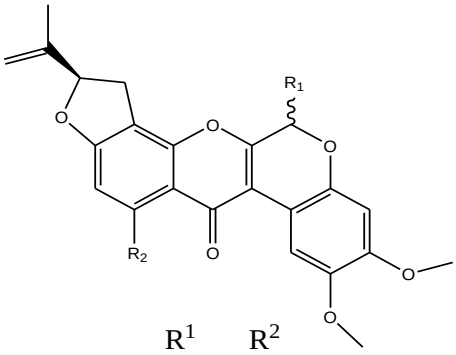
56



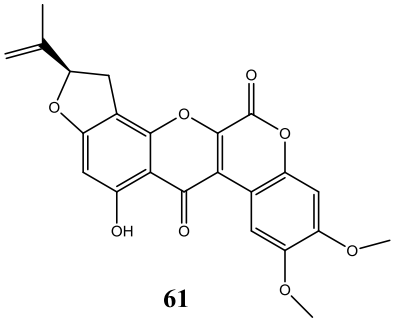
57



58



	R^1	R^2
59	H	OH
60	OMe	OH



61

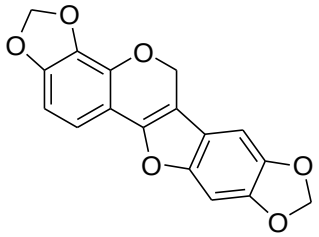
2.10.1.7 Pterocarpanoids of the Genus *Tephrosia*

Pteracarpanoids are known as the second largest group of naturally occurring isoflavonoids; they have a number of medicinal values including antifungal, antiviral and antimalarial activities (Maurich *et al*., 2006). Depending on the level of oxidation in the B/C ring junction , they can be categorised into four sub-groups: 6a-hyrdoxypterocarpans, pterocarpenes, pterocarpanes and coumestans (Maurich *et al*., 2006). Some of the pterocarpanoids isolated from various *Tephrosia* species are listed in Table 2.10.

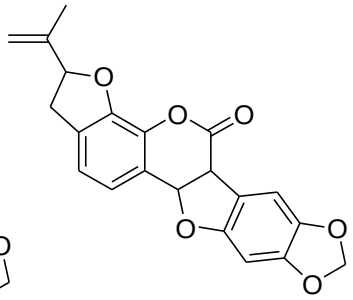
Table 2.10: Pterocarpanoids of the Genus *Tephrosia*

Pterocarpanoid	Plant source	Reference
3,4:8,9-Di-methylene-dioxypterocarpene (**62**)	*T. aequilata* (RT)	Atilaw *et al*., 2017
Tephcalostan (**63**)	*T.calophylla* (WP)	Kishore *et al*., 2003
Emoroidocarpan (**64**)	*T.emoroides* (RT)	Machocho *et al*., 1995
Hildecarpidin (**65**)	*T.hildebrandtii* (RT)	Lwande *et al*., 1987
2-O-Methyllucernol (**66**)	*T,hamiltonii* (RT)	Rajani & Sarma, 1988
Acanthocarpan (**67**)	*T.bidwilli* (LV)	Ingham & Markham, 1980
Tephrocarpin (**68**)	*T.bidwilli* (LV)	Ingham & Markham, 1980

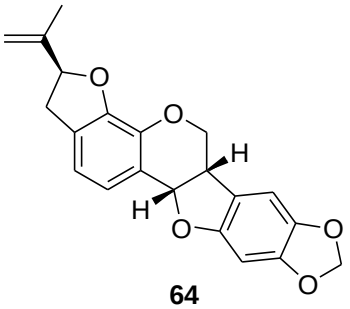
Key: RT- roots, WP- whole plant, LV-Leaves



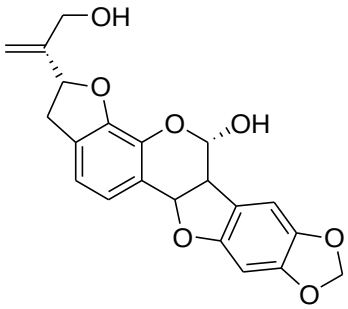
62



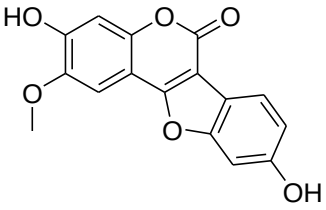
63



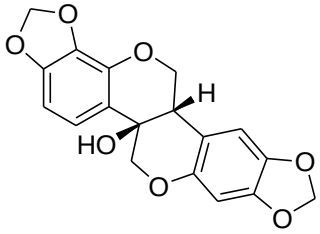
64



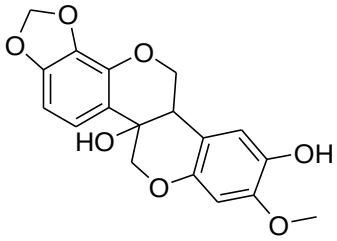
65



66



67



68

2.10.1.8 Chalconoids of the Genus *Tephrosia*

Chalcones and chalcanes are open chain flavonoids with two aromatic rings bound by α,β−unsaturated carbonyl group. They are among the main constituent of *Tephrosia* species. Most of them have modified prenyl group in ring A. Aequichalcone A (**69**) and

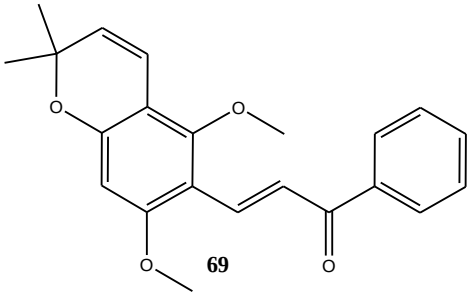
aequichalcone B (**70**) isolated from roots of *Tephrosia aequilata* (Atilaw *et al*., 2017) have modified prenyl ring at C-5'.

The Table 2.11 shows the list of some chalcones from the genus *Tephrosia*.

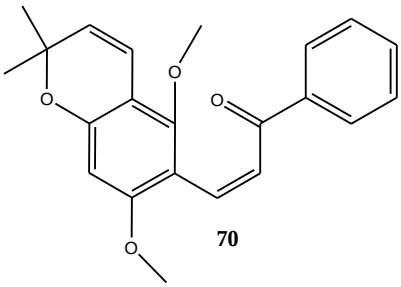
Table 2.11: Chalconoids of the Genus *Tephrosia*

Chalconoid	Plant source	Reference
Aequichalcone A (**69**)	*Tephrosia aequilata* (RT)	Atilaw *et al*., 2017
Aequichalcone B (**70**)	*Tephrosia aequilata* (RT)	Atilaw *et al*., 2017
Aequichalcone C (**71**)	*Tephrosia aequilata* (RT)	Atilaw*et al*., 2017
Obovatachalcone (**72**)	*Tephrosia obovata* (AP)	Chen *et al*., 1978
Obovatin (**73**)	*Tephrosia obovata* (AP)	Chen *et al*., 1978
Tunicatachalcone (**74**)	*Tephrosia tunicate* (RT)	Andrei *et al*., 2000
Obovatachalcone (**75**)	*Tephrosia tunicate* (RT)	Andrei *et al*., 2000
Epoxy-obovatachalcone (**76**)	*Tephrosia carrollii* (AP)	Gómez-Gariba *et al*.,2001
Oaxacacin (**77**)	*Tephrosia carrollii* (AP)	Gómez-Gariba *et al*.,2001
(+)-Tephrosone (**78**)	*Tephrosia purpurea* (WP)	Chang *et al*., 2000

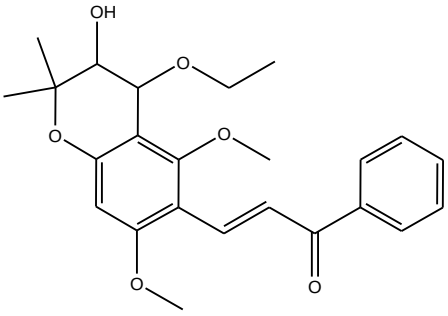
Key: RT- roots, AP- aerial parts, WP- whole plant



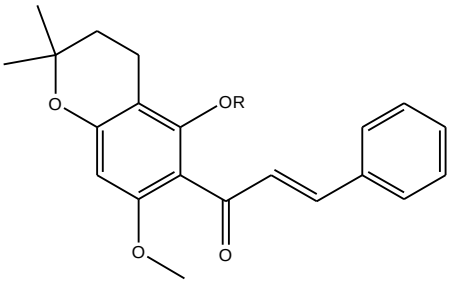
69



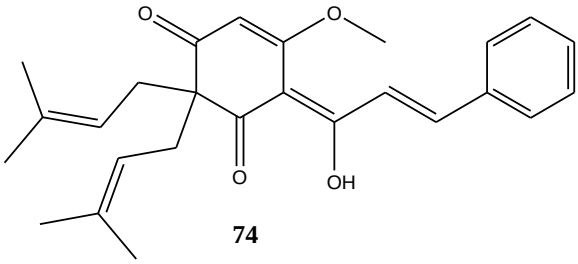
70



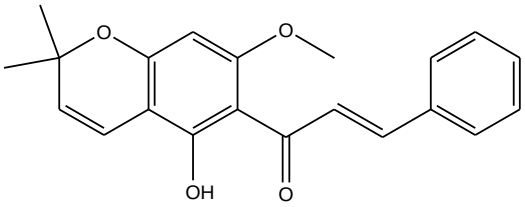
71



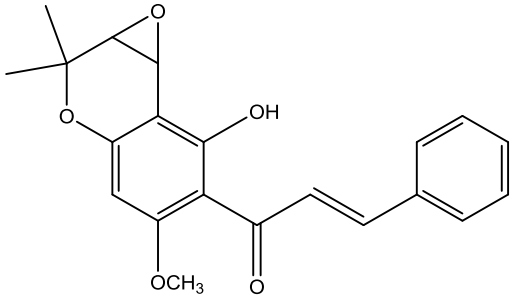
72, R=H
73, R=COCH3



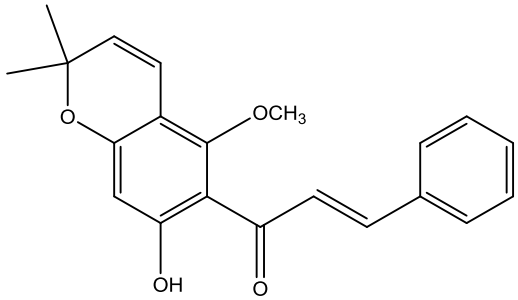
74



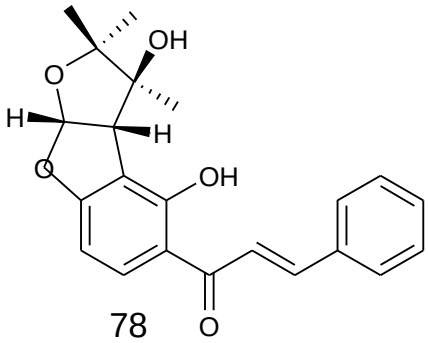
75



76



77



78

2.11 Biological Activities of Compounds from the Genus *Tephrosia*

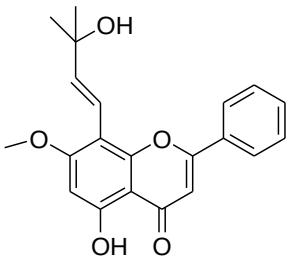
In the search for alternative drugs that are cheaper and accessible, many species from the genus *Tephrosia* have been investigated. The compounds isolated from genus *Tephrosia* were found to be active as antilarval, antiplasmodial, antimicrobial, estrogenic antitumor, antileshmanial and antifeedant. These compounds were found among different sub classes of flavonoids such as flavanones, flavanonols, flavones, flavonols, chalcones, pterocarpans and rotenoids. Some of the compounds with known activities (Table 2.12) include; (*E*)-5-hydroxyteprostachin (**30**) isolated from *T.purpurea*, which showed good antiplasmodial activity against D6 strain, of *Plasmodium falciparum* (IC_{50}=1.7µM) (Atilaw *et al*., 2017); tepurlepflavone (**33**) isolated from *T. purpurea*, showed a good antiplasmodial activity against chloroquine sensitive strains of *P*.

falciparum (IC_{50}=14.8 µM) (Atilaw *et al*., 2017); 6α-hydroxy-α-toxicarol (**81)** isolated from *T. villosa*, showed antiplasmodial activity against D6 strain of *P. falciparum* (IC_{50}=7.97 µM) (Muiva-Mutisya *et al*., 2014)

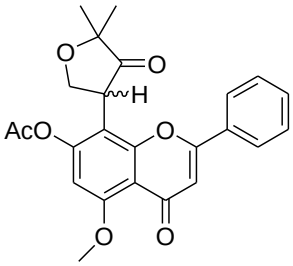
Compounds from this genus also showed other biological activities (Table. 2.12) , for example 2',6'-dimethoxy-4',5'-(2",2"-dimethyl)-pyranochalcone (**82**), a compound isolated from *T. pulcherrima* was found to have antimicrobial activity (MIC=5.90 µM) (Ganapaty *et al*.,2008). Amorpholone (**83**), isolated from stems and leaves of *T. candida*, was tested for larvacidal activity against the larvae of *Spodoptera litura* and was found to be active (LD_{50}=3.1 µM) (Kole *et al*., 1992). Two compounds isolated from *T. pupurea*, (+)-tephrorine A (**82**) and (+)-tephrosone (**78**) were found to have cancer chemopreventive effects with CI of 4.0 µM and 4.1 µM, respectively (Chang *et al*., 2000).

Table 2.12: Biological activities of some compounds from *Tephrosia* species

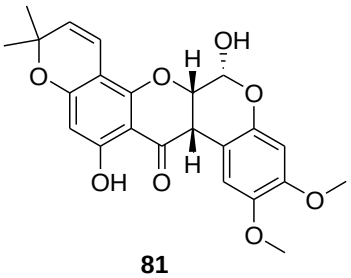
Compound	Biological activity	*Tephrosia* species	Reference
(*E*)-5-Hydroxyteprostachin (**79**)	Antiplasmodial	*T.purpurea*	Atilaw *et al*., 2017
Tepurlepflavone (**80**)	Antiplasmodial	*T.purpurea*	Atilaw *et al*., 2017
6α-Hydroxy-α-toxicarol (**81**)	Antiplasmodial	*T.villosa*	Muiva-Mutisya *et al*., 2014
2',6'-Dimethoxy-4',5'-(2",2"-dimethyl)-pyranochalcone (**82**)	Antimicrobial	*T.pulcherrima*	Ganapaty *et al*., 2008
Amorpholone(iii) (**83**)	Insecticidal	*T. candida*	Kole *et al*., 1992
(+)-TephrorineA (**84**)	Chemopreventive	*T.pupurea*	Chang *et al*., 2000
(+)-Tephrosone (**85**)	Chemopreventive	*T.pupurea*	Chang *et al*., 2000



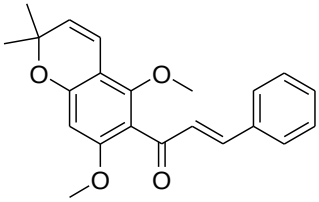
79



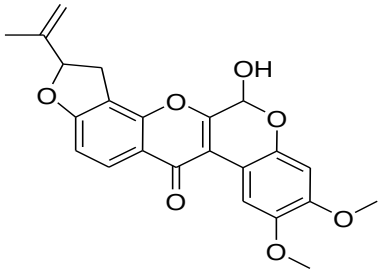
80



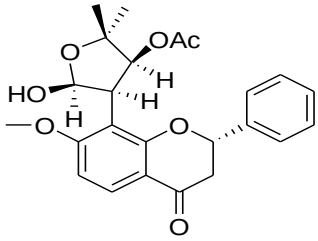
81



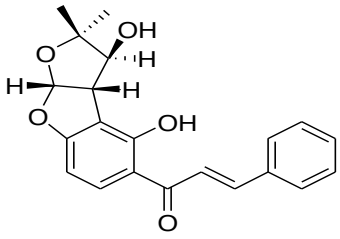
82



83



84



85

CHAPTER 3: MATERIALS AND METHODS

3.1 General Experimental Procedures

Several experiments were handled with different analytical instruments. Centrifugal chromatography was done on chromatotron Model 7924T; NMR data was obtained from 500 MHz Bruker Advance III HD; UV spectra from Specord S600; CD data was obtain from Jasco J-715 spectropolarimeter and other data were obtained as shown in the (Table 3.1).

The specifications and details of analytical instruments used are in the Table 3.1 below.

Table 3.1: Instrumentation involved

Experiment name	Instrument used
TLC	Merck pre-coated silica gel 60 F254 plates
Column Chromatography	Silica gel 60 (70-230 mesh)
Gel filtration	Sephadex LH-20
Centrifugal TLC	Chromatotron Model 7924T
NMR spectra	500 MHz Bruker Advance III HD Spectrometers and 400 MHz MR400-DD2
UV spectra	Specord S600 (Analytic Jena AG) Spectrometer
X-ray Single Crystal Structures	Bruker D8
ECD	Jasco J-715 spectropolarimeter
LCMS	1200 HPLC / 6490LC MS/ G1159A.
HRMS	Micromass GC-TOFmicro mass spectrometer

3.2 Plant Materials

Tephrosia rhodesica was collected in Kwale, coastal region of Kenya and *Tephrosia polyphylla* was collected in Kakamega County in July, 2017. The plants were identified with the help of a taxonomist, Mr. Patrick C. Mutiso of School of Biological Sciences, the University of Nairobi where vouchers specimens (MUP 001/July/2017 for roots of *T. rhodesica*; MUP 002/July/2017 for seedpods of *T. rhodesica* and MUP 003/July/2017 for stems of *T. polyphylla*) were deposited. Roots, seedpods and stems were air-dried at room temperature and ground to fine powder.

3.3 Extraction and Isolations of Compounds

3.3.1 Extraction and Isolations of compounds from the roots of *T. rhodesica*

The air dried and ground roots (1.7 Kg) of *T. rhodesica* were extracted with CH_2Cl_2/CH_3OH (1:1) (4×1.5 L) by cold percolation at room temperature. The extract was concentrated *in vacuo* using a rotary evaporator to yield 113 g of dark brown paste. A portion of the extract (100 g) was subjected to column chromatography over silica gel (700 g) eluting with hexane containing increasing amounts of EtOAc. The eluent with 2% EtOAc in hexane was crystallized from methanol to yield white crystals of 7-methylglabranin (**90**, 50 mg). The eluent with 4% EtOAc (8g) in hexane was further subjected to column chramotography on silica gel (80 g), eluting with hexane containing increasing amounts of CH_2Cl_2. The eluent at 30% CH_2Cl_2 gave white crystals of rhodimer (**88**, 20 mg) after crystallization from methanol.The fractions eluted with 6% EtOAc in hexane from the second column were combined (3.3 g) separated by column chromatography over silica gel (85 g) eluting with CH_2Cl_2 containing increasing amounts of EtOAc, The fractions at 30% CH_2Cl_2, were combined and further purified on a chromatotron using solvent system Hexane: CH_2Cl_2: EtOAc (5:1:0.5) to yield rhodbenzofuran (**86**, 28mg). The fractions eluted with 15 % EtOAc were combined and subjected to column chromatography on Sephadex LH-20 (CH_2Cl_2/CH_3OH (1:1) to give brown amorphous solid of flemichapparin (**92**, 20 mg) and 2-hydroxy-6-

methoxybenzoic acid (**89**, 23 mg). The less polar samples were combined and crystallized in methanol; Candidone (**91**, 40 mg) was also isolated from this column.

3.3.2 Extraction and Isolations of Compounds from the Seedpods of *T. rhodesica*

The air dried seedpods (550 g) of *T. rhodesica* was ground and extracted with CH_2Cl_2/CH_3OH (1:1) (4×1.5 L) by percolation at room temperature. The extract was concentrated *in vacuo* on a rotary evaporator to yield 28.7 g of a dark green solid. A portion of extract 1.7 g was kept for bioassay and other portion of the extract 26.0 g was subjected to column chromatography over silica gel (190 g) eluting with hexane containing increasing amount of EtOAc. The eluent with 6% EtOAc in hexane gave rhoflavononol (**87**, 50 mg) after column chromatography on Sephadex LH-20 (CH_2Cl_2/CH_3OH (1:1) and eluent at 10% were combined and purified by preparative TLC to obtained Teprowatsin (**93**, 18mg).

3.3.3 Extraction and Isolations of Compounds from the Stem of *T. polyphylla*

The air-dried stem (205 g) of *T. polyphylla* was ground and extracted with CH_2Cl_2/CH_3OH (1:1) (4×1.0 L) by cold percolation at room temperature. The extract was concentrated *in vacuo* on a rotary evaporator to yield 21 g of a dark green paste. A portion of the extract (20 g) was subjected to column chromatography over silica gel (200 g) eluting with petroleum ether containing increasing amounts of EtOAc. The eluent with 8-10% EtOAc in petroleum ether were combined and crystallized from methanol to yield white crystals of 4'-demethyltoxicarol isoflavone (**49**, 45 mg). The eluent with 30% EtOAc in petroleum ether gave 2',4',5,5'-tetramethoxy-2",2"-dimethylpyrano-[6',5"-h]isoflavone (**50**, 15mg).

3.4 Structure Modification

3.4.1 Oxime Derivative of Candidone (**91**)

To a 20 mg portion of Candidone (**91**) was added 6.0 mg of hydroxylamine hydrochloride and 7.0 mg of anhydrous sodium acetate in 1.0 ml of methanol as a solvent and heated at 60 ^{0}C for six hours. The sample mixture crystallized in acetonitrile to form white crystals of candidone-oxime (**94**, 19.60mg).

3.5 *Plasmodium falciparum* Growth Inhibition Assay

The preceding method of asexual *P. falciparum* imaging assay was used to determine parasite growth inhibition in accordance with the procedure described by Duffy and Avery (Duffy & Avery, 2012). In this method, 2-3% parasite (D6) and 0.3% hematocrit was incubated in total assay volume of 50 µL in presence of the tested compound for 72 hrs at 37 ^{o}C and 5% $CO_{2,}$ in a poly-D-lysine-coated cell carrier imaging plates. Plates were stained with DAPI (6,4'-diamidino-2-phenylindole) after incubation in presence of saponin and Triton X-100 and incubated for a further 5hrs at room temperature in the dark before obtaining digital images. The digital images obtained were then analyzed using the PerkinElmer Acapella spot detection software, where spots that fulfilled the function were counted. The percentage inhibitions of parasite replication were then calculated using DMSO and artemisinin control data.

3.6 **Properties of isolated and modified compounds**

7-methylglabranin (**90**)

White crystals. UV (MeOH) λ_{max}: 290 and 341 nm. CD (MeCN) λnm (Δε; $M^{-1}cm^{-1}$): $(-13.63)_{283}$; $(-3.62)_{240}$; $(20.49)_{222}$;$(1.30)_{250}$;$(4.25)_{309}$,^{1}H and ^{13}C NMR (Table 4.2). HR-ESI-MS ($[M+H]^{+}$m/z 339.4,calcd for $C_{21}H_{22}O_4$ 339.1551.

Candidone (**91**)

Yellow amorphous solid. UV (MeOH) λ_{max}: 286 and 322 nm. . CD (MeCN) λnm (Δε; $M^{-1}cm^{-1}$) $(-15.16)_{250}$; $(116.60.58)_{338.}$ ^{1}H and ^{13}C NMR (Table 4.3). LC-ESI-MS ($[M+H]^{+}$m/z 353.3, $C_{22}H_{24}O_4$.

Rhodbenzofuran (**86**)

Yellow amorphous solid. UV (MeOH) λ_{max}:260, 310 and 365 nm. ^{1}H and ^{13}C NMR (Table 4.1). HR-ESI-MS ($[M+H]^{+}$*m/z* 351.1596,calcdfor $C_{22}H_{22}O_4$351.1600.

Rhodimer (**88**)

White crystals. UV (MeOH) λ_{max}:235, 282 and 340 nm.CD (MeCN) λnm (Δε; $M^{-1}cm^{-1}$) $(-0.42)_{282}$; $(3.89)_{219.}$ ^{1}H and ^{13}C NMR (Table 4.4). LC-ESI-MS ($[M+H]^{+}$m/z 661.1, $C_{42}H_{44}O_7$.

2-Hydroxy-6-methoxybenzoic acid (**89**)

Brown amorphous solid.^{1}H NMR ($CDCl_{3,}$ 500 MHz):δ_H 6.85 (1H,d, *J*=8.0 Hz, H-3);7.48 (1H,dd, *J*=8.0, 2.0 Hz, H-4),7.57 (1H,d, *J*=2.0 Hz, H-5), 6.99 (1H,s, 2-OH),8.16 (1H,s,1'-OH), 3.90 (3H,s,6-OMe);^{13}C NMR ($CDCl_{3,}$ 125 MHz): δ_C127.2 (C-1),128.1 (C-2),114.3 (C-3),123.8(C-4),112.4 (C-5),147.2 (C-6),151.0 (C-1'),61.4 (6-OMe).

Flemichapparin (**92**)

Brown amorphous solid. UV (MeOH) λ_{max}:286 and 322 nm. ^{1}H and ^{13}C NMR (Table 4.6). LC-ESI-MS ($[M+H]^{+}$m/z 297.3, $C_{17}H_{12}O_5$.

Rhodflavononol (**87**)

Yellow oily substance. UV (MeOH) λ_{max}:216 and 280 nm. CD (MeCN) λ nm (Δε; $M^{-1}cm^{-1}$) $(-5.30)_{222}$; $(-1.05)_{222}$; $(1.58)_{219,}$ ^{1}H and ^{13}C NMR (Table 4.8). HR-ESI-MS ($[M+H]^{+}$*m/z* 369.1702,calcd for $C_{22}H_{24}O_5$ 369.1700).

Tephrowatsin (**93**)

Brown amorphous solid. UV (MeOH) λ_{max}: 235 and 282 nm. CD (MeCN) λnm (Δε; $M^{-1}cm^{-1}$) $(-9.86)_{224}$; $(-1.73)_{280}$; $(2.56)_{249,}$ ^{1}H and ^{13}C NMR (Table 4.7). LC-ESI-MS ($[M+H]^{+}$*m/z* 337.2, $C_{22}H_{24}O_3$.

4'-Demethyltoxicarol isoflavone (**49**)

White crystals. UV (MeOH) λ_{max}:216 and 280 nm. ^{1}H and ^{13}C NMR (Table 4.9). hr-ESI-MS ($[M+H]^{+}$*m/z* 397.0000,calcd for $C_{22}H_{20}O_7$397.1200.

2',4',5,5'-Tetramethoxy-2",2"-dimethylpyrano-[6',5"-h]isoflavone (**50**)

Yellow amorphous solid. UV (MeOH) λ_{max}:230 and 285 nm. ^{1}H and ^{13}C NMR (Table 4.10). LC-ESI-MS ($[M+H]^{+}$m/z 425.0000,calcd for $C_{24}H_{24}O_7$425.1600.

Candidone-oxime (**94**)

White crystals. UV (MeOH) λ_{max}:237 and 280 nm. CD (MeCN) λnm (Δε; $M^{-1}cm^{-1}$) $(-1.76)_{267}$; $(-1.16)_{234}$; $(6.13)_{210}$;$(1.24)_{250.}$ ^{1}H and ^{13}C NMR (Table 4.11). LC-ESI-MS ($[M+H]^{+}$m/z 368.3, $C_{22}H_{25}NO_4$.

CHAPTER 4: RESULTS AND DISCUSSIONS

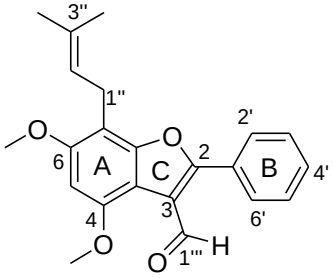
4.1: Characterization of Isolated Compounds

4.1.1: Compounds from roots of *Tephrosia rhodesica*

Phytochemical investigation of the roots of *Tephrosia rhodesica* resulted in the isolation of six compounds of which one is new. The characterization of these compounds is discussed in the following sections.

4.1.1.1: Rhodbenzofuran (**86**)

Compound **86** was isolated as a yellow amorphous solid whose molecular formula was deduced as $C_{22}H_{22}O_4$ based on HR-ESI-MS ($[M+H]^+$ m/z 351.1596) and NMR analyses (Table 4.1).The UV spectrum (λ_{max} 260, 310 and 365 nm) along with the ^{13}C NMR signals at δ_C 158.6 (C-2) and δ_C 117.6 (C-3) were indicative of a 2-arylbenzofuran skeleton (Fukai *et al*., 1996; Iinuma *et al*., 1994). The presence of a formyl group at C-3 was evident from HMBC correlation of the formyl proton at δ_H 10.60 (H-1$^{'''}$) with δ_C 158.6 (C-2) and δ_C 117.6 (C-3).



86

The presence of two methoxy and a prenyl groups was evident from the NMR spectra (Table 4.1). The placement of two methoxy (δ_H 3.89, δ_C 55.9 and δ_H 3.85, δ_C 57.0) groups were established by HMBC correlations (Table.4.1) of these groups with C-4 (δ_C 152.7) and C-6 (δ_C 156.4), respectively, on ring A. In support of this, both methoxy groups

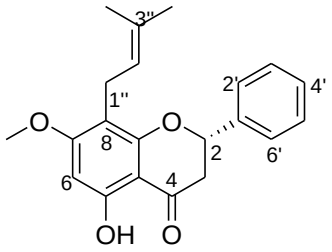
showed NOE correlation with H-5 (δ_H 6.50). Finally the prenyl group was placed at C-7 from the HMBC correlations of CH_2-1" (δ_H 3.56) to C-6 (δ_C 156.4),C-7 (δ_C 106.8), C-7a (δ_C 154.1), C-2" (δ_C 122.3) and C-3" (δ_C 132.0).The singlet at (δ_H 6.50) in ring A was assigned to H-5 due to its HMBC correlations (Table.4.1) to C-3a (δ_C 110.4), C-4 (δ_C 152.7) , C-6 (δ_C 156.4) and C-7 (δ_C 106.8). The NMR spectral data showed that, δ_C 130.7 (C-1'); δ_C 128.5 (C-2'/6'), δ_H 8.20 (H-2'/6'); δ_C 128.9 (C-3'/5'), δ_H 7.50 (H-3'/5'); δ_C 129.6 (C-4'), δ_H 7.48 (H-4') and further showed ring B is unsubstituted. The attachment was further supported by HMBC correlations (Table.4.1) of H-2'/6' (δ_H 8.20) to C-2 (δ_C 158.6), C-1'(δ_C 130.7) and C-3'/5' (δ_C 128.9). Based on the above spectroscopic data, this new compound was characterized as 4,6-dimethoxy-7-(3-methylbut-2-en-1-yl)-2-phenyl-1-benzofuran-3-carbaldehyde and given the trivial name rhodbenzofuran.

Table 4.1: ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data for compound (**86)**, $CDCl_3$

Position	δ_C	$\delta_{H,}$ *m*, (*J* in Hz)	HMBC
2	158.6		
3	117.6		
3a	110.4		
4	152.7		
5	92.4	6.50 *s*	C-3, C-4, C-4a, C-6, C-7
6	156.4		
7	106.8		
7a	154.1		
1'	130.7		
2'/6'	128.5	8.20 *m*	C-1', C-2, C-3', C-5',
3'/5'	128.9	7.50 *m*	C-2', C-6'
4'	129.6	7.48 *m*	C-3' , C-5'
1"	22.4	3.56	C-2", C-3", C-6, C-7, C-7a
2"	122.3	5.32	C-1", C-3", C-4",C-5"
3"	132.0		
4"	18.0	1.85 *s*	C-2",C-3",C-5"
5"	25.9	1.69 *s*	C-2",C-3",C-4"
4-OMe	55.9	3.89 *s*	C-4
6-OMe	57.0	3.85 *s*	C-6
1"'	187.8	10.60 *s*	C-2, C-3

4.1.1.2: 7-Methylglabranin (**90**)

Compound **90** was isolated as white crystals and had characteristic UV absorption maxima at (290 and 341 nm) of flavanones. The formula $C_{21}H_{22}O_4$ was established from the molecular ion peak ($[M+H]^+$ m/z 339.4 in the LC-ESI-MS) and NMR spectra (Table 4.2). In addition, the NMR data (Table.4.2) showed the presence of oxygenated methine signal for H-2 (δ_H 5.41, 1H, dd, *J*=12.7, 3.2 Hz; δ_C 78.8,), the methylene signals for H-3ax (δ_H 3.05, δ_C 43.6, 1H, dd, *J*=17.1, 12.7 Hz) and H-3eq (δ_H 2.85, δ_C 43.6, 1H, dd, *J*=17.1, 3.2 Hz) with AMX system, typical of ring C of flavanones. This was also supported by the HMBC correlations (Table.4.2) of H-3eq to C-2 (δ_C 78.8), C-4 (δ_C 196.4) and C-1' (δ_C 139.1). That ring B is unsubstituted was apparent from the ^{1}H [δ_H 7.42 for H-2'/6'; δ_H 7.45 for H-3'/5' and δ_H 7.39 for H-4] and ^{13}C [δ_C 139.1 for C-1'; δ_C 128.9 for C-2'/6'; δ_C 126.1 for C-3'/5' and δ_C 128.7 for C-4'] NMR spectra. The nature of ring B was supported by HMBC correlations (Table.4.2) of H-2'/6' (δ_H7.42) to C-2 (δ_C 78.8), C-1'(δ_C 139.1) and C-3'/5' (δ_C 126.1). The proposed flavanone skeletal structure was also evident in COSY experiment. In the COSY experiment, the oxygenated methine proton at δ_H 5.41 (H-2) showed cross peaks with methylene protons at δ_H 3.05 (H-3ax) and δ_H 2.85 (H-3eq) confirming the flavanone ring C structure.



90

In ring A, the presence of signals for methylene group (δ_C 21.8, δ_H 3.24, 2H, d, *J*=7.3 Hz for CH_2-1''), a prenyl group was established by (δ_H 5.15, 1H, and δ_C 122.6) for CH-2'') and two methyls (δ_H1.66, δ_C 17.8 for Me-4''; and δ_H 1.63, δ_C 25.9, for Me-5'').The location of this prenyl at C-8 on ring A was supported by HMBC correlations (Table.4.2) of H-1'' to C-8 (δ_C 109.2),C-8a (δ_C 158.9), C-7 (δ_C 165.9),C-2'' (δ_C 122.6) and C-3'' (δ_C 132.5).

Additional substituents in ring A were the hydrogen bonded hyroxy (δ_H 12.2) and methoxy (δ_H 3.86,;δ_C 56.1) groups located on C-5 and C-7 respectively. The locations of these substituents were supported by HMBC correlations (Table.4.2), of 5-OH to C-5 and a prenyl group at C-8. In the ^{1}H NMR spectrum, one aromatic proton signal was observed at δ_H 6.10 (δ_C 92.6, 1H, s, H-6) only with the oxygenation of aromatic ring A at C-5 (δ_C 162.8) and C-7 (δ_C 165.9). The nature of ring A was confirmed by HMBC correlations (Table.4.2) of H-6 to C-5 (δ_C 162.8), C-7 (δ_C 165.9), C-4a (δ_C 103.1) and C-8 (δ_C 109.2). The absolute configuration of C-2 was determined to be (2*S*) from X-ray single crystal structure (Figure 4.1) and positive Cotton effect at 339, and a negative Cotton effect at 283 nm (Figure 4.2) in the CD spectrum (Slade *et al*., 2005). Through forgoing discussion and comparing with literature, compound **90** was identified as (2*S*)-5-hydroxy-7-methoxy-8-(3-methylbut-2-en-1-yl)-2-phenyl-2,3-dihydro-4H-chromen-4-one, with a trivial name, 7-methylglabranin, which was previously isolated from *Tephrosia vilosa* (Jayaraman *et al*., 1980).

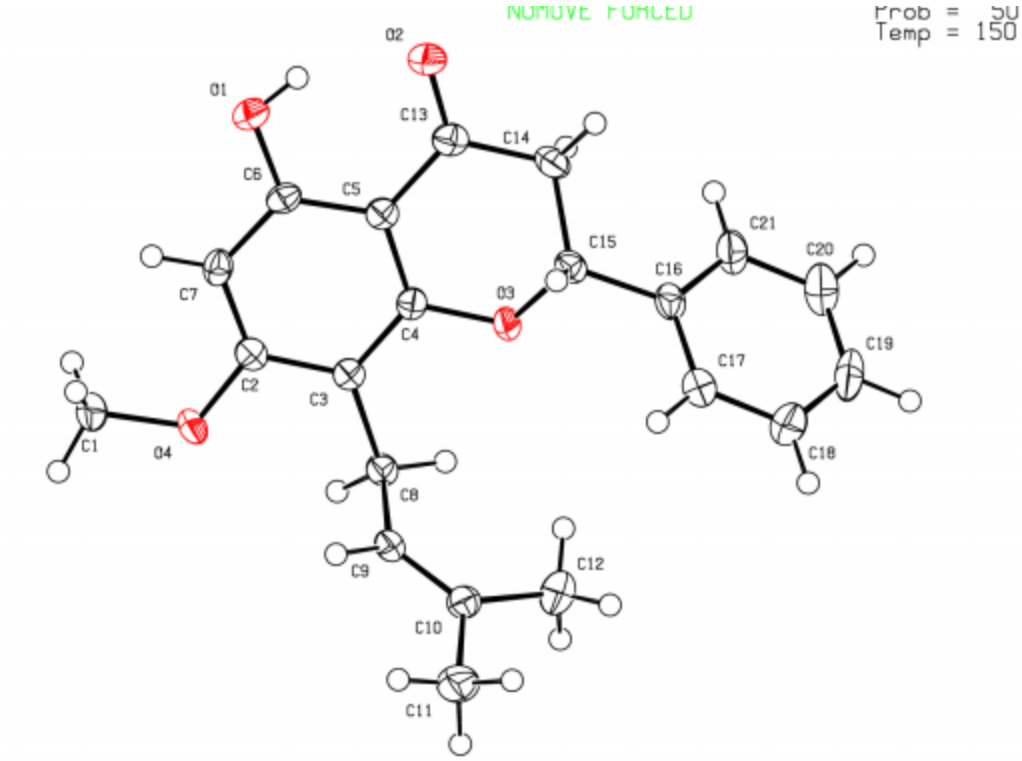
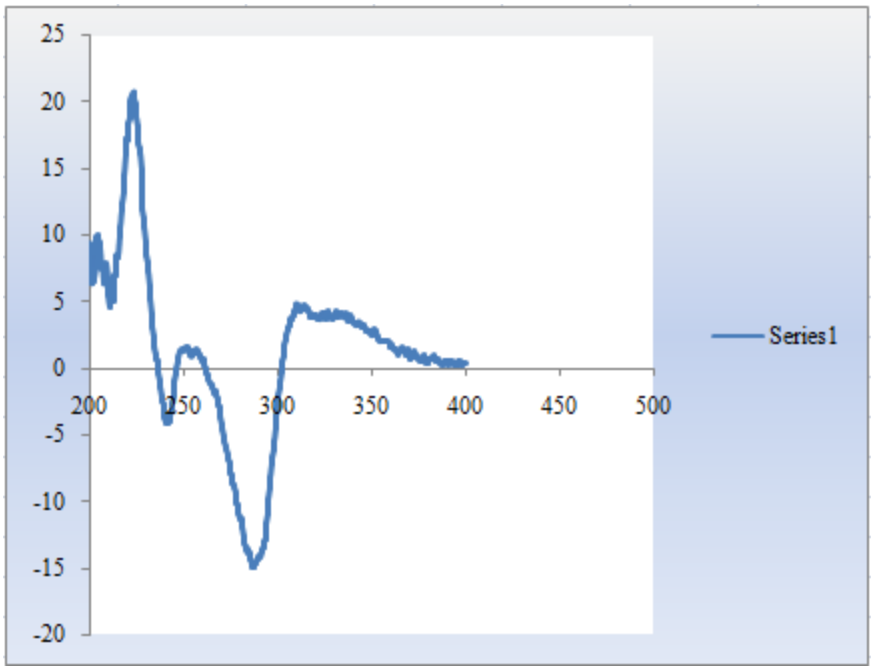


Figure 4.1: X-ray single crystal structure of compound **90**

mdeg



(Wave length) nm

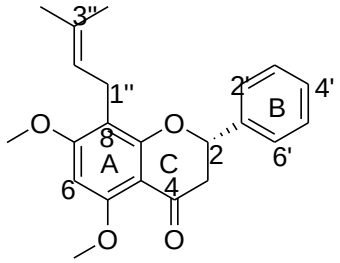
Figure 4.2: ECD spectrum of compound (**90)**

Table 4.2:^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data for compound (**90)**, $CDCl_3$

Position	δ_C	δ_H, *m*, (J in Hz)	HMBC
2	78.8	5.41, *dd* (12.7, 3.2)	C-4, C-1'
3	43.6	3.05, *dd* (17.1, 12.7)	C-4
		2.85, *dd* (17.1, 3.2)	C-2, C-4, C-1'
4	196.4		
4a	103.1		
5	162.8		
6	92.6	6.10 *s*	C-4a, C-5, C-7, C-8
7	165.9		
8	109.2		
8a	158.9		
1'	139.1		
2'/6'	128.9	7.42 *m*	C-1'
3'/5'	126.1	7.45 *m*	C-4'
4'	128.7	7.39 *m*	C-3'/5'
1''	21.8	3.24*d*,(7.3)	C-2'', C-3'', C-7, C-8, C-8a
2''	122.6	5.15	C-4'', C-5''
3''	131.5		
4''	17.8	1.66 *s*	C-2'', C-3'', C-5''
5''	25.9	1.63 *s*	C-2'', C-3'', C-4''
5-OH		12.2 *s*	C-5, C-6, C-4a
7-OMe	56.1	3.86 *s*	C-7

4.1.1.3: Candidone (**91**)

Compound **91** was isolated as a yellow amorphous solid and showed characteristics UV (λ_{max} 286 and 322 nm) and NMR [(δ_H 5.40, dd, *J*=12.8, 3.1 Hz for H-2; δ_C 78.6,), (δ_H 2.98, dd, *J*=16.6, 12.8 Hz) for H-3_{ax} and (δ_H 2.84, dd, *J*=16.6, 3.1 Hz; H-3_{eq}; δ_C 45.8)]; δ_C 190.0 for C-4 spectra of a flavanone. That this compound is a flavanone derivative was also supported by the HMBC correlations of H-3_{eq} to C-2 (δ_C 78.6), C-4 (δ_C 190.0) and C-1' (δ_C139.3). In the HH-COSY experiment, the oxygenated methine at δ_H 5.40 (H-2) showed correlations with methylene protons at δ_H 2.98 (H-3_{ax}) and δ_H 2.84 (H-3_{eq}), again typical of ring C protons of flavanones. The molecular formula $C_{22}H_{24}O_4$ was proposed from the LC-ESI-MS spectrum which showed a protonated molecular ion peak ($[M+H]^+$ at *m/z* 353.3, and NMR spectral data (Table 4.3). As in compound **91**, NMR spectral data (Table 4.3) support unsubstituted ring B.



91

In ring A, the presence of a prenyl group at C-8 [H-1'' (δ_H3.29, δ_C21.9, 2H, dd, J=7.4,3.6 Hz) and two methyl groups (δ_H 1.65, δ_C 17.7, CH_3-4'') and (δ_H 1.63, δ_C 25.8, 3H,s)] and two methoxy groups at C-5 (δ_H 3.90, δ_C 55.7) and C-7 (δ_H 3.93, δ_C 55.7) was apparent from the NMR spectral data (Tabe 4.3). The location of this prenyl at C-8 was established from HMBC correlations (Table 4.3) of H-1'' to C-8 (δ_C 110.3), C-8a (δ_C 161.1), C-7 (δ_C 163.3), C-2'' (δ_C 122.5) and C-3'' (δ_C 131.5). The location of the methoxy group at C-5 (δ_C 160.7) and C-7 (δ_C 163.3) was established from HMBC spectrum (Table 4.3). The absolute configuration of C-2 was determine to be (2S) from positive

Cotton effect at 338 in the ECD spectrum (Figure 4.3.) (Slade *et al*., 2005). Based on these spectroscopic data and comparing with the literature, compound **91** was identified as (2S)-2,3-dihydro-5,7-dimethoxy-8-(3-methylbut-2-enyl)-2-phenylchromen-4-one, with a trivial name as candidone which was previously isolated from *Tephrosia candida* (Roy *et al*., 1986).

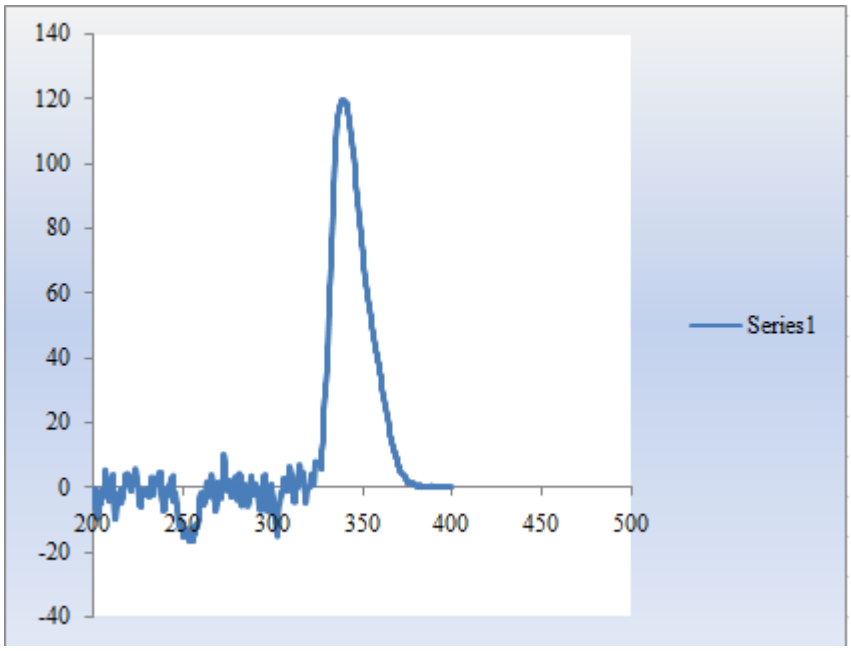


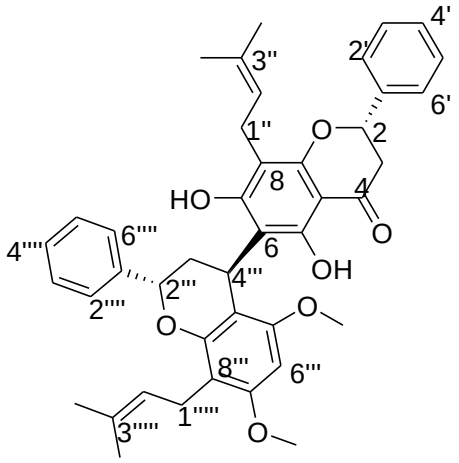
Figure 4.3: ECD spectrum of compound (**91)**

Table 4.3: ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data for compound (**91**), $CDCl_3$

Position	δ_C	δ_H, *m*, (*J* in Hz)	HMBC
2	78.6	5.40, *dd* (12.8, 3.1)	C-1', C-4
3	45.8	2.98, *dd* (16.6, 12.8)	C-4
		2.84, *dd* (16.6, 3.1)	C-1', C-2, C-4
4	190.0		
4a	106.1		
5	160.7		
6	88.7	6.13 *s*	C-4a, C-5, C-7, C-8
7	163.3		
8	110.3		
8a	161.1		
1'	139.3		
2'/6'	128.6	7.40 *m*	C-1'
3'/5'	125.9	7.46 *m*	C-4'
4'	128.3	7.35 *m*	C-3'/5'
1''	21.9	3.29*bt*	C-2'', C-3'', C-7, C-8, C-8a
2''	122.5	5.16*bt*	C-4'',C-5''
3''	131.5		
4''	17.7	1.65 *s*	C-2'', C-3'', C-5''
5''	25.8	1.63 *s*	C-2'', C-3'', C-4''
5-OMe	56.1	3.90 *s*	C-5
7-OMe	55.7	3.93 *s*	C-7

4.1.1.4: Rhodimer (**88**)

Compound **88** was isolated as white crystals and showed a characteristics UV absorption maxima at (235, 282 and 340 nm) of flavanones It was also apparent from the MS and NMR data that this compound is a dimeric flavonoid. In one half of the molecule, the ^{1}H and ^{13}C NMR spectra showed signals for oxygenated methine (δ_H 5.43, 1H, dd, *J*=13.2, 3.0 Hz for H-2; δ_C 79.0), methylene (δ_H 3.07, 1H, dd, *J*=17.0, 13.2 Hz for H-3_{ax} and δ_H 2.84, 1H, dd, *J*=17.0, 3.0 Hz for H-3_{eq}; δ_C 44.1,) of a flavanone skeletone. Another half of the molecule is attached at C-4‴, showing that compound **88** is a flavanone-flavan dimmer.



88

The NMR data (Table 4.4) showed two sets of unsubstituted aromatic rings δ_C 141.8 (C-1′); δ_C 129.1 (C-2′/6′), δ_H 7.48 (H-2′/6′); δ_C 126.4 (C-3′/5′), δ_H 7.37 (H-3′/5′); δ_C 128.9 (C-4′), δ_H 7.34 (H-4′) and δ_C 139.5 (C-1‴); δ_C 126.5 (C-2‴/6‴), δ_H 7.39 (H-2‴/6‴); δ_C 128.7 (C-3‴/5‴), δ_H 7.44 (H-3‴/5‴); δ_C 128.0 (C-4‴), δ_H 7.29 (H-4‴), two sets of prenyl groups δ_C 22.4 (C-1″), δ_H 3.20 (H-1″); δ_C 123.0 (C-2″), δ_H 5.15 (H-2″); δ_C 131.3 (C-3″); δ_C 18.2 (C-4″), δ_H 1.66 (H-4″); δ_C 26.3 (C-5″), δ_H 1.58 (H-5″) and δ_C 26.2 (C-1⁗), δ_H 3.36 (H-1⁗); δ_C 123.6 (C-2⁗), δ_H 5.06 (H-2⁗); δ_C 131.9 (C-3⁗); δ_C 22.2 (C-4⁗), δ_H 1.69 (H-4⁗); δ_C 27.3 (C-5⁗), δ_H 1.65 (H-5⁗). The important HMBC correlations observed were H-3ax and H-

3eq to C-2 (δ_C 79.0); C-3 (δ_C 196.7) and C-1$^{'}$ (δ_C 141.8), H-3ax$^{'''}$ and H-3eq$^{'''}$ to δ_C 75.8 (C-2$^{''''}$) and δ_C 110.2 (C-4a$^{''''}$).

The relative configuration (2$^{'''}$S*, 4$^{'''}$R*) was established from the X-ray structure (Figure 4.4) of compound **88**. Comparing these spectroscopic data with the available literature, compound **88** was found to be a rhodimer isolated from roots of *Tephrosia rhodesica.*

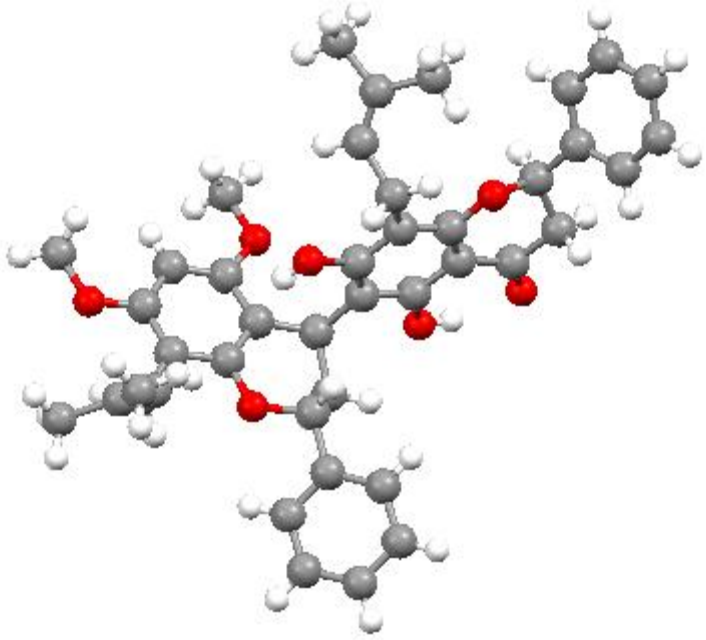


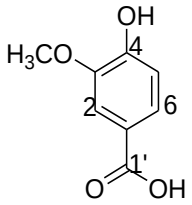
Figure 4.4: X-ray single crystal structure of compound (**88)**

Table 4.4: ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data for compound (**88**), $CDCl_3$

Position	δ_C	$\delta_{H,}$ *m*, (J in Hz)	HMBC	Position	δ_C	$\delta_{H,}$ *m*, (J in Hz)	HMBC
2	79.0	5.43,*dd* (13.2, 2.9)	C-4, C-1', C-2'/6'	2'''	75.8	5.24,*dd* (11.4, 2.1)	C-3'''
3	44.1	2.84,*dd* (17.0, 2.9) 3.07,*dd* (17.0,13.2)	C-2, C-4,C-1' C-4	3'''	22.4	2.18,*dt* (14.0,11.4,5.80),2.32,*dt* (14.0,3.1,2.2)	C-4''', C-6 C-4'''
4	196.7			4'''	37.4	4.66,*dd* (5.8,2.1)	C-5,C-7,C-4a, C-8'''a, C-2'''
4a	100.8			4a'''	110.2		
5	159.7			5'''	157.9		
6	103.2			6'''	88.8	6.17 *s*	C-5''',C-7''',C-8''', C-4''', C-4'''a
7	159.1			7'''	163.2		
8	108.5			8'''	111.3		
8a	158.0			8'''a	154.7		
1'	141.1			1''''	139.5		
2'/6'	129.1	7.47 *m*	C-2, C-3'/5'	2''''/6''''	126.5	7.39 *m*	C-2'''', C-3''''/5''''
3'/5'	126.4	7.37 *m*	C-2'/6'	3''''/5''''	128.7	7.44 *m*	C-2''''/6''''
4'	128.9	7.34 *m*	C-3'/5'	4''''	128.0	7.29 *m*	C-3''''/5''''
4''	18.2	1.66 *s*	C-2'',C-3'', C-5''	4'''''	22.2	1.69 *s*	C-2''''',C-3''''', C-5'''''
5''	26.3	1.58 *s*	C-2'',C-3'', C-4''	5'''''	27.3	1.65 *s*	C-2''''',C-3''''', C-4'''''
5-OH		12.64 *s*	C-5	5'''-OMe	56.3	3.74 *s*	C-5'''
7-OH		6.81 *s*	C-6	7'''-OMe	56.2	3.87 *s*	C-7'''

4.1.1.5: 4-Hydroxy-3-methoxybenzoic acid (**89**)

Compound **89** was isolated as brown amorphous solid, with molecular formula $C_8H_8O_4$ which was assigned based on LC-ESI-MS spectrum ($[M+H]^+$ m/z 169.1) together with ^1H and ^{13}C data (Table 4.5). The ^1H NMR spectral data showed a 1,3,4-trisubstituted benzene ring with signals appearing at (δ_H 7.57, d, 2.0 Hz for H-2, δ_H 6.85, d, *J*=8.0 Hz, for H-5), (δ_H 7.48, dd, *J*=8.0, 2.0 Hz, for H-6).These substituents being carboxylic acid at C-1 (δ_C 168.3), methoxy at C-3 (δ_H 3.90, δ_C 54.9) and and hydroxy at C-4 (δ_C 150.9). The methoxy group was placed at C-3 from the HMBC correlation of the methoxy proton (δ_H 3.90) to C-3 (δ_C 147.1). The substitution pattern was supported by HMBC spectrum (Table 4.5). Thus this compound was characterized as 4-hydroxy-3-methoxybenzoic (**89**). (Pouchert and Behnke, 1993).



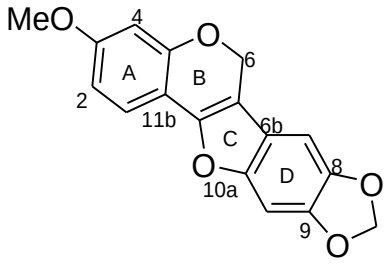
89

Table 4.5: ^1H (400 MHz) and ^{13}C (100 MHz) NMR data for compound (**89**), $CDCl_3$

Position	δ_C	δ_H, *m*, (J in Hz)	HMBC
1	128.1		
2	112.3	7.57, *d* (2.0)	C-1', C-3, C-4, C-6
3	147.1		
4	150.9		
5	114.3	6.84, *d* (8.0)	C-3, C-4, C-6,
6	123.7	7.50, *dd* (8.0, 2.0)	C-1', C-2, C-4
1'	168.3		
2-OH		6.99 *s*	
1'-OH		8.16 *s*	
6-OMe	54.9	3.90 *s*	C-6

4.1.1.6: Flemichapparin-B (**92**)

Compound **92** was isolated as brown amorphous solid and showed UV absorption maxima at 286 and 322 nm. The molecular formula $C_{17}H_{12}O_5$ was proposed from the $[M+H]^+$ peak at *m/z* 297.3 from the LC-ESI-MS and NMR spectra (Table 4.6). The ^1H (δ_H 5.51, 2H, s, H_2-6) and ^{13}C (δ_C 66.1 for C-6, 107.6 for C-6a and 150.9 for C-11a) NMR spectra indicated a pterocarpene skeleton. In support of this, the HMBC spectrum showed correlations of H-2 to C-6a (δ_C 107.6), C-6b (δ_C 119.6), C-4a (δ_C 155.5) and C-11a (δ_C 150.9). The presence of methylenedioxy group (δ_H 5.99, 2H, s, δ_C 102.2) and methoxy group (3-OMe, δ_H 3.79, δ_C 56.0) were evident from the NMR spectra (Table 4.6).



92

The ^1H NMR and HH-COSY spectra revealed the presence of three aromatic protons with an AXY spin system, assigned to H-1 (δ_H 3.5, 1H, d, *J*=8.4 Hz), H-2 (δ_H 6.53, 1H, dd, *J*=2.4, 8.4 Hz) and H-4 (δ_H 6.49, 1H, d, *J*=2.4),with the corresponding carbon signals appearing at δ_C 121.3 (C-1), δ_C 107.1 (C-2) and δ_C 102.9 (C-4), respectively of ring A which is oxygenated at C-3, as expected biogenetically. In the HMBC spectrum, H-1 showed correlation with C-11a (δ_C 150.9), C-4a (δ_C 155.5) and C-3 (δ_C 161.5), H-2 to C-11b (δ_C 110.6) and C-4 (δ_C 146.3), H-4 to C-11b (δ_C 146.3) and 3-OMe to C-3 (δ_C 161.5). In ring D, the presence of two para-oriented aromatic protons at δ_H 7.03 for H-7 (δ_C 94.4) and δ_H 6.67 for H-10 (δ_C 97.8) is consistent with the placement of the methylenedioxy group between C-8 and C-9. The substitution pattern in ring D was confirmed by HMBC experiments where H-7 showed correlation with C-8 (δ_C 145.4), C-9 (δ_C 146.3) and C-10a (δ_C 148.2). Putting all these together and comparing the data with literature, this

compound was identified as 6a,11a-dehydropterocarpan given a trivial name flemichapparin-B (**92**) previously isolated from *Flemingia chappar* (Adityachaudhury& Gupta, 1973). However, this is the first report from *Tephrosia* species.

Table 4.6: ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data for compound (**92**), $CDCl_3$

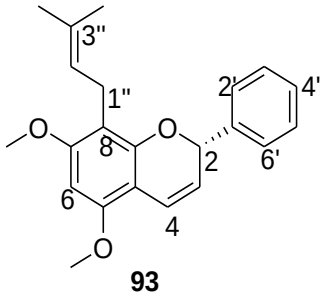
Position	δ_C	$\delta_{H,}$ *m*, (*J* in Hz)	HMBC
1	121.3	7.35 ,*d* (8.4)	C-3,C-4a,C-11a
2	107.1	6.53 ,*dd* (2.42, 8.4)	C-4,C-11b
3	161.5		
4	102.9	6.49, *d* (2.4)	C-11b
4a	155.5		
6	66.1	5.51 *s*	C-4a,C-6a,C-6b,C-11a
6a	107.6		
6b	119.6		
7	94.4	7.03 *s*	C-8,C-9,C-10a
8	145.4		
9	146.3		
10	97.8	6.67 *s*	C-6a
10a	148.2		
11a	150.9		
11b	110.3		
OCH_2O	102.2	5.99 *s*	C-8,C-9
OMe	56.0	3.79 *s*	C-3

4.1.2: Compounds from seedpods of *Tephrosia rhodesica*

From the seedpods of *Tephrosia rhodesica* two compounds were isolated, of which one is new. The characterization of these compounds is discussed below.

4.1.2.1: Tephrowatsin B (**93**)

Compound **93** was obtained as brown amorphous solid. The LC-ESI-MS ($[M+H]^+$ peak at *m/z* 337.2), UV (λ_{max} 235 and 282 nm), ^{1}H NMR [which showed the presence of oxygenated methine protons at δ_H 5.84 (1H, dd, *J*=3.7, 1.8 Hz, for H-2) and two mutually coupled olefinic protons δ_H 5.66 (1H, dd, *J*=9.9, 3.7 Hz, for H-3) and δ_H 6.83 (1H, dd, *J*=9.9, 1.8 Hz for H-4)] and the ^{13}C NMR spectral data [δ_C 77.0 (for C-2), δ_C 120.1 (for C-3) and δ_C 119.1(for C-4)] suggested that the compound is a flav-2-ene derivative with molecular formula $C_{22}H_{24}O_3$. The presence of unsubstituted ring B [δ_C 141.2 (C-1′); δ_C 127.1 (C-2′/6′), δ_H 7.43 (H-2′/6′); δ_C 128.5 (C-3′/5′), δ_H 7.33 (H-3′/5′); δ_C 128.1 (C-4′), δ_H 7.20 (H-4′)], a prenyl [δ_H 3.21(δ_C 21.8, for CH_2-1″), δ_H 5.06 (δ_C 123.3 (for CH-2″)] and two methyl groups at [δ_H 1.63 (δ_C 17.8, for CH_3-4″) and δ_H 1.57 (δ_C 25.9, for CH_3-5″)] and two methoxy groups [δ_H 3.81 (δ_C 55.9) and δ_H 3.82 (δ_C 55.8, 3H, s)] was also evident from the NMR spectra (Table 4.7).



93

In ring A, only one aromatic proton signal was observed at δ_H 6.05 (δ_C 88.7), a ring which otherwise is substituted with two methoxy groups (at C-5 and C-7, from biogenetic grounds), a prenyl (either be placed at C-6 or C-8). The location of prenyl at C-8 was established by HMBC correlations (Table 4.7) of H-1″ to C-8 (δ_C 110.6), C-8a (δ_C 152.1), C-7 (δ_C 158.6), C-2″ (δ_C 123.3) and C-3″ (δ_C 130.8). The 5-OMe and 7-OMe

location on ring A was supported by HMBC correlations (Table 4.7) for these groups to C-5 (δ_C 154.1) and C-7 (δ_C 158.6) respectively. The other observed key HMBC correlations (Table 4.7) include H-6 to C-5 (δ_C 154.1), C-7 (δ_C 158.6), C-4a (δ_C 104.8) and C-8 (δ_C 110.6), H-2 to C-3 (δ_C 120.1), C-4 (δ_C 119.1) and C-1' (δ_C 141.1), H-3 to C-4a (δ_C 104.8), H-4 to C-2 (δ_C 77.0) and C-8a (δ_C 152.1). The absolute configuration at C-2 was determine to be (2S) from positive Cotton effect at 250 and the negative Cotton effect at 224 and 280 nm) (Slade *et al*., 2005) in the ECD spectrum (Figure 4.5). Hence from the spectral data and comparing with literature, compound **93** was identified as (2*S*)-5,7-dimethoxy-8-(3-methylbut-2-enyl)-2-phenyl-2H-chromene, trivial name tephrowatsin B, previously reported from *Tephrosia watsoniana* (Gomez *et al*., 1985), however, this is the first report from *Tephrosia rhodesica.*

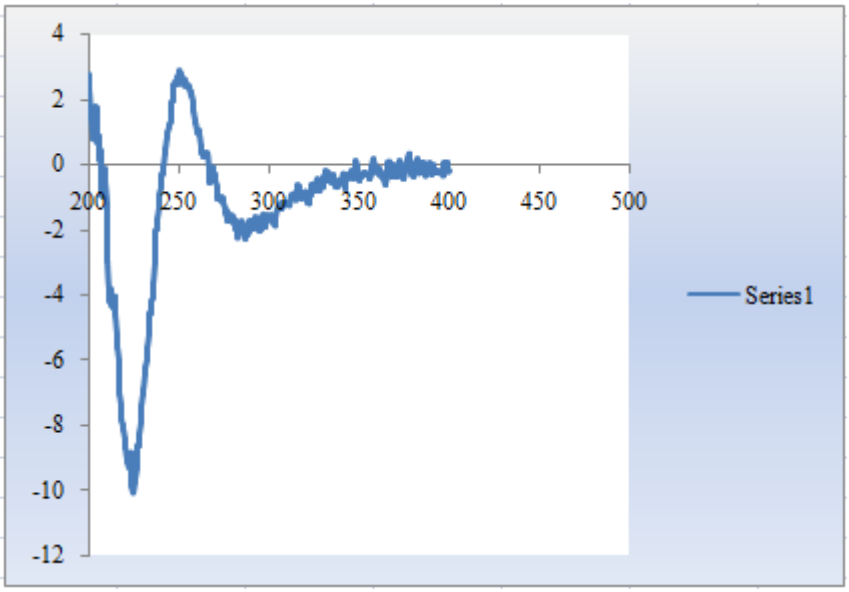


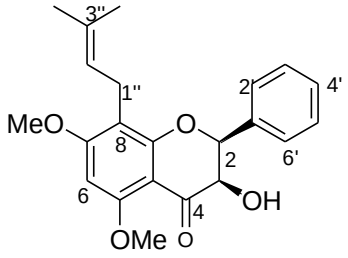
Figure 4.5: ECD spectrum of compound (**93**)

Table 4.7: ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data for compound (**93**), $CDCl_3$

Position	δ_C	δ_H, *m*, (J in Hz)	HMBC
2	77.0	5.84, *dd* (1.8, 3.7)	C-3,C-4, C-1'
3	120.1	5.66 ,*dd* (3.7, 9.9)	C-4a
4	119.1	6.83 ,*dd* (1.8, 9.9)	C-2, C-8a
4a	104.8		
5	154.1		
6	88.7	6.05 *s*	C-5, C-7, C-8 ,C-4a
7	158.6		
8	110.6		
8a	152.1		
1'	141.2		
2'/6'	127.1	7.43 *m*	
3'/5'	128.5	7.33 *m*	C-4'
4'	128.1	7.29 *m*	C-3'/5'
1''	21.8	3.21	C-2'',C-3'',C-7,C-8,C-8a
2''	123.3	5.06	
3''	130.8		
4''	17.8	1.63 *s*	C-2'',C-3'',C-5''
5''	25.9	1.57 *s*	C-2'',C-3'',C-4''
5-OMe	55.9	3.81 *s*	C-5
7-OMe	55.8	3.82 *s*	C-7

4.1.2.2: Rhodflavononol (**87**)

Compound **87** was isolated as a yellow oily substance. HRMS showed a $[M+H]^+$ peak at *m/z* 369.1702, which along with NMR data (Table 4.8) was consistent with the molecular formula $C_{22}H_{24}O_5$. The UV (λ_{max} 216 and 280 nm), ^{1}H NMR which showed an AX spin system [δ_H 5.24, 1H, d, *J*=12.5 Hz (for H-2) and at δ_H 4.55, 1H, d, *J*=12.5 Hz (for H-3)] and ^{13}C NMR which showed signals at [δ_C 87.9 (C-2), δ_C 72.8 (C-3) and δ_C 170.9 (C-4)] spectral data suggested that compound **87** is a flavononol derivative.



87

In the HMBC spectrum (Table 4.8), correlations of H-2 to C-3 (δ_C 72.8), C-4 (δ_C 170.9) and C-1$^{'}$ (δ_C 141.1) supported the flavononol skeleton. The NMR spectral data (Table 4.8) showed that ring B in compound **87** is unsubstituted as in compound **93**.The presence of prenyl group (Table 4.8) and two methoxy groups [(δ_H 3.83, δ_C 55.5 for 5-OMe) and (δ_H 3.86, δ_C 55.9 for 7-OMe] in ring A was also evident from NMR spectral data (Table 4.8). The only aromatic proton in this ring was observed at δ_H 6.13 (δ_C 88.2). As in compound **93**, the two methoxy groups were fixed at C-5 and C-7 based on HMBC correlations (Table 4.8) of 5-OMe and 7-OMe to C-5(δ_C 153.6) and C-7(δ_C 158.2) respectively (Table 4.8). The location of prenyl group at C-8 was established from HMBC correlations of H-1$^{''}$ to C-8 (δ_C 119.8), C-2$^{''}$ (δ_C 123.3) and C-3$^{''}$ (δ_C 130.3).

The large coupling constant ($J_{2,3}$ = 12.5 Hz) showed that H-2 and H-3 are trans-oriented together with the negative Cotton effect at 290 nm in the ECD spectrum (Figure 4.6) (Slade *et al*., 2005). The absolute configuration at C-2 and C-3 was determined to be (2*S*, 3S). This new compound was characterized as (2*S*, 3*S*)-3-hydroxy-5,7-dimethoxy-8-

(3-methylbut-2-en-1-yl)-2-phenyl-2,3-dihydro-4H-chromen-4-one, and given the trivial name rhodflavononol (**87**).

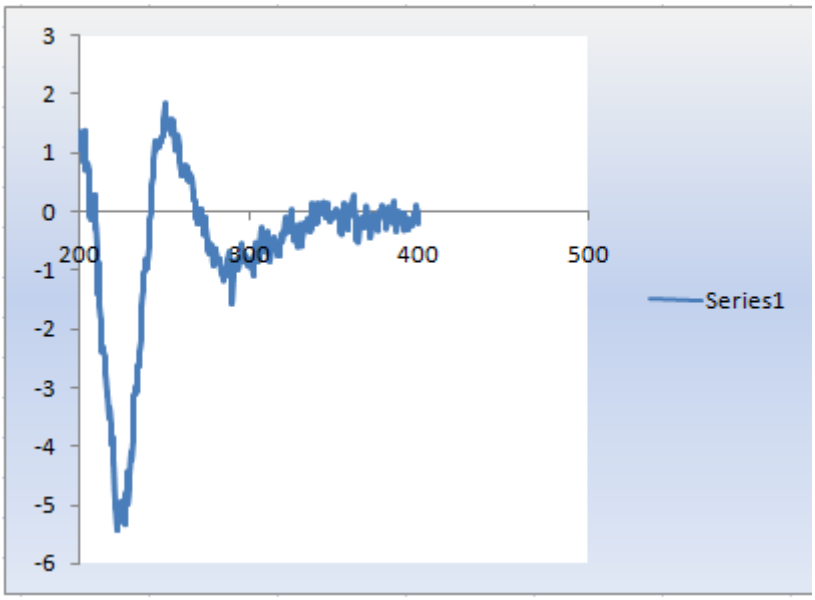


Figure 4.6: ECD spectrum of compound (**87**)

Table 4.8: ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data for compound (**87**), $CDCl_3$

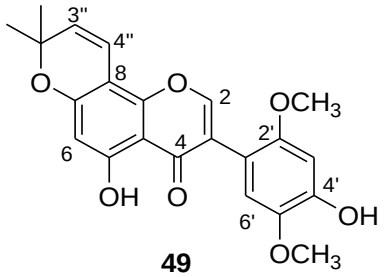
Position	δ_C	δ_H, *m*, (J in Hz)	HMBC
2	87.9	5.24, *d* (12.5)	C-3,C-1'
3	72.8	4.55, *d* (12.5)	
4	170.9		
4a	118.8		
5	153.6		
6	88.2	6.13 *s*	C-5,C-7, C-8, C-4a
7	158.2		
8	119.8		
8a	158.2		
1'	141.8		
2'/6'	127.4	7.47 *m*	
3'/5'	128.1	7.38 *m*	
4'	125.9	7.31 *m*	
1''	21.6	3.48	
2''	123.3	5.18	
3''	130.3		
4''	17.6	1.63 *s*	C-2'',C-3'',C-5''
5''	25.6	1.60 *s*	C-2'',C-3'',C-4''
3-OH		4.12 *s*	
5-OMe	55.5	3.83 *s*	C-5
7-OMe	55.9	3.86 *s*	C-7

4.1.3. Compounds from the stem of *Tephrosia polyphylla*

Two compounds were isolated from the stems of *Tephrosia polyphylla*. The characterization of these compounds has been discussed below.

4.1.3.1.4'-Demethyltoxicarolisoflavone **(49)**

Compound **49** was isolated as white crystals. The molecular formula $C_{22}H_{20}O_7$ was determined from the HRMS ($[M+H]^+$ m/z 397.1306) together with ^1H and ^{13}C NMR data (Table 4.9).The UV (λ_{max} 216 and 280 nm), ^1H NMR (δ_H 7.91, for H-2) and ^{13}C NMR (δ_C 154.6 for C-2, 120.1 for C-3 and 180.8 for C-4) is consistent that compound **49** is an isoflavone derivative.



49

In support of this isoflavone skeletal structure, the HMBC spectrum (Table 4.9) showed correlations of H-2 to C-3 (δ_C 120.1), C-4 (δ_C 180.8) and C-8a (δ_C 152.1). In ring A, the ^1H and ^{13}C NMR spectra further showed the presence of an intramolecularly bonded hydroxy group at δ_H 12.96 and 2,2-dimethylchromene ring (Table 4.9); The only aromatic proton in ring A appeared at δ_H 6.29 (δ_C 99.7). The placement of these substituents was established based on HMBC correlations (Table 4.9). Thus HMBC correlations of H-3" to C-2" (δ_C 77.8), H-4" to C-7 (δ_C 159.2) and C-8a (δ_C 152.1) and 2"-Me_2 to C-2" (δ_C 77.8) is consistent with the placement at C-7/C-8. In support of this, H-6 showed HMBC correlations to C-5 (δ_C 161.9), C-7 (δ_C 159.2), C-4a (δ_C 105.9) and C-8 (δ_C 101.1).

In ring B, the ^1H NMR spectrum revealed a *para*-oriented aromatic protons signals for H-2' (δ_H 6.67, δ_C 100.1) and H-5' (δ_H 6.88, δ_C 109.6), with substituents at C-3'(OMe,

δ_H3.87, δ_C 56.1, 3H, s), C-6(6-OMe, δ_H 3.74, δ_C 56.4, 3H, s)] and C-4' (OH, δ_H 5.78) groups. The substitution pattern in this ring was confirmed by HMBC spectrum (Table 4.9), H-5' to C-3 (δ_C 120.1), C-3' (δ_C 140.1), C-4' (δ_C 147.6) and C-6' (δ_C 152.0), H-2 to C-3'(δ_C 140.1) and C-6'(δ_C 152.0). One methoxy group was placed at C-5' from the HMBC correlation of the methoxy proton (δ_H 3.74) to C-5' (δ_C 109.6). The identity of this compound was confirmed by comparison of the spectroscopic data with literature (Dagne *et al*., 1992), and single crystal X-ray analysis (Figure 4.7).

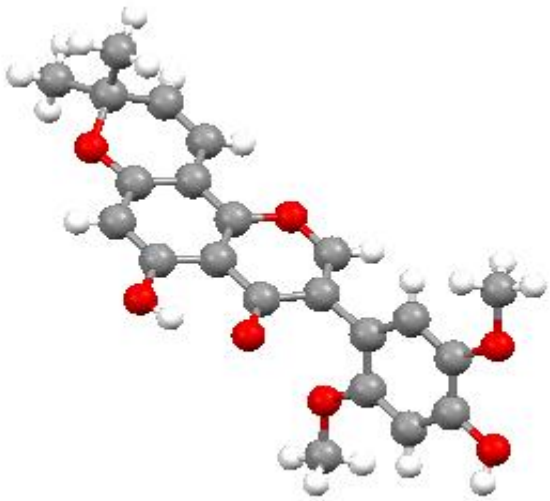


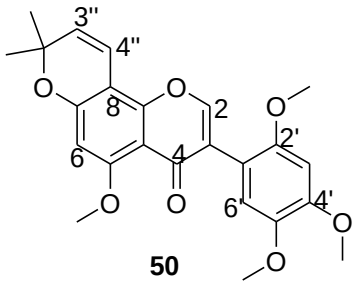
Figure 4.7: X-ray single crystal structure of compound (**49**)

Table 4.9: ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data for compound (**49**), $CDCl_3$

Position	δ_C	δ_H, *m*, (J in Hz)	HMBC
2	154.6	7.91 *s*	C-3, C-4, C-8a
3	120.1		
4	180.8		
4a	105.9		
5	161.9		
6	99.7	6.29 *s*	C-5, C-7, C-8, C-4a
7	159.2		
8	101.1		
8a	152.1		
1'	114.1		
2'	100.1	6.67 *s*	C-3', C-4'
3'	140.1		
4'	146.7		
5'	109.6	6.88 *s*	C-3', C-4', C-6'
6'	152.0		
2''	77.8		
3''	127.1	6.69 ,*d* (10.0 Hz)	C-7, C-8a, C-2''
4''	114.5	5.58 ,*d* (10.0 Hz)	C-2''
2''-Me_2	28.0	1.47 *s*	
5-OH		12.96 *s*	
4'-OH		5.78 *s*	
3'-OMe	56.1	3.87 *s*	C-3'
6'-OMe	56.4	3.74 *s*	C-6'

4.1.3.2:5-Methyltoxicarolisoflavone (**50**)

Compound **50** was isolated as a yellow amorphous solid. The molecular formula $C_{24}H_{24}O_7$ was determined from the LC-ESI-MS ($[M+H]^+$ peak at *m/z* 409.20) together with ^1H and ^{13} C NMR data (Table 4.10), the UV (λ_{max} 230 and 285 nm), ^1H NMR (δ_H 7.81, H-2) and ^{13}C NMR (δ_C 150.4 for C-2, δ_C 123.1 for C-3 and δ_C 171.3 for C-4) showed that compound **50** is an isoflavone derivative.



50

In support of this isoflavone skeletone H-2 showed HMBC correlation (Table 4.10) to C-3 (δ_C 123.1), C-4 (δ_C 171.3) and C-8a (δ_C 148.9). The ^1H and ^{13}C NMR spectra revealed the presence of a 2,2-dimethylchromene ring and four methoxy substituents (Table 4.10). As in compound **49**, the ^1H NMR spectrum showed a single aromatic proton at δ_H 6.31 for H-6 (δ_C 99.9) with C-5 substituted with methoxy (δ_H 3.91, δ_C 53.4) and C-7/C-8 with 2,2-dimethylchromene group in ring A. The placement of these substituents was based on HMBC correlations (Table 4.10). In comparison to compound **49**, the chemical shift value of C-4 (δ_C 171.3) in compound **50** is shielded which showed that it is not involved in intramolecular hydrogen bonding and supports that C-5 is substituted with methoxy group.

In ring B, the ^1H NMR spectral data revealed the para-oriented aromatic protons H-3' (δ_H 6.95, C-3' δ_C 115.3) and H-6' (δ_H 6.62, C-6' δ_C 102.8) signal typical of ring B substituted with four substituents. The other groups attached to ring B were three methoxy groups, 2'-OMe (δ_H 3.71, δ_C 60.6), 4'-OMe (δ_H 3.90, δ_C 55.9) and 5'-OMe (δ_H 3.84, δ_C 56.5) with HMBC correlations (Table 4.10) to C-2' (δ_C 152.4), C-4' (δ_C 149.0), C-

5' (δ_C 141.6) and C-5 (δ_C161.6) respectively. Thus compound **50** was identified as 5-methyltoxicarol isoflavone, previously isolated from *Tephrosia polyphylla* (Dagne *et al*, 1992).

Table 4.10: ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data for compound (**50**), $CDCl_3$

Position	δ_C	$\delta_{H,}$ *m*, (J in Hz)	HMBC
2	150.4	7.81 *s*	C-3, C-4, C-8a,
3	123.1		
4	171.3		
4a	109.0		
5	161.6		
6	96.7	6.31 *s*	C-5, C-7, C-8, C-4a
7	155.7		
8	102.6		
8a	148.9		
1'	118.0		
2'	152.4		
3'	115.0		
4'	149.0		
5'	141.6		
6'	102.8		
2''	73.9	5.57, *d* (10.0)	C-2''
3''	127.4	6.73, *d* (10.0)	C-7, C-8a, C-2''
4''	114.5		
2''-Me_2	28.3	1.48 *s*	
5-OMe	53.4	3.91 *s*	C-5
2'-OMe	60.6	3.71 *s*	C-2'
4'-OMe	55.9	3.90 *s*	C-4'
5'-OMe	56.5	3.84 *s*	C-5'

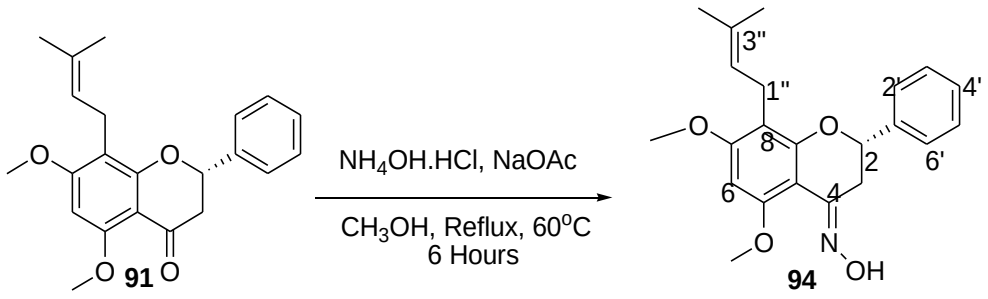
4.2 Characterization of Structurally Modified Compounds

4.2.1 Oxime derivative

Compound **91** (Candidone) was treated with hydroxylamine hydrochloride and unhydrous sodium acetate in presence of methanol as a solvent to give candidone-oxime (**94**).

4.2.1.1: Candidone–oxime (**94**)

Compound **94**, a derivative of compound **91**, was obtained as white crystals with UV absorption maxima at 237 and 280 nm. The molecular weight was determined to be 367 with molecular formula $C_{22}H_{25}NO_4$ from the molecular ion peak ($[M+H]^+$ m/z 368.3) in the HR-ESI-MS spectrum. In addition, The NMR data (Table 4.11), ^1H and ^{13}C NMR spectra, the oxygenated methine signal at H-2 (δ_H 5.40, δ_C 78.6, 1H, dd, J=12.8, 3.1 Hz), the methylene proton signals at H-3ax (δ_H 2.98, δ_C 45.8, 1H, dd, J=16.6, 12.8 Hz) and H-3eq (δ_H 2.84, δ_C 45.8, 1H, dd, J=16.6, 3.1 Hz) is indicative of the AMX system typical of flavanone ring C. This was also supported by the HMBC correlations (Table 4.11) of H-3eq to C-2 (δ_C 78.6); C-4 (δ_C 190.0) and C-1' (δ_C 139.3) which in turn support the presence of unsubstituted ring B. In the COSY experiment, the oxygenated methine proton at δ_H 5.40 (H-2) showed across peaks with methylene protons at δ_H 2.98 (H-3ax) and δ_H 2.84 (H-3eq) confirming the flavanone ring C structure.



20.00 mg

19.60 mg
98.0 % yield

The derivatization of oxime was supported by the conversion of C=O in deshileded C-4 (δ_C 190) in compound **91** to shielded C-4 (δ_C 149.8), C=NOH in compound **94**. The shielding was also observed in compound **94** at C-3 (δ_C 31.1) and H-2 (δ_H 5.17) compared to deshielding in compound **91** at C-3 (δ_C 45.8) and H-2 (δ_H 5.40)

Putting all these spectroscopic data together, the new compound **94** was characterized as (2S**)** -**(**4E)-4-(hydroxyimino)-5,7-dimethoxy-8-(3-methylbut-2-en-1-yl)-2-phenyl-3,4-dihydro-2H-chromene. It was given a trivial name candidone-oxime.

Table 4.11: ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data for compound (**94**), $CDCl_3$

Position	δ_C	δ_H, *m*, (J in Hz)	HMBC
2	76.8	5.17, *dd* (12.5, 3.2)	C-4, C-1′
3	31.1	3.72, *dd* (16.0, 12.5)	C-4
		2.80, *dd* (16.0, 3.2)	C-2, C-4, C-1′
4	149.8		
4a	111.3		
5	156.6		
6	89.3	6.20 *s*	C-5, C-7, C-8, C-4a,
7	159.9		
8	100.7		
8a	157.9		
1′	140.2		
2′/6′	128.7	7.42 *m*	C-1′
3′/5′	126.2	7.50 *m*	C-4′
4′	128.3	7.37 *m*	C-3′/5′
1″	22.1	3.29	C-7,C-8,C-8a, C-2″,C-3″
2″	123.0	5.06	C-4″,C-5″
3″	131.1		
4″	18.9	1.64 *s*	C-2″,C-3″,C-5″
5″	25.3	1.61 *s*	C-2″,C-3″,C-4″
5-OMe	55.9	3.88 *s*	C-5
7-OMe	55.8	3.94 *s*	C-7
4-NOH		3.80 *s*	

4.3 Biological Actvity

4.3.1: *In-vitro* Antiplasmodial Activity

The crude CH_2Cl_2/CH_3OH (1:1) extract of *T. rhodesica* roots, seedpods and stems of *T. Polyphylla* together with the isolated compounds were tested for antiplasmodial activities against the chloroquine-resistant clone (W2) and chloroquine-sensitive clones (3D7 and D6) strains of *Plasmodium falciparum*. The crude extract of the seedpods of *T. rhodesica* was the most active with IC_{50} value of 0.8 mg (against the W2) and IC_{50} value of 1.9 mg against D6 strains of *Plasmodium falciparum* (Table 4.12). Among the pure compounds tested, the dimeric flavonoid rhodimer (**88**) and candidone (**91**) showed good activity with IC_{50} values below 3 µM against the W2 strain of *Plasmodium falciparum*.

Table 4.12: *In-vitro* antiplasmodial activities of the named compounds.

Crude extracts	IC_{50} (mg)		
	W2	3D7	D6
T.rhodesica (Roots extract)	4.9	10.8	2.2
T.rhodesica (Seedpods extract)	0.8	4.5	1.9
Pure compunds	IC_{50} (µM)		
Rhodimer (**88**)	2.3±0.2		
4-Hydroxy-3-methoxybenzoic acid (**89**)	9.5	20.8	
Candidone (**91**)	1.2±0.1	3.5±0.4	18.6
Candidone-oxime (**94**)			13.1
Rhodbenzofuran (**86**)			17.0
Flemichapparin (**92**)	27.3±3.8		16.3
Chloroquine	0.05900	0.0075	0.0070
Mefloquinine	0.00058	0.0220	0.0006

CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Phytochemical investigation of the roots of *Tephrosia rhodesica* led to the isolation and characterization of six compounds including a new compound, rhodbenzofuran (**86**). The seedpods of *Tephrosia rhodesica* gave two compounds, of which one is new, rhodflavononol (**87**). From the stem of *Tephrosia polyphylla* two known compounds were isolated. In antiplasmodial assay against chloroquine-resistant (W2) and Chloroquine-sensitive (3D7 and D6) clones. The extract of the seedpods of *Tephrosia rhodesica* was the most active. Among the isolated compounds, candidone (**91**) was the most active against chloroquine resistant clone (W2) and chloroquine sensitive clone (3D7). The rest of the compounds showed moderate activities against chloroquine sensitive clone (D6).

An oxime derivative, candidone-oxime (**94**), was prepared from candidone (**91**), it was found to be active against chloroquine-resistance clone (D6) than Candidone (**91**).Thus, the introduction of oxime (NOH) from carbonyl (C=O) position has improved the activity of the flavonoid.

5.2.1 Recommendations

The following recommendations are made based on the gaps in this study:

I. The dervitization should be done on other compounds to form more oximes.
II. Candidone -oxime should be tested against chloquine resistance (W2) strain of *P. falciparum*

5.2.2 Suggestion for further studies

The following suggestions are made based on the results of this study:

I. Further investigation of stem and leaves of *T. rhodesica,* seedpods and leaves of *T. polyphylla* to isolate more active compounds is necessary.

II. The cytotoxicity of the crude extracts and of the isolated compounds should be done.

III. The *in vivo* antiplasmodial activity tests should be carried out on extracts and isolated compounds to established their potency and efficacy.

IV. Structure-Activity relationship of compounds isolated is necessary.

REFERENCES

Abreu, P. M., & Luis, M. H. (1996). Constituents of *Tephrosia uniflora*. *Natural Product Letters*, ***9***, 81–86.

Adinarayana, K., Jayaveera, K. N., Madhu, K. B., & Mallikarjuna, R. P. (2009). Growth inhibition and induction of apoptosis in estrogen receptor positive and negative human breast carcinoma cells by *Tephrosia calophylla* roots. *J Pharm Chem*, ***3***, 35–41.

Adityachaudhury, N., & Gupta, P. K. (1973). A new pterocarpan and coumestan in the roots of *Flemingia chappar*. *Phytochemistry*, ***12***, 425–428.

Agrawal, P. K. (2013). *Carbon-13 NMR of flavonoids* (Vol. **39**). Elsevier.

Ammar, M. I., Nenaah, G. E., & Mohamed, A. H. H. (2013). Antifungal activity of prenylated flavonoids isolated from *Tephrosia apollinea* L. against four phytopathogenic fungi. *Crop Protection*, **49**, 21–25.

Andrei, C. C., Ferreira, D. T., Faccione, M., De Moraes, L. A. B., De Carvalho, M. G., & Braz-Filho, R. (2000). C-prenylflavonoids from roots of *Tephrosia tunicata*. *Phytochemistry*, ***55***, 799–804.

Atilaw, Y., Duffy, S., Heydenreich, M., Muiva-Mutisya, L., Avery, V., Erdélyi, M., & Yenesew, A. (2017). Three Chalconoids and a Pterocarpene from the Roots of *Tephrosia aequilata*. *Molecules*, ***22***, 318.

Atilaw, Y., Muiva-Mutisya, L., Ndakala, A., Akala, H., Yeda, R., Wu, Y., Yenesew, A. (2017). Four Prenyl flavone Derivatives with Antiplasmodial Activities from the Stem of *Tephrosia purpurea* subsp. *leptostachya*. *Molecules*, ***22***, 1514.

Balakrishnan, B. R., Sangameswaran, B., Sufiyan, A., & Bhaskar, V. H. (2007). Anti-hyperglycemic activity of roots of *Tephrosia villosa* Pers. *Plant Archives*, ***7***, 729–731.

Beentje, H. J., Adamson, J., & Bhanderi, D. (2^{nd} Ed.). Kenya trees, shrubs and lianas. 1994. *National Museums of Kenya, Nairobi Google Scholar*.

Belmain, S. R., Amoah, B. A., Nyirenda, S. P., Kamanula, J. F., & Stevenson, P. C. (2012). Highly variable insect control efficacy of *Tephrosia vogelii* chemotypes. *Journal of Agricultural and Food Chemistry*, ***60***, 10055–10063.

Bodeker, G., & Ong, C.-K. (2005). *WHO global atlas of traditional, complementary and alternative medicine* (Vol. **1**). World Health Organization.

Bojang, K. A., Milligan, P. J., Pinder, M., Vigneron, L., Alloueche, A., Kester, K. E., Gothard, P. (2001). Efficacy of RTS, S/AS02 malaria vaccine against *Plasmodium falciparum* infection in semi-immune adult men in The Gambia: a randomised trial. *The Lancet*, ***358***, 1927–1934.

Butler, M. S. (2004). The role of natural product chemistry in drug discovery. *Journal of Natural Products*, ***67***, 2141–2153.

Chakradhar, V., Babu, Y. H., Ganapaty, S., Prasad, Y. R., & Rao, N. K. (2005). Anti-inflammatory activity of a flavonol glycoside from *Tephrosia spinosa*. *Natural Product Sciences*, ***11***, 63–66.

Chang, L C, Chávez, D., Song, L. L., Farnsworth, N. R., Pezzuto, J. M., & Kinghorn, a D. (2000). Absolute configuration of novel bioactive flavonoids from *Tephrosia purpurea*. *Organic Letters*, ***2***, 515–518.

Chang, L. C., Gerha, C., Song, L., Farnsworth, N. R., Pezzuto, J. M., & Kinghorn, A. D. (1997). Activity-guided isolation of constituents of *Tephrosia purpurea* with the potential to induce the phase II enzyme, quinone reductase. *Journal of Natural Products*, **60**, 869-873

Chen, Y., Yan, T., Gao, C., Cao, W., & Huang, R. (2014). Natural Products from the Genus *Tephrosia*. *Molecules*, ***19***, 1432–1458.

Chen, Y.L., Wang, Y.S., Lin, Y.L., Munakata, K., & Ohta, K. (1978). Obovatin, Obovatin Methyl Ether and Obovatachalcone, New Piscicidal Flavonoids from *Tephrosia obovata*. *Agricultural and Biological Chemistry*, ***42***, 2431–2432.

Choudhary, G. P. (2007). In vitro antioxidant studies of the ethanolic extract of *Tephrosia purpurea* L. *Ancient Science of Life*, ***27***, 26.

Crombie, H. J., Chengappa, S., Hellyer, A., & Reid, J. G. (1998). A xyloglucan oligosaccharide-active, trans glycosylating-D-glucosidase from the cotyledons of nasturtium *Tropaeolum majus* L seedlings–purification, properties and characterization of a cDNA clone. *The Plant Journal*, ***15***, 27–38.

Cunningham, A. B., Shanley, P., & Laird, S. (2008). Health, habitats and medicinal plant use. *Human Health and Forests: A Global Overview of Issues, Practice and Policy*, 374p.

Dagne, E., Dinku, B., Gray, A. I., & Waterman, P. G. (1988). Pumilaisoflavones A and B from the seed pods of *Tephrosia pumila*. *Phytochemistry*, ***27***, 1503–1505.

Dagne, E., Mammo, W., & Sterner, O. (1992). Flavonoids of *Tephrosia polyphylla*. *Phytochemistry*, ***31***, 3662–3663.

Dagne, E., Yenesew, A., & Waterman, P. G. (1989). Flavonoids and isoflavonoids from *Tephrosia fulvinervis* and *Tephrosia pentaphylla*. *Phytochemistry*, ***28***, 3207–3210.

Dewick, P. M. (2002). *Medicinal natural products: a biosynthetic approach*. John Wiley & Sons.

Duffy, S., & Avery, V. M. (2012). Development and optimization of a novel 384-well anti-malarial imaging assay validated for high-throughput screening. *The American Journal of Tropical Medicine and Hygiene*, ***86***, 84–92.

Dundorp, A. M., & Nosten, F. (2009). Artemisinin resistance in *Plasmodium falciparum*. *New Eng J Med*, ***361***, 455–467.

Dutt, S. K., & Chibber, S. S. (1983). Candidol, a flavonol from *Tephrosia candida*. *Phytochemistry*, ***22***, 325–326.

Dzenda, T., Ayo, J. A., Adelaiye, A. B., & Adaudi, A. O. (2007). Ethno-medical and veterinary uses of *Tephrosia vogelii* hook. F: A review. *Nigerian Veterinary Journal*, ***28***, 24–39.

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

Flannery, E. L., Chatterjee, A. K., & Winzeler, E. A. (2013). Antimalarial drug discovery—approaches and progress towards new medicines. *Nature Reviews Microbiology*, ***11***, 849.

Fong, I. W. (2013). Challenges in the Control and Eradication of Malaria. *Challenges in infectious diseases* (pp. 203–231). Springer.

Fukai, T., Sheng, C.-B., Horikoshi, T., & Nomura, T. (1996). Isoprenylated flavonoids from underground parts of *Glycyrrhiza glabra*. *Phytochemistry*, ***43***, 1119–1124.

Gakuya, D. W., Itonga, S. M., Mbaria, J. M., Muthee, J. K., & Musau, J. K. (2013). Ethnobotanical survey of biopesticides and other medicinal plants traditionally used in Meru central district of Kenya. *Journal of Ethnopharmacology*, ***145***, 547–553.

Gamble, C. L., Ekwaru, J. P., & ter Kuile, F. O. (2006). Insecticide-treated nets for preventing malaria in pregnancy. *Cochrane Database of Systematic Reviews*, **2**, 1465–1858

Ganapaty, S., Pannakal, S. T., Srilakshmi, G. V. K., Lakshmi, P., Waterman, P. G., & Brun, R. (2008). Pumilanol, an antiprotozoal isoflavanol from *Tephrosia pumila*. *Phytochemistry Letters*, **1**, 175–178.

Ganapaty, S., Srilakshmi, G. V., Pannakal, S. T., & Laatsch, H. (2008). A pyranochalcone and prenylflavanones from *Tephrosia pulcherrima* (Baker) drumm. *Natural Product Communications*, ***3***, 49–52.

Gomez, F., Quijano, L., Calderón, J. S., Rodriquez, C., & Rios, T. (1985). Prenylflavans from *Tephrosia watsoniana*. *Phytochemistry*, **24**, 1057–1059.

Gómez-Garibay, F., Arciniega, M. D. L. O., Céspedes, C. L., Taboada, J., & Calderón, J. S. (2001). Chromene Chalcones from *Tephrosia carrollii* and the Revised Structure of Oxacacin. *Zeitschrift Für Naturforschung C*, **56**, 11–12.

Gupta, M., Mazumder, U. K., Gomathi, P., & Selvan, V. T. (2008). Antimicrobial activity of methanol extracts of *Plumeria acuminata* leaves and *Tephrosia purpurea* (Linn.) Pers. roots. *Nat Radiance*, **7**, 102–105

Horie, T., Kawamura, Y., Kobayashi, T., & Yamashita, K. (1994). Revised structure of a natural flavone from *Tephrosia candida*. *Phytochemistry*, **37**, 1189–1191.

Hussain, T., Siddiqui, H. H., Fareed, S., Vijayakumar, M., & Rao, C. V. (2012). Chemopreventive evaluation of *Tephrosia purpurea* against N-nitrosodiethylamine-induced hepatocarcinogenesis in Wistar rats. *Journal of Pharmacy and Pharmacology*, **64**, 1195–1205.

Iinuma, M., Okawa, Y., Tanaka, T., Kobayashi, Y., & Miyauchi, K. (1994). Phenolic compounds in *Erythrina bidwillii* and their activity against oral microbial organisms. *Heterocycles*, ***2***, 687–692.

Ingham, J. L., & Markham, K. R. (1980). Tephrocarpin, a pterocarpan phytoalexin from *Tephrosia bidwilli* and a structure proposal for acanthocarpan. *Phytochemistry*, **21**, 2969–2972.

Jang, D. S., Park, E. J., Kang, Y.-H., Hawthorne, M. E., Vigo, J. S., Graham, J. G., Kinghorn, A. D. (2003). Potential cancer chemopreventive flavonoids from the stems of *Tephrosia toxicaria*. *Journal of Natural Products*, **66**, 1166–1170.

Jayaraman, I., Ghanim, A., & Khan, H. A. (1980). A new prenylated flavanone [7-methylglabranin] from *Tephrosia villosa*. *Phytochemistry*,**19**, 1267–1268.

Juma, W. P., Akala, H. M., Eyase, F. L., Muiva, L. M., Heydenreich, M., Okalebo, F. A., Yenesew, A. (2011). Terpurin flavone: An antiplasmodial flavone from the stem of *Tephrosia Purpurea*. *Phytochemistry Letters*, ***4***, 176–178.

Kare, M., Kone, M. E. K., Boulanger, A., Niassy, B., Lenouen, D., Muckensturm, B., & Nongonierma, A. (2006). Isolation, identification and antibacterial tests of chalcones and rotenoids of *Tephrosia deflexa* Baker. *J. Soc. Ouest-Afr. Chim*, **11**, 41–45.

Kigen, G. K., Ronoh, H. K., Kipkore, W. K., & Rotich, J. K. (2013). Current trends of traditional herbal medicine practice in Kenya: a review. *African Journal of Pharmacology and Therapeutics*, ***2***, 32–37

Kishore, P. H., Reddy, M. V. B., Gunasekar, D., Murthy, M. M., Caux, C., & Bodo, B. (2003). A new coumestan from *Tephrosia calophylla*. *Chemical and Pharmaceutical Bulletin*, ***51***, 194–196.

Kokwaro, J. O. (2009). Medicinal Plants of East Africa. 3rd ed (Kenya Literature Bureau), 185–187.

Kole, R. K., Satpathi, C., Chowdhury, A., Ghosh, M. R., & Adityachaudhury, N. (1992). Isolation of amorpholone, a potent rotenoid insecticide from *Tephrosia candida*. *Journal of Agricultural and Food Chemistry*, ***40***, 1208–1210.

Lu, F., Culleton, R., Zhang, M., Ramaprasad, A., Von Seidlein, L., Zhou, H., Wang, W. (2017). Emergence of indigenous artemisinin-resistant *Plasmodium falciparum* in Africa. *New England Journal of Medicine, **376***, 991–993.

Lwande, W., Bentley, M. D., & Hassanali, A. (1986). The structure of hildecarpin, an insect antifeedant 6a-hydroxypterocarpan from the roots of *Tephrosia hildebrandtii* Vatke. *International Journal of Tropical Insect Science, **7***, 501–503.

Lwande, W., Bentley, M. D., Macfoy, C., Lugemwa, F. N., Hassanali, A., & Nyandat, E. (1987). A new pterocarpan from the roots of *Tephrosia hildebrandtii*. *Phytochemistry, **26***, 2425–2426.

Lwande, W., Greene, C. S., & Bentley, M. D. (1985). Flavonoids from the roots of *Tephrosia elata*. *Journal of Natural Products*, ***48***, 1004–1005.

Machocho, A. K., Lwande, W., Jondikoa, J. I., Moreka, L. V. C., & Hassanali, A. (1995). Three new flavonoids from the root of *Tephrosia emoroides* and their antifeedant activity against the larvae of the spotted stalk borer Chilo partellus Swinhoe. *International Journal of Pharmacognosy*, ***33***, 222–227.

Martinez, R. M., Zarpelon, A. C., Zimermann, V. V., Georgetti, S. R., Baracat, M. M., Fonseca, M. J., Verri Jr, W. A. (2012). *Tephrosia sinapou* extract reduces inflammatory leukocyte recruitment in mice: effect on oxidative stress, nitric

oxide and cytokine production. *Revista Brasileira de Farmacognosia*, ***22***, 587–597.

Maurich, T., Iorio, M., Chimenti, D., & Turchi, G. (2006). Erybraedin C and bitucarpin A, two structurally related pterocarpans purified from *Bituminaria bituminosa*, induced apoptosis in human colon adenocarcinoma cell lines MMR-and p53-proficient and-deficient in a dose, time, and structure-dependent fashion. *Chemico-Biological Interactions*, ***159***, 104–116.

Mohan, C. U. (2011). Antihyperlipidemic effect of *Tephrosia calophylla* against acute and chronic hyperlipidemia in wistar albino rats. RGUHS.

Muiva, L. M., Yenesew, A., Derese, S., Heydenreich, M., Peter, M. G., Akala, H. M., Keriko, J. M. (2009). Antiplasmodial β-hydroxydihydrochalcone from seedpods of *Tephrosia elata*. *Phytochemistry Letters*, ***2***, 99–102.

Muiva-Mutisya, L., Macharia, B., Heydenreich, M., Koch, A., Akala, H. M., Derese, S., Yenesew, A. (2014). 6α-Hydroxy-α-toxicarol and (+)-tephrodin with antiplasmodial activities from *Tephrosia species*. *Phytochemistry Letters*, **10,** 179–183.

Murthy, M. S. R., & Rao, E. V. (1985). Maxima isoflavone J: A new O-prenylated isoflavone from *Tephrosia maxima*. *Journal of Natural Products*, ***48***, 967–968.

Mutabingwa, T. K. (2005). Artemisinin-based combination therapies (ACTs): Best hope for malaria treatment but inaccessible to the needy! *Acta Tropica*, ***95***, 305–315.

Parine, N. R., Lakshmi, P., Kumar, D., Shaik, J. P., Alanazi, M., & Pathan, A. A. K. (2015). Development and characterisation of nine polymorphic microsatellite markers for *Tephrosia calophylla* Bedd.(Fabaceae). *Saudi Journal of Biological Sciences*, ***22***, 164–167.

Parmar, V. S., Jain, R., Simonsen, O., & Boll, P. M. (1987). Isolation of candirone: A novel pentaoxygenation pattern in a naturally occurring 2-phenyl-4h-1-benzopyran-4-one from. *Tetrahedron*, ***43***, 4241–4247.

Pavana, P., Manoharan, S., Renju, G. L., & Sethupathy, S. (2007). Antihyperglycemic and antihyperlipidemic effects of *Tephrosia purpurea* leaf extract in streptozotocin induced diabetic rats. *Journal of Environmental Biology*, ***28***, 833.

Phillips, D. A., & Kapulnik, Y. (1995). Plant isoflavonoids, pathogens and symbionts. *Trends in Microbiology*, ***3***, 58–64.

Polhill, R. M. (1981). Raven PH (ed.). 1981 Advances in legume systematics: parts 1 & 2. *Kew, Royal Botanic Gardens*, **2**, 1–7.

Pouchert, C. J., & Behnke, J. (1993). The Aldrich library of ^{13}C and ^{1}H FT NMR spectra. Aldrich Chemical Comp.1115.

Prashant, A& Krupadanam, G. D. (1993). Dehydro-6-hydroxyrotenoid and lupenone from *Tephrosia villosa*. *Phytochemistry*, ***32***, 484–486.

Rajani, P& Sarma, N. (1988). A coumestone from the roots of *Tephrosia hamiltonii*. *Phytochemistry*, ***27***, 648–649.

Rao, E. V& Prasad, Y. R. (1992). Prenylated flavonoids from *Tephrosia spinosa*. *Phytochemistry*, ***32***, 183–185.

Rao, E. Ventakata., Rajendra Prasad, Y., & Sree Rama Murthy, M. (1994). prenylated flavanone from *Tephrosia maxima*. *Phytochemistry*, ***37***, 111–112.

Rao, E. V., Venkataratnam, G., & Vilain, C. (1985). Flavonoids from *Tephrosia fulvinervis*. *Phytochemistry*, ***24***, 2427–2430.

Rao, E. Ventakata, & Raju, N. R. (1984). Two flavonoids from *Tephrosia purpurea*. *Phytochemistry*, ***23***, 2339–2342.

Ratsimamanga-Urverg, S., Rasoanaivo, P., Rabemanantsoa, C., Ratsimamanga, A. R., & Frappier, F. (1994). Antibacterial activity of flavonoids isolated from *Mundulea monantha* and *Tephrosia linearis*. *Fitoterapia*, **65**, 551–553.

Roy, M., Mitra, S. R., Bhattacharyya, A., & Adityachaudhury, N. (1986). Candidone, a flavanone from *Tephrosia candida*. *Phytochemistry*, ***25***, 961–962.

Rungsung, W., Ratha, K. K., Dutta, S., Dixit, A. K., & Hazra, J. (2015). Secondary metabolites of plants in drugs discovery. *World Journal of Pharmaceutical Research*, ***4***, 604–613.

Samanta, A., Das, G., & Das, S. K. (2011). Roles of flavonoids in plants. *Carbon*, **6**, 12-35

Sarin, J. P. S., Singh, S., Garg, H. S., Khanna, N. M., & Dhar, M. M. (1976). A flavonol glycoside with anticancer activity from *Tephrosia candida*. *Phytochemistry*, ***15***, 232–234.

Schijlen, E. G., De Vos, C. R., Van Tunen, A. J., & Bovy, A. G. (2004). Modification of flavonoid biosynthesis in crop plants. *Phytochemistry*, ***65***, 2631–2648.

Shah, R., Parmar, S., Bhatt, P., & Chanda, S. (2011). Evaluation of hepatoprotective activity of ethyl acetate fraction of *Tephrosia purpurea*. *Pharmacologyonline*, ***3***, 188–194.

Sharma, P., Rastogi, S., Bhatnagar, S., Srivastava, J. K., Dube, A., Guru, P. Y., Dhawan, B. N. (2003). Antileishmanial action of *Tephrosia purpurea* linn, extract and its fractions against experimental visceral leishmaniasis. *Drug Development Research*, ***60***, 285–293.

Shawl, A. S., Zaman, A., & Kalla, A. K. (1984). Isoflavones of *Iris spuria*. *Phytochemistry*, ***23***, 2405–2406.

Sikolia, S., Beck, E., Kinyamario, J. I., Onyango, J. C., Ouma, G., Agnew, A. D., Farguhar, G. (1994). Distribution of the C3 and C4 species of the Centrospermeae along an altitudinal gradient in Western Kenya. *International Journal of Botany*, ***4***, 1239–1244.

Slade, D., Ferreira, D., & Marais, J. P. (2005). Circular dichroism, a powerful tool for the assessment of absolute configuration of flavonoids. *Phytochemistry*, ***66***, 2177–2215.

Smalberger, T. M., Vleggaar, R., & Weber, J. C. (1974). Flavonoids from *Tephrosia lupinifolia*. *Tetrahedron*, ***30***, 3927–3931.

Stauffer, W., & Fischer, P. R. (2003). Diagnosis and treatment of malaria in children. *Clinical Infectious Diseases*, ***37***, 1340–1348.

Tarus, P. K., Machocho, A. K., Lang'at-Thoruwa, C. C., & Chhabra, S. C. (2002). Flavonoids from *Tephrosia aequilata*. *Phytochemistry*, ***60***, 375–379.

Tavares, M., da Silva, M. R. M., de Siqueira, L. B. de O., Rodrigues, R. A. S., Bodjolle-d'Almeira, L., dos Santos, E. P., & Ricci-Júnior, E. (2018). Trends in insect repellent formulations: A review. *International Journal of Pharmaceutics*, ***539***, 190–209.

Trampuz, A., Jereb, M., Muzlovic, I., & Prabhu, R. M. (2003). Clinical review: Severe malaria. *Critical Care*, ***7***, 315.

Veberic, R., Jurhar, J., Mikulic-Petkovsek, M., Stampar, F., & Schmitzer, V. (2010). Comparative study of primary and secondary metabolites in 11 cultivars of persimmon fruit *Diospyros kaki* L. *Food Chemistry*, ***119***, 477–483.

Venkata R. E., Rajendra P. Y., & Sree M. M. (1994). prenylated flavanone from *Tephrosia maxima*. *Phytochemistry*, ***37***, 111–112.

Venkataratnam, G., Rao, E. V., & Vilain, C. (1987). Flavonoids of *Tephrosia procumbens*—revised structure for praecansone A and conformation of praecansone B. *J. Chem. Soc., Perkin Trans.* ***1***, 2723–2727.

Wanga, B. N., Akenga, T., Imbuga, M., Gitonga, L., Olubayo, F., & Namungu, P. (2006). Antimicrobial acitivity of extracts from *Tephrosia vogelii* Hook F. *Journal of Agriculture, Science and Technology*, ***8***, 1–14.

Waterman, P. G., & Khalid, S. A. (1980). The major flavonoids of the seed of *Tephrosia apollinea*. *Phytochemistry*, ***19***, 909–915.

Wheate, N. J., Walker, S., Craig, G. E., & Oun, R. (2010). The status of platinum anticancer drugs in the clinic and in clinical trials. *Dalton Transactions*, ***39***, 8113–8127.

W. H.O (2015). World malaria report *2015*.

W. H.O (2017). World malaria report 2017

W. H.O (2018). World malaria report 2018

Willcox, M. L., Graz, B., Falquet, J., Diakite, C., Giani, S., & Diallo, D. (2011). A "reverse pharmacology" approach for developing an anti-malarial phytomedicine. *Malaria Journal*, ***10***, 58.

Winkel-Shirley, B. (2001). Flavonoid biosynthesis. A colorful model for genetics, biochemistry, cell biology, and biotechnology. *Plant Physiology*, ***126***, 485–493.

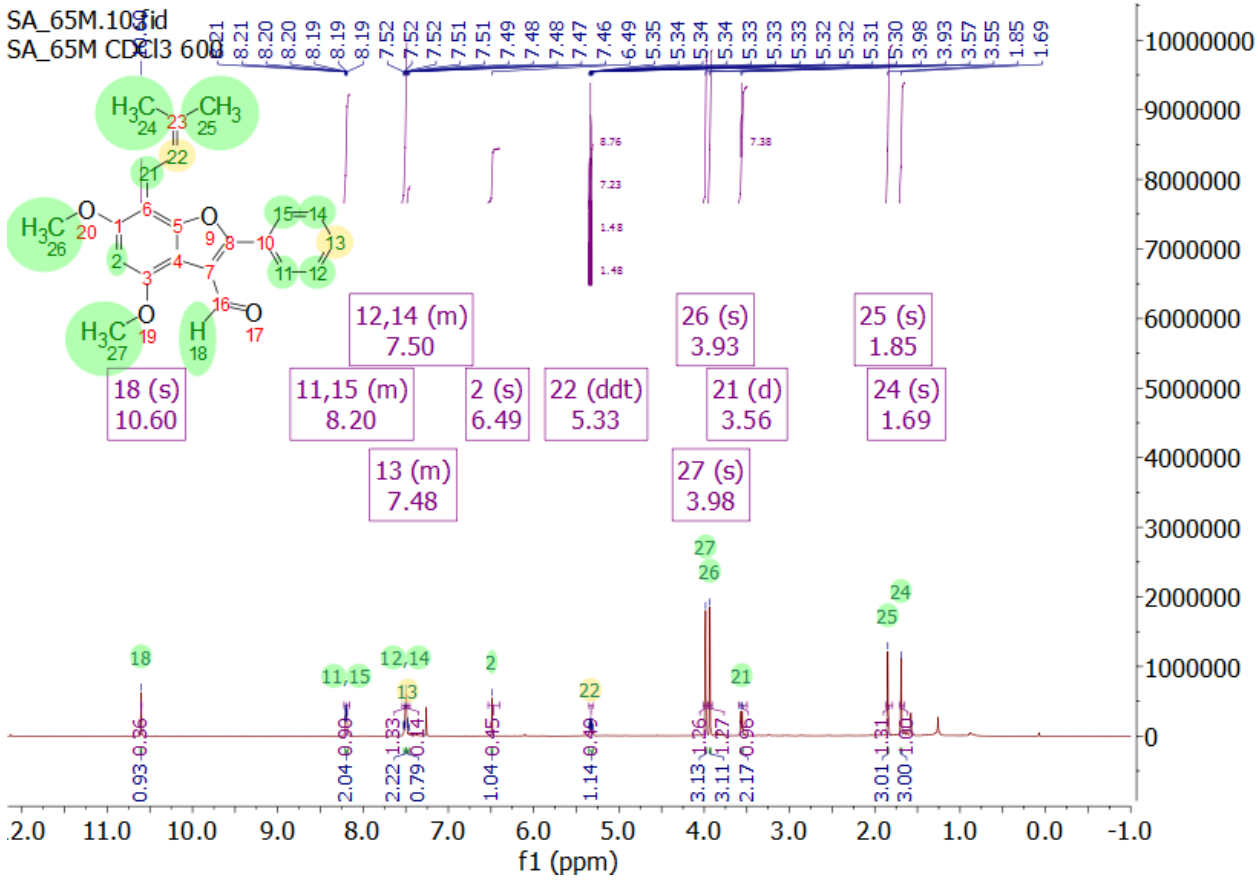
Yenesew, A., Dagne, E., & Waterman, P. G. (1989). Flavonoids from the seed pods of *Tephrosiapumila*. *Phytochemistry*, ***28***, 1291–1292.

Yenesew, A., Irungu, M., Desres,S., Midiwo, JO., Heydenreich, M., Peter, MG., Akala, H., Layla, P., & Waters, NC. (1989). Flavonoids and isoflavonoids with antiplasmodial activities from root bark of *Erthrina abyssinica*. *Planta Med*, ***69***, 658–661.

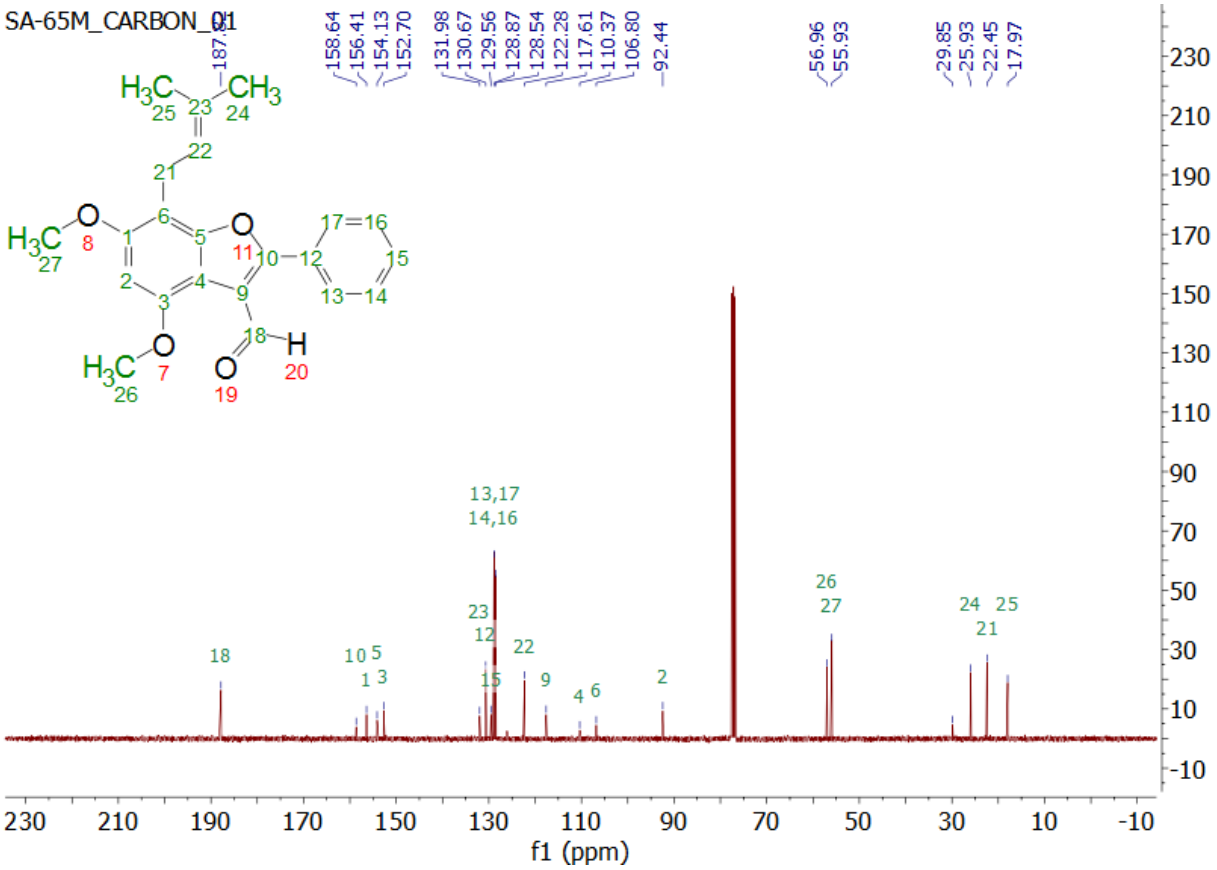
Zhi, W., & Pedley, L. (2010). *Tephrosia Pers*. *Flora of China*, **10**, 190–193.

APPENDICES

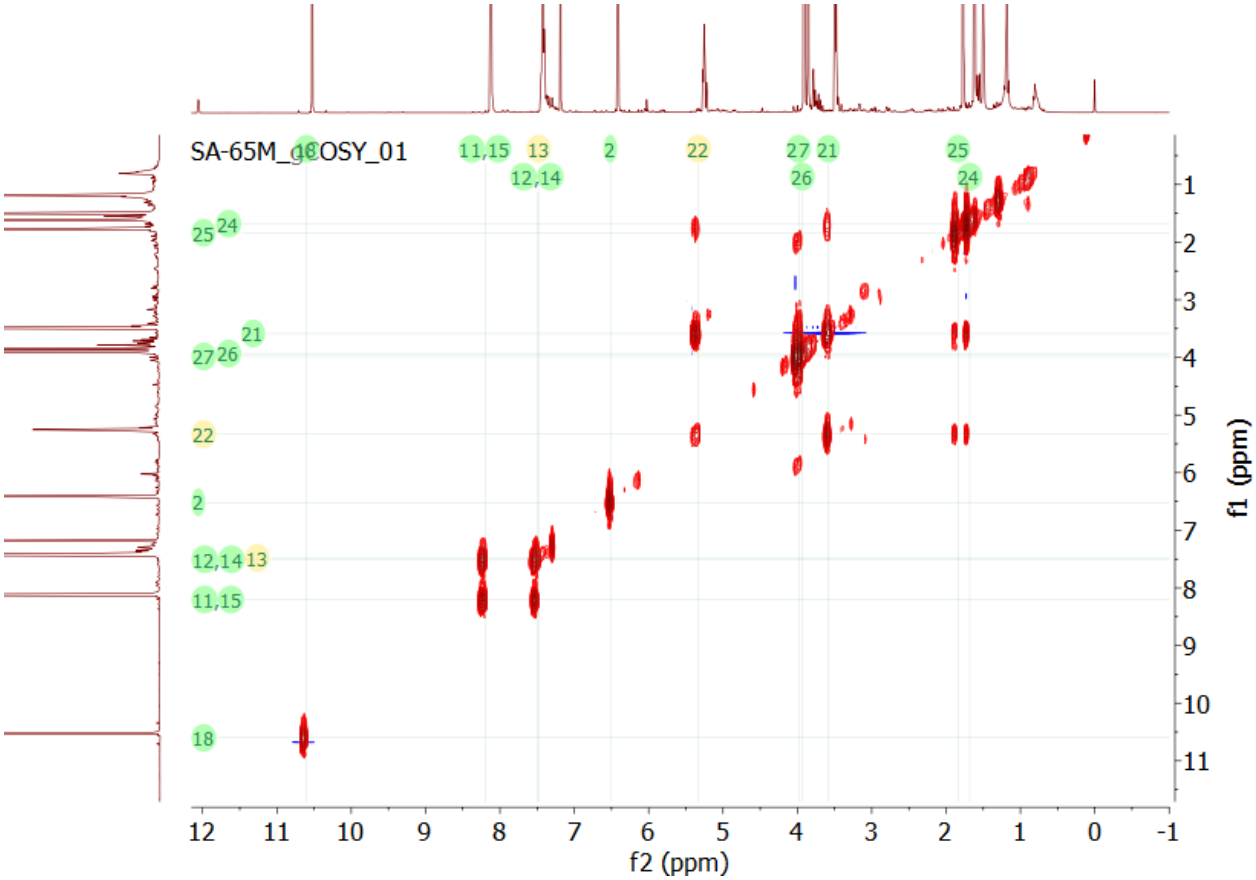
APPENDIX 1A: ^{1}H NMR Spectrum of Rhodbenzofuran (**86**) (500MHz; $CDCl_3$)



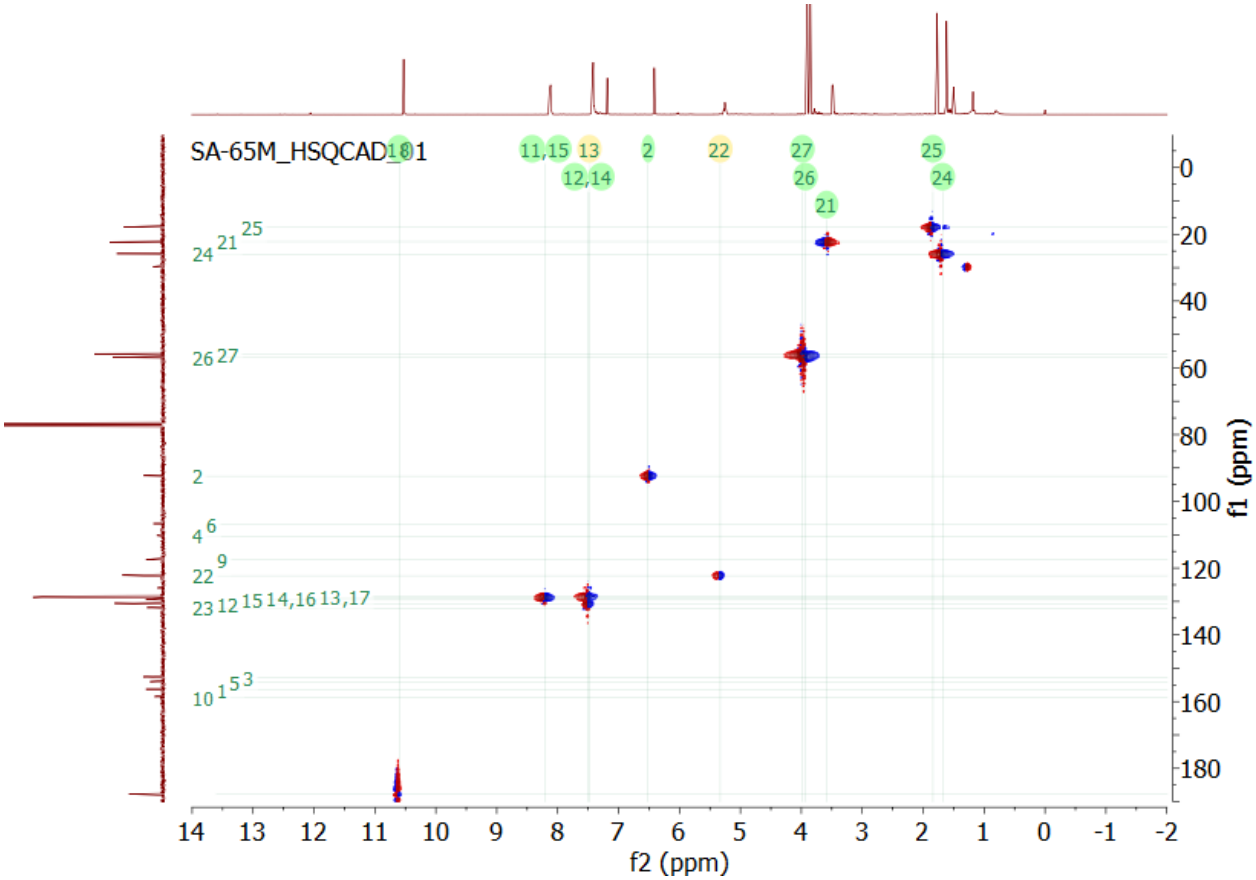
APPENDIX 1B: ^{13}C NMR Spectrum of Rhodbenzofuran (**86**) (125 MHz; $CDCl_3$)



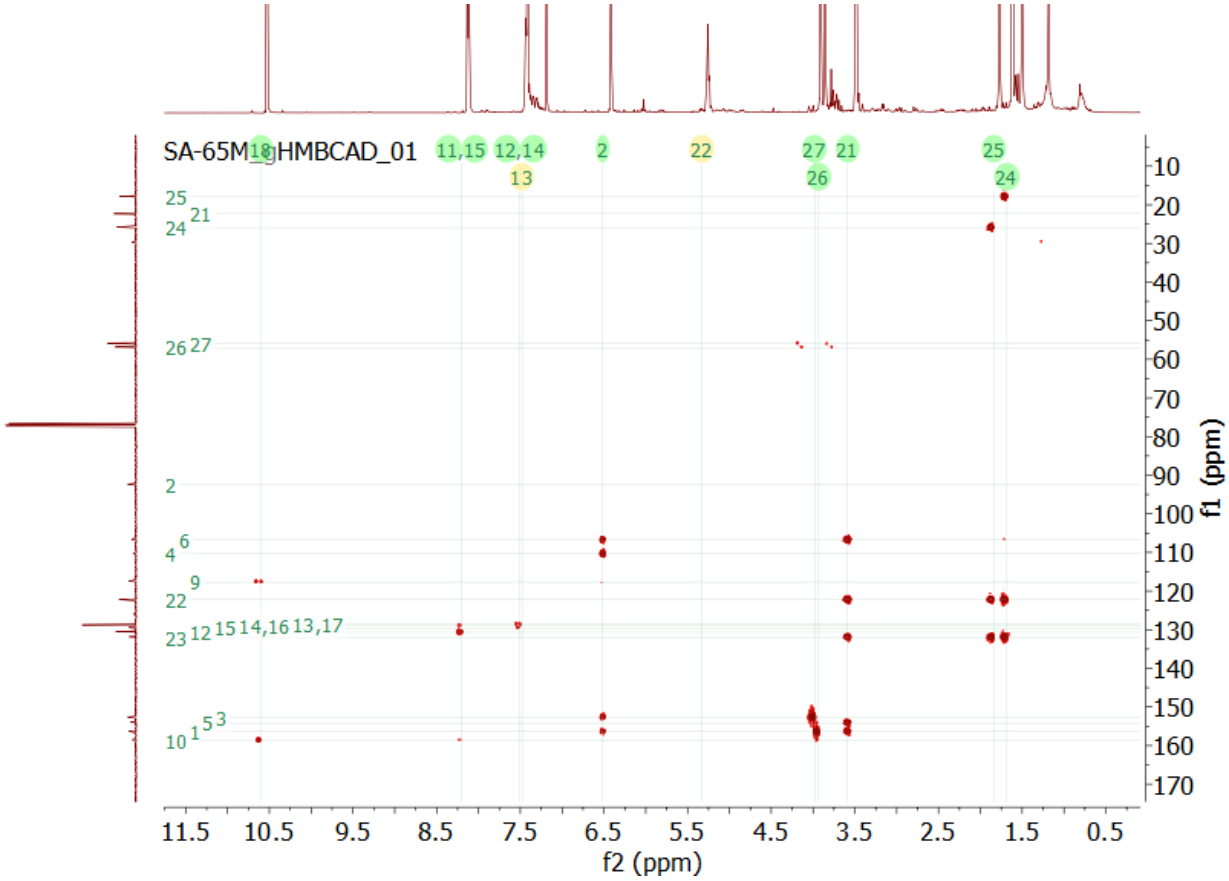
APPENDIX 1C: HH-COSY Spectrum of Rhodbenzofuran (**86**) (500MHz; $CDCl_3$)



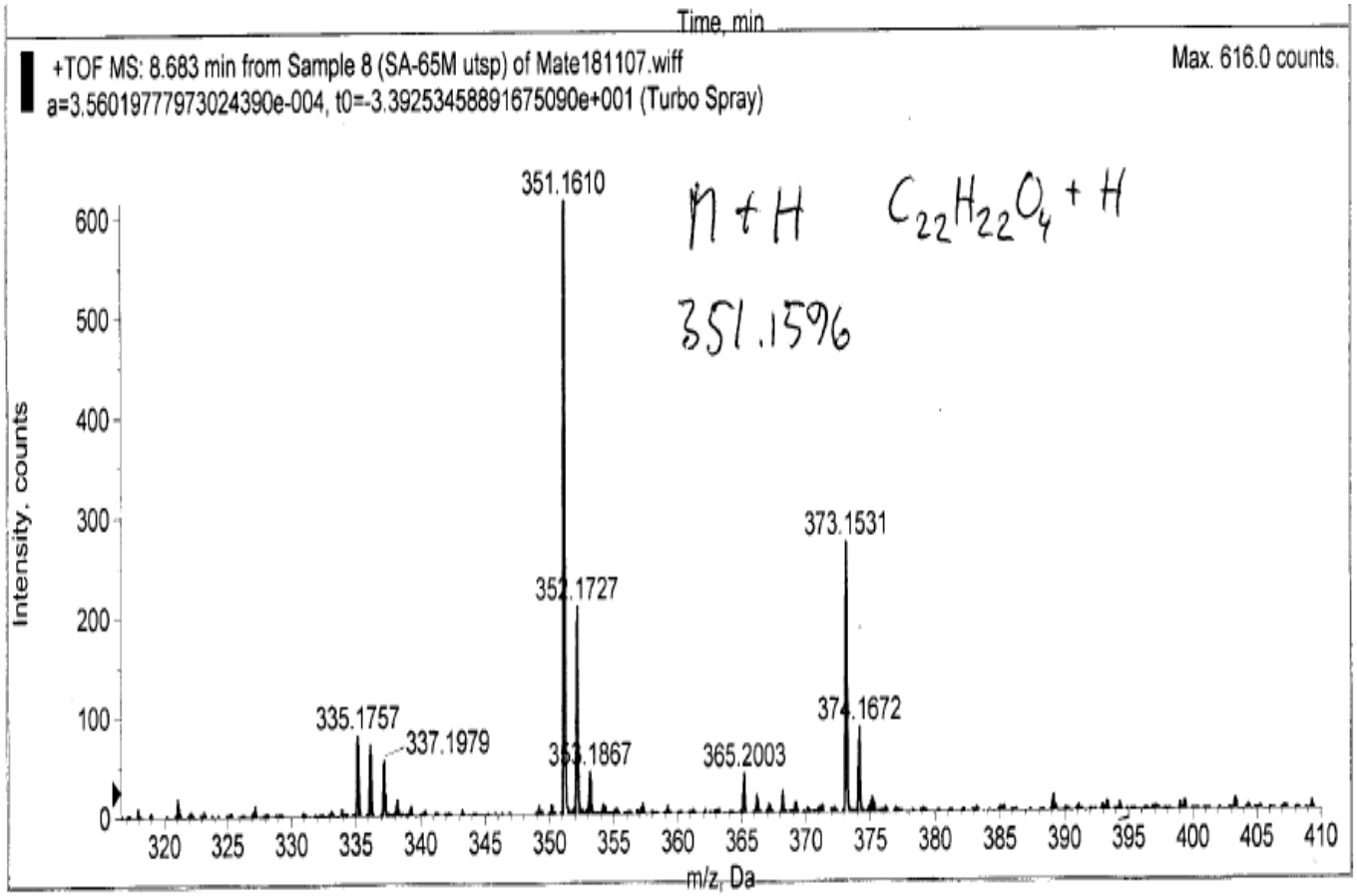
APPENDIX 1D: HSQC Spectrum of Rhodbenzofuran (**86**) (500MHz; $CDCl_3$)



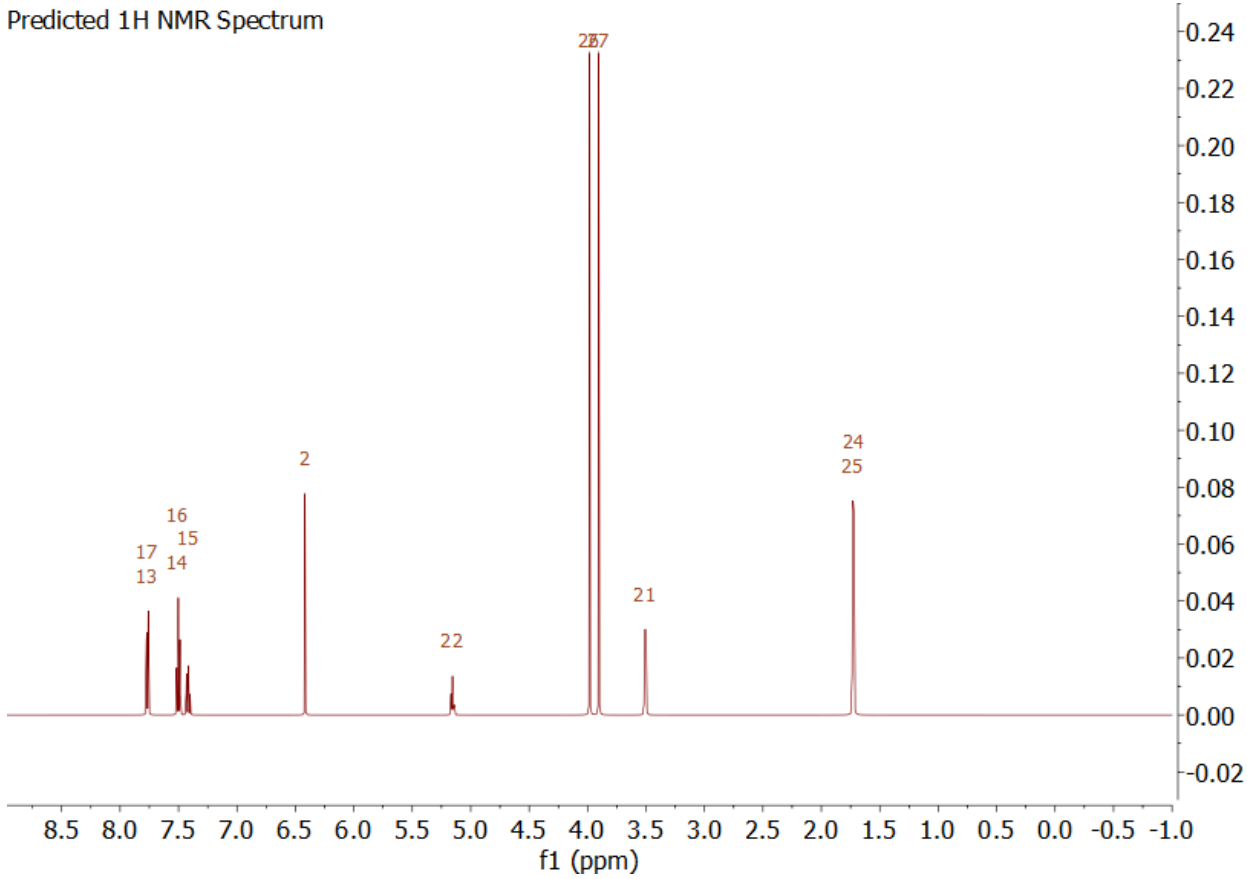
APPENDIX 1E: HMBC Spectrum of Rhodbenzofuran (**86**) (500MHz; $CDCl_3$)



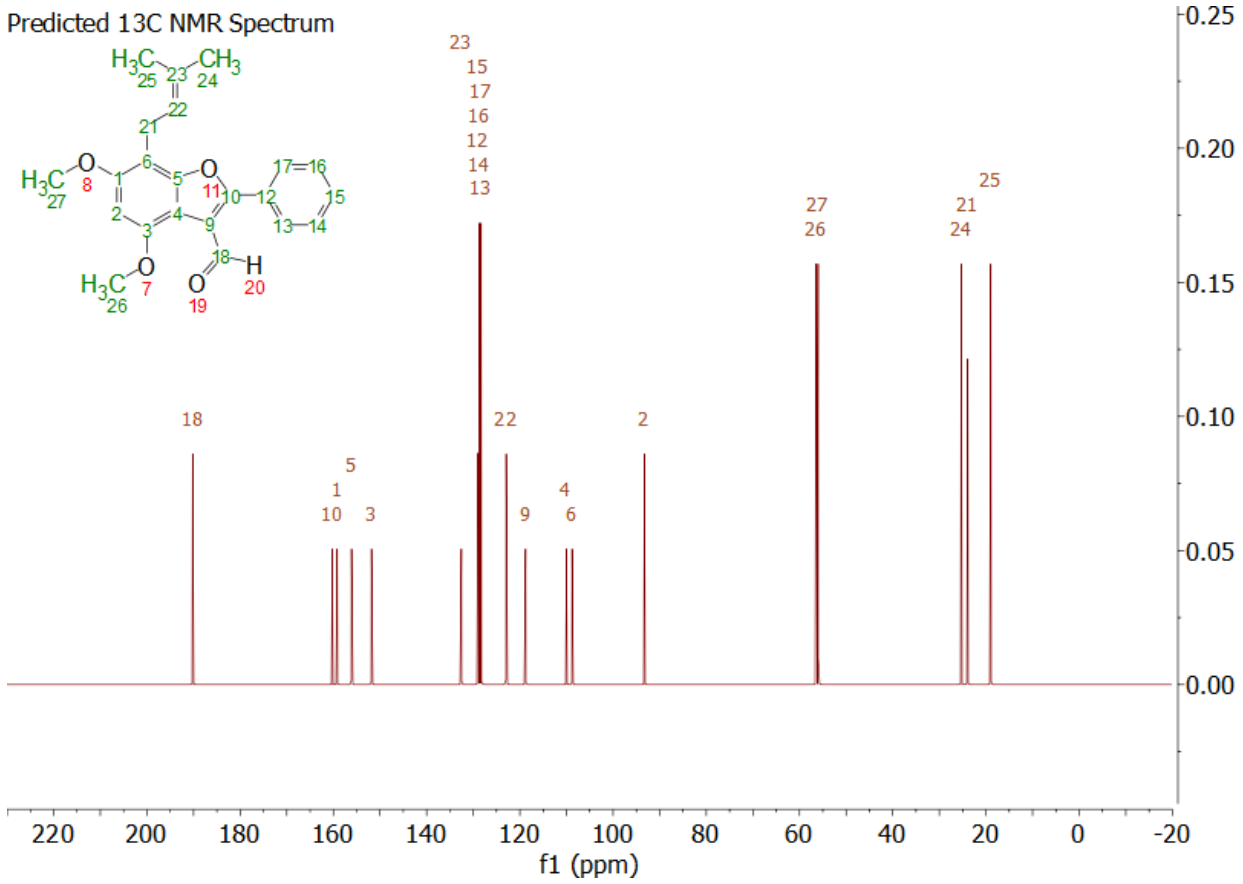
APPENDIX 1F: HRMS Spectrum of Rhodbenzofuran (**86**)



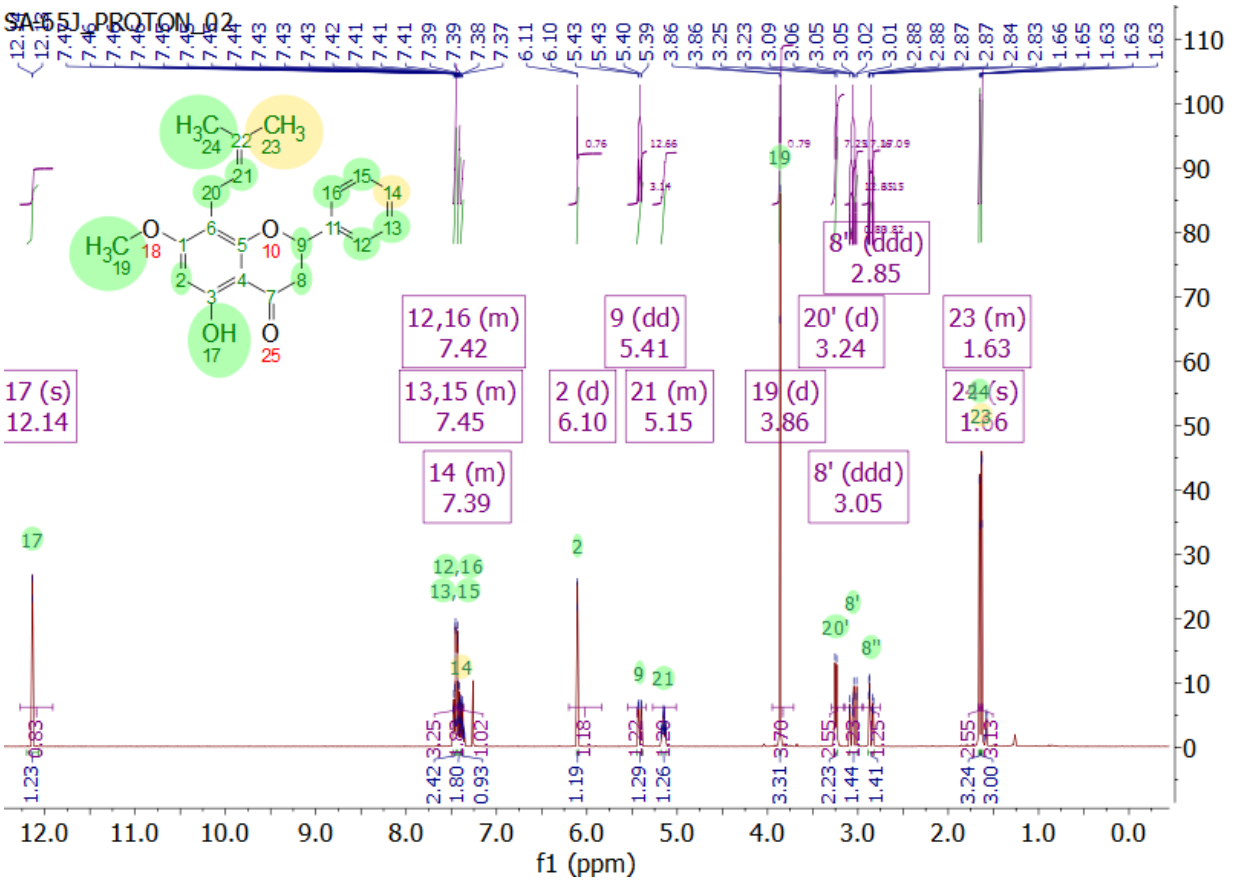
APPENDIX 1G: Predicted 1H NMR Spectrum of Rhodbenzofuran (**86**)



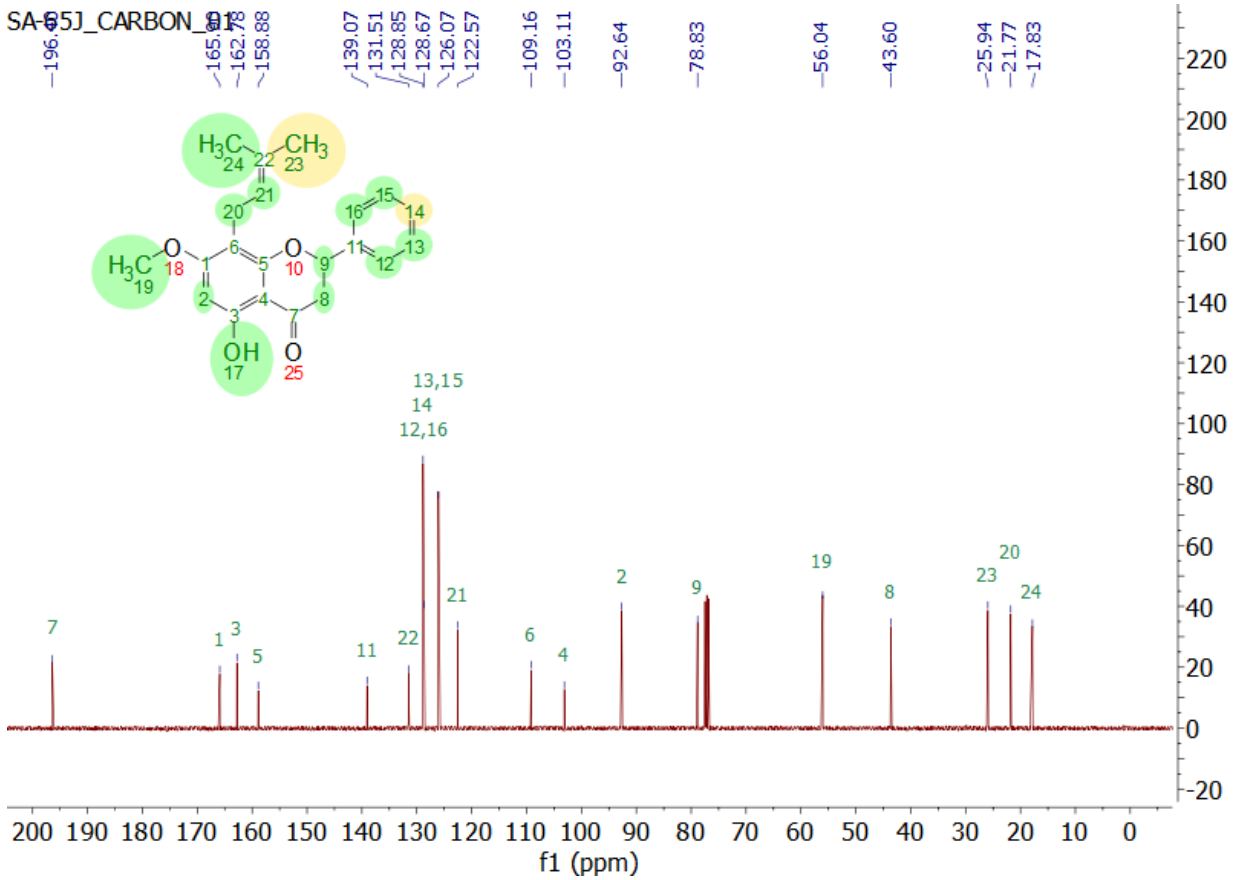
APPENDIX 1H: Predicted ^{13}C NMR Spectrum of Rhodbenzofuran (**86**)



APPENDIX 2A: ^{1}H NMR Spectrum of 7-methylglabranin (**90**) (500MHz; $CDCl_3$)

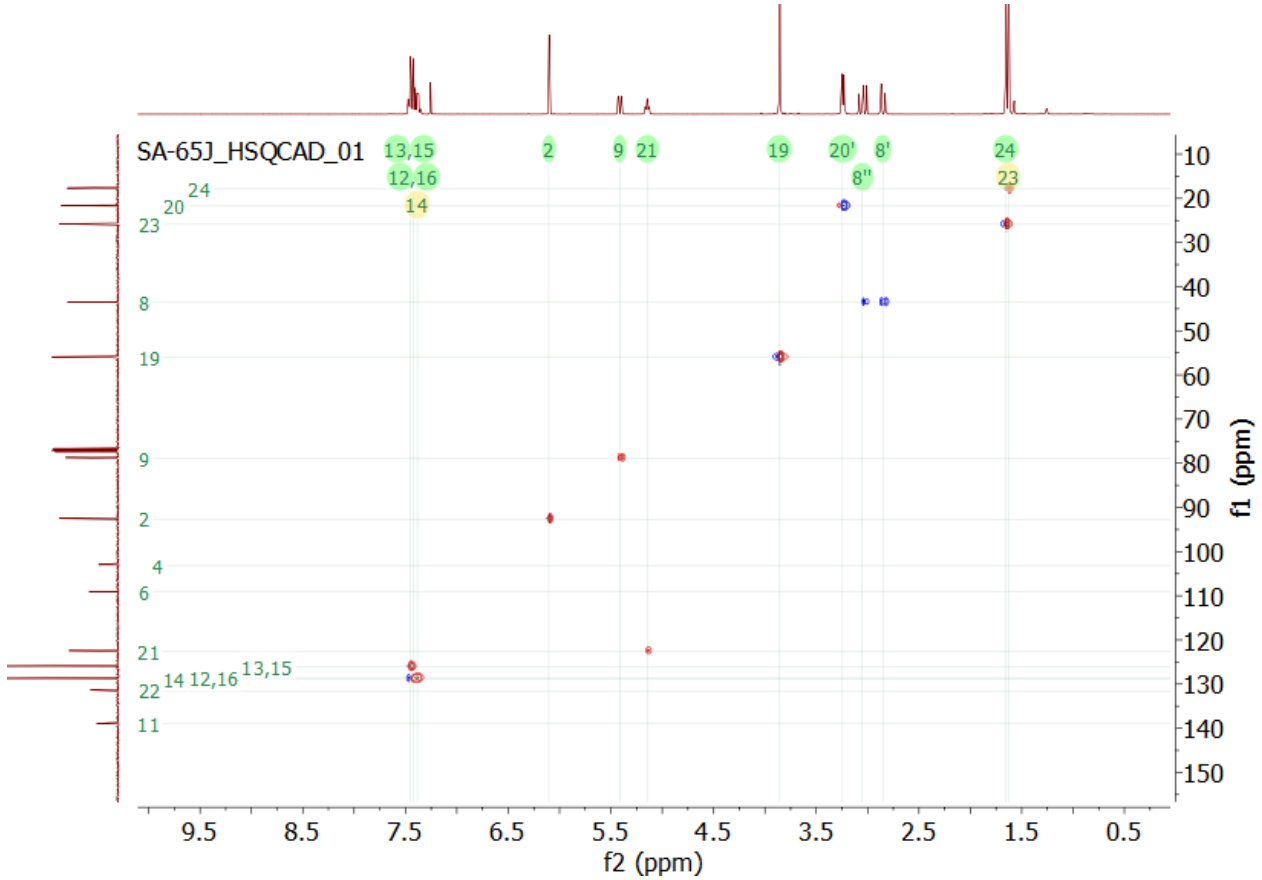


APPENDIX 2B: ^{13}C NMR Spectrum of 7-methylglabranin (**90**) (125 MHz; $CDCl_3$)

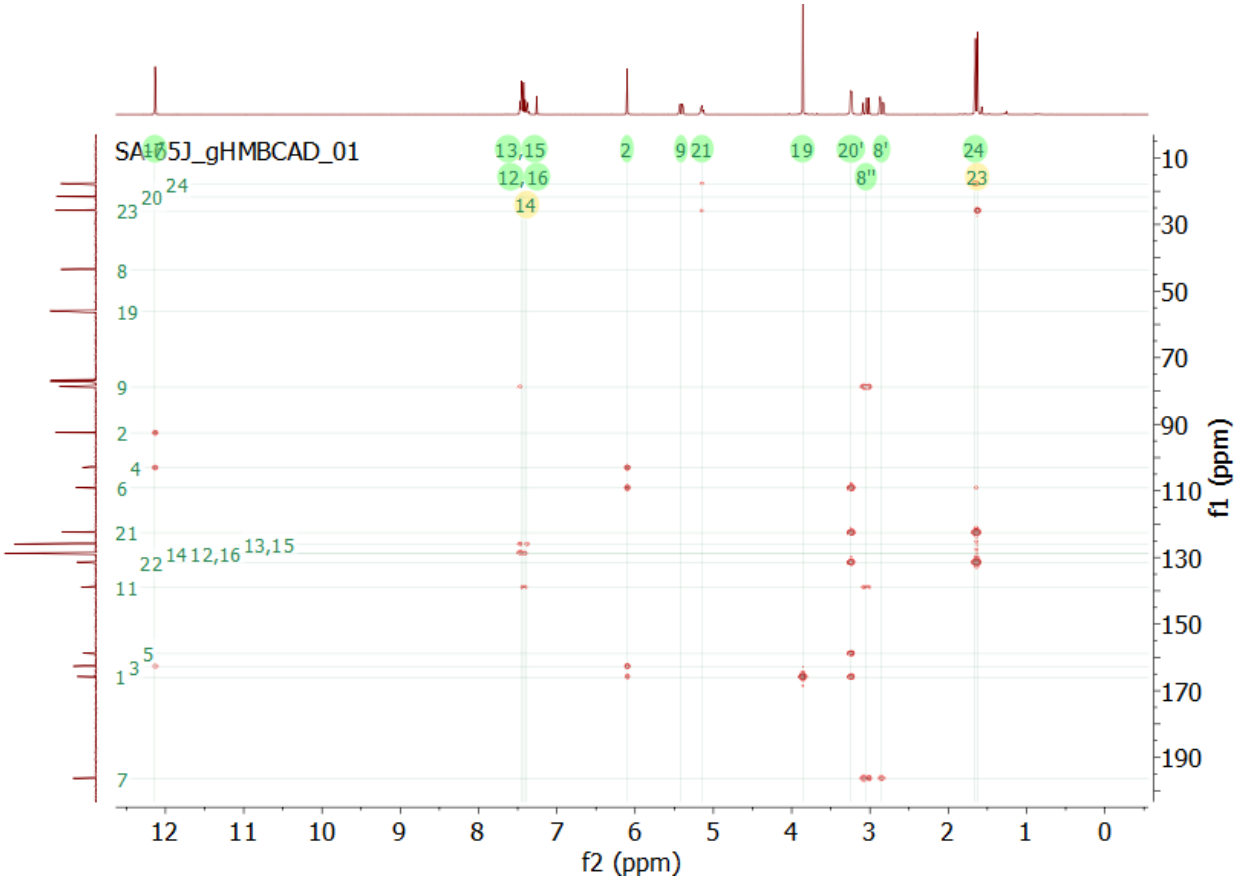


APPENDIX 2C: HH-COSY Spectrum of 7-methylglabranin (**90**) (500 MHz; $CDCl_3$)

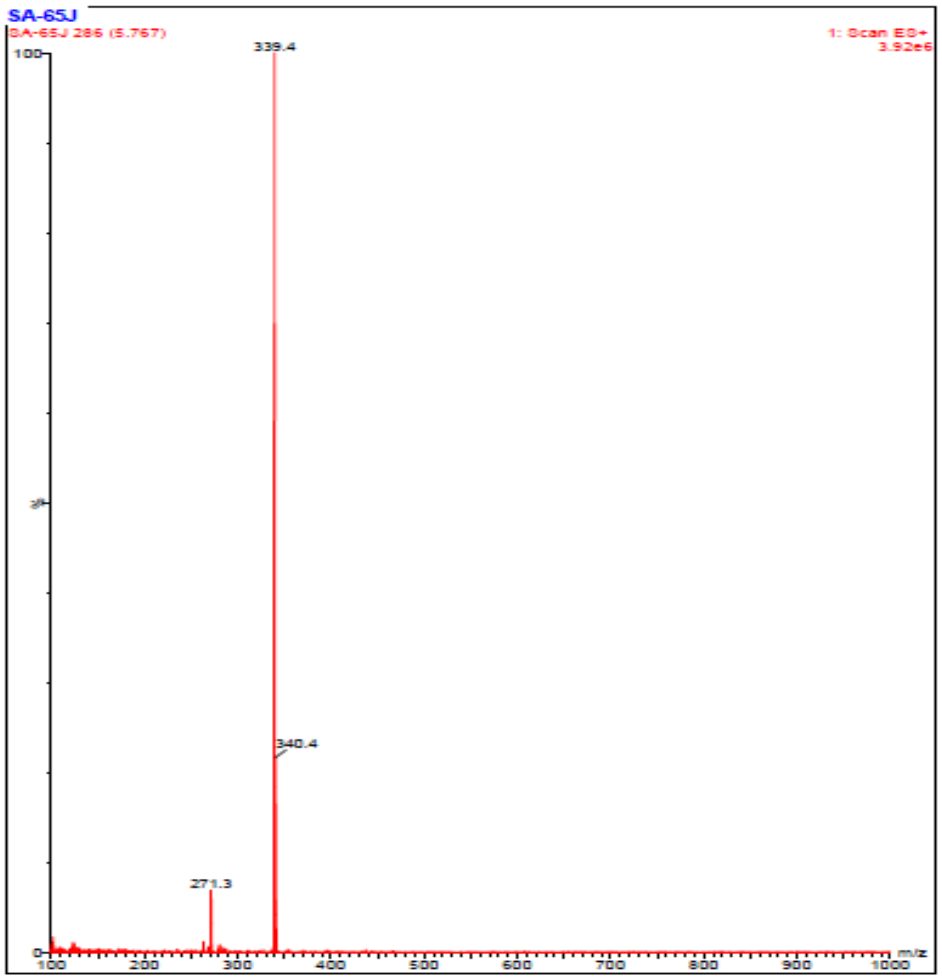
APPENDIX 2D: HSQC Spectrum of 7-methylglabranin (**90**) (500 MHz; $CDCl_3$)



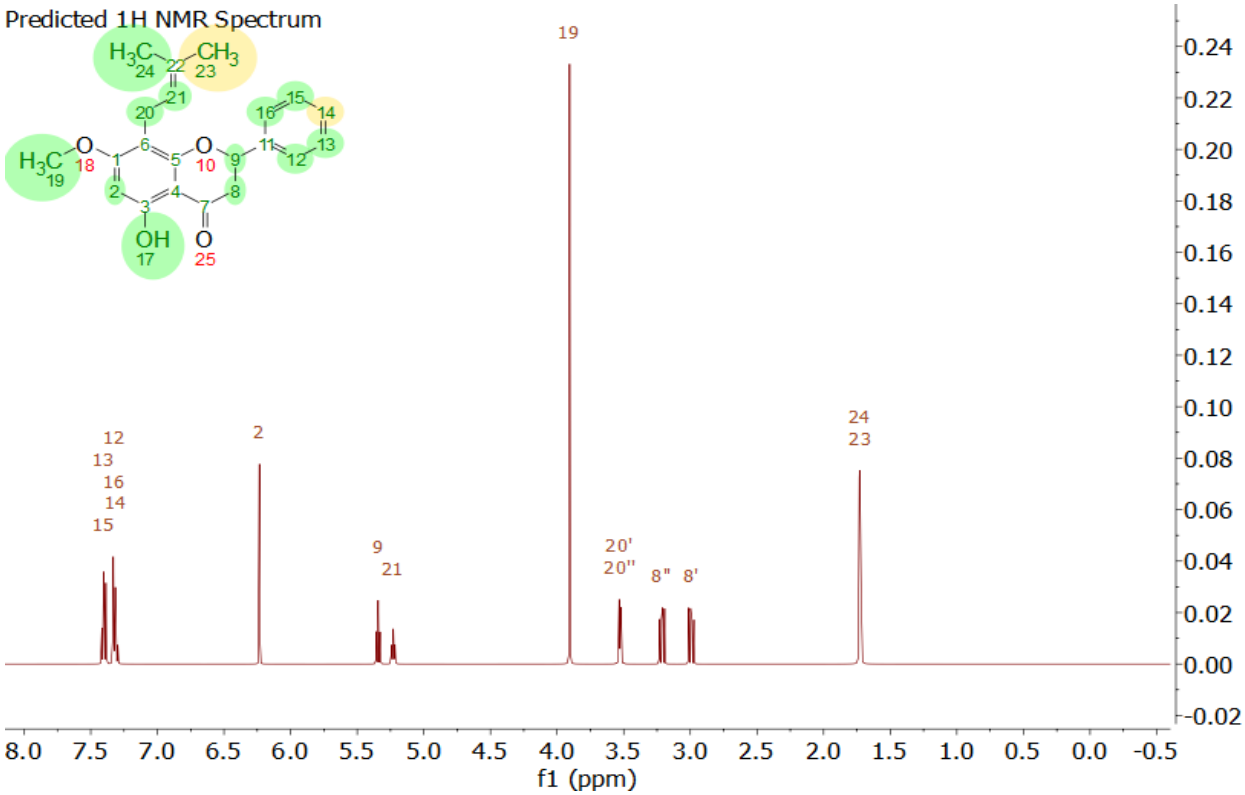
APPENDIX 2E: HMBC Spectrum of 7-methylglabranin (**90**) (500 MHz; $CDCl_3$)



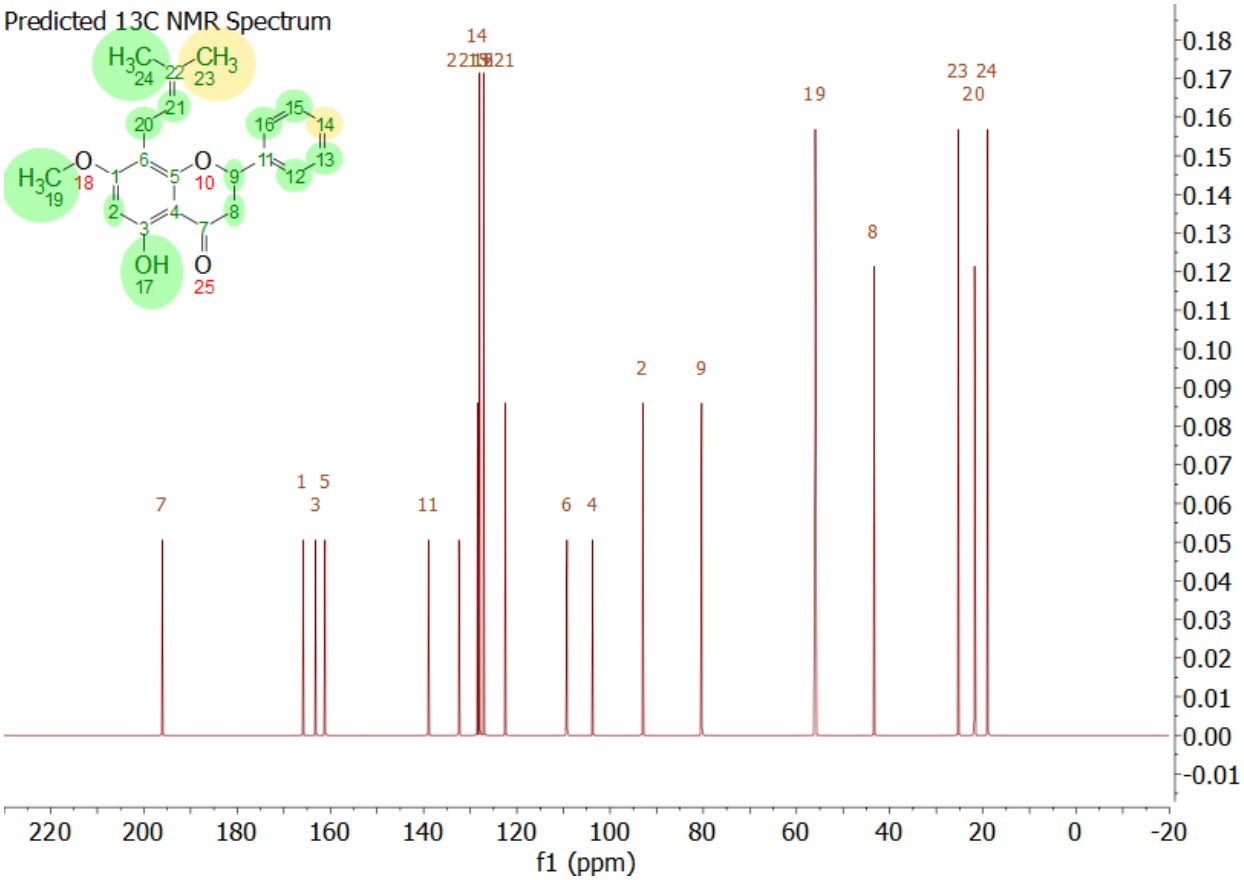
APPENDIX 2F: LCMS Spectrum of 7-methylglabranin (**90**)



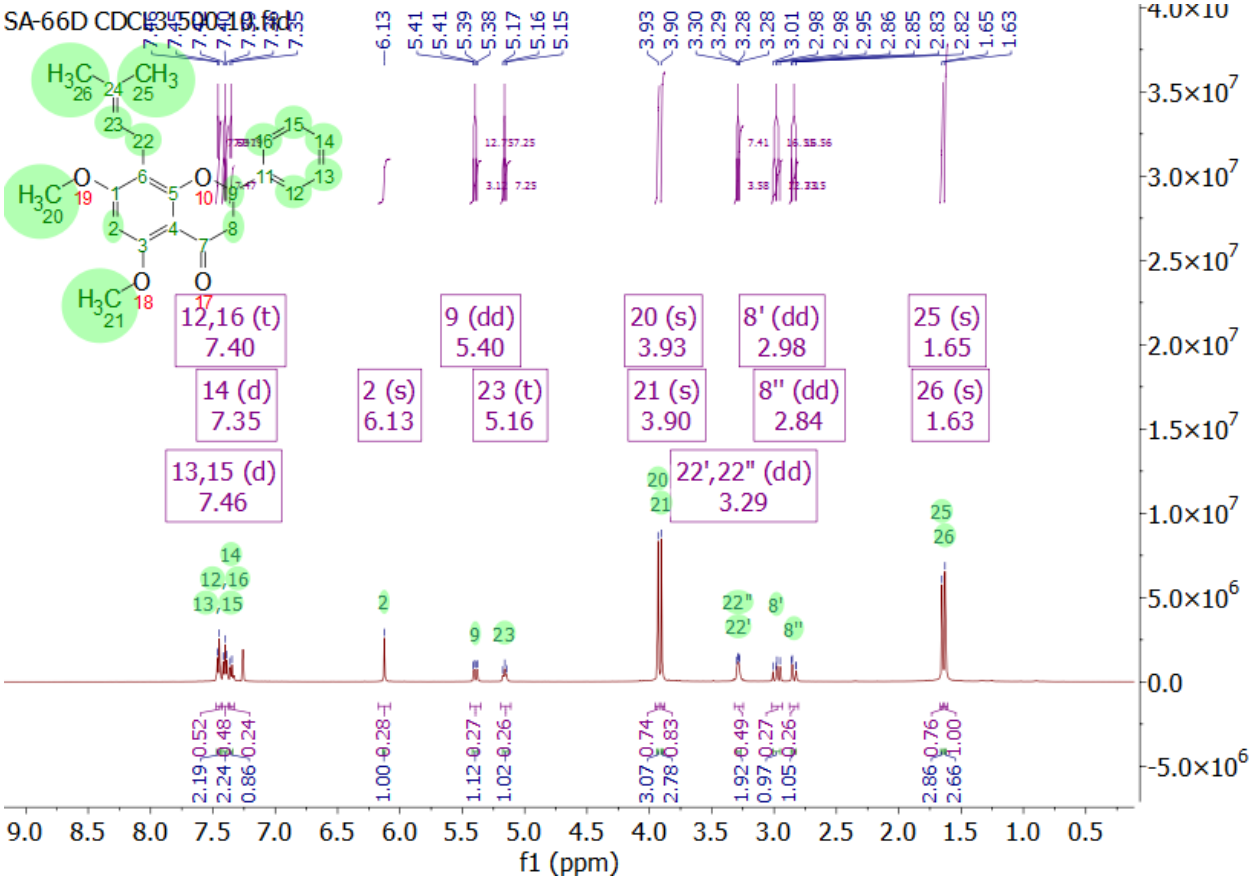
APPENDIX 2G: Predicted ^{1}H NMR Spectrum of 7-methylglabranin (**90**)



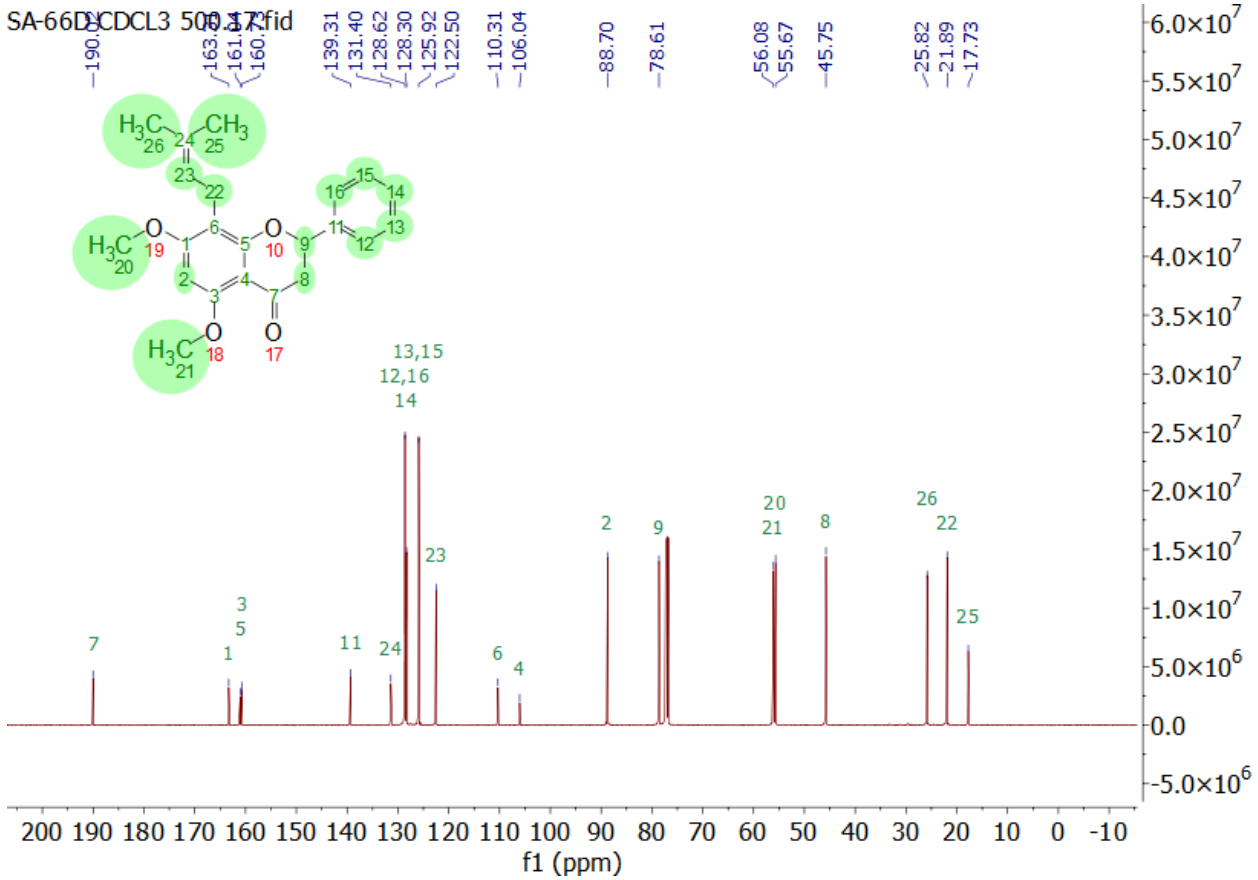
APPENDIX 2H: Predicted ^{13}C NMR Spectrum of 7-methylglabranin (**90**)



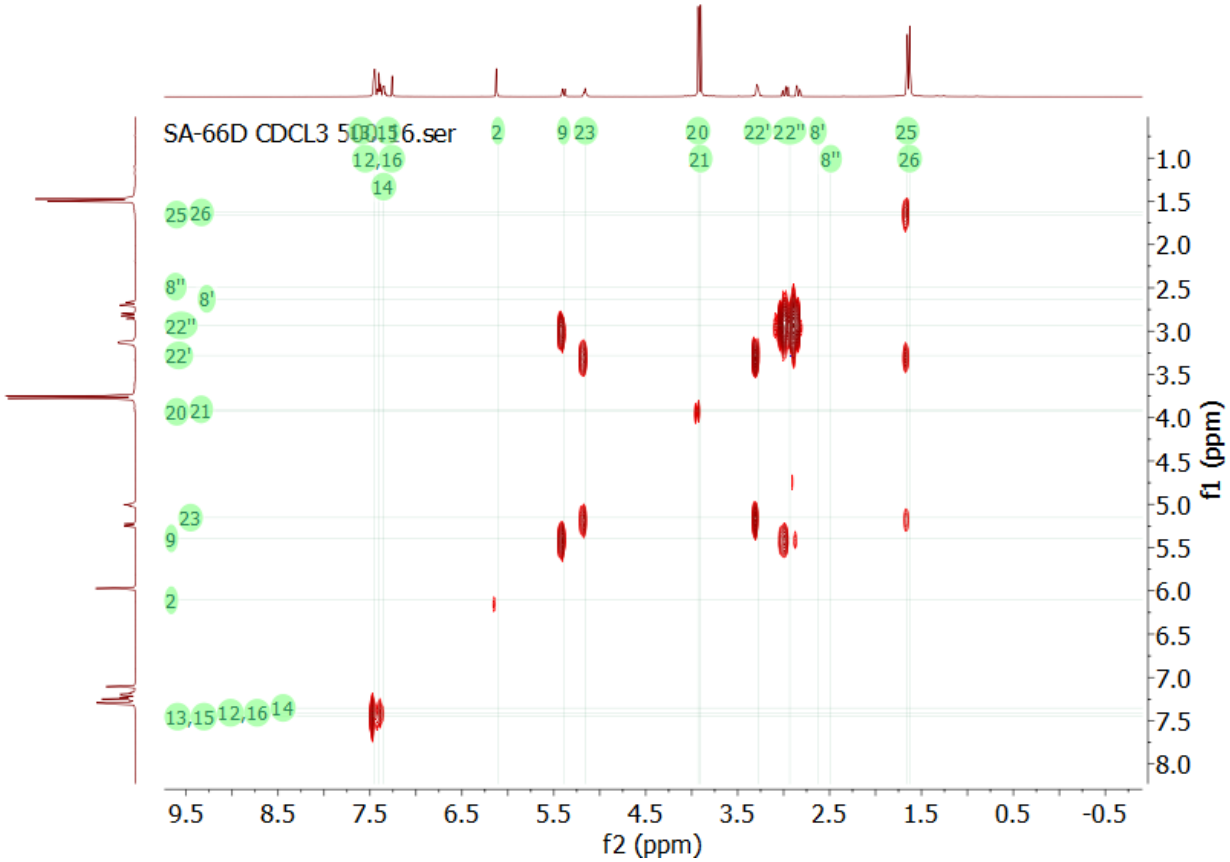
APPENDIX 3A: ^{1}H NMR Spectrum of Candidone (**91**) (500MHz; $CDCl_3$)



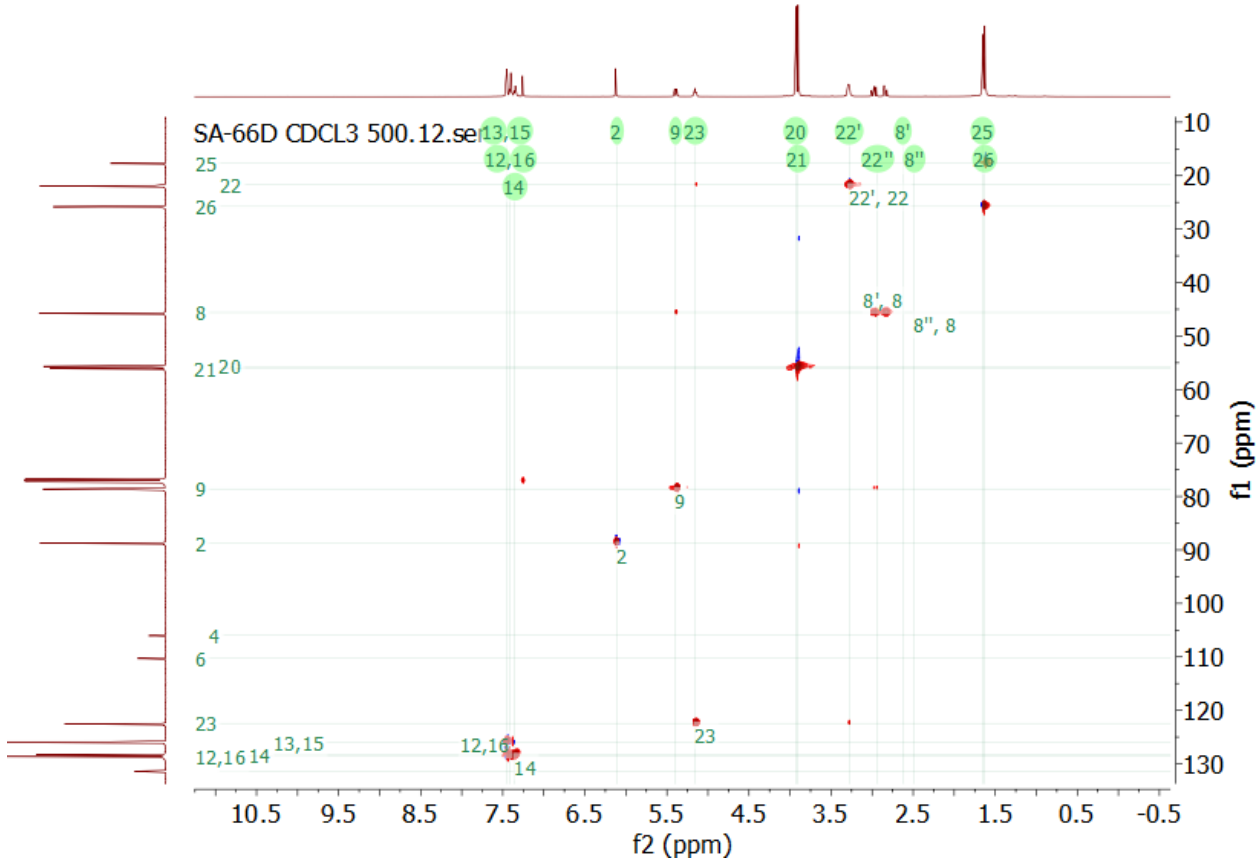
APPENDIX 3B: ^{13}C NMR Spectrum of Candidone (**91**) (125 MHz; $CDCl_3$)



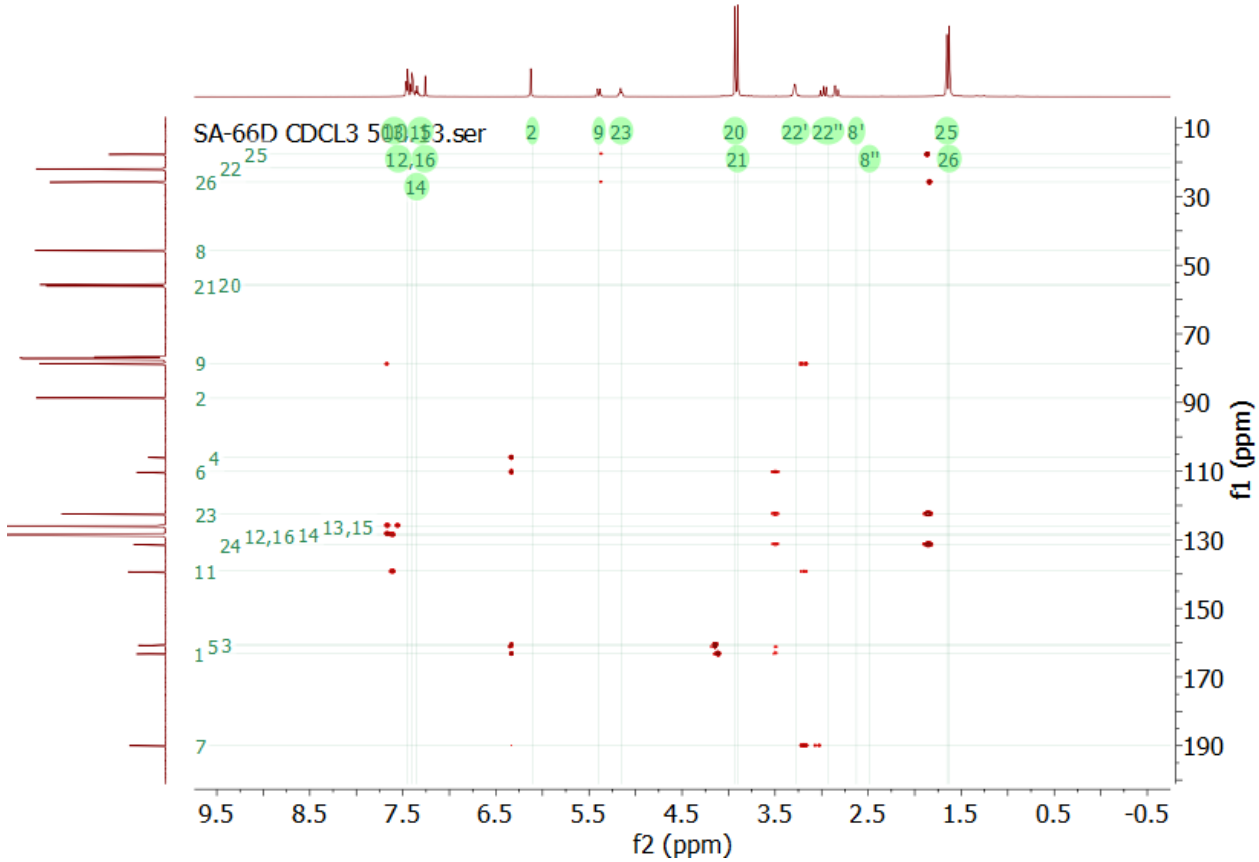
APPENDIX 3C: HH-COSY Spectrum of Candidone (**91**) (500 MHz; $CDCl_3$)



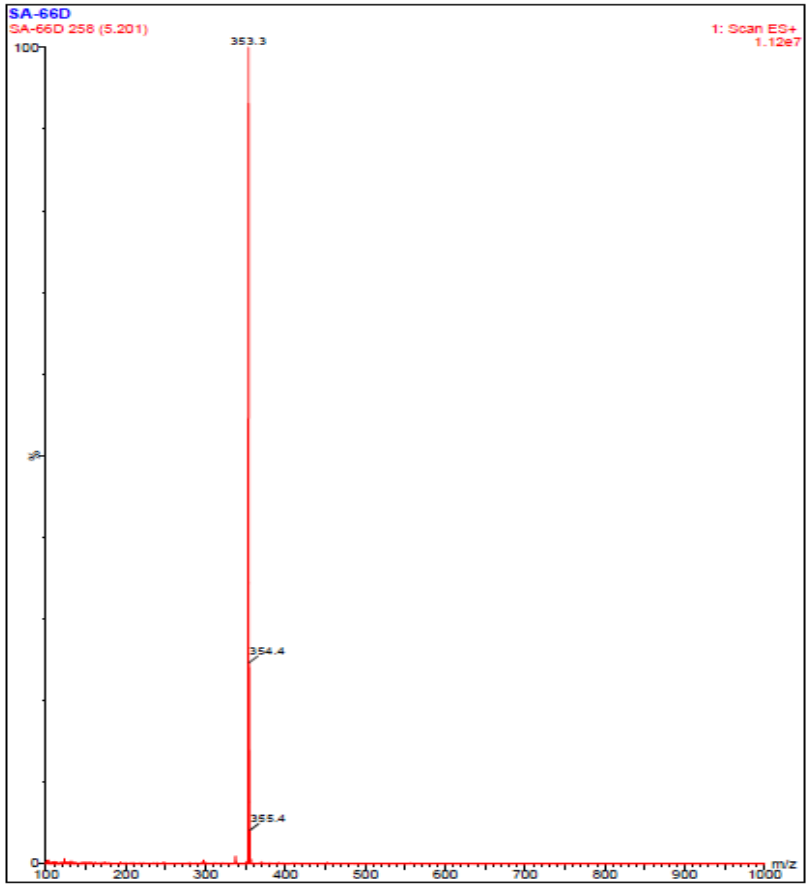
APPENDIX 3D: HSQC Spectrum of Candidone (**91**) (500 MHz; $CDCl_3$)



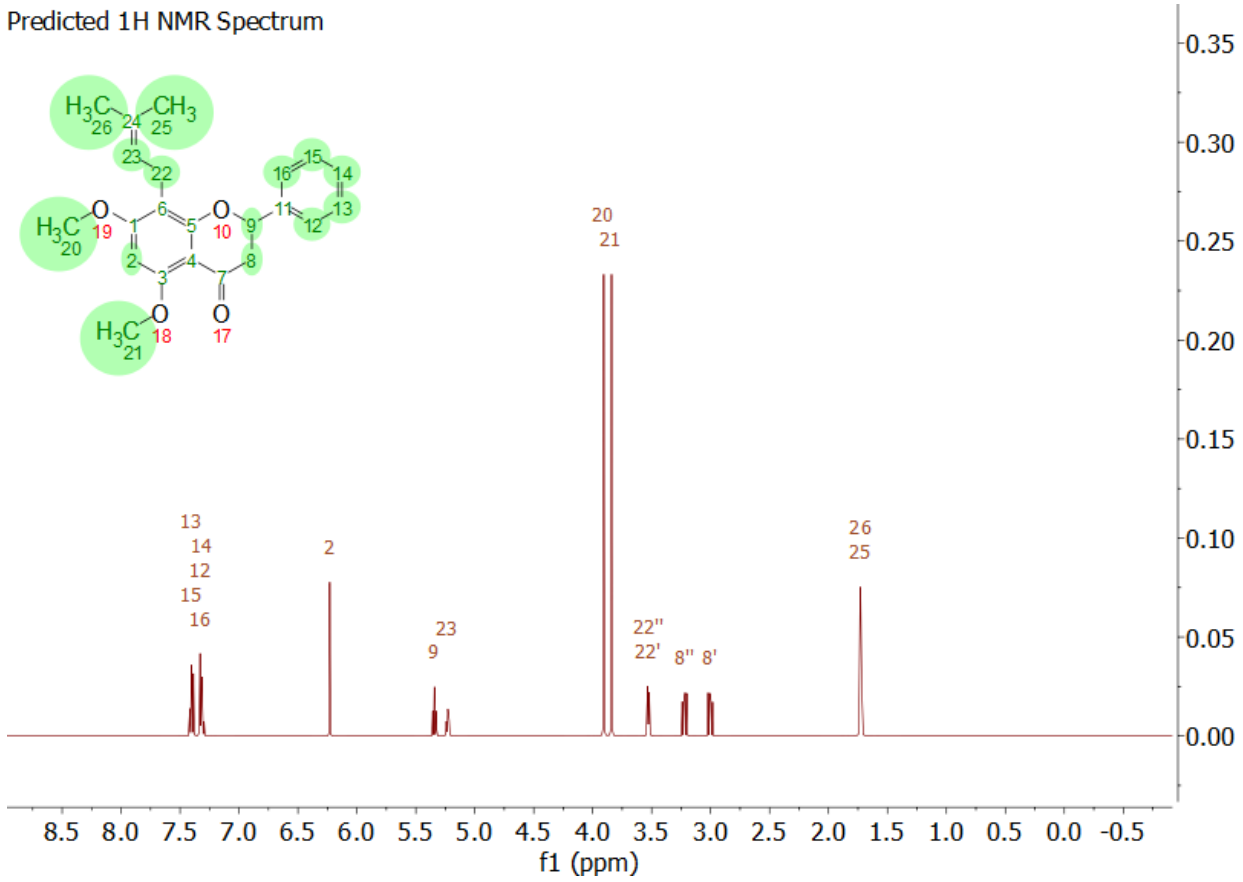
APPENDIX 3E: HMBC Spectrum of Candidone (**91**) (500 MHz; $CDCl_3$)



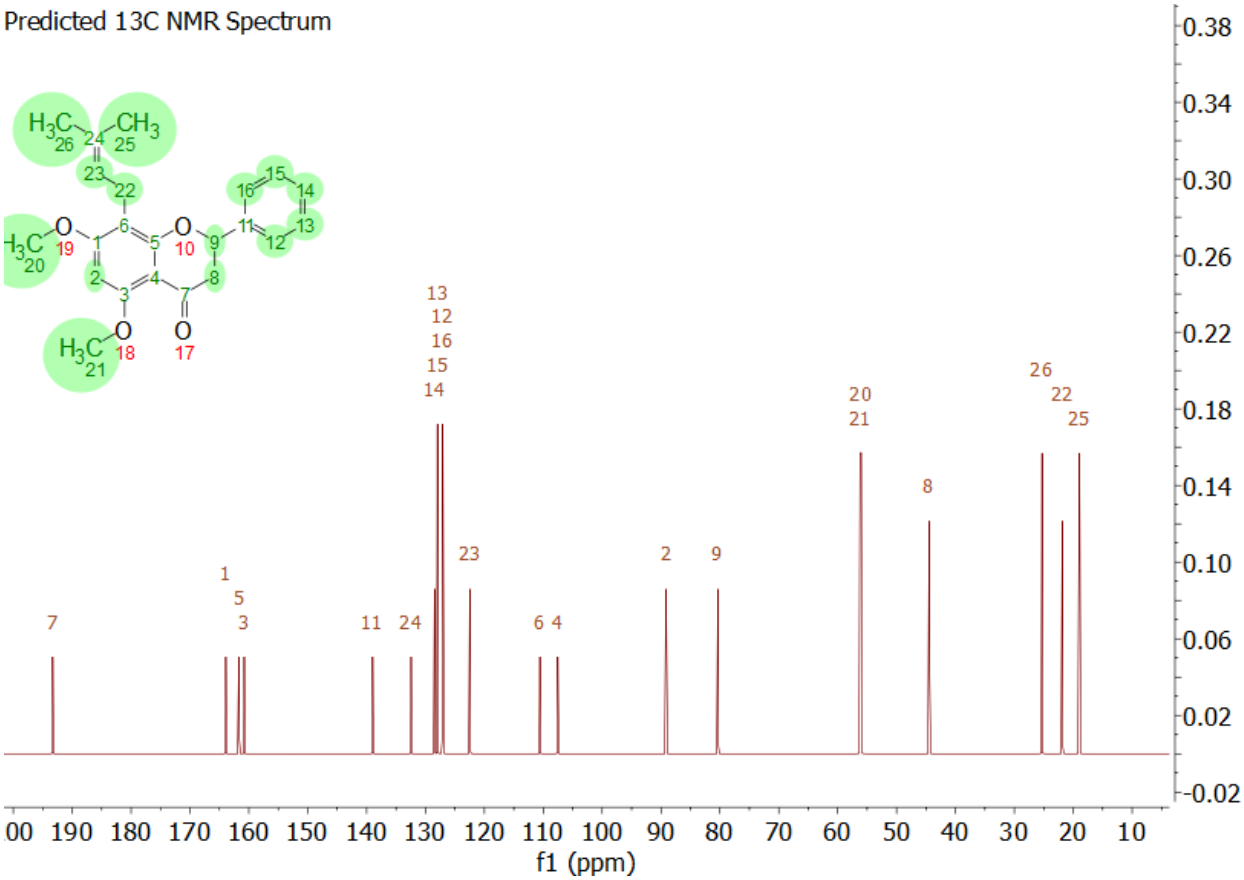
APPENDIX 3F: LCMS Spectrum of Candidone (**91**)



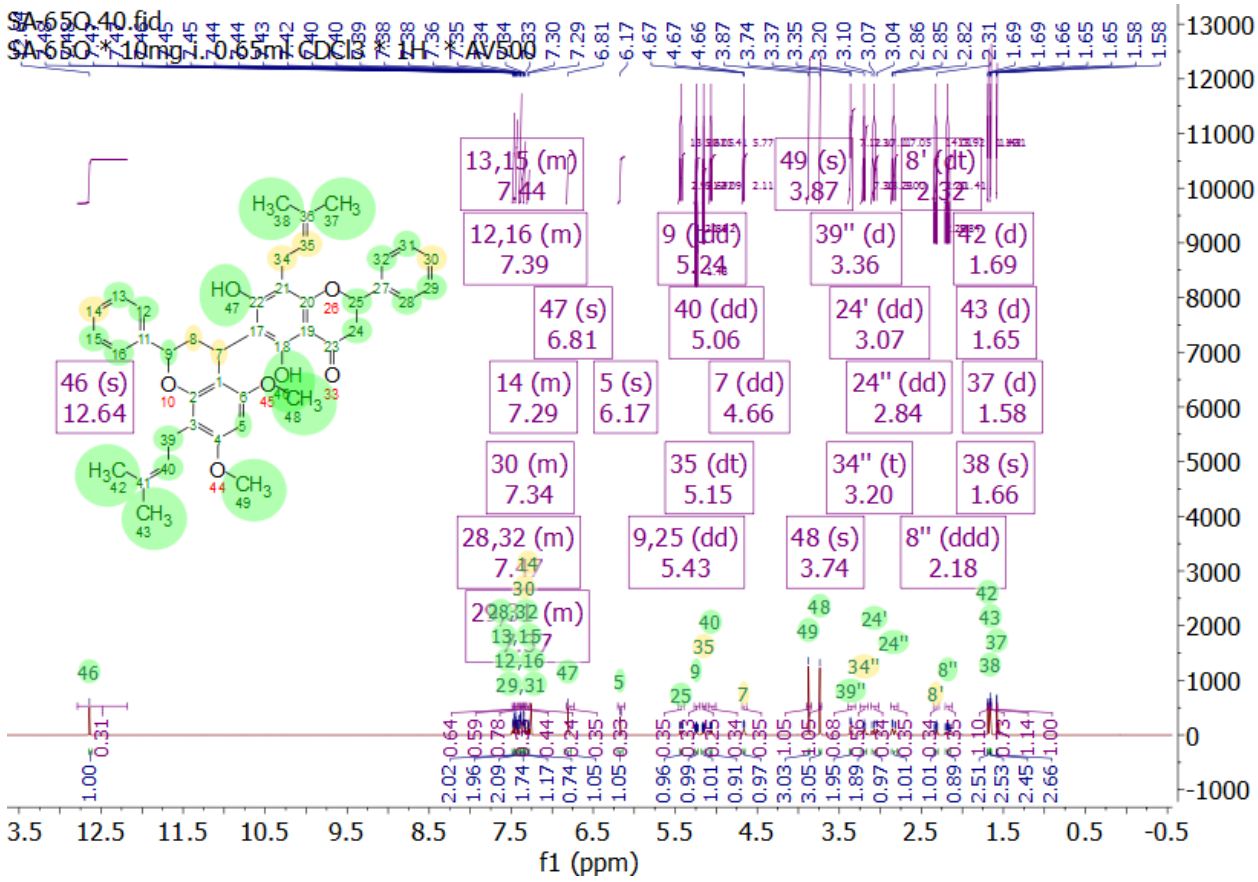
APPENDIX 3G: Predicted[1]H NMR Spectrum of Candidone (**91**)



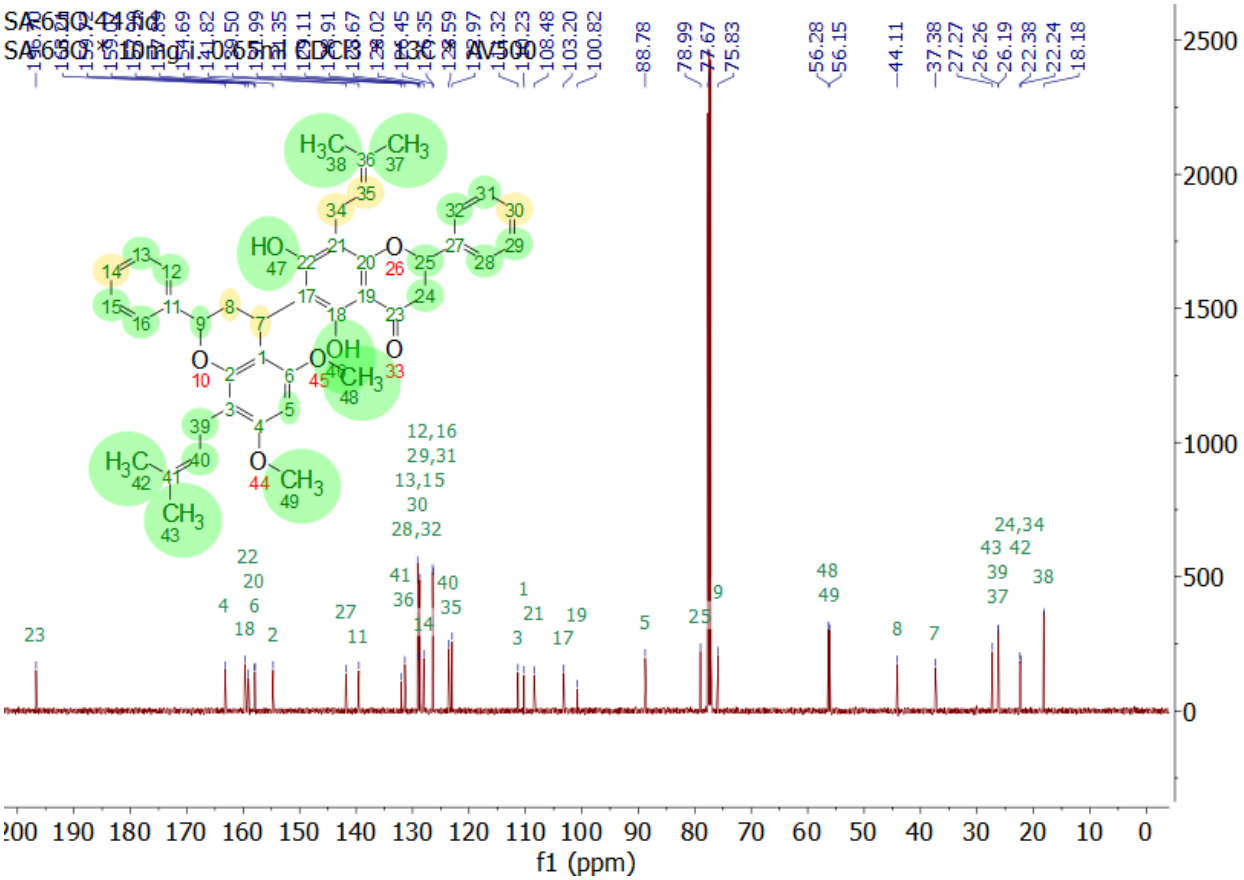
APPENDIX 3H: Predicted[13] C NMR Spectrum of Candidone (**91**)



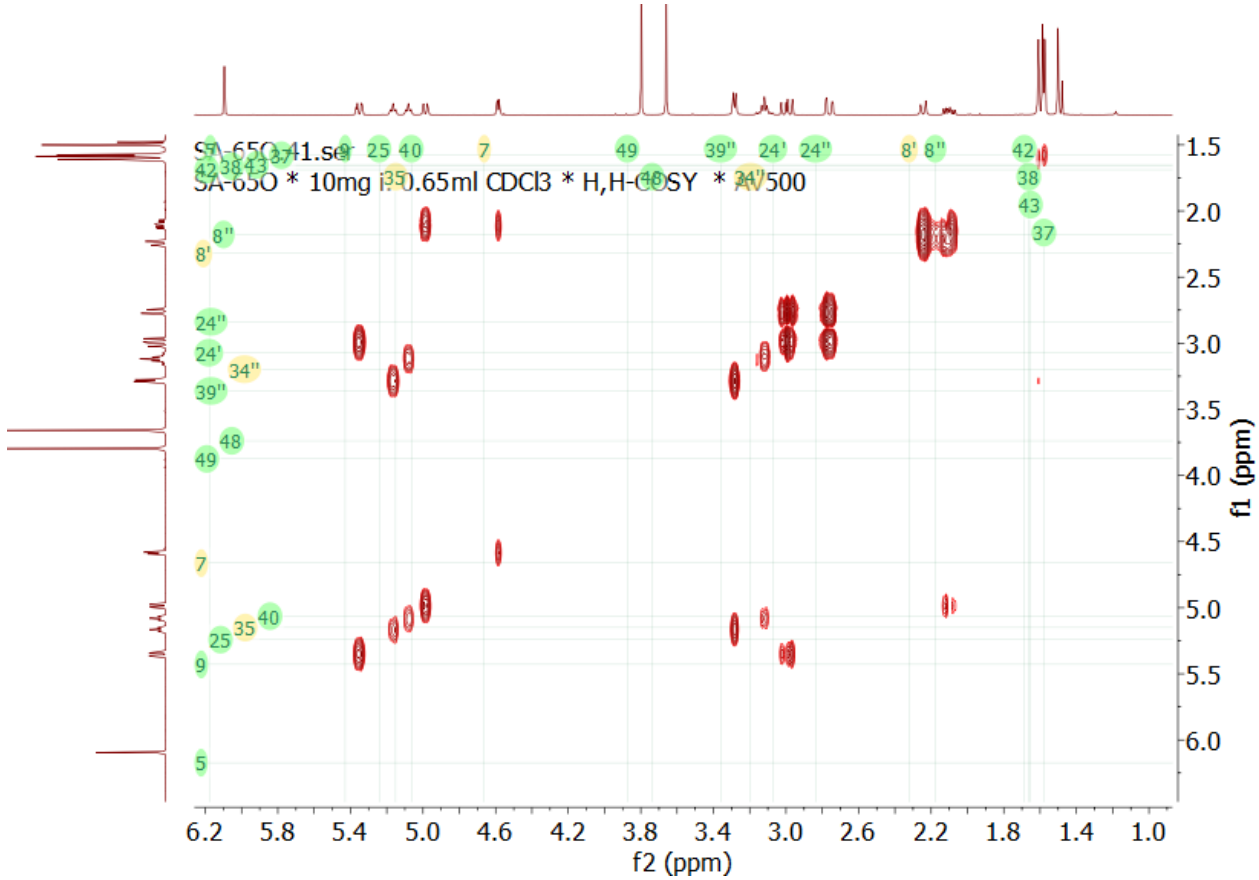
APPENDIX 4A: ^{1}H NMR Spectrum of rhodimer (**88**) (500 MHz; $CDCl_3$)



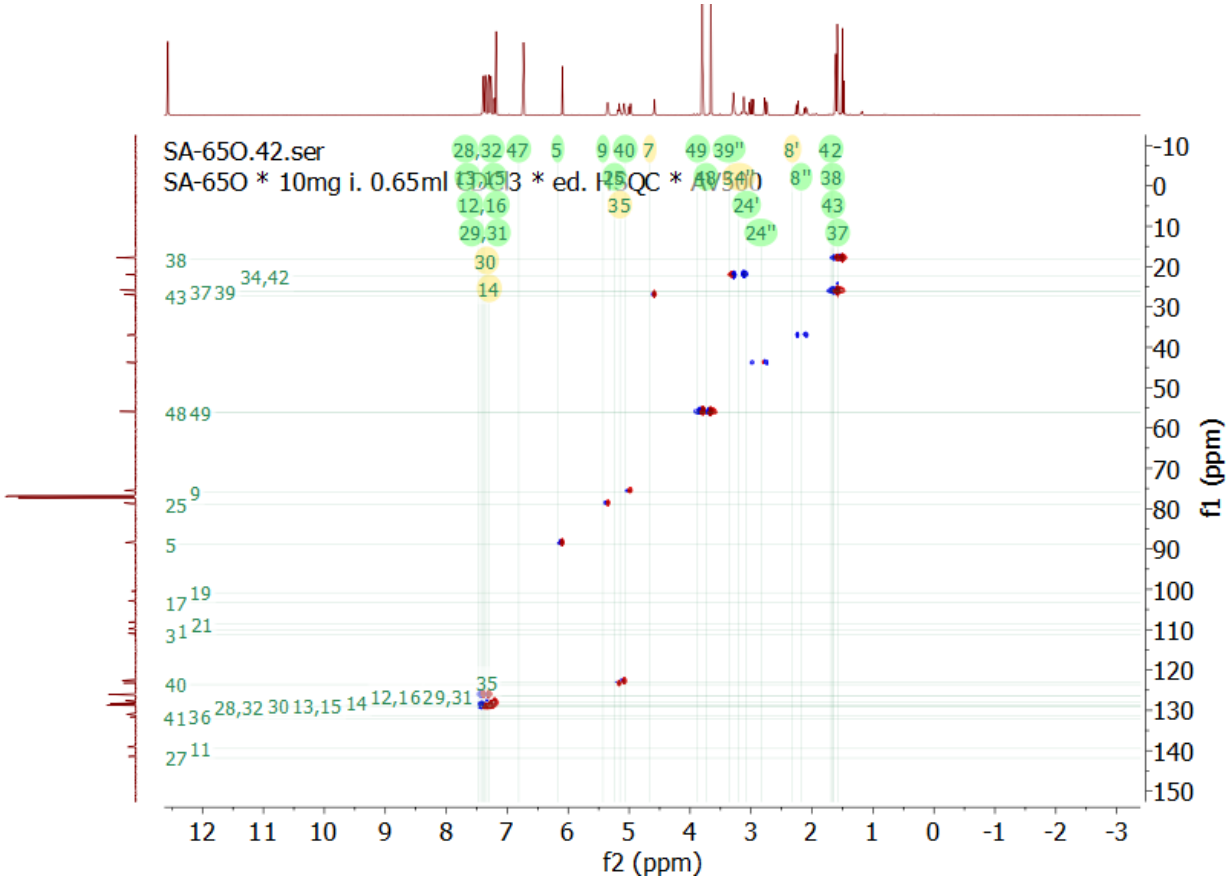
APPENDIX 4B: ^{13}C NMR Spectrum of rhodimer (**88**) (125 MHz; $CDCl_3$)



APPENDIX 4C: HH-COSY Spectrum of rhodimer (**88**) (500 MHz; $CDCl_3$)

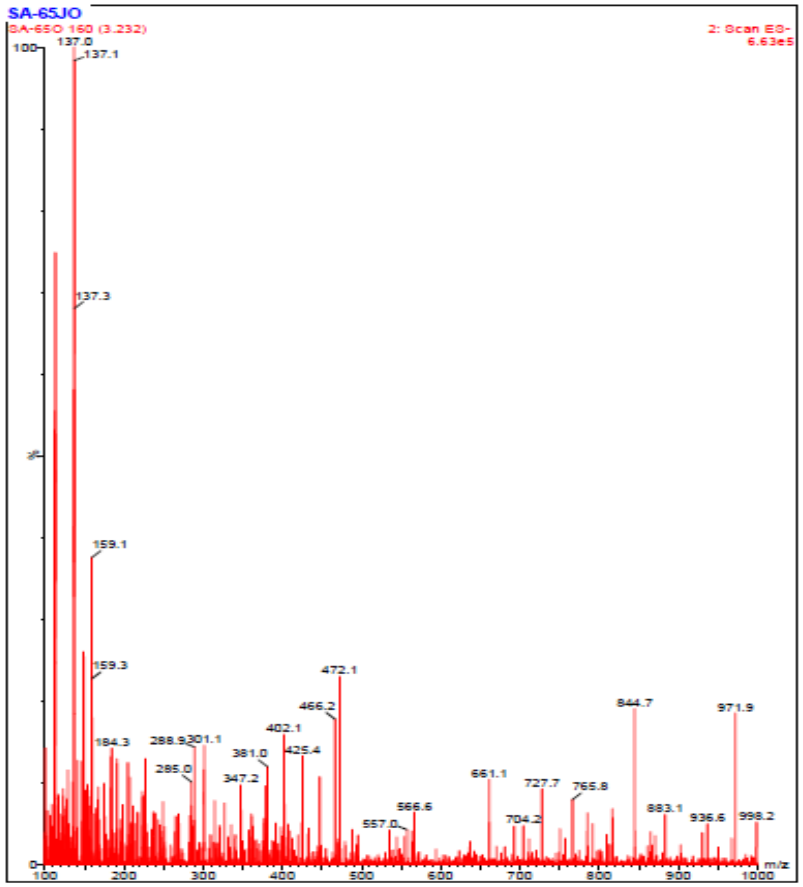


APPENDIX 4D: HSQC Spectrum of rhodimer (**88**) (500 MHz; $CDCl_3$)

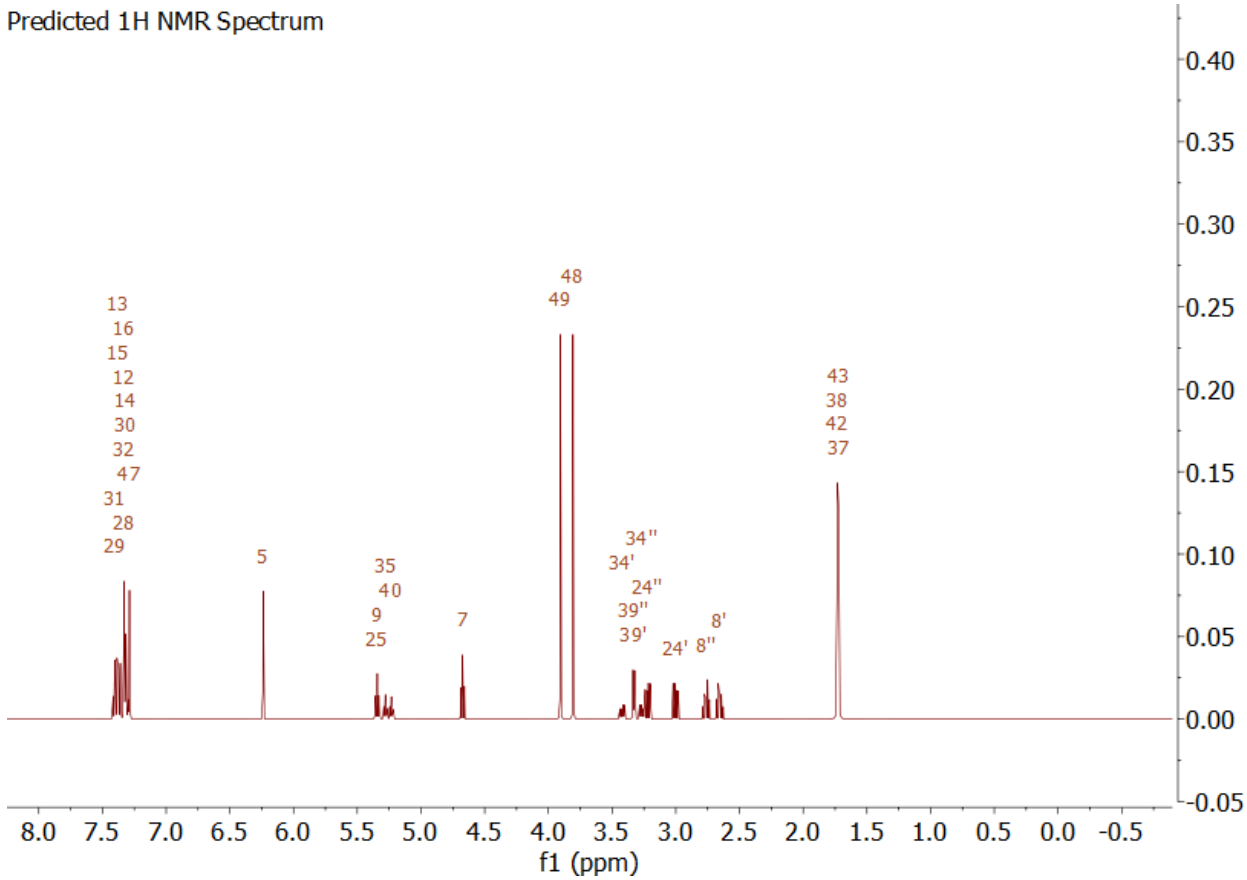


APPENDIX 4E: HMBC Spectrum of rhodimer (**88**) (500 MHz; $CDCl_3$)

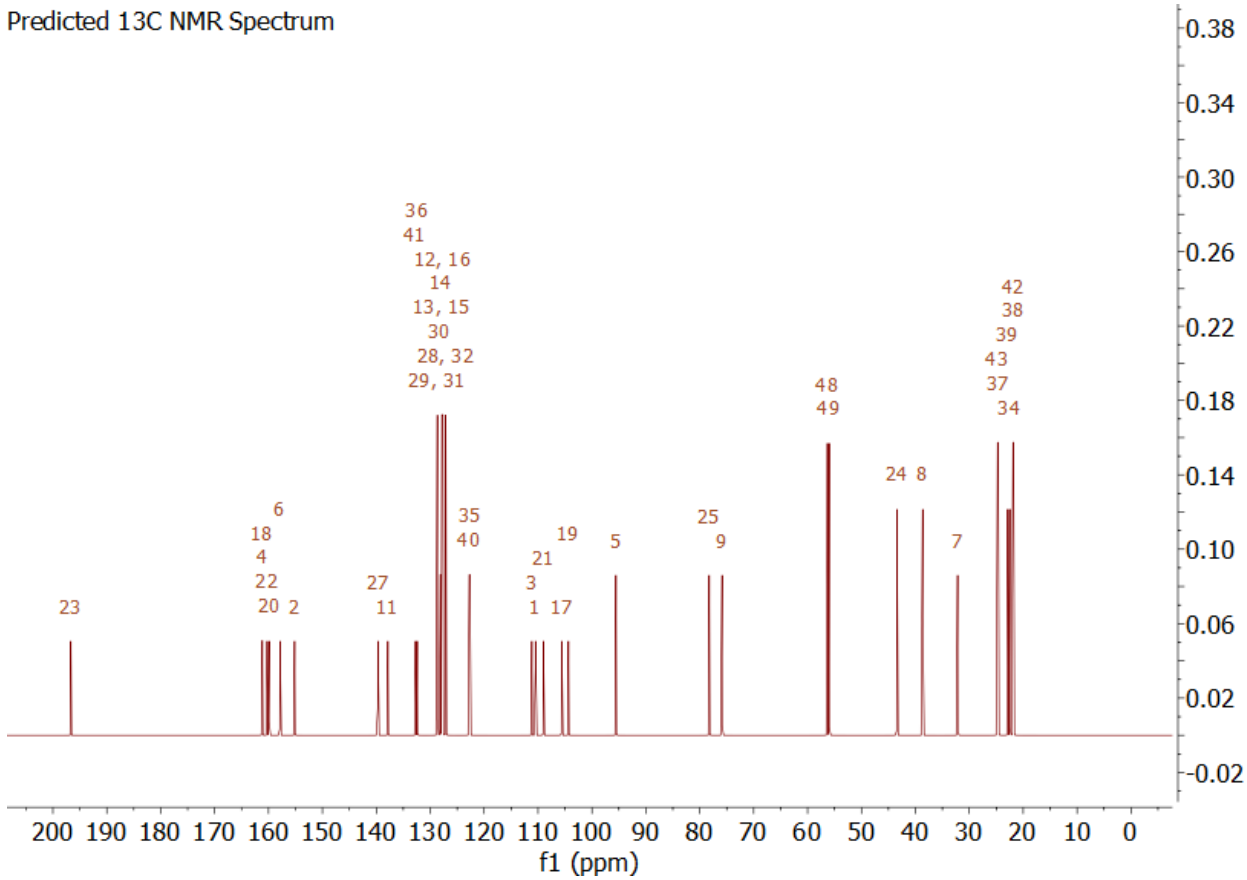
APPENDIX 4F: LCMS Spectrum of rhodimer (**88**)



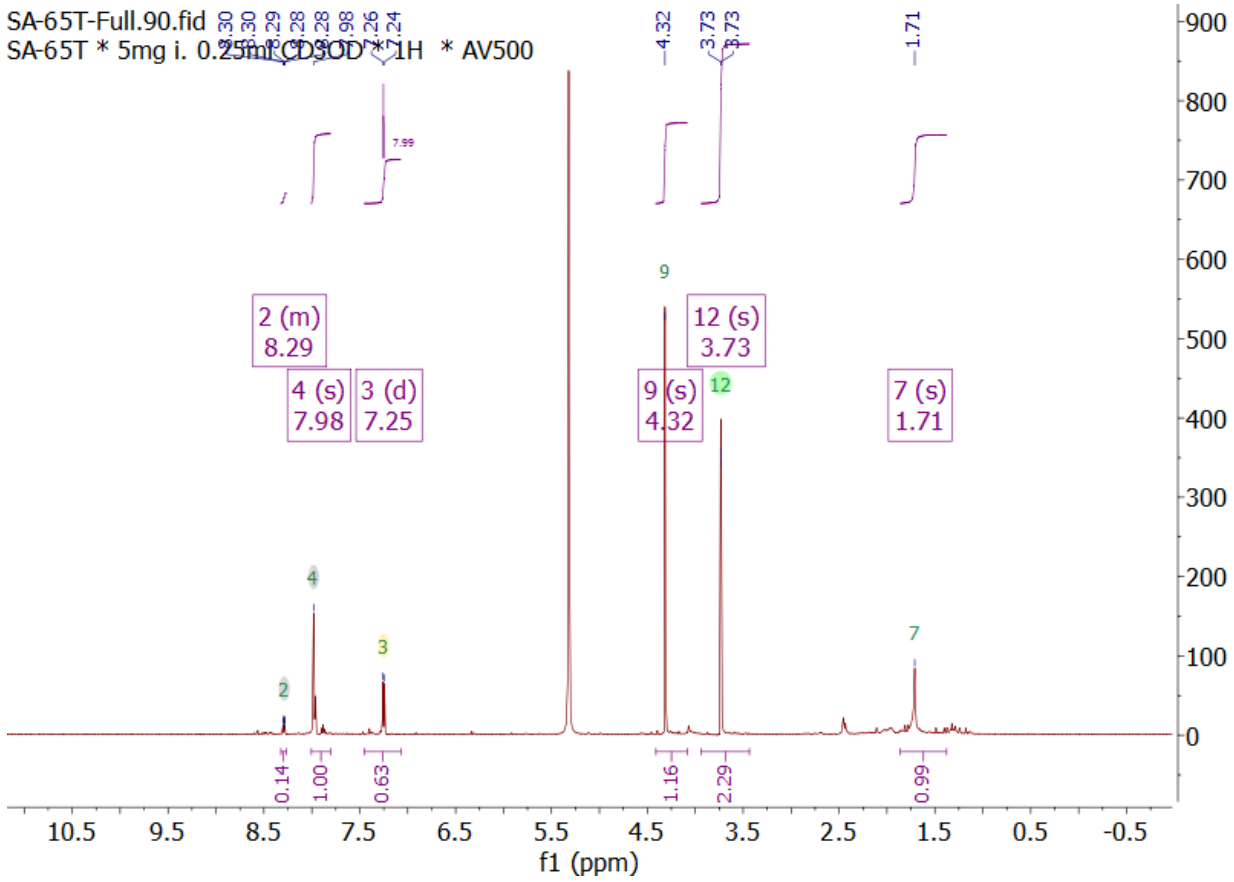
APPENDIX 4G: Predicted ^{1}H NMR Spectrum of rhodimer (**88**)



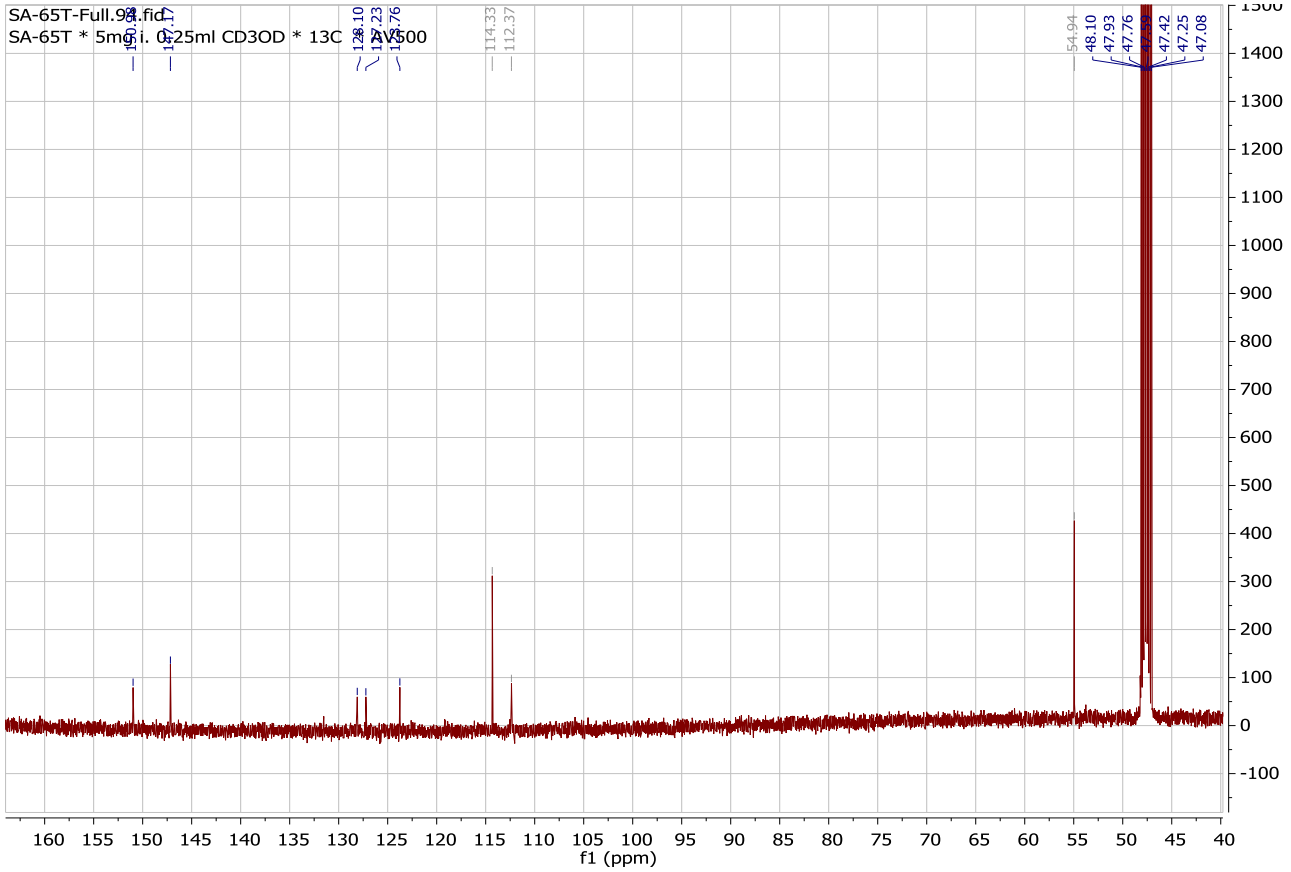
APPENDIX 4H: Predicted ^{13}C NMR Spectrum of rhodimer (**88**)



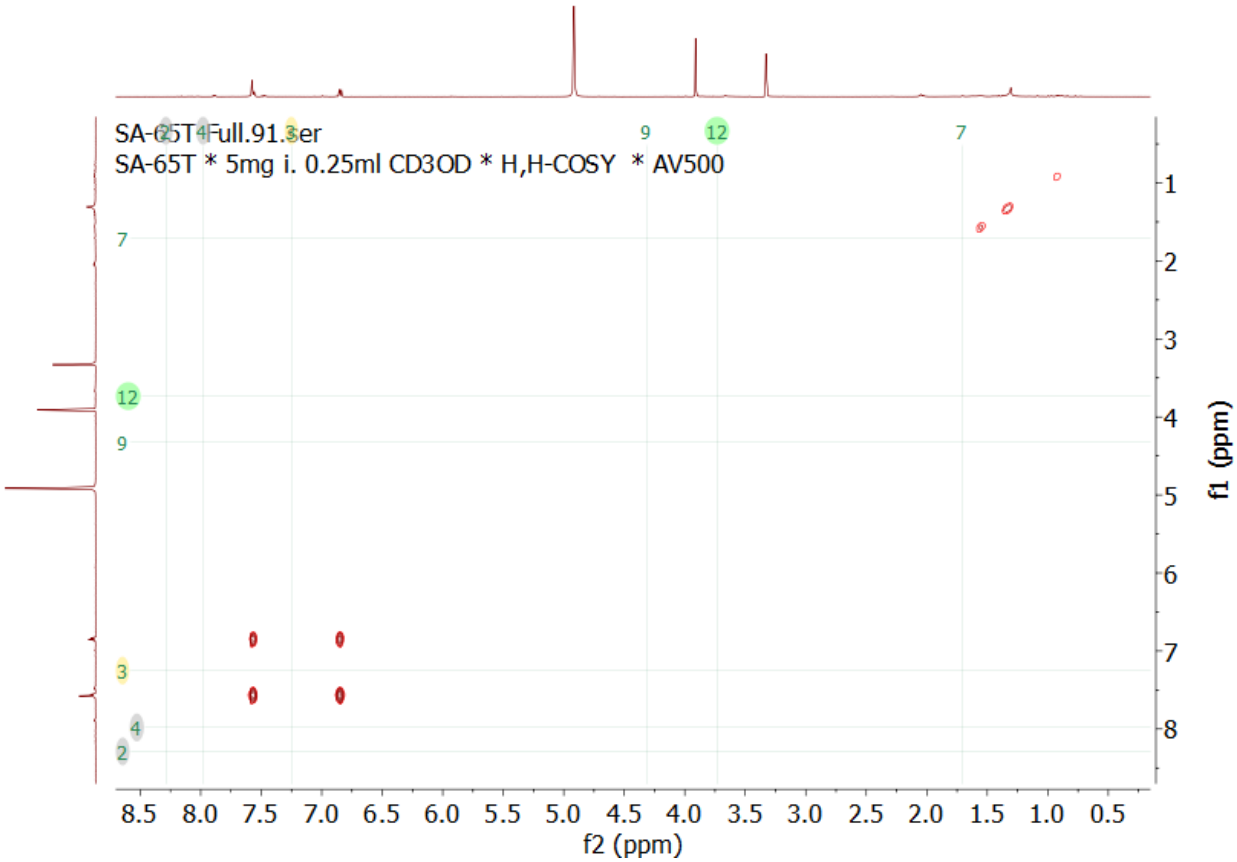
APPENDIX 5A: ^{1}H NMR Spectrum of compound (**89**) (400 MHz; $CDCl_3$)



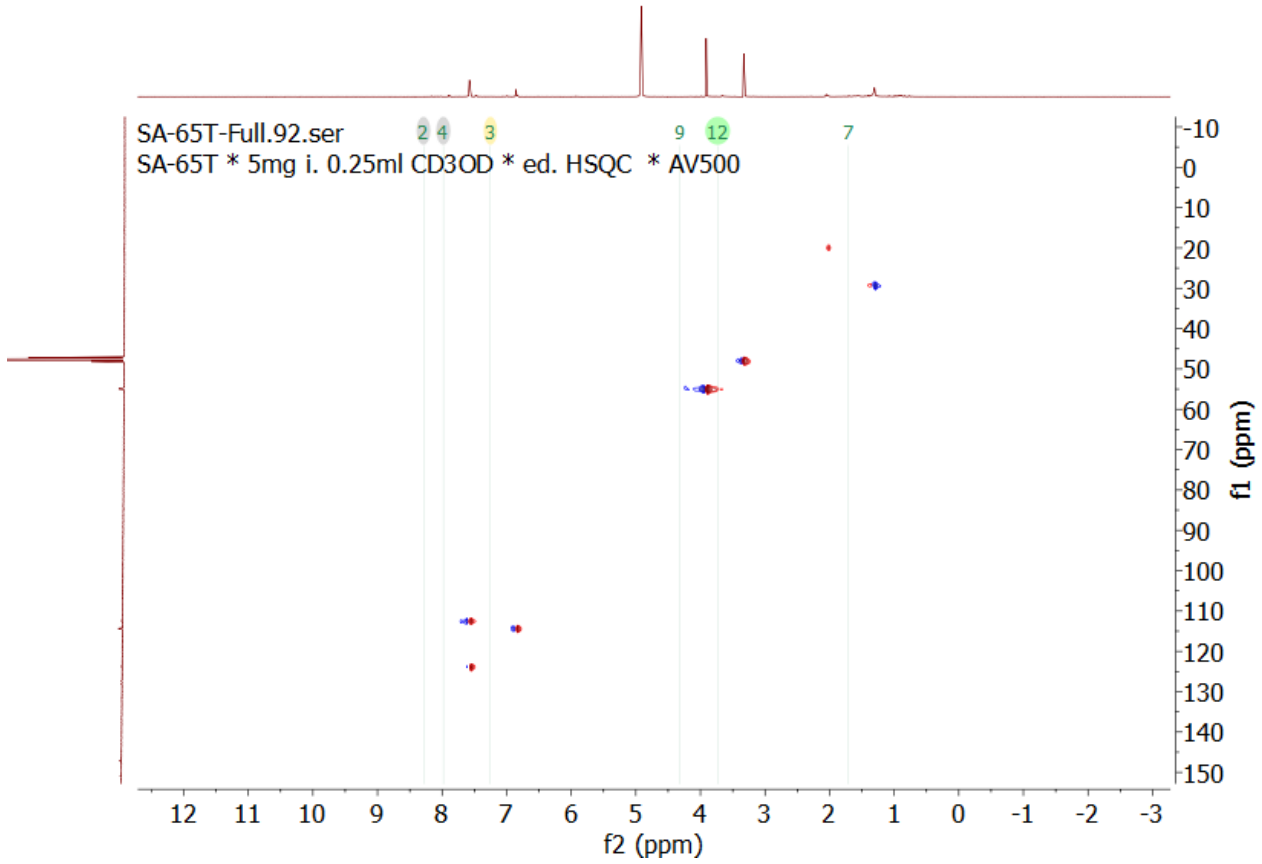
APPENDIX 5B: ^{13}C NMR Spectrum of compound (**89**) (100 MHz; $CDCl_3$)



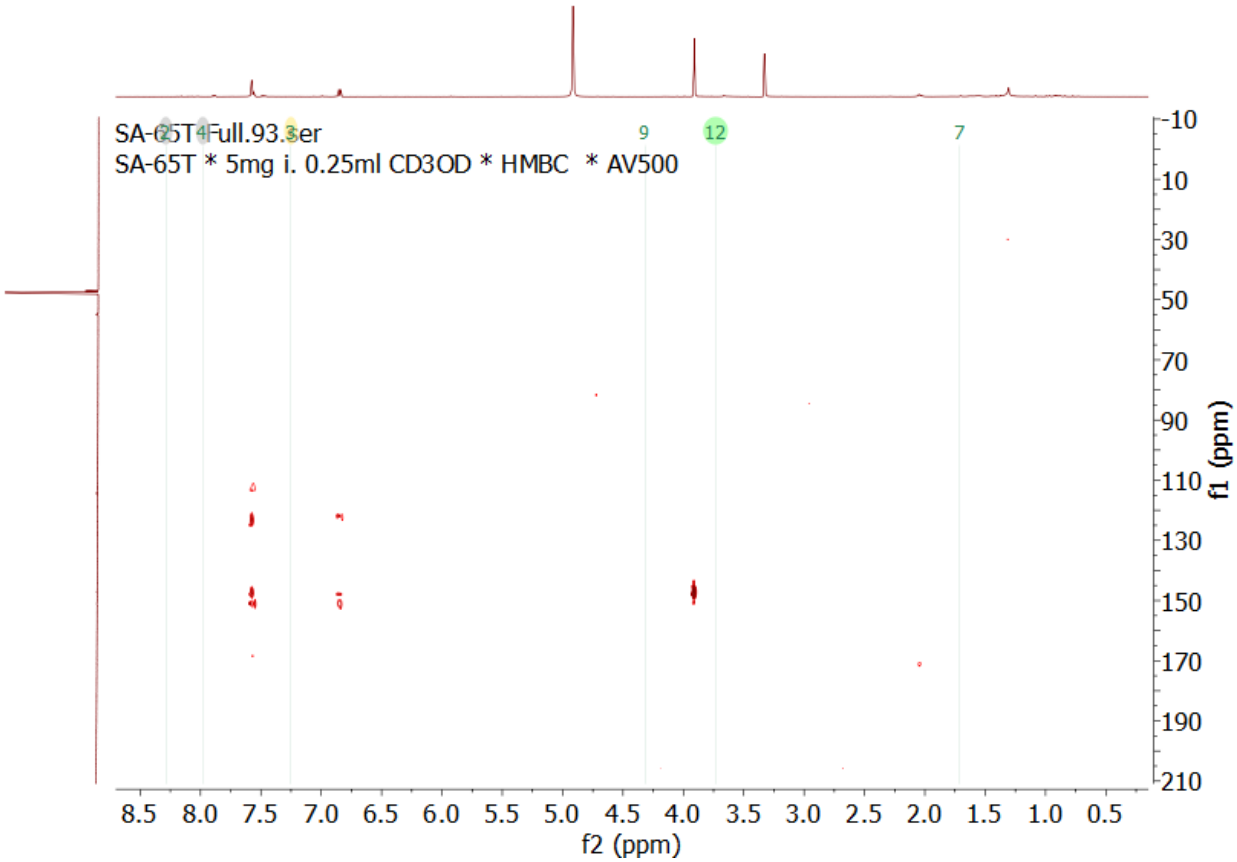
APPENDIX 5C: HH-COSY Spectrum of compound (**89**) (400 MHz; $CDCl_3$)



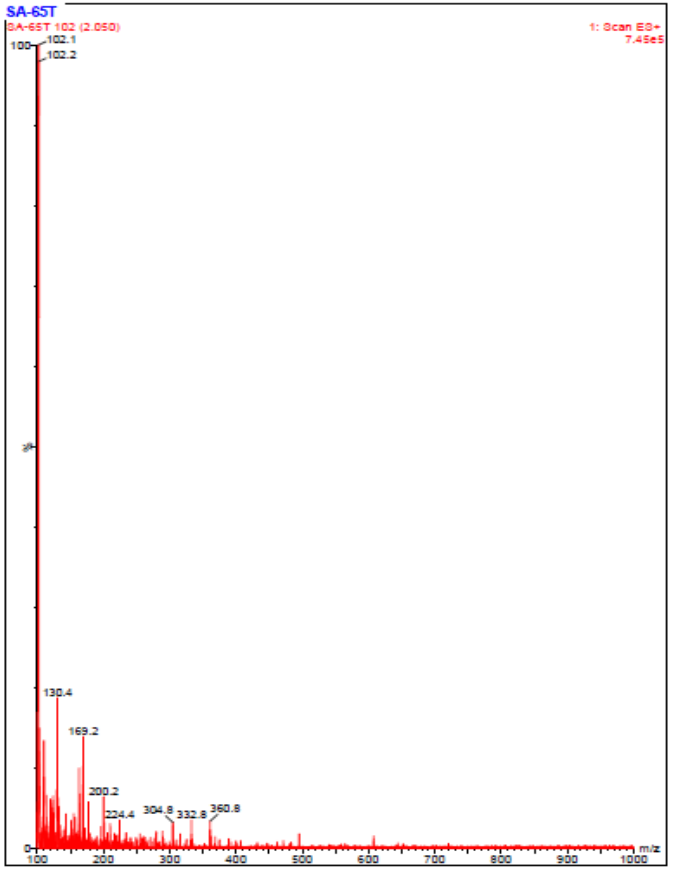
APPENDIX 5D: HSQC Spectrum of compound (**89**) (400MHz; $CDCl_3$)



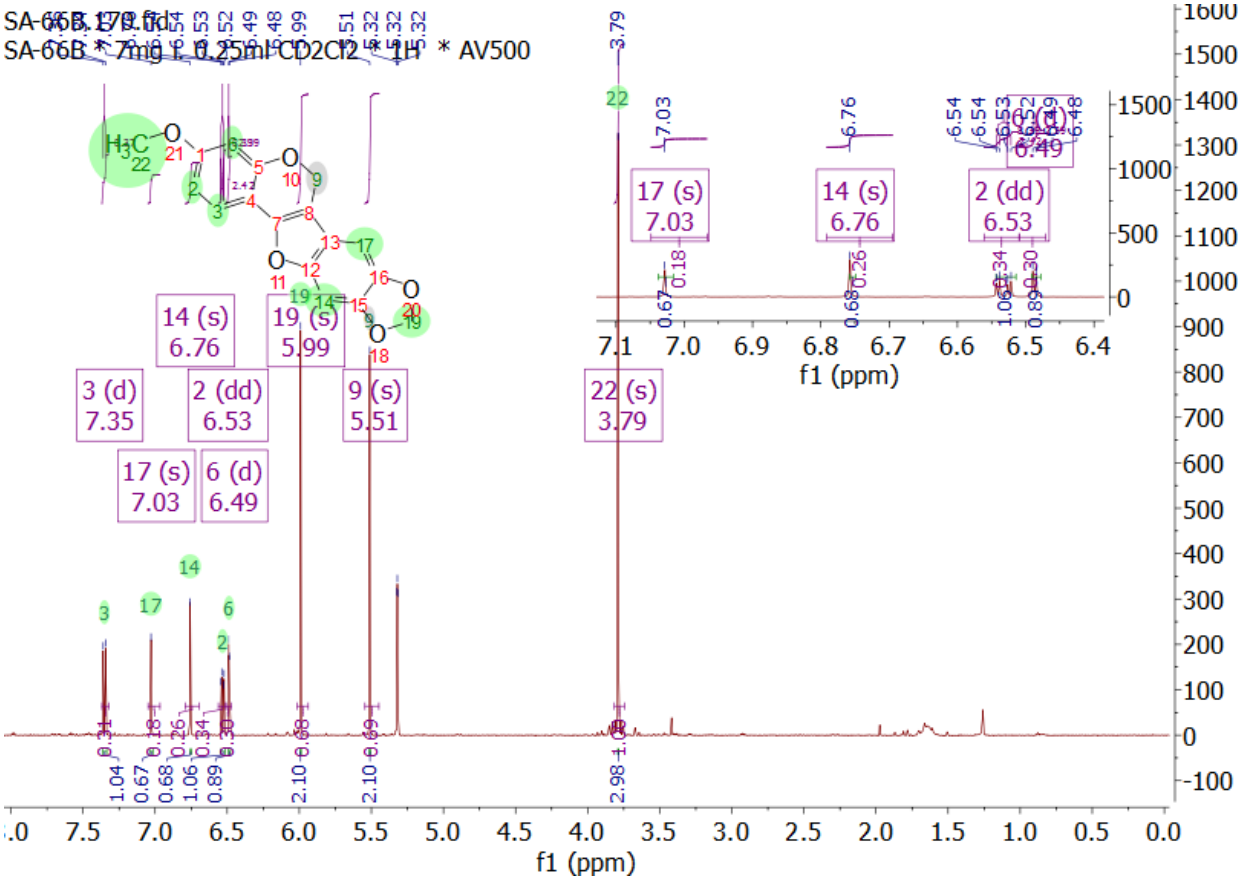
APPENDIX 5E: HMBC Spectrum of compound (**89**) (400 MHz; $CDCl_3$)



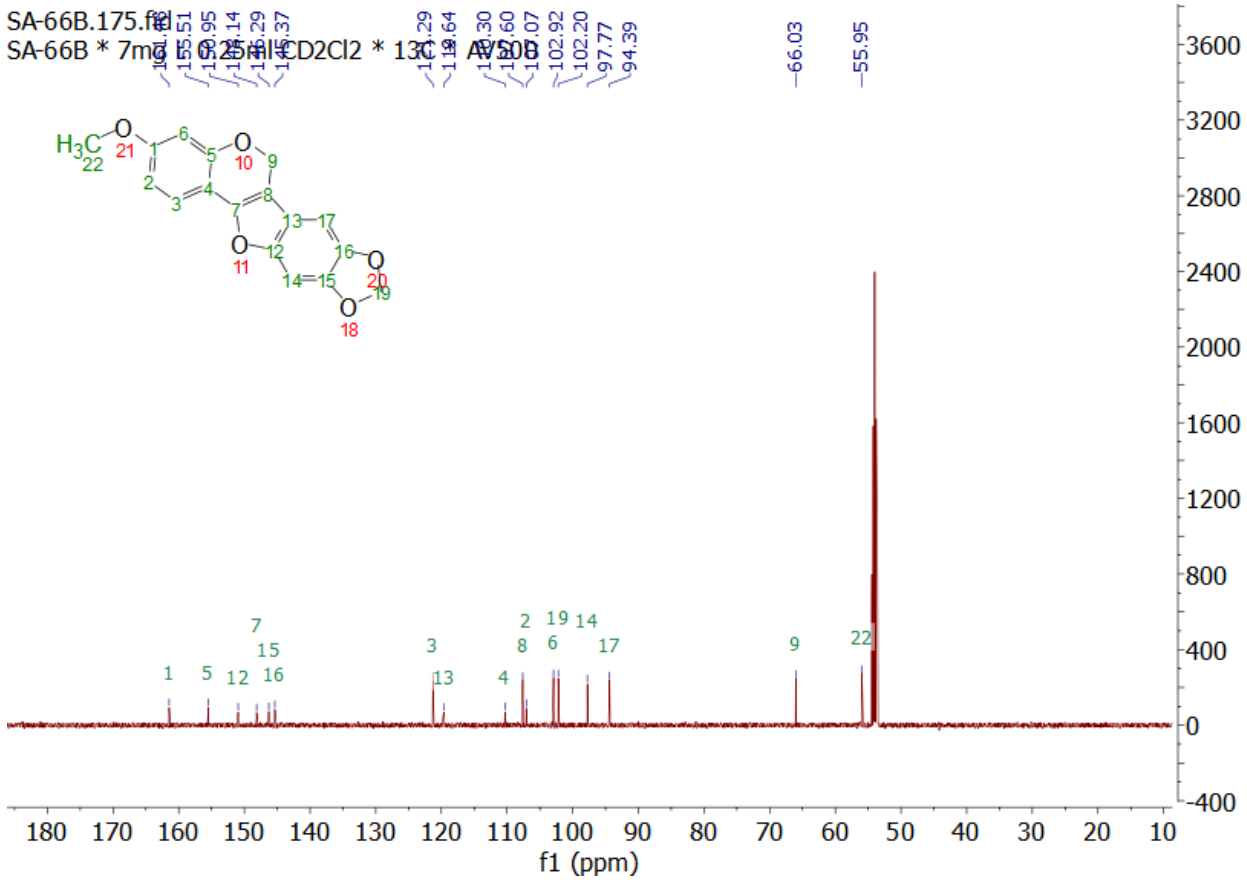
APPENDIX 5F: LCMS Spectrum of compound (**89**)



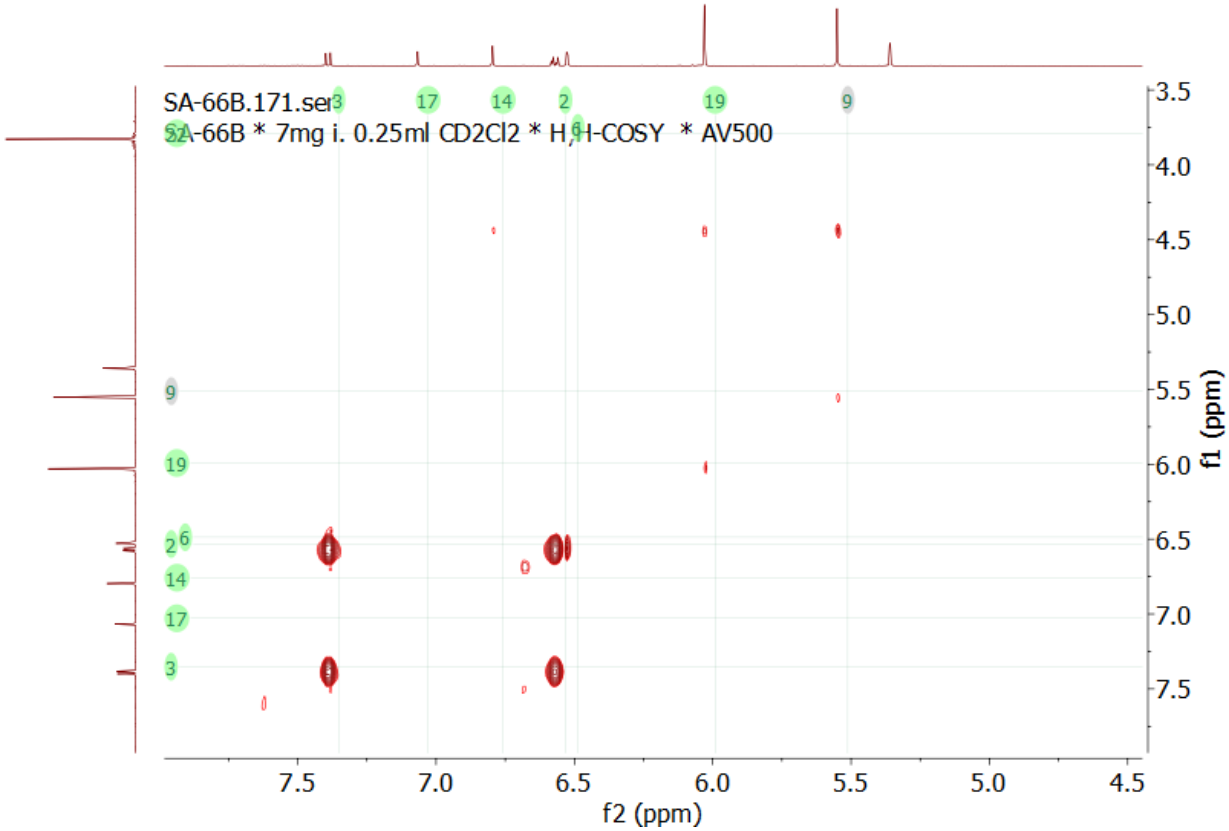
APPENDIX 6A: ^{1}H NMR Spectrum of flemichapparin (**92**) (500 MHz; $CDCl_3$)



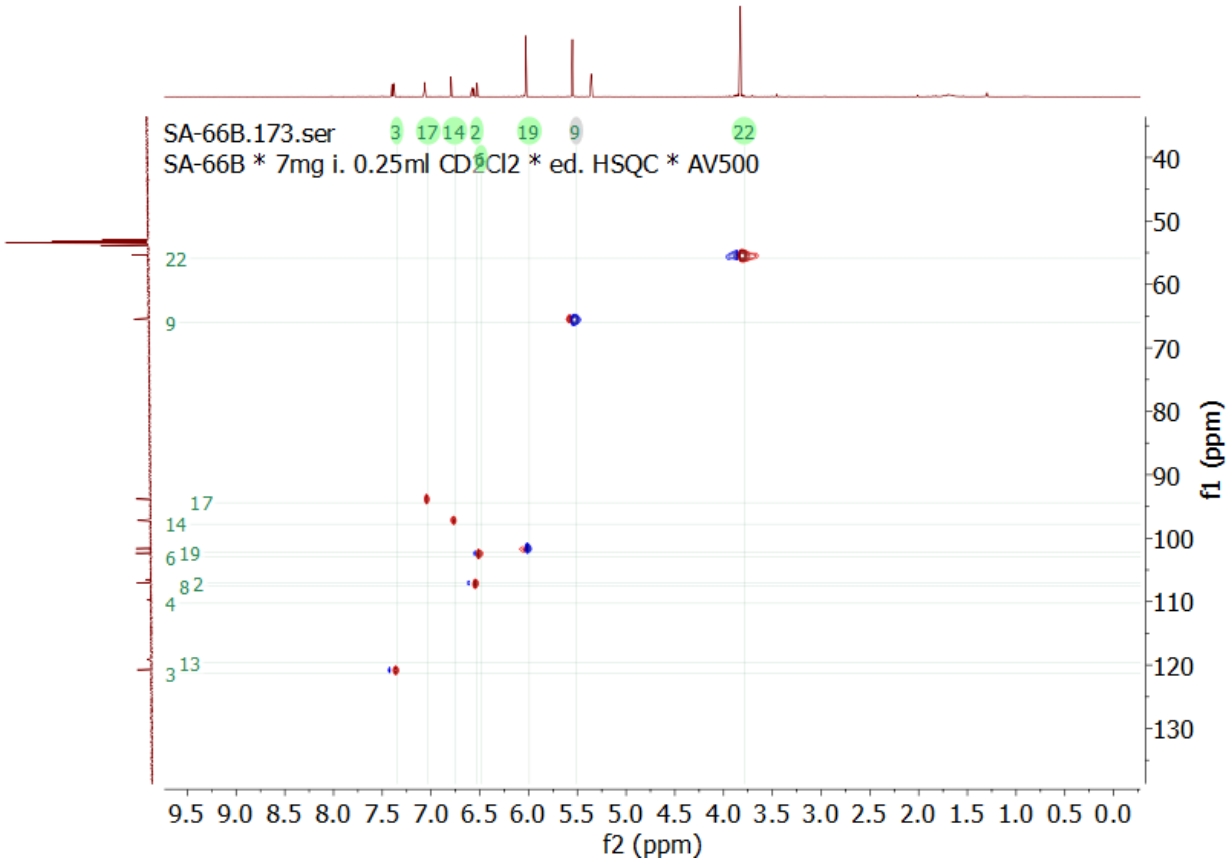
APPENDIX 6B: ^{13}C NMR Spectrum of flemichapparin (**92**) (500 MHz; $CDCl_3$)



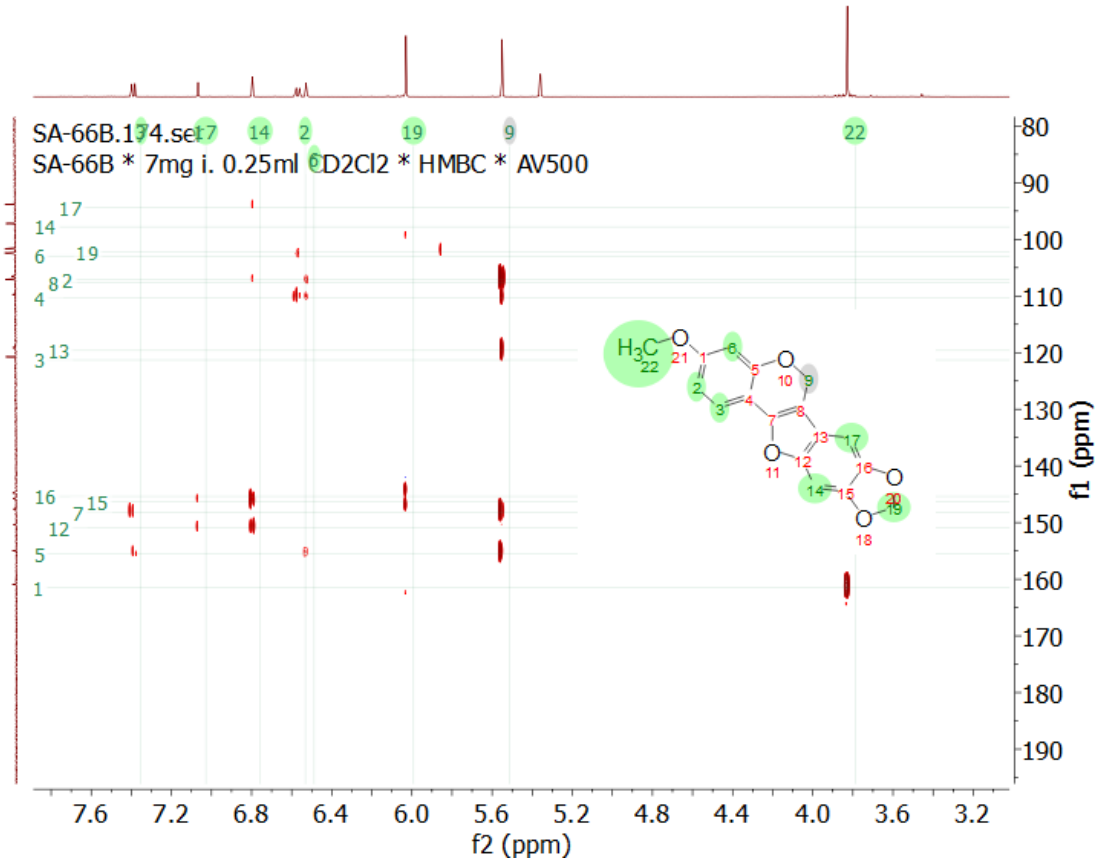
APPENDIX 6C: HH-COSY Spectrum of flemichapparin (**92**) (500 MHz; $CDCl_3$)



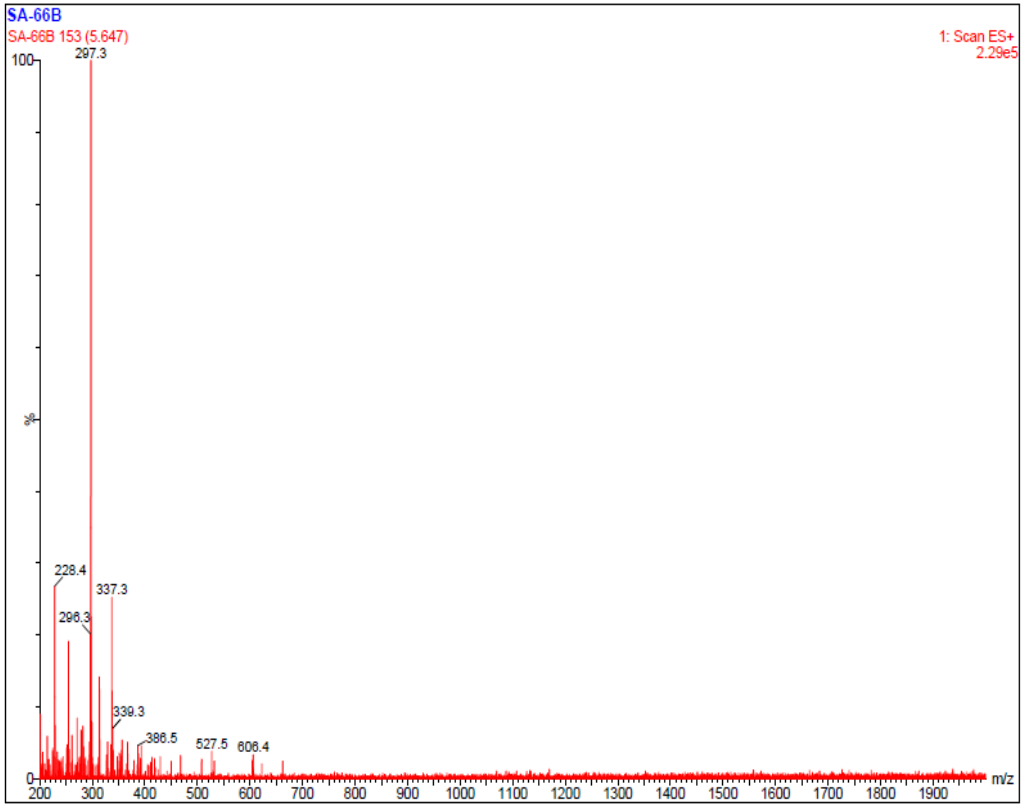
APPENDIX 6D: HSQC Spectrum of flemichapparin (**92**) (500MHz; $CDCl_3$)



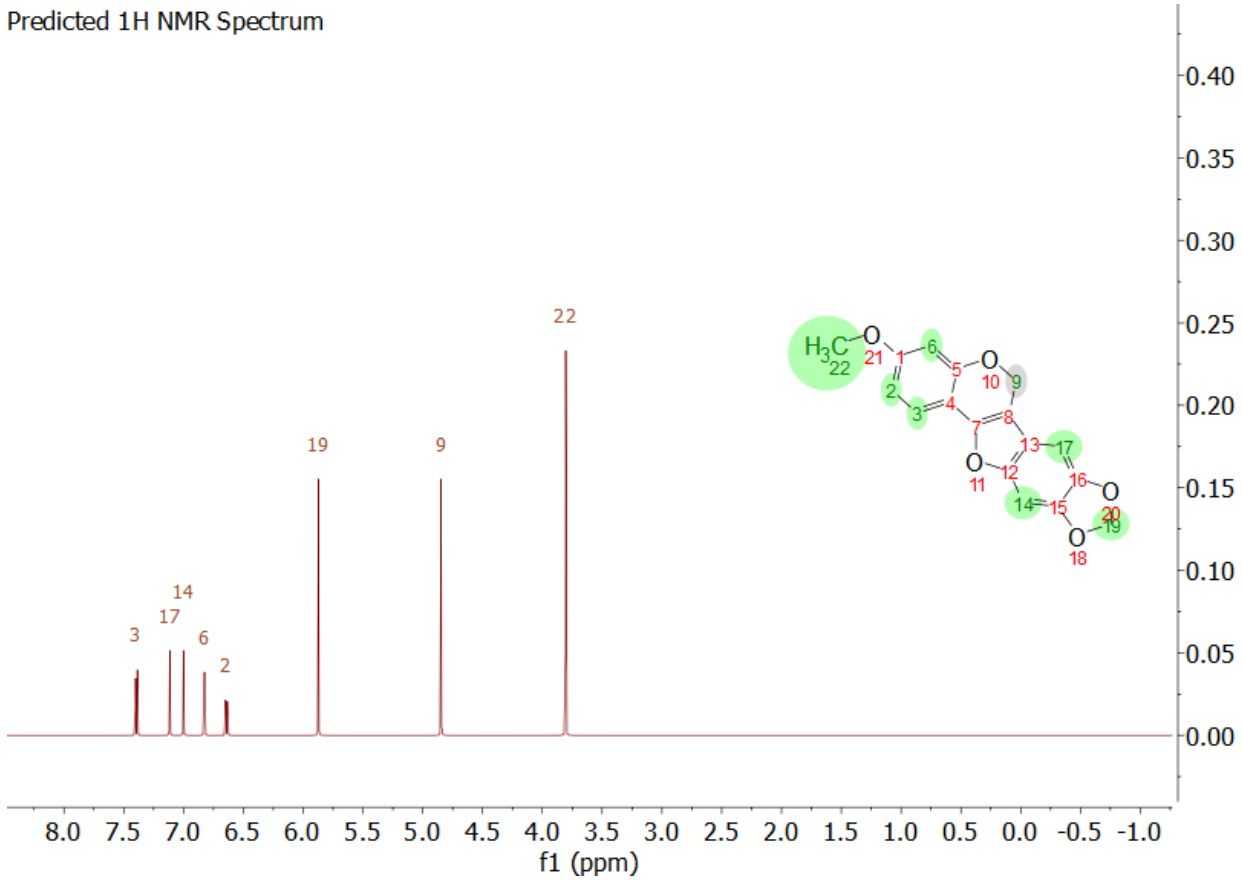
APPENDIX 6E: HMBC Spectrum of flemichapparin (**92**) (500 MHz; $CDCl_3$)



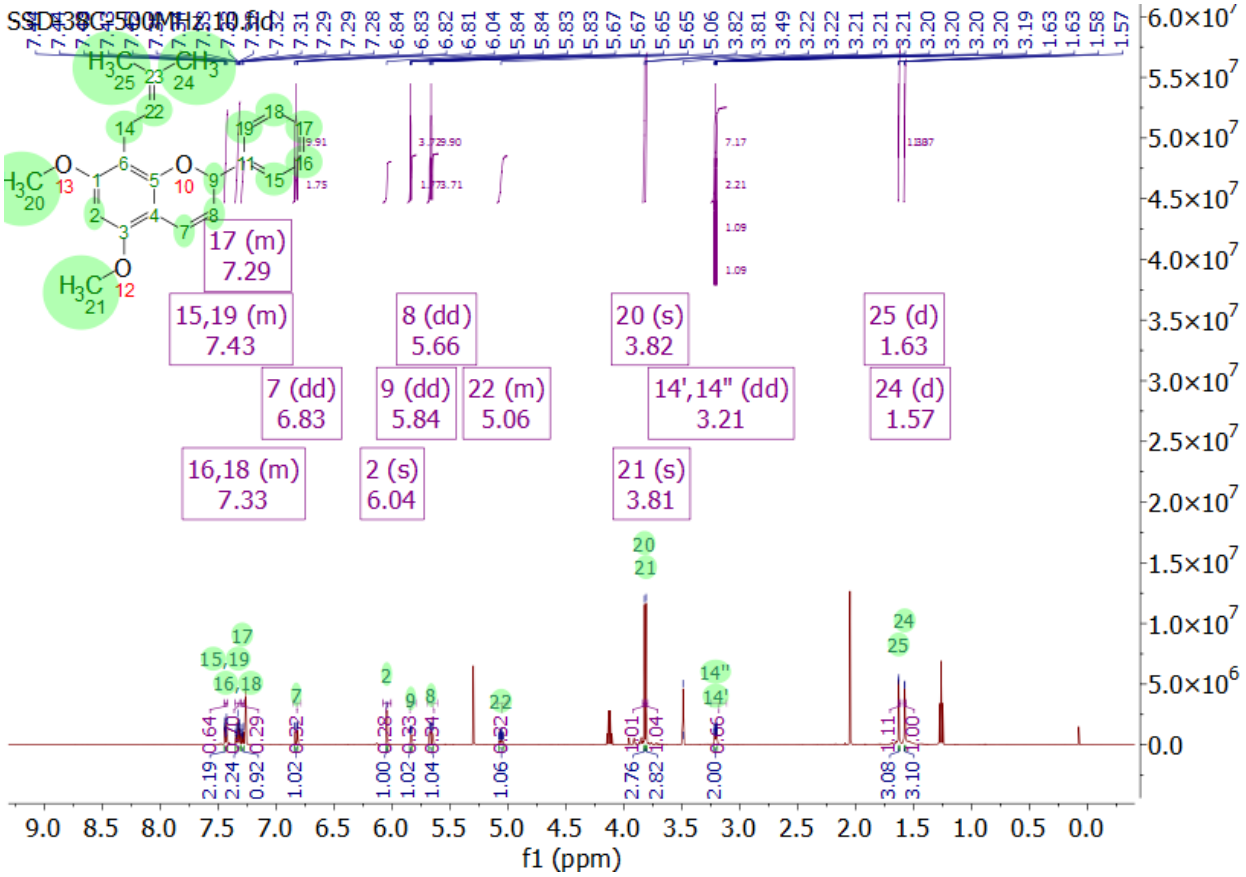
APPENDIX 6F: LCMS Spectrum of flemichapparin (**92**)



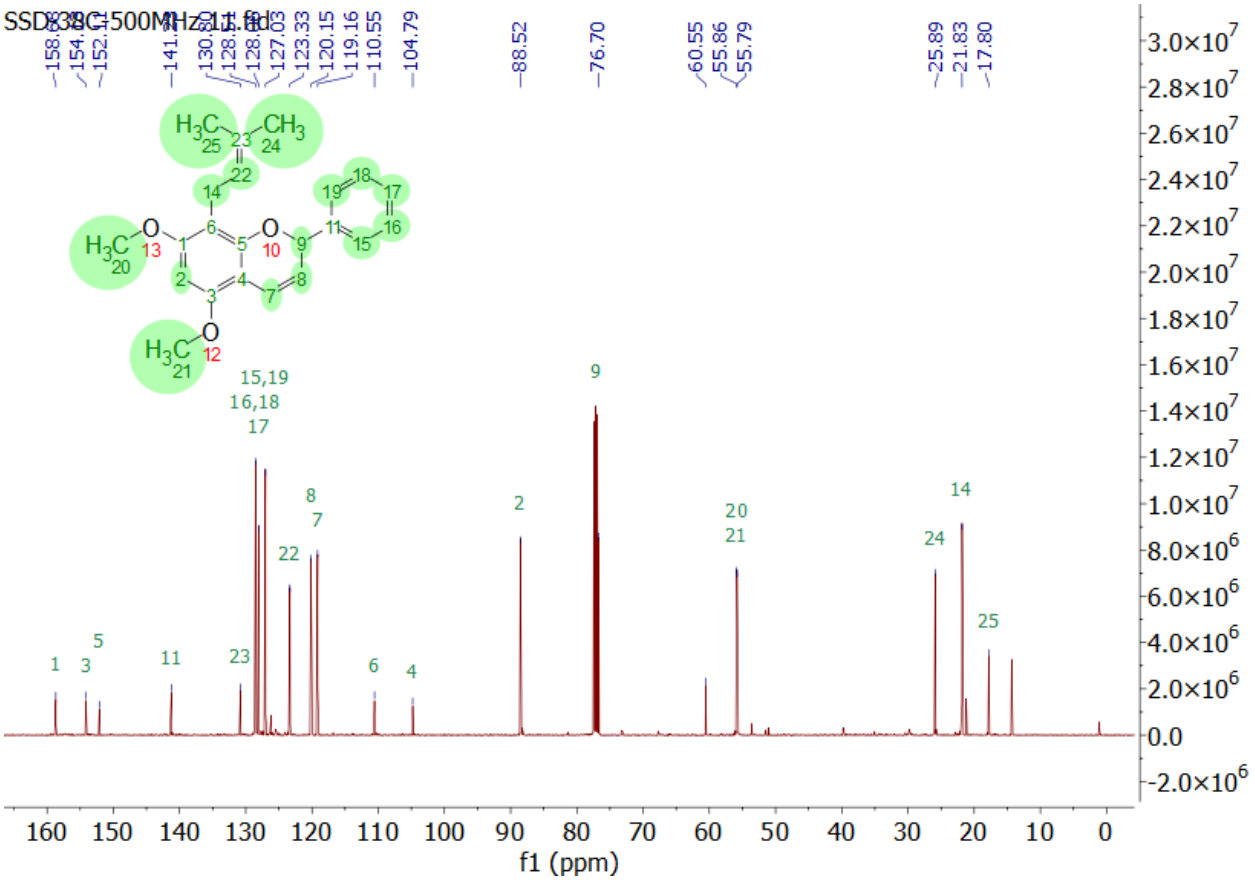
APPENDIX 6G: Predicted[1]H NMR Spectrum of flemichapparin (**92**)



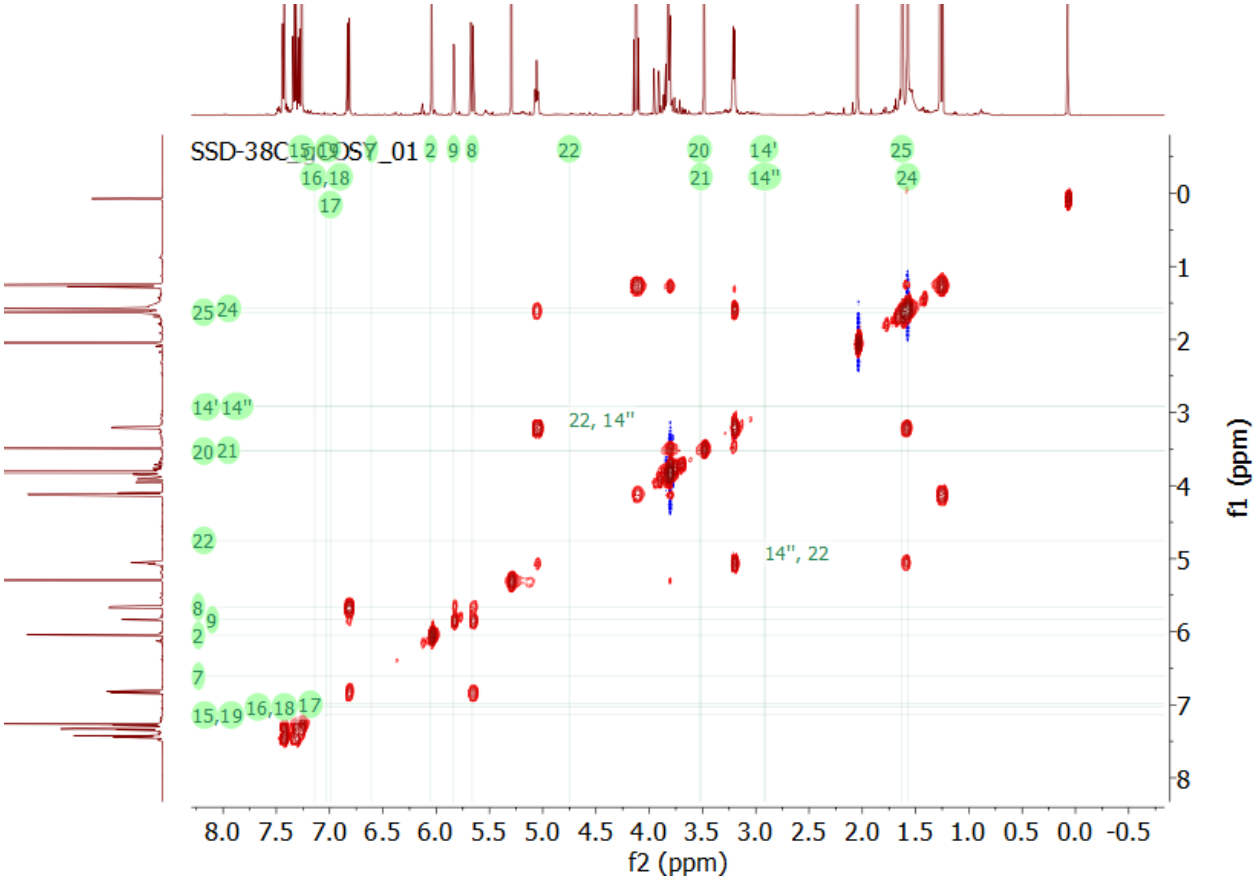
APPENDIX 7A: ^{1}H NMR Spectrum of Tephrowatsin B (**93**) (500MHz; $CDCl_3$)



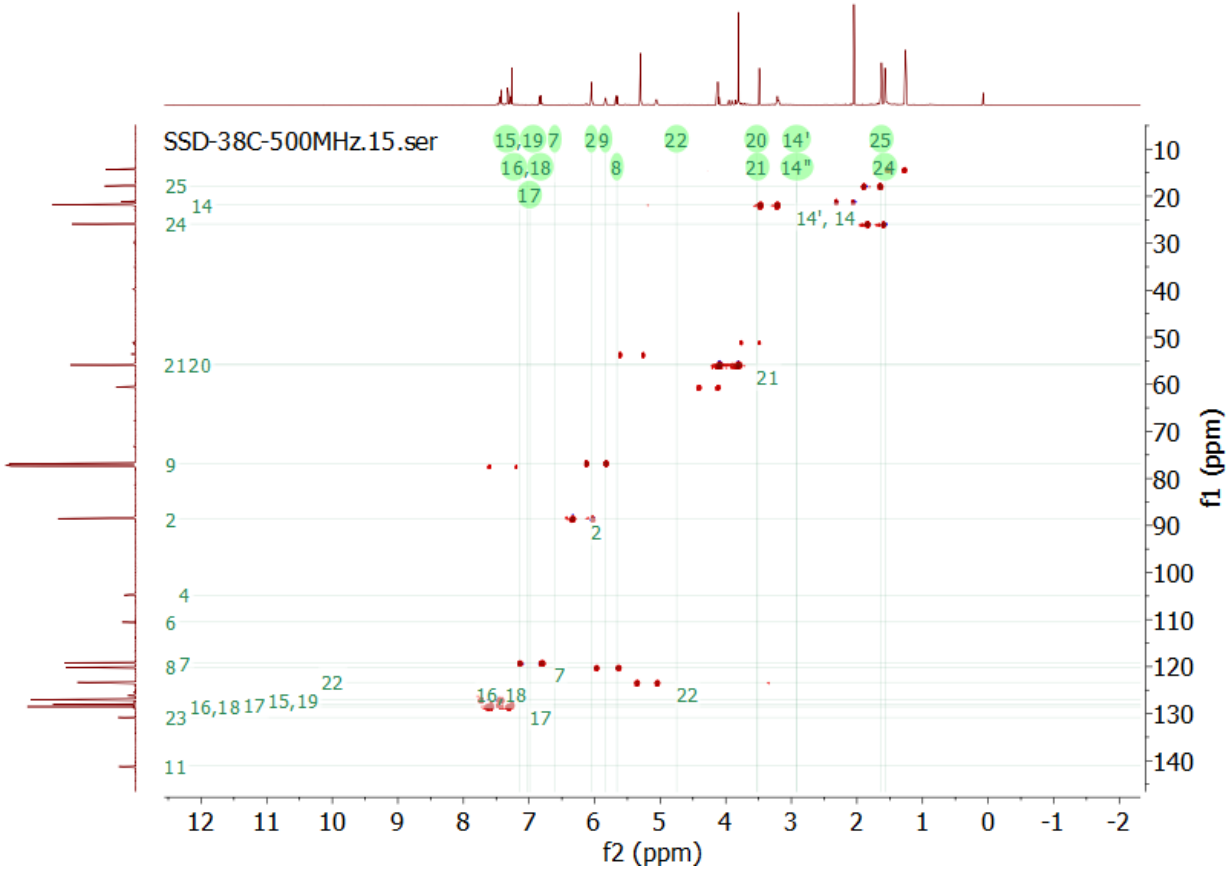
APPENDIX 7B: ^{13}C NMR Spectrum of Tephrowatsin B (**93**) (500 MHz; $CDCl_3$)



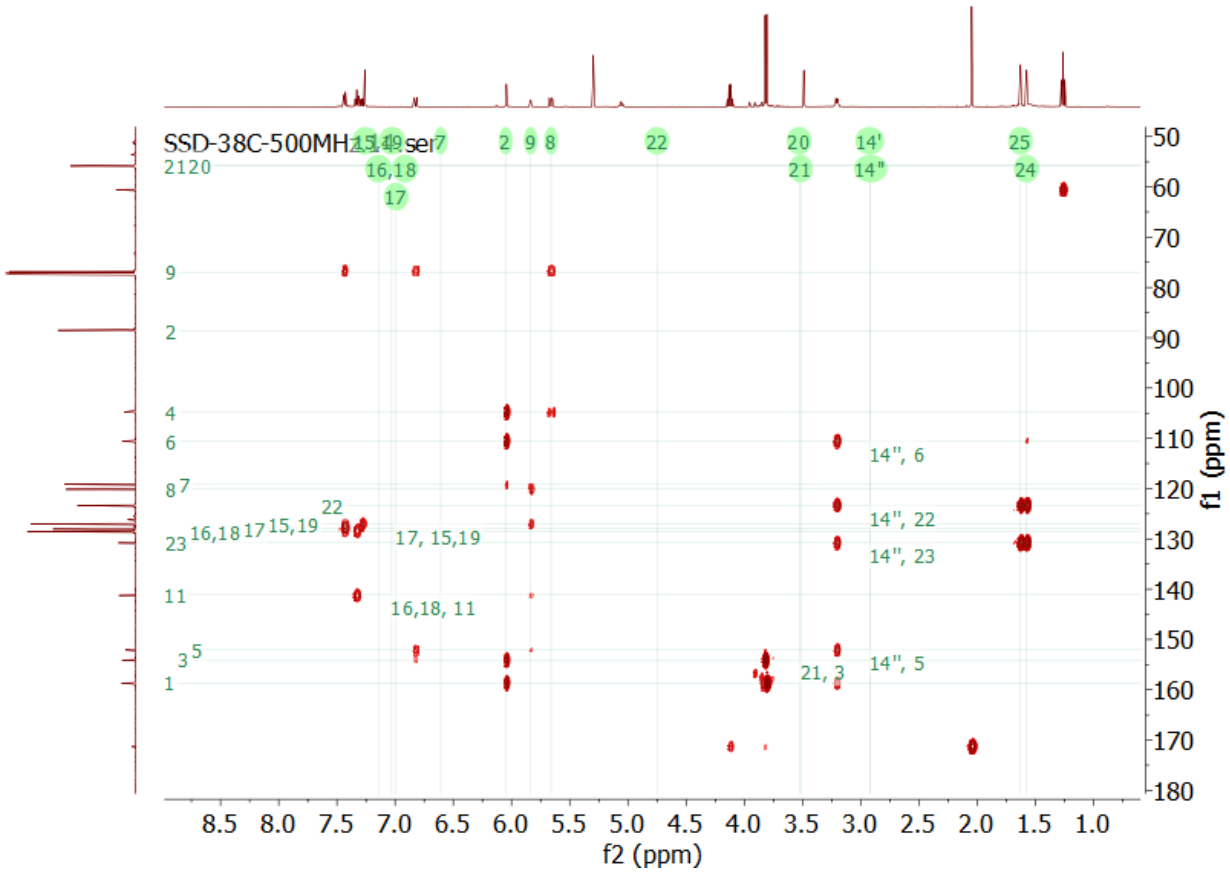
APPENDIX 7C: HH-COSY Spectrum of Tephrowatsin B (**93**) (500 MHz; $CDCl_3$)



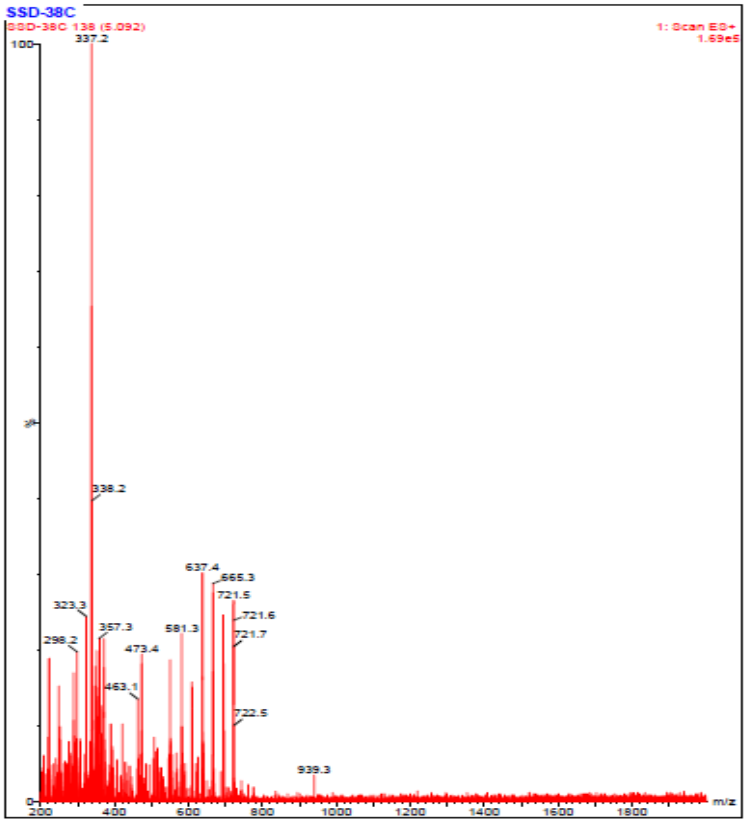
APPENDIX 7D: HSQC Spectrum of Tephrowatsin B (**93**) (500 MHz; $CDCl_3$)



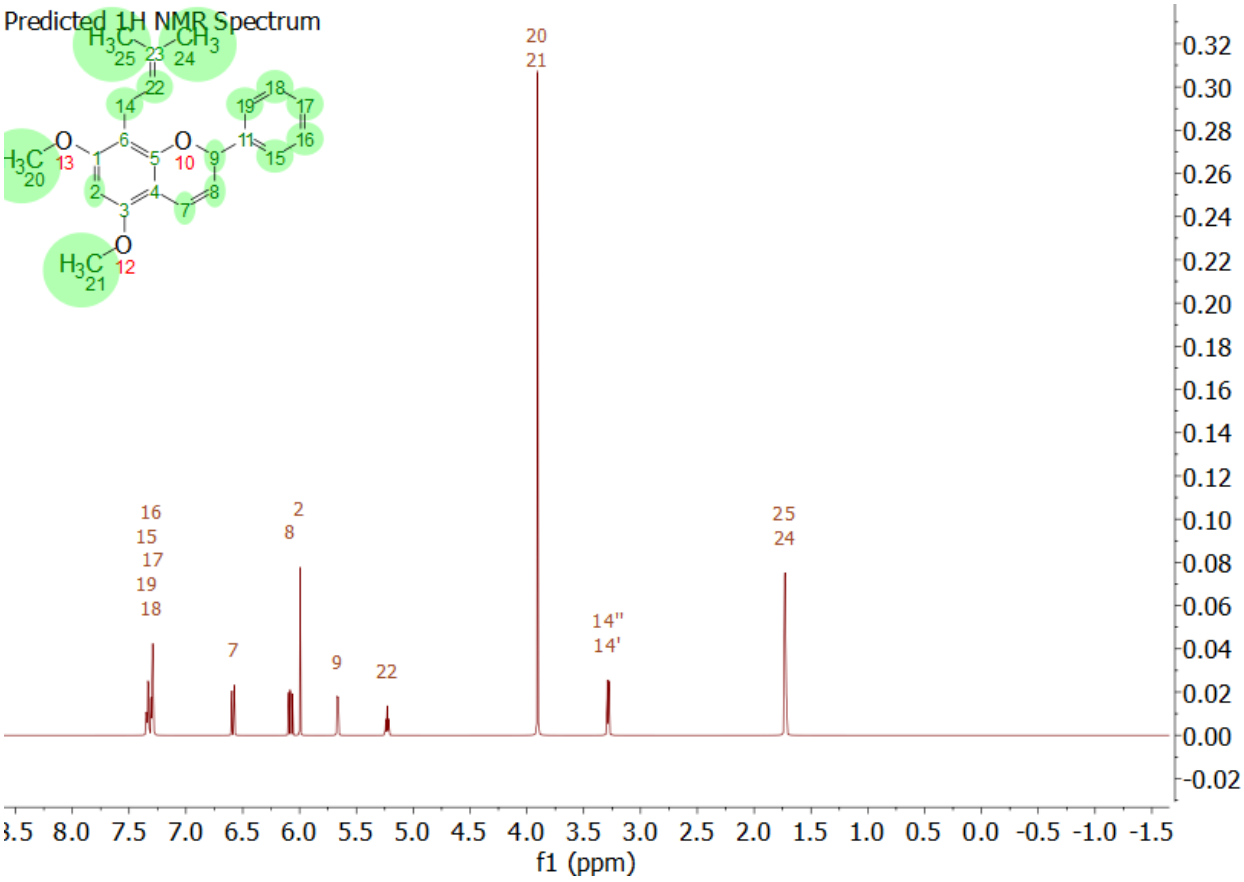
APPENDIX 7E: HMBC Spectrum of Tephrowatsin B (**93**) (500 MHz; $CDCl_3$)



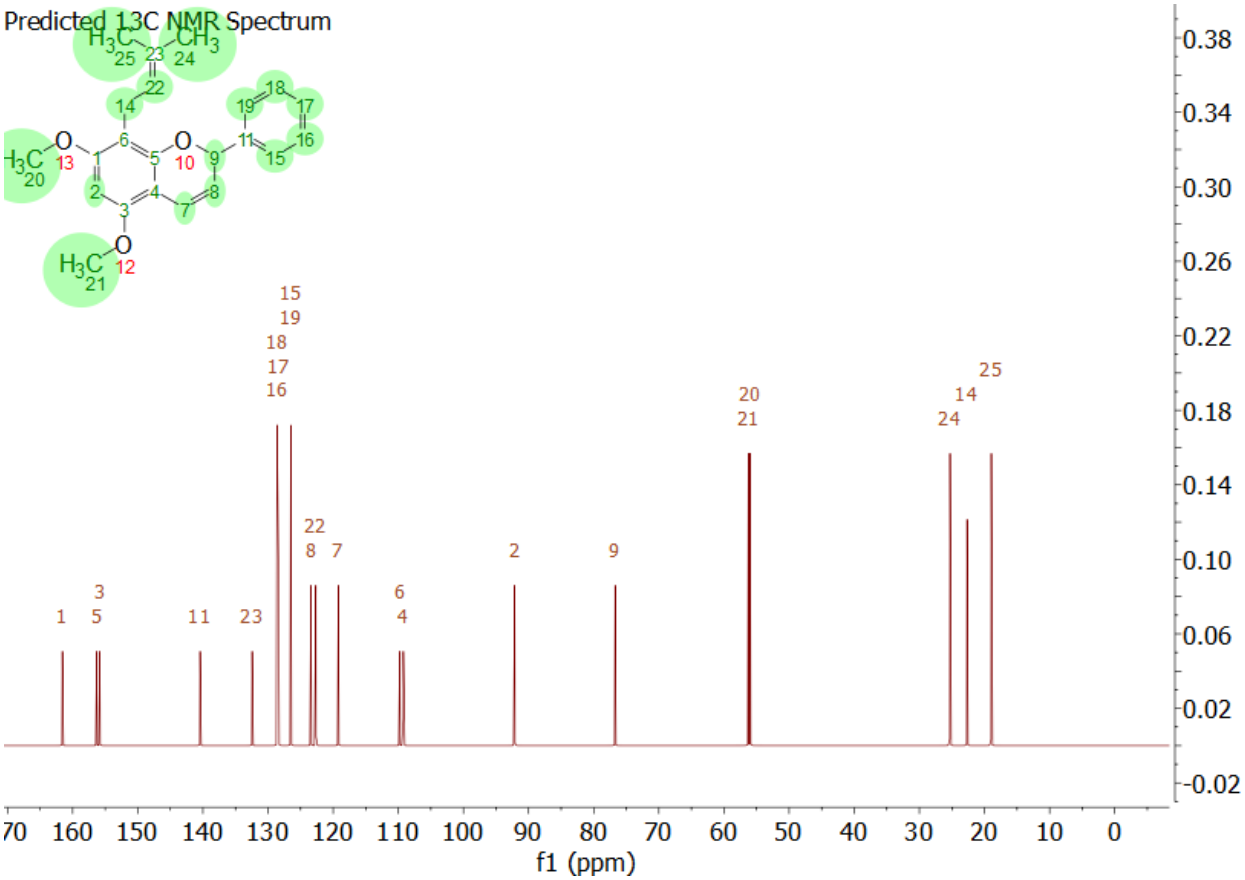
APPENDIX 7F: LCMS Spectrum of Tephrowatsin B (**93**)



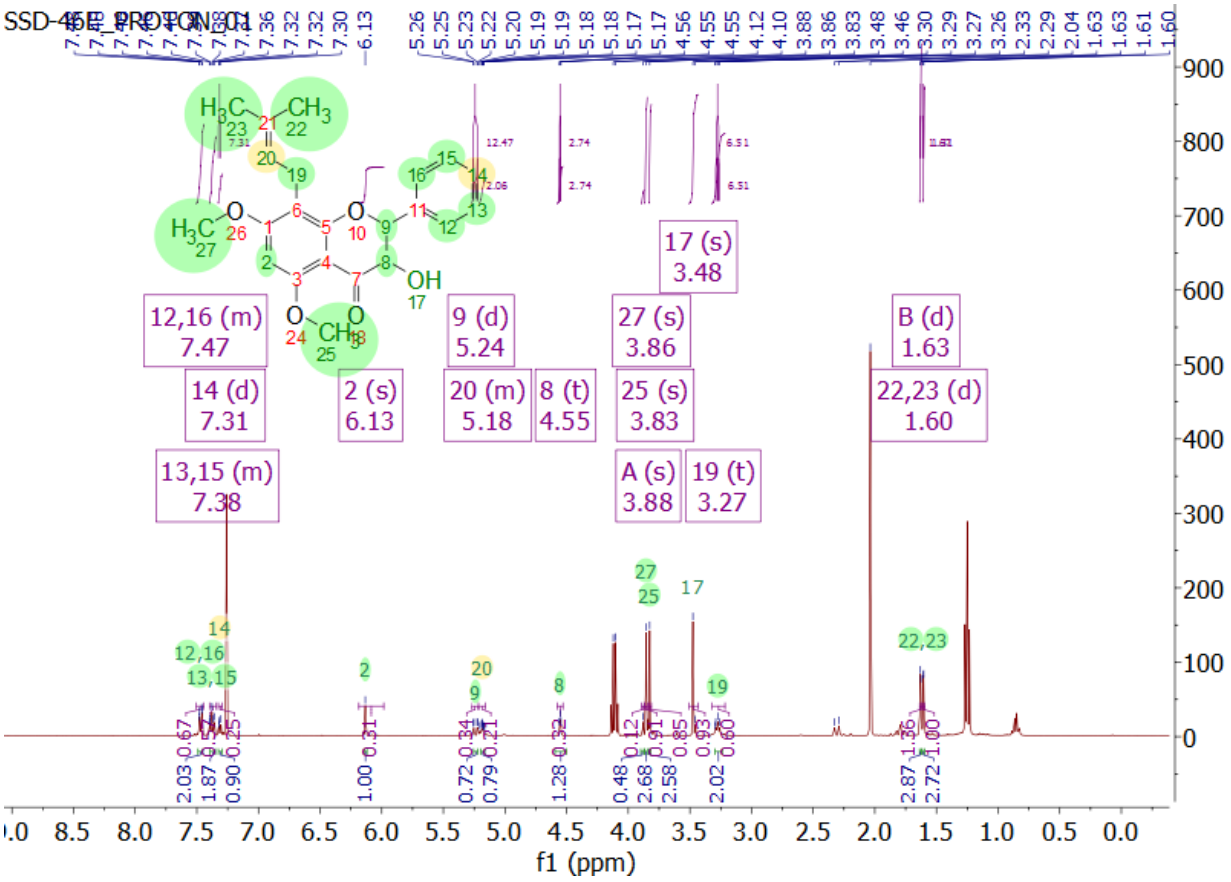
APPENDIX 7G: ^{1}H NMR Predicted Spectrum of Tephrowatsin B **(93)**



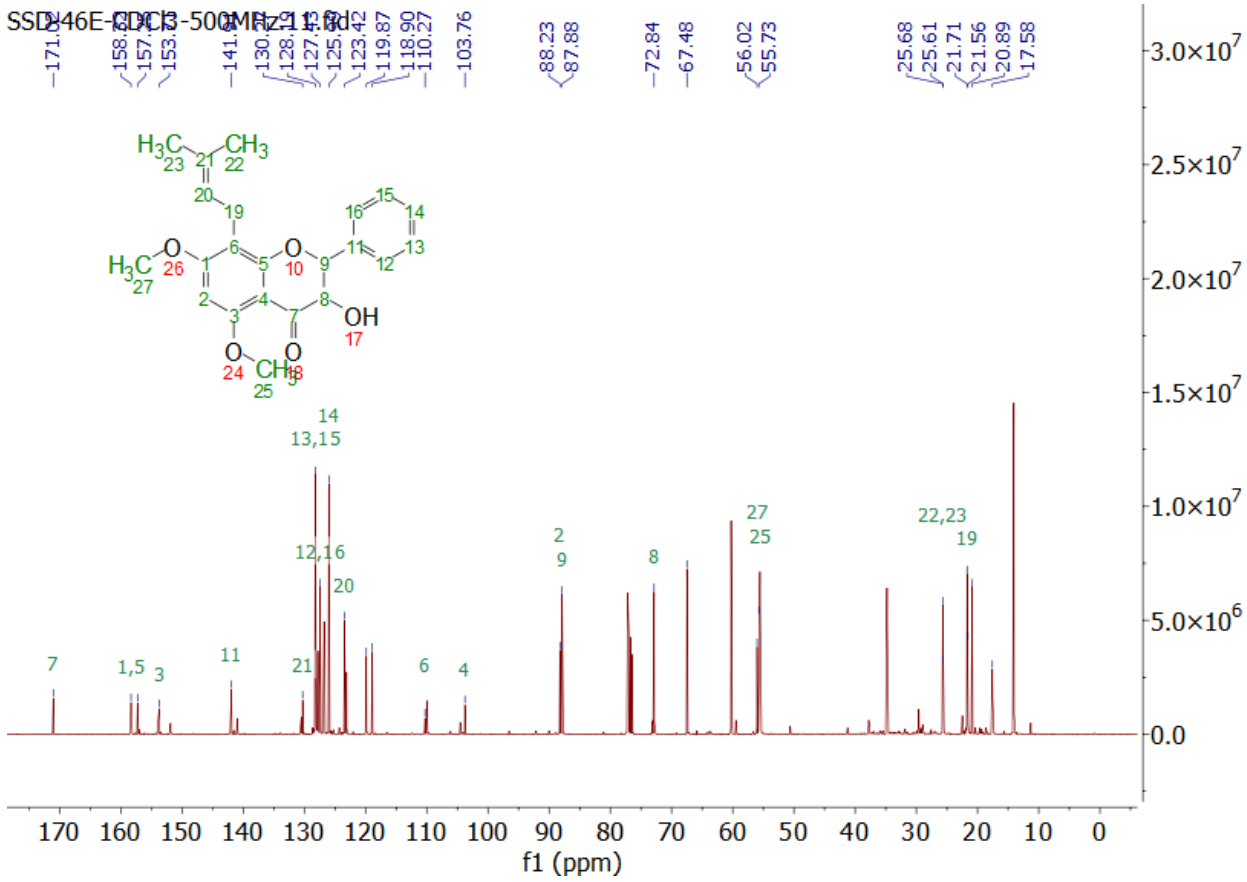
APPENDIX 7H: Predicted ^{13}C NMR Spectrum of Tephrowatsin B (**93**)



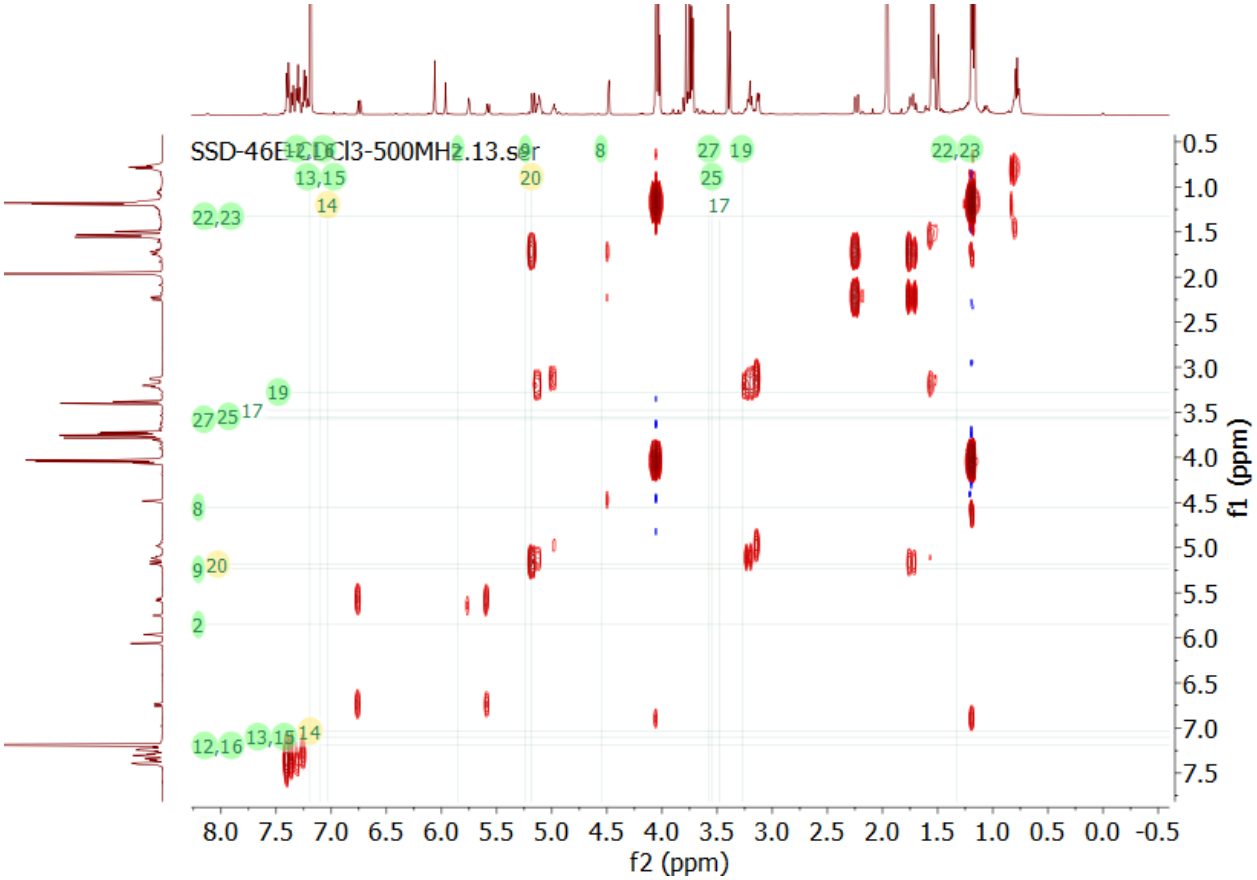
APPENDIX 8A: ^{1}H NMR Spectrum of Rhodflavononol (**87**) (500 MHz; $CDCl_3$)



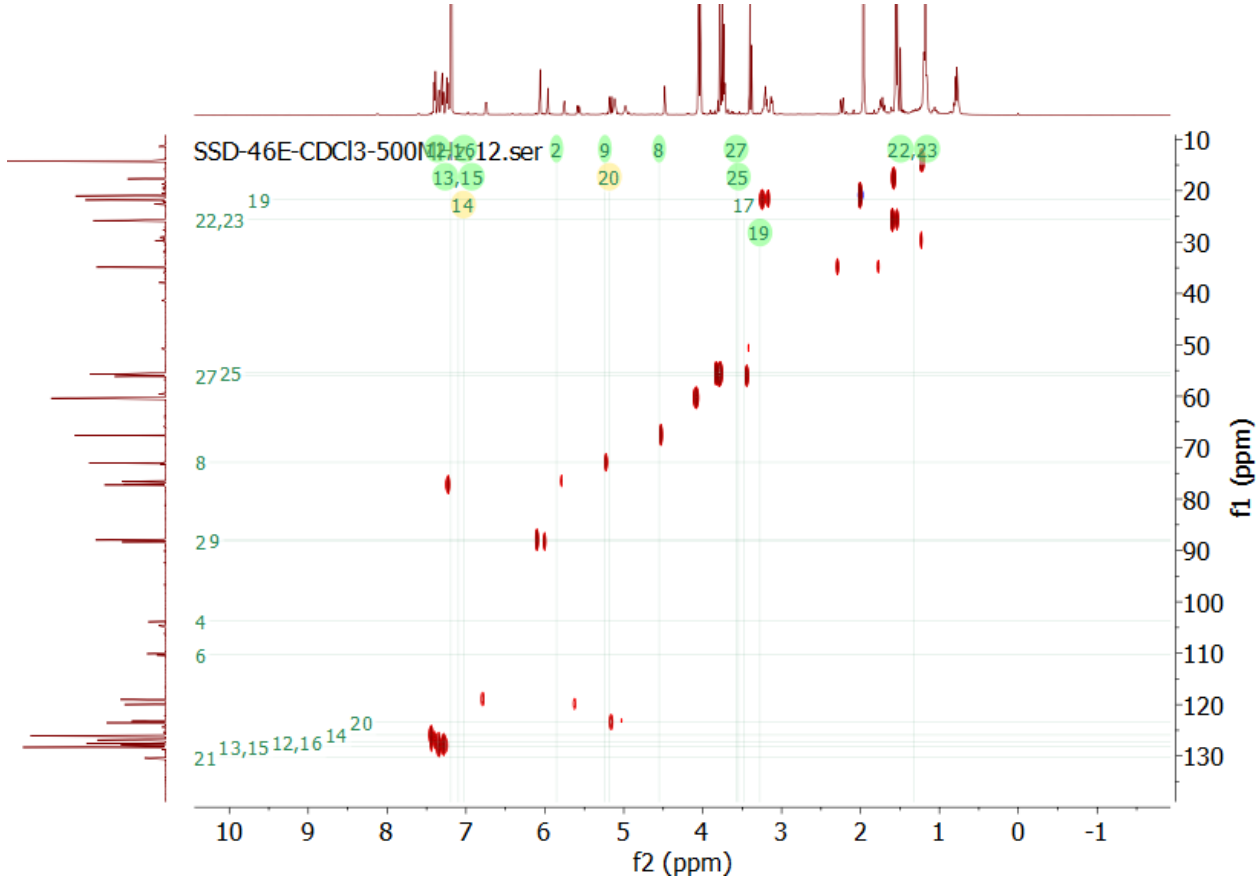
APPENDIX 8B: ^{13}C NMR Spectrum of Rhodflavononol (**87**) (500 MHz; $CDCl_3$)



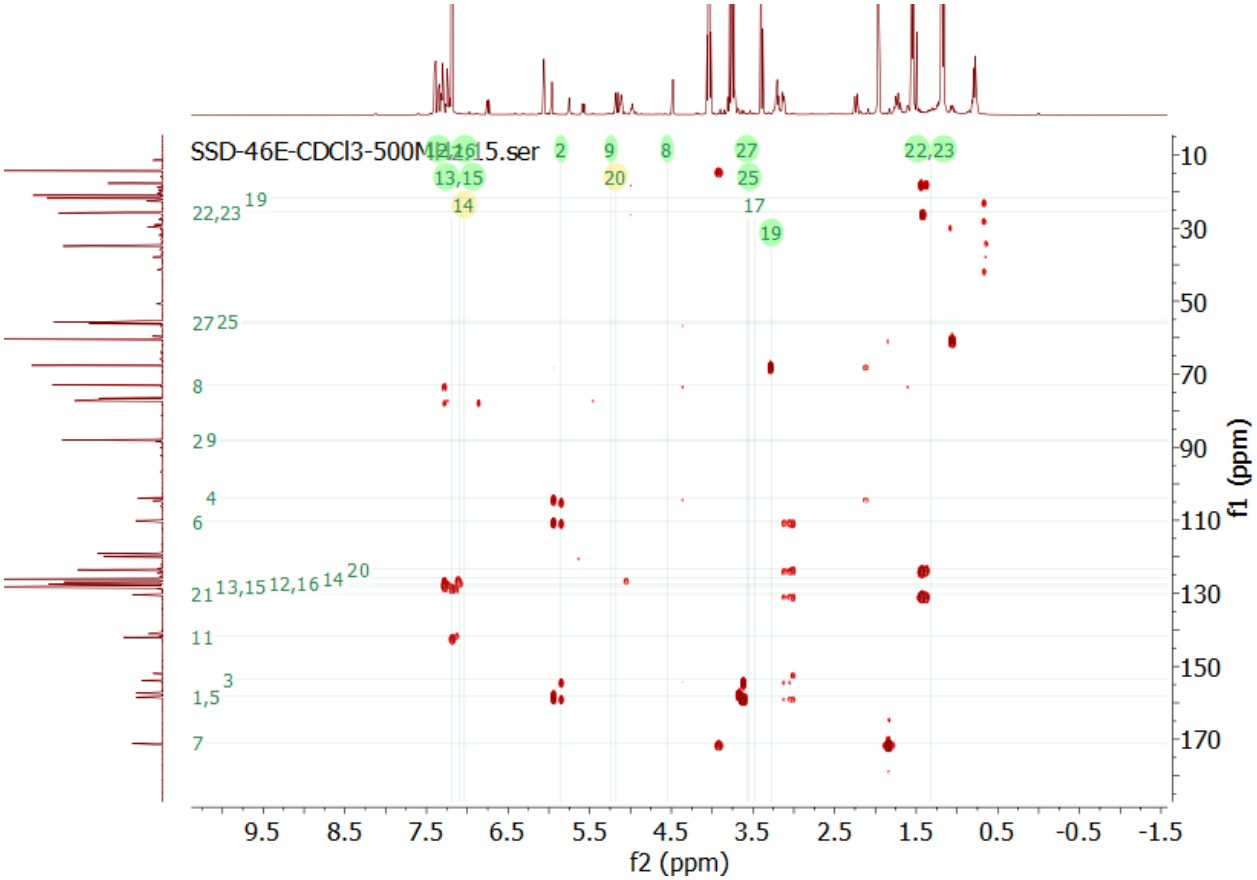
APPENDIX 8C: HH-COSY Spectrum of Rhodflavononol (**87**) (500 MHz; $CDCl_3$)



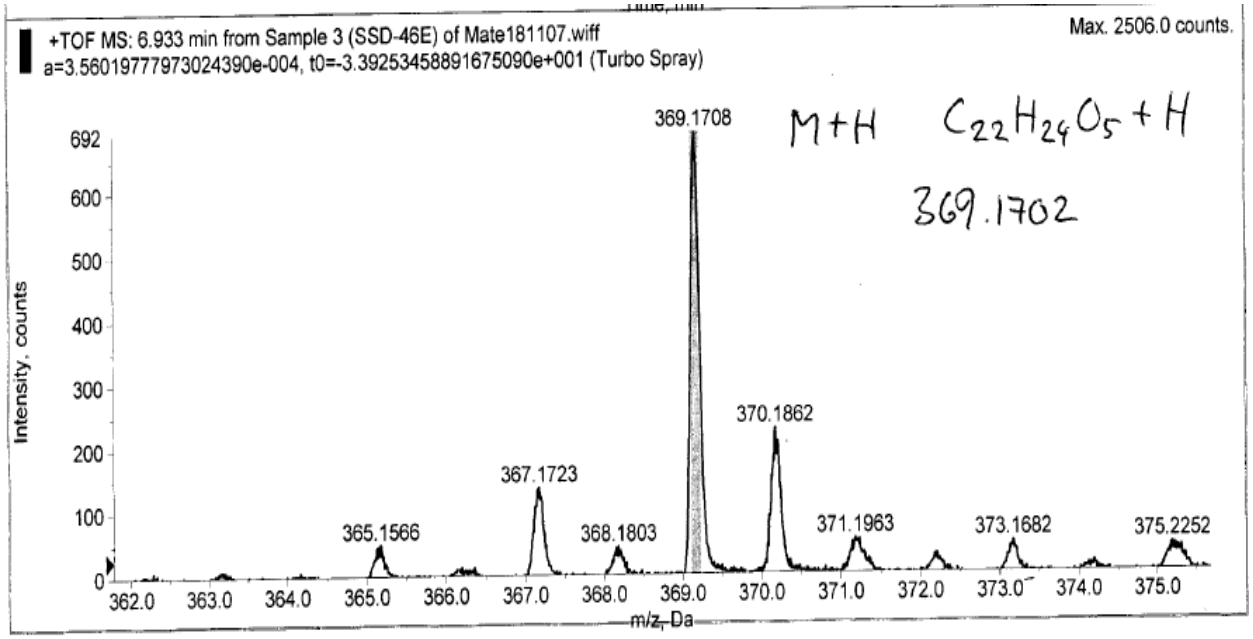
APPENDIX 8D: HSQC Spectrum of Rhodflavononol (**87**) (500MHz; $CDCl_3$)



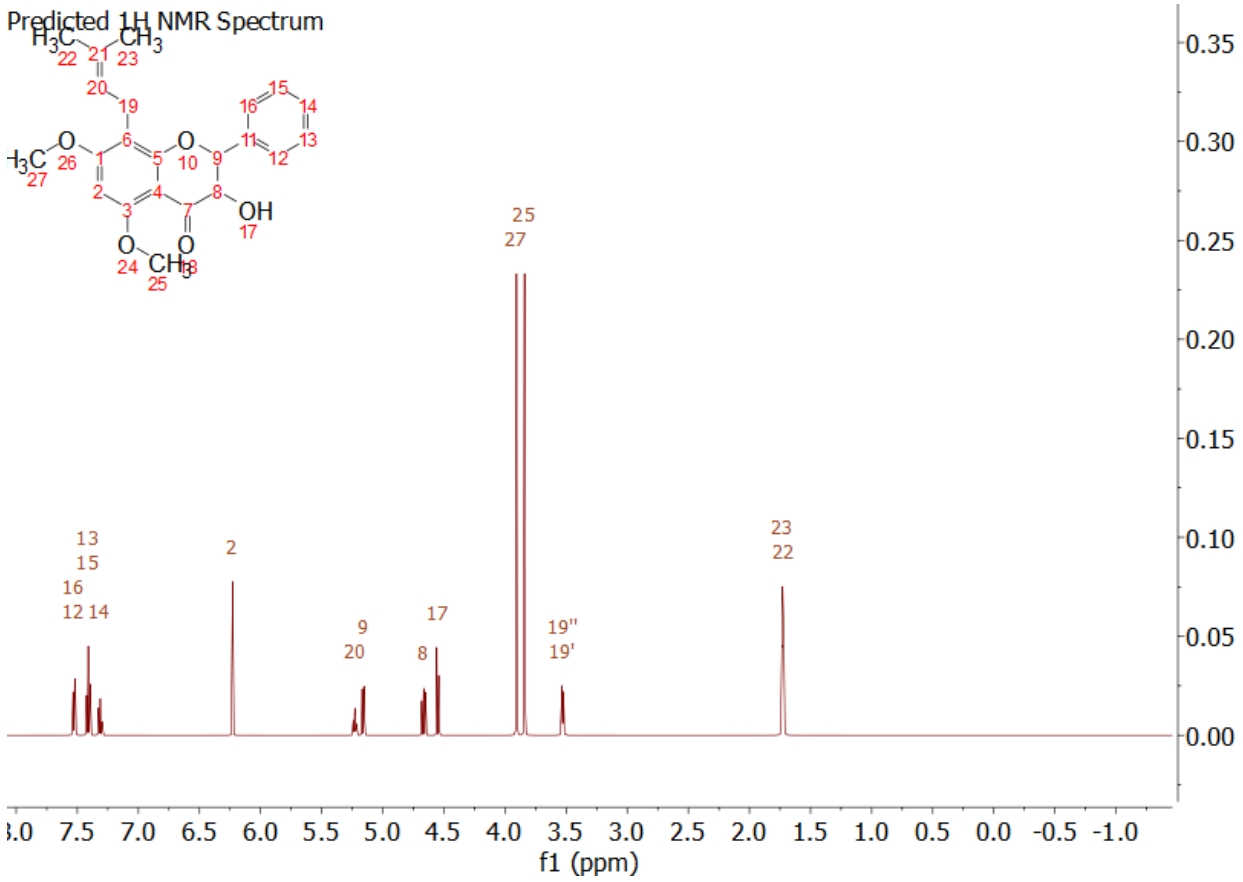
APPENDIX 8E: HMBC Spectrum of Rhodflavononol (**87**) (500 MHz; $CDCl_3$)



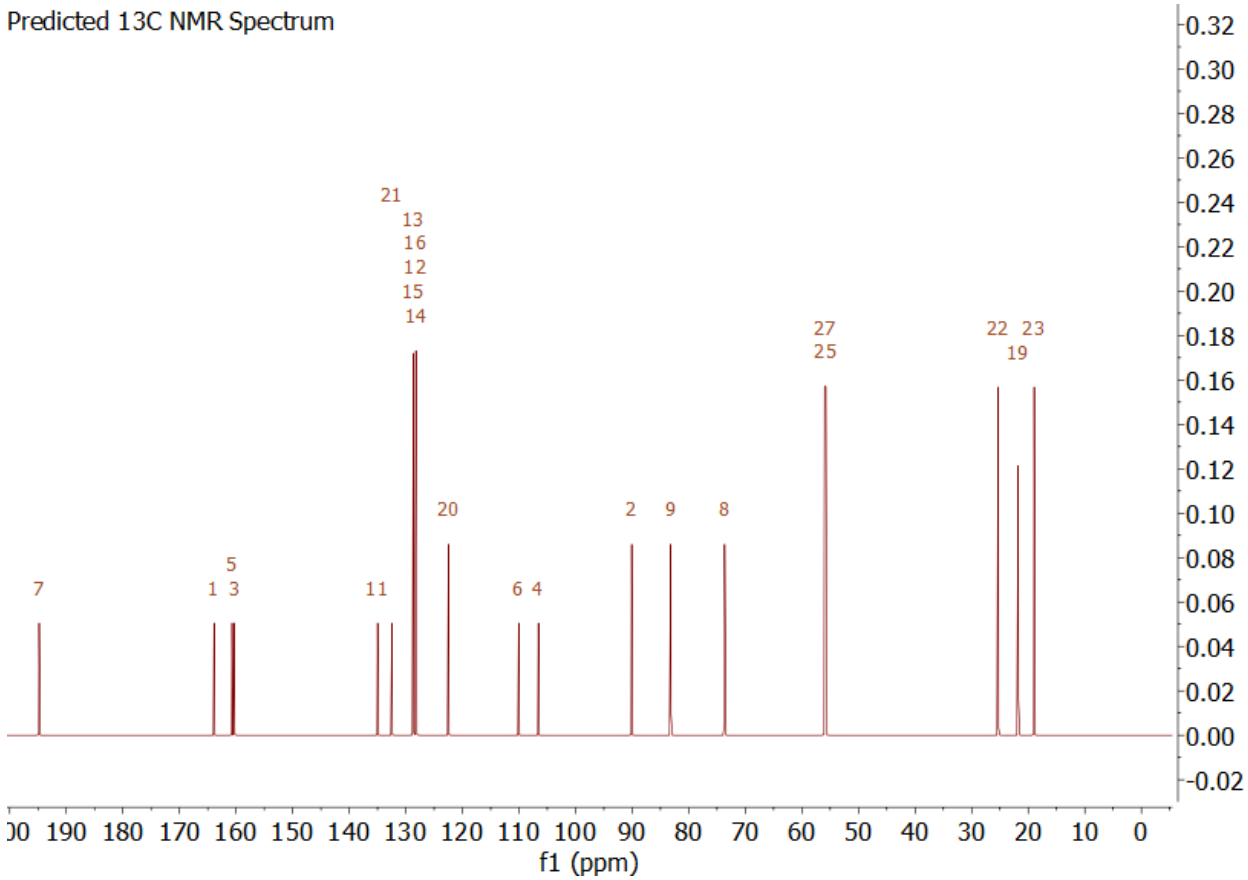
APPENDIX 8F: HRMS Spectrum of Rhodflavononol (**87**)



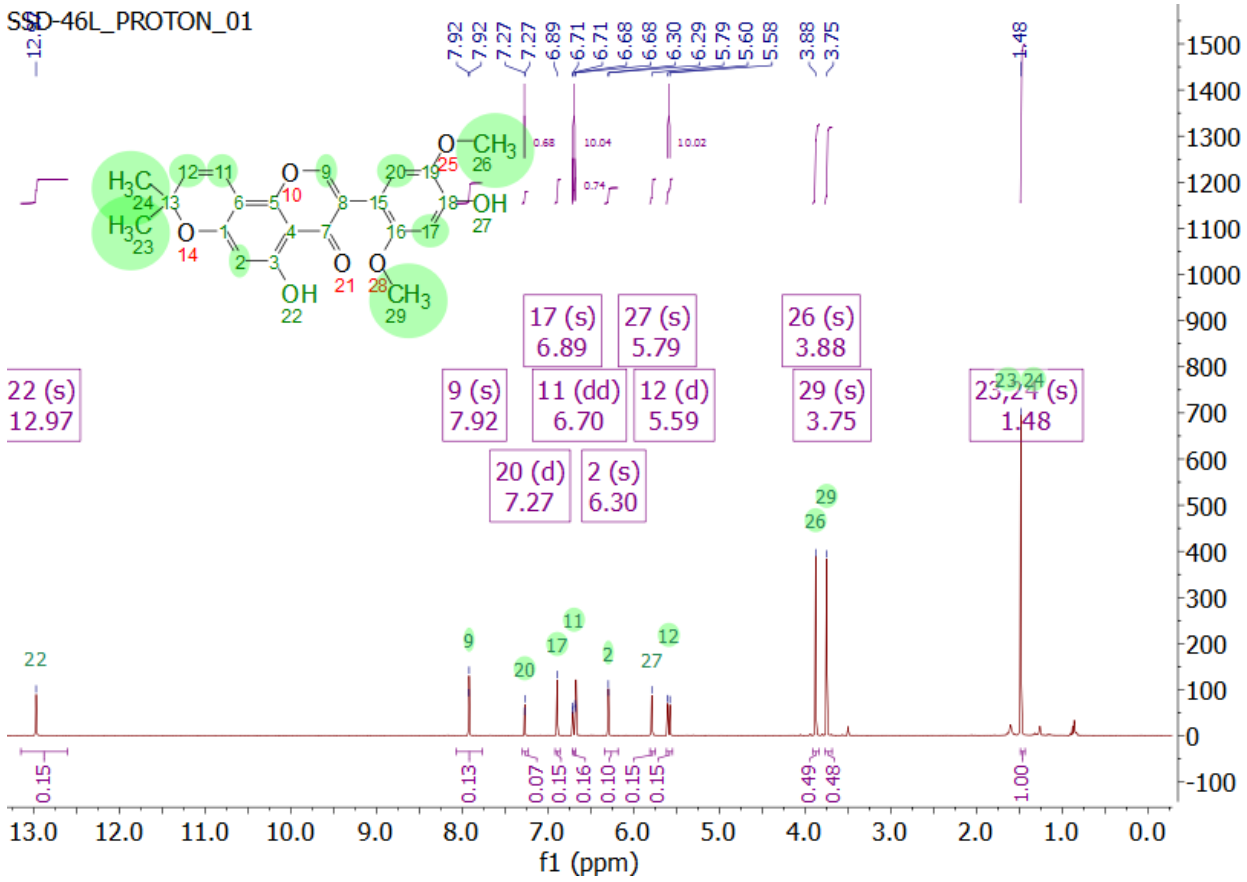
APPENDIX 8G: Predicted ^{1}H NMR Spectrum of Rhodflavononol (**87**)



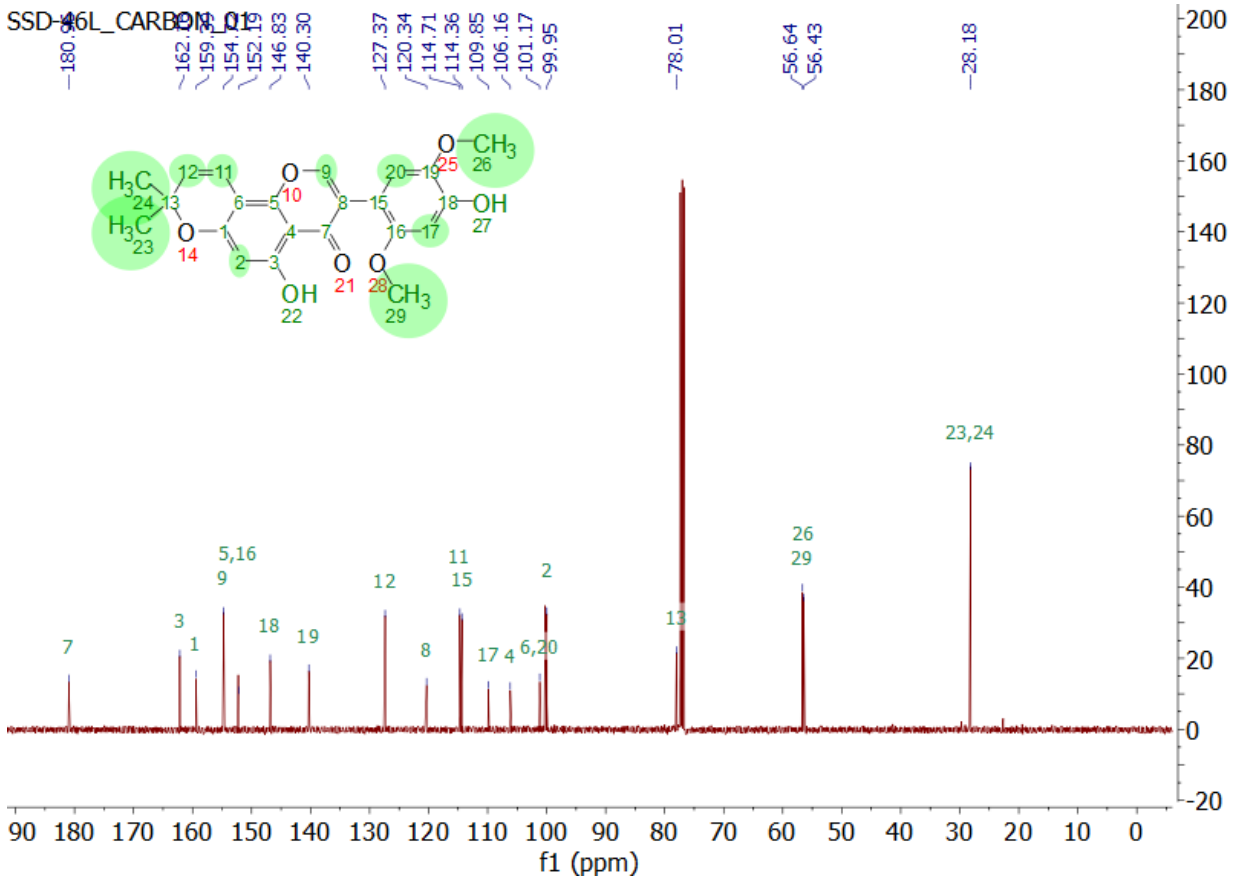
APPENDIX 8H: Predicted ^{13}C NMR Spectrum of Rhodflavononol (**87**)



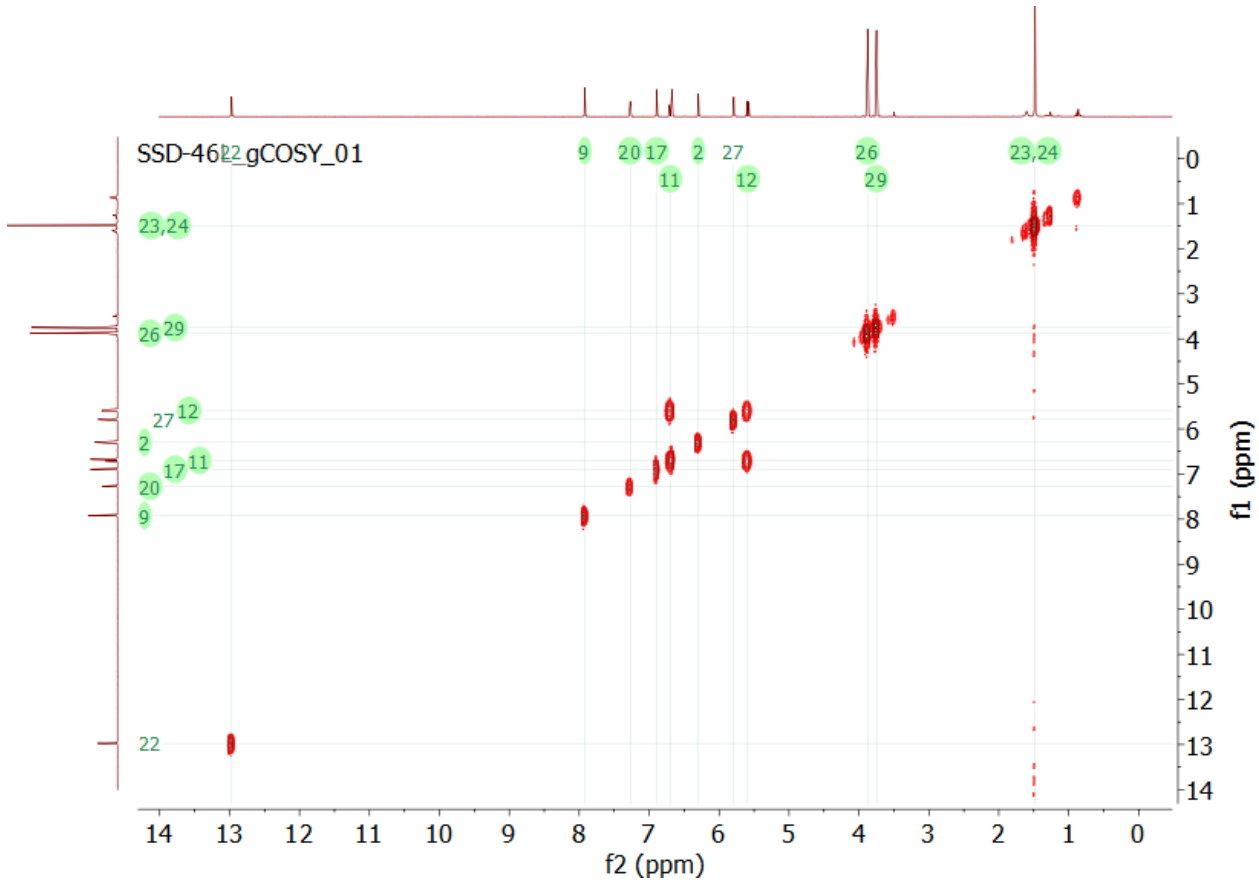
APPENDIX 9A: ^{1}H NMR Spectrum of compound (**49**) (500 MHz; $CDCl_3$)



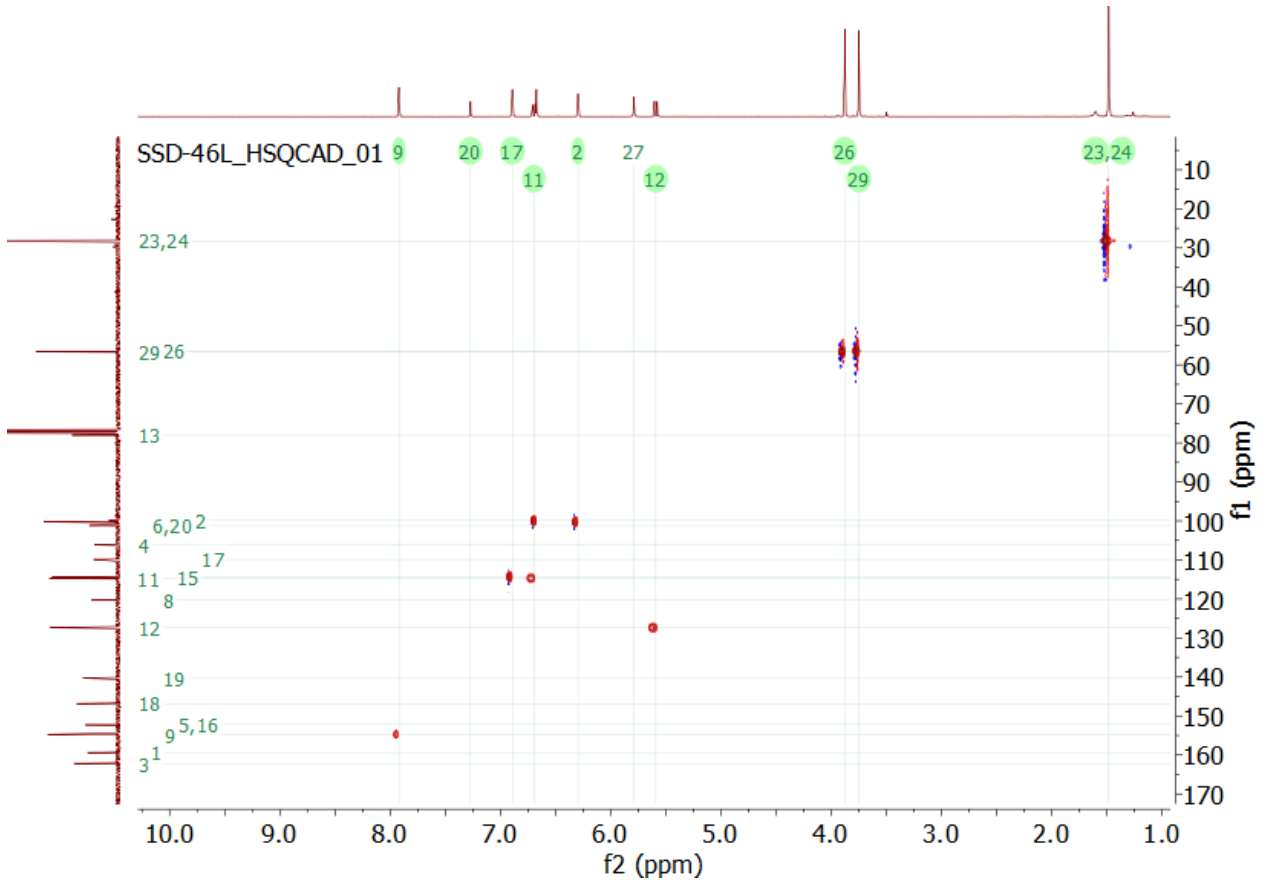
APPENDIX 9B: ^{13}C NMR Spectrum of compound (**49**) (500 MHz; $CDCl_3$)



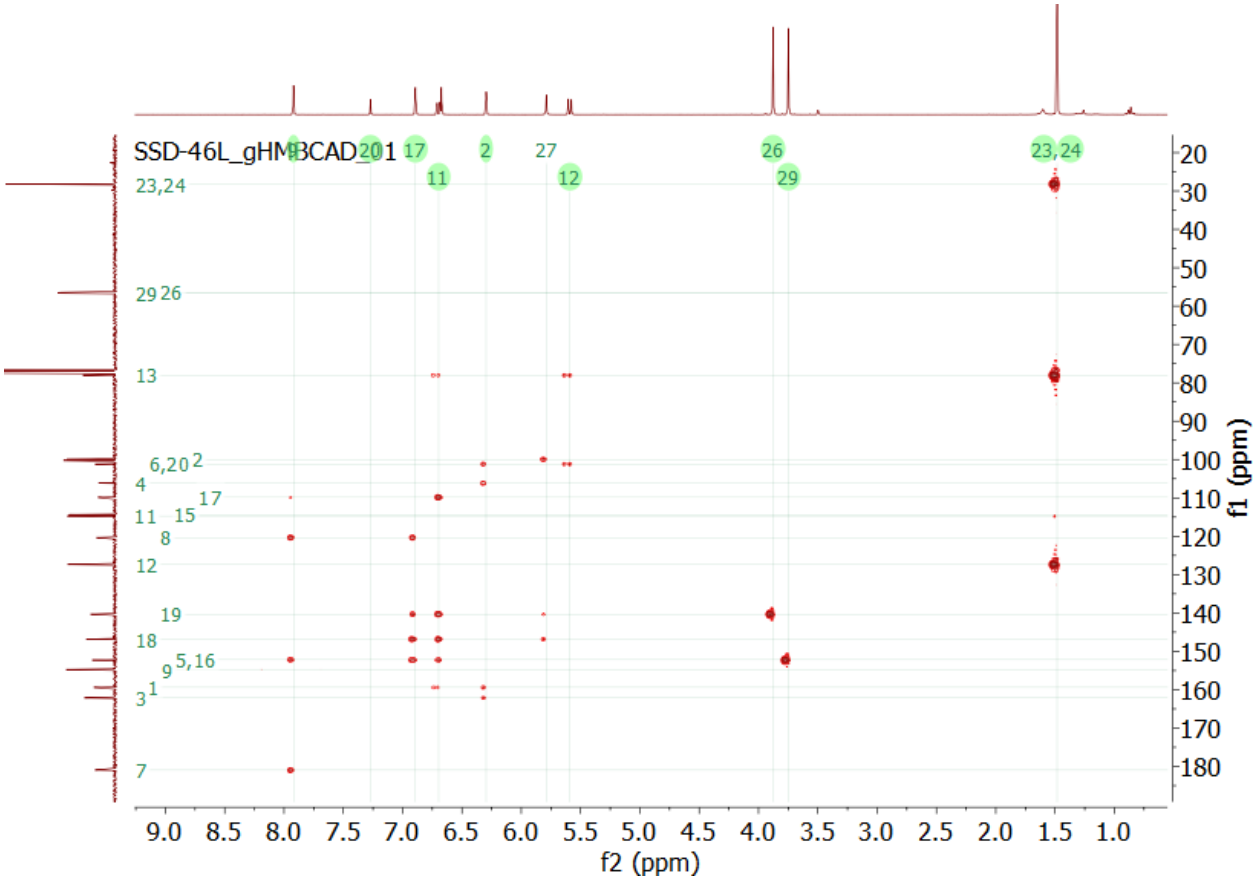
APPENDIX 9C: HH-COSY Spectrum of compound (**49**) (500 MHz; $CDCl_3$)



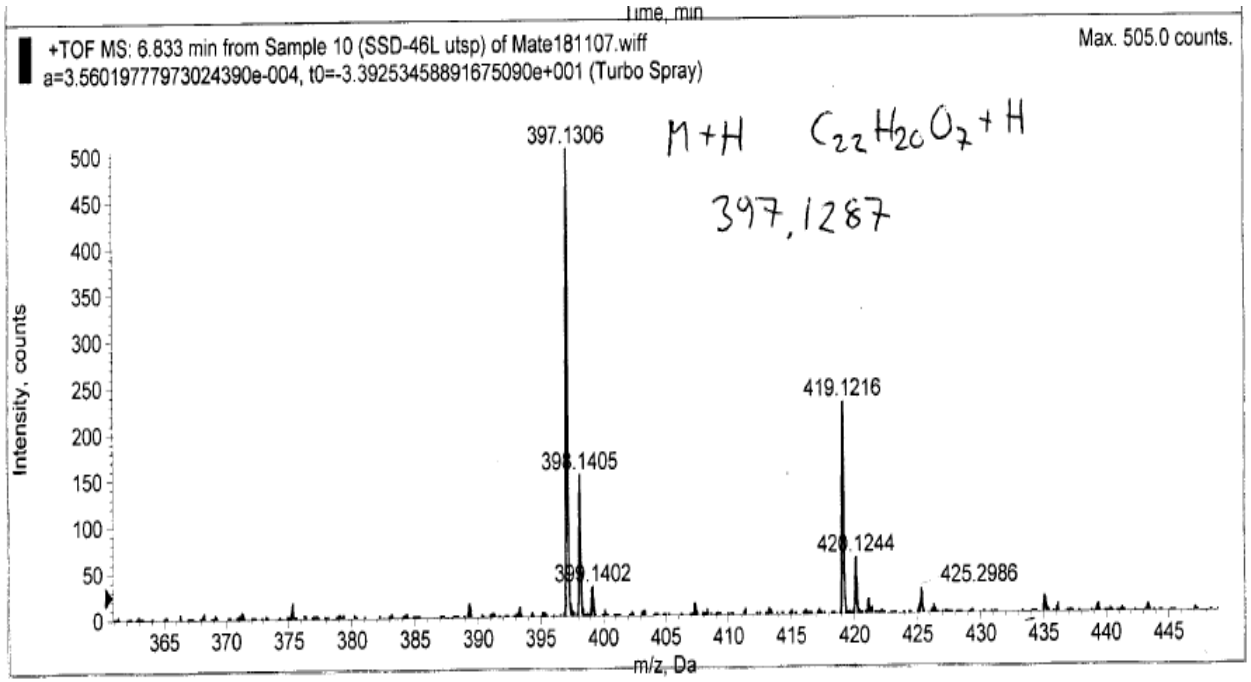
APPENDIX 9D: HSQC Spectrum of compound (**49**) (500 MHz; $CDCl_3$)



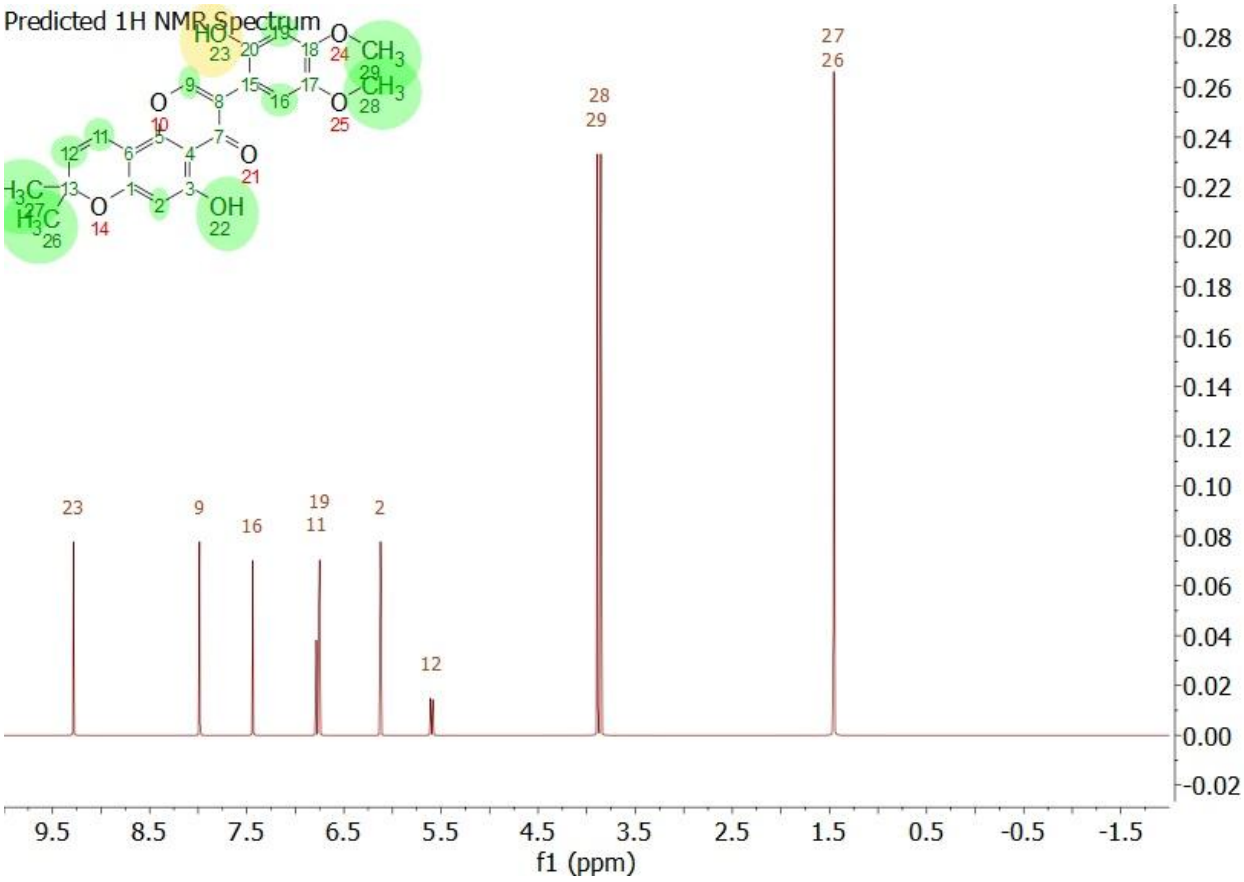
APPENDIX 9E: HMBC Spectrum of compound (**49**) (500 MHz; $CDCl_3$)



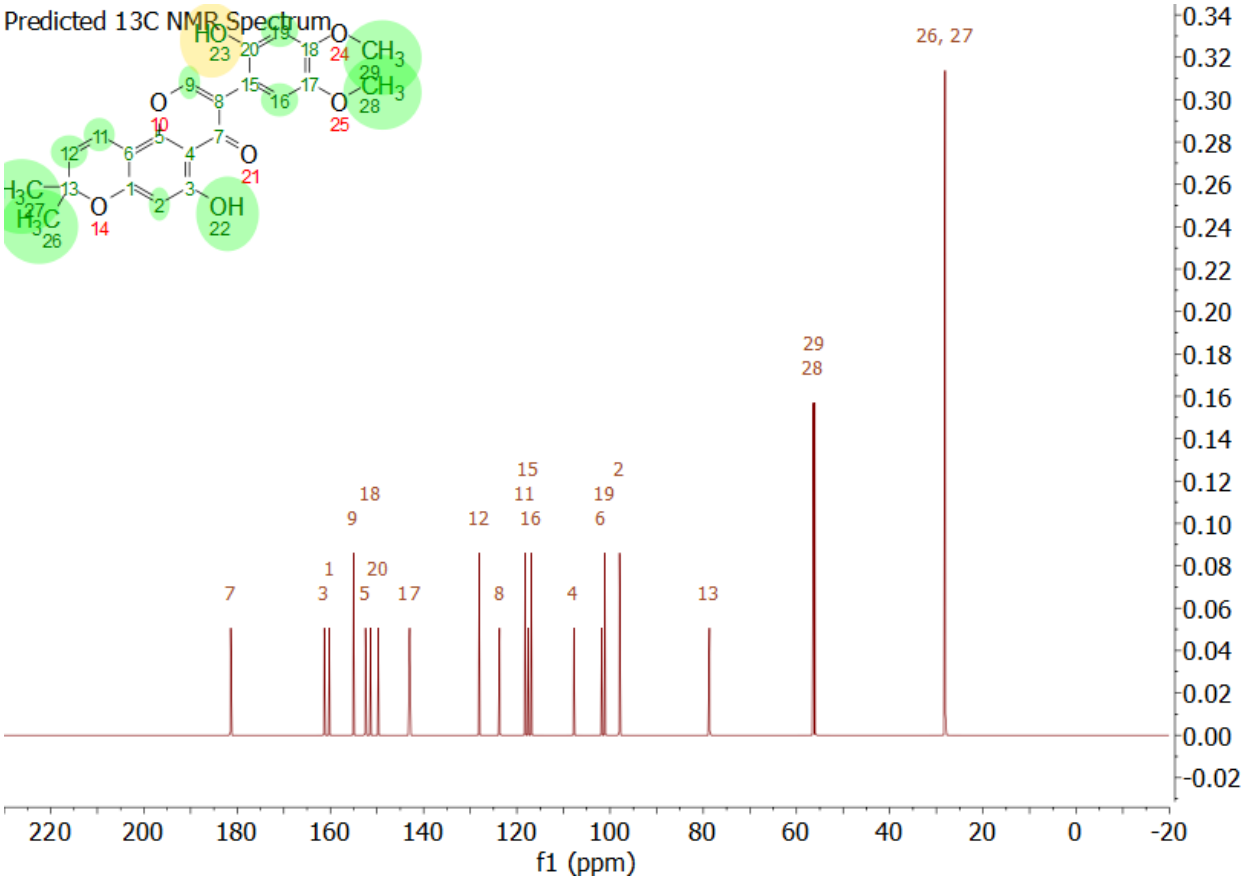
APPENDIX 9F: HRMS Spectrum of compound (**49**)



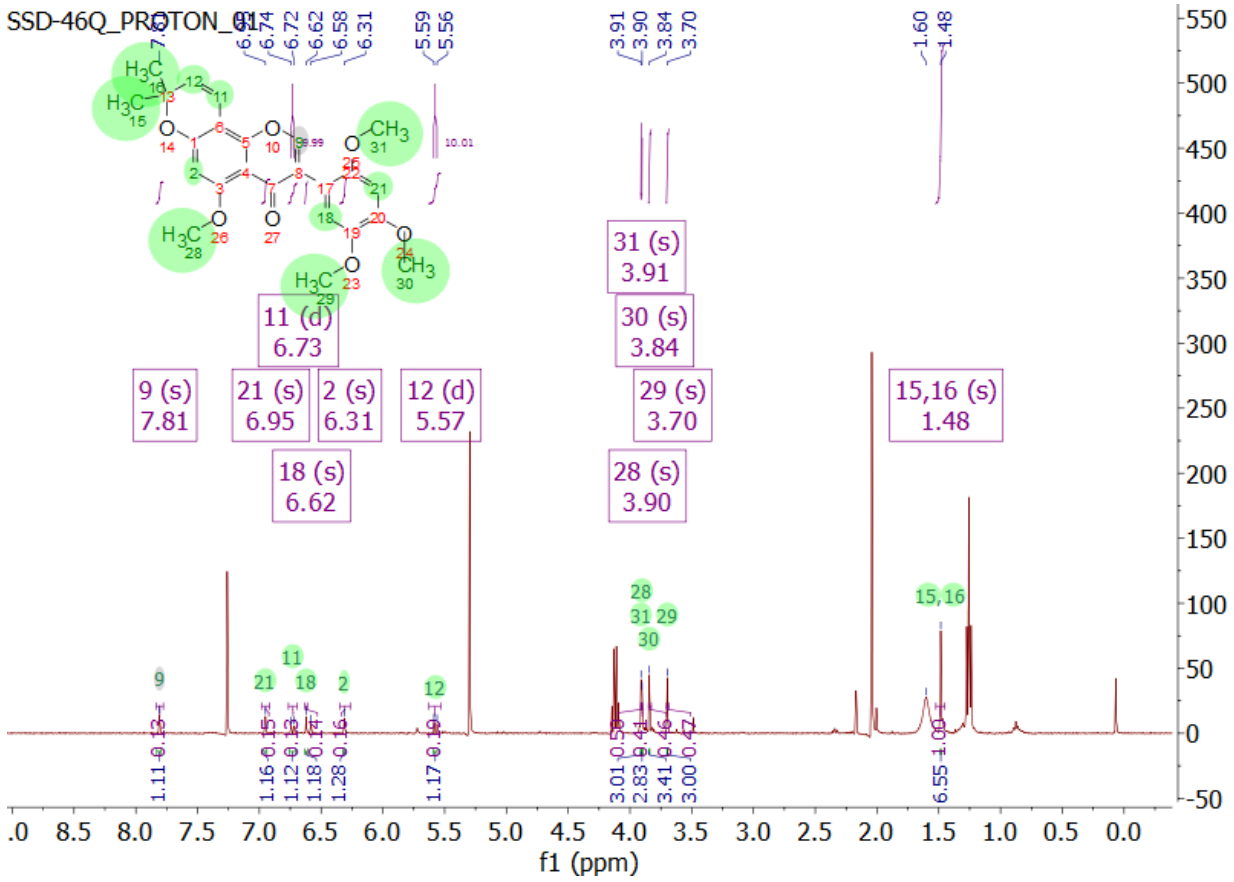
APPENDIX 9G: Predicted[1]H NMR Spectrum of compound (**49**)



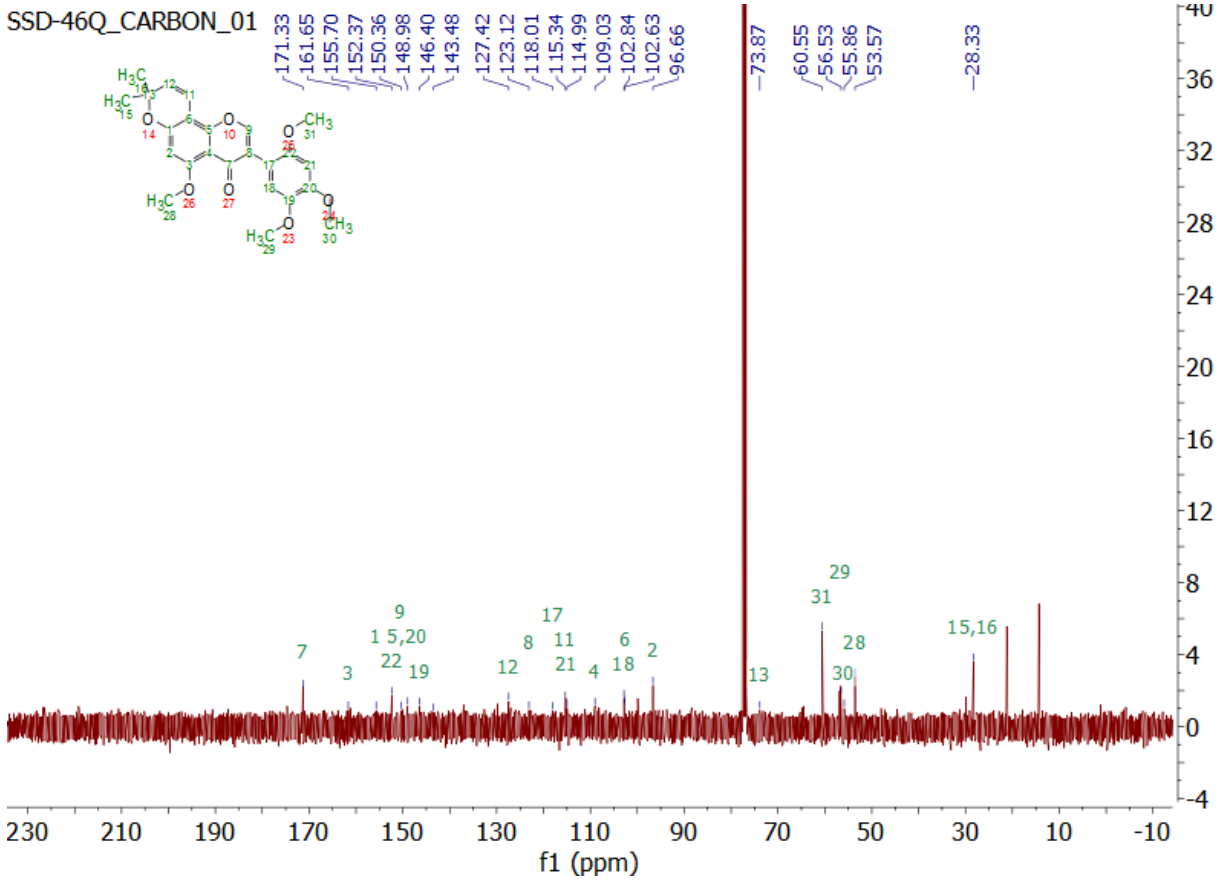
APPENDIX 9H: Predicted^{13}C NMR Spectrum of compound (**49**)



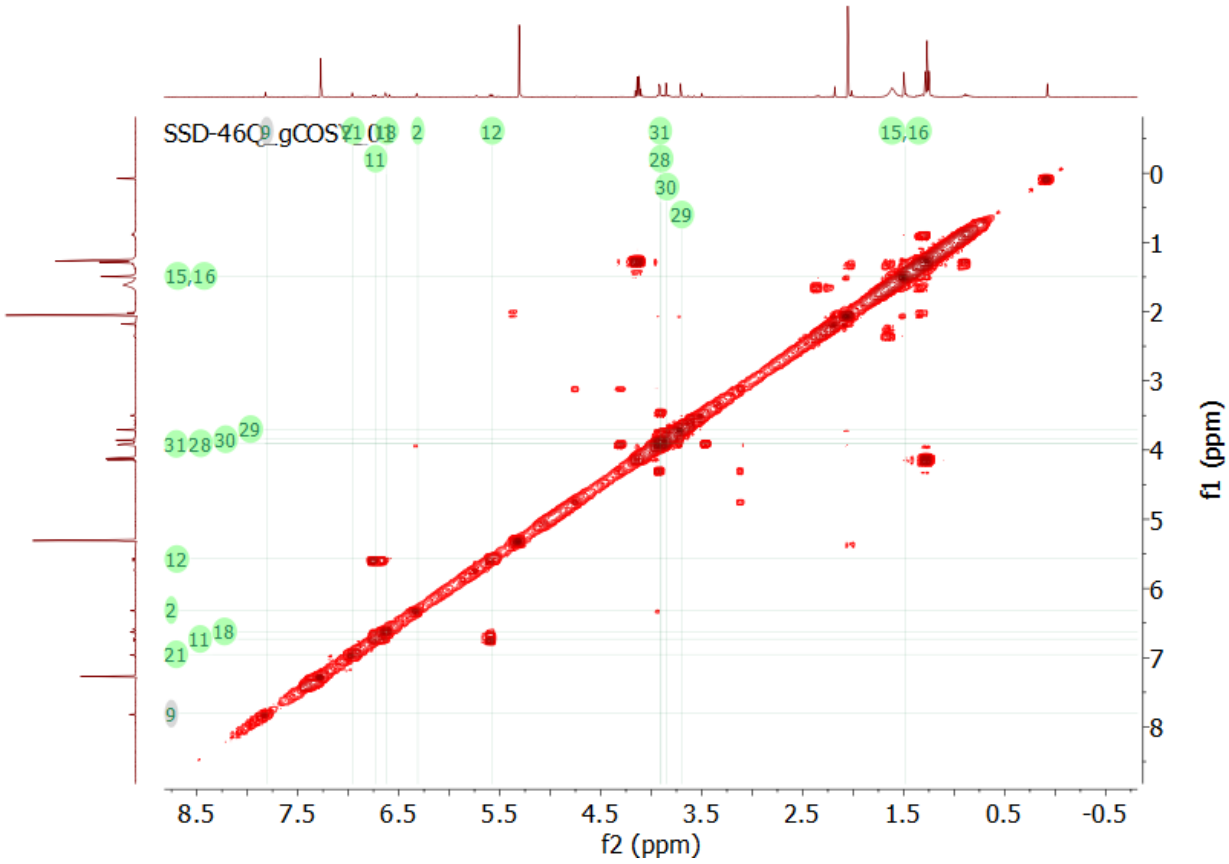
APPENDIX 10A: ^{1}H NMR Spectrum of compound (**50**) (500 MHz; $CDCl_3$)



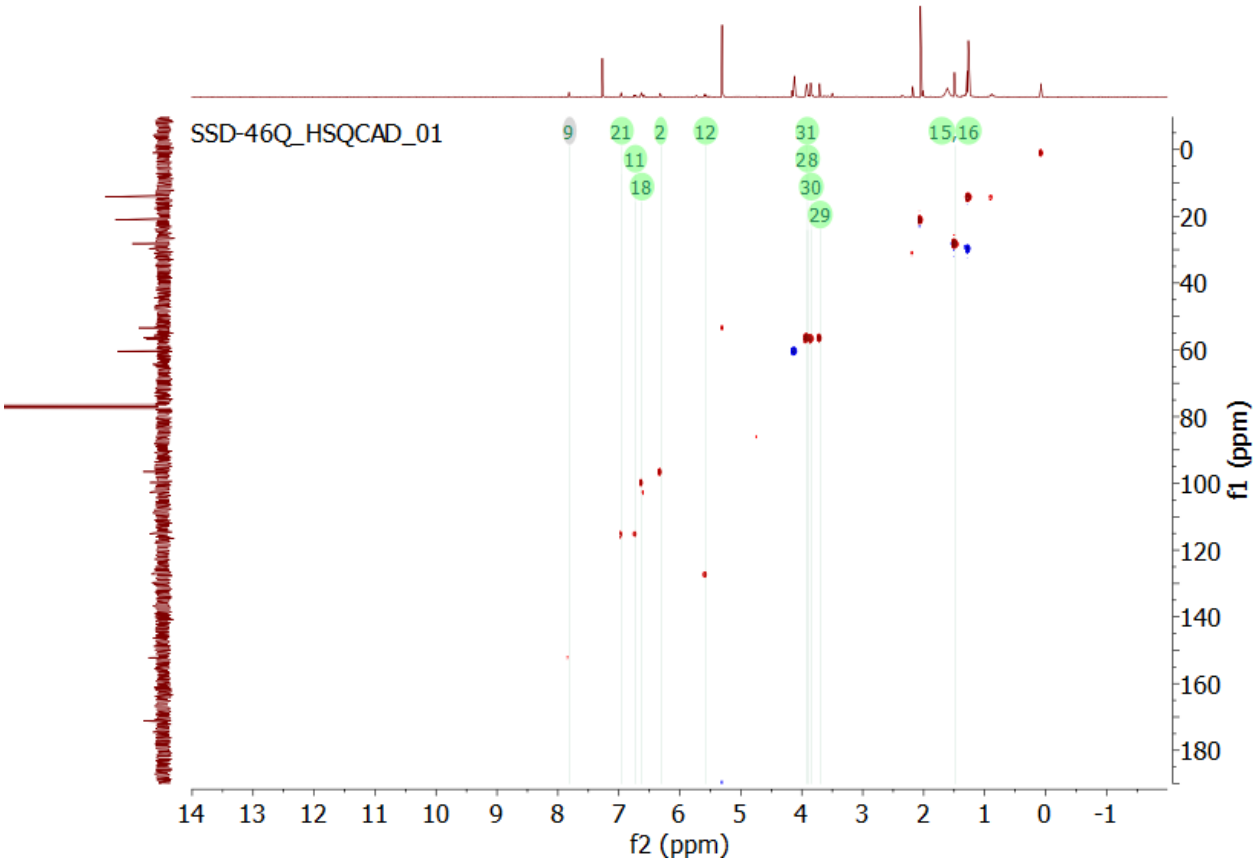
APPENDIX 10B: ^{13}C NMR Spectrum of compound (**50**) (125 MHz; $CDCl_3$)



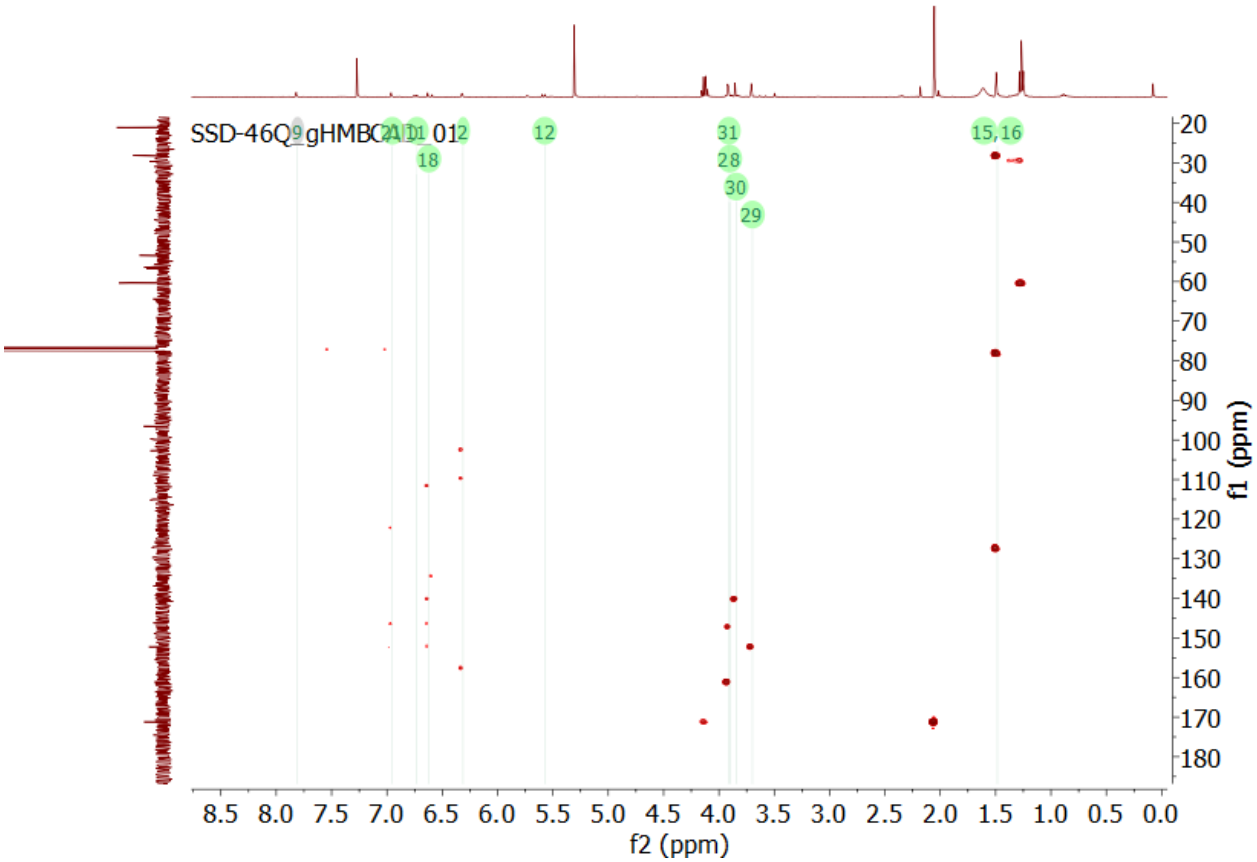
APPENDIX 10C: HH-COSY Spectrum of compound (**50**) (500 MHz; $CDCl_3$)



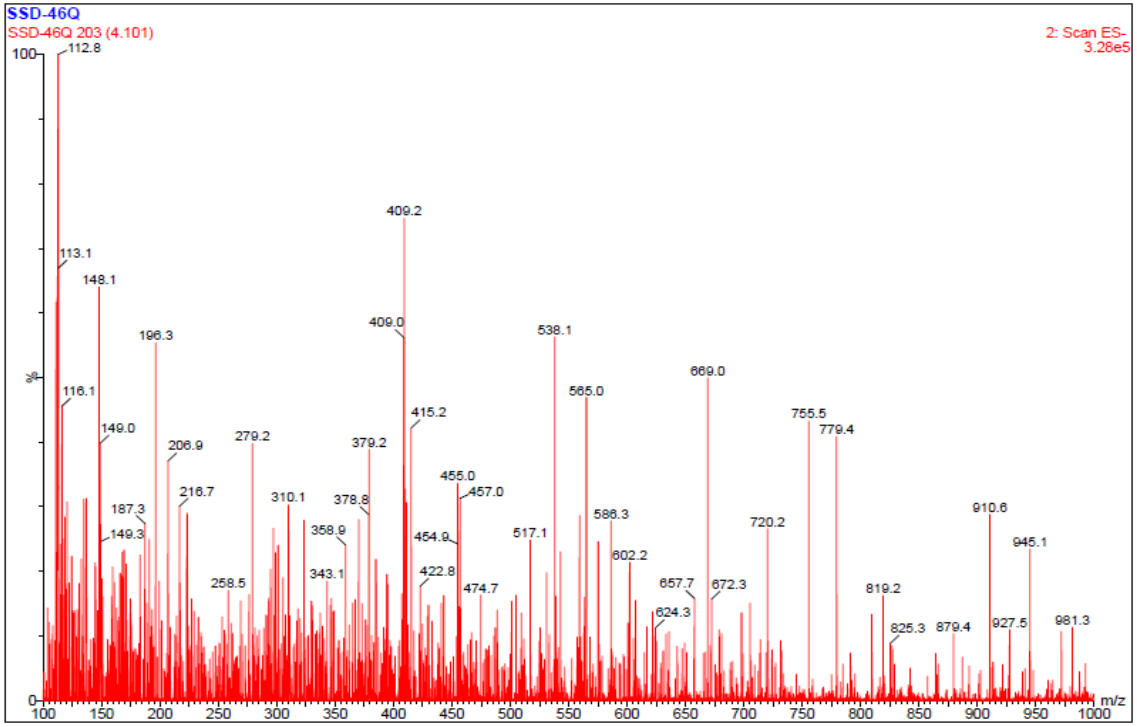
APPENDIX 10D: HSQC Spectrum of compound (**50**) (500 MHz; $CDCl_3$)



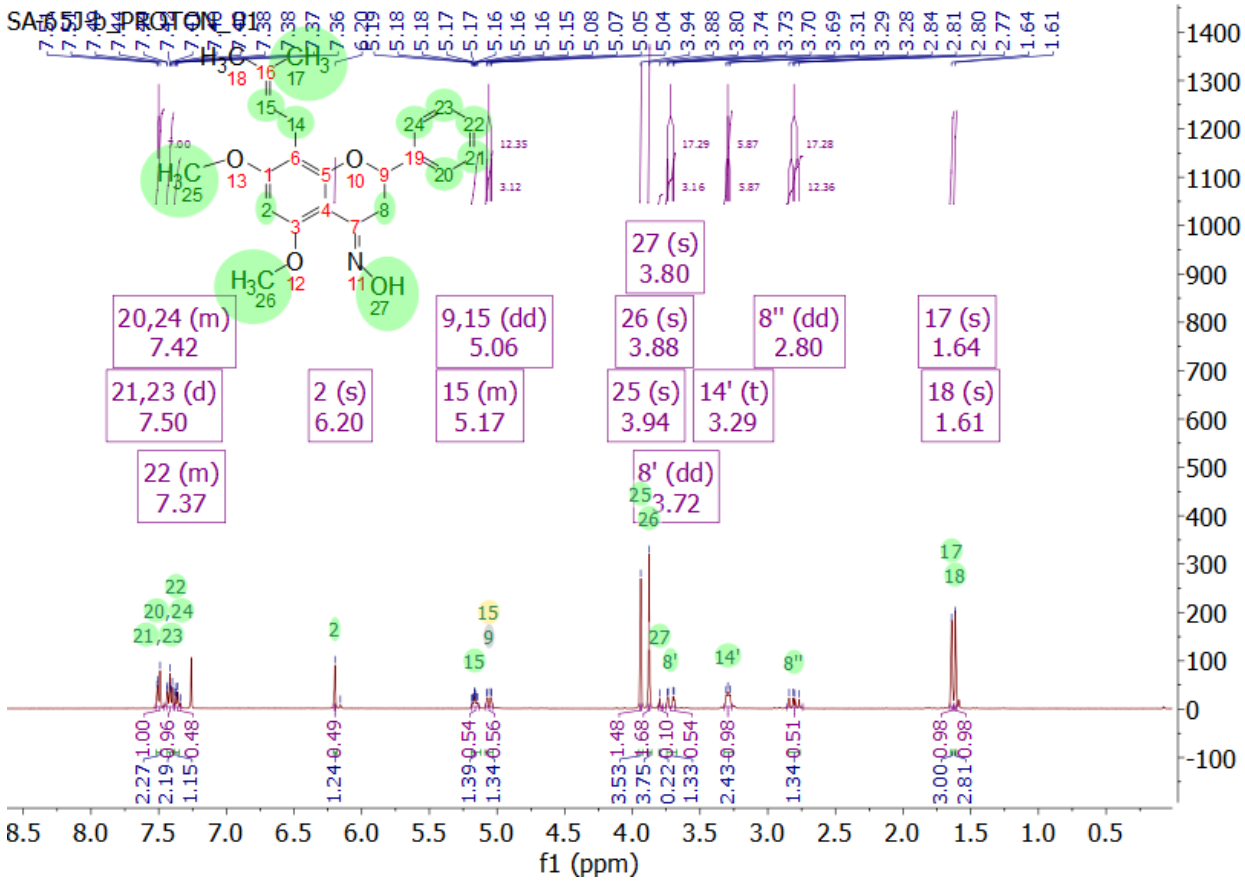
APPENDIX 10E: HMBC Spectrum of compound (**50**) (500 MHz; $CDCl_3$)



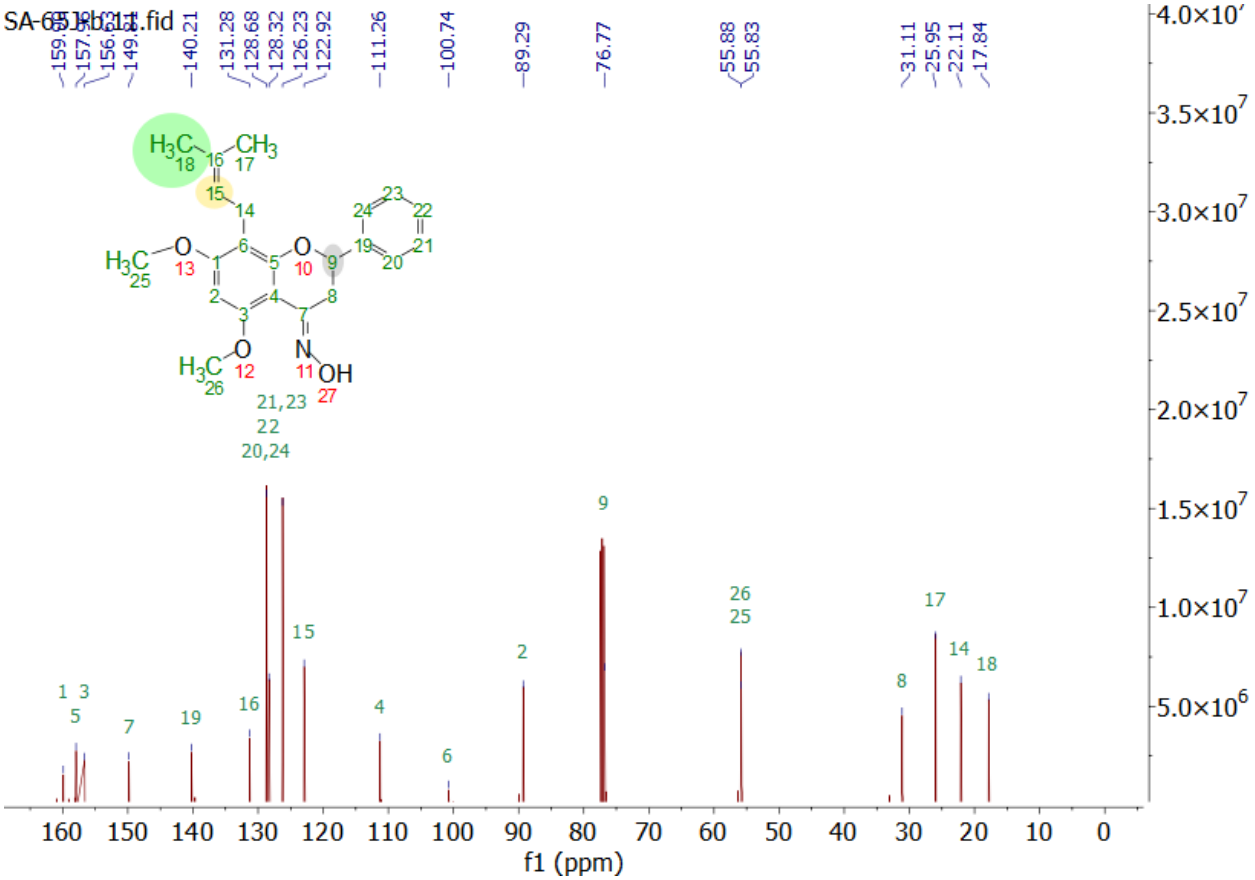
APPENDIX 10F: LCMS Spectrum of compound (**50**)



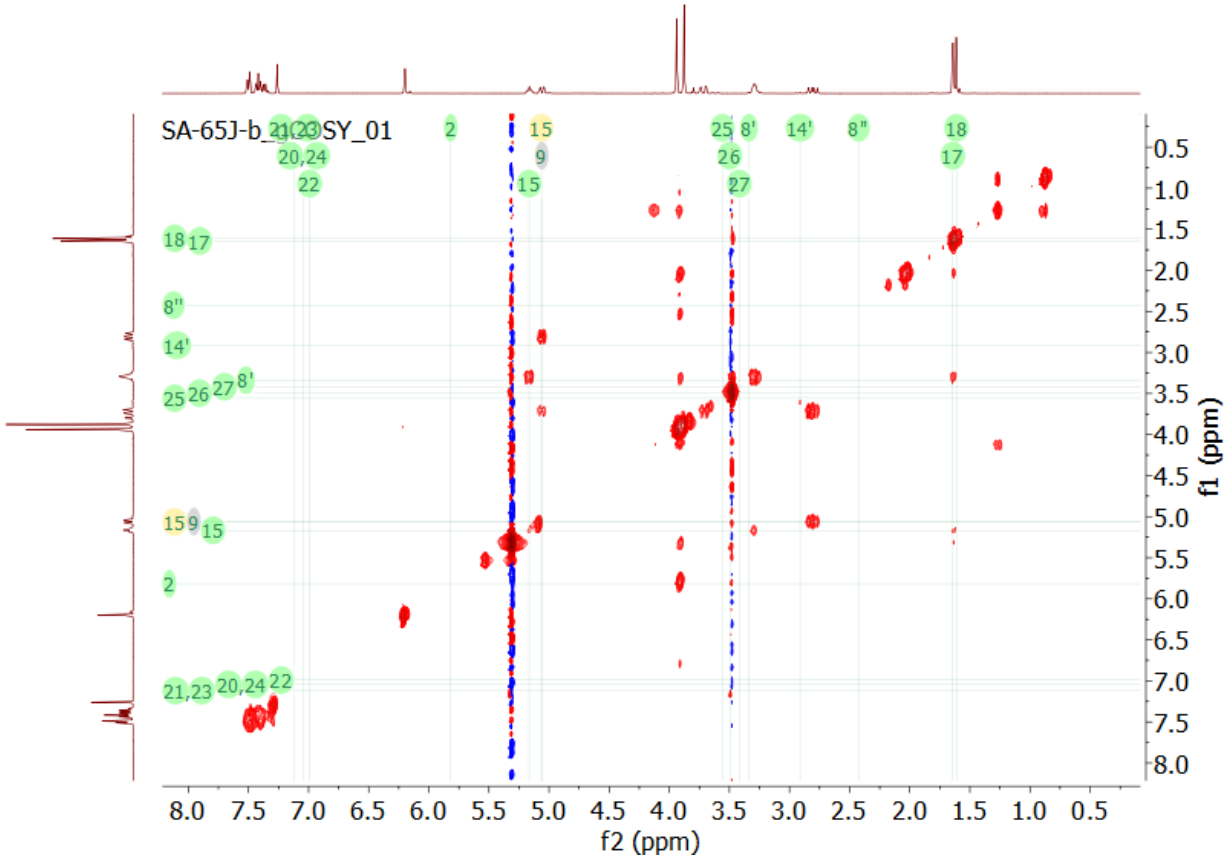
APPENDIX 11A: ^{1}H NMR Spectrum of candidone-oxime (**94**) (500 MHz; $CDCl_3$**)**



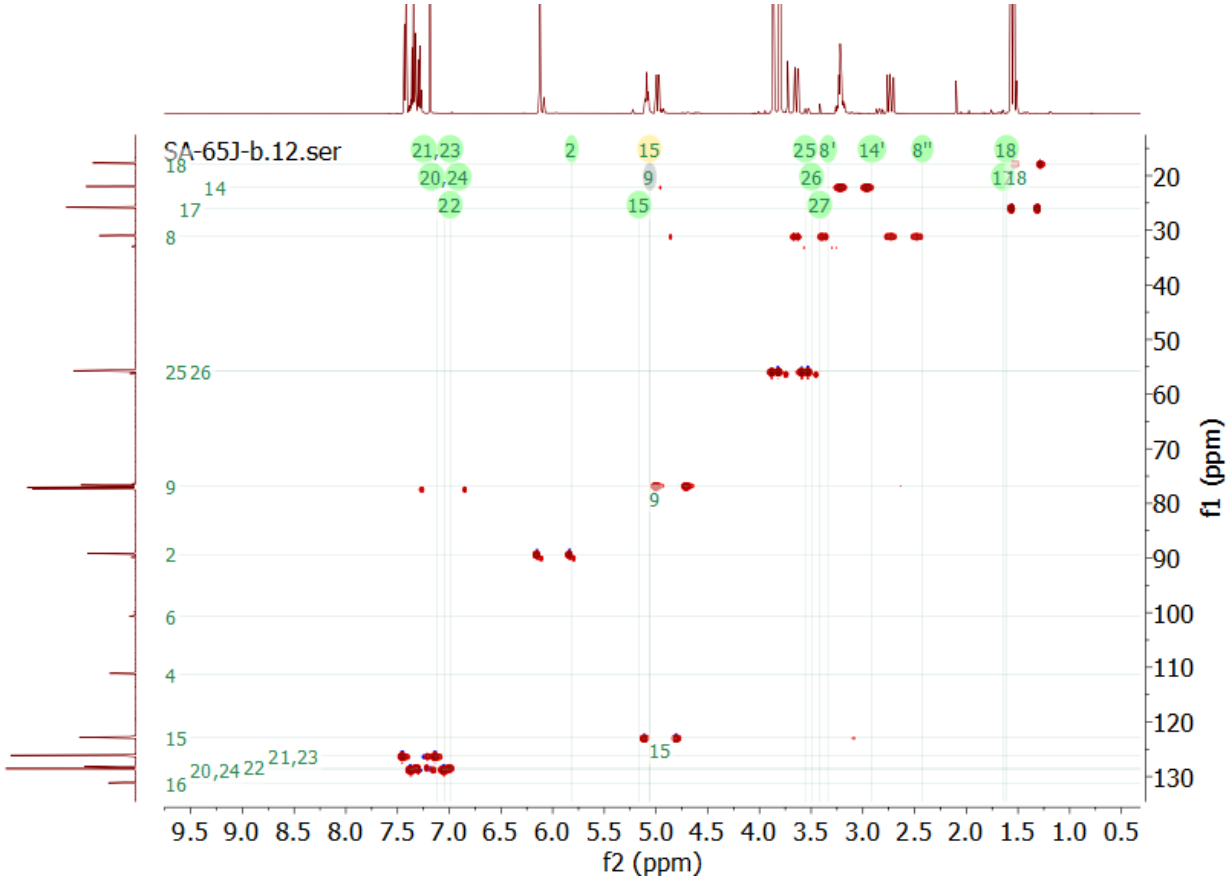
APPENDIX 11B: ^{13}C NMR Spectrum of candidone-oxime (**94**) (125 MHz; $CDCl_3$)



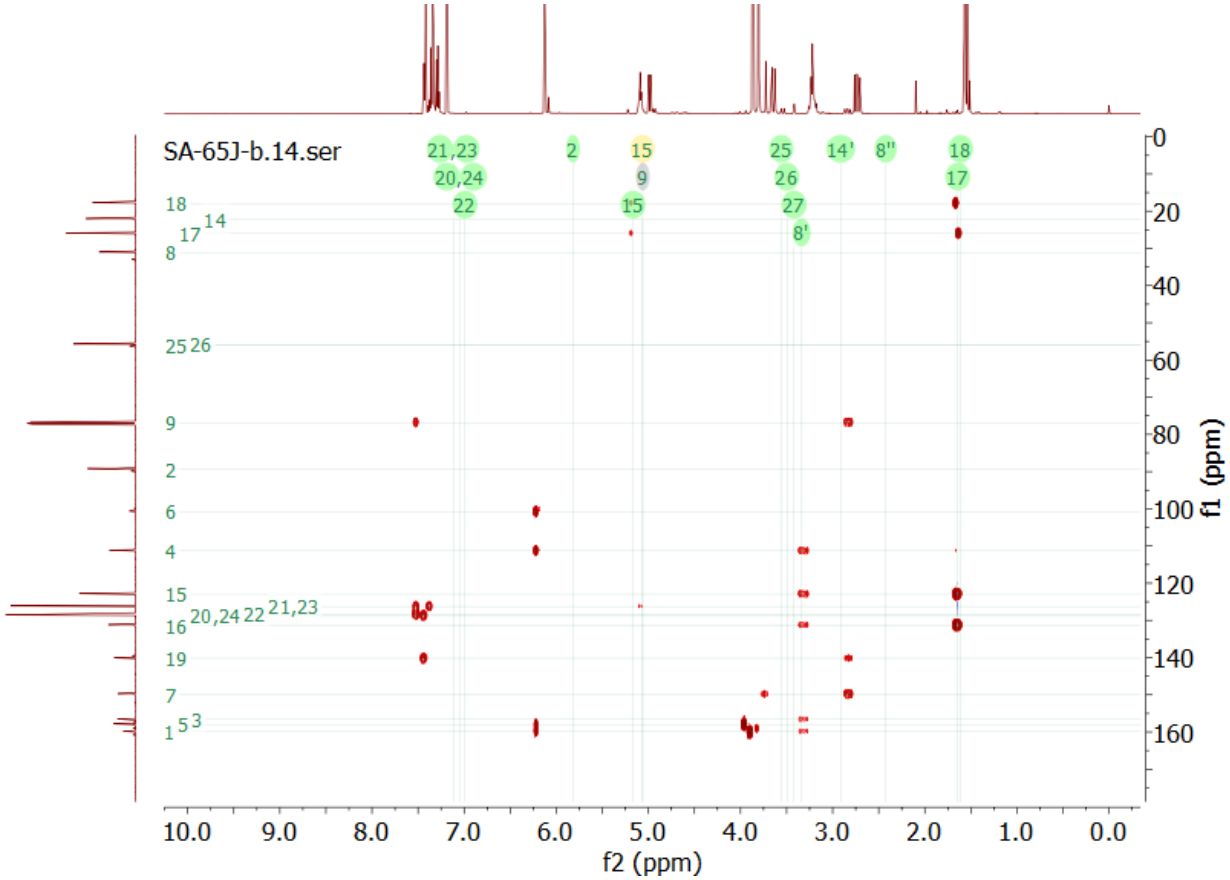
APPENDIX 11C: HH-COSY Spectrum of candidone-oxime (**94**) (500 MHz; $CDCl_3$)



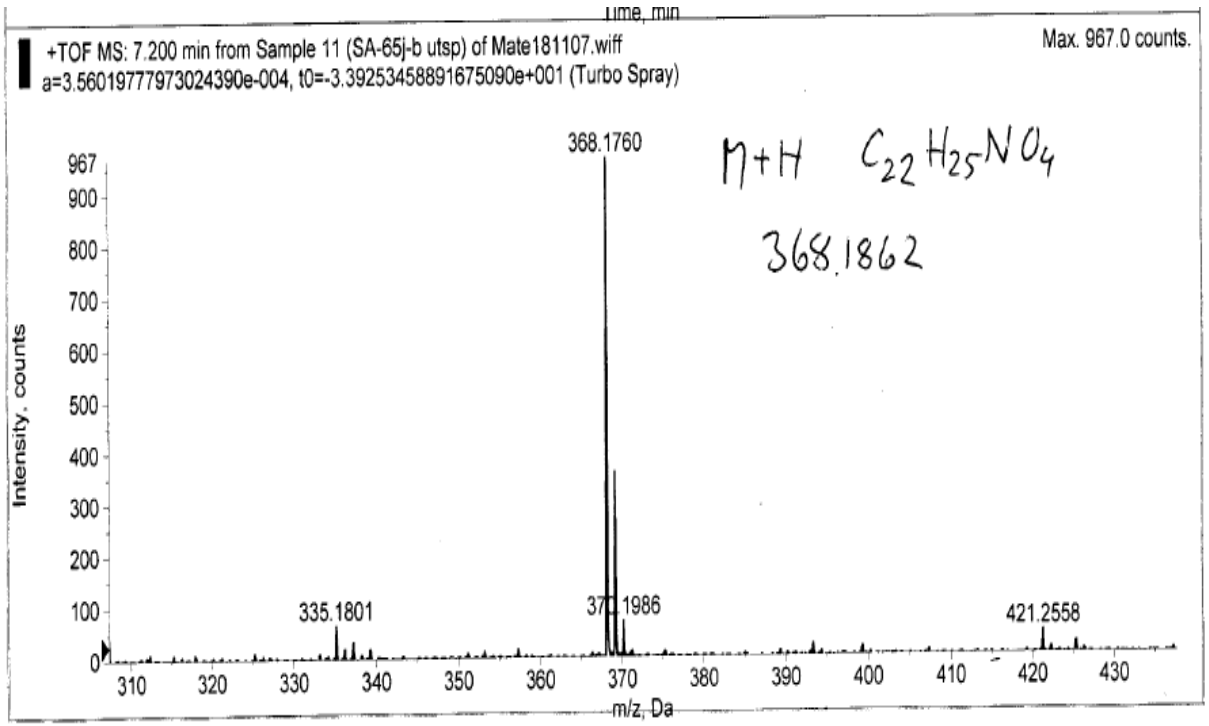
APPENDIX 11D: HSQC Spectrum of candidone-oxime (**94**) (500 MHz; $CDCl_3$)



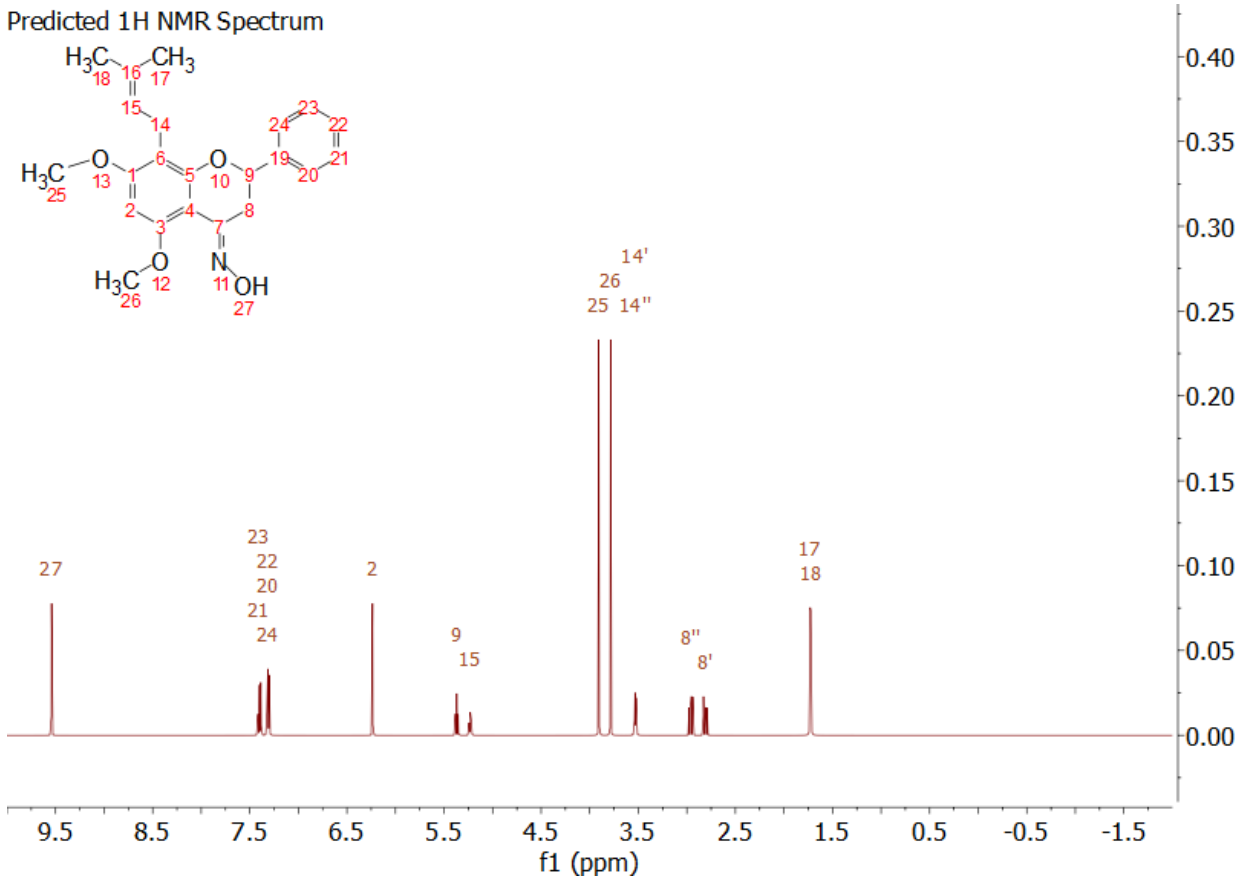
APPENDIX 11E: HMBC Spectrum of candidone-oxime (**94**) (500MHz; $CDCl_3$)



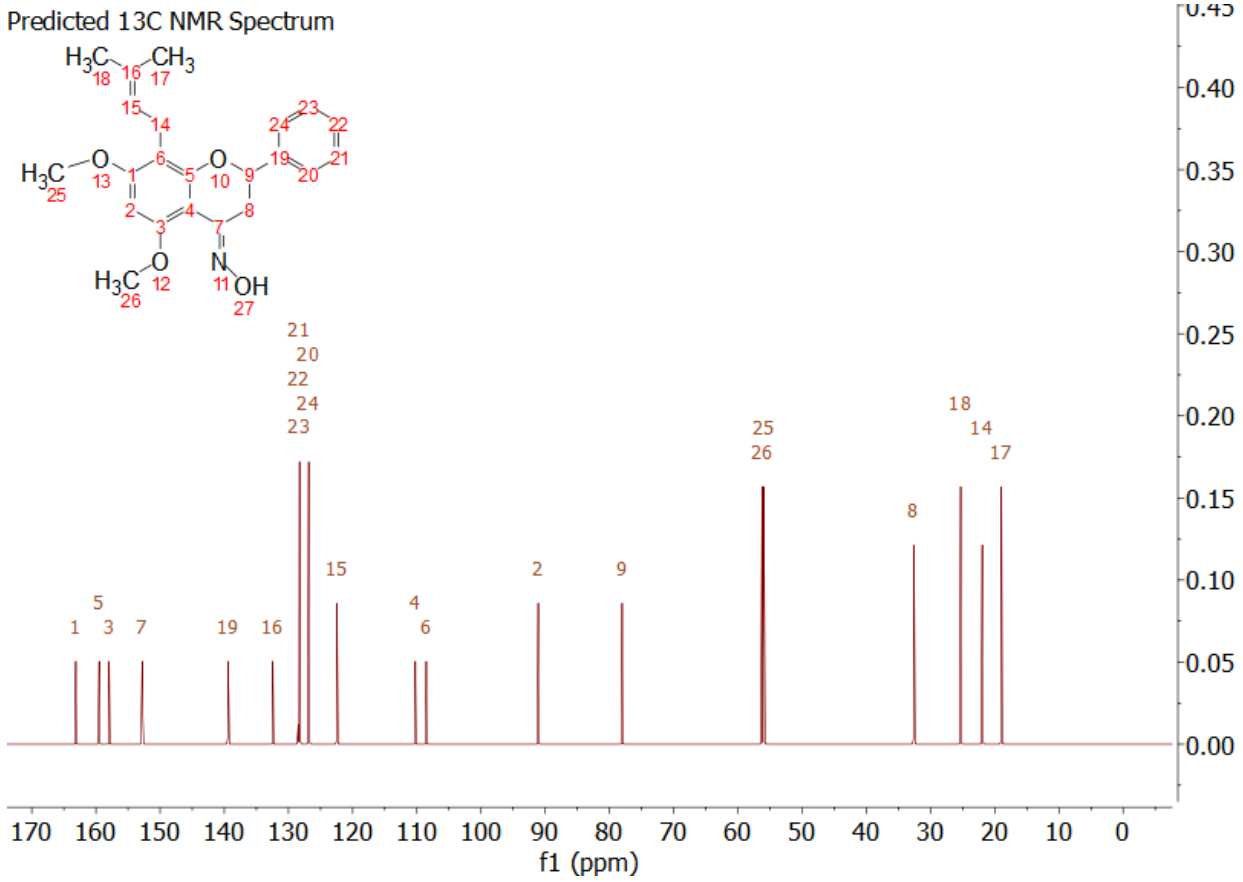
APPENDIX 11F: HRMS Spectrum of candidone-oxime (**94**)



APPENDIX 11G: Predicted ^{1}H NMR Spectrum of candidone-oxime (**94**)



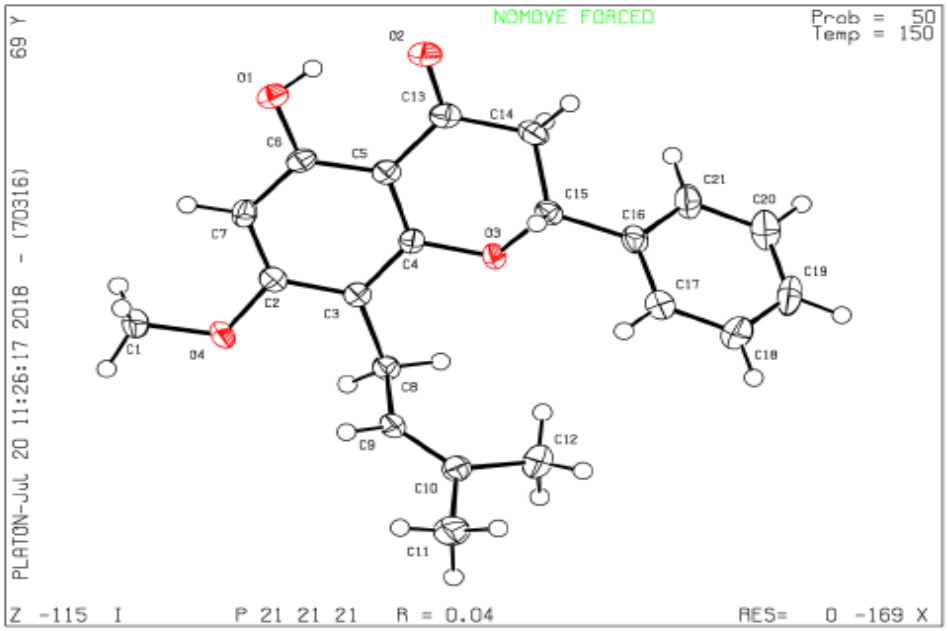
APPENDIX 11H: Predicted ^{13}C NMR Spectrum of candidone-oxime (**94**)



If you wish to submit your CIF for publication in Acta Crystallographica Section C or E, you should upload your CIF via the web. If you wish to submit your CIF for publication in IUCrData you should upload your CIF via the web. If your CIF is to form part of a submission to another IUCr journal, you will be asked, either during electronic submission or by the Co-editor handling your paper, to upload your CIF via our web site.

PLATON version of 14/07/2018; check.def file version of 05/06/2018

Datablock I - ellipsoid plot



APPENDIX 13: Originality Report of This Thesis.

Turnitin Originality Report

Processed on: 26-Nov-2019 16:35 EAT
ID: 1222108187
Word Count: 12029
Submitted: 1

PHYTOCHEMICAL INVESTIGATION OF TEPHROSIA RHODESICA AND TEPHROSIA POLYPHYLLA FOR ANTIPLASMODIAL PRINCIPLES By Paul Mach

Similarity Index	Similarity by Source	
10%	Internet Sources:	9%
	Publications:	2%
	Student Papers:	6%

1% match (Internet from 23-Mar-2015)
http://www.cmd.gov.hk/html/b5/service/hkcmms/vol3/Vol3_pdf_chinese/D_Monographs/049-062_FG_C.pdf

1% match (Internet from 27-Jun-2015)
http://projects.nri.org/adappt/docs/PhytochemistryTephrosia.pdf

1% match (Internet from 28-Dec-2015)
http://www.mdpi.com/1420-3049/19/2/1432/pdf

1% match (Internet from 18-Nov-2013)
http://soccer.55555.to/saposon/jefunited/index_e.htm

1% match (Internet from 06-Aug-2013)
http://www.chem.tamu.edu/rgroup/wooley/chem466/130131%20Giles%20Marco%20Polymer%20Chem%20Guest%20Lecture%20Notes%20for%20Students.pdf

1% match (Internet from 16-Sep-2017)
http://www.mdpi.com/1420-3049/22/9/1514/htm

< 1% match (Internet from 05-Sep-2019)